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Transcranial direct current stimulation: a potential modality for stroke rehabilitation

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Declarations

Statement of Originality

I Jodie Marquez hereby declare that this 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.

Statement of Authorship

I Jodie Marquez hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of this thesis a written statement from each co-author, and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Statement of Ethical Conduct

In addition, ethical approval from the Hunter New England Area Health Service Ethics Committee, and co registration from the University of Newcastle Human Ethics Committee was granted for the clinical studies presented in this thesis. In each instance, participants were required to read an information statement and provide informed written consent prior to the collection of any data.

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List of Abbreviations

Activities of daily living	ADLs
Arterial spin labelling	ASL
Blood-oxygenated dependent signal	BOLD
Brain derived neurotrophic factor	BDNF
Central nervous system	CNS
Cerebrospinal fluid	CSF
Constraint induced movement therapy	CIMT
Contingent negative variation	CNV
Cortical silent period	CSP
Diffusion tension imaging	DTI
Dorsolateral prefrontal cortex	DLPFC
Echo time	TE
Echoplanar imaging	EPI
Electroconvulsive therapy	ECT
Electroencephalogram	EEG
Event related potential	ERP
Food and drug administration	FDA
Fuggyl meyer assessment	FM
Functional electrical stimulation	FES
Functional magnetic radiation imaging	fMRI
Glutamate	Glu
Glutamine + glutamate	Glx
High definition direct current stimulation	HD-tDCS
Inositol	Ins
Intra-cortical facilitation	ICF
Intrinsic connectivity contrast	ICC
Jebsen Taylor function test	JTT
Lactate	Lac
Long term potentiation	LTP
Low field magnetic stimulation	LFMS
Magnetic resonance imaging	MRI
Magnetic resonance spectroscopy	MRS
Montreal cognitive assessment	MoCA
Motor evoked potentials	MEPs
N-acetylaspartic acid	NAA
N-methyl-d-aspartate	NMDA
Paired associative stimulation	PAS
Primary motor cortex	M1

Proprioceptive neuromuscular facilitation	PNF
Purdue peg board test	PPBT
Reaction time	RT
Regional cerebral blood flow	rCBF
Repetition time	TR
Repetitive Transcranial magnetic stimulation	rTMS
Resting membrane threshold	RMT
Resting state functional Magnetic resonance imaging	Rs-fMRI
Short interval intra-cortical inhibition	SICI
Simple reaction time	SRT
Supplementary motor area	SMA
Theta burst stimulation	TBS
Total creatin	Cr+Pcr
Transcranial alternating current stimulation	tACS
Transcranial direct current stimulation	tDCS
Transcranial magnetic stimulation	TMS
Transcranial random noise stimulation	tRNS
Voxel-based morphometry	VBM
Wolf motor function test	WMFT

Thesis Abstract

Transcranial Direct Current Stimulation (tDCS) is a form of non-invasive brain stimulation which has been investigated in a broad range of neuropsychiatric conditions and as a method to modulate cognitive performance in healthy individuals. It is generally accepted that the main mechanism by which tDCS modulates brain function is via a neural membrane polarization shift which can, in turn, lead to diverse changes in single neuron, synaptic and network activity (Peterchev, Wagner et al. 2012). However, the direction of polarization shift is sensitive to the stimulation dose, the state of brain activity at the time of stimulation and individual anatomy (Bikson and Rahman 2013). This results in a large inter individual variability to the neurophysiological and behavioural response to tDCS. Given the simplicity of tDCS and the complexity of brain function, we sought to unveil some of the physiological mechanisms underpinning the effects of tDCS in order to better our understanding of the variability in response to tDCS and to allow us to predict those most likely to respond. Ultimately our objective was to direct the translation of the research evidence into therapeutic applications of tDCS for stroke patients.

The aim of this research was to determine the potential application of tDCS in the stroke population. At the commencement of this PhD research project, keen interest in the use of tDCS as a potential therapeutic tool in neuromotor conditions, such as stroke, was emerging. As tDCS is portable, relatively inexpensive, free from major adverse effects, and easily applied concurrently with other interventions, it is ideally suited for use in stroke rehabilitation therapy. The goal of tDCS in stroke is to increase cortical excitability of the lesioned hemisphere and/or reduce excitability on the non-lesioned hemisphere to restore interhemispheric balance (Mordillo-Mateos, Turpin-Fenoll et al. 2012).

The vast majority of literature investigating tDCS has been conducted in young, healthy subject. As stroke patients are typically more senior and have age related changes in cortical structure, function and excitability, we began our investigation into the functional and physiological effects of tDCS in a healthy, aged population. We found that the hemispheres responded differently to tDCS and the response appeared to be task specific, but it was not mediated by age. However, a subsequent multimodal imaging study did not support these findings and failed to reveal a difference when tDCS was applied to the dominant or non-dominant hemisphere but showed that the effects were diffuse and determined by the type of stimulation.

In a systematic review of the stroke literature we synthesised the evidence from 15 studies and confirmed the safety and acceptability of this modality in the stroke population.

We concluded that tDCS may be effective in enhancing motor performance, atleast in the short term. Those most likely to benefit were patients with chronic stroke and/or mild to moderate impairments. However these positive findings were not consistent across all studies and the size of the treatment effect was at best modest and may not translate to clinically meaningful change for some or all patients. We used this evidence to conduct a randomised controlled trial in chronic stroke patients and found that neither anodal nor cathodal stimulation resulted in statistically significant improvement in upper limb performance. A secondary analysis was performed and identified that those with moderate or severe disability responded positively to cathodal stimulation with improved gross motor function.

This thesis, in conjunction with the rapidly growing body of evidence in this field, highlights the inconsistency in the effects of tDCS at both an intraindividual level and between subjects, and the transient nature of these effects which limits the clinical value of this intervention. Further scrutiny of the mechanisms underpinning the effects of tDCS is required for the rational advancement of tDCS as a clinical modality in stroke rehabilitation.

Chapter 1: Introduction

1.1 History of transcranial stimulation

Investigation into the merits of non-invasive brain stimulation as a therapeutic tool has been gaining momentum over the past two decades. However use of this modality is not a contemporary concept. Application of electrical currents to modify brain function was first documented centuries ago. Reports exist of the physician to the Roman Emperor Claudius placing live torpedo fish over the scalp to deliver an electric shock to relieve headache (Largus 1959 cited in: Brunoni, Nitsche et al. 2012), as well as the use of electric catfish in an attempt to cure epilepsy (Priori 2003). The invention of the electric battery in the 18th century led to more systematic applications of electricity to evoke physiological effects. The first widespread therapeutic use of cranial electrical stimulation was initially documented in 1902 where it was applied to treat sleep disorders and depression (Schlaug, Renga et al. 2008).

From this early research several differing electric current modalities have emerged. The term transcranial electrical stimulation encompasses all forms of electrical currents applied to the brain for experimental or therapeutic purposes using at least one scalp electrode or magnetic coil (Bikson, Dmochowski et al. 2013). Variations in dose, including electrode parameters (electrode number, position, shape and composition) and stimulation parameters (waveform, repetition, intervals, duration, number of sessions), as well as the physiological mechanisms of action, vary significantly between the different forms of stimulation. Although research in transcranial stimulation mechanisms in clinical settings has been active for over a century, most approaches gained initial interest which grew over several decades, then diminished, and then were largely abandoned (Guleyupoglu, Schestatsky et al. 2013). Transcranial stimulation techniques include, but are not limited to, transcranial direct current stimulation (tDCS), high definition tDCS (HD-tDCS),transcranial alternating current stimulation (tACS), transcranial random noise stimulation (tRNS), electroconvulsive therapy (ECT) transcranial magnetic stimulation (TMS) repetitive TMS (r-TMS) and low field magnetic stimulation (LFMS) (Peterchev, Wagner et al. 2012). Apart from TMS, tDCS is the most well-known and most studied modality used to modulate brain excitability (Moliadze, Atalay et al. 2012).

1.1.2 Emergence of direct current stimulation

tDCS involves stimulating the brain with weak direct currents of electricity. Initial studies referred to tDCS as ‘galvanization’ and the initial applications were in the treatment of psychiatric disorders.

One of the first reports of tDCS dates back to 1804 when it was used to treat 'melancholia' (Aldini 1804, cited in Brunoni, Nitsche et al. 2012). In the 1880s German psychiatrists described tDCS methods almost identical to current day techniques to recommend this modality for acute psychoses and anxieties (Arnt 1878 cited in Steinberg 2013). Anodal stimulation was applied to reduce depressive symptoms and cathodal stimulation used to manage manic symptoms. Brain polarization was shown to be beneficial to patients who were resistant to other forms of treatment including electroconvulsive therapy (Redfearn, Lippold et al. 1964). In 1926, Bishop and Erlanger discovered that anodal polarization of motor neurones increased the potential differences across the nerve sheath and that cathodal polarization decreased the differences (Bishop and Erlanger 1926). In the 1960's animal studies using cats and rats demonstrated that a weak anodal current increased the firing rate of tonically discharging neurones and cathodal polarization had the inverse effect (Bindman, Lippold et al. 1964). In 1969 direct current stimulation was also investigated as a method for inducing anaesthesia (Brown, 1975).

While several pilot studies reported positively on the effects of tDCS, a controlled trial with outcomes disputing its efficacy was published in 1970 (Arfai, Theano et al. 1970). Due to these inconsistent reports, as well as a poor understanding of its basic mode of action, the technique was practically discontinued in the 1970's and growing attention was given to electroconvulsive therapy and psychopharmacologic drugs (Steinberg 2013). However, 'galvanic' current has been used by physiotherapists unremittingly throughout the last 70 years on the trunk and limbs to treat pain, musculoskeletal and neurological disorders. The role of cortical applications of tDCS has been gaining increasing interest over the last few decades in parallel with advances in neurophysiological testing techniques (such as magnetic resonance imaging, transcranial magnetic stimulation, and electroencephalogram methods). This resurgence in interest has been led by the research of Priori et al, 2003 (Priori 1998) and extended by Nitsche and Paulus (2000 and 2001).

The tDCS device has changed very little over the last century and its clinical applicability has been limited by the conventional technology used to assess its affects. Factors such as focality, depth of penetration and targeting the tissue of interest have been raised as limiting factors. Yet, tDCS is more focally targeted than most pharmacological therapies, therefore it may be able to circumvent many of the undesired side effects of drug therapies (Fregni and Pascual-Leone 2007). Because the device can be used by multiple people for multiple treatment sessions it may be more cost effective than typical pharmaceutical interventions (Valldeoriola, Coronell et al. 2011) and has the potential to be implemented in environments with limited resources such as developing countries (Cabrera, Evans et al. 2014).

1.1.3 Mechanism of action

The transcranial application of weak direct current is believed to induce intracerebral current flow which is capable of transiently altering neuronal activity and behaviour. It provides a subthreshold stimulus that modulates the likelihood that neurons will fire by manipulating the balance of ions inside and outside the neural membrane thus modulating the resting membrane potential, hyperpolarizing or depolarizing tissue, without direct neuronal depolarization (Bikson, Inoue et al. 2004, Schlaug and Renga 2008). In this way it differs from TMS in that it does not yield the rapid depolarization required to produce action potentials in neural membranes. Therefore it is considered a neuromodulatory device rather than neuroexcitatory (Nitsche, Cohen et al. 2008).

It has been consistently shown that anodal tDCS increases motor evoked potentials (MEPs) and cathodal tDCS decreases MEPs by tonic depolarization or hyperpolarisation (respectively) of the resting membrane potential and that these effects occur within several seconds of the stimulation onset (Ziemann, Paulus et al. 2008). The current flows from the negatively charged cathode to the positively charged anode. With the anode positioned over the primary motor cortex (M1) and the cathode over the contralateral orbit the current is directed in an anterior-posterior flow enhancing cortical excitability. Whereas reversing the electrode position with the cathode over the M1 a posterior-anterior current flow reduces excitability (Nitsche, Cohen et al. 2008).

It is this primary polarization mechanism that underlies the acute effects of tDCS on cortical excitability (Brunoni, Nitsche et al. 2012). However tDCS elicits after-effects that cannot be attributed solely to changes in the resting membrane potential. These effects are similar to those observed in long term potentiation (LTP) whereby there is a lasting increase in postsynaptic excitatory potentials. LTP in turn is capable of inducing cortical reorganisation most likely by increasing local synaptic efficiency which may alter deficient network processing (Krause, Marquez-Ruiz et al. 2013). This is supported by evidence suggesting that tDCS modifies neurotransmitter activity and/or neuronal metabolism by altering synaptic N-methyl-d-aspartate (NMDA) receptors and GABAergic activity (Stagg, Best et al. 2009). This altered excitability may be propagated in anatomically or functionally connected distant cortical and subcortical regions (Lang, Siebner et al. 2005). Although the mechanism of activation of unrelated cortical regions remains unclear, it is likely to be mediated through its action on cortico-subcortical /cortico-cortical projections onto pyramidal tract neurones and corticospinal neurones (Di Lazzaro, Ranieri et al. 2013, Kim, Kim et al. 2013).

In addition to effecting changes in neuronal membrane polarization there are also non-neural cells and tissues (such as blood vessels and connective tissues) within the CNS that are subjected to tDCS and may influence the effects of stimulation. These non-neural effects are thought to influence physiologic mechanisms such as vascular motility, inflammation and cell migration (Brunoni, Nitsche et al. 2012). Other proposed mechanisms include activation of glial cells, changes in blood-brain barrier permeability, electroporation, joule heating, electrophoresis, effects on inorganic transport, protein signalling and transcription and effects on cell division (Peterchev, Wagner et al. 2012). tDCS may also affect brain activity via non-electromagnetic interactions such as placebo effect, scalp pressure from the electrodes, secondary afferent effects from muscle, cranial and peripheral nerve stimulation, and environmental conditions such as comfort (Peterchev, Wagner et al. 2012).

Animal studies have helped to illuminate the physiological mechanisms underpinning the behavioural effects of tDCS. Yet, these findings are not without controversy, and there is conflicting evidence between studies using differing species of animals. For example, a study demonstrated long-lasting effects of tDCS on subcortical neurons in the rat but that the effect was evoked by the opposite polarity of tDCS to that found to be effective on subcortical neurones in the cat (Bolzoni, Pettersson et al. 2013), or for cortical neurones in humans. That is, in the rat, excitatory subcortical effects were evoked by cathodal, not anodal, stimulation (Bolzoni, Baczyk et al. 2013). For this reason, as well issues of positioning of the electrodes and the differing cortical architecture of the animal brain compared to the human brain, animal studies in tDCS are relatively rare and of limited value.

Several pharmacological studies have aimed to clarify the cellular mechanisms of tDCS. By using pharmacological agents to block or enhance the activity of neurotransmitters, the effects of tDCS on cortical excitability can be examined. These studies have identified that tDCS appears to affect the NMDA receptors, GABAergic receptors, monoaminergic neurotransmitters, dopaminergic system and serotonergic system. A complex interaction between pharmacotherapeutic agents and tDCS exists such that the effects of tDCS can be delayed, enhanced, prolonged or abolished by the concurrent use of these agents and therefore careful consideration must be given when selecting participants for tDCS interventions and when interpreting the outcomes of studies (for a detailed description refer to Brunoni, Nitsche et al. 2012).

In summary, tDCS is thought to modulate motor cortex excitability via both synaptic and non-synaptic mechanisms and although both of these mechanisms are facilitated by tDCS they may have a different time course and the corticospinal activation has been shown to be facilitated for a longer time (Di Lazzaro, Ranieri et al. 2013; Das, Holland et al. 2016).

More recently computational models have been developed which have improved our knowledge into the mechanisms involved in the effects of tDCS on single neurones, yet the physiological mechanisms of how tDCS affects and interacts with a network of neuronal populations remains obscure (Molaee-Ardekani, Marquez-Ruiz et al. 2013) and founded on vicariation models (De Pino, Pellegrino et al. 2014). Most pertinent is the lack of information regarding how tDCS affects the behaviour of non-pyramidal neurones (typically inhibitory interneurons) that modulate the activity of the pyramidal cells which are themselves known to be strongly influenced by electrical stimulation (Radman, Ramos et al. 2009).

1.1.4 Alternate forms of non-invasive brain stimulation

tDCS and TMS are the most studied techniques used to modulate brain excitability. More recently, other modalities have been introduced but remain relatively novel and as such there is a lot less data available regarding efficiency, protocols and safety. To date, there appears to be consensus that the effects of each of these modalities tend to be comparable when applied at equivalent intensities and dosage (Moliadze, Antal et al. 2010).

1.1.4.1 Transcranial Magnetic Stimulation (TMS)

TMS is delivered to the brain by passing a strong, brief electrical current through an insulated wire coil placed on the skull. This generates a transient magnetic field which passes relatively unimpeded through the layers of tissue and bone and reaches the brain where it in turn induces a secondary current, in a parallel plane to the plane of the stimulation coil, which is capable of depolarizing neurones (Bolognini, Pascual-Leone et al. 2009). A variety of TMS coils are available with the two most common being circular and figure-8. Circular coils induce a broad electric field peak under the coil perimeter whereas figure-8 type coils produce a focused electric field peak under the centre of the figure-8 (Paulus, Peterchev et al. 2013). The coil is generally held in place by the researcher for the duration of the stimulation. The subject's head is partially immobilized in a padded head rest or chin rest to prevent movement of the head relative to the coil. Conventional TMS protocols employ simple trains of evenly spaced pulses usually consisting of stimuli applied at either low (1-2Hz) or high (5-20Hz) frequency (Bolognini, Pascual-Leone et al. 2009). Low frequency TMS (≤1Hz) results in decreased cortical excitability and high frequency TMS (>1Hz) increases cortical excitability (Bolognini, Pascual-Leone et al. 2009). It should be noted the effects are unlikely to be reflective of normal physiological activation (Paulus, Peterchev et al. 2013).

The majority of studies have investigated TMS stimulation of the motor cortex as there are clear effects in muscle responses as measured by MEPs (Paulus, Peterchev et al. 2013).

When delivered to this region TMS induces efferent volleys along the corticospinal tracts (Barker, Jalinous et al. 1985). The resultant effect is dependent on the stimulation duration and frequency, output pulse shape, coil geometry and positioning, and the strength of the magnetic field (Bolognini, Pascual-Leone et al. 2009). The exact mechanism of neural stimulation via TMS remains unclear but the response is generally small, relatively short-lasting, and highly variable and dependent on the same intersubject and intrasubject factors as tDCS (Maeda, Keenan et al. 2000, Gangitano, Valero-Cabre et al. 2002, Paulus, Peterchev et al. 2013). A direct comparison of TMS and tDCS is provided in Table 1.1.

Table 1.1 Comparison of tDCS and TMS modalities

tDCS	TMS
Direct current applied with cathode and anode electrodes	Magnetic field generated by coil
Modifies excitability thresholds	Generates depolarisation
Low temporal resolution	High temporal resolution
Diffuse spread of current	Focal current distribution
Skull shunts current	Magnetic field passes through scalp unimpeded
Easy to sham	Difficult to produce sham
Portable	Large and immobile
Inexpensive	10x more expensive than tDCS
No external indicator of effectiveness	Immediate external indicator of effectiveness
Easy to apply with concurrent therapy	Unable to be used with concurrent therapy
Potential to both increase or inhibit excitability	
Low cutaneous sensation – high patient tolerance	
Able to target various brain tissues	
Intrasubject and intersubject variability in response	

1.1.4.2 Theta Burst Stimulation (TBS) and Paired Associative Stimulation (PAS)

Theta burst stimulation (TBS) is a variation of TMS which uses repeated high-frequency (50Hz) bursts of pulses applied at theta frequency (5Hz). By varying the train duration and temporal spacing of the bursts it is possible to produce enduring changes in cortical excitability using shorter application times and lower intensities than conventional TMS (Paulus, Peterchev et al. 2013). Several single session studies reported promising findings however a larger trial with repeated sessions found no beneficial effect for either inhibitory or excitatory TBS (Talelli, Wallace et al. 2012). Another technique known as Paired Associative Stimulation (PAS) combines TMS with peripheral nerve stimulation at fixed time intervals. Excitatory increases or decreases can be achieved depending on the interval between the stimulation. For example, if the sensory stimulus is applied 10ms before the TMS pulse, inhibition is induced, when applied with a 25ms interval excitation is induced (Wolters, Sandbrink et al. 2003). PAS-induced after-effects are thought to be synapse specific which is in contrast to the nonspecific plasticity induced by tDCS (Kuo, Paulus et al. 2008).

1.1.4.3 Transcranial Alternating Current Stimulation (tACS)

tACS refers to electrical stimulation where the current is not constant (as in tDCS) but alternates between the anode and cathode (switching polarity) with a sinusoidal waveform. tACS can be applied in a wide frequency range and may include a direct current offset (Marshall and Binder 2013). Although not entirely understood, the prevailing hypothesis is that the alternating fields can increase or decrease the power of the oscillatory rhythms in the brain in a frequency-dependent manner by synchronizing or desynchronising neuronal networks (Reato, Rahman et al. 2013). It is unclear whether tACS induces spikes in fibre tracts or modulates cortical excitability in the same proposed manner as tDCS, by lowering the neuronal membrane thresholds (Hermann, Rach et al. 2013).

A recent study compared the effects of cortical stimulation when administering tACS at three different frequencies and a sham condition. Each was applied in a random order for 10 minutes to 21 healthy young adults. The results showed that 140Hz stimulation increased M1 excitability for up to 1 hour after the stimulation period, at 250Hz the effect and its duration were reduced and there was no effect for sham and stimulation at 80Hz (Moliadze, Antal et al. 2010). These results suggest that at 140Hz tACS stimulation is at least as effective as anodal tDCS applied at the same intensity and duration. It has the added advantage of avoiding the polarity specificity of tDCS and as it is passes undetected by subjects, has better blinding potential.

Furthermore, with tACS, the after effects are comparatively robust after changing muscle activity from a resting to an active state. It was previously thought that (unlike cathodal tDCS), tACS offers no feasible way of reducing the excitability of the motor cortex (Moliadze, Antal et al. 2010). However it has recently been shown that tACS induces excitation at an intensity of 1mA, whereas at an intensity of 0.4mA inhibition is induced (Moliadze, Atalay et al. 2012).

In accordance with the evidence available for other electrical stimulation modalities, the support for tACS is currently inconsistent. Although several authors have demonstrated the ability of tACS to modulate cortical excitability (Antal and Paulus 2013, Herrmann, Rach et al. 2013, Marshall and Binder 2013) others dispute these findings (Brignani, Ruzzoli et al. 2013). There seems to be consensus that active networks are very sensitive to alternating current stimulation but the effects are highly dependent on specific network dynamics and in this regard there are still large gaps in our understanding (Reato, Rahman et al. 2013).

In terms of safety there have been reports of perceived retinal phosphenes or flashes in the visual field when using frequencies between 1Hz and 45Hz which although harmless may cause concern in participants and may be an obstacle to blinding (Antal, Boros et al. 2008). However the use of frequencies above 40Hz appears to prevent this visual experience and also reduces the perception of cutaneous sensations (Turi, Ambrus et al. 2013).

1.1.4.4 Transcranial Random Noise Stimulation (tRNS)

tRNS is an adapted form of tACS, and there have only been a few studies published to date investigating this modality. tRNS uses frequencies in the range of 0.1 to 100Hz (low frequency tRNS) and high frequency ranges from 101-640Hz (Chaieb, Antal et al. 2012). It utilizes an alternating current with random amplitude and frequency variation. In contrast to tDCS, the current flow has no directionality (Ambrus, Paulus et al. 2010). Yet the functional after effects appear to be comparable to tDCS. A recent study reported that tRNS applied for 10 minutes over M1 (frequency 0.1-640Hz) led to significant improvements in performance of an implicit motor learning task and increased motor cortex excitability for up to 60 minutes post stimulation with no adverse effects (Terney, Chaieb et al. 2008). This suggests that tRNS, like tDCS, can change cortical excitability by inducing depolarisation and tRNS has the advantage of higher cutaneous perception thresholds when compared to tDCS (Ambrus, Paulus et al. 2010).

A recent study investigated the importance of the timing of application of tRNS relative to the task and revealed that unlike tDCS, tRNS facilitated task performance only when it was applied during

task execution and not when it was applied prior to the task (Pirulli, Fertonani et al. 2013). This suggests that different modulatory effects at a neuronal level mediate the effects of these modalities (Pirulli, Fertonani et al. 2013).

1.1.4.5 High Definition tDCS (HD-tDCS)

High-definition tDCS involves the use of small, gel-based electrodes (25cm^2 of total area) arranged in arrays. This allows categorical increases in anatomical targeting by increasing the focality of current flow (Datta, Elwassif et al. 2009). Although the position and size of sponge electrodes can shape the effects of tDCS, overall the current flow is presumably diffuse (Moliadze, Antal et al. 2010) and HD-tDCS has been developed in an attempt to overcome this. Similar to conventional tDCS, HD-tDCS induces polarity-specific changes in motor cortical excitability, but the effect may be superior to the former montage in that the magnitude or the time-course of the after-effects may be greater (Kuo, Bikson et al. 2013). For example a 4x1 ring montage uses a centre electrode which determines the polarity of stimulation (anode or cathode) and 4 return electrodes in an effort to rationally guide the current flow (Guleyupoglu, Schestatsky et al. 2013).

A few preliminary studies assessing different montages and individualised rays have reported positive findings (Minhas, Bansal et al. 2010, Dmochowski, Datta et al. 2011, Dmochowski, Datta et al. 2013). Edwards et al. (2013) investigated the current distribution of HD-tDCS when using the 4x1 ring configuration using an individualised MRI-based model and found that this was an effective way to modulate the motor cortex and that the current was focalised within the stimulation area. Others demonstrated that with the ring electrode centred over M1, a more profound and durable change occurred in motor evoked potential amplitude than with traditional tDCS (Kuo, Bikson et al. 2013). The extent to which a more circumscribed field can improve clinical effects is yet to be determined (Edwards, Cortes et al. 2013). Still more sophisticated high definition montages, using 64 electrodes to focus the current to brain structure even further, have been proposed and are currently under investigation (Dmochowski, Datta et al. 2011). A further recent advancement of tDCS is transcranial micropolarisation. This technique uses small electrodes instead of pads and is being pioneered in Russia (Shelyakin, Preobrazhenskaya et al. 2000).

As HD-tDCS typically evokes a peak cortical field which is comparable to conventional tDCS it is proposed that clinical differences would result from the elimination of non-target tissue and therefore removal of antagonistic side-effects or regional interactions or variation in current flow patterns within the target (Edwards, Cortes et al. 2013).

This suggests that HD-tDCS provides benefit in terms of well controlled, focalised cortical activation however research in this field is in its infancy and further investigation is required, particularly with regard to the relationship with potential therapeutic benefit in comparison to conventional tDCS.

1.1.4.6 Comparison of different modalities

Contemporary modalities of non-invasive brain stimulation techniques continue to emerge and rival investigations of tDCS and TMS. Comparisons between different modalities of stimulation are limited due to inherent differences in application and physiological response. At this stage, in healthy adults, no one modality appears to be superior to another in terms of the effects produced. However, tDCS does have several advantages in terms of simplicity of application, portability and cost.

1.2 Applications of tDCS

1.2.1 The device

The tDCS stimulating device is comprised of a portable, lightweight box and a set of surface electrodes. Whilst in operation the device constantly monitors the resistance in the system (resulting from dryness of the electrodes, loss of contact etc.) which allows it to provide a steady flow of direct current. If the resistance/impedance is too high a safety function is activated to terminate the stimulation preventing the voltage/current density from being increased beyond safe limits.

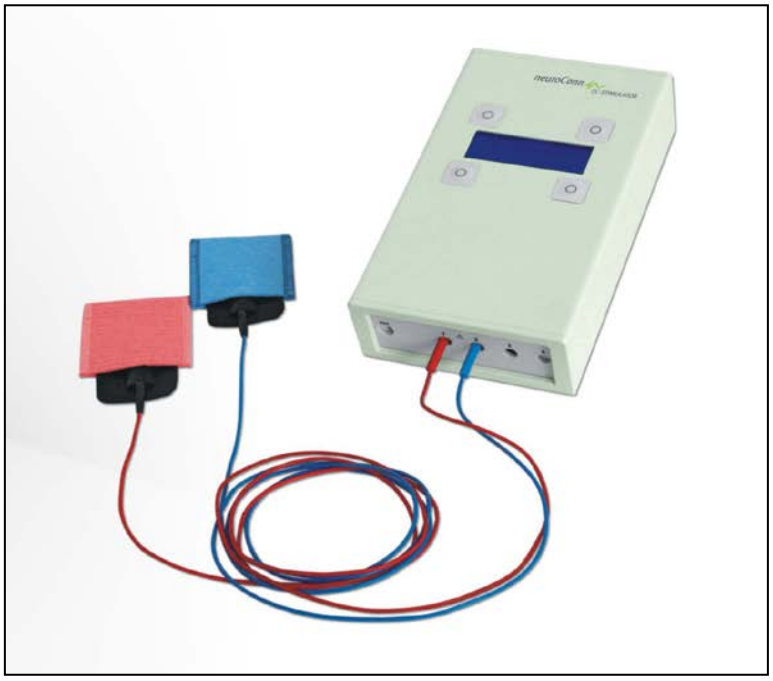


Figure 1.1: Neuroconn DC stimulator

The device should guarantee a constant current strength because this determines the intensity of the electrical field in the tissues and a constant voltage device could result in unwanted increases in tissue strength if resistance decreases. Stimulators have voltage settings from 0-4mA and can supply up to 80 mA/min per session (Schlaug and Renga 2008).

The current is delivered via a pair of sponge covered rubber electrodes which are moistened with tap water or sodium chloride solution and held in position by rubber straps. Debate continues about which wetting agent is preferable. Some authors advocate the use of tap water as this causes less discomfort and itching than saline soaked sponges and allows EEG recording to occur immediately after the tDCS (Palm, Keeser et al. 2008). Furthermore, decreasing sponge salinity decreases peak current density at the electrode corners (Minhas, Datta et al. 2011). However others recommend the use of a weak sodium chloride solution as tap water leads to higher impedance and therefore greater thermal side effects (Dundas, Thickbroom et al. 2007). These cutaneous sensations are thought to be induced by electrochemical reactions in which electrons are transferred between the electrode and the stimulated tissue as well as additional factors such as the concentration of the saline solution, the electrode position and differences in skin- microstructure. (Turi, Ambrus et al. 2014). Other efforts to minimise the intensity of the cutaneous sensations include a fade-in fade-out phase at the beginning and end of the stimulation and using relatively large electrodes to maintain a low level of current density (Nitsche, Liebetanz et al. 2003).

The size of the electrodes varies in different studies but is generally in the range of 25-35cm^2 with currents 1-2mA which generates current densities ranging from 0.28-0.80 A/m^2 (Brunoni, Nitsche et al. 2012). The saline/water soaked electrodes are placed on the region of interest and the direction of the current flow determines the effect on the underlying tissue. The most commonly used protocol for tDCS stimulation was introduced by Nitsche & Paulus (Nitsche and Paulus 2000). This entails the use of 1mA of continuous current through two rectangular shaped electrodes positioned on the scalp for a duration of up to 20 minutes.

The applied current is first distributed throughout the scalp and then passes across the skull and cerebrospinal fluid before entering the brain (Datta, Bansal et al. 2009). At the commencement of stimulation the subject may feel a slight itching/tingling or warming sensation which will abate in most cases after 30 seconds to 1 minute owing to tolerance. To decrease cutaneous sensation even further the current is generally ramped up over several seconds at the commencement of the stimulation period and ramped down at the end of the session.

1.2.2 Sham/Control condition

It is commonly reported that because the perception of sensory stimulation fades after approximately 30 seconds it is relatively easy to conduct sham-controlled studies with tDCS. For sham stimulation the tDCS is ramped up for several seconds and then ramped down again after 30 seconds to give the subject the initial tingling sensation and then turned off without the subject being aware that the stimulation has ceased.

This mode can be programmed into the stimulator to further standardize the application and is selected in a masked manner so as to enable double-blinded experimental designs (Nitsche, Cohen et al. 2008). This brief period of stimulation does not appear to affect brain function and makes real tDCS and sham stimulation difficult to distinguish (Gandiga, Hummel et al. 2006, Brunoni, Nitsche et al. 2012). A recent study supports the use of this sham protocol but argues that it is successful not because the cutaneous sensations fade after 30 seconds but, on the contrary, the sensations persist after the stimulator has shut-off (Ambrus, Al-Moyed et al. 2012). Another study directly evaluated whether sham and active stimulation are indistinguishable (Kessler, Minhas et al. 2013) and reported that the sensory side effects are more frequent and severe in active stimulation compared with sham tDCS and therefore may not be an adequate control condition for some studies, particularly in studies where sensory side effects may interfere with task performance. However, although the experience of active and sham stimulation may be different, subjects were unable to explicitly discriminate between sham and active conditions when directly asked, thus it appears that the masking of the stimulation condition was successfully achieved (Kessler, Minhas et al. 2013). This finding has been reproduced by numerous authors (Gandiga, Hummel et al. 2006, Poreisz, Boros et al. 2007, Ambrus, Al-Moyed et al. 2012, Russo, Wallace et al. 2013).

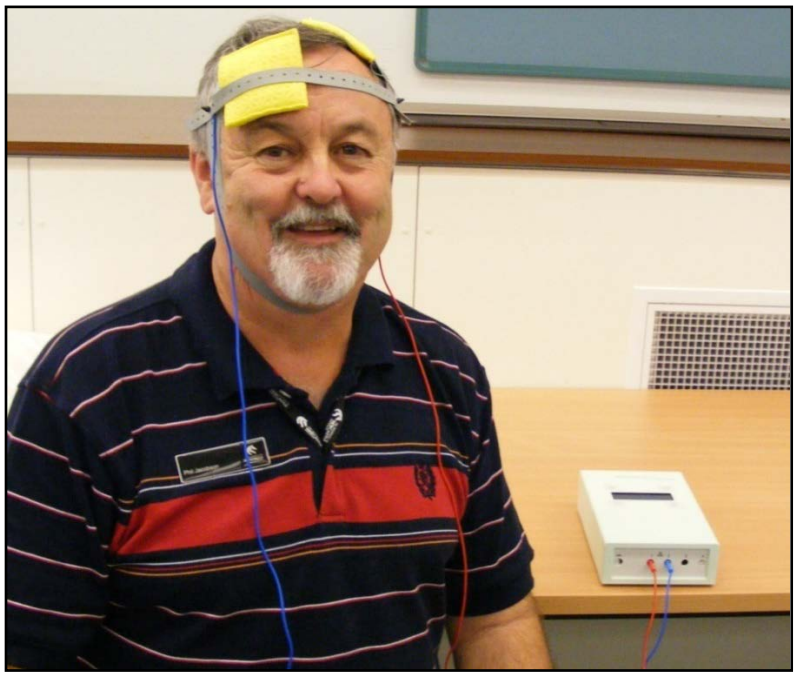
An additional blinding issue may be the temporary redness that occurs in the area under the electrodes with active stimulation and not necessarily with the sham condition. In clinical protocols, particularly when repeated sessions of stimulation are required, this redness may be evident after several days and be obvious to not only the participant but the researchers/outcome assessors (Brunoni, Nitsche et al. 2012).

1.2.3 Electrode positioning

Electrode position and orientation will affect the outcome produced by tDCS as different neuronal populations will be stimulated. As different areas of the brain have different tissue characteristics the induced currents may be distorted and possibly alter the amount of current delivered to the brain tissue (Nitsche, Cohen et al. 2008). The direction of the excitability shift might be divergent, depending not only on the stimulation polarity but also the specific electrode montage.

Electrode positioning is usually described according to the International 10/20 Electroencephalogram (EEG) system. Therefore to stimulate M1 the active electrode is placed over C3/C4 which approximately corresponds to the location of the motor cortex. This position can be further confirmed by the induction of motor-evoked potentials in the contralateral first dorsal interosseous muscle using TMS (Cincotti, Babiloni et al. 2004).

Figure 1.2: Application of anodal tDCS to the left M1 region



The M1 hand area has been used almost exclusively in the literature due to its superficial location on the cortex. One paper reports successfully using anodal stimulation of the leg area of M1 leading to sustained increases of MEPs in the tibialis anterior muscle (Jeffery, Norton et al. 2007) and another demonstrated improved ankle control in a small sample of stroke patients following tDCS (Madhavan and Stinear 2010). tDCS has also been applied over noncortical tissues such as the cerebellum (Ferrucci, Marceglia et al. 2008) and spinal cord (Winkler, Hering et al. 2010) with results indicating that these sites can be successfully used to modulate neuronal excitability. Ultimately the choice of montage will be application specific but clearly further systematic investigation of the behavioural and neural effects of different electrode montages is warranted (Schlaug and Renga 2008).

Several authors have explored locations for the reference electrode placement and although there appears to be an accepted consensus, some debate continues. The reference electrode determines

the current path from the active electrode through the brain. Importantly, the region of brain modulation is not simply beneath the electrode of interest but is a function of the position and properties of both electrodes (Peterchev, Wagner et al. 2012).

The most commonly reported arrangement is with the reference electrode positioned over the contralateral supraorbital region (Nitsche and Paulus 2000).The term reference electrode does not necessarily indicate that this electrode is functionally inert. It just infers that the neuronal excitability under this electrode is not of interest in the experimental set up. However with this configuration the potential exists for stimulation to occur at both electrode sites, that is, unwanted excitability changes under the reference electrode in addition to stimulation of M1, which could complicate interpretation of the results. In principle, this can be circumvented by increasing the size of the reference electrode to reduce local current density (Nitsche, Doemkes et al. 2007). To avoid this issue other authors recommend the use of a non-cephalic location for the reference electrode such as the right shoulder or the leg (Cogiamanian, Marceglia et al. 2007). Yet with this arrangement current could potentially affect the brainstem and flow out through the foramen magnum and cause negative effects such as apnoea, nausea and respiratory depression (Bindman, Lippold et al. 1964). Although several studies have been conducted with this configuration, with no ill-effects, it has been considered controversial since a reported episode of transient respiratory depression in a study participant. (Redfearn, Lippold et al. 1964). It was inferred from this episode that this electrode configuration had produced modulation of the activity of the cardio-respiratory centres in the brainstem. Subsequent authors have quantitatively investigated current density with extracephalic reference electrodes on both the right deltoid and the right tibia and found that effects at the level of the brainstem was limited and resulted in no effect on cardio respiratory and autonomic function (Parazzini, Rossi et al. 2013, Vandermeeren, Lefebvre et al. 2013).

Moliadze et al. (2010) compared the efficacy of various montages using different reference electrode positions and found that the distance between the two electrodes correlated negatively with the duration of the after-effects. Although there were no serious side effects the stimulation intensity needed to be increased to account for larger inter-electrode distances. This distance appears to affect the fraction of the applied current that reached the brain or is shunted through the scalp (Datta, Elwassif et al. 2009). That is, increasing the distance between the electrodes on the scalp increases the relative amount of current entering the brain rather than shunted across the scalp. Another relevant aspect of inter-electrode distance is that more closely spaced electrodes produce more superficial stimulation (Miranda, Lomarev et al. 2006).

In contrast, the ability to increase and decrease activity in different brain regions simultaneously, through application of both electrodes on the scalp, may be advantageous particularly in conditions such as stroke where an imbalance in excitability may exist. This configuration is often termed 'bihemispheric' stimulation. It has been consistently demonstrated in healthy subjects that simultaneous modulation of cortical excitability in different directions in the two motor cortices is achievable with this bihemispheric montage (Lindenberg, Renga et al. 2010, Bolognini, Vallar et al. 2011) and several authors suggest that the effect size is greater with this bilateral montage (Vines, Cerruti et al. 2008, Williams, Pascual-Leone et al. 2010, Mordillo-Mateos, Turpin-Fenoll et al. 2012). A further study directly comparing simultaneous bilateral montage to the traditional unilateral configuration, using healthy subjects, demonstrated that simultaneous bilateral tDCS induces similar effects to the unilateral montage on the cathode stimulated side but that the effect is less robust on the anode side and concluded that corticospinal excitability was not differentially modulated by the different electrode configurations (Mordillo-Mateos, Turpin-Fenoll et al. 2012). This was supported by a study using healthy aged subjects where both anodal and bihemispheric montages were beneficial but there was no significant differences between them (Kidgell, Goodwill et al. 2013).

1.2.4 Electrode size

Electrode size is an important factor contributing to the final output of stimulation. Electrode size determines spatial focality of the applied current, whereby the larger the size of the electrode the poorer the focality (Nitsche, Doemkes et al. 2007). This has been substantiated by computer modelling studies which demonstrate that relatively large electrodes result in diffuse electrical activation in regions under and between the electrodes (Datta, Bansal et al. 2009). It is known that smaller electrodes result in a reduction in current which is almost proportional to the reduction of the electrode area (Miranda, Faria et al. 2009). Therefore the penetration depth is presumably reduced with smaller electrodes (Vollmann, Conde et al. 2013). However this does not denote a reduced functional effect. One study investigated the effects of tDCS using three differently sized electrodes on cortical excitability. A constant reference electrode size of $35cm^2$ was used but active electrode size was varied from 12, 24 and $35cm^2$ and the current density was kept constant ($0.029mA/cm^2$). The authors reported that the corticospinal excitability of the extensor carpi radialis muscle was greatest with the $12cm^2$ ($p = 0.002$ when compared to $24cm^2$ and $p=0.000$ when compared to $35cm^2$), and there was no significant difference between the two larger sized electrodes. Therefore reducing the active electrode size may result in spatially more focused stimulation and increases the efficiency of tDCS to produce larger corticospinal excitability. The authors speculate that this may be due to the fact that larger electrodes stimulate nearby cortical functional areas which can have an inhibitory effect on the M1. For example the motor association

cortex has inhibitory effects on M1, while the premotor cortex facilitates M1 (Bastani and Jaberzadeh 2013).

Cutaneous discomfort is also thought to be associated with electrode size such that larger electrodes with a lower current density will result in milder cutaneous sensations. This was highlighted by Ambrus et al. (2010) who reported that the perception of stimulation tends to concentrate under the forehead electrode and underscores their recommendation for using a larger electrode in this position. However, this theory was examined by Turi et al. (2014) who investigated the relationship between current density, current intensity and perceived cutaneous sensations during tDCS. Contrary to the generally accepted opinion that cutaneous discomfort diminishes as the current density is reduced, they found that large electrodes (35cm^2) were associated with greater cutaneous discomfort when compared to smaller electrodes at a given current density. Also the levels of cutaneous perception were similar for small and large electrodes when current intensity was kept constant. This may be because although larger electrodes have lower current densities than smaller electrodes at any given intensity, more cutaneous receptors are affected due to the extended electrode–skin interface (Turi, Ambrus et al. 2014).

An important consideration with regard to electrode size is the excessive use of saline/water to moisten the scalp. The region where the current enters or exits the body through the electrode is defined by the area covered by the electrolyte. Therefore it is the electrolyte-skin interface which defines the functional electrode position/size rather than the dimension of the solid-conductor in the electrode. This is important to note in the application of tDCS where it is common practice to increase the moistness of the electrode pads with solution to decrease impedance. In the process the hair and straps holding the electrodes in position may become wet and therefore the effective electrode area is extended beyond the perimeter of the electrode and may affect current density (Peterchev, Wagner et al. 2012).

1.2.5 Electrode Shape

Rectangle-shaped electrodes are routinely used. Computational modelling studies have demonstrated that the current distribution underneath these traditional electrodes is non-uniform and tends to be concentrated at the perimeter of the electrodes. This is particularly evident with larger electrodes (Wagner, Fregni et al. 2007, Moliadze, Antal et al. 2010) and the edge of the electrode closest to the reference electrode (Minhas, Datta et al. 2011).

The concentration of current may lead to lowered cutaneous perception thresholds adding to discomfort or impaired blinding. It has been theorized that this may be overcome by eliminating the corners and reducing the perimeter length by using circle-shaped electrodes. To date, no difference

between round and rectangular electrode geometry has been determined and therefore changing to a round shape electrode is unwarranted (Ambrus, Antal et al. 2011).

More recently, in an attempt to increase the focality and the efficiency of tDCS, novel types and shapes of electrodes have emerged in trials. This includes ring electrodes and concentric electrodes, and montages using varied sizes of electrodes. In one study tDCS was applied using two anodes and one ring shaped cathode (Kuo, Bikson et al. 2013). Others have used one anode and several cathodes (Datta, Elwassif et al. 2008, Datta, Elwassif et al. 2009). Using a computational model the 4x1 HD-tDCS montage was predicted to allow unprecedented targeting of cortical regions (Datta, Bansal et al. 2009). Clinical studies to validate this prediction are ongoing (Bikson, Rahman et al. 2012). At this stage, the safety of using these novel paradigms and effects on non-target areas are yet to be established (Bastani and Jaberzadeh 2013).

1.2.6 Current Intensity

The majority of reported studies have used a current intensity of 1mA and current intensities at or above 3mA are too painful for routine application (Furubayashi, Terao et al. 2008). However it is thought that increasing the intensity may increase the efficacy of the stimulation and may lead to increases in the improvement of behavioural performance (Cuypers, Leenus et al. 2013). A study directly comparing the effects of anodal stimulation applied at 1mA and 1.5mA in 13 healthy subjects found a significant improvement in hand function with 1.5mA tDCS compared to sham but not with 1mA (Cuypers, Leenus et al. 2013). A study with Parkinson Disease patients reported that 2mA of tDCS was required to improve working memory performance whereas 1mA failed to produce significant effects (Fregni, Boggio et al. 2006). However, a study investigating the effects of different cathodal stimulation intensities found that when the intensity was doubled from 1mA to 2mA the stimulation effect was reversed from inhibition to excitation (Batsikadze, Moliadze et al. 2013). This may be linked to early investigations which showed that direct current intensities target different cortical neurones, with weak stimulation modulating predominantly nonpyramidal neurons and stronger intensities presumably modulating pyramidal neurones (Purpura and McMurtry 1965). However this has not been substantiated in more recent studies. Using TMS to measure cortical excitability and short interval intra-cortical inhibition (SICI) of the contralateral wrist muscles Kidgell et al. (2013) investigated the differential effects of 0.8, 1.0 and 1.2mA of anodal tDCS in healthy subjects. Interestingly, they found that increased cortical excitability and decreased intra-cortical inhibition was facilitated in all conditions and this was independent of current intensity, implying that lower current intensities are just as effective in modulating cortical plasticity as higher intensities.

Clearly, the question of optimal current intensity remains unanswered. It is most likely inappropriate to consider current intensity in isolation from other stimulation parameters which renders comparison across studies difficult due to the variation in applications reported. Therefore many authors are pleading for standardization of current strength, electrode size, stimulation duration, and electrode orientation so that study results can be directly compared (Hunter et al, 2013).

1.2.7 Current Density

When considering the mechanisms of tDCS it is important to consider the magnitude and location of the current induced in cortical tissues (Brunoni, Nitsche et al. 2012). Current density is a quotient of current intensity and electrode size (Nitsche, Cohen et al. 2008) whereby the treatment effect is amplified with increased current strength or reduced electrode size. The amount of current that effectively reaches the target brain region is dependent on personal factors such as skin and skull resistance, the resistance of intracranial structures such as blood vessels, cerebrospinal fluid and meninges, and the resistance of the brain tissue which varies according to cell type, structure and orientation (e.g. glial cells, white matter etc.) (Brunoni, Nitsche et al. 2012).

Individual anatomical characteristics and morphology of subjects will have an influence on field distributions and the potential efficacy of tDCS (Parazzini, Rossi et al. 2013). One study demonstrated that significant variation in current density occurs between subjects when the same electrode configuration is applied and that this difference may be ten-fold in the area near the electrodes and two-fold in distant regions such as the hypothalamus (Russell, Goodman et al. 2013). This is particularly relevant for people with skull fractures or stroke lesions. For example, a lesion caused by stroke will become filled with cerebrospinal fluid (cystic) and will preferentially shunt current flow leading to distortions in guiding the current to the target region and may lead to safety concerns such as current hot spots (Brunoni, Nitsche et al. 2012). Despite the difference in the conductivities of the scalp and the skull, about 50% of the applied current is thought to reach the brain (Miranda 2013) and is sufficient to produce neuronal excitability shifts (Wagner, Fregni et al. 2007).

We know that current does not flow uniformly through the skin, but concentrates near the edges of the electrodes or where the skin is irregular (Miranda, Lomarev et al. 2006). In the brain, current tends to concentrate on the edge of gyri (Datta, Bansal et al. 2009). Computer based modelling studies have been conducted to provide more accurate insight into detailed current flow patterns. These models rely on assumptions about tissue impedance and the representation of the head in computational models can range in detail from concentric geometric graphics to high resolution models based on individual magnetic resonance images (Peterchev, Wagner et al. 2012).

Despite the variation in complexity and difficulty with model validation this work has the goal of correctly predicting brain current flow to guide clinical therapeutic delivery (Bikson, Rahman et al. 2012). Although it is reasonable to assume that regions with more current flow are more likely to be affected by stimulation than those regions with little current flow, it is important to acknowledge that the intensity of current flow in any specific brain region does not translate in a simple linear manner to the degree of brain activation or modulation (Bikson, Rahman et al. 2012).

Current density and flow patterns may change throughout the course of the stimulation. Electrode resistance of the skin decreases over time during tDCS provided wet electrodes are used – low resistance is a pre-requisite for avoiding side effects. If the electrodes dry out unevenly a resultant increase in resistance leads to a more focal, intense and variable current flow through the scalp which may cause electrochemical burns (Paulus and Opitz 2013). A drop in skin resistance has some influence on the current flow through to the underlying skull regions and it is plausible that brain tissue resistance, like skin resistance, may also change during the course of tDCS stimulation (Paulus and Opitz 2013). Investigation of the temporal course of brain tissue resistance during stimulation is currently lacking.

Several authors have demonstrated that increasing current density will result in longer lasting and stronger effects (Nitsche and Paulus 2000, Nitsche, Nitsche et al. 2003). However, if prolonged tDCS effects are desired it is recommended that the stimulation duration be increased and not the current density. The reason for this is two-fold. Firstly, an increased current density will increase cutaneous sensation, which may become unpleasant or painful for the subject (Nitsche, Cohen et al. 2008). Secondly, as current density increases the depth of penetration of the effective electrical field increases. As different areas of the brain have different tissue characteristics the induced currents may be distorted and possibly alter the amount of current delivered to the brain tissue (Nitsche, Cohen et al. 2008). Thus the excitability of the cortical neurones not affected by lower stimulation intensities may be different to the superficial ones. To date there is no universal relationship between current density and brain excitability for the spectrum of possible electrode montages (Miranda, Lomarev et al. 2006).

1.2.8 Duration of stimulation

Apart from intensity, another important parameter of stimulation is its duration. Taking MEPs as the criterion, the minimal duration for induction of after-effects was demonstrated to be three minutes (Nitsche and Paulus 2000). A series of studies examining the effects of different durations of stimulation on corticomotor excitation indicated a linear relationship between the duration of application and the increase in the duration of after-effects up to a stimulation duration of 20

minutes (Nitsche and Paulus 2001, Nitsche, Schauenburg et al. 2003). That is, five minutes of stimulation leads to five minute changes in MEPs. When applied for greater periods of time tDCS produces lasting effects in the motor cortex which are stable for up to 90 minutes (Ziemann, Paulus et al. 2008).

The required duration of stimulation to produce prolonged effects appears to be different for each type of tDCS. For example, anodal stimulation with 13 minute stimulation duration was necessary to produce a sustained excitability increase of 90 minutes post stimulation (Nitsche and Paulus 2001). In contrast, only nine minutes of cathodal tDCS was required to induce similar durable excitability decreases, implying that cathodal may be a more efficient form of stimulation (Moliadze, Atalay et al. 2012). However the effects of longer durations of stimulation are a lot less predictable. Monte-Silva et al found that doubling anodal stimulation time from 13 minutes to 26 minutes reversed the effects of the stimulation from excitation to inhibition (Monte-Silva, Kuo et al. 2013). Prolongation of cathodal stimulation session from 60 to 90 minutes resulted in no additional benefit. The optimal stimulation duration remains poorly defined and further investigation of longer stimulation durations is needed.

1.2.9 Frequency of stimulation sessions

It has been proposed that the repetition of tDCS sessions over a period of days/weeks has the potential to enhance the efficacy of the stimulation by cumulating or stabilizing the effects. Several studies whereby tDCS was applied in a repetitive design support the theory that the effects derived from tDCS are cumulative both at a cortical and a behavioural level in healthy and clinical populations (Fregni and Pascual-Leone 2006, Reis, Schambra et al. 2009, Alonzo, Brassil et al. 2012). The mechanisms underlying the long-lasting effects of tDCS have yet to be elucidated, especially those with regard to consecutive sessions. It has been postulated that these long lasting functional changes occur via synaptic plasticity changes in the motor cortex or long term potentiation (Stagg and Nitsche 2011). This would also explain the why cortical excitability increases are cumulative between stimulation sessions rather from an increased responsiveness to each successive tDCS session (Galvez, Alonzo et al. 2013).

Yet the optimal frequency at which tDCS sessions should be administered is under debate. Several authors have investigated the use of different session intervals in healthy adults. Boggio et al. (2007) demonstrated that the behavioural effects of anodal tDCS can be stabilized by applying tDCS on a daily basis over a period of one week. Similarly, Alonzo, et al. (2012) found that tDCS applied at 2mA for 20 minutes induced changes in excitability that lasted for at least two hours with further cumulative increases in excitability when sessions were repeated on five consecutive days.

However, the cumulative effects were not observed when the stimulation was given second daily. This suggests that one day is within the window for consolidation of the excitatory effects whereas a two day interval is outside the time-frame for these benefits to occur. If carry-over effects are not desired, an inter-sessional interval of 48 hours to one week has been suggested (Nitsche, Cohen et al. 2008).

Another study attempted to explore the optimal inter-stimulation interval for applications of cathodal stimulation, ranging from no break to 24 hours in healthy subjects (Monte-Silva, Kuo et al 2010). Doubling the stimulation period (no break between nine minute sessions, i.e. one 18 minute session) prolonged the duration and amplitude of the after effects of the stimulation on cortical excitability. However, when the second stimulation occurred after a longer intermission (three or 24 hours), when the after-effects of the first stimulation had returned to baseline, the effects were diminished (Monte-Silva, Kuo et al. 2010). But not only can the timing of the successive stimulation effect the longevity of the effects, repeated stimulation may also lead to antagonistic effects (Siebner, Lang et al. 2004). When cathodal stimulation was applied at an interval of three minutes after the first session (applied during the after-effects) then it had the opposite effect to the first session. That is, instead of inhibition, cathodal stimulation resulted in facilitatory effects (Fricke, Seeber et al. 2011). This is consistent with the rules of homeostatic plasticity whereby the effects of repeated short periods of tDCS follow a time dependent principle, whereby the ease of facilitating or inhibiting synaptic activity is dependent on the previous network activity (Fricke, Seeber et al. 2011).

Galvez et al. (2013) investigated whether current intensity should be incrementally increased over multiple sessions as is the recommendation with electroconvulsive therapy (ECT) where the dose is commenced at a submaximal level rather than keeping the current intensity constant across all sessions. They found no difference in the degree of excitability produced when tDCS was applied in successive sessions at a constant strength over five consecutive days or when the dosage was gradually increased over the same timeframe. Therefore there is currently no evidence to advocate or denounce commencing tDCS at a submaximal intensity and increasing it over the treatment period.

Overall, repeated sessions appear to have a cumulative effect associated with greater magnitude and duration of behavioural effects, however the mechanism of this tDCS induced consolidation remains postulative (Reis, Schambra et al. 2009). The effects of tDCS can be enhanced, negated, or unaffected by the interstimulation period but the optimal timing, repetition rate and duration of sessions to induce these lasting effects remains undetermined (Brunoni, Nitsche et al. 2012). It should also be noted that response reliability at the level of the individual has not yet been fully

established and studies exploring tDCS effects in healthy subjects across multiple days have suggested that the response pattern may be unpredictable (Horvath, Carter et al. 2014). More data investigating the effects of tDCS across time, with consideration given to circadian and metabolic cycles, is required (Horvath, Carter et al. 2014). Understanding the interaction of consecutive stimulation protocols is crucial in order to implement effective applications of tDCS in clinical populations.

1.3 Proposed effects of tDCS

tDCS has been applied to different cortical regions to affect perceptual, cognitive and behavioural functions. Clinical and functional studies propose beneficial effects of tDCS in several neurological and psychiatric disorders including **:** epilepsy (Fregni, Thome-Souza et al. 2006, Liebetanz, Klinker et al. 2006), intractable pain (Fregni, Boggio et al. 2006, Lefaucheur, Antal et al. 2008, DosSantos, Love et al. 2012), major depressive disorders (Fregni, Boggio et al. 2006, Boggio, Rigonatti et al. 2008, Blumberger, Tran et al. 2012, Knotkova, Rosedale et al. 2012), Parkinsons Disease (Fregni, Boggio et al. 2006, Kaski, Dominguez et al. 2014, Manenti, Brambilla et al. 2014), cerebellar ataxia (Pozzi, Minafra et al. 2014), fibromyalgia (Marlow, Bonilha et al. 2013), migraine (Chadaide, Arlt et al. 2007, Antal, Lang et al. 2008), addictions (Boggio, Liguori et al. 2009, Fraser and Rosen 2012), cognitive performance (Iyer, Mattu et al. 2005, Floel 2014, Scheldrup, Greenwood et al. 2014) , tinnitus (De Ridder and Vanneste 2012), Alzheimers Disease (Ferrucci, Mameli et al. 2008, Hansen 2012) Multiple Sclerosis (Cuypers, Leenus et al. 2013, Ferrucci, Vergari et al. 2014), spinal cord injury (Fregni, Boggio et al. 2006) stroke (Hesse, Werner et al. 2007, Stagg, Bachtiar et al. 2012, Zimerman, Heise et al. 2012) and ageing (Hummel, Heise et al. 2010, Zimerman and Hummel 2010).

However, the overwhelming majority of studies have been conducted in healthy young adults and therefore any inferences made to broader clinical groups from these findings must be made cautiously. In healthy subjects, tDCS has been shown to perturb initial motor learning and consolidation (Robertson, Press et al. 2005). A single application of anodal tDCS delivered over M1 has been shown to induce transient improvements in various motor and cognitive tasks (Nitsche, Schauenburg et al. 2003, Antal, Kincses et al. 2004, Vines, Nair et al. 2006, Reis, Schambra et al. 2009). Some of these facilitatory effects on learning processes include: visuo-motor function (Antal, Kincses et al. 2004), implicit motor learning (Nitsche, Schauenburg et al. 2003), and probabilistic classification learning (Kincses, Antal et al. 2004). Others include: visuo-motor memory (Heimrath, Sandmann et al. 2012), working memory (Jones and Berryhill 2012), colour discrimination (Costa, Nagy et al. 2012), automatic verbal processes (Vannorsdall, Schretlen et al. 2012), and emotional processing (Nitsche et al 2012).

1.3.1 Specificity of effects

tDCS is often criticised for its lack of specificity. Specificity of stimulation refers broadly to the ability of tDCS to produce precise, as opposed to diffuse, changes in brain function. This is ultimately determined by both anatomical and functional factors (Bikson and Rahman 2013). Anatomical specificity derives from guiding the current to the targeted brain structures whereas functional specificity stems from task specificity. Thereby neuronal networks which are already activated

are enhanced, while separate neuronal networks that are inactive are not modulated. Although task-specific effects of tDCS have been shown, the mechanism remains poorly understood (Saucedo Marquez, Zhang et al. 2013) (Bikson and Rahman 2013).

Several studies have demonstrated that tDCS induces relatively focal effects which are limited to the area under the electrode (Uy and Ridding 2003, Miranda, Lomarev et al. 2006). Repositioning the electrode a few centimetres towards the premotor cortex can abolish the effects of tDCS in a motor task (Nitsche, Schauenburg et al. 2003). Yet the discussion of specificity may be unwarranted. Many brain regions are involved in even basic motor tasks. Therefore, conceptually, it would seem illogical to expect stimulation to produce specific functional changes by passing a current through a multi-tasking complex brain region (Bikson and Rahman 2013).

1.3.2 Duration of effects

The persistence of the effects after the cessation of the intervention varies in studies and is largely dependent on the duration and the intensity of the stimulation. Short applications of anodal or cathodal stimulation on the motor cortex (e.g. a few minutes) result in cortical excitability changes during the stimulation but no after-effects (Nitsche and Paulus 2000). In contrast 10 minutes or more of stimulation can elicit prolonged after-effects which can be sustained for over an hour depending on the cortical area and on the outcome/variable measured (Brunoni, Nitsche et al. 2012). For example, researchers have used TMS to show that 10-20 minutes of anodal tDCS over the motor cortex may lead to an increase in excitability up to 150% lasting for approximately 90 minutes (Nitsche and Paulus 2001).

For clinical purposes, longer lasting effects are crucial and although preliminary findings indicate therapeutic potential, the after-effects of tDCS to date have generally been limited in duration to a few hours (Liebetanz, Koch et al. 2009). Options to prolong the effects include: prolongation of the stimulation duration, enhancing stimulation strength, or repetition of tDCS sessions. As previously discussed the effects of longer duration stimulation is nonlinear and unpredictable, and it is generally considered good practice to keep current strength as low as possible to decrease

cutaneous discomfort and minimize stimulation of neuronal populations at a deeper depth. Therefore, it appears that multiple stimulation sessions, are required and the magnitude of the behavioural change has been associated with the number of sessions received (Monte-Silva, Kuo et al. 2010).

The physiology underpinning the prolonged sensory, motor and cognitive effects of tDCS has been attributed to persistent bidirectional modification of postsynaptic connections similar to long term potentiation/depression effects (Nitsche and Paulus 2000). The brain derived neurotrophic factor (BDNF) gene is involved in mechanisms of synaptic plasticity in the adult brain and has been demonstrated to play a significant role in shaping plasticity induced by electric stimulation (Antal, Chaieb et al. 2010). That is, enhancement or weakening of the NMDA receptor activity depending on the polarity of the stimulation (Nitsche, Seeber et al. 2005). This is supported by studies using the NMDA-receptor antagonist dextromethorphan which demonstrated that antagonizing NMDA receptors did not alter the excitability changes created by tDCS during the stimulation but prevented the formation of after-effects independent of their direction. Therefore the glutamatergic system, in particularly NMDA receptors, seem to be necessary for the induction and maintenance of the tDCS neuroplastic after effects (Liebetanz, Nitsche et al. 2002).

Yet the mechanism of the lasting effects of tDCS after the cessation of stimulation is not without debate. Nitsche et al. (2003) have shown that the after effects of tDCS are associated with an involvement of intra-cortical synaptic mechanisms. Anodal stimulation has been shown to have no effect on short-interval intra-cortical facilitation during tDCS (or SICI). Yet after the cessation of anodal tDCS: SICI is decreased, intra-cortical facilitation (ICF) is increased. With cathodal stimulation the ICF decreases both during and after the stimulation and the SICI increases after the stimulation. The complexity of these effects may be due to the different locations and orientations of interneurons in each pathway (Nitsche, Nitsche et al. 2003).

1.3.3 Measures used to assess the effects of tDCS

The effects of tDCS have been measured using a variety of outcome tools ranging from localised physiological measures to more global effects on cognitive and motor functioning, and the relationship between these different types of measures remains unresolved. There is widespread assumption that the changes in corticomotor excitability are associated with the magnitude of the behavioural improvement. However studies which directly compare these outcomes have produced mixed results. Several authors have reported that changes in cortical excitability are correlated with behavioural changes (Hummel, Celnik et al. 2005, Hummel, Voller et al. 2006) yet others dispute the existence of an association between the changes in corticomotor plasticity (increased MEP

amplitude and reduced SICI) and improved motor function (Rogasch, Dartnall et al. 2009, Cirillo, Rogasch et al. 2010, Kidgell, Goodwill et al. 2013). Williams et al. (2010) reported a strong relationship between changes in MEP amplitude and motor performance but no association between SICI and motor performance. What is becoming increasingly evident is that the relationship between neurophysiologic and behavioural changes is not linear (Vines, Cerruti et al. 2008, Kidgell, Daly et al. 2013) and that all the outcome measurement tools used to date have limitations.

TMS has been the most widely used method for evaluating the effects of tDCS on changes in cortical excitability and can be used to provide information about the effects of tDCS on cortico-cortical as well as cortico-spinal excitability, intra-cortical inhibition and facilitation, as well as inter-hemispheric interactions (Nitsche, Seeber et al. 2005, Stefan, Kunesch et al. 2000, Nair, Renga et al. 2008)**.** However this method does not provide information about multifocal brain activation or neural network properties that will have an effect on tDCS outcomes (Saiote, Turi et al. 2013). MEPs are also commonly used whereby an increase in MEP amplitude of the target muscle following tDCS is thought to reflect cortical elements of plasticity via intrinsic changes in the excitability of the corticospinal cells (Kidgell, Daly et al. 2013).

A change in MEP amplitude that remains elevated for up to 60 minutes has been reported and confirmed by mathematical models that show that tDCS can modify transmembrane potential to influence the excitability of individual neurones (Wagner, Fregni et al. 2007, Stagg and Nitsche 2011). Resting membrane threshold (RMT) is also reported in the literature and can be defined as the minimum stimulus intensity required to evoke MEPs of at least 50uV in 50% of trials in a series. RMT is dependent upon the intrinsic excitability of the intra-cortical elements in the circuit responsible for MEP generation as well as the excitability of the inputs to the corticospinal neurones (Paulus, Peterchev et al. 2013). Another related measure often reported is the cortical silent period (CSP). The CSP is the interruption of voluntary electromyographic activity in the target muscle. This form of inhibition is GABA mediated and it usually follows an MEP but can also be seen in the absence of an MEP with low stimulus intensities (Paulus, Peterchev et al. 2013).

Electroencephalogram (EEG) recordings represent a surrogate measure of neurophysiological effects of tDCS. EEG records spontaneous neuronal firing, known as the event related potential (ERP), which is modified according to the brain area provoked. EEG recordings have demonstrated that cathodal tDCS increases power in the delta and theta bands of the EEG (Nitsche, Cohen et al. 2008). Yet, these measures may lack specificity, that is, the measured ERP can be an epiphenomenon of another brain region rather than a relevant firing (e.g. noise rather than signal).

Also the measurement is collated after, but not during, the delivery of the tDCS (Brunoni, Nitsche et al. 2012). This is explored further in Chapter 2 of this thesis.

Another surrogate measure is the use of biomarkers. The one of note in tDCS is brain derived neurotrophic factor (BDNF) which plays a role in synaptogenesis and neuroplasticity. A recent study showed the BDNF expression increases following tDCS (Fritsch, Reis et al. 2010)**.** The use of biomarkers is limited as they do not cross the blood-brain barrier; therefore serum levels may not reflect actual brain activity. Furthermore, biomarker levels reflect net brain activity and do not represent a specific area.

Assessment of motor functional performance is often undertaken when tDCS is applied to the motor cortex. The use of one dimensional behavioural tasks, such as reaction time and measures of strength, do not seem to correlate with more composite/compound measures of performance (Hummel, Heise et al. 2010) and in high functioning individuals may be limited by a ceiling effect of the task itself or of the assessment tool (Furuya, Klaus et al. 2014).

In recent years, research interest has grown in the use of magnetic resonance imaging (MRI) to attempt to expose the intrinsic mechanisms underpinning the effects of tDCS. This technique affords high spatial resolution which allows evaluation of subtle changes in areas not only in close proximity to the electrodes but also in remote brain regions (Jang, Ahn et al. 2009). It is popular as it can be performed repeatedly and is free from ionizing radiation. Several approaches are available and include functional MRI (fMRI), Diffusion Tensor Imaging (DTI), Magnetic Resonance Spectroscopy (MRs) and Voxel-based morphometry (VBM). However, the reliability of some methods of MRI are currently under dispute and it is not typically used simultaneously with tDCS therefore visualization of the brain during stimulation is not possible. Refer to Chapter 3 of this thesis for further information.

1.3.4 Factors which may influence the effects of tDCS

The effects of tDCS on the cortex are generally uniform across studies in healthy subjects in that anodal stimulation increases and cathodal stimulation decreases excitability. However, the size of the effect varies and it is generally not understood why some participants show more improvement with less stimulation current, or why others show no difference in effect size with respect to different current levels (Kim, Kim et al. 2014). For example, Fricke et al. (2011) reported an average improvement in MEP amplitude of 93.2% following five minutes of anodal stimulation in one group but only 9.2% enhancement in the second group. Substantial within-group variability also exists but is often masked by group averaging. For example, one study reports that following nine minutes of anodal stimulation one subject demonstrated a 295% increase in MEP amplitude whilst another

subject in the study demonstrated a mere 5% increase (Nitsche and Paulus 2001). In order for tDCS to be effectively applied in clinical populations, it may be necessary to examine response characteristics at the individual level.

Furthermore, inconsistencies in reports regarding which type of montage is more beneficial and mixed findings between indices of corticomotor plasticity and motor function, are rendering standardisation and optimization of the application of tDCS difficult. There is currently no consensus regarding stimulation parameters to maximize therapeutic outcomes and a protocol for maximum therapeutic efficacy remains undetermined (Galvez, Alonzo et al. 2013). Clearly, stimulation parameters are critical but cannot fully explain the extensive between group and within-group variation that exists. This inconsistent effect between individuals is likely to be the result of many factors that are known to influence corticomotor plasticity as outlined below.

Anatomical differences

Even when identical electrode montages are applied the size of the treatment effect has not been consistent across studies or subjects. This is most likely due to anatomical differences amongst individuals. A recent study with young healthy participants demonstrated that the current density in the brain is significantly influenced by different anatomical properties of each individual such as skull thickness and shape and the cortical folding pattern (Kim, Kim et al. 2013). This implies that individualized stimulation paradigms, considering anatomical data, would enhance the potential benefit of tDCS.

Another individual factor which may affect current flow is hair thickness. Hair is not a conductor and to combat this, a large amount of saline solution is often used to saturate thick hair which can spread and drip beyond the target area of interest. This may guide current in unwanted directions as the number and size of contact points at the scalp becomes unknown (Horvath, Carter et al. 2014). Similarly sweat on the scalp may impact current dynamics. Because sweat increases skin conductivity it is possible that the salts, oils and electrolytes in the pores of the scalp will prevent current from entering the cortex (Horvath, Carter et al. 2014).

Time of day

The response to stimulation appears to be affected by circadian rhythms and therefore by the time of day it is administered. Studies using PAS protocols have demonstrated that physiological outcomes are improved when the stimulation is applied in the afternoon rather than in the morning and one contributing factor is diurnal variation in cortisol levels (Sale, Ridding et al. 2008).

Several studies have looked at the influence of the time of the day that tDCS is applied with regard to memory outcomes. Marshall et al. (2004) demonstrated that tDCS enhanced declarative memory when applied during slow wave sleep but not when applied during the wake retention interval. Slow wave sleep is a known key factor for consolidation of plasticity and memory and the enhancing effect was associated with a tDCS induced increase in EEG activity considered to facilitate plasticity processes (Ridding and Ziemann 2010).

Genetic factors

The most studied genetic influence on brain plasticity is the brain derived neurotrophic factor (BDNF) gene which is released in an activity dependant manner and has a significant role in promoting changes in synaptic efficacy (Ridding and Ziemann 2010). It has also been suggested that changes induced by brain stimulation techniques are likewise influenced by this genetic variation. A study by Cheeran et al. (2008) demonstrated that the response of healthy subjects to different plasticity-inducing protocols in the motor cortex is associated with the polymorphism of the BDNF gene that they carry. These differences were significant in the after-effects of TMS protocols but less evident with cathodal tDCS. This indicates that different brain stimulation protocols act on different neural circuits which are differentially responsive to the BDNF polymorphism (Liebetanz, Nitsche et al. 2002). The implication is that genotype is one factor that can influence the effects of brain stimulation, and it may be necessary to include this as a potential covariate in data analysis.

Regular physical activity

Regular aerobic exercise has been shown to modify brain plasticity and improve learning and memory. This is theorised to occur via increased cerebral blood flow, angiogenesis and an increase in neurotrophic factors (Ridding and Ziemann 2010). These mechanisms may enhance the effects of brain stimulation in cardio-vascularly fit individuals. Consistent with this theory, one study using a PAS protocol reported that the size of the treatment effect was greater in highly active subjects than those who were sedentary (Cirillo, Lavender et al. 2009).

Influence of age

Evidence that ageing may be associated with a reduced capacity for motor plasticity, and therefore a reduced benefit to be gained from brain stimulation techniques, has been investigated in studies using TMS and PAS protocols (Tecchio, Zappasodi et al. 2008, Fathi, Ueki et al. 2010, Todd, Kimber et al. 2010). These studies consistently demonstrate a decreased LTP-like response in elderly subjects. To date there have been no studies directly investigating the size of the physiological treatment effect in different age groups using tDCS. Therefore, age dependency for tDCS efficacy has not been

established but cannot be excluded in light of the findings of age related studies in TMS (Pitcher, Ogston et al. 2003).

Gender differences

Several studies have highlighted that gender differences may influence response to tDCS. A study investigating sex differences in motor stimulation found that cathodal tDCS was more effective in females (Kuo, Nitsche et al. 2007). Likewise, another study reports that anodal tDCS over the visual cortex, was more effective in women (Chaieb, Antal et al. 2008). These gender differences are likely due to the influence of ovarian hormones on task performance (Pirulli, Fertonani et al. 2013). Therefore some researchers have attempted to control for gender differences by testing females during the follicular menstrual phase where progesterone levels are low and oestrogen levels are high and female cortical excitability is thought to be similar to that of males (Inghilleri, Conte et al. 2004).

Region stimulated

The effects of tDCS appear to be region specific and are possibly related to the orientation of fibres originating from or connecting to the stimulated region (Schlaug and Renga 2008). For example, the duration of the effects appear to be briefer in the visual cortex compared to the motor cortex (Antal, Kincses et al. 2004). Hence the response of one cortical region cannot be extrapolated to other regions. Other factors such as the initial state of excitation of the neurones prior to stimulation and the intrinsic properties of the neural network in which the stimulated neurones are integrated will influence the final outcome of stimulation (Miranda 2013). As the effects of tDCS are critically dependent on the direction of current flow through the neurones, small populations of neurones, oriented differently to those causing the net effects, may have an opposite effect and may eliminate all current generated excitability enhancements (Nitsche, Fricke et al. 2003).

Motor and cognitive interference

When stimulating the motor cortex, unrelated cognitive effort, as well as prolonged muscle contraction, can abolish the effects of the stimulation (Antal, Terney et al. 2007). That is, the plasticity induced by tDCS is dependent on the physiological state of the subject during the stimulation. It has been suggested that mental effort or motor activation may change the membrane potential or the post synaptic calcium ion concentration and therefore affect the response to external electrical stimulation (Huang, Rothwell et al. 2008).

The effect of attentional focus on the influence of the magnitude of induced plasticity has been examined in experimental models. Quartarone et al. (2004) reported this interference effect using a motor imagery task following five minutes of cathodal tDCS stimulation. This paradigm prolonged the effects of stimulation on MEP amplitude, conversely, it abolished the effects of anodal stimulation. Others demonstrated that the response to both cathodal and anodal stimulation was reduced when subjects concentrated on a cognitive task during the stimulation. It is speculated that the activation of cortical areas for the cognitive task may lead to deactivation of other areas of the brain which may interfere with neuroplasticity processes (Antal, Terney et al. 2007).

Similarly, several studies have demonstrated that motor activity undertaken during tDCS can negatively interfere with the effects of the stimulation. A simple motor task (moving a ball) conducted during stimulation led to reduced effects in MEP amplitude with both cathodal and anodal stimulation (Antal, Terney et al. 2007). A bilateral montage using stimulation applied for 10 minutes with concurrent active or passive finger abduction/adduction led to reduced MEP amplitudes to the same degree as performing the motor task alone which also suggests the opposing effects of motor activity on stimulation (Miyaguchi, Onishi et al. 2013).

Unfortunately, the findings to date are not consistent and interest in the relationship between timing of tDCS application and activity/therapy tasks has been ignited (Guleyupoglu, Schestatsky et al. 2013). Several authors have demonstrated that the application of tDCS during the execution of an implicit learning task leads to an improvement in the rate of learning (Nitsche, Schauenburg et al. 2003). This is supported by Stagg et al. (2011) who utilised an explicit motor learning task whereby the performance was only improved when the stimulation was applied during the execution of the task. Yet others have reported that behavioural facilitation is enhanced when the stimulation is applied before the task execution (Fertonani, Rosini et al. 2010, Vallar and Bolognini 2011). The most recent evidence involving 90 participants Pirulli at al. (2013) demonstrated that anodal tDCS facilitated greatest performance improvements in a visual perceptual learning task when applied before the task execution.

These findings intimate that simple thoughts or behaviours during or following stimulation may negate or interfere with the effects of tDCS. If the resting muscle is voluntarily activated or if attentional processes are enforced, anodal excitatory effects may even be converted into inhibition (Paulus, Peterchev et al. 2013). During administration protocols it is difficult to control for these factors and they may at least partially explain the conflicting findings reported in the literature (Nitsche, Cohen et al. 2008). This may also, in part, be explained by the differences in the task

examined whereby some studies used a visual task and others a motor task (Pirulli, Fertonani et al. 2013).

Dominant versus non-dominant hemisphere

Transcallosal inhibition from the dominant hemisphere can be different to that from the non-dominant hemisphere. Fregni et al. (2005) explored this in a study by evaluating the effects of anodal and cathodal stimulation in a sample of three dominant hemisphere and three non-dominant hemisphere strokes. Improvement was similar for both hemispheres with anodal stimulation, however, following cathodal tDCS the improvement was higher when the dominant hemisphere was inhibited (13.7% mean change) than when the non-dominant hemisphere was inhibited (9.7%). Other authors confirm that, with anodal stimulation, there is no significant difference between the after-effects measured on either side of M1 at any time point post stimulation, however the findings with cathodal stimulation require further validation (Boggio, Castro et al. 2006, Moliadze, Antal et al. 2010).

Asymmetric use of the non-dominant hand compared to the dominant hand is associated with reduced motor performance and skill. Similarly, asymmetry in motor function between the dominant and non-dominant limbs is a likely consequence of hemispheric differences in corticomotor excitability and inhibition (De Genarro, Cristiani et al. 2004, Cirillo, Rogasch et al. 2010). Chapter 2 of this thesis explores this issue further.

Handedness

One study has directly investigated whether tDCS induces different responses in those who are right handed compared to those who are left handed or ambidextrous (Schade, Moliadze et al. 2012). This study revealed that right handed subjects responded in an anticipated manner, that is, increased cortical excitability with anodal stimulation and decreased excitability with cathodal stimulation. However left handers and ambidextrous subjects had a greatly reduced amplitude of effect indicating that the effects of tDCS differ according to hemispheric lateralization. As the majority of research has been conducted in right handed subjects caution should be applied when making inferences to those who are not right limb dominant.

Pharmacology

Baseline cortical excitability can differ in individuals due to a range of factors. People using pharmacotherapy (e.g. anticonvulsants, antidepressants) as well as those who smoke may affect the uniformity of effects seen in studies (Brunoni, Nitsche et al. 2012).

Numerous studies have investigated the effects pharmacological agents on the response to cortical stimulation in healthy subjects and found that the size and direction of the effects can be highly modulated by pharmacology (for a review see Ziemann, Meintzschel et al. 2006). As many forms of long-term plasticity are dependent on NMDA receptor activity, antagonistic drugs, such as dextromethorphan, can block the effects of stimulation and NMDA agonists can facilitate the effects (Liebetanz, Nitsche et al. 2002). Neuromodulating neurotransmitters such as dopamine, acetylcholine, noradrenaline and serotonin have also been shown to modify stimulation induced plasticity. Therefore, pharmacotherapeutic agents which target the release or uptake of these neurotransmitters may influence cortical stimulation outcomes (for a summary see Ridding and Ziemann 2010).

1.3.5 Summary of the effects of tDCS

There is extensive variability in the response to tDCS that is evident between subjects but also within the same subject when tested on separate occasions. Neither the chemical factors nor the stimulation dose fully determines the biologic or therapeutic outcome. As with pharmacology, replication of the stimulation dose across subjects does not guarantee that the outcomes will be identical and dose selection factors cannot fully determine the physiologic response (Peterchev, Wagner et al. 2012). Therefore, in the context of clinical research, these individual factors may produce variability in findings and to avoid this, careful standardization of the sample and technique must be performed.

Table 1.2 Summary of factors affecting response to tDCS

Variable Description	Examples
Stimulation parameters	frequency, intensity, polarity, duration, interval between, total number of sessions
Electrode configuration	size, shape, distance between, orientation
Conductivity and geometry of the structures being stimulated	the scalp and skull thickness and electrical impedance, orientation and location of axons within the white matter, neural pathways with respect to the electric field
Individual neural morphology	neurotransmitter concentrations and receptor expression
Individual factors	age, gender, cognitive and affective state, concomitant pharmacologic interventions, baseline hormone levels, genetics, circadian rhythm and time of day, chronic and acute physical exercise

A summary of factors thought to affect the outcome of tDCS stimulation is provided in Table 1.1. The relative contribution of each of these factors is impossible to determine at this stage as there is a lack of studies which systematically manipulate each factor while controlling for the other parameters (Saiote, Turi et al. 2013). An additional issue for researchers is sample heterogeneity, particularly in disease conditions such as stroke where variations in the presentation of the condition can add to the unpredictability of the response to tDCS. In practice, stimulation parameters are the easiest to control and assess (Szelenyi, Journee et al. 2013). But the immense range of possible dose parameters, which provides for exceptional flexibility, also presents a challenge in determining the optimal dose for specific applications and may hinder interpretation and replication of the findings (Peterchev, Wagner et al. 2012).

1.4 Safety

Extensive animal and human studies and theoretical evidence indicate that the tDCS protocols which are currently used are safe (Nitsche, Cohen et al. 2008, Liebetanz, Koch et al. 2009). Furthermore, the induced outcomes, both electrophysiological and behavioural effects, appear to be totally reversible (Liebetanz, Koch et al. 2009). tDCS has been tested in thousands of subjects worldwide and there have been very few reported adverse events associated with its application. The most severe documented adverse event occurred in one of the earliest scientific studies whereby the patient experienced a transient respiratory and motor paralysis which resolved when the current was stopped (Redfearn, Lippold et al. 1964). In this case the reference electrode was positioned on the leg and the current intensity was in the order of 3mA which is not typically used in current practice. However to date, there have been no human studies aimed at systematically exploring and defining the safety limits of tDCS. Safety considerations are based on measurements of neuron-specific enolase (a marker of neuronal damage), MRI data, questionnaires asking about side effects, cognitive testing, and observation of clinical symptoms (Iyer, Mattu et al. 2005, Liebetanz, Koch et al. 2009).

1.4.1 Safety protocols

Nitsche and colleagues have described general safety limits for tDCS and emphasize current density and total charge as the most important parameters for judging tDCS safety (Nitsche, Liebetanz et al. 2003). McCreary et al. (1990) found that current density must exceed 25mA/ cm^2 before brain tissue is at risk of harm. The current density in protocols that apply 1mA through an electrode with a size of 15-25cm^2 is approximately 0.1mA/cm^2 . This translates into 0.004% of the magnitude at which stimulation could be potentially damaging to tissue (Schlaug and Renga 2008).

The exclusion criteria are very general and apply to all types of electrical stimulation: unstable medical conditions, epilepsy, acute eczema at the electrode sites, metallic implants near the electrodes (e.g. cochlear implants, aneurysm clips), pacemaker, and pregnancy.

Concerns for safety are heightened when paradigms with increased intensities or prolonged durations are applied. Yet as more intensified tDCS regimes are potentially more therapeutically potent, our present incomplete knowledge of the potentially dangerous effects of tDCS may be fettering the development of more beneficial paradigms (Liebetanz, Koch et al. 2009). On the contrary, a concern has been raised that in an attempt to further progress our understanding and the implementation of tDCS researchers may be using unorthodox experimentation outside the tested norms (Bikson, Bestmann et al. 2013). Improvised devices and/or practices that apply electricity to the brain without reference to established protocols may distort the long term validation of tDCS and put participants at risk of injury (Bikson, Bestmann et al. 2013).

Safety considerations have been built into the modern day tDCS commercially available units. With high resistance, a tDCS device may reach a programmed, pre-defined upper voltage limit to shut down the unit and abort the session. If this occurs, typically the operator will add more saline solution/remoisten the electrodes and recommence the session. This allows the operator to use impedance measures to insure stimulation efficacy by guaranteeing constant current strength and keep safety measures within a defined range (Paulus, Peterchev et al. 2013).

1.4.2 Sensory discomfort

Sensory side effects are commonly reported with tDCS and include: tingling, itching and burning, yet these are typically categorised by participants as mild and do not limit continued involvement in studies (Poreisz, Boros et al. 2007, Kessler, Minhas et al. 2013). A mild, transitory redness beneath the electrodes can be expected and is most likely due to local vasodilatation rather than skin damage (Brunoni, Nitsche et al. 2012). The frequency and severity of sensory effects appears to be age-related whereby age seems to have a protective effect. Several authors report that healthy young volunteers reported higher rates of tingling sensations with both active and sham stimulation than older healthy volunteers and patients with chronic stroke and the severity of discomfort was significantly greater in younger cohorts (Gandiga, Hummel et al. 2006, Kessler, Minhas et al. 2013).

1.4.3 Adverse events

Global symptoms such as headache, impaired concentration, nausea and fatigue are reported much less commonly in the literature and are more likely to be linked to the experimental tasks involved in the study rather than the tDCS itself (Tadini, El-Nazer et al. 2011, Kessler, Minhas et al. 2013).

The risk of epileptic seizures is often raised in the literature but as tDCS does not cause seizure nor reduce the seizure threshold in animals, this does not appear to be a valid risk for human subjects. However this may not be true for patients with a diagnosis of epilepsy (Nitsche, Cohen et al. 2008). Another concern is that the misplacement of the electrodes can at least transiently impair specific cognitive functions (Kadosh, Soskic et al. 2010) or induce maladaptive behavioural changes (Fumagalli, Vergari et al. 2010). Yet it is generally accepted that if consideration is given to the montage and dosage and patient monitoring, then these effects should not be anticipated (Poreisz, Boros et al. 2007).

However, several isolated cases of adverse reactions to tDCS have been reported. A single case of monocular eyelid myokymia (fasciculations of the lower orbicularis oculi muscle) in an older subject after five consecutive sessions of tDCS has been reported (Wessel, Zimerman et al, 2013). Erythemata consistent with an allergic event caused by DC-iontophoresis or a photodermatitis type reaction has been reported in a healthy young male adult (Riedel, Kabisch et al. 2012). A greater concern is the possibility of skin irritation and breakdown. Skin lesions were first reported by Palm et al. (2008) in all five subjects with major depressive symptoms, who received 2mA of anodal tDCS for 20mins five days per week for two weeks. The lesions occurred under the cathode (on the forehead) and showed extensive redness and intracutaneous changes up to 2cm in diameter. The lesions occurred after the fourth or fifth tDCS session and healed within three weeks after the final session. Similarly five cases of skin lesions were reported under the cathode by authors who applied 2mA of tDCS for the management of pain following spinal cord injury (Rodriguez, Opisso et al. 2014). Another study where 15 patients received 30mins of 1.5mA anodal tDCS in the management of chronic tinnitus for two days per week for three weeks reported three cases of skin lesions (Frank, Wilfurth et al. 2010). However, there are two important distinctions in this study in that the lesions occurred under the anode and the electrode sponges were soaked in tap water not saline. A more recent study (Palm, Feichtner et al. 2014) directly compared the effects of using different solutions (tap water, saline, or electrode gel) in healthy young volunteers. With 2mA of bifrontal tDCs applied for 20mins, five of the 10 subjects developed blistered skin lesions when tap water was used, and three out of four participants developed crusty ulcerations in the electrode gel condition. These occurred independently of the skin type, impedance, and electrode type (anode/cathode).

It is speculated that potential contributing factors to skin lesions include: properties of the skin (Rodriguez, Opisso et al. 2014), excessive drying out of the electrode sponges, uneven drying of electrode gels (Palm, Feichtner et al. 2014), electrochemical reactions in the skin, accumulation of electrochemically produced toxins in the sponges, or toxic reaction by metallic particles in tap water which can be transferred to the skin (Frank, Wilfurth et al. 2010).

Only one reported incident of a skin lesion occurring after a single session of tDCS exists in the literature. In this case a skin burn occurred in a healthy young male, beneath the cathode, following 2mA of anodal stimulation over a 26 minute period. Hot spots of current density around the edges of the electrodes and perhaps around skin inhomogeneities (e.g. sweat glands) are considered to lessen tolerability to stimulation and efforts to increase uniformity of current density are rational (Minhas, Datta et al. 2011) Therefore, although infrequent in occurrence, skin lesions have to be considered as a potential side-effect , especially when tDCS is applied repeatedly, and subjects should be warned about this possible outcome (Frank, Wilfurth et al. 2010).

1.4.4 Regulations of use

The qualities that offer the wide clinical applicability of this device – ease of use and access – simultaneously give rise to safety concerns. It is relatively easy to purchase a tDCS device or alternatively just as easy to source online information about building your own device where essentially only a 9V battery and easily purchased electronic parts are required. (Brunoni, Nitsche et al. 2012). A rudimentary tDCS can be put together for less than $100 and there are currently people involved in self-experimentation and self-treatment using home-made units (Cabrera, Evans et al. 2014). Combined with media coverage promoting this modality as an all-purpose cognitive enhancer, interest has been fuelled in the do-it-yourself community (Fitz and Reiner 2015). Among the safety concerns are the flexibility of the configuration of the device (e.g. electrode placement, current thresholds, polarity etc.) and interaction with other therapies especially psychoactive agents. This notion of unregulated self-usage is worrisome given that so much remains unknown about the neural mechanisms and long term effects of tDCS and there is fear that home experimentation may lead to adverse consequences (Cabrera, Evans et al. 2014).

It is worth noting that the Food and Drug Administration (FDA) which regulates the marketing of medical devices in the United States, as well as recent European Union legislation, has permitted commercial use of tDCS for research purposes only.The regulatory status of tDCS remains in its infancy (Guleyupoglu, Schestatsky et al. 2013). Despite this, devices are already being sold to the public in the USA (Fitz and Reiner 2015).

1.4.5 Summary of safety considerations

The reports of skin lesions are isolated and rare and tDCS is generally considered safe with little side effects when used within defined parameters. However, although single and multiday sessions have been found to be safe, the safety of prolonged periods of stimulation requires further investigation (Schlaug, Renga et al. 2008). Similarly most safety data has been obtained from studies with single-stimulation sessions in healthy, young adults without medications. Less is known about the adverse

effects of consecutive stimulation sessions in the elderly or those using concurrent pharmacotherapy (Brunoni, Nitsche et al. 2012).

Therefore, although tDCS appears to be safe, there is no evidence that this modality is unequivocally benign in that it may produce occult effects, particularly in cognitive function, which have not been anticipated or measured (Fitz and Reiner 2015). For example, a study investigating stimulation of the parietal cortex demonstrated enhanced numerical competence, however it was only when automaticity was assessed that a deficit was noted (Iuculano and Cohen Kadosh 2013). It is these inadvertent effects that cause uneasiness in the widespread clinical application of tDCS at this stage.

1. 5 Stroke and tDCS

1.5.1 Stroke in Australia

Stroke is defined as a focal neurological impairment of sudden onset and presumed vascular origin lasting more than 24 hours or resulting in death (World Health Organisation, 2006). Stroke may be ischaemic or haemorrhagic in nature resulting in an area of neuronal death, which leads to a loss of brain function and impairments specific to the area of damage which may include communication, motor, or cognitive compromise (Australian Institute of Health and Welfare, 2010). Based on epidemiological research, one in five Australians who suffer their first-ever stroke die within the first month of the event, and one third die within the first year (Thrift, Dewey et al. 2009). These death rates have continually declined over the past few decades because of advances in acute stroke management together with improvements in primary and secondary stroke prevention strategies (Grefkes and Ward 2014). Despite this, in the last 10 years the prevalence of stroke has risen by 18.2% and this trend is expected to continue due to the aging of the population (Australian Institute of Health and Welfare, 2006).

In 2012 there were over 42,000 Australian stroke survivors and this number is projected to be 709,000 by the year 2030 (Deloitte Access Economics, 2013). This growing prevalence is accompanied by increasing financial costs. The lifetime costs of first stroke episodes are reportedly more than $2billion dollars per annum in Australia (Cadilhac, Carter et al. 2009). In addition, the informal care provided to stroke survivors represents a significant hidden cost to society (Dewey, Thrift et al. 2002).

1.5.2 Stroke morbidity

Despite advances in acute management, stroke remains a major cause of disability in western countries such as Australia (Sturm, Donnan et al. 2004). In 2009, it was estimated that over a third of Australians with stroke continued to live with disability as a result of the stroke and that these

people were much more likely to be profoundly limited in daily activities than people with other disabilities (Australian Institute of Health and Welfare, 2013). Two in three stroke survivors are reliant on others for assistance for daily living and this dependency tends to worsen over time (Dhamoon, Moon et al. 2009). This creates an extensive burden on stroke survivors, caregivers, the health system, and society and encompasses personal, financial, physical, emotional, psychological and social distress. Furthermore, evidence suggests that this burden is greatest in the chronic stages of recovery once the stroke survivor is living back in the community (Carod-Artal and Egido 2009). Even patients subjected to effective acute interventions such as thrombolysis or thrombectomy often nonetheless experience persistent neurological deficits. Therefore, at this current time, neurorehabilitation remains the most important pillar supporting recovery of function post stroke (Grefkes and Fink 2012).

1.5.3 Stroke recovery

Recovery from stroke is attributed to a dynamic neuroplastic process of regeneration and cortical reorganization in both the perilesional and contralesional hemispheres. This involves an increase in the excitability of the perilesional areas mediated by excitatory neurotransmitters in the acute and subacute phase (Kreisel, Bazner et al. 2006). This subsides in the chronic phase which is more characterized by changes in inter-hemisperic communications. Regeneration involves axonal and dendritic sprouting and formation of new synapses which is stimulated by the release of various growth factors in the perilesional cortex (Carmichael 2006).

Reorganization involves remapping of the lesioned area representations onto non-lesioned cortex either in the perilesional cortex or in the contralesional hemisphere (Schlaug and Renga 2008). This is evidenced by increased activation of the contralesional cortex during movement of the stroke affected limb. The significance of this remains undetermined. It has been proposed that it is an epiphenomenon of recovery, an adaptive neuroplastic process, or alternatively a maladaptation that may impair recovery (Schlaug, Renga et al. 2008). These processes seem to occur collectively and can be enhanced by rehabilitation therapies, even long after the stroke has occurred (Zhao, Wang et al. 2009).

Incomplete motor recovery is associated with abnormal brain activity in both the ipsilesional and contralesional hemisphere. This knowledge has been extended by fMRI- based studies which show that abnormal interhemispheric coupling between the primary motor cortices is a key feature of more severely impaired patients even if the cortex has been spared by the lesion (van Meer, van der Marel et al. 2010). Early reactivation of the remaining intact regions of the lesioned cortex correlates with improved recovery (Schlaug, Renga et al. 2008). Restoration of motor function after stroke

begins with improvements in repetitive, simplistic activities such as force production, evolving to relearning of more complex motor synergies and skilled tasks required for functional use (Hummel, Voller et al. 2006).

1.5.4 Stroke rehabilitation

Although some degree of recovery may occur spontaneously, evidence suggests that intensive practice is necessary to maximize recovery potential (Nudo and Friel 1999). To date the best approach seems to be rigorous physical therapy which is an eclectic combination of facilitation approaches (e.g. proprioceptive neuromuscular facilitation (PNF), weight bearing, functional electrical stimulation (FES), etc.), task specific training (e.g. finger tracking), task oriented training (e.g. Constraint Induced Movement Therapy (CIMT) and bilateral arm training (Bolognini, Pascual-Leone et al. 2009).

Yet clearly traditional methods are inadequate with large inter-individual variation in response to therapy and large numbers of stroke survivors living with residual deficits (Wade, Langton Hewer et al. 1983). Our growing understanding of neural plasticity and its relationship to stroke recovery has led to the emergence of several novel stroke rehabilitative methods. These include, body weight support treadmill training, virtual reality training, and robotic therapy. These modalities increase the volume and intensity of motor training that is attainable post stroke and are thought to direct use-dependent plasticity (Takeuchi and Izumi 2013). However, use-dependent plasticity is known to be impaired post stroke (Carmichael 2006) and therefore other methods, aimed at augmenting the response, such as tDCS, may be advantageous (Takeuchi and Izumi 2013).

1.5.5 Proposed mechanism of tDCS in stroke recovery

The use of tDCS to facilitate stroke recovery is based on the hypothesis that a focal lesion of the motor cortex disrupts cortical activity and creates an imbalance between the hemispheres. This produces disinhibition of the contralesional areas suppressing neural activity in the lesioned hemisphere which already has decreased excitability due to the stroke lesion (Grefkes and Fink 2012). This hypothetical model suggests that tDCS can facilitate a shift of the imbalance towards a more equal state (Schlaug and Renga 2008). In this context, tDCS appears to be the ideal tool as it can non-invasively exert an inhibitory influence on the contralesional motor cortex (via cathodal stimulation) or an excitatory influence on the perilesional motor regions (via anodal stimulation) or exert both effects concurrently (via bihemispheric stimulation). Hence, all modes of tDCS (anodal, cathodal and bihemispheric) may have a role in enhancing stroke recovery (Schlaug and Renga 2008).

The hand area of the M1 has been used almost exclusively in the stroke literature due to its superficial location on the cortex, but several studies report successfully stimulating the cortical leg area (Madhavan, Weber et al. 2011, Sohn, Jee et al. 2013, Chang, Kim et al. 2015), the language centres (Elsner, Kugler et al. 2015), and the cerebellum (Ferrucci, Cortese et al. 2015, Pozzi, Minafra et al. 2014).

Furthermore, brain imaging studies have revealed that motor skill learning is associated with the recruitment of large scale neuronal circuits involving the supplemental motor area (SMA) and dorsolateral prefrontal cortex (DLPFC) (Dayan and Cohen 2011). It has been shown that anodal tDCS over SMA can facilitate visuomotor learning in a similar manner to M1 stimulation, possibly due to an indirect facilitatory effect of SMA to M1 via the dense efferent cortico-cortical connections (Vollmann, Conde et al. 2013). A few studies have examined the effects of tDCS on the posterior parietal cortex and demonstrated that anodal stimulation can improve attentional processing and cathodal stimulation impairs function (Ko, Han et al. 2008, Sparing, Thimm et al. 2009, Bolognini, Olgiati et al. 2010). Medina et al. (2013) found that anodal stimulation of the right posterior parietal cortex improves reaction times for allocentric processing which may inform stimulation paradigms for patients with visuospatial neglect.

1.5.5.1 Anodal tDCS

The most commonly reported protocol for tDCS stimulation in stroke is the anodal montage with the active electrode positioned over the area of scalp corresponding to the lesioned motor cortex. Here the implication is that increasing the excitability of the affected perilesional region can increase functional performance (Ward, Cohen, 2004). The earliest randomised trial in stroke consisted of a single application of 20mins of anodal tDCS (1mA/25cm^2) to assess its effect on hand function (Hummel, Celnik et al. 2005). The study used a sham controlled, double blind, cross over design and demonstrated a significant improvement in the anodal group. The magnitude of the improvement was modest (≈12% improvement) but robust as it occurred in each of the six participants. The effect outlasted the stimulation period by at least 25 minutes, but had returned to baseline when retested 10 days later. The results showed a trend for more improvement to be detected in the Jebsen Taylor Test of Hand Function (JTT) subtests requiring fine distal motor control (compared to those needing only proximal/gross motor control, where an effect was not seen).

Hummel et al. (2006) extended this work by investigating whether anodal stimulation had an effect on simple tests which may be more appropriate for severely impaired patients. Both simple reaction time and pinch force, which predominantly rely on M1 functioning rather than extensive brain networks, had significant improvements compared to sham tDCS following a single 20min session of

anodal 1mA/25cm^2 tDCS. Stratification of the 11 subjects according to impairment revealed that tDCS improvement was greater in the more impaired group. Several studies have followed but have not been consistently able to reproduce these results. For a systematic review of the literature refer to Chapter 4.

1.5.5.2 Cathodal tDCS

Cathodal application, that is, stimulation of the non-lesioned hemisphere to down-regulate excitability and release the lesioned hemisphere from excessive transcallosal inhibition, may be inherently more advantageous than anodal stimulation for several reasons (Boggio, Nunes et al. 2007). Anatomy changes following stroke in the affected hemisphere could disturb the electric current administered by the tDCS and therefore the results from stimulating the affected hemisphere may be less predictable (Fregni, Boggio et al. 2005). Stimulating the non-affected hemisphere has the benefits of a normal cortical topography, intact intra-cortical connections, reduced risk of triggering a "scar" seizure, and reliance on a model of distribution of current density that is not disturbed by a lesion where there is non-homogenous tissue (Schlaug, Renga et al. 2008).Yet this theory has yet to be substantiated and it remains unclear which mode of stimulation is superior.

Several studies with small samples directly comparing cathodal and anodal stimulation to explore the differences in results have been conducted. Fregni et al. (2005) applied 1mA/35cm^2 for 20 minutes in a cross over, sham controlled trial. The six subjects received sham, anodal or cathodal stimulation in a randomized order to reveal significant differences in upper limb function for both the cathodal and anodal paradigms as compared to sham. The authors report no statistically significant difference between the anodal and cathodal groups. However, there was a larger absolute improvement for the cathodal group (cathodal mean improvement from baseline = 11.7%, anodal mean improvement = 6.8%). These findings were replicated in a similar study where four subjects received the same treatment conditions but weekly over a four week period for each condition (sham, cathodal, anodal). Here the mean improvement from baseline was 9.5% for cathodal and 7.3% for anodal stimulation (Boggio, Nunes et al. 2007).

Conversely, detrimental behavioural effects have been reported in upper limb motor tasks following cathodal tDCS (Stagg, Jayaram et al. 2011). During the performance of unimanual tasks, contralateral motor regions are positively modulated, while ipsilateral areas are inhibited which may lead to efficient synaptic transmission and ultimately faster motor execution (Grefkes, Nowak et al. 2008). Therefore, the application of cathodal tDCS may interfere with this inter-hemispheric decoupling

mechanism by synchronizing the activity between the two hemispheres resulting in impaired motor performance (Amadi, Ilie et al. 2013).

1.5.5.3 Bihemispheric tDCS

This technique is thought to have the most potential in stroke and other conditions producing unilateral brain injury whereby the behavioural effects occur not only through dysfunction at the lesion site, but also from increased inhibition arising from the contralesional side of the brain (Priori, Hallett et al. 2009). The hypothesis is that simultaneous excitation of the lesioned hemisphere and inhibition of the contralesional hemisphere may provide additive efficacy (Kidgell, Goodwill et al. 2013). This was first tested in healthy subjects by Vines et al. (2008) who reported that bilateral tDCS facilitated motor performance in the anode stimulated hemisphere to a greater level than when the same hemisphere was stimulated using the typical unilateral anodal montage. Subsequently, Lindenberg et al. (2010) examined a sample of chronic stroke patients to demonstrate that bihemispheric stimulation can facilitate motor recovery. This finding was recently confirmed by others (Lefebvre, Laloux et al. 2012, Lefebvre, Thonnard et al. 2014). Yet the superiority of this montage has been refuted and it remains relatively under investigated compared to other tDCS applications.

1.5.6 Additional considerations for tDCS in stroke

There are many variables which have the potential to affect the outcome of tDCS as discussed in Chapter 1.3.4. In the case of stroke, additional consideration must be given to disease related factors which may also impact on the effects produced by cortical stimulation.

1.5.6.1 Time since stroke

The optimal post stroke time at which tDCS should be initiated to enhance recovery has not yet been established. As recovery of motor function after stroke is associated with changes in inhibitory and facilitatory circuits within the motor cortex, which occur over time, corticospinal excitability in the subacute stage may be different from that in the chronic stage (Wittenberg, Bastings et al. 2007). For instance, it is suspected that the NMDA receptor plays an important role in the acute phase of stroke to prevent cell death in the penumbra and it is postulated that over activation of NMDA, which may occur via tDCS, may be detrimental. Yet conversely in the later stages increased activation of the NMDA may be essential for recovery (Adeyemo, Simis et al. 2012). Consequently it is not realistic to presume the effects of tDCS at different stages of recovery would be equal.

To date, the majority of studies have focused on chronic stroke patients in outpatient settings.

The few available subacute stroke studies (Kim, Ohn et al. 2009, Kim, Lim et al. 2010, Rossi, Sallustio et al. 2013) report similar findings however further research directly comparing the effects of tDCS in subacute patients with chronic stroke applications is required to determine the optimal time point for tDCS interventions.

1.5.6.2 Stroke severity and localization

Ideal electrode montage and dosage parameters are poorly defined in the healthy population. This is even more problematic in clinical populations such as stroke where it is expected that the lesioned brain tissue influences current flow. As a result of stroke scar tissue, larger cerebrospinal spaces form with corresponding increases in cerebrospinal fluid, which has a conductance 4-10 times higher than brain tissue (Wagner, Valero-Cabre et al. 2007). These factors modify the geometry and the pattern of current flow both in the perilesional areas and wider cortical regions with dramatic effects resulting from the positioning of the reference cathode electrode (Datta, Baker et al. 2011). It has been suggested that electrode configuration should be individually tailored to leverage current flow to the target tissue in stroke patients and that computational models are critical for the rational design of individualised tDCS therapy with this pathology. However, the practicality of this suggestion is questionable.

Due to the heterogeneity of patients included in stroke studies it is difficult to elucidate the effect of stroke severity and location on the effects of tDCS. Hesse et al (2007) reported within-group differences in tDCS response according to stroke severity. This non-case-controlled study combined six weeks of robot-assisted arm training with tDCS in 10 subjects. Three of the subjects demonstrated significantly improved arm function as measured by the Fugyl Meyer test, but in contrast, seven patients with severe paresis and cortical lesions demonstrated no significant improvement.

This result supports the suggestion that an intact pyramidal tract may be an important consideration for patient selection. Similarly, others report an increased benefit from tDCS when it is applied to patients with subcortical as opposed to cortical strokes. It is possible that in this scenario, where the cortex is preserved, the tDCS has greatest capacity to facilitate neuroplasticity (Adeyemo, Simis et al. 2012). However this has not been consistently reported in the literature and it is currently not clear whether the severity of the stroke provides better or worse potential for recovery.

The concept of inter-hemispheric balance is considered in most brain stimulation studies involving stroke patients. The restoration of this balance is the prevailing hypothesis which underpins rehabilitative therapies. But several authors are now contemplating whether the contralesional hyperactivity post stroke is not maladaptive but instead denotes an additional role in recovery

(Adeyemo, Simis et al. 2012). It may, infact, be beneficial in a particular subset of strokes and consideration of the level of stroke recovery may be necessary before applying tDCS. Recent insights into stroke recovery suggest that increased participation of the sites adjacent to the infarct as well as reorganisation of function in the homologous regions of the intact hemisphere is important (Levin 2006). This also raises the issue of how stimulation should be applied in the case of bilateral strokes. Clearly the excitability relationship between the recent stroke and previous lesion would need to be considered when developing the stimulation paradigm.

1.5.6.3 tDCS as an adjunct to therapy

The effects of cortical stimulation in stroke recovery may be enhanced by combining it with other rehabilitative techniques as provided by Physiotherapists or Occupational Therapists. Motor training is thought to have a similar impact on synaptic and network plasticity as cortical electrical stimulation therefore it may be possible that coupling the two approaches can potentiate relearning of motor skills to a level unattained by either intervention alone (Schlaug, Renga et al. 2008).
This concept is based on the theory that peripheral sensorimotor activity combined with central brain stimulation can enhance synaptic plasticity and motor relearning by modulating afferent inputs to the cortex (Schlaug and Renga 2008).

Furthermore, cortical stimulation activates neural circuits in a non-specific manner, therefore motor training may be able to guide the activation of the specific neural networks to facilitate recovery of the desired functional ability (Bolognini, Pascual-Leone et al. 2009). Several authors have investigated the effects of combining traditional, peripheral electrical stimulation therapies with tDCS to examine whether the effects are summative, i.e. increase corticomotor excitability beyond that of tDCS alone, or competitive, i.e. block or reverse the effects of tDCS. The results are inconclusive (Nitsche, Roth et al. 2007, Bolognini, Vallar et al. 2011).

The timing of the application of tDCS in relation to physical therapy is contentious. Most research has focused on sequential application of modalities. That is, the tDCS stimulation is followed by adjunctive therapy. When the application is sequential the effects generally adhere to the principles of homeostatic plasticity, that is, the application of two modalities which each separately enhance excitability leads to reduced cortical excitability, whereas the combination of a suppressive and excitatory modality increases excitability (Lang, Siebner, 2004).

The effects on synaptic function induced by concurrent neuromodulatory techniques are poorly understood and less reliable. One study has shown that PAS and repetitive median nerve stimulation

at the arm increased motor cortical excitability to a greater extent than cortical stimulation alone (Stefan, Kunesch et al. 2000). Others have demonstrated that tDCS has an additional benefit when applied with constraint induced therapy in patients with stroke (Bolognini, Vallar et al. 2011). Schabrun et al. (2013) built on this work by investigating the effects of concurrent tDCS and neuromuscular electrical stimulation of the abductor pollicis brevis muscle. They found that concurrent application of the two modalities failed to induce summative effects on cortical excitability as would be predicted by homeostatic plasticity mechanisms. Combined cathodal tDCS and peripheral muscle stimulation suppressed the increase in cortical excitation induced by the peripheral stimulation alone. This is not consistent with the principles of homeostatic plasticity and the authors suggest an anti-gating hypothesis to explain this finding (Schabrun, Chipchase et al. 2013).

Heterogeneity of adjuvant therapies, dosage and timing make comparison of studies and a determination of benefits difficult to establish. Clearly the complex interaction whereby the neural effects are non-summative, and even competing when modalities are applied concurrently requires further investigation to ensure that beneficial effects on behaviour are facilitated, and deleterious effects avoided (Schabrun, Chipchase et al. 2013). Specifically, we have no clear indication as to whether implementing therapy pre, post or co-stimulation alters the long-term motor effects (Adeyemo, Simis et al. 2012).

1.5.7.4 Risk of seizure

According to Olsen (2001) the risk of developing seizures is 35 times greater in the first year post stroke compared to the general population and 19 times more likely in the second year post stroke. This risk is thought to be greater in those who have experienced a haemorrhagic stroke as opposed to an ischaemic event (Reith, Jorgensen et al. 1997). Although there have been no documented reports of tDCS related seizure, consideration is warranted. Currently, most research excludes stroke participants with a history of seizure or usage of anti-seizure medication therefore the lack of seizure episodes reported in the tDCS literature cannot be generalised to this subgroup of the population.

1.6 Conclusion

Stroke is undeniably one of the largest health issues faced by contemporary Australians. It contributes some of the largest burden of illness on patients, their families, the health care system, and the community. Indeed, motor impairments following stroke are the leading cause of disability in adults (Bolognini, Pascual-Leone et al. 2009). Of those who survive approximately half will live the remainder of their lives with residual disabilities (Clarke, Black et al. 1999).

It is these stroke realities which compel the urgent search for more effective interventions to maximize the rehabilitation potential of people with stroke and to prevent subsequent complications. Indeed, further development of rehabilitation strategies has been set as a priority for research by the National Stroke Foundation (2010).

tDCS has re-emerged in the past two decades as a potential therapeutic modality and researchers have ardently begun to investigate its application using a range of protocols and in a range of conditions. Early published research demonstrated positive and robust excitability changes in the stimulated cortex of healthy adults but as the number of clinical studies has increased, obvious intra and inter trial variability has become increasingly apparent. This inconsistency in the effects produced by tDCS as well as disparity in trial design, participant characteristics and stimulation protocols has made it almost impossible to interpret the merit of this modality. To determine whether tDCS has potential as a therapeutic intervention in stroke rehabilitation is an even more difficult task given the heterogeneity of the stroke population.

Set on this background, this thesis was undertaken with the aim of contributing to the growing body of research investigating tDCS with the goal of facilitating its transition from research to clinical applications in stroke rehabilitation. Due to the additional highlighted complexities of stimulating the lesioned brain we commenced our research of tDCS with healthy aged participants. This laid the foundations for the subsequent study investigating the application of tDCS in people with stroke.

Chapter 2: Effects of tDCS in the Healthy Aged

2.1 Publication Details and Author Affiliations

Marquez J[1 2], Conley A[2 4], Karayanidis F[2 4], Lagopoulos J[3], and Parsons M[1 2 5] (2015). Anodal direct current stimulation in the healthy aged: Effects determined by the hemisphere stimulated. *Restorative Neurology and Neuroscience* 33 (4):509-19.

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2.2 Co-author Statement of Contribution

As co-authors, we attest that the Research Higher Degree candidate, Jodie Marquez, contributed to the development of the research design, data collection, data analysis, and writing of the journal submission for the publication entitled: Anodal direct current stimulation in the healthy aged: effects determined by the hemisphere stimulated*. Restorative Neurology and Neuroscience* 33(4):509-19.

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2.3 Synopsis

The bulk of research investigating the effects of tDCS has been conducted in healthy young subjects, predominantly university student volunteers. This sampling approach limits the generalisability of findings to those not represented in these samples, namely those who are older. This is a clinically important distinction as neurodegeneration and neuromuscular control decline as a natural part of the ageing process. Furthermore, evidence suggests that the effectiveness of motor training alone in older adults is reduced when compared to the young (Rogasch, Dartnall et al. 2009). As our interest is in the application of tDCS in people who have residual stroke deficits, and who are predominantly comprised of old patients, extrapolation of findings from young subjects may be flawed.

Yet, studies investigating the effects of tDCS in the elderly are lacking. In the two previous studies conducted using aged participants (Hummel, Heise et al. 2010, Zimerman, Nitsch et al. 2013) the samples were small and the tDCS was limited to dominant hemisphere stimulation and performance measures of the dominant hand. Hence our aim was to build on this earlier work to more comprehensively assess if:

1. The effects of tDCS are mediated by age
2. The results are influenced by which hemisphere is stimulated
3. Measures of upper limb functional performance are associated with physiological cortical measures
4. tDCS produced effects in the ipsilateral (to hemisphere stimulated)upper limb

As part of this study we also collected comprehensive EEG data. Full analysis and inclusion of this data was outside the scope of this publication. Although it does not form part of this thesis, this analysis has been conducted and published, and is included as Appendix I.

We believe it is important to investigate these factors in the healthy aged brain which is anatomically intact, prior to developing a research protocol for investigating stroke patients where there are inherently many more confounding variables.

2.4 Abstract

Purpose: Research popularity and scope for the application of transcranial direct current stimulation have been steadily increasing yet many fundamental questions remain unanswered. We sought to determine if anodal stimulation of either hemisphere leads to improved performance of the contralateral hand and/or altered function of the ipsilateral hand, or affects movement preparation, in older subjects.

Method: In this cross-over, double blind, sham controlled study, 34 healthy older participants (age range 40-86) were randomised to receive 20 minutes of stimulation to either the dominant or non-dominant motor cortex. The primary outcome was functional performance of both upper limbs measured by the Jebsen Taylor Test and hand grip strength. Additionally, we measured motor planningusing electrophysiological (EEG) recordings.

Results: Anodal stimulation resulted in statistically significantly improved performance of the non-dominant hand ($p<0.01$) but did not produce significant changes in the dominant hand on any measure ($p >0.05$). This effect occurred irrespective of the hemisphere stimulated. Stimulation did not produce significant effects on measures of gross function, grip strength, reaction times, or electrophysiological measures on the EEG data.

Conclusion: This study demonstrated that the hemispheres respond differently to anodal stimulation and the response appears to be task specific but not mediated by age.

Keywords

tDCS, upper limb function, transcranial direct current stimulation, ageing

2.5 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, Kincses et al. 2004), implicit learning (Nitsche, Schauenburg et al. 2003, Kincses, Antal et al. 2004, Kang and Paik 2011), procedural learning (Tecchio, Zappasodi et al. 2010, Stagg, Jayaram et al. 2011), working memory (Zaehle, Sandmann et al. 2011), reaction time (Nitsche, Schauenburg et al. 2003), fine motor skills (Vines, Nair et al. 2006, Vines, Cerruti et al. 2008, Reis, Schambra et al. 2009), functional performance (Boggio, Castro et al. 2006), and muscle endurance (Cogiamanian, Marceglia 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. 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 et al. 2010). Ageing leads to alterations in the excitability of the motor cortex (Oliviero, Profice 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 are altered with age such that movement patterns become more rigid and reaction times are increased (Bennett and Castiello 1995). Secondly, healthy aging is associated with a successive decline in cognitive and motor abilities which impair independent functioning (Burke and Barnes 2006). It has been speculated (Zimerman and 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/no-go 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 et al. 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.

2.6 Methods

2.6.1 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, Pan et al. 2004). Left handed subjects were excluded as laterality in the motor hand function tests might not be present in these subjects (Ozcan, Tulum 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, Phillips et al. 2005), reported history of neurological disease or muscular dysfunction, psychiatric illness, use of CNS-acting medication, epilepsy, pregnancy, metal implants in the cranium or upper torso, unstable medical conditions, or skin lesions on the scalp.

2.6.2 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/no-go 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. no-go 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 (no-go 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.

2.6.3 tDCS

Anodal tDCS was delivered using a commercially available, programmable, direct current stimulator (neuroConn DC-stimulator). Two saline-soaked 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 et al, 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 and Cohen 2010, Hummel, Heise 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, Celnik et al. 2005, Gandiga, Hummel et al. 2006, Nitsche, Cohen et al. 2008). As several studies have demonstrated that the physiological state of the subject during stimulation can impede the effects of tDCS (Antal, Terney et al 2007, Quartarone Morgante 2004), subjects were instructed to sit quietly during the stimulation to avoid interference from cognitive or physical activity.

2.6.4 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/no-go 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/no-go 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 (W.R. Fulham). 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.

2.7 Results

2.7.1 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; χ^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 2.1). At baseline, all measures were consistent with age matched normative data (Jebsen, Taylor et al. 1969).

2.7.2 *Functional Motor Measures*

Total JTT: As shown in Figure 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.

Table 2.1 Demographic and Baseline Characteristics

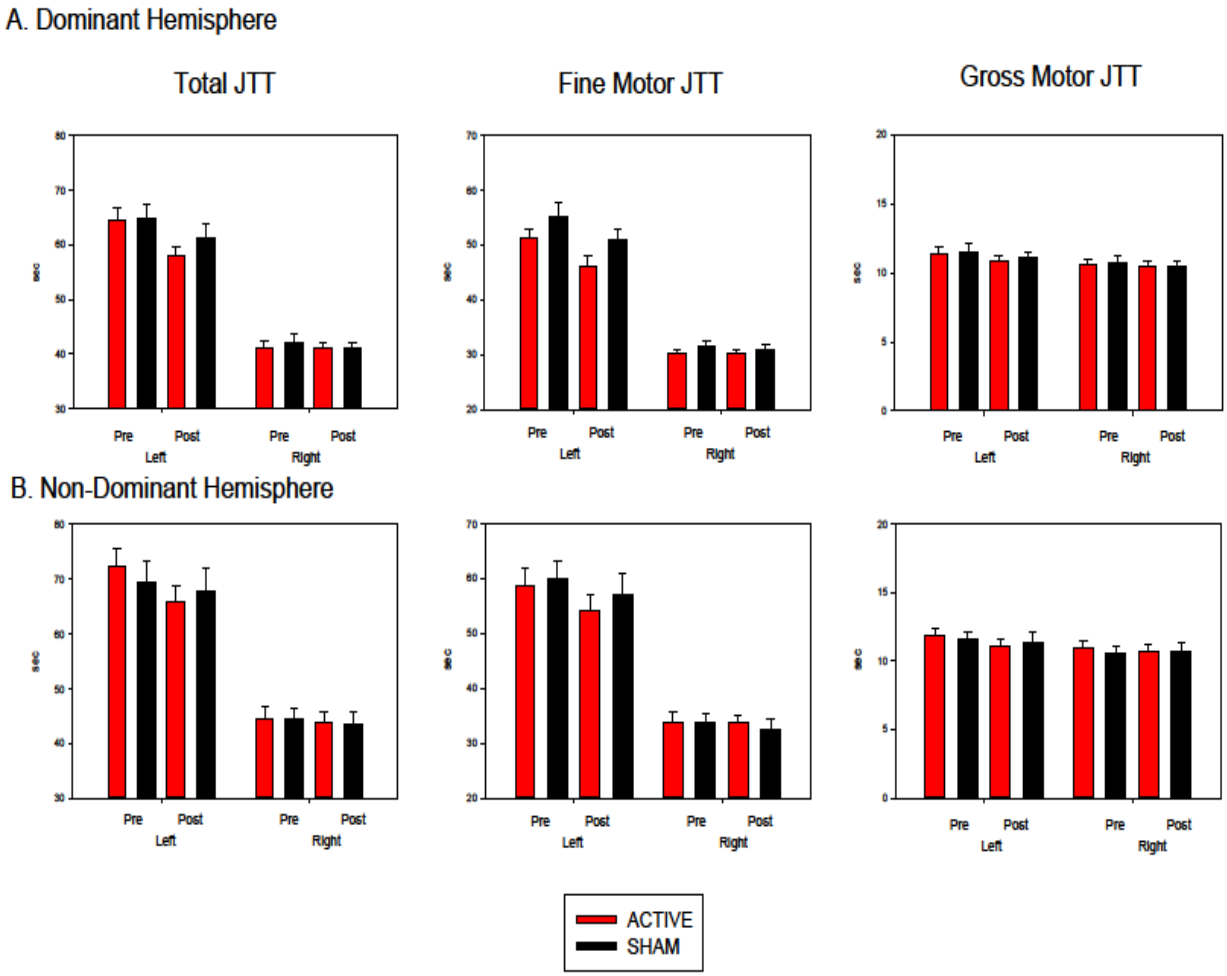
Characteristic	
Gender	Males 19 (56%)
Age	61.4 ± 12.2
MoCA	27.9 ± 2.0
JTT dominant hand	43.2 ± 7.7
JTT non- dominant hand	67.2 ± 13.5
Key grip dominant hand	18.2 ± 5.6
Key grip non- dominant hand	17.7 ± 4.7
Tip grip dominant hand	14.1 ± 4.1
Tip grip non- dominant hand	13.8 ± 4.0

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

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.

Fine motor JTT: Figure 2.1 (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 2.1 (centre) suggest that, like total JTT, stimulation improved performance for the left hand. Again, there was no effect of Hemisphere of stimulation.

Figure 2.1. 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.

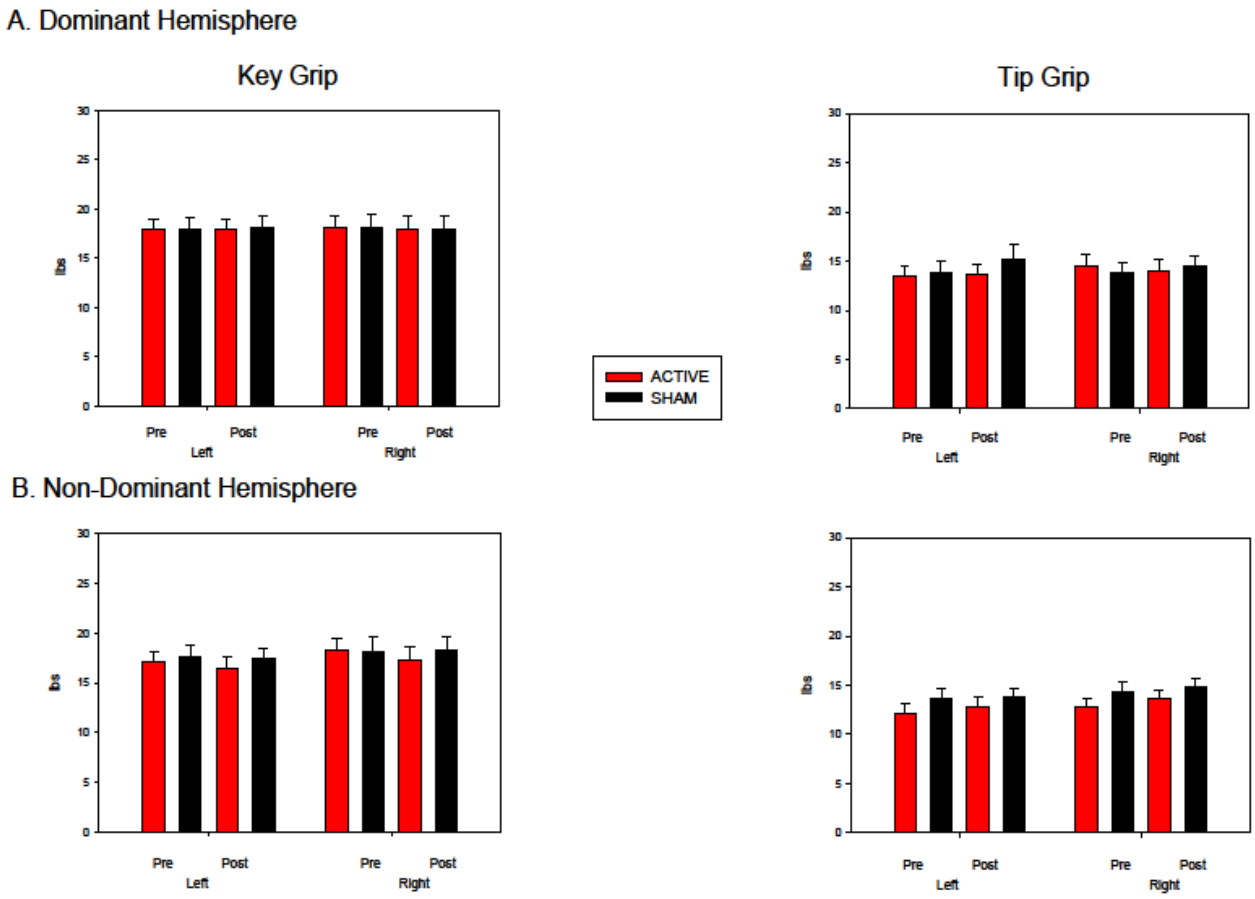
Gross motor JTT**:** As shown in Figure 1 (right), gross JTT was faster for right than left hand (F (1, 32) = 20.8, p<0.001), post-intervention compared to pre-intervention (F (1, 32) = 5.5, p=0.026), and this practice effect was greater for left than right hand responses (F (1, 32) = 4.6, p=0.04). However,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

Grip measures: Grip measure scores are shown in Figure 2.2. There was no stimulation x time interaction on either measure.

2.7.3 Go/No-go Task Behavioural Results

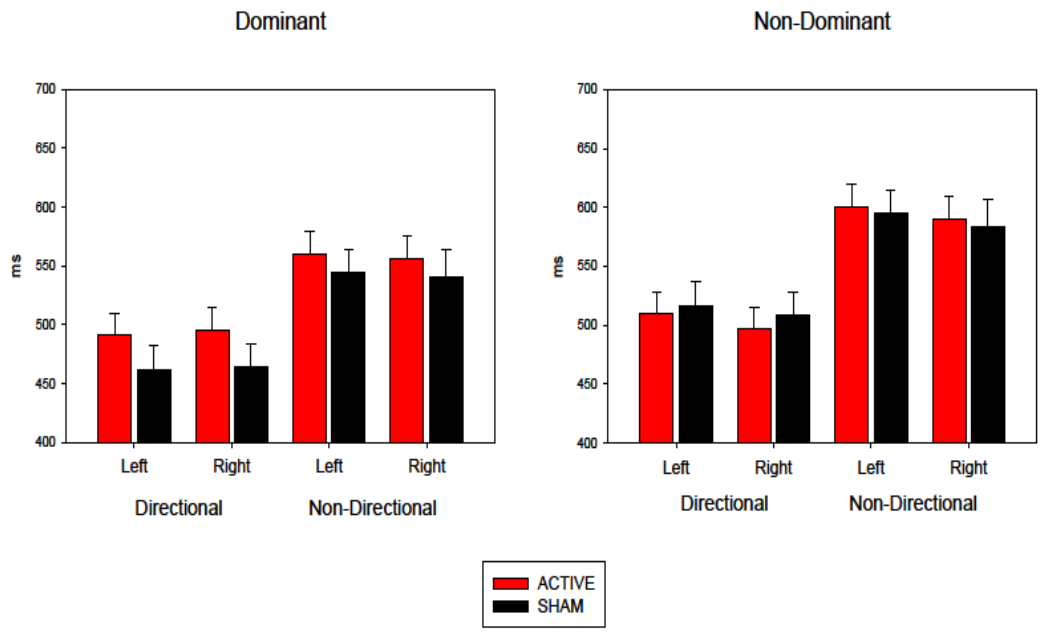
Both dominant and non-dominant hemisphere stimulation groups responded faster to directional compared to non-directional cues (F (1, 32) = 153.7, p<0.001) consistent with use of cues to prepare a motor response. As evident in Figure 2.3, anodal tDCS stimulation did not reduce reaction time (p>0.2). In 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 2.2 Effects of (A) dominant and (B) non dominant hemisphere stimulation on grip strength



Force measured in pounds per centimetre of pressure (lbs) exerted using key grip and tip pinch grip, pre and post stimulation.

Figure 2.3 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

2.7.4 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).

2.7.5 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.

2.8 Discussion

2.8.1 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, Castro et al. 2006, Vines, Nair et al. 2006, Williams, Pascual-Leone et al. 2010) 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, Cristiani 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 confer no additional benefit on function (Zimerman and 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, Cristiani 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, Heise 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/no-go task. While on the functional tasks, stimulation produced some improvement in non-dominant hand performance, on the cued go/no-go 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/no-go 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 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, Mason-Parker 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 2013, Stagg & Jayaram 2011) yet others state that tDCS must be applied prior to the task (Fertonani 2010, Vallar 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, McIntosh 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.

2.8.2 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, Siebner et al. 2005). Furthermore, we used relatively large electrodes ($35cm^2$) which cover not only the primary motor cortex but also the adjacent cortices reducing the focality of the stimulation. In particular, 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 used electrode montage, future studies using an extracephalic reference or smaller anode electrode may overcome this concern.

2.8.3 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 et al. 2013) and warrants further investigation.

2.9 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.

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2.11 Disclosure Statement

The authors have no conflicts of interest to disclose.

The study was approved by the ethics committee of Hunter New England Health and ratified by the University of Newcastle (Ref: 10/11/17/4.02). All participants gave their written informed consent. The study was conducted according to the declaration of Helsinki Principles.

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Chapter 3: Physiological Effects of tDCS in the Healthy Aged

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3.3 Synopsis

The mechanisms and neural correlates underlying the effects of tDCS have not been fully explored and are currently poorly understood. It is not be possible to predict or fully interpret the disparity reported in the tDCS literature until we have a more comprehensive understanding of the physiological effects underlying tDCS. Previously, most information was garnered from animal and pharmacological research but the evolution of brain imaging techniques allows us to examine tDCS from a new perspective and extend that knowledge.

Several imaging approaches are available with high spatial resolution to allow the evaluation of subtle changes in the stimulated regions, however the reliability of some methods is currently under dispute (Stagg and Johansen-Berg 2013). Furthermore, no individual technique directly measures neuronal activity but rather their metabolic results, that is, changes of blood flow, oxygen content, or glucose consumption (Grefkes and Fink 2014). Several fMRI studies have been conducted to characterize both the local and distant effects of tDCS on cortical activity (Baudewig, Nitsche et al. 2001, Stagg, O'Shea et al. 2009, Polania, Paulus et al. 2012). These studies are generally limited by small sample sizes, single modalities of imaging, and recruitment of young, healthy participants. Therefore to extend this work, and obtain comprehensive and valid information, we conducted a multimodal imaging study in healthy aged participants investigating the effects of both cathodal and anodal stimulation, on both the dominant and non-dominant hemisphere, using functional upper limb performance measures for both arms, resting state fMRI analysis, quantitative spectroscopic analysis, and functional connectivity.

The purpose of this study was to expand the work commenced in our previous study in the healthy aged to gain a complex depiction of the physiological processes associated with direct current cortical stimulation. This will bring us closer to the overarching goal of determining the potential of tDCS and furthering its translation in stroke rehabilitation.

3.4 Abstract

Background: Transcranial direct current stimulation of the motor cortex has been shown to produce variable results supporting its efficacy.

Objective: The aim of this study was to use multimodal magnetic resonance imaging (MRI) to investigate the mechanisms behind the cortical response to both anodal and cathodal stimulation on both hemispheres in relation to hand function.

Methods: Twenty healthy, aged adults received 20 minutes of stimulation of the primary motor cortex on separate occasions in a randomised order.

Results: Following anodal stimulation there were statistically significant increases in dominant hand ($P = 0.002$) and bilateral hand function ($P = 0.03$), as well as increases in MRI spectroscopy measured concentrations of N-Acetylaspartic acid ($P = 0.03$), and an increase in regional cerebral blood flow ($P=0.02$) as measured using arterial spin labelling. Cathodal stimulation resulted in significantly improved bimanual hand performance ($P=0.03$), decreased concentrations of glutamate ($P<0.001$) decreased cerebral blood flow ($P =0.032$). There were no other significant differences between dominant and non-dominant hemisphere stimulation with the exception of hand performance and connectivity measures.

Conclusions: These results present a complex account of the neural correlates of tDCS effects in the healthy aged, which are varied and widespread. We demonstrated analogous findings across various measures but inconsistencies with previous studies conducted in the young and people with stroke.

Keywords: magnetic resonance imaging (MRI), motor cortex, rehabilitation, transcranial direct current stimulation (tDCS), aging

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3.5 Introduction

Interest in the use of transcranial electrical brain stimulation (tDCS) in the management of neurological conditions, such as stroke, continues to flourish. However as research becomes more available inconsistency in the effects of tDCS at both an intra-individual level and between subjects has become more apparent, such that we are currently unable to predict those most likely to respond or the anticipated size of the effect (Marquez, van Vliet et al. 2015). The successful translation of findings from proof-of concept studies to clinical trials involving neurological populations is hindered by our current lack of understanding of the physiological mechanisms underpinning the effects of tDCS. Magnetic resonance imaging (MRI) has the potential to unveil some of these mechanisms to further our understanding and direct the successful use of tDCS in therapeutic applications (Amadi, Ilie et al. 2013).

Transcranial direct current stimulation (tDCS) is the generation of a weak electrical current through the cortex via non-invasive electrodes positioned on the scalp (Nitsche and Paulus 2000). Sustained applications, of typically 20minutes, have been shown to generate changes at the synaptic level that persist following the cessation of stimulation (Nitsche and Paulus 2000). tDCS provides a subthreshold stimulus that modulates the resting membrane potential in accordance with the direction of current flow. Hence it can produce hyperpolarization to decrease the excitability of neurones beneath the cathode (cathodal stimulation), or increased excitability by depolarizing the neurones beneath the anode (anodal stimulation) (Schlaug and Renga 2008). Although the area of stimulation is discrete, the physiological effects of tDCS are thought to be large scale and reach distal networks (Polania, Nitsche et al. 2011, Sehm, Schafer et al. 2012, Amadi, Ilie et al. 2013). Yet the specific pattern of the effects produced has not been established.

Recent advances in magnetic resonance imaging techniques have led to the development of several approaches capable of investigating tDCS induced brain effects, although individually each technique has its limitations. Resting state functional MRI (rs-fMRI) can indirectly capture functional changes produced by neuronal activity through measurements of the vascular response (Biswal, Yetkin et al. 1995). This can be complimented by Arterial Spin Labelling (ASL) which is used to non-invasively assess cerebral blood flow (rCBF) by magnetically labelling inflowing blood (Petersen, Lim et al. 2006). Cerebral blood flow is used as a surrogate measure of brain activity to examine regional brain tissue and brain network effects in functionally related, but potentially distant brain regions (Zheng, Alsop et al. 2011). Due to the close link between brain metabolism and perfusion the combination of ASL and rs-fMRI can be used to disentangle vascular and neuronal contributions to stimulation (Haller, Zaharchuk et al. 2016). Magnetic resonance spectroscopy (MRS) allows us to quantify the

total amount of a particular neurotransmitter within a localised region of the brain and is therefore useful for detecting physiological change in response to tDCS, however there is limited ability to directly relate these measures to synaptic activity (Stagg and Johansen-Berg 2013). Hence studies combining more than one imaging modality with tDCS are required to assess neuroplastic changes across these different scales (Hunter, Coffman et al. 2013).

Previous neuroimaging studies have typically used single modalities of imaging to investigate the effects of tDCS in young adults, with less than 10 subjects and one modality of tDCS (Clark, Coffman et al. 2011, Amadi, Ilie et al. 2014, Hunter, Coffman et al. 2015). The paucity of research evaluating the effects of tDCS in the aged is problematic as cognitive and motor functions decline, and cortical excitability and structure change with age. Therefore extrapolating the effects of tDCS from studies in young adults to make inferences for neurological conditions, such as stroke, which has increased prevalence in the aged, may not be valid (Marquez, Conley et al. 2015). This lack of evidence is compounded by the difficulty of making comparisons across studies due to differences in subject selection, tDCS stimulation paradigms, and data acquisition and methods of analysis.

By combining several neuroimaging techniques, as well as an examination of hand performance, we aim to examine the effects of anodal and cathodal stimulation of the motor cortex, when applied to either the dominant or non-dominant hemisphere, in an elderly sample. This will give us a complex account of neurochemical and functional connectivity changes as a result of tDCS that will have relevance to therapeutic applications in the aged and neurological populations.

3.6 Materials and Methods

3.6.1 Subjects

Subjects were recruited from the Hunter Medical Research volunteer registry**.** To be eligible subjects were required to be right handed, over the age of 40 years, and have no history of neurological disease. Left handed subjects were ineligible as the laterality of brain function may not be present in these subjects (Ozcan, Tulum et al. 2004). Forty years was used as a cut-off age as although the specific time in life when brain ageing begins is not defined, genetic studies suggest measurable decline after the age of 40 years (Lu, Pan et al. 2004). Subjects were excluded if they did not meet the standard criteria for tDCS: psychiatric illness, use of CNS-acting medication, pregnancy, metal implants in the cranium or upper torso, unstable medical conditions, skin lesions on the scalp; or for receiving MRI including: claustrophobia, metal in the body, pacemaker, defibrillator, aneurysm clips, artificial heart valves, implants, brain shunts, neurostimulator or drug infusion pumps.

The protocol for this project was approved by the Hunter New England Health Human Research Ethics Committee, and complied with the declaration of Helsinki.

3.6.2 Study design

Subjects were allocated via computer generated randomisation on a 1:1 ratio to the order of intervention: anodal/cathodal or cathodal/anodal. They were then randomised to receive the intervention to either their dominant or non-dominant hemisphere. The two intervention sessions were separated by two weeks to prevent contamination by residual stimulation effects (Nitsche and Paulus 2001). All assessments were conducted prior to the first intervention in order to establish a baseline, and immediately following each application of tDCS. This was conducted using the Purdue Pegboard test (PPBT) which is a validated test of both unilateral and bimanual fingertip dexterity and gross movement of the fingers, hand and arm (Tiffin and Asher 1948). It is comprised of 4 subtests and takes approximately 5 minutes to administer. Following this, patients underwent MRI examinations.

3.6.3 tDCS

Stimulation was delivered using a NeuroConn programmable direct current stimulator. The 1mA of current was applied via two 5x7cm surface electrodes, soaked in saline, positioned on the scalp. For anodal stimulation the active electrode was 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 (Herwig, Schonfeldt-Lecuona et al. 2001) and the cathode was positioned on the contralateral supraorbital region. This is the most typically reported electrode montage for stimulating the cortical region which represents hand function (Floel and Cohen 2010). The electrode arrangement was similar for cathodal stimulation whereby the current direction was reversed by exchanging the electrode positions.

For both anodal and cathodal stimulation, 1mA of current was applied for 20minutes. The stimulator was programmed to slowly increase the current strength over 30 seconds at the beginning of the intervention and likewise at the end of the session to maximise tolerance. Participants were told to expect a tingling sensation under the electrodes that would ease so that little or no perception of the stimulation would persist after approximately 2 minutes.

3.6.4 MRI

All patients underwent MRI investigation at 3-Tesla on the same Siemens Magnetom Prisma scanner (Siemens Healthcare, Erlangen, Germany), with a Siemens 32-channel phased-array head-coil.

The imaging protocol consisted of; High-resolution structural imaging involved a T1-weighted axial-MPRAGE (slice thickness 1mm, 0.5mm x 0.5mm voxels, TR 1900msec, TE 2.82msec, resolution matrix 256x246), Short echo-time single voxel spectroscopy (voxel dimensions 20x20x20mm, TR 3000msec, TE 30msec), pseudo continuous atrial spin labelling (fast gradient echo acquisition with 45° flip angle, 8.98 ms repetition time (TR), 2.96 ms echo time (TE), 0.4 × 0.4 × 1.8 mm^3 voxel size, and 512 × 512 × 54 matrix size.) and resting state functional MRI (rs-fMRI) with visual fixation (3mm slices, TR 2500msec, TE 30msec, resolution matrix 64x64, acquisition time 7min35sec). ASL imaging was post processed using MiStar (Apollo, Melbourne Australia).

Quantitative Spectroscopic Analysis

Raw time-domain ^1H MRS data from 4.0 to 1.0ppm in the spectral dimension were analyzed using LCModel with the unsuppressed water scan as a concentration reference (figure 1C) (Provencher 2001). As a quality-assurance measure, LCModel produces a Cramer–Rao lower bound of the fit to the peak of interest. If this value was greater than 15%, the fit was deemed unreliable and was excluded from analysis. We quantitatively assessed concentrations of N-Acetylaspartic acid (NAA), lactate (Lac), Total Creatine (Creatine + Phosphocreatine [Cr+PCr]), Inositol (Ins), Glutamate (Glu), and the composite complex formed by glutamine and glutamate (Glx). Correction for tissue water content as well as tissue water and metabolite relaxation correction were not performed, and hence all spectroscopy measurements are expressed in institutional units.

Functional connectivity analysis

Functional connectivity changes over time were assessed using the functional connectivity toolbox for correlated and anticorrelated brain networks (Conn; Whitfield-Gabrieli and Nieto-Castanon 2012). Prior to processing of functional images, each participant's MPRAGE volume was normalised to Montreal Neurological Institute (MNI) - 152 standard space using a non-linear registration approach and subsequently segmented into grey matter, white matter, and cerebrospinal fluid (CSF) tissue classes in order to generate a whole brain mask, and for use in extracting confounding factors related to physiological noise. Slice timing correction was performed and all volumes were realigned to the first volume using a six-parameter (rigid body) spatial transformation. Realignment transformation matrices and global signal intensities were then analysed using the Artifact Detection Tool (ART; www.nitrc.org/projects/artifact_detect/) to identify signal and motion outliers. Functional volumes were subsequently normalised to the MNI Echoplanar Imaging (EPI) template using a non-linear registration approach, and were then smoothed with an 8mm full-width half maximum kernel.

Temporal confounding factors, such as cardiac, respiratory, and other physiological noise, were removed using the aCompCor method (Behzadi, Restom et al. 2007). This approach removes such noise by identifying significant principal components derived from regions of interest that are unlikely to contain any signal modulated by neural activity (in this case, white matter and CSF regions). Motion parameters and outliers were also removed at this stage. Finally, the residual time series were band pass filtered (0.008 – 0.09 Hz).

Voxel-to-voxel connectivity was calculated in the form of the intrinsic connectivity contrast (ICC) metric which measures the absolute strength of the global connectivity pattern between each voxel and the rest of the brain (Martuzzi, Ramani et al. 2011). The ICC value for each voxel was normalised by transforming to *Z* scores. We specifically targeted mean ICC within a region of interest corresponding to the contralesional thalamus in MNI space as an indicator of the connectivity between this structure and the rest of the brain.

3.6.5 Data analysis

Statistical analysis was performed using IBM SPSS Statistics 21 (IBM Corp. Armonk, NY). Given the modest sample size, we used conservative non-parametric statistical methods for all analyses. Differences in metabolite concentration, ASL perfusion and rsfMRI data were compared between the baseline scans and those of the opposite stimulation (anodal vs cathodal), using Wilcoxon Signed Rank Test taking into account hand dominance. Given the multiple correlations tested in this analysis, we used a Benjamini-Hochberg procedure to obtain a significance threshold and control for the false discovery rate. In all other analyses, a *p*-value <0.05 was considered to indicate statistical significance.

3.7 Results

3.7.1 Participant characteristics

A total of 20 participants (11 male, mean age 70.5± 10.2 years) were enrolled into the study. At baseline, PPBT scores were consistent with age matched normative data (Desrosiers, Herbert et al. 1995) and there were no differences in hand function between those who received stimulation to the dominant compared to non-dominant hemisphere on any PPBT measure ($p>0.05$). All enrolled participants completed the study and there were no adverse events.

3.7.2 Functional Performance

When anodal tDCS was applied to the dominant hemisphere there was a significant improvement in right hand performance on the PPBT compared to baseline (mean change =1.7, $p=0.002$).

When the dominant hemisphere was stimulated with cathodal stimulation there was a significant improvement in the bimanual PPBT task (mean change = 1, p=0.03) (refer to Figure 3.1a). When anodal stimulation was applied to the non-dominant hemisphere there was an improvement in the non-dominant hand but this failed to reach significance (mean change = 1.2, p=0.08), there was a significant improvement in the bimanual assembly task (mean change = 0.6, p = 0.02). Cathodal tDCS also produced a significant improvement in the assembly task (mean difference = 0.8, p=0.003) (refer to Figure 3.1b).

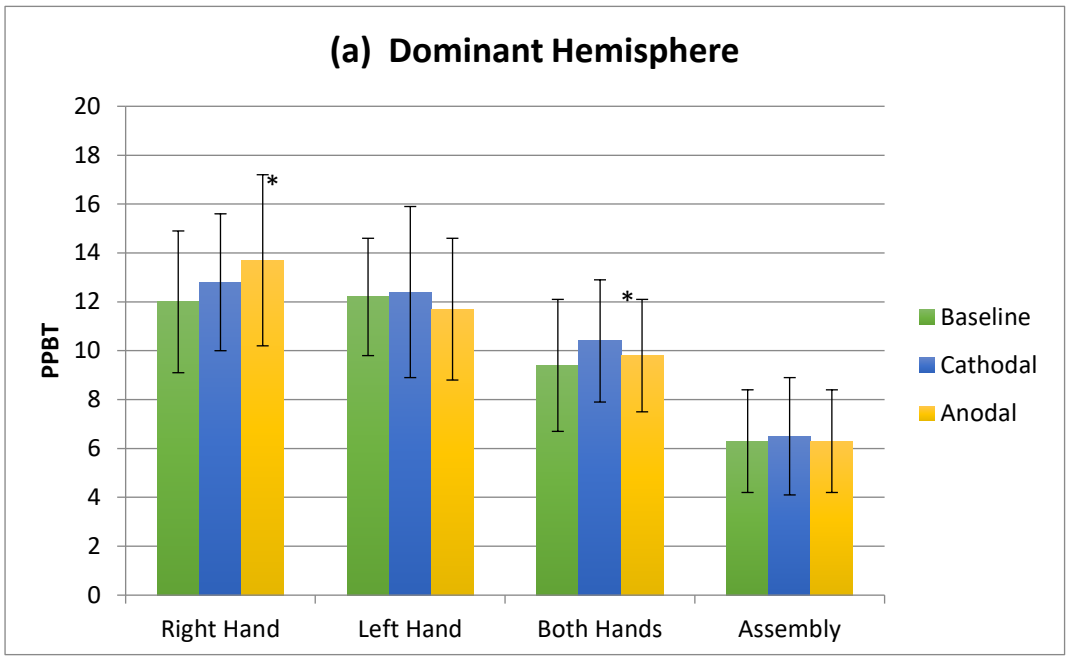
3.7.3 Spectroscopy Findings

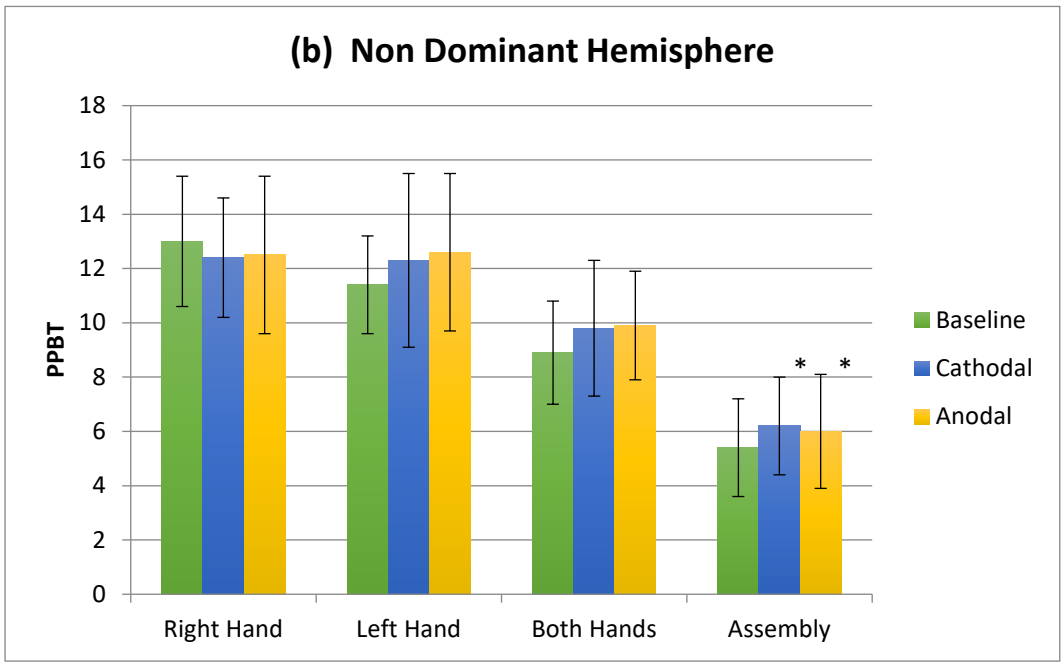
Participants administered anodal stimulation to the dominant hemisphere showed an increase in NAA compared to the contralateral hemisphere and the baseline scan (7.6mM anodal ipsilateral vs 6.3mM contralateral, p<0.001, 7.09mM baseline p=0.031). This result was also present in patients who received anodal stimulation to the non-dominant hemisphere (NAA 8.3mM anodal ipsilateral vs 7.6mM contralateral, p=0.032, 7.19mM baseline p=0.042). Anodal stimulation did not result in any significant changes to GLU results. Stimulation did not significantly alter the concentration of lactate or creatine to either the dominant or non-dominant hemispheres. Cathodal stimulation decreased the concentration of Glutamate (GLU) compared to the contralateral hemisphere in patients administered stimulation to the dominant (4.8mM Cathodal ipsilateral vs 5.7mM contralateral, p<0.001, 4.8mM baseline p<0.001) and non-dominant hemispheres (6mM cathodal ipsilateral vs 4.8mM contralateral, p<0.001, 4.8mM baseline p<0.001). Cathodal stimulation did not result in any significant changes to NAA results (Refer to Table 3.I).

3.7.4 Cerebral Blood Flow

Cerebral blood flow, as measured on atrial spin labelling, showed significant increases in the ipsilateral and contralateral hemispheres with anodal stimulation (mean 45mL/100g/min vs baseline mean 39mL/100g/min, p=0.022). Cathodal stimulation resulted in a decrease of CBF as measured on ASL (mean 33 mL/100g/min vs baseline mean 39mL/100g/min, p=0.032).

Figure 3.1. Effects of stimulation on upper limb functional performance





PPBT: Purdue Pegboard test recorded in number of successful completions in 30 seconds, assembly recorded as number of completions in 60 seconds

Figures reported as mean ± standard deviation

* indicates statistically significant

Table 3.1. Stimulation effects on neurotransmitters and regional cerebral blood flow

a. Dominant Hemisphere Stimulation

	NAA			GLU			ASL		
	Ipsi	Contra	P	Ipsi	Contra	P	Ipsi	Contra	P
Baseline	7.10	6.90	0.38	4.80	5.85	0.39	39.00	38.00	0.30
Anodal	7.60	6.30	<0.001	4.90	5.36	0.10	41.00	48.00	0.20
Cathodal	6.90	6.80	0.51	4.80	5.73	<0.001	33.00	32.00	0.24

b. Non-Dominant Hemisphere Stimulation

	NAA			GLU			ASL		
	Ipsi	Contra	P	Ipsi	Contra	P	Ipsi	Contra	P
Baseline	6.90	7.20	0.60	4.80	5.02	0.28	40.00	40.00	0.32
Anodal	7.30	6.60	0.03	5.50	5.28	0.12	38.00	42.00	0.48
Cathodal	7.10	6.50	0.18	4.80	6.05	<0.001	34.00	33.00	0.51

MRI measures of spectroscopy derived N-Acetylaspartic acid (NAA) and Glutamate (GLU) levels. Cerebral blood flow measures derived from Arterial Spin Labelling (ASL).

3.7.5 Functional Connectivity

Resting state functional MRI showed a significant increase in the activity in the executive network due to cathodal stimulation (t=4.86, p=0.0142. Refer to table 3.2). The visual medial network showed a strong trend to increased activity with cathodal stimulation (p=0.0522). Otherwise no other network was showed a significant change.

Table 3.2 Resting state functional connectivity changes following stimulation

Network	Anodal stimulation		Cathodal stimulation	
	T value	P	T value	P
Executive	5.93	0.24	4.87	0.01
Visual Medial	5.17	0.21	5.60	0.65
Dorsal DMN	5.39	0.57	5.59	0.57
Visual Lateral	5.27	0.52	5.57	0.55
Visual Occipital	6.06	0.38	5.33	0.29
Auditory	5.49	0.69	5.07	0.75
Motor	6.09	0.30	5.21	0.61

3.8 Discussion

The principal finding of this study was that a single session of direct current stimulation produced similar effects when applied to either the dominant or non-dominant motor cortex of healthy aged subjects. The results were determined by the polarity of the stimulation (anodal or cathodal) and included multiple effects. Anodal stimulation resulted in improved contralateral hand function which was associated with increased NAA and diffuse and bilateral increases in rCBF. No functional connectivity changes were seen. With cathodal stimulation, there was improvement in bimanual upper limb performance with decreased GLU and rCBF, and changes in the activity of the executive function network with rs-FMRI.

3.8.1 Dominant versus non-dominant hemisphere stimulation

Asymmetry in motor function between the dominant and non-dominant hand is likely a consequence of hemispheric differences in corticomotor excitability and transcallosal inhibition between the two hemispheres. Therefore we were interested in investigating whether there was a difference in response according to which hemisphere was stimulated. Several studies have demonstrated no significant difference between the effects produced by stimulating either hemisphere with anodal stimulation (Boggio, Castro et al. 2006, Moliadze, Antal et al. 2010). Yet in a previous investigation of a healthy aged sample we found that fine motor performance of the dominant hand could be improved by anodal stimulation of either the dominant or non-dominant hemisphere but no benefit was conferred on the dominant hand (Marquez, Conley et al. 2015). A differential effect for cathodal stimulation has also been surmised whereby inhibitory stimulation of the dominant hemisphere has been reported to be reap greater benefit than cathodal stimulation of the non- dominant hemisphere (Fregni, Boggio et al. 2005). In this current study the only differential effects were noted on the functional performance measure. Here stimulation of the non-dominant hemisphere with either cathodal or anodal stimulation improved performance on the assembly task but this was not observed for dominant hemisphere stimulation. In agreement with previous findings dominant hemisphere anodal stimulation resulted in improved dominant hand performance but this was not observed with anodal stimulation of the non-dominant hemisphere as previously shown in the aged (Marquez, Conley et al. 2015). We can only speculate the reason for this disparity but it may reflect differences in the measurement tool used to assess functional performance as the effects of tDCS are thought to be task dependent (Marquez, Conley et al. 2015) but equally may be a consequence of the high level of inter subject variability in stimulation response.

3.8.2 Spectroscopy findings

MRS has been used in several studies to assess neurochemical changes as a result of tDCS. Our results demonstrate that these metabolic effects are polarity and spatially specific irrespective of the hemisphere stimulated. More specifically, anodal stimulation resulted in increased levels of NAA in the stimulated hemisphere which is thought to be related to increased neuronal energy status. This observation has only been reported in one previous tDCS study using anodal stimulation of the parietal region in young healthy volunteers (Clark, Coffman et al. 2011). Reductions in GLU concentrations following cathodal stimulation are inferred to indicate reduced neural activation. This supports the findings of Stagg (Stagg, Jayaram et al. 2011) who found similar reductions in GLU with cathodal stimulation and a relationship with global cortical excitability as measured by transcranial magnetic stimulation. However, again this finding is not typical with other authors reporting no change in this transmitter (Rango, Cogiamanian et al. 2008, Clark, Coffman et al. 2011, Kim, Stephenson et al. 2014). This inconsistency is observed for lactate, creatine, inositol and combined glutamine/glutamate measures where we failed to show any significant effect of stimulation but others have reported change (Rango, Cogiamanian et al. 2008, Stagg, O'Shea et al. 2009, Clark, Coffman et al. 2011, Stagg, Jayaram et al. 2011, Kim, Stephenson et al. 2014, Hunter, Coffman et al. 2015).

3.8.3 Resting connectivity changes

Previous studies investigating the effects of motor tDCS paradigms have shown that anodal stimulation increases local functional connectivity within the motor network (Polania, Paulus et al. 2012, Sehm, Schafer et al. 2012) as well as increased coupling with the rest of the brain both within and beyond the motor system (Polania, Nitsche et al. 2011, Sehm, Schafer et al. 2012). This modulation suggests that it increases local spontaneous activity and functional activity across the brain may be increased in some networks and decreased in others (Polania, Paulus et al. 2012). Similarly stimulation of the pre frontal cortex led to modulation of large scale patterns of resting state connectivity close to the stimulating electrode but also in distant regions (Keeser, Meindl et al. 2011, Pena-Gomez, Sala-Lonch et al. 2012). A further study compared the effects of anodal stimulation to bihemispheric stimulation and found widespread, bilateral alterations with bihemispheric tDCS and more local modulations of functional motor networks with anodal stimulation (Lindenberg, Sieg et al. 2016). These authors did however report highly variable results across the group. Our findings are less convincing and we were only able to detect a significant change in resting connectivity in the executive network following cathodal stimulation. One reason for this disparity may be that we examined aged subjects rather than healthy young participants.

Plasticity induced by tDCS is likely to be reduced in the aged brain due to decreased segregation of neural networks (Chan, Park et al. 2014) and more diffuse brain activity which may reflect differences in motor cortex organisation of both brain hemispheres in the aged brain (Bernard and Seidler 2012). Another contributing factor may be the electrode montage we used. The position of the return electrode has previously been noted to have a significant impact on the patterns of functional connectivity (Sehm, Schafer et al. 2012). With cathodal stimulation of the motor cortex the anode was positioned over the contralateral orbit and therefore may have directly influenced the frontal cortex and affected the outcomes of our analysis.

3.8.4 Cerebral Blood Flow changes

The most common method for analysing rCBF is the BOLD technique (blood- oxygen level dependent signal) (Schlaug and Renga 2008). We preferentially used ASL which is a relatively new technique with excellent temporal stability which has advantages over BOLD as tDCS is usually applied over a period of up to 20 minutes. We found diffuse changes in blood flow which were determined by the polarity but not the side of stimulation. That is, anodal stimulation resulted in an increase in resting state rCBF and cathodal led to a decrease both ipsilateral and contralateral to the side of stimulation This is consistent with the findings of Zheng et al. who examined rCBF in a sample of young subjects simultaneously with the application of tDCS. They report that although both anodal and cathodal tDCS increased rCBF during stimulation the increase was almost three times greater in the anodal group, and then decreased post stimulation in both groups but to a greater extent in the cathodal group possibly reflecting persistent inhibition (Zheng, Alsop et al. 2011). Similar findings have been reported following stimulation of the dorsolateral prefrontal cortex (Stagg 2013). As such, rCBF as measured by ASL may be a reliable surrogate marker of the after-effects of tDCS. Furthermore, it may be less susceptible than BOLD response to the vascular changes associated with aging.

3.8.5 Strengths and Limitations

The use of multiple imaging modalities coupled with a functional measure of hand performance, using multiple stimulation paradigms, allowed us to extract a complex account of the effects of tDCS in a clinically relevant sample of healthy aged subjects. The modest sample size may have reduced the statistical power of our analysis however recruitment of 20 subjects is greater than most comparative studies and the use of a cross over design meant that group sizes were preserved and not allocated to smaller groups. Another potential limitation is the use of a bicephalic montage. We used this configuration for anodal and cathodal stimulation as it is the most frequently used and therefore allows for comparison with other studies. However in this scenario anodal tDCS to the motor cortex simultaneously delivers cathodal stimulation to the prefrontal cortex and vice versa for

cathodal stimulation. Therefore it is difficult to interpret whether the measured outcomes are driven by the effects at the motor cortex or the prefrontal cortex. Future studies using an extracephalic reference electrode would help decipher this (Amadi, Ilie et al. 2014).

3.9 Conclusion

This multi modal imaging study has furthered our understanding of the neuroplastic effects of tDCS through measurements of functional performance, brain neurochemistry, cerebral blood flow, and functional connectivity within the same subjects. This comprehensive analysis included comparison of both anodal and cathodal stimulation paradigms and stimulation of both the dominant and non-dominant hemispheres of aged brains. The lack of consensus across different studies, particularly with analysis of neurotransmitters through spectroscopic analysis, is striking. This may be due to differences in study design, data acquisition and analysis, participant and stimulation characteristics. It may also in part reflect the unreliability of tDCS. This inconsistency in response has become increasingly obvious as the body of tDCS research has grown and it is clear that our understanding of the effects of tDCS is far from complete. Further multimodal imaging studies are required to probe these findings further and may combine resting state imaging with task based paradigms. Studies to quantify neurophysiological processes are critical to understand how the aged brain responds to tDCS and how this interacts with underlying disease conditions such as stroke in order to potentiate the use of tDCS as a therapeutic modality.

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3.11 Disclosure Statement

This project was supported financially by a small projects grant awarded by the National Stroke Foundation (Australia).

The authors have no conflicts of interest to disclose.
The study was approved by the ethics committee of Hunter New England Health and ratified by the University of Newcastle (Ref: 10/11/17/4.02). All participants gave their written informed consent. The study was conducted according to the declaration of Helsinki Principles.

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Chapter 4: Systematic Review and Meta-Analysis of the Stroke Literature

4.1 Publication Details and Author Affiliations

Marquez J[1 2 3], van Vliet P[1 2 3], McElduff P[3], Lagopoulos J[5], Parsons M[2 3 4]. (2015).Transcranial direct current stimulation (tDCS): Does it have merit in stroke rehabilitation? A systematic review. *International Journal of Stroke* 10 (3):306-16

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4.2 Co-author Statement of Contribution

As co-authors, we attest that the Research Higher Degree candidate, Jodie Marquez, contributed to the development of the research design, data collection, data analysis, and writing of the journal submission for the publication entitled: : Transcranial direct current stimulation (tDCS): Does it have merit in stroke rehabilitation? A systematic review. 2015. *International Journal of Stroke* 10(3): 306-16.

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4.3 Synopsis

Current clinical practice for stroke rehabilitation is based on the principles of motor learning and neural plasticity. However the response to therapy varies greatly between individuals and is thought to be affected by numerous individual, stroke and recovery variables (Takeuchi and Izumi 2013). This variability in effects has also been reported in the tDCS literature. In order to attempt to understand this inconsistency a systematic review of randomised, controlled trials was conducted. We pooled data to conduct meta-analyses to identify if overall tDCS was effective in improving functional performance after stroke, but also whether there were differential effects according to time since stroke, tDCS montage, or stroke severity.

This review was primarily concerned with outcomes of functional performance. But stroke can produce many symptoms which may theoretically be altered by cortical stimulation. These include: cognition, mood, swallowing and language disorders. Appendix II contains an excerpt from a peer-reviewed book chapter (completed by this researcher) which provides information relating to these additional outcomes that were not included in the systematic review conducted as part of this thesis.

4.4 Abstract

Background and Purpose: Transcranial direct current stimulation has been gaining increasing interest as a potential therapeutic treatment in stroke recovery. We performed a systematic review with meta-analysis of randomized controlled trials to collate the available evidence in adults with residual motor impairments as a result of stroke. The primary outcome was change in motor function or impairment as a result of transcranial direct current stimulation, using any reported electrode montage, with or without adjunct physical therapy.

Results: The search yielded 15 relevant studies comprising 315 subjects. Compared to sham, cortical stimulation did not produce statistically significant improvements in motor performance when measured immediately after the intervention (anodal stimulation: facilitation of the affected cortex: SMD = 0.05, p = 0.71; cathodal stimulation: inhibition of the non-affected cortex: SMD = 0.39, p = 0.08; bihemispheric stimulation: SMD = 0.24, p = 0.39). When the data was analysed according to stroke characteristics statistically significant improvements were evident for those with chronic stroke (SMD = 0.45, p = 0.01) and subjects with mild to moderate stroke impairments (SMD = 0.37, p = 0.02).

Conclusion: Transcranial direct current stimulation is likely to be effective in enhancing motor performance in the short term when applied selectively to stroke patients. Given the range of stimulation variables and heterogeneous nature of stroke, this modality is still experimental and further research is required to determine its clinical merit in stroke rehabilitation.

Key words: stroke, rehabilitation, physical therapy modalities, electric stimulation therapy

4.5 Introduction

Recovery from stroke remains suboptimal and drives the compelling search for effective methods of stroke rehabilitation which are accessible, safe, and easy to administer. This has led to increasing interest in non-invasive cortical stimulation. Cortical stimulation may have a role in promoting both contralesional and ipsilesional plastic changes after stroke. This is based on the hypothesis that a focal lesion leads to reduced output from the lesioned hemisphere and disrupts the balance of interhemispheric communication. Electrical stimulation may be able to facilitate a shift of this imbalance towards the pre-stroke equilibrium by downregulating excitability via application to the non-lesioned hemisphere thus releasing the lesioned hemisphere from excessive transcallosal inhibition (Boggio, Nunes et al. 2007). Conversely it may be applied to the lesioned hemisphere to increase the excitability of the perilesional regions (Ward and Cohen 2004).

Transcranial direct current stimulation (tDCS) has emerged as one of the primary techniques under investigation. The tDCS stimulating device is a 13cm x21cm portable box with 2 rubber electrodes applied with conductive gel or water soaked pads. Typically, the protocol for tDCS utilizes 1-2mA of continuous current for a duration of 10-20 minutes with one electrode placed in the region of the motor cortex and the other on the contralateral supraorbital region. At a cost of US$8,000, the price, ease of application and small size of the unit render it a practical concurrent therapy option for rehabilitation clinicians. The studies performed in healthy adults to date have consistently shown that cortical activity, including motor function, can be temporarily altered by tDCS and the effects depend on the polarity and position of the electrodes, whereby brain activity is increased by anodal stimulation and conversely decreased by cathodal stimulation. The tDCS technique has been in existence since the 1960's with a body of evidence in psychological conditions where reports have indicated it to be a safe technique that is well tolerated by patients (Redfearn, Lippold et al. 1964).

Several narrative reviews have been conducted describing the effects of tDCS in a range of conditions including stroke (Hummel and Cohen 2006, Alonso-Alonso, Fregni et al. 2007, Harvey and Nudo 2007, Schlaug and Renga 2008, Schlaug, Renga et al. 2008, Bolognini, Pascual-Leone et al. 2009, Williams, Imamura et al. 2009). The purpose of this review was to systematically review the potential of tDCS to enhance the motor recovery of stroke survivors. The research questions were:

1. What are the effects of tDCS on body function or activity limitation in patients with stroke compared to no treatment or standard physical therapy?
2. Are the effects of tDCS in patients with stroke dependant on stimulation parameters (e.g. anodal versus cathodal) or patient characteristics (e.g. chronic versus acute stroke)?
3. Is tDCS a safe modality for use in the stroke population?

4.6 Methods

4.6.1 Identification of studies

A literature search was undertaken to locate all eligible published studies. Electronic searches of MEDline, PubMed, CINAHL and ProQuest were performed using keywords for the health condition, stroke, and terms for the intervention, tDCS, in various combinations to locate the relevant papers. There was no methodological filter used for the study design and no time constriction applied to the literature search which was completed in September 2012. In addition, the reference lists of all relevant articles were hand searched for further studies. Duplicates were removed manually. On the basis of titles and abstracts, the principal author (JM) retrieved relevant studies after which two authors (JM and PvV) independently evaluated the studies for inclusion in this review. Any uncertainties regarding inclusion were clarified through discussion (see Table 4.1 for inclusion criteria).

Table 4.1. Inclusion Criteria

Design
- Randomised or quasi -randomised controlled trials

Participants
- Adults > 18years
- Diagnosis of stroke (haemorrhagic or infarct, any location, acute or chronic, any level of disability)

Intervention
- Transcranial direct cu rrent stimulation (either polarity, any configuration, single or multiple sessions, alone or as an adjunct to other interventions)

Outcome measures
- Impairment or Functional measures (any validated tool of physical function or impairment eg Fugyl -Meyer asse ssment, Jebsen -Taylor test of hand function, grip strength, reaction time)

Comparisons
- tDCS versus sham stimulation
- tDCS in addition to other therapy versus other therapy
- Anodal tDCS versus cathodal tDCS

4.6.2 Selection of studies

Studies involving adults with a diagnosis of stroke as defined by the original authors irrespective of lesion location, severity, or classification, were eligible. Included literature was limited to full text publications in English which utilized a controlled experimental design on human subjects and reported original data.

Because the aim of our study was to assess benefits of tDCS in terms of movement performance studies were excluded if they did not include at least one measure of impairment, body function or activity limitation.

Two reviewers (JM and PvV) worked collaboratively to assess the methodological quality of the studies using the criteria from the Physiotherapy Evidence Database scale (PEDro) (see Table 4.2 for results). Studies were only included if they used a randomized and controlled design. This included studies with random allocation to treatment groups as well as cross-over research design with randomization to treatment order.

Table 4.2 Included studies: summary of research design

Study	Random allocation	Concealed allocation	Groups similar at baseline	Participant blinding	Therapist blinding	Assessor blinding	<15% dropouts	Intention to treat analysis	Between group difference	Point estimate & variability	Total Pedro Score
Fregni et al. 2005	✓	X	✓	✓	X	✓	✓	✓	✓	✓	8
Boggio et al. 2007	✓	X	X	✓	x	✓	✓	✓	✓	✓	7
Kim et al. 2009	✓	X	✓	✓	X	X	✓	✓	✓	✓	7
Celnik et al. 2009	✓	X	✓	✓	X	✓	✓	✓	✓	✓	8
Lindenberg et al. 2010	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	9
Mahmoudi et al 2011	✓	X	X	✓	X	✓	✓	✓	✓	✓	7
Kim et al. 2010	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	9
Madhavan et al 2011	✓	X	✓	✓	X	✓	✓	✓	✓	✓	8

Nair et al 2011	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	9
Geroin et al. 2011	✓	X	✓	X	X	✓	✓	✓	✓	✓	7
Bolognini et al. 2011	✓	X	✓	✓	✓	✓	X	X	✓	✓	7
Hesse et al. 2011	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	9
Zimerman et al 2012	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	8
Rossi et al. 2012	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	9
Stagg et al. 2012	✓	X	✓	✓	X	X	✓	✓	✓	✓	7

All included studies specified eligibility criteria

4.6.3 Interventions

Several different applications of tDCS to modulate cortical excitability are described in the literature and we did not restrict our inclusion of studies based on this factor. For the purposes of this review the following descriptions apply:

***Anodal tDCS*:** the active electrode (anode) is positioned over the lesioned (stroke) motor cortex and the cathode (reference) over the contralesional supraorbital region with the intent of upregulating the excitability of the perilesional regions

Sham tDCS: the electrodes are positioned as per anodal stimulation.

Cathodal tDCS: The cathode is positioned over the contralesional motor cortex and the anode over the ipsilesional supraorbital region with the intent of downregulating the excitability of the contralesional cortex

Bihemispheric tDCS: The anode is positioned over the lesioned motor cortex and the cathode over the contralesional cortex with the intent of simultaneously upregulating the excitability of the perilesional cortex and downregulating the excitability of the contralesional cortex

Extracephalic tDCS: The anode is positioned over the lesioned motor cortex and the cathode over the contralesional deltoid muscle with the intent of upregulating the excitability of the perilesional regions.

In each of these montages the intensity of the stimulation is steadily increased to the selected

threshold over a period of 30 seconds at the commencement of the intervention and similarly decreased over 30 seconds at the conclusion of the intervention. In the situation of Sham tDCS the stimulation is ramped up to give the patient the initial tingling sensation and then turned off without the subject being aware that the stimulation has ceased (Gandiga, Hummel et al. 2006).

4.6.4 Outcome measures

The primary outcome measures were motor impairment, body function and activity limitation. Typical measures of these outcomes include: simple reaction time (SRT), Motor Assessment Scale, the Jebsen Taylor test of hand function (JTT), the Fugyl Meyer Assessment (FM) and the Wolf Motor function test (WMFT). Included studies were also examined for reports of adverse events such as discomfort, fatigue and headache.

4.6.5 Data analysis

Details from each study including sample size, participant characteristics, tDCS parameters and reported findings were extracted using a standardized form. The results extracted relate to outcomes measured immediately following the application of tDCS and not longer term effects due to either the lack of follow-up data in the majority of studies or large variation in follow-up periods post intervention. Our results therefore indicate the presence or absence of immediate responses to tDCS and do not address change in long term performance. Authors were contacted where there was difficulty extracting the data from the published paper.

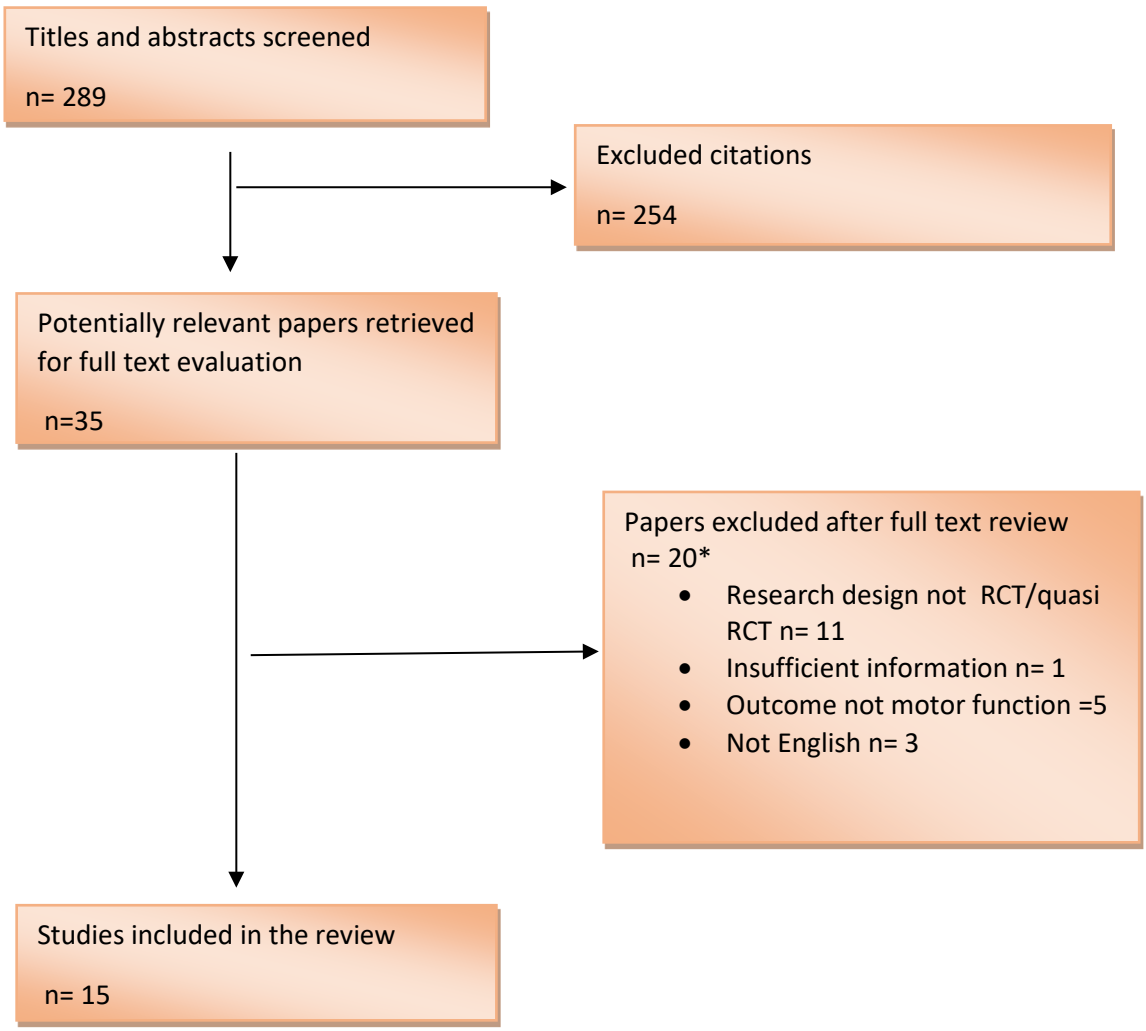
Where means and standard deviation values were provided for pre-post intervention conditions, the standardized mean difference (SMD) was calculated. This allowed us to convert all outcomes to a common scale to compare studies which used different tools to measure the same outcome. We followed general practice to interpret a value of 0.2 to indicate a small effect, and 0.8 a large effect (Valentine and Cooper 2008). Changes from baseline were used as the primary outcome. In the case where a decreased score on the assessment tool used in the original research represented an improvement, the positive form of the difference score was used to allow for comparison across the different scales. A meta-analysis was then conducted to obtain the average effect of the tDCS interventions and to compare the effects against sham intervention. Inter-trial heterogeneity was quantified using I^2 (Higgins and Green 2006). Trials in the meta-analysis were considered to have low statistical heterogeneity if I^2 was equal or less than 25% (Higgins, Thompson et al. 2003), in which case a fixed-effect model was used. If I^2 was greater than 25% a random-effects model was used to incorporate inter-trial heterogeneity (Higgins and Green 2006).

We calculated 7 separate meta-analyses. Firstly we analysed the effects of different types of tDCS:

anodal, cathodal and bihemispheric stimulation compared to sham stimulation. Then, we analysed the effect of tDCS on different patient subgroups, those with chronic stroke and those with acute/subacute stroke. According to customary convention we defined acute stroke as within the first 3 days of symptom onset, subacute stroke as less than 3 months and chronic stroke as greater than 3 months since the initial symptoms. Finally we pooled the data from studies which included subjects with mild-moderate impairments and those with moderate-severe impairments. As no standard definition of these categories exists we used the definitions and criteria provided by the original authors to determine groupings. We were unable to include the results of 2 studies in any of the meta-analyses due to insufficient data (Madhavan, Weber et al. 2011, Stagg, Bachtiar et al. 2012).

4.7 Results

Figure 4.1. Flow of studies through the review.



**Papers may have been excluded for failing to meet more than one inclusion criteria.*

4.7.1 Flow of studies through the review

The search resulted in the identification of 289 articles after the removal of duplicates. Of these 35 were deemed potentially relevant. On closer scrutiny a further 20 were excluded due to research design (11 studies failed to meet the randomization criteria), no inclusion of an impairment, function or activity limitation measure (5 studies), insufficient information (1 study), Russian or Chinese text only (3 studies). This left 15 studies for inclusion in the review (see Figure 4.1 for the flow of studies through the review).

4.7.2 Characteristics of studies

Quality: The methodological quality of the 15 studies meeting all inclusion criteria was consistently high with a mean PEDro score of 7.9 out of 10 (SD 0.9, range 7-9). All studies used randomization however only one reported concealed allocation (Kim, Lim et al. 2010). Each study, with the exception of one (Geroin, Picelli et al. 2011) used participant blinding, all but one (Kim, Ohn et al. 2009) blinded the assessors, but only 6 of the 15 studies reported therapist blinding. All studies had excellent retention rates with only two studies (Kim, Lim et al. 2010, Bolognini, Vallar et al. 2011) reporting dropouts but this remained less than 15% of each sample.

Participants: The mean age of the 315 participants across the studies was 59.3 years with a range from 28 to 87 years and a preponderance of males (61%). Time since stroke varied with 10 out of 15 studies recruiting participants with chronic stroke, 4 recruiting subacute subjects, and 1 study recruiting a mixed sample of chronic and subacute subjects. However, the majority of participants were less than 12 months post stroke (58%). Both cortical and subcortical strokes were included in the samples but cortical strokes predominated (cortical n = 137, subcortical n = 110, both n = 68).

Several different scales were used to classify stroke severity. This included grip strength, upper limb score of the Fugyl Meyer, and the ability to perform all items on the JTT. According to the classifications provided by the original authors 11 studies included participants with mild/moderately affected participants, and only 4 studies recruited subjects with moderate/severe impairments.

Intervention: The majority of studies investigated the effects of anodal stimulation compared to the sham condition (11 studies). In addition, many studies analysed the effect of several different applications of tDCS including cathodal stimulation (8 studies), bihemispheric stimulation (3 studies), and extracephalic stimulation (1 study). All studies used a sham control condition. Stimulation intensity was most typically 1mA (10 studies) with a range of 0.5mA to 2mA and stimulation duration ranged from 7- 40minutes. Seven studies reported the effects of tDCS following a single session of

stimulation, others trialled weekly sessions or consecutive daily sessions. Concurrent physical therapy or training during stimulation was administered in 9 of the studies.

Outcome measures: Several standardized tools were used to assess body function and activity limitation. This included the JTT (4 studies), the FM (5 studies) SRT tasks (4 studies), six minute walk test (1 study) and the BBT (1 study). Time points for data collection were not consistent across the trials. Most studies provide outcomes immediately following the stimulation period with 3 exceptions where there was a delay of up to 7 days after the cessation of the intervention prior to re-assessment (Kim, Lim et al. 2010, Lindenberg, Renga et al. 2010, Nair, Renga et al. 2011). Four studies collected short term follow up data out to at least 7 days post intervention and only 3 studies collected long term data out to at least 3 months (Kim, Lim et al. 2010, Hesse, Waldner et al. 2011, Rossi, Sallustio et al. 2012). Patient tolerance of the intervention was reported in all studies and discomfort, fatigue and attention data were formally collected via visual analogue scales or questionnaires in 5 studies (Fregni, Boggio et al. 2005, Celnik, Paik et al. 2009, Kim, Ohn et al. 2009, Stagg, Bachtiar et al. 2012, Zimerman, Heise et al. 2012).

Table 4.3. Summary of included studies

Study	Design	Participants	Stimulation Protocol	Intervention description	Outcome measures
Fregni et al. 2005	Cross-over, sham controlled, double blind Randomized, counterbalanced	n = 6 Mean age: 53.7 range: 28-75 chronic mixed cortical & subcortical mild-mod deficits	Cathodal and anodal 1mA/35cm^2 20mins	Single session of anodal tDCS, cathodal tDCS and sham separated by ≈48hours	JTT
Boggio et al. 2007	Study #1 Cross-over, sham controlled, double blind	n = 4 Mean age: 57.4 range: 38-75 chronic subcortical lesions mild deficits	Anodal and cathodal 1mA/35cm^2 20mins	1 session weekly for 4 weeks	JTT
	Study#2 Open label study	n = 5 chronic subcortical lesions mild deficits	Cathodal 1mA/35cm^2 20mins	5 consecutive daily sessions	
Kim et al. 2009	Single (patient) blinded, sham controlled,	n = 10 Mean age: 62.8 range: 28-75	Anodal 1mA/25cm^2 20mins	Single sessions of tDCS and sham separated by	BBT Finger acceleration

	counterbalanced, randomized	subacute subcortical (1 cortical) mild deficits		≈24hours	
Celnik et al. 2009	Cross-over, sham controlled, double blind Randomized	n = 9 Mean age: 55.3 range: 40-73 chronic mixed cortical & subcortical lesions mild deficits	Anodal 1mA/57cm^2 20mins PNS median and ulnar nerve of paretic hand (1hz for 2hours)	PNS + tDCS PNS + tDCS$_{sham}$ tDCS + PNS$_{sham}$ PNS$_{sham}$ + tDCS$_{sham}$ Sessions separated by ≈6days 28mins of finger task practice followed by stimulation	Finger sequence task
Kim et al. 2010	Double blind, sham controlled, stratified randomization and long term follow up	n= 18 Mean age: 57.2 range: 34-77 subacute mixed cortical & subcortical mild - moderate deficits	Cathodal and anodal 1mA/25cm^2 20mins	10 sessions 5x/week over 2 weeks combined with concurrent 30mins OT	FM(UE) BI
Lindenberg et al. 2010	Cross-over, sham controlled, double blind Block randomization & stratification for impairment	n= 20 Mean age: 56.2 range: 34-77 chronic MCA territory infarct Mild-moderate deficits	Bihemispheric 1.5mA/16.3 cm^2 30mins	5 consecutive daily sessions of either bihemispheric tDCS or sham tDCS combined with 60mins concurrent OT	FM(UE) WMFT
Mahmoudi et al. 2011	Cross-over, sham controlled, double blind Counterbalanced and randomized to order	n = 10 Mean age: 60.8 range: 40-87 mixed chronic & subacute mixed:cortical/ subcortical mild-moderate deficits	1. Bihemispheric anodal & cathodal 2. Anodal 3. Cathodal 4. Extracephalic tDCS 5. Sham 1mA/35 cm^2 20min	Single sessions separated by >96 hours	JTT
Madhavan et al. 2011	Cross-over, sham controlled, double blind Randomized	n = 9 Mean age: 65.4 range: 50-87 chronic subcortical except 1 cortical mild-moderate	Anodal tDCS and anodal over non-lesioned cortex, 0.5mA/8cm^2	3x15 min sessions separated by >48hrs Concurrent practice of ankle-tracking task using paretic	Tracking error in visuo-motor ankle tracking task FM(LE)

		deficits		ankle	
Nair et al. 2011	Sham controlled, double blind, randomized	n = 14 Mean age: 55.8 range: 40-76 chronic mixed:cortical/ subcortical mod-severe deficits	Cathodal tDCS 1mA 30 mins	5 days/ consecutive sessions Concurrent OT 60mins	ROM FM (UE)
Geroin et al. 2011	Sham controlled, single blind, randomized	n = 30 Mean age: 62.7 range: 51-73 chronic mixed: subcortical/cortical mild-mod deficits	Anodal tDCS 1mA/35cm^2 7 mins	10 sessions 5x week for 2 weeks Concurrent robotic gait training	6 MWT 10MWT
Bolognini et al. 2011	Sham controlled, double blind, randomized	n = 14 Mean age: 46.7 range: 26-75 chronic mixed:cortical/ subcortical mod-severe deficits	Bihemispheric 2mA/35cm^2 40mins	10 sessions 5x week for 2 weeks Concurrent CIMT: 90% of waking hours + 4 hours shaping	JTT Grip strength FM(UE)
Hesse et al. 2011	Sham controlled, double blind, randomized multicentre	n = 96 Mean age: 64.9 range: 39-79 subacute mixed:cortical/ subcortical severe deficits	Cathodal and anodal 2mA/35cm^2 20mins	30 sessions 5x week for 6 weeks Concurrent robot training and regular therapy	FM (UE) Grip strength BI
Zimerman et al 2012	Sham controlled, double blind, crossover psuedorandomized order	n = 12 Mean age: 58.3 range: 31-73 chronic mixed:cortical/ subcortical mild deficits	Cathodal tDCS 1mA/25cm^2 20 mins	Concurrent bilateral motor sequence training 15-20mins Separated by 1 week	Finger sequence task
Rossi et al. 2012	Sham controlled, double blind, randomized	n = 50 Mean age: 68.2 range: ?-80 acute mixed:cortical/ subcortical mod-severe deficits	Anodal tDCS 2mA/35cm^2 20 mins	5 sessions 5 x week for 1 week	FM (UE) BI

Stagg et al. 2012	Sham controlled, single blind, randomized	n = 13 Mean age: 64 range: 30-80 chronic subcortical: 7cortical: 6 mild-mod deficits	Cathodal and anodal 1mA/35cm^2 20mins	Single sessions separated by one week Concurrent blocks of visually cued response time task and grip strength task	Grip strength Response time

JTT: Jebsen Taylor Test of hand function, BI: Barthel Index, ROM: Range of joint motion, 6MWT: 6 minute walk test, 10MWT: 10 metre walk test, PNS: Peripheral nerve stimulation, PNSsham, PNS delivered with to the deep peroneal and posterior peroneal nerves for 2 hours, FM: Fugyl Meyer Test (UE: upper extremity component, LL: lower extremity component), WFMT: Wolf Motor function test, BBT: Box and Block test

4.7.3 Effect of tDCS

Physical Function: The effect of anodal tDCS on motor performance was examined by pooling the data from 9 studies involving 224 subjects. When compared with sham controls, anodal tDCS did not significantly alter significantly motor performance (SMD = 0.05, CI = -0.25 to 0.31, p = 0.71, see figure 2). This finding was similar for cathodal stimulation when the data from 7 studies involving 154 subjects was pooled (SMD = 0.39, CI = -0.05 to 0.82, p = 0.08, see figure 4.3) and bihemispheric stimulation using the data from 3 studies involving 54 subjects (SMD = 0.24, CI = -0.3 to 0.77, p = 0.39, see figure 4.4). Only one author investigated the use of an extracephalic reference electrode and report a non-significant effect relative to sham (p = 0.82) (Mahmoudi, Haghighi et al. 2011). Two studies conducted follow-up assessments 3 months after the intervention and both report no between-group differences (p>0.05) (Hesse, Waldner et al. 2011, Rossi, Sallustio et al. 2012). Kim et al. (2010) were the only authors to report long-term outcomes which were measured 6 months after the intervention. Functional performance was significantly better 6 months post cathodal stimulation compared to sham (p < 0.05) whereas anodal stimulation showed a trend towards improvement relative to sham but this did not reach statistical significance at 6 months follow up.

Figure 4.2 SMD (95% CI) of effect of anodal stimulation on motor performance compared to sham by pooling data from 9 studies (n = 224).

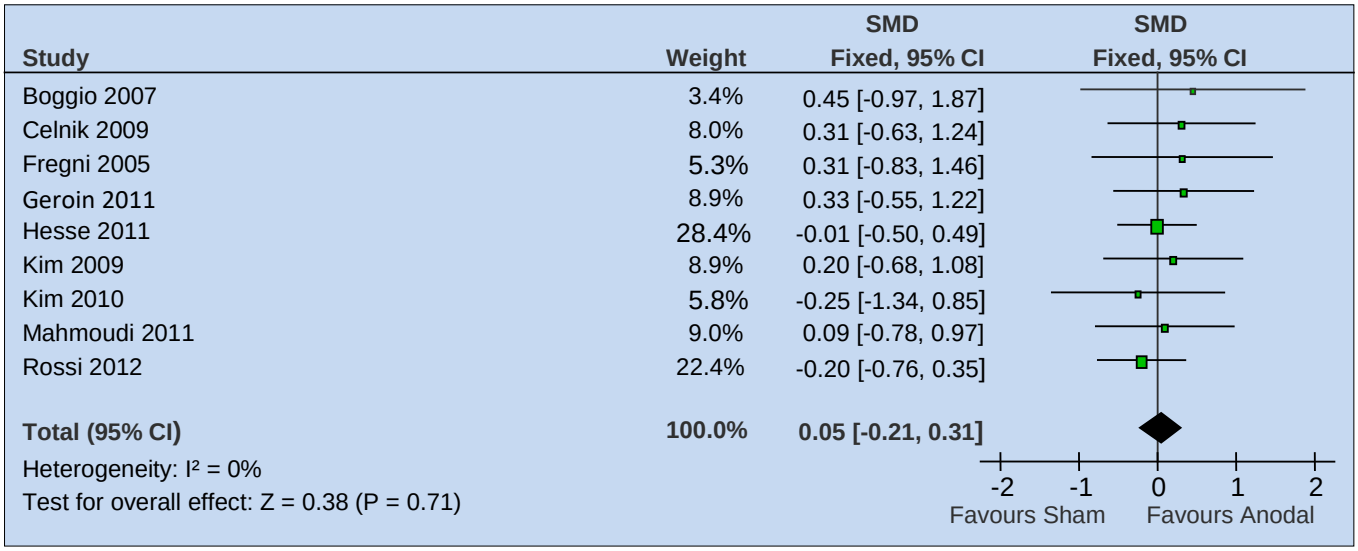


Figure 4.3 SMD (95% CI) of effect of cathodal stimulation on motor performance compared to sham by pooling data from 7 studies (n = 154).

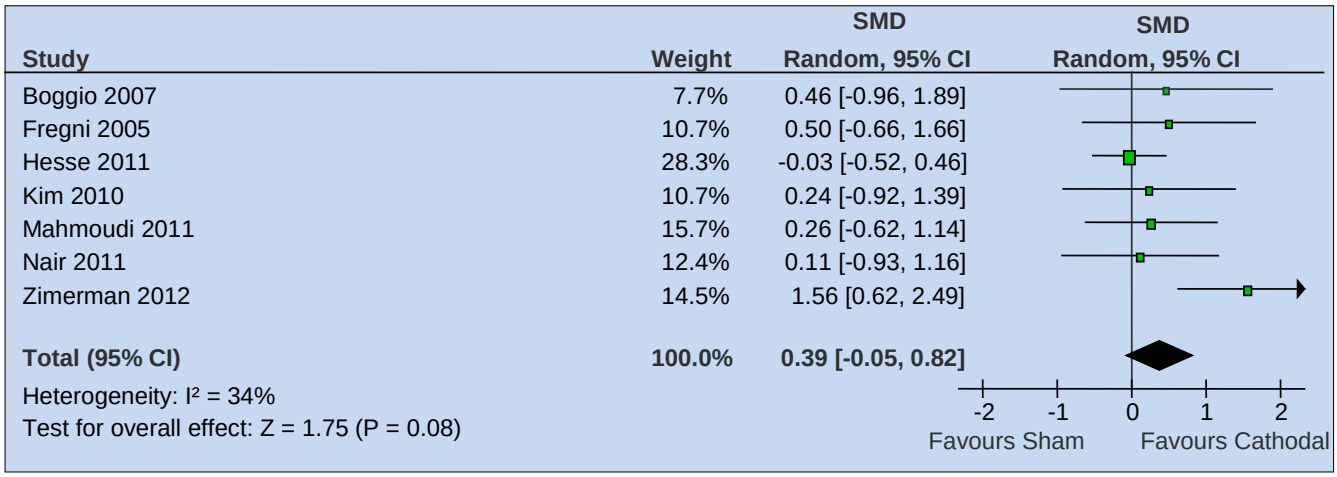
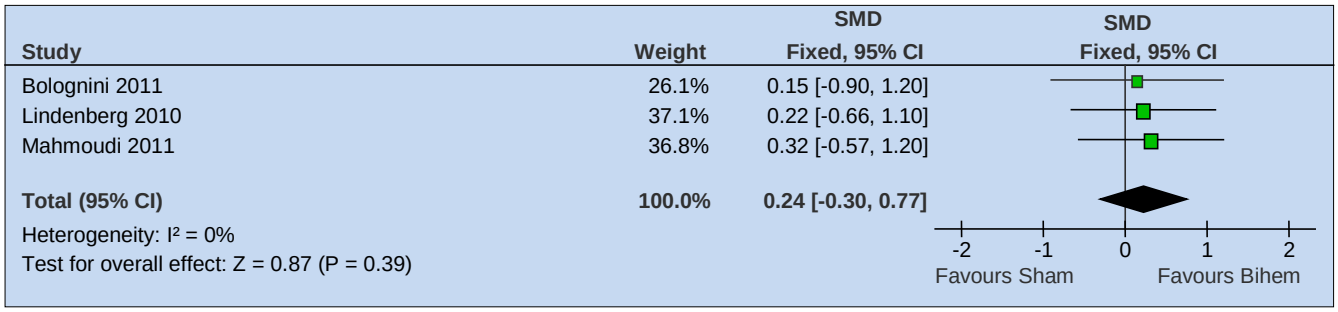


Figure 4.4 SMD (95% CI) of effect of bihemispheric stimulation on motor performance compared to sham by pooling data from 4 studies (n = 38).



Further analysis was conducted by comparing acute, subacute and chronic stroke samples. Only one study investigated subjects with acute stroke (Rossi, Sallustio et al. 2012). These authors report a non-significant effect associated with tDCS. The effect of tDCS on motor performance in people with chronic stroke was evaluated by pooling the data from 8 studies involving 130 subjects. When compared with sham controls, tDCS significantly improved performance (SMD = 0.45, CI = 0.09 to 0.80, p = 0.001, see figure 4.5). This positive finding was not replicated when we pooled the data from the 3 studies (n = 49) which used subacute stroke samples (SMD = 0.01, CI = -0.39 to 0.42, p = 0.94, see figure 4.6). The final analyses revealed a statistically significant benefit of tDCS in 9 studies involving 155 subjects who demonstrated mild/moderate impairment (SMD = 0.37, CI = 0.05 to 0.70, p = 0.02, see figure 4.7) but not those classified with moderate/severe impairment in 4 studies with 141 subjects (SMD = -0.05, CI = -0.38 to 0.28, p = 0.78, see figure 4.8).

Figure 4.5 SMD (95% CI) of effects of tDCS on motor performance of people with chronic stroke compared to sham by pooling data from 8 studies (n = 130).

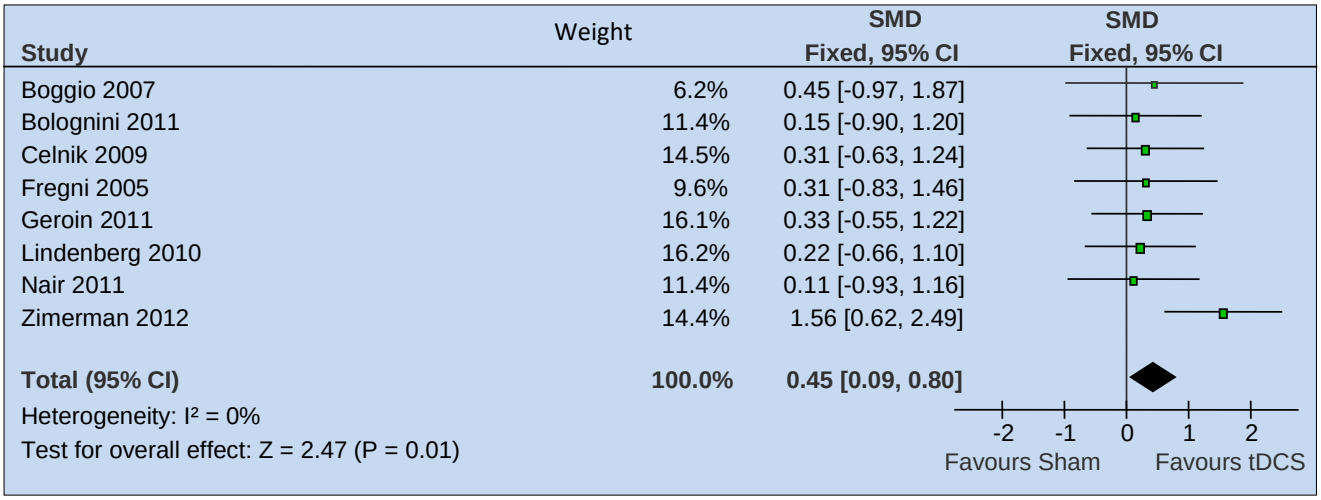


Figure 4.6 SMD (95% CI) of effects of tDCS on motor performance of people subacute stroke compared to sham by pooling data from 3 studies (n=49).

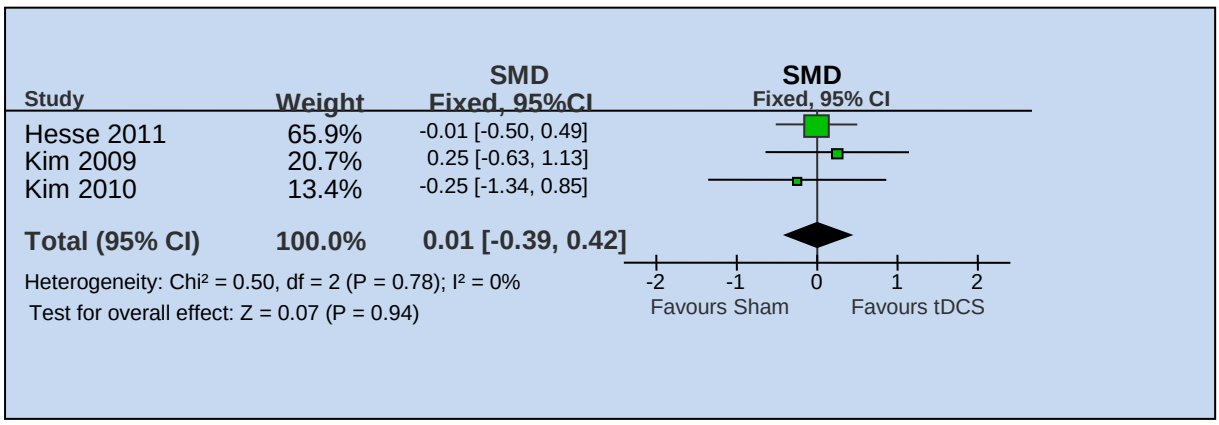


Figure 4.7 SMD (95% CI) of effects of tDCS on motor performance of people with mild/moderate stroke impairments compared to sham by pooling data from 9 studies (n= 155).

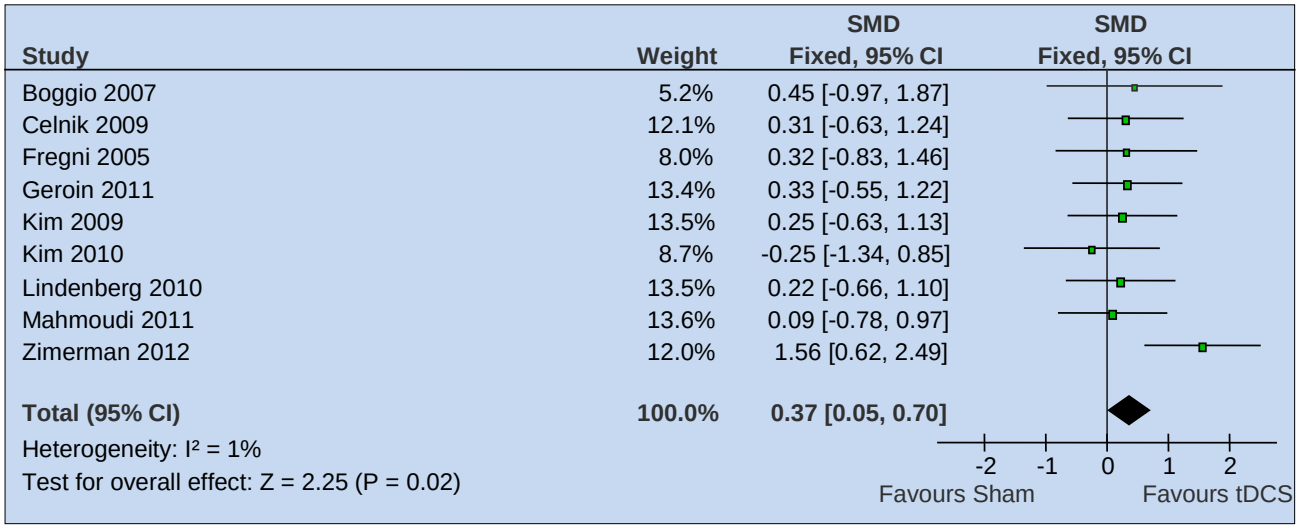


Figure 4.8 SMD (95% CI) of effects of tDCS on motor performance of people with moderate/severe stroke impairments compared to sham by pooling data from 4 studies (n= 141)

4.7.4 Safety

The absence of adverse events was recorded in 14 of the 15 studies; the remaining study (Kim, Lim et al. 2010) recorded 2 adverse episodes whereby one participant experienced a headache (during anodal stimulation) and another dizziness (during cathodal stimulation). These symptoms lead the participants to discontinue their involvement in the study. There was no significant effect of stimulation on attention or fatigue.

Table 4.4 Included studies: summary of findings

Study	Outcome measures	Findings (mean % improvement relative to sham)
Fregni et al. 2005	JTT	Cathodal 15.3% (p<0.05) Anodal 10.7% (p<0.05) No significant difference between anodal & cathodal
Boggio et al. 2007	JTT	Cathodal 9.5% (p=0.016) Anodal 7.3% (p=0.046) No significant difference between anodal and cathodal (p=0.56)
Kim et al. 2009	BBT	Anodal: 17.8% (p<0.05)
Celnik et al. 2009	Finger sequence task	Anodal: 18.6% (p<0.05) Anodal + peripheral nerve stimulation = 41.3% (p<0.05)
Kim et al. 2010	FM(UE)	Anodal: 27.3% Cathodal: 16.3% No significant difference between groups at 1 day post intervention Cathodal better than sham at 6mths in FM measure (p<0.05)
Lindenberg et al. 2010	FM(UE)	Bihemispheric: 11.7% (p<0.001)
Mahmoudi et al. 2011	JTT	Bihemispheric 13.9% (p= 0.011) Anodal 9.3% (p = 0.016) Cathodal 6.8% (p = 0.010) No significant difference b/w these 3 groups Extracephalic 2.4% (p = 0.82)
Madhavan et al. 2011	Visuo-motor ankle tracking task	Anodal = 10.8% (p=0.0001)
Nair et al. 2011	FM (UE)	Cathodal: FM: 7.5 % (p=0.048) ROM: 13.3% (p=0.002)
Geroin et al. 2011	6MWT 10MWT	Anodal: 6MWT : 9% (p=0.14) 10MWT: 3.4% (p=0.32)
Bolognini et al. 2011	JTT FM(UE) HS	Bihemispheric: JTT: 19% (p<0.01) FM: 16.2% (p<0.03) HS: 41.6% (p<0.01)
Hesse et al. 2011	FM(UE) HS	Anodal: Cathodal FM: 10.9% 3.9% HS: -1% 1.4% No significant difference b/w these 2 groups or sham
Zimerman et al. 2012	Correct finger sequences	Anodal: 35.7% (p=0.04)
Rossi et al. 2012	FM	Anodal: FM: -25.8% (p = 0.82)
Stagg et al. 2012	Response time	Anodal: Cathodal: 12.6% (p=0.002) 7.9% (p=0.04)

4.8 Discussion

The results of this systematic review provide evidence from 15 studies with relatively high methodological quality in support of tDCS when applied to selected stroke patients. These positive findings are not consistent across all the included studies possibly due to the heterogeneity of the participant characteristics and stimulation paradigms. Those most likely to benefit are patients with chronic stroke and/or mild to moderate motor impairments. Likewise the size of the treatment effect is variable and at best modest with a maximum effect size of 35.7% improvement relative to sham.

Based on the current findings we are unable to decipher if one form of tDCS is superior to another. Pooled data demonstrates a lesser treatment effect for the anodal stimulation montage (anodal SMD = 0.05; cathodal SMD = 0.39; bihemispheric SMD 0.39) and whilst several individual studies demonstrate greater treatment effect following cathodal stimulation compared to anodal (Fregni, Boggio et al. 2005, Boggio, Nunes et al. 2007), these differences fail to reach statistical significance and others report the effects of cathodal stimulation to be significantly weaker than anodal (Mahmoudi, Haghighi et al. 2011). Although cathodal, anodal and bihemispheric stimulation appear to have merit, there is currently no evidence to support the use of extracephalic stimulation paradigms in stroke patients (Mahmoudi, Haghighi et al. 2011). Furthermore, the optimal number of tDCS sessions and session duration has not yet been well defined with conflicting reports in the literature. It has been proposed that repetition of tDCS in consecutive sessions can enhance the efficacy of the stimulation by cumulating or stabilizing the effects. Several authors have demonstrated the positive effects of tDCS enduring beyond the intervention period by 1 week (Lindenberg, Renga et al. 2010, Bolognini, Vallar et al. 2011, Nair, Renga et al. 2011), however this is disputed by other authors who utilized daily tDCS stimulation ranging from 1 to 6 weeks with no reported benefit (Geroin, Picelli et al. 2011, Hesse, Waldner et al. 2011, Rossi, Sallustio et al. 2012). The ideal number and timing of sessions and the sustainability of the effects remain undetermined and requires long term prospective investigation.

There is consensus that for motor improvements to be lasting tDCS must occur in conjunction with training (Hummel, Celnik et al. 2008). This may enhance skill acquisition by increasing afferent inputs to the cortex while its intrinsic excitability is being enhanced by tDCS. tDCS has been shown to beneficially enhance the effects of peripheral nerve stimulation (Celnik, Paik et al. 2009), constraint induced movement therapy (Bolognini, Vallar et al. 2011), and Occupational Therapy training (Kim, Lim et al. 2010, Lindenberg, Renga et al. 2010, Nair, Renga et al. 2011). In contrast, Rossi et al 2012, were unable to show any additional benefit of tDCS when supplementing routine therapy in acute

stroke patients. Likewise there is no evidence to support tDCS as an adjuvant to bilateral robot-assisted limb training in either the upper limb (Hesse, Waldner et al. 2011) or lower limb (Geroin, Picelli et al. 2011). These conflicting findings suggest that several factors may influence the outcome of the combined treatment approach and may include the temporal delivery of tDCS in relation to the training as well as the type of training.

Stroke is a heterogenous disease affecting a diverse population. The establishment of participant selection criteria based on lesion location, time after stroke, and/or integrity of the corticospinal pathway, may assist in determining which patients are most likely to benefit from tDCS. Corticospinal excitability in the subacute stages of stroke recovery may be different from that in the chronic stages and therefore neuromodulatory agent such as tDCS may have differential effects. This is supported by our meta-analysis whereby there was a difference in the size of the treatment effect when the sample was comprised of people with chronic stroke (SMD= 0.45) compared to subacute stroke (SMD = 0.01). However, Mahmoudi et al. (2011) dispute this premise and found no correlation between time post stroke and outcome in their randomized controlled trial ($p>0.02$). Furthermore, cautious interpretation is required as the positive findings in chronic stroke have predominantly been reported in mild to moderately affected patients who were able to grasp and manipulate objects with the affected hand. In contrast the acute/subacute samples often included participants with severe deficits. Similarly, cortical excitability and current flow is likely to differ according to stroke location and lesion volume. Several authors support this theory reporting that patients with subcortical lesions had significantly greater improvements than those with cortical lesions (Hesse, Waldner et al. 2011, Mahmoudi, Haghighi et al. 2011). Indeed there could be merit in defining damaged brain areas and networks more specifically through imaging in future studies to determine the presence of differential responses to tDCS according to lesion characteristics. It has been proposed that tDCS may be effective for a wide range of stroke types and motor deficits as long as part of the corticospinal output is preserved (Kim, Lim et al. 2010). Future research may need to consider matching controls for lesion characteristics as at this stage we are unable to conclusively determine if the effects of tDCS are independent of these factors.

This review indicates that tDCS is well tolerated by patients with only 2 documented drop outs in all the reported studies. These drop outs occurred following adverse events in the study with the highest dosage of tDCS (Kim, Lim et al. 2010). Although headache and dizziness are relatively minor symptoms, it may suggest that in subacute stroke 10 sessions of 2mA direct current is reaching the threshold of patient tolerance. This highlights the need for the determination of stimulation parameters which maximize benefit but limit the associated risks.

A strength of this review is that it included functionally relevant outcome measures related to motor performance, which is often the major clinical focus of stroke rehabilitation. It presents an impartial synthesis of all the high quality English publications in the field which provides a more robust estimate of the likely effect of tDCS than individual studies alone. Although 15 studies were identified by this review the total number of participants was small (n = 315) and further caution with interpretation of these findings is warranted given the likelihood of publication bias towards positive findings. While this data confirm that tDCS has the potential to improve motor performance in stroke patients, long term effects on motor function remain unclear as only one study evaluated effects after 6 months.

4.9 Conclusion

It is becoming increasingly apparent that the best intervention for stroke recovery will incorporate a combination of techniques to maximize neuronal plasticity (Hummel, Celnik et al. 2008). Research investigating a possible role for tDCS is mounting but remains inconclusive and hampered by both the heterogeneity of the patient and stimulation characteristics. It appears that in order to maximize the potential of this modality, prudent selection of patients and stimulation parameters will be required. Factors such as lesion patterns, severity of paresis, time course post stroke and the type of adjuvant therapy are methodological issues which require further attention.

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Chapter 5: The Effects of tDCS on Arm Function in People with Chronic Stroke

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5.3 Synopsis

The global incidence of stroke is increasing while at the same time the incidence of death from stroke is declining. Consequently stroke is transitioning more into a disease of chronically disabled survivors (Dobkin and Carmichael 2016). Therefore the search for improved methods of rehabilitation must continue. Physiologically tDCS seems like a plausible option and evidence from healthy populations demonstrates that cortical excitability can be altered at least transiently by tDCS. However, evidence for functional, clinically meaningful improvements in the healthy population is less consistent and even less reliable in people with stroke.

We modelled our research protocol on our previous study with healthy aged participants whereby we used a cross-over study design with 20 minutes of stimulation, a sham controlled condition, and assessment of both upper limbs. Evidence from our systematic review indicates that those most likely to benefit are those with chronic stroke but there was no significant difference in benefit between cathodal and anodal stimulation montages. We used this information to direct the recruitment of people with chronic stroke into the study and included both anodal and cathodal montage conditions.

5.4 Abstract

Background and Aims: Transcranial direct current stimulation (tDCS) has been proposed as a tool to enhance stroke rehabilitation however evidence to support its use is lacking. The aim of this study was to investigate the effects of anodal and cathodal tDCS on upper limb function in chronic stroke patients.

Methods: Twenty five participants were allocated to receive 20mins of 1mA of anodal, cathodal or sham cortical stimulation in random, counterbalanced order. Patients and assessors were blinded to the intervention at each time point. The primary outcome was upper limb performance as measured by the Jebsen Taylor Test of Hand Function (total score, fine motor subtest score and gross motor subtest score) as well as grip strength. Each outcome was assessed at baseline and at the conclusion of each intervention in both upper limbs.

Results: Neither anodal nor cathodal stimulation resulted in statistically significantly improved upper limb performance on any of the measured tasks compared to sham stimulation ($p>0.05$). When the data was analysed according to disability, participants with moderate/severe disability demonstrated significantly improved gross motor function following cathodal stimulation compared to sham ($p=0.014$). However this was accompanied by decreased key grip strength in the unaffected hand ($p=0.003$).

Conclusion: We are unable to endorse the use of anodal and cathodal tDCS in the management of upper limb dysfunction in chronic stroke patients. While, there appears to be more potential for the use of cathodal stimulation in patients with severe disability, the effects were small and must be considered with caution, as they were accompanied by unanticipated effects in the unaffected upper limb.

Key words: tDCS, upper limb function, chronic stroke

5.5 Introduction

Despite advances in conventional rehabilitation over the past twenty years, 60% of stroke patients do not regain functional use of their paretic upper limb (Bartolo *et al*., 2014). This compels the need for more effective methods of stroke rehabilitation, ones which will not only result in greater motor recovery but are accessible, safe and easy to administer. One method which meets these criteria and has experienced mounting interest over the past decade is transcranial direct current stimulation (tDCS).

tDCS is said to be neuromodulatory as it delivers a subthreshold stimulation that affects the neuronal firing rate by altering the balance of ions inside and outside the neuronal membrane. This produces hyperpolarisation in the region of the cathode, and depolarisation of the resting membrane potential beneath the anode (Nitsche *et al*., 2003). Therefore, depending on the placement of the electrodes, cortical excitability can be increased or inhibited according to the desired effect of the intervention.

The use of tDCS to facilitate stroke recovery is based on the premise that a focal lesion produces a region of decreased neuronal output and disrupts the balance of interhemispheric inhibition. The premorbid hemispheric balance may be restored in two ways: either through the use of anodal stimulation to the lesioned hemisphere to cause a sub-threshold excitatory effect, or by applying the cathode to the non-lesioned hemisphere and using a negatively charged current to inhibit neuronal excitability of that hemisphere (Bolognini *et al*., 2009). Both applications have the potential to restore a more equal interaction between hemispheres (Harvey & Stinear, 2010) and therefore improve motor performance of the affected limb (Alonso-Alonso *et al*., 2007).

Whilst there has been burgeoning research investigating the use of tDCS in neurological conditions, there is currently inconsistent and imprecise evidence available on its effectiveness as a modality to aid rehabilitation. Several systematic reviews have synthesized the available evidence for tDCS in the stroke population (Bastani & Jaberzadeh, 2012; Butler *et al*., 2013; Elsner *et al*., 2013; Marquez *et al*., 2015) with variable outcomes. All of these reviews are hampered by the small number of quality studies that are available for inclusion in the review and the heterogeneity of stimulation and patient characteristics. A Cochrane review involving a total of 286 patients found no significant benefit of tDCS in activities of daily living (Elsner *et al*., 2013). One review supports the use of tDCS to improve upper limb function (Butler *et al*., 2013) but this was not supported by other reviews when only high quality evidence was included in the meta-analysis (Elsner *et al*., 2013; Marquez *et al*., 2015). When the data were analysed according to stroke chronicity, there appears to be differential effects in favour of those who received tDCS more than 5 months after stroke. Data from

130 subjects were combined to demonstrate a significant improvement in motor performance of the upper limb compared to sham (SMD=0.45, CI = 0.09-0.80, P=001) however this benefit was not evident for subacute patients (Marquez *et al*., 2015). Although this effect appears promising, the studies included in this meta-analysis employed different stimulation paradigms and different patient inclusion criteria making interpretation of the results difficult. The purpose of this current study was to examine the effects of tDCS in chronic stroke patients with further scrutiny to determine whether tDCS can improve upper limb function when applied for 20 minutes at a current strength of 1mA; whether one type of montage (anodal or cathodal) is superior to the other, and whether stroke severity plays a role in the subsequent effects.

5.6 Methods

5.6.1 Subjects

People with chronic stroke living in the Hunter Region were identified from a local database and invited to participate via a mailed information letter. Others self-referred following an advertisement in a local disability information newsletter. Inclusion criteria consisted of first episode of ischaemic stroke at least 6 months prior to enrolment, with residual upper limb deficits. Exclusion criteria included evidence of cognitive impairment (defined as a Montreal Cognitive Assessment score of less than 21), a self-reported current or previous significant psychiatric disorder, serious medical condition which could interfere with assessment, CNS- acting medication, metal implants in the cranium or upper torso, skin lesions on the scalp, a flaccid affected upper limb, pregnancy, or age below 18 years. The protocol for this project was approved by the University of Newcastle's Human Research Ethics Committee (H-2010-1339), and complied with the Declaration of Helsinki.

5.6.2 Study Design

Patients were allocated via computer generated randomisation to an order in which to receive the three stimulation interventions (sham, anodal & cathodal tDCS). This order was counterbalanced across patients. In the initial session, subjects were interviewed to obtain demographic, stroke and medical information, and baseline data were collected. The three stimulation sessions were each separated by two weeks to prevent contamination by residual stimulation effects (Nitsche & Paulus, 2001). During each session, assessment of function and strength was conducted immediately after the intervention in an identical manner to baseline measurement. Both patients and assessors were blinded to the intervention received at each time point.

5.6.3 Transcranial Direct Current Stimulation

tDCS was applied using a NeuroConn programmable direct current stimulator. A 1mA current was applied via two 5x7cm surface electrodes positioned on the scalp in a previously validated montage (DaSilva *et al*., 2011). One electrode was positioned over the primary motor cortex of the desired

hemisphere, and the other electrode on the contralateral supraorbital area. Anodal stimulation consisted of a 20 minute application of 1mA stimulation to the ipsilesional primary motor cortex, and cathodal stimulation was applied to the corresponding contralesional area. The current strength was slowly increased over 30 seconds at the beginning of treatment and decreased likewise at the end of the session. This causes a transient sensation of tingling or prickling which subsides after a number of seconds. The sham setup was identical and consisted of a 30 second increase in current strength after which stimulation was ceased. Sham tDCS has a transient sensation of tingling identical to actual stimulation, and has previously been validated, with patients being unable to distinguish between real and sham tDCS (Gandiga *et al*., 2006).

5.6.4 Outcome Measures

The primary outcome of interest was change in function assessed using the Jebsen Taylor Test (JTT). The JTT is a valid and reliable method of determining upper limb function through a series of seven timed functional tasks (Jebsen *et al*., 1969). After familiarisation with the tasks, patients completed six of the seven JTT items using standardised instructions (i.e., the writing task was excluded). Subtest completion was restricted to 45 seconds and this maximum score was allocated when the patient failed to complete the task within this time. Individual task times were recorded and were used to calculate scores for gross motor and fine motor subtests as well as total JTT.
In addition, pinch and grip force testing was performed using a hand-held dynamometer. This was conducted using standardised instructions, with the shoulder in neutral and elbow flexed at 90 degrees so that the hand lay close to the stomach. Three attempts were allowed and the mean force was recorded.

5.6.5 Data Analysis

For both JTT and grip measures, results for each stimulation session were calculated on the difference score from the baseline session. The five difference scores (JTT (total, fine, gross), force (pinch, grip) were analysed using a 3 Stimulation (anodal, cathodal, sham) x 2 Response Hand (affected, unaffected) repeated measures Analysis of Variance. Where there was a significant main effect or interaction with Stimulation, simple comparisons of each stimulation condition against Sham were performed separately for each hand using t-tests.

5.7 Results

5.7.1 Participant characteristics

A total of 25 patients (15 male, mean age 64.28±11yrs) were enrolled into the study. A description of the sample is provided in Table 5.1. Disability varied but the majority of the subjects (17/25) were classified as having minor disability according to the modified Rankin Score. There was a wide range

in function of the affected upper limb as highlighted in the baseline outcome measure scores as shown in Table 5.2. All enrolled participants completed the study and there were no adverse reactions.

Table 5.1. Description of chronic stroke patients

Measure	
Age (mean ± SEM)	64.28±2.2
Sex (Male/Female)	15/10
Handedness (Left/Right)	5/20
Lesioned Hemisphere (Left/Right)	15/10
Affected Hemisphere (Dominant/Non-dominant)	11/14
MoCA Score (mean ± SEM)	25.25±0.6
MRS Level (1/2/3)	17/5/3
Months Post-Stroke (mean ± SEM)	80.4±8.7

Abbreviations: standard error of the mean (SEM), Montreal Cognitive Assessment (MoCA), Modified Rankin Scale (MRS).

Table 5.2. Mean scores and interquartile ranges for each outcome measure at baseline

Measure	**Affected UL Mean (SEM)**	**IQR**	**Non-affected UL Mean (SEM)**	**IQR**
Total JTT	63.97 (6.4)	42.7	34.74 (1.1)	9.9
Fine tasks JTT	35.88 (3.8)	25.8	22.12 (1.0)	6.6
Gross tasks JTT	24.52 (3.4)	15.25	11.7 (0.48)	3.65
Key Grip	15.93 (1.1)	7.9	18.7 (1.2)	11.1
Tip Grip	14.44 (2.2)	6.15	15.33 (1.0)	6.6

Abbreviations: Jebsen Taylor Test (JTT), standard error of the mean (SEM), interquartile range (IQR)

5.7.2 JTT performance

Figure 1(a) shows the Total JTT difference scores from the baseline session for each experimental session and both the affected and unaffected hands. While there was no main effect of either stimulation or hand (both $p>0.09$), there was a small but significant stimulus by hand interaction ($p=0.048$). As shown in Figure 1(a), the unaffected hand showed no effect of stimulation, relative to baseline ($F<1$). The affected hand showed enhanced performance compared to baseline for both sham and cathodal stimulation sessions, and no improvement relative to baseline for anodal stimulation. Although there was a marginally significant effect of stimulation ($p=0.08$), simple comparisons of each active condition against sham showed that neither the relative deterioration under anodal tDCS nor the relative improvement under cathodal tDCS were significant (both $p>0.1$). For the unaffected hand, there was no influence of stimulation condition on performance ($F<1$). Figures 1(b) shows Fine Motor JTT difference scores from the baseline session. Again the affected hand appeared to have delayed completion time under anodal stimulation relative to baseline, however there was no significant effect of hand or stimulation. Gross Motor JTT scores (Figure 1c) also showed no significant effects of stimulation or hand (all $p>0.1$).

5.7.3 Grip Strength Measures

Figure 1 shows Key Grip (d) and Pinch Grip (e) strength difference scores from the baseline session. For the affected hand, Pinch Grip scores were better at test relative to baseline. However, the effect did not differ across stimulation conditions. There were no significant effects of stimulation, hand or their interaction on either key grip or pinch strength (all $p>0.2$).

5.7.4 Effects of tDCS according to disability

To examine whether tDCS differentially improves performance only in patients with greater or lesser disability, we reran the above analyses with a group factor based on MRS score. The majority of patients (n=17) had only mild disability (i.e., MRS score of 1), with only five scoring in the moderate and three in the severe range (i.e., MRS scores of 2 and 3, respectively). Therefore, the between-subjects factor had two levels: mild and moderate/severe disability. Figures 2 and 3 show the effect of the intervention when the data was dichotomised according to severity. For both Total JTT and Fine Motor JTT scores, the more disabled group showed a pattern that more closely resembles the whole group analyses discussed above and shown in Figures 3(a),(b),(c). However there was no main effect or interaction between group and stimulation or hand.

Figure 1. Change scores from baseline for each of the outcome measures

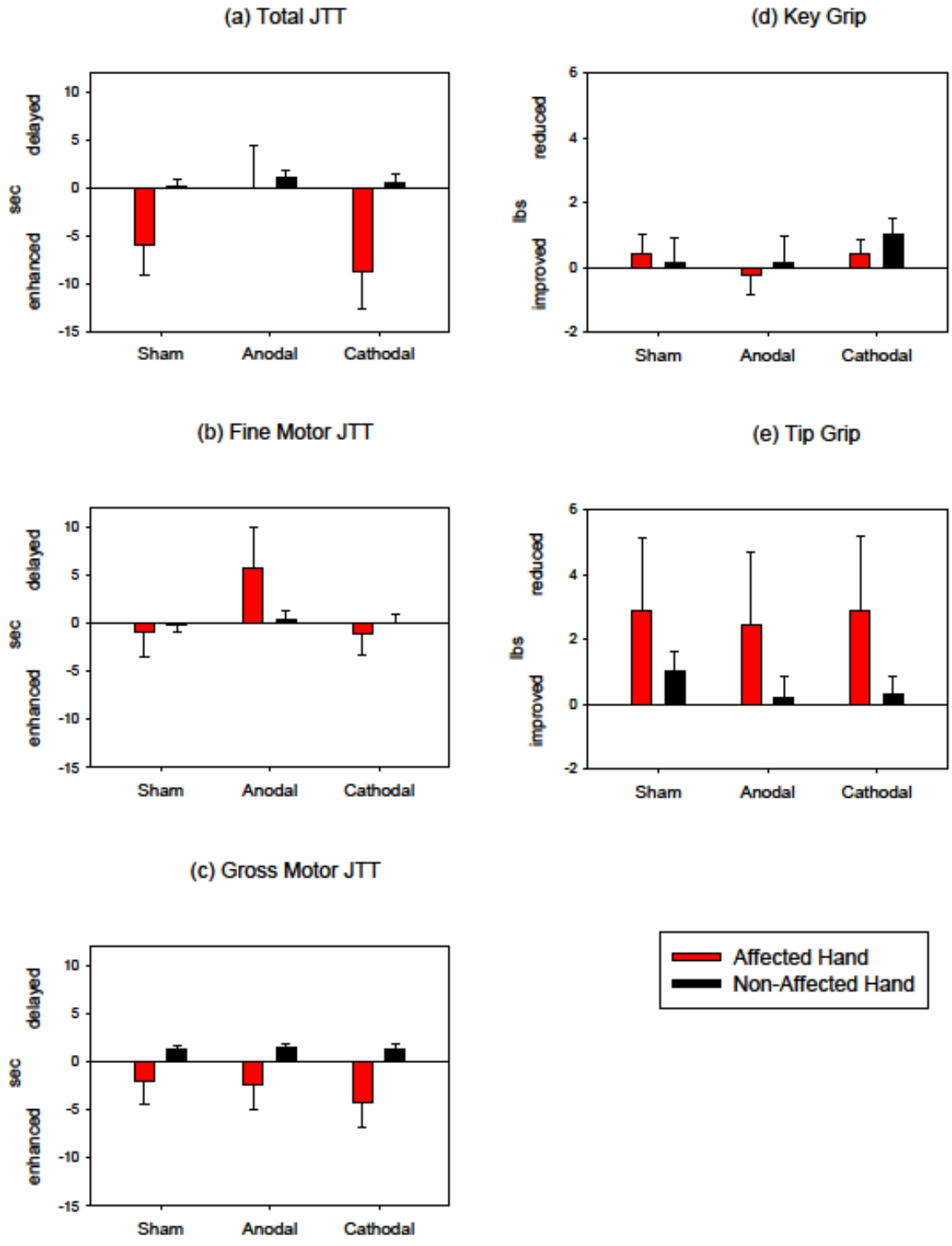


Figure 2. Change scores from baseline for participants with mild disability

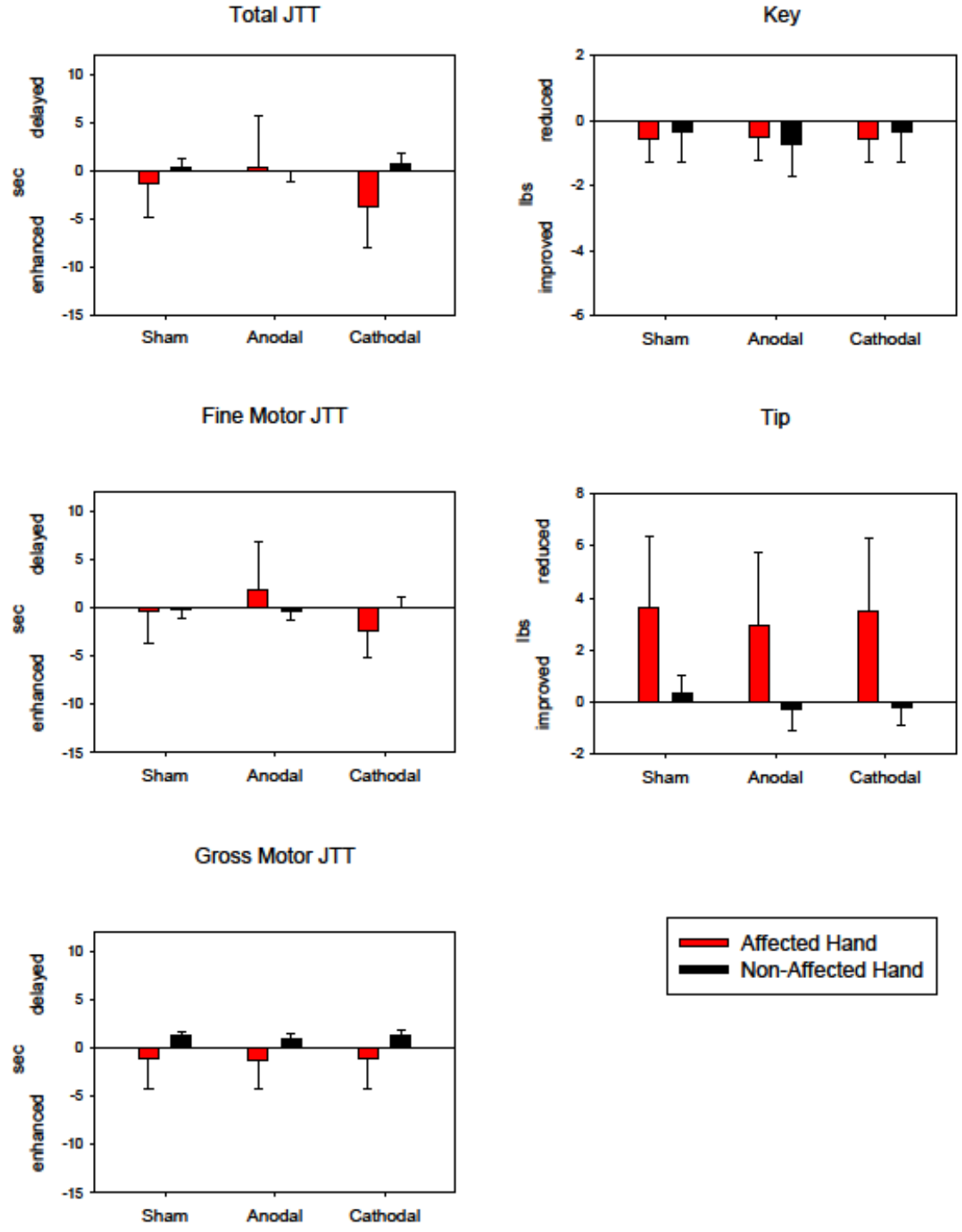
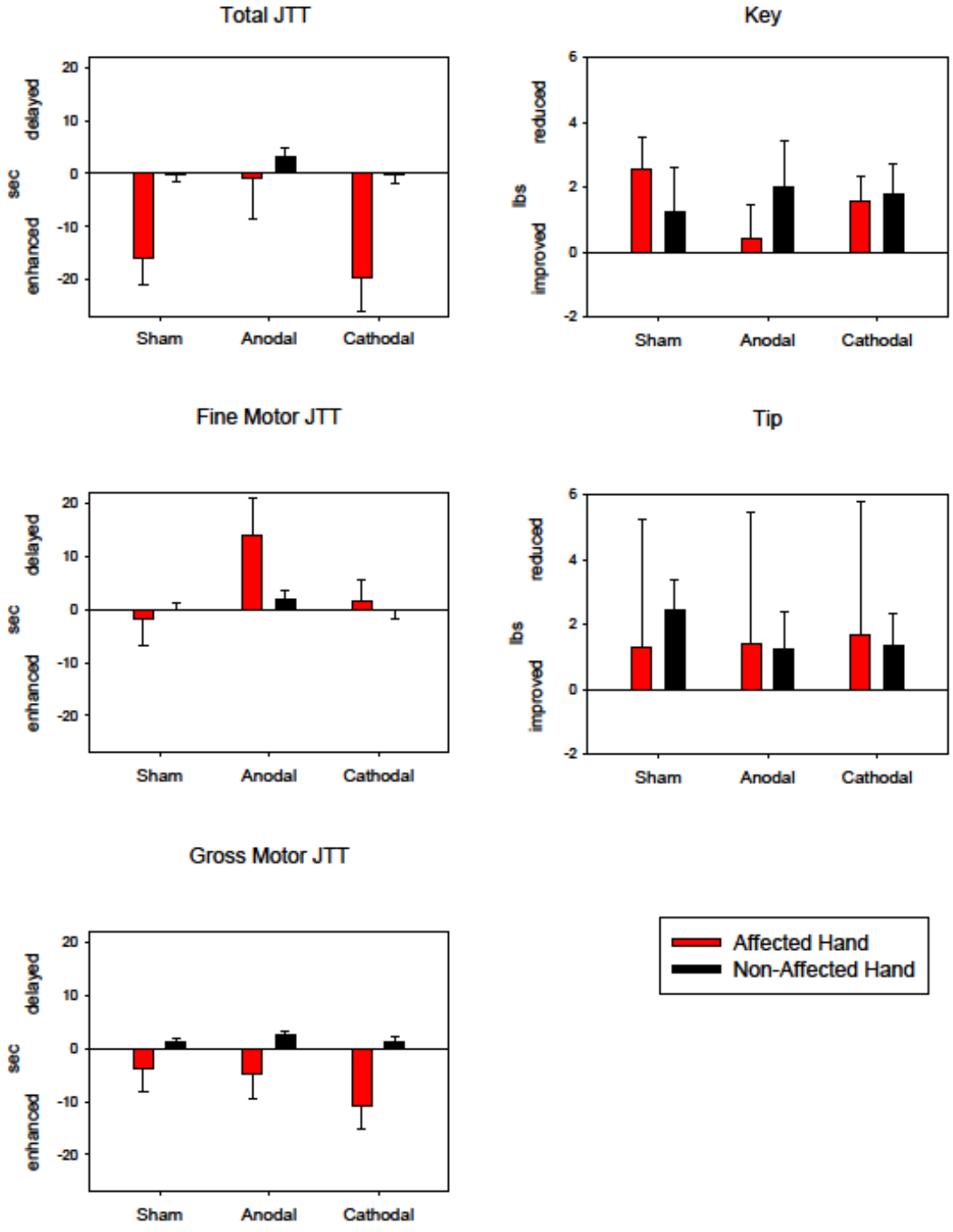


Figure 3. Change scores from baseline for participants with moderate/severe disability



The Gross Motor JTT score showed a significant interaction between group and stimulation (F (2, 46) = 4.7, p=0.014), as well as between group, stimulation and hand (F (2, 46) = 4.6, p=0.02). As shown in Figure 2(c), gross motor JTT performance was significantly better following cathodal tDCS compared to sham for the more disabled group (P=0.014). Moreover, this improvement following cathodal tDCS in more disabled patients was stronger for the affected compared to the unaffected hand.

A significant three-way interaction between disability, stimulation and hand was also found on the Key Grip measure (F (2, 46) 6.5, p=0.003). As shown in Figure 2(d), for the moderate/severe disability group, cathodal tDCS over the unaffected motor area resulted in decreased key grip performance of the unaffected hand.

5.8 Discussion

In this study we used a double blind randomised controlled design in 25 chronic stroke patients and failed to reveal any significant effects of tDCS on upper limb function. This finding was irrespective of the type of stimulation used (anodal vs cathodal), the hand assessed (affected vs unaffected) or the type of task examined (gross function vs fine motor function vs strength). However, following cathodal stimulation, patients with moderate/severe disability showed enhanced gross motor function of the affected upper limb, but diminished strength in the unaffected upper limb. Thus cathodal stimulation produced an inconsistent effect across affected and unaffected limbs in moderately to severely disabled indicating a disparate effect for this subgroup of patients to this type of stimulation.

Interest in tDCS in chronic stroke has grown following the publication of a single case study reporting benefits in both grip strength and JTT scores in a chronic hemiparetic 84 year old male (Hummel & Cohen, 2005). Since then there have been 8 published studies investigating the effects of anodal or cathodal tDCS on upper limb function in this population. Seven of these studies reported significant positive effects of stimulation compared to sham (Boggio *et al*., 2007; Cha *et al*., 2014; Fregni *et al*., 2005; Hummel *et al*., 2006; Nair *et al*., 2011; O'Shea *et al*., 2014; Zimerman *et al*., 2012). It is important to note that all of these studies had small samples (n≤14 except one study which had a sample of 20 subjects; (Cha *et al*., 2014). Comparison between studies is difficult due to different paradigms, combined therapies, and stroke characteristics. Our findings are consistent with those of Au Yeung et al who found no significant difference between tDCS and sham on measures of grip strength and the Purdue Pegboard test in mildly affected chronic stroke sample. (Au-Yeung *et al*., 2014). Clearly some chronic stroke patients appear to benefit from tDCS, but this result is

inconsistent. Conclusions drawn from these few small studies must be made with caution given the likelihood of publication bias towards positive findings.

We revealed an unequal response to cathodal compared to anodal stimulation. Our findings are consistent with those of previous studies that reported greater treatment effect following cathodal stimulation compared with anodal stimulation in chronic stroke patients although these differences failed to reach significance (Au-Yeung *et al*., 2014; Boggio *et al*., 2007; Fregni *et al*., 2005). Another study reported that the effect of cathodal stimulation was weaker than anodal stimulation in a mixed sample of sub-acute and chronic patients (Mahmoudi *et al*., 2011). Cathodal application, that is, stimulation of the non-lesioned hemisphere may be inherently more advantageous for several reasons. Anatomical changes in the lesioned hemisphere following stroke could disturb the electric current administered by the tDCS and therefore the effects may be less predictable (Fregni *et al*., 2005). Stimulating the non-lesioned hemisphere has the benefit of normal cortical topography, intact intra-cortical connections, and more uniform current density that is not disturbed by non-homogenous/damaged tissue (Schlaug & Renga, 2008).

In contrast to patients with mild disability, more disabled patients showed improvement in gross motor function following cathodal stimulation. This supports the findings of a recent study by Lefebvre *et al* who used bihemispheric tDCS and found that patients with the most disability reaped the greatest benefits (Lefebvre *et al*., 2014). However, this finding is not consistent throughout the literature. In a recent meta-analysis that pooled data from nine available studies, it was those with mild/moderate impairment that derived benefit from stimulation (SMD =0.37, CI = 0.05 – 0.70, P = 0.02) and there was no significant benefit for patients with severe stroke (Marquez *et al*., 2015). Similarly, others found that both anodal and cathodal stimulation had a negative impact on a reaction time task in patients with severe disability whereby the gain was less than with sham or even detrimental to performance (O'Shea *et al*., 2014). This is consistent with the theory that poorly-recovered stroke patients may require ipsilateral cortical activity to compensate for their injury and therefore cathodal stimulation may be disruptive to function (Bradnam *et al*., 2012). Currently it remains unclear whether tDCS holds greater promise for different levels of stroke severity.

Negative effects of cathodal stimulation on non-affected upper limb strength have not previously been reported. This may be in part because the majority of studies neglect to measure the bilateral effects of tDCS and therefore potential clandestine effects in the unaffected limb are not reported. As cathodal stimulation is thought to increase inhibition of the contralateral cortex, it is plausible that it increases inhibition to the contralateral hand – in this scenario, the unaffected limb. In the case of severe stroke, patients are heavily reliant on the unaffected upper limb for activities of daily

living and this negative effect could have serious consequences for function. This effect requires further investigation.

5.9 Conclusion

The effects of tDCS in stroke are inconsistent and there is wide variation in the size of the effects. In this study, we examined the effects of tDCS in patients with chronic stroke, who have previously been shown as the most likely to respond (Marquez *et al*., 2015). However, our results failed to demonstrate an overall benefit of tDCS on upper limb function. Interindividual variation is extensive in people with stroke and it is reasonable to assume that patients with different characteristics will respond differently to variations in stimulation paradigms. We found that for cathodal stimulation, the effect of tDCS correlated negatively with residual hand function, a finding that is not commonly supported by previous research. Given the simplicity of tDCS and the complexity of brain function, further analysis of the mechanisms underpinning tDCS is required to unravel whether and how tDCS protocols can be optimised to increase efficiency and predictability in stroke patients.

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The authors have no conflicts of interest to disclose.

The study was approved by the ethics committee of Hunter New England Health and ratified by the University of Newcastle (Ref: 12/04/18/4.01). All participants gave their written informed consent. The study was conducted according to the declaration of Helsinki Principles.

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Chapter 6. Discussion

6.1 Summary of principal findings

This thesis comprehensively reports research which systematically analyses the functional and physiological effects of tDCS in the healthy aged and chronic stroke patients. Some of our findings support those reported in other published work, however other aspects of our findings are novel and conflict with previous reports. In summary, we have identified:

1. The hemispheres respond differently to tDCS. In healthy aged participants we recorded an improvement in performance of the non-dominant upper limb as a result of dominant and non-dominant hemisphere stimulation. There was no change in performance in the dominant upper limb.
2. Age was negatively associated with upper limb functional performance but it did not mediate the size of the effect of tDCS. That is, despite the changes in cortical activity and motor learning that accompany age, the brain responds to stimulation in a manner consistent with the young.
3. Task specificity appears to exist in the response to tDCS. Our research suggests measurable effects of stimulation are more pronounced on fine motor tasks but not evident on gross motor tasks or measures of strength.
4. The effects of tDCS are diffuse. Although the electrodes are placed over specific scalp regions it cannot be assumed that the underlying cortical region is selectively or specifically stimulated.
5. In stroke patients, further scrutiny of electrode montage is necessary to maximise benefit and limit adverse effects. We found that the performance of the affected upper limb deteriorated with anodal stimulation and for those with the greatest disability cathodal stimulation may be preferential.
6. The systematic review of the literature revealed a lack of quality research in stroke patients with a total pooled sample of 315 participants. Differences in study design and subjects render direct comparisons problematic. Overall:
    I. All studies are limited by small sample sizes
    II. The majority of studies investigate people with chronic stroke and those with subcortical lesions. There has been no investigation of cathodal stimulation in subacute stroke

III. No studies have long term follow up past 3 months. Therefore any benefits may be transient

IV. It is not possible to determine if one montage is superior to another however there was a slightly greater treatment effect for those with moderate/severe disability to cathodal stimulation.

V. The majority of findings are modest inducing, at best, 10-20% functional improvement

6.2 Research implications

Research is continuing to expand to involve an increasing number of variations in procedures as well as research populations, but the core understanding of the physiological effects produced by tDCS, even in healthy subjects, remains poorly understood. This variability in research conditions makes comparison of results across studies, and interpretation of findings, very difficult. For this reason, we commenced our research with healthy aged participants and comprehensively assessed both hemispheres and both upper limbs in a well-defined manner. As reproducibility is low in the reported literature, it remains to be seen if our findings could be substantiated. Clearly further clinical studies are required to determine if methodological or patient characteristics, or both, are the reason for the inconsistent findings across studies. Specifically, researchers must determine the optimal approach regarding:

1. Study design: sample requirements, eligibility criteria, electrode positioning, dosage, timing, adjunctive therapies, pharmacotherapy, measurement of effects
2. Patient characteristics: dominant hemisphere, age, gender, level of fitness, comorbidities/medications, genetics, etc
3. Stroke characteristics: site and size of lesion, pyramidal tract involvement, time since stroke, degree of impairment

Our multimodal imaging study demonstrated consistent findings across several different measures of physiological function and hand performance. This was particularly evident for changes in metabolite concentrations and blood flow. This indicates a potential for tDCS to be used to facilitate longer-term changes with repeated applications, particularly if combined with adjunct therapies such as physiotherapy. A multimodal imaging study investigating this approach, with long term follow-up, is an avenue warranted in subsequent research.

In the future, a more coordinated research effort is necessary and this may require taking a step back to systematically vary one variable at a time to gain a deeper understanding of the unpredictability of tDCS findings. There is also a likelihood of publication bias and studies with negative findings should be published and available for review. This coincides with efforts to improve transparency, with full reporting of data on all analyses conducted with data sharing through repositories (Buch, Santarnecchi et al. 2016).

6.3 Clinical implications

There appears to be a theoretical clinical basis for the use of tDCS as an adjunct treatment whereby it may work in synergy with established therapies to be able to boost the treatment affects. tDCS can be paired with pharmacotherapy for conditions such as depression and pain and various forms of physical or cognitive training (Guleyupoglu, Schestatsky et al. 2013). Alternatively, as a substitutive treatment for other therapies which are contraindicated or ineffective. For example, as a replacement for pharmacologic management for patients who did not respond to or with poor drug tolerance such as the elderly (Brunoni, Nitsche et al. 2012) However, currently there is inconsistent and imprecise evidence for the effectiveness of tDCS and we have not demonstrated that tDCS is capable of producing reliable or meaningful clinical change.

Brain reorganisation after stroke is a dynamic process which differs considerably between patients and motor relearning itself is a complex process with probable different underlying neural substrates for different tasks (Buch, Santarnecchi et al. 2016). Indeed, changes in MEP amplitude are the only consistent outcome reported by motor tDCS studies (Horvath, Forte et al. 2014). A better understanding of motor learning processes, and the tasks used to assess them is critical to determine whether tDCS can manipulate the specific features of skill learning and behaviours that are meaningful to everyday life (Buch, Santarnecchi et al. 2016).

A major limitation to clinical transferability appears to be generalisability. Clearly not all people respond in the same way, or to the same magnitude. It is unlikely that one stimulation protocol exists which is suitable for all patients and at this stage we are unable to define patient selection criteria.

6.4 Conclusion

Rehabilitation strategies for motor recovery after stroke remain unsatisfactory. In view of our ageing societies, the burden of stroke is expected to rise further in the next decades, thus an urgent need exists for the further development of tailored interventions to augment recovery. This has led to staunch interest in tDCS. This modality has become an increasingly popular method of

neuromodulation and there is a body of evidence to support its use. However, our work has also identified many concerns which limit the wide scale adoption of this tool. Namely inconsistency between subjects within our studies and across trials, unknown sustainability of effects, inability to predict those most likely to respond, and lack of clinically meaningful benefit. Indeed, in the current wave of research enthusiasm for tDCS its potential to augment stroke recovery may have been overgeneralised.

It seems we have reached a gap between pre-clinical research and consistent evidence to support application of tDCS as a rehabilitation modality. Individual patient data has shown that some patients do benefit from cortical stimulation however, research must unravel how stimulation protocols can be optimized with the goal of increasing efficacy, and producing durable effects, while limiting undesired side-effects, as well as determining stroke characteristics amenable to these protocols. The complexity of this task cannot be overstated with many factors capable of influencing the final outcome of the stimulation. Until this has been established, through future research, tDCS cannot be considered for routine use in stroke rehabilitation.

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Chapter 7. Appendices

Appendix I: Additional publication related to chapter 2

Publication details

Conley AC, Fulham WR, **Marquez JL**, Parsons MW, & Karayanidis F. (2016). No Effect of Anodal Transcranial Direct Current Stimulation over the Motor Cortex on Response-Related ERPs during a Conflict Task. *Frontiers in Human Neuroscience*, *10*, 384.

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This publication relates to addition EEG analysis. This data was collected as part of the study investigating the physiological response of the healthy aged to anodal tDCS. Here we report no effect of tDCS on reaction time or response-related potentials during the cued go/no-go task. This effect was replicated in a sample of healthy young subjects.

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ORIGINAL RESEARCH
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No Effect of Anodal Transcranial Direct Current Stimulation Over the Motor Cortex on Response-Related ERPs during a Conflict Task

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Anodal transcranial direct current stimulation (tDCS) over the motor cortex is considered a potential treatment for motor rehabilitation following stroke and other neurological pathologies. However, both the context under which this stimulation is effective and the underlying mechanisms remain to be determined. In this study, we examined the mechanisms by which anodal tDCS may affect motor performance by recording event-related potentials (ERPs) during a cued go/nogo task after anodal tDCS over dominant primary motor cortex (M1) in young adults (Experiment 1) and both dominant and non-dominant M1 in older adults (Experiment 2). In both experiments, anodal tDCS had no effect on either response time (RT) or response-related ERPs, including the cue-locked contingent negative variation (CNV) and both target-locked and response-locked lateralized readiness potentials (LRP). Bayesian model selection analyses showed that, for all measures, the null effects model was stronger than a model including anodal tDCS vs. sham. We conclude that anodal tDCS has no effect on RT or response-related ERPs during a cued go/nogo task in either young or older adults.

Keywords: transcranial direct current stimulation, event-related potential, contingent negative variation, lateralized readiness potential, P300, ageing

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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 article, 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.

tDCS involves the application of a weak current across the surface of the cortex via scalp electrodes (Nitsche and Paulus, 2000; Utz et al., 2010). When applied over the motor cortex, this current generates changes to motor output (Nitsche and 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 and Paulus, 2001; Nitsche et al., 2003a; 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., 2003b; 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 characterized by motor dysfunction (for a review see Flöel, 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 (PFC) 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 behavioral performance and changes in ERP waveforms in older compared to young adults (Polich, 1997; Sterr and 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 behavioral 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 behavioral 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 and Schröter, 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 lateralized 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 M1, 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 300 ms 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 PFC (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 cue-target interval (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.

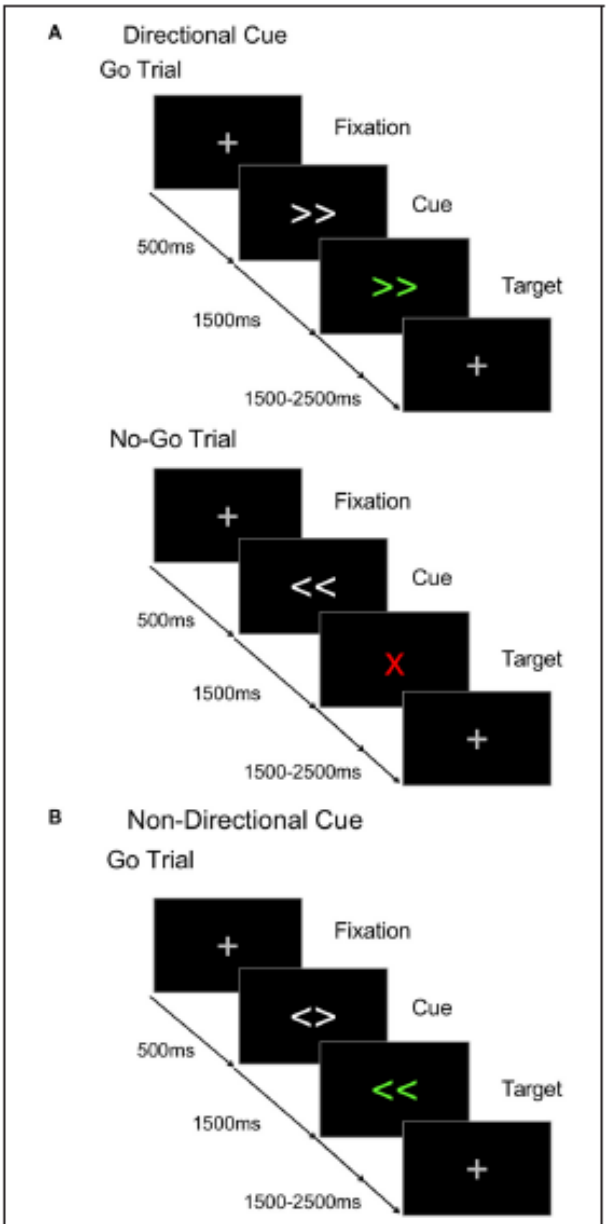

FIGURE 1 | Cued go/nogo task. The time course for **(A)** go and nogo trials in directional cue condition, and **(B)** go trials in non-directional cue condition.

EXPERIMENT 1

In Conley et al. (2015), we showed that anodal tDCS stimulation over the dominant M1 in young adults had no effect on behavioral 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 RT.

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.

METHOD

Participants

Twenty-four healthy young adults completed active and sham stimulation sessions[1]. One participant was removed from the analyses due to excessive artifact in their electrophysiological recording, so the remaining analysis was performed on 23 participants (9 males, mean age 21.2 ± 2.5 years). All participants were right handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). All participants were screened for non-suitability for DCS, 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.

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 1 mA current continuously for 20 min (with 10 s ramp-up/down at the beginning and end of the intervention) using two rubber electrodes (35 cm^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 (Nitsche and Paulus, 2000, 2001). The sham stimulation condition involved the application of a 1 mA current for 50 s (10 s ramp up and 40 s application) followed by 20 min 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 3 weeks apart to avoid any carryover effects of stimulation.

[1]The behavioral results were presented in Experiment 1 of Conley et al. (2015). Here we report the behavioral data very briefly, to allow comparison with data from older adults in Experiment 2.

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 CTI (1500 ms, **Figure 1**). Each trial began with a fixation cross (500 ms) that was replaced by the cue (1500 ms) which was, in turn, replaced by the target. Directional and non-directional cues were presented in separate randomized 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 1000 ms and the subsequent target-cue interval was jittered (mean 2000 ms, random sequence, 1500–2500 ms). 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.

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 20 min 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, 2004) 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 40 min 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 3 weeks later to complete the second session, in which they received the other stimulation intervention.

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 0.02–30 Hz bandpass filter and a 50 Hz notch filter to remove line noise. EEG data were processed and analyzed using EEG Display 6.3.12 (Fulham, 2012).

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

tLRP and rLRPs were extracted from the C3/C4 electrode pair using the averaging method explained by Coles (1989):

$$LRP = \left[Mean(C4 - C3)_{LH\ response} + Mean(C3 - C4)_{RH\ response}\right]/2 \quad (1)$$

Both target- and response-locked waveforms were filtered using a 30 Hz zero-phase, low pass filter to reduce high frequency noise. tLRP waves were baselined across 200 ms prior to the onset of the target, whereas rLRP waves were baselined between 500 and 700 ms 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 100–600 ms post-target onset for tLRPs and over 300–100 ms before the response for rLRPs.

Data Analyses

Both the RT and the mean ERPs for go trials were analyzed using a repeated measures generalized 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 analyzed statistically. LRP analysis included only the Stimulation and Cue factors.

RESULTS

Behavioral Results

Mean RTs for young adults are displayed in **Table 1** (top). As shown in **Figure 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; $F_{(1,22)} < 1$).

Electrophysiological Results

Mean amplitudes for CNV and P300 ERP components for young adults are displayed in **Tables 2**, **3** (top), respectively. Peak P300 latencies are displayed for young adults in **Table 4** (top). **Figure 3A** shows cue-locked ERP waveforms at Cz and Pz electrodes following anodal tDCS and sham stimulation. The CNV emerged around 500 ms post-cue onset, peaking just before target onset. CNV amplitude was larger for directional than non-directional cues (−4.9 vs. −2.8 μV; $F_{(1,22)} = 18.9$, $p < 0.001$) but did not vary with response hand ($F < 1$). As shown in **Figure 3A**, stimulation did not affect CNV amplitude or interact with cue or response hand (all $F < 1$).

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 $p > 0.05$).

Mean tLRP and rLRP onset latencies for young adults are displayed in **Table 5** (top). As shown in **Figure 4A** (left), tLRP emerged earlier for directional than for non-directional cues, with the latter showing an early positive dip (cue: 271 vs. 313 ms; $F_{(1,22)} = 38.0$, $p < 0.001$). rLRP had a later onset for

TABLE 1 | Mean response time (RT, milliseconds) for young and old adults for each cue and hand following anodal tDCS and sham.

Group/Stimulation	Directional left	Directional right	Non-directional left	Non-directional right
Young adult				
Active	392.8 (14.0)	388.0 (12.7)	470.5 (11.1)	451.6 (10.5)
Sham	396.1 (13.8)	388.1 (12.0)	473.3 (13.8)	447.2 (11.0)
Old adult				
Dominant				
Active	500.5 (14.4)	499.0 (14.8)	570.2 (16.2)	565.1 (16.4)
Sham	477.3 (14.3)	474.6 (12.9)	558.1 (15.9)	549.1 (16.3)
Non-Dominant				
Active	497.8 (16.5)	483.3 (16.9)	584.1 (18.5)	576.7 (18.8)
Sham	497.7 (16.3)	489.6 (14.7)	578.7 (18.2)	563.5 (18.7)

Standard error of the mean is in parentheses.

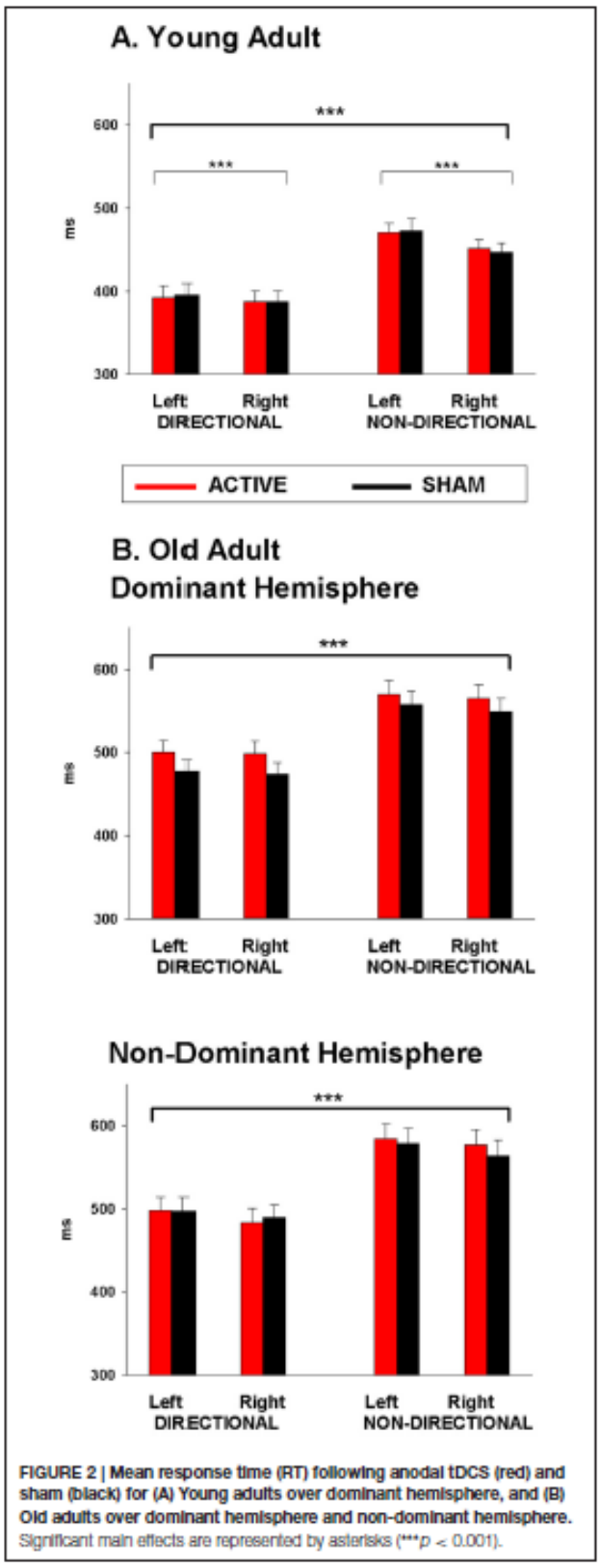

FIGURE 2 | Mean response time (RT) following anodal tDCS (red) and sham (black) for (A) Young adults over dominant hemisphere, and (B) Old adults over dominant hemisphere and non-dominant hemisphere. Significant main effects are represented by asterisks (***$p < 0.001$).

directional than non-directional cues (**Figure 4A**, right; −109 ms vs. −131 ms; $F_{(1,22)} = 25.4$, $p < 0.001$). Stimulation did not significantly affect the onset latency of either tLRP or rLRP (all $F < 1$).

DISCUSSION

Overall, behavioral 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. LRPs 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 tLRP, 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 behavioral 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 and Hallet, 2005).

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 (Cespón et al., 2013). Compared to young adults, old adults tend to show slower stimulus evaluation, as evidenced by increased P300 latency across the lifespan (for a review see Polich, 1996). 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; Golob et al., 2005; Sterr and Dean, 2008).

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

TABLE 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.

Group/Stimulation	Directional left	Directional right	Non-directional left	Non-directional right
Young adult				
Active	−5.0 (0.9)	−5.0 (0.9)	−3.1 (0.8)	−2.8 (0.7)
Sham	−5.3 (0.97)	−4.2 (1.0)	−2.9 (0.5)	−2.6 (1.0)
Old adult				
Dominant				
Active	−5.4 (1.1)	−4.0 (1.0)	−4.2 (0.9)	−3.9 (1.0)
Sham	−5.5 (0.97)	−6.1 (0.97)	−3.9 (1.3)	−4.1 (0.9)
Non-Dominant				
Active	−7.4 (1.2)	−6.4 (1.2)	−4.5 (1.0)	−4.5 (1.2)
Sham	−5.8 (1.1)	−6.8 (1.1)	−4.9 (1.4)	−4.3 (1.1)

Standard error of the mean is in parentheses.

dominant and non-dominant hemisphere stimulation, the effect should be greater for the contralateral than the ipsilateral hand.

METHOD

Participants

Thirty-nine right-handed healthy older adults[2] completed testing under anodal tDCS and sham stimulation in separate sessions. Due to excessive EEG artifact, two participants were removed from further analyses, resulting in a final sample of 37 participants (19 males, mean age 59.9 ± 10.9 years). 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: 21 participants (12 males, mean age 58.8 ± 9.9 years) received anodal tDCS over their dominant motor area, whereas the remaining 16 participants (7 males, mean age 61.2 ± 12.2 years) received active tDCS over their non-dominant motor area. Participants in both groups were randomly assigned to stimulation order as described in Experiment 1. This study was approved by the University of Newcastle's Human Research Ethics (H-2010-1339).

[2]Data from most of these participants contributed to Marquez et al.'s (2015) which focused on clinical measures of motor performance (e.g., JTT and grip tasks).

Design and 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 JTT (Jebsen et al., 1969) and pinch grip tests (Hinson and 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 RT in older adults (mean 3000 ms, random sequence, 2500–3500 ms). The statistical analyses of both the behavioral 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.

RESULTS

Behavioral Results

Results of the JTT and the pinch-grip tasks are presented in Marquez et al. (2015). For the cued go/nogo task, error

TABLE 3 | Peak P300 amplitude at Pz (microvolts) for young and old adults for each cue and hand following anodal tDCS and sham.

Group/Stimulation	Directional left	Directional right	Non-directional left	Non-directional right
Young adult				
Active	13.8 (1.0)	15.1 (1.1)	17.9 (1.1)	18.9 (0.9)
Sham	14.6 (1.1)	15.0 (1.1)	17.7 (1.0)	17.9 (1.1)
Old adult				
Dominant				
Active	15.0 (1.2)	15.0 (1.2)	19.0 (1.4)	17.6 (1.4)
Sham	15.9 (1.1)	15.1 (1.1)	19.3 (1.3)	18.3 (1.3)
Non-Dominant				
Active	13.9 (1.4)	14.5 (1.4)	17.2 (1.6)	15.9 (1.6)
Sham	13.9 (1.2)	13.8 (1.3)	15.5 (1.5)	14.9 (1.5)

Standard error of the mean is in parentheses.

TABLE 4 | P300 peak latencies (milliseconds) at Pz for young and old adults for each cue and hand following anodal tDCS and sham.

Group/Stimulation	Directional left	Directional right	Non-directional left	Non-directional right
Young adult				
Active	342.4 (16.9)	337.45 (18.5)	344.2 (10.6)	357.4 (6.9)
Sham	317.1 (14.3)	336.7 (15.9)	345.0 (14.3)	344.8 (10.4)
Old adult				
Dominant				
Active	465.6 (22.1)	470.0 (22.6)	442.4 (12.7)	448.2 (12.5)
Sham	469.5 (24.6)	432.7 (22.9)	452.9 (11.3)	470.0 (12.7)
Non-Dominant				
Active	420.4 (25.3)	429.3 (25.9)	447.7 (14.5)	465.0 (14.3)
Sham	424.4 (28.2)	431.7 (26.3)	468.6 (13.0)	454.4 (14.6)

Standard error of the mean is in parentheses.

rate for go trials and false alarm rate for nogo trials were very low and not statistically analyzed (1.61% and 0.1%, respectively). As shown in **Figure 2B**, RT was 338 faster for directional than for non-directional cues ($F_{(1,35)} = 144.04$, $p < 0.001$; 490 vs. 568.2 ms). 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 ± 11, Sham: 523.6 ± 10; $F_{(1,35)} = 2.12$, $p > 0.1$). 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 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.

Electrophysiological Results

CNV

Cue-locked ERP waveforms for healthy older adults are shown in **Figure 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 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.

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.

LRP

The target-locked and response-locked LRPs for the old adults (**Figure 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. 381.3 ms; $F_{(1,35)} = 35.36$, $p < 0.001$; −118.6 vs. −163.5 ms; $F_{(1,35)} = 34.6$, $p < 0.001$, respectively). There was no main effect of stimulation or interaction between stimulation and other factors for either tLRP or rLRP (both $p > 0.2$).

DISCUSSION

Both behavioral 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 RTs and P300 latencies than young adults (**Tables 1**, **4**), consistent with a disruption of motor processes with increasing age. Nevertheless, again, we found no effect of anodal tDCS over M1 on behavioral performance, ERP or LRP waveforms that would be consistent with enhancement of motor processes.

BAYESIAN ANALYSIS

Across both experiments, we found no evidence that anodal tDCS over M1 has a beneficial effect on either behavioral 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 favor of a beneficial effect

TABLE 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.

Group/Stimulation	tLRP		rLRP	
	Directional	Non-directional	Directional	Non-directional
Young adult				
Active	273.8 (10.3)	312.9 (7.4)	−107.7 (8.5)	−134.0 (9.1)
Sham	268.1 (10.0)	313.3 (6.9)	−109.2 (9.1)	−128.6 (7.8)
Old adult				
Dominant				
Active	335.1 (13.9)	376.9 (6.8)	−130.6 (12.5)	−166.2 (10.8)
Sham	348.1 (11.1)	371.9 (7.7)	−106.0 (7.6)	−159.0 (9.9)
Non-Dominant				
Active	335.4 (15.9)	387.4 (7.8)	−115.8 (14.3)	−165.1 (12.4)
Sham	341.7 (12.7)	389.1 (8.8)	−122.0 (8.8)	−163.6 (11.4)

Standard error of the mean is in parentheses.

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 et al. (2012) to create Bayes factors for each possible model. Bayesian analysis was performed using the BayesFactor package in R (Morey and Rouder, 2013), assuming the default setting for the fixed-effect prior ($r = 0.5$).

For Experiment 1, Bayesian analysis for RT 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 amplitudes, target and rLRP 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 four 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 RTs showed that the most likely model to predict the data had an effect of

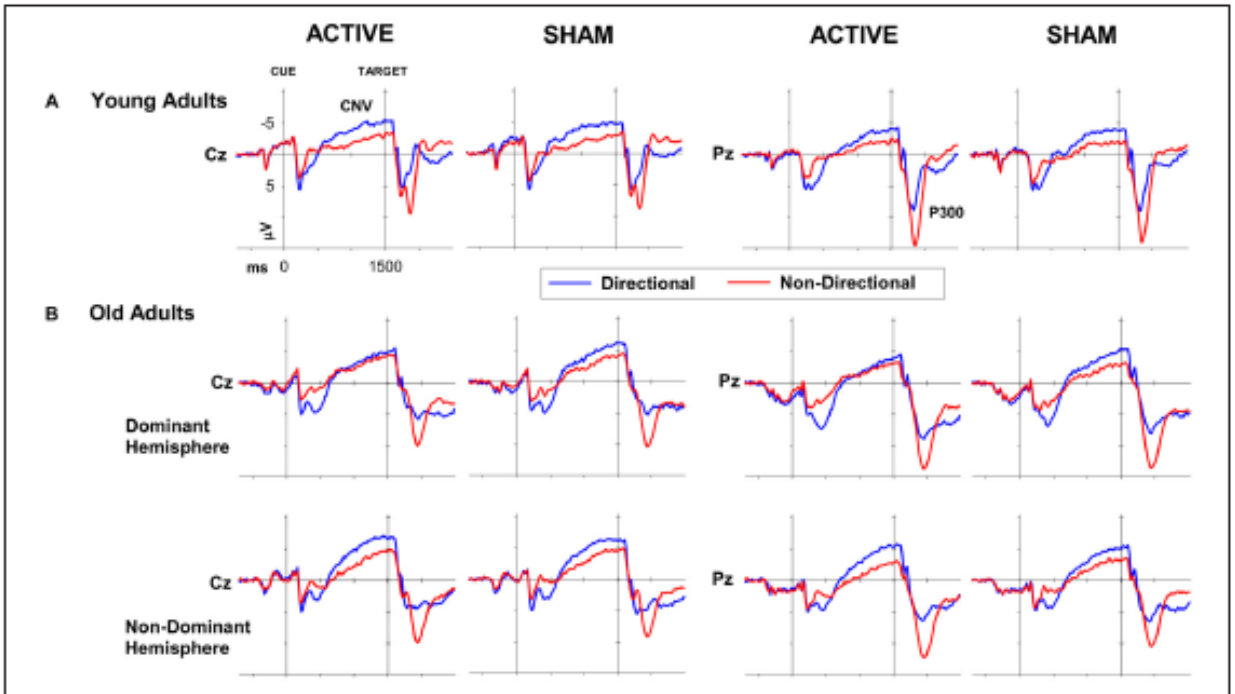

FIGURE 3 | ERP waveforms for directional (blue) and non-directional (red) conditions at Cz (left) and Pz (right) following active (i.e., anodal tDCS) and sham stimulation for (A) young and (B) old adults.

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 (ie, 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.

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 behavioral 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 RT 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 and Hallet, 2005). We therefore conclude that anodal tDCS over M1 does not affect motor ERPs in either healthy younger or older adults.

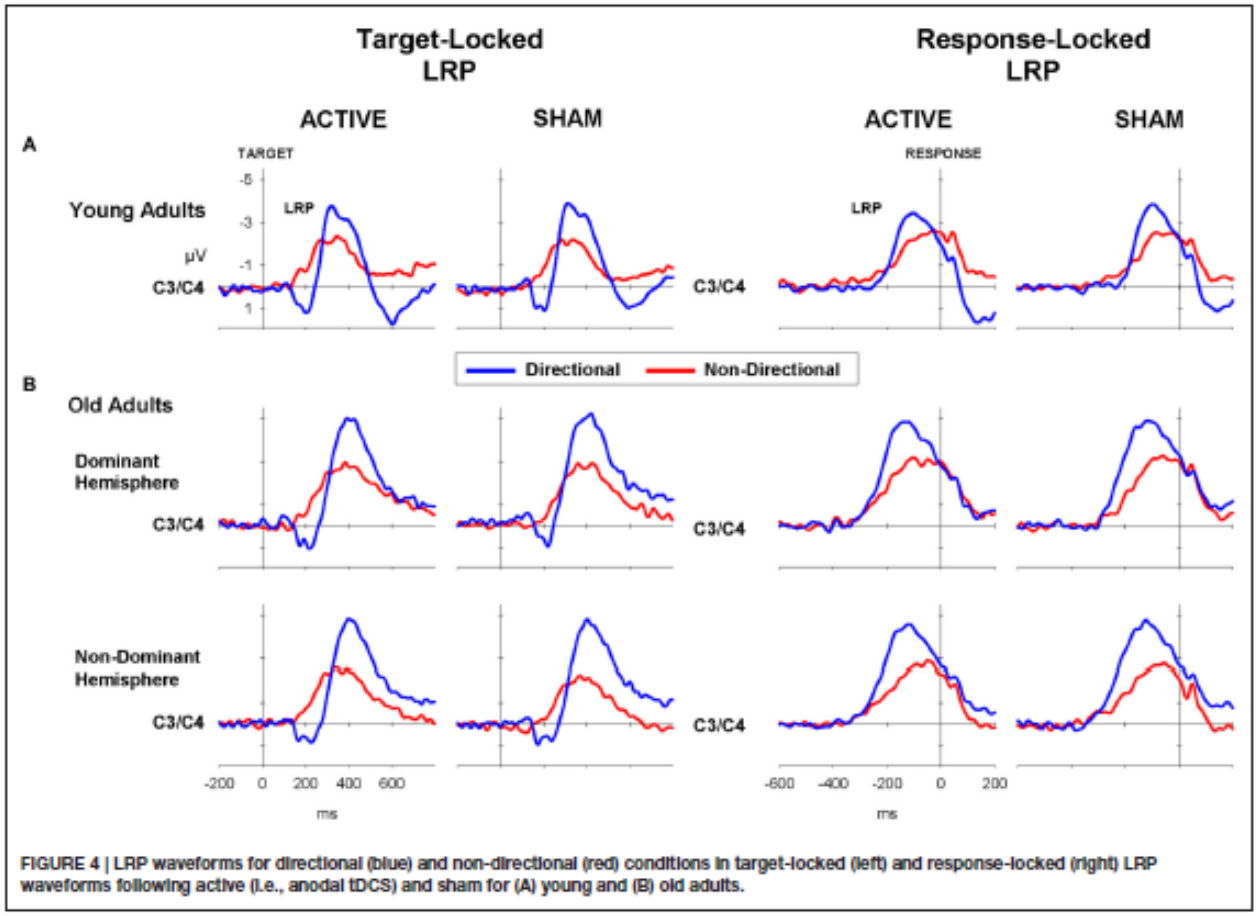

FIGURE 4 | LRP waveforms for directional (blue) and non-directional (red) conditions in target-locked (left) and response-locked (right) LRP waveforms following active (i.e., anodal tDCS) and sham for (A) young and (B) old adults.

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 30–40 min delay may have abolished any effect of anodal tDCS over M1 on behavior or ERPs. However, this is unlikely. Firstly, Conley et al. (2015) found no effect on behavioral 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 (20 min) should elicit sustained post-stimulation effects lasting a minimum of 1 h (Nitsche and Paulus, 2001). 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., 13–20 min). 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., 2013; Lapenta et al., 2014 respectively). Additionally in Lapenta et al. (2014), 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 and 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 1 mA 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 5 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 et al., 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.

AUTHOR CONTRIBUTIONS

Data was collected by ACC and JLM. ACC, WRF and FK were involved with the processing and analysis of data. All authors contributed to the interpretation of the data as well as the writing and finalization of the manuscript.

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Appendix II: Additional review information related to chapter 4

Publication details

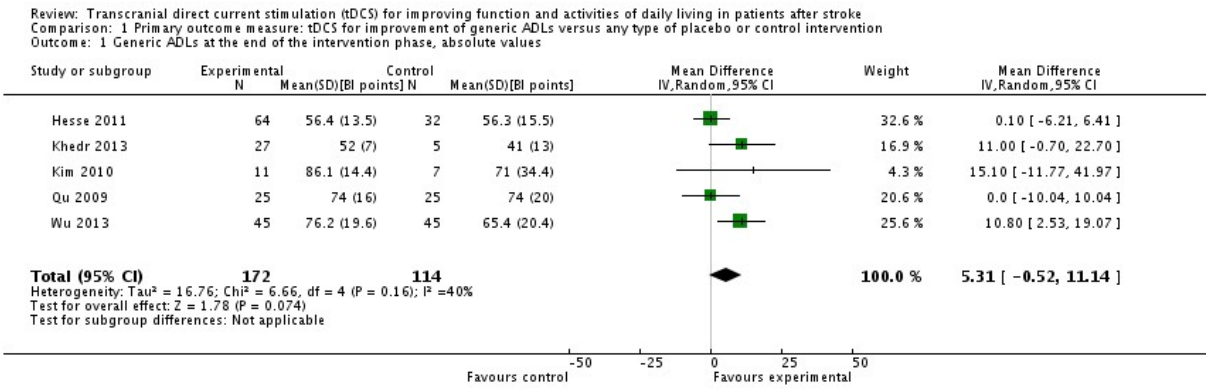
Marquez J, and Parsons M. (2017). Chapter 26: Electrical and magnetic brain stimulation to enhance recovery post stroke. In: Hankey G. and Saver J. (eds) *Stroke Treatment and Prevention: An Evidenced-Based Approach* (2nd edition). Cambridge University Press. In Press.

The purpose of this book chapter was to collate all existing evidence of all forms of non-invasive brain techniques in stroke patients. This excerpt from the chapter supplements the information provided in chapter 4 of this thesis. It provides information relating to additional outcomes that have not been included in the systematic review conducted as part of this thesis.

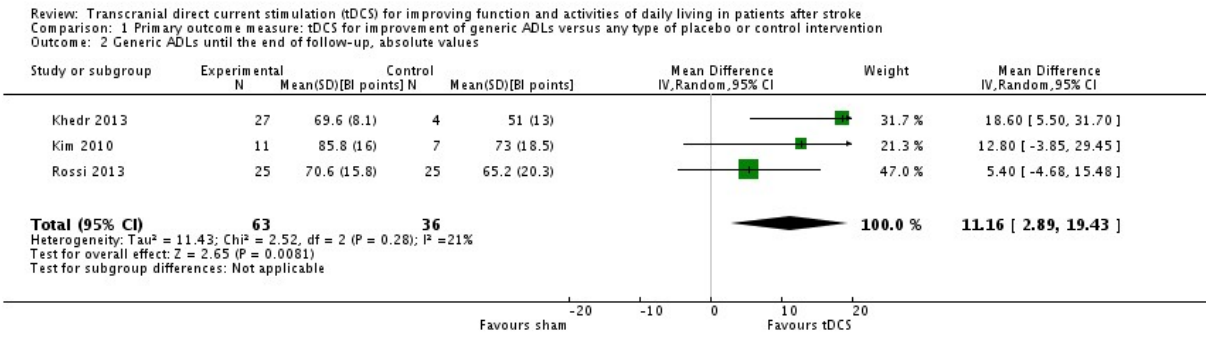
Effects of tDCS according to outcome

Activities of Daily Living (ADLs)

A Cochrane review involving a total of 286 stroke patients found no significant benefit of tDCS in ADL outcomes following tDCS with a mean improvement of 5.31 on the Barthel Index (95% CI = -0.52 -11.14) (Elsner, Kugler et al. 2013).



Three of these studies, with 99 participants in total, assessed the effects of tDCS on ADL at 3 month follow-up and demonstrated there was evidence of an effect (mean improvement in Barthel Index = 11.13, 95% CI = 2.89-19.37). It should be noted that the confidence interval was wide and this benefit was not sustained when only high quality studies were included in the analysis (figure 3) (Elsner, Kugler et al. 2013)



Upper limb function

A meta-analysis of 7 studies with 302 stroke patients found evidence to support the use of tDCS to improve UL function as measured by the upper extremity Fugyl Meyer Score (mean difference= 3.45, 95% CI = 1.24 – 5.67) however this benefit was not maintained at 3 month follow-up when the data from two studies with 68 participants was pooled (MD = 9.23, 95% CI = -13.47 – 31.96) and the effect was not sustained when studies at risk of bias were excluded (figure 4) (Elsner, Kugler et al. 2013)

Lower limb function

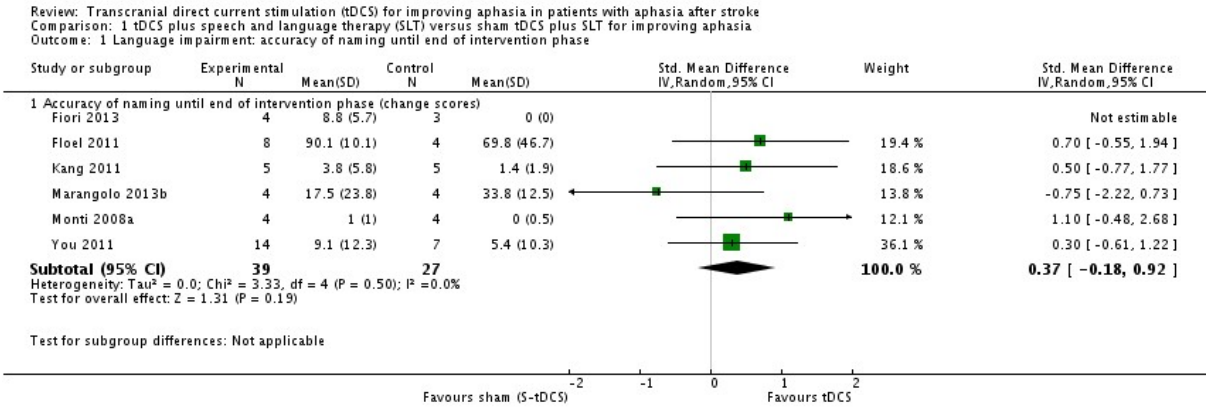
Studies investigating the effects of tDCS on lower limb function are few and no systematic reviews have been conducted mainly because the somatotopy of the legs is located deep in the central sulcus and technically difficult to isolate. However there is consistent evidence that this area can be reached and is a feasible target for tDCS stimulation (Chang, Kim et al. 2015). Several RCTs support the use of anodal tDCS to improve postural stability (Sohn, Jee et al. 2013, Koyama, Tanaka et al. 2014), lower limb muscle strength (Tanaka, Hanakawa et al. 2009, Tanaka, Takeda et al. 2011) and gait function (Tahtis, Kaski et al. 2014). Conversely studies have failed to show beneficial effects on gait parameters, balance control (Chang, Kim et al. 2015), and lower limb biomechanics (Tahtis, Kaski et al. 2014). These isolated reports from small studies require further validation.

Aphasia

Many published trials report positive findings in favour of tDCS in the management of post-stroke aphasia, however the only systematic review of this topic found no evidence to support the effectiveness of tDCS. A meta-analysis of data from six RCTs, including 66 subjects, found no evidence of benefit regarding markers of language function such as the relative

change in naming accuracy as a result of tDCS compared to sham (SMD = 0.37, 95% CI -0.18 to 0.92, P = 0.19) (figure 5) (Elsner, Kugler et al. 2015).

This review could not locate any appropriate studies which used a standardised measure of functional communication, that is, an objective measure of real-life communication. Most studies use picture naming as a surrogate measure of aphasia. Similarly the authors were unable to find studies examining the effects of tDCS on cognition in stroke patients with aphasia.



Cognition

Studies in healthy adults have shown tDCS to be capable of improving attention, memory and executive functions (Smith and Clithero 2009). In stroke, research is limited to two studies with conflicting findings. One RCT reported significant improvement in auditory memory in patients with post stroke cognitive impairment, but no effect on visual memory or attention (Yun, Chun et al. 2015), yet another reported that anodal tDCS improved attention (Kang, Baek et al. 2009). Due to the prevalence of cognitive impairment post stroke and its association with poor rehabilitation outcomes, further research in this field is warranted.

Mood

tDCS has induced mood improvements in several neuropsychiatric conditions but has not been assessed in controlled trials for post stroke depression. A single case study reported considerable improvement in mood following 10 sessions of 2mA tDCS applied daily for 30mins per day over the left dorsolateral prefrontal cortex in a patient who was refractory to antidepressants (Bueno, Brunoni et al. 2011). Although this case report is incapable of providing efficacy data, it supports the merit of further investigations.

Dysphagia

Several small studies have examined the effects of tDCS as a means to rehabilitate post stroke swallowing problems. Data from three studies with a total of 50 subjects was pooled to demonstrate a non-significant effect of tDCS on dysphagia (SMD = 0.52 , 95% CI = -0.13 – 1.16)(Pisegna, Kaneoka et al. 2015).

Summary of tDCS evidence

Currently, there is inconsistent and imprecise evidence available on the effectiveness of tDCS for improving ADL performance, function and language after stroke. We are currently unable to decipher if one form of tDCS is superior to another and no comment can be made on the effects of tDCS on mood and cognition due to the lack of evidence in these areas. There are currently numerous ongoing registered large scale randomised controlled trials and this further evidence is necessary to substantiate whether tDCS has merit as a therapeutic adjunct in stroke rehabilitation.

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