Investigating transcranial direct current stimulation and its therapeutic potential

Katherine Dyke, BSc

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

April 2017

Abstract

Transcranial direct current stimulation (tDCS) is a popular non-invasive brain stimulation technique, which has the potential to modulate cortical excitability. The effects of tDCS are known to outlast the stimulation period, and in some cases, repeated applications have been found to produce long lasting clinically relevant effects. The primary aim of this thesis was to explore the reliability and therapeutic potential of this technique.

In Chapters 3 and 4 transcranial magnetic stimulation (TMS) was used to measure tDCS effects. These experiments revealed substantial variability regarding the way in which healthy adults responded to stimulation. Notably, there were differences between participants regarding the direction and magnitude of change in cortical excitability. Furthermore, even when group level effects were found reliably, there was substantial intra-subject variability across repeated testing sessions.

Subsequent experiments in Chapters 5 and 6, explored the biological and behavioural effects of tDCS in individuals with Gille de la Tourette's syndrome (GTS). GTS is a neurodevelopmental disorder characterised by motor and phonic tics which have been linked to hyper excitability within motor-cortical regions. Therefore, these experiments aimed to reduce cortical excitability of targeted regions in the hope that this would impact on tics. Disappointingly, no such effects were found immediately after a single session of tDCS (Chapter 5). Consequently, it was hypothesised that repeated applications may be necessary for significant reductions in tics to occur. This was investigated in Chapter 6 using an in-depth case study. The results were encouraging, in particular there was a substantial drop in tics following 10 days of tDCS at 1.5mA intensity. The stimulation was well tolerated and the treatment regimens were closely adhered to, despite tDCS being delivered in the participants own home with remote supervision. A weaker stimulation intensity was not as effective. The findings of Chapters 3-6 highlight that the optimal stimulation parameters may vary from person to person, and that exploration of individual data is critical in therapeutic contexts. The results also suggest that tDCS may be helpful as a treatment for GTS and furthermore highlight the feasibility of home use stimulation.

Acknowledgements

I am very grateful to my supervisors Stephen Jackson and Georgina Jackson for their enthusiastic support, encouragement and guidance throughout my PhD. I have learned so much and am indebted to you both for giving me this opportunity. I am also very grateful to Jane Fowlie for all her help with recruitment, to Elena Nixon for her advice on tic counting and to the participants themselves who made this research possible.

I would like to thank my office mates Miguel Espirito Santo, Christina Ralph-Nearman and Beverly Brown for making it such a lovely place to work and for our many interesting conversations. I am also very grateful to my friends in the department for their support and advice, in particular Sophia Pépés and Hilmar Sigurdsson for help with all things scanner related, Amelia Draper for introducing me to Nottingham, and Soyoung Kim for discussing ideas with me and helping with my never ending Matlab issues.

I would like to say a huge thankyou to my wonderful parents for supporting me in all I do and for their tireless efforts as thesis proof readers. Finally, a special thankyou to James who has been there for me throughout, with an endless supply of encouragement, patience and understanding.

Table of contents

List of figures

List of tables

Chapter 1: General Introduction

Key words: *transcranial direct current stimulation (tDCS), electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS), long term potentiation (LTP), long term depression (LTD), supplementary motor area (SMA), magnetic resonance spectroscopy (MRS), Gilles de la Tourette's syndrome (GTS), cortical excitability.*

In this thesis, a number of studies are discussed in which the effects of transcranial direct current stimulation (tDCS) were explored in neurologically typical individuals and individuals with Gille de la Tourette's syndrome (GTS). This thesis also explores the relationship between different methods used to measure neurotransmitter function including Gamma-Aminobutyric Acid (GABA) and glutamate.

1.1 A brief history of therapeutic brain stimulation

The use of electrical stimulation to influence and study the brain is not new. In fact, there are historical references dating as far back as 131-401 AD which document the uses of fish with electrical properties to alleviate headaches (Priori, 2003). Although these early examples exist, prior to the 17th century little was understood about the body's natural relationship with electricity. An individual who is often credited with greatly enhancing our understanding of this is Luigi Galvani. Galvani revolutionized scientific thinking by demonstrating that lifeless bodies could be made to move using electrical stimulation (Piccolino, 1998). In doing so he was the first person to identify the electrical nature of muscle contraction and nerve conduction (Piccolino, 1998). Galvani's nephew Giovanni Aldini developed this by conducting experiments in which electrical currents were applied to the heads of executed prisoners to induce muscular contractions (Piccolino, 1998). Aldini also applied electrical currents to the heads of living individuals as a treatment for complaints such as 'melancholia' (Priori, 2003). These experiments are arguably the origin of modern therapies using electrical stimulation to treat neuropsychiatric disorders.

Throughout the 17th century understanding and experimentation with electrical stimulation grew, and with it, application of electrical currents to treat mental health conditions increased (Elliott, 2014). During this time perhaps one of the most important developments was the use of electro convulsive therapy (ECT) to treat patients under psychiatric care. Initially ECT was used to treat a range of conditions, however, with improved psychiatric care and the introduction of psychotropic drugs in 1950s and 1960s it's use began to decline (Fink, 2001). Today ECT is still used to successfully alleviate symptoms in conditions such as severe treatment resistant depression (Fink, 2001), however, it is not without side effects or complications. Even with the use of general anaesthesia and more efficient use of electrical wave forms the treatments can result in memory impairment and other cognitive deficits (Sackeim et al., 2007). The nature of the technique makes it unpopular with many and has heavily restricted its therapeutic use. Nevertheless, ECT provides a powerful demonstration of the potential of electrical stimulation in treating the symptoms of psychiatric illness.

A number of less invasive brain stimulation techniques have since emerged which use electrical currents to influence the brain. These include transcranial magnetic stimulation (TMS), and transcranial direct current stimulation (tDCS).

1.2 Modern day methods: Transcranial Direct Current Stimulation (tDCS)

tDCS is a non-invasive brain stimulation technique which has been shown to effectively modulate cortical excitability in humans. The application of tDCS involves running a low density electrical current between two electrodes – at least one of which is placed on the scalp (see Figure 1.1). The intensity of the currents used are typically 1-2mA, which is substantially below those found to be safe in animals (Mccreery et al. (1990) Liebetanz et al. (2009)). The electrodes used are typically made from conductive rubber and are connected to a battery powered device capable of delivering continuous current. When the device is

switched on direct current flows from one electrode (Anode) to the other (Cathode); some of this current will be shunted by the scalp and cerebral spinal fluid (CSF), however, a proportion will penetrate through to reach the cortical surface of the brain (Moreno-Duarte et al., 2014).

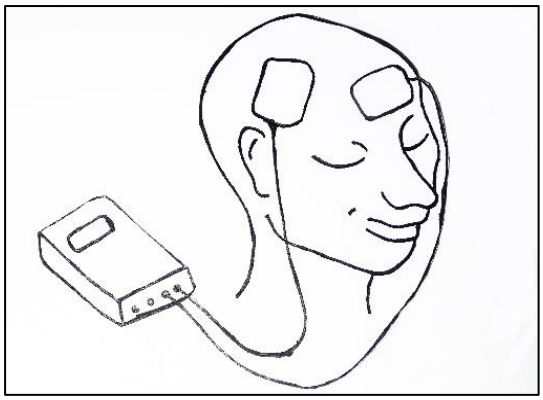


Figure 1.1. Illustration *of tDCS application.*

Within the tDCS literature the two electrodes are often referred to as 'active' and 'reference' rather than anode and cathode. The active electrode is typically placed on the scalp above the area of interest, whereas the reference electrode can be placed in a number of different locations (including on the contralateral forehead, opposite hemisphere or at an extra-cephalic location such as the shoulder). It is important to note that the term 'reference' is somewhat misleading, as the current will affect tissue at this location in addition to at the active electrode and areas between. It should also be noted that when researchers refer to anodal or cathodal stimulation they are typically referring to the electrode placed above the area of interest i.e. the 'active' electrode.

tDCS is a particularly interesting technique as it has the ability to both increase and decrease cortical excitability depending on the electrode polarity. A number of studies have demonstrated that cortical excitability typically decreases in the areas under the cathode and increases in areas beneath the anode (Jacobson et al., 2012). These induced changes in excitability are typically short lived, lasting up to 120 minutes following stimulation cessation (Batsikadze et al., 2013). However, more long term effects may be achievable with repeated

applications through activation of long term potentiation (LTP) and long term depression (LTD) type changes in plasticity. The potential to induce longer lasting changes in cortical excitability with minimal adverse effects has led to a dramatic increase in interest of the technique's therapeutic potential, particularly in recent years (Brunoni et al., 2012).

tDCS has a number of additional advantages over other forms of stimulation which make it an appealing tool for therapeutic use. The machine itself is small, portable and relatively easy to use which makes home use possible. This is particularly advantageous when compared with other non-invasive brain stimulation techniques such as TMS, as these often involve repeated visits to a clinic for treatment which may be inconvenient and/or distressing.

When recognised guidelines are followed, tDCS is considered to be a safe and low risk technique (see Fregni et al. (2014)) with considerably less adverse effects reported than those occurring as a result of other forms of stimulation or psychoactive medication. An extensive review carried out on 567 tDCS sessions in healthy controls and individuals with symptoms of migraine, tinnitus or stroke, found the most common side effect was tingling under the electrode; moderate fatigue and an itching sensation were also reported by some during stimulation. Reported side effects occurring after stimulation included headache which was reported by approximately 11.8%, nausea which was reported in less than 2% and insomnia which was reported by less than 1% (Poreisz et al, 2007). It is possible that some of these effects may be circumstantial and unrelated to tDCS itself. A more recent review revealed that although side effects such as tingling and itching sensations below the electrodes were slightly higher in active stimulation conditions, this was not significantly different to sensations reported during sham. In addition to this, reports of headache, burning sensation or discomfort were similar for the two conditions (Brunoni, Amadera, et al., 2011).

A number of studies have highlighted the potential effectiveness of tDCS in treating a range of conditions including depression (Boggio et al., 2008;

Brunoni, Ferrucci, et al., 2011; Fregni, Boggio, et al., 2006), and schizophrenia (Brunelin, 2012). tDCS has also been found to be helpful in reducing chronic pain (Antal et al., 2010), pain caused by fibromyalgia (Fregni, Gimenes, et al., 2006) and reducing fatigue caused by multiple sclerosis (Tecchio et al., 2014). Small scale case studies also suggest that tDCS may be helpful in reducing seizure in individuals with epilepsy (Yook et al., 2011) and in reducing tics in Tourette's syndrome (Carvalho et al., 2015; Mrakic-Sposta et al., 2008).

Although the reports of therapeutic application are generally positive, they are limited in number and, therefore, few extensive reviews of their effectiveness have been carried out. Studies into the effects of tDCS within healthy neurologically typical individuals reveal a somewhat mixed picture. Although many studies report that tDCS is able to influence cortical excitability and behavioural outcomes, this is not always found. In a recent meta-analysis Horvath et al. (2015) made the claim that tDCS has little or no reliable neurophysiological effects beyond changes in specific TMS measures (Horvath et al., 2015). This claim and the methodology used in reaching it has been met with a strong backlash and criticism (for example see Antal et al. (2015)). However, it does serve to highlight that tDCS effects are not always simple and predictable.

In the excitement surrounding tDCS and its clinical potential, important factors have often been ignored and there are a number of unexplored issues which require attention in order to build stronger conclusions about its effectiveness. In particular, there is uncertainty about the exact mechanisms underlying the effects (although see section 2.3). There is also little understanding of the effectiveness of stimulation across and within participants, and regarding issues relating to parameter selection and the setup of stimulation protocols.

1.3 Gilles de la Tourette Syndrome

Gilles de la Tourette syndrome (GTS) is a childhood onset disorder, characterized by the presence of motor and phonic tics which are present for a minimum of 1 year (Leckman, 2002). Tics are involuntary, brief, stereotyped behaviours of a limited duration and can involve movement (motor tic) or the production of sound (phonic tic). The disorder is thought to affect approximately 1% of children and has been reported almost globally (Robertson, 2008). GTS often follows a time course whereby tics are typically at their worst aged 10- 12 years; for three quarters of children these will then diminish by early adulthood with over a third becoming seemingly tic free adults (Bloch & Leckman, 2009). Many individuals with GTS will also have a co-occurring diagnosis such as Attention Deficit Hyperactivity Disorder (ADHD) or Obsessive Compulsive Disorder (OCD). Estimates suggest that approximately 86.5% of children with GTS have also met the criteria for one or more additional diagnoses, with ADHD being the most common (Bitsko et al., 2014). This can make understanding the aetiology of GTS particularly complex, as instances of 'pure GTS' are less common.

The most common forms of treatment for GTS are behavioural therapies such as 'habit reversal training' and pharmacological interventions including antipsychotics. Although these work for some, they are not always ideal. In particular, the side effects associated with antipsychotics make them unappealing for many. For example, a study of 51 participants with GTS who were prescribed the antipsychotic Haloperidol found that 41% discontinued its use due to intolerable side effects (Silva et al., 1996). There is a clear need for the development of additional treatments and it is possible that non-invasive brain stimulation techniques may play an important role.

The aetiology and neurobiology of Tourette syndrome is not yet fully understood, however, genetic factors are implicated and differences in the

structure and function of different brain regions are also likely to be involved. Although the evidence is far from conclusive, a number of structures and circuits have been identified including the basal ganglia (Worbe et al., 2012), corpus callosum and caudate nucleus (see Albin & Mink (2006) for review). Thinning in motor cortical areas has also been reported (Worbe et al., 2010), and dysfunction in the cortico-striato-thalamo-cortical circuits suggested (Mink, 2001).

1.4 Non-invasive brain stimulation and Tourette Syndrome

In order for tDCS to be a developed as a potential treatment for GTS, it is important that an appropriate stimulation site can be identified. As previously discussed a number of different regions have been implicated, however, some of these such as the basal ganglia are deep within the brain. Direct stimulation of these deep sub-cortical structures using a standard 1-2mA intensity is not possible as the current density is strongest at the cortical surface and rapidly decreases with distance from the electrodes. However, a number of studies have suggested that it may be possible to reach these structures using stimulation which targets cortical areas connected to structures of interest (Kadosh, 2015). Fortunately, one cortical area seems to be particularly implicated in the production of tics. This location known as the supplementary motor area (SMA), has been found to have altered metabolic activity in individuals with Tourette's syndrome (Eidelberg et al., 1997). In addition to this, levels of an inhibitory neurotransmitter known as Gamma-Aminobutyric Acid (GABA) within the SMA have been found to correlate with tic scores (Draper et al., 2014). The connectivity of this area also makes it an appealing target for stimulation, as it has extensive connections to areas relating to motor control and cognitive processing (Picard & Strick, 2001).

To date, it seems that non-invasive stimulation may be useful in reducing tics when applied to the SMA. Application of repetitive TMS (rTMS) using pulse configurations known to reduce cortical excitability has been found to reduce tic

severity scores over a 12 week period following 10 sessions of stimulation (Kwon et al., 2011). A similar finding was published by Le et al. (2013) who reported an improvement in tic symptoms following 20 days of rTMS which lasted up to 6 months in some participants; and by Mantovani et al. (2007) who reported significant reductions in self-reported measures of tics following 10 days of stimulation. The application of cathodal tDCS to the SMA also shows therapeutic promise, Carvalho et al. (2015) found that tic severity and frequency reduced significantly following 10 sessions of cathodal tDCS. These effects were still present at a 6 month follow up and changes in the resting state network of the brain were also identified. Finally a study by Mrakic-Sposta et al. (2008), reported a significant reduction in tics following 5 days of cathodal tDCS that was greater than that which occurred following a sham condition.

These results are all very promising, but, with the exception of Mrakic-Sposta et al. (2008) none were sham controlled, which makes possible placebo effects difficult to distinguish from those due to stimulation. Furthermore, the studies have often focused on a limited pool of outcome measures (for example self-report alone) therefore making it impossible to draw strong conclusions about how the effects may be occurring.

1.5 Research aims and summary

Through a series of experiments my aim was to better understand the effects of tDCS in order to explore its potential as a therapeutic intervention for reducing tics in individuals with Tourette's syndrome. The initial two studies (Chapters 3 and 4) were designed to investigate the neural effects of tDCS in neurologically typical individuals to establish and identify patterns of response relating to different parameter selections. In the third study (chapter 5) I explored the neural and behavioural changes related to cathodal stimulation of the SMA in individuals with Tourette's syndrome. This was expanded into experiment four (Chapter 6) in which the effects of prolonged application were investigated in a single case. Finally, an experiment investigating the use of

magnetic resonance spectroscopy was conducted (Chapter 7) to explore what neurochemical markers are being measured by the technique and how this relates to previous findings about the neurobiology of tDCS effects.

In this thesis the following questions are addressed:

1. What is the time course of the effects of a single session of tDCS? Does this differ depending on the intensity and polarity of stimulation used?

2. How stable are the effects of tDCS within and between individuals?

3. Does a single session of tDCS applied to the SMA influence cortical excitability or tics in individuals with Tourette's syndrome?

4. Does 10 days of tDCS applied to the SMA have any short term or lasting effects on tics in an individual with Tourette's syndrome.

5. What do past studies using MRS tell us about the biological underpinnings of tDCS? How do we know what MRS is measuring?

Chapter 2: Non-invasive stimulation and investigation of the human motor cortex

Key words: *transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS),* direct waves (D-wave), indirect waves (I-wave), corticospinal neurones (CSNs), pyramidal tract neurones (PTNs), *motor evoked potential (MEP), motor threshold (MT), input output curve (IO curve) short interval intracortical inhibition (SICI), long interval intracortical inhibition (LICI), intracortical facilitation (ICF), magnetic resonance spectroscopy (MRS).*

In recent years a number of different techniques have been used to explore the physiological effects of tDCS. One of the most popular is transcranial magnetic stimulation (TMS) which can be used to make inferences about cortical excitability within the motor cortex. This technique is used throughout the experiments discussed in this thesis and is therefore covered in depth in this section. Other methods including animal work and pharmacological manipulations have also been important; as has work conducted using Magnetic Resonance Spectroscopy (MRS).

2.1 Transcranial Magnetic Stimulation

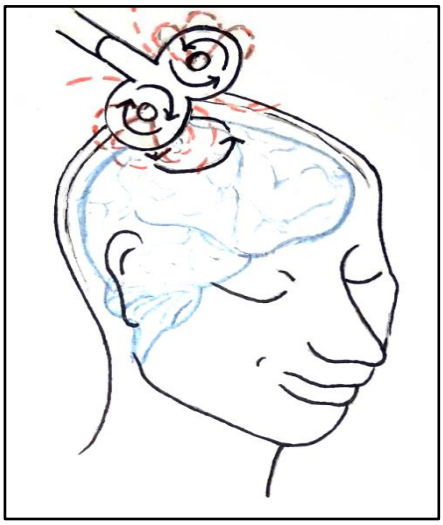


Figure 2.1. *Illustration of TMS application. Black arrows indicate electrical current flow, red dashed line indicates magnetic field.*

Transcranial Magnetic Stimulation (TMS) is a safe, non-invasive stimulation technique which was first demonstrated in 1985 (Barker et al., 1985). This was five years after Merton and Morton (1980) demonstrated that areas of the human motor cortex could be stimulated through the intact scalp using a brief high voltage electric shock. The development of TMS as a technique revolutionised the field of non-invasive brain stimulation as, in comparison to its predecessors, it causes minimal discomfort. This is because it is not necessary to

apply high currents directly to the scalp (which can cause strong and unpleasant sensations at high intensities); instead TMS works via the principles of electro-magnetic induction.

During stimulation a brief but strong electrical current is delivered from an electrical capacitor to the TMS coil. The TMS coil contains windings of copper wire which conduct the current. Different TMS machines are capable of delivering this current using two main pulse configurations, known as mono and biphasic. A monophasic pulse involves the production of a strong initial current which is then balanced by a dampened return current. Whereas a biphasic pulse is characterised by an initial current rise followed by a reverse in current and then again by a subsequent increase, meaning the direction of the current is reversed twice (Rossini et al., 2015). Both of these pulses are capable of inducing a fluctuating magnetic field which is perpendicular to the coil. The resultant fluctuating magnetic fields (which can be at strengths of 1-2 Tesla (Rossini et al., 2015) are able to pass through the scalp and skull relatively unaltered to induce an electrical current in the brain. This induced current is able to interact with the neuronal tissue and influence electrical signalling of neuronal populations; in particular the induced current can depolarize neurones or their axons (Hallett, 2000).

The effects of TMS are dictated by a number of important factors, including properties relating to the hardware such as the coil type (L. G. Cohen et al., 1990), pulse configuration (Brasil-Neto et al., 1992; Kammer et al., 2001) and possibly even differences between stimulator models (Kammer et al., 2001). These factors can easily be controlled within studies, although may cause some issue when comparing across them.

The shape of the TMS coil influences the magnetic field generated, which in turn influences the strength and localization of the induced electrical current below. The two most prototypical coils are single round coils and figure-of-eight/'butterfly' coils (See Figure 2.2). Single round coils create the strongest induced electrical field at the circumference, whereas the peak field strength for

figure of eight coils is created at the intersection between the two round components (L. G. Cohen et al., 1990); this makes figure-of-eight coils more focal than round coils. In addition to coil shape, the size of the coil is also important, with smaller coils resulting in more spatially restricted electrical fields (L. G. Cohen et al., 1990).

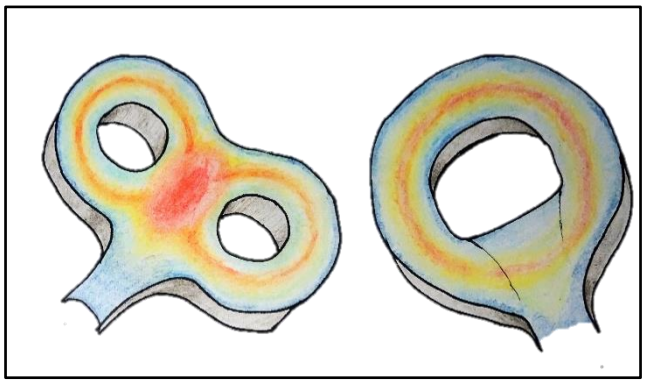


Figure 2.2. *Illustration of figure of eight coil (left) and single round coil (right) with peak magnetic field shown as heat map.*

As previously noted the nature of the TMS pulse (mono or biphasic) can also influence the effects of the stimulation (Brasil-Neto et al., 1992; Kammer et al., 2001). A TMS pulse results in a minimum of two phases (dependant on the pulse configuration), which are positive when the current in the coil is increasing and negative when the current is decreasing (Brasil-Neto et al., 1992). The differences in the wave forms appears to result in differences in the optimal coil orientation for stimulation of the motor cortex (Brasil-Neto et al., 1992; Kammer et al., 2001), and differences in motor threshold (Kammer et al., 2001). It is, therefore, important to maintain these as constants within any study and be mindful when comparing between.

2.1.1 TMS and the motor cortex

TMS can be used to study a range of cortical areas and functions; however, by far the most popular area of study is the motor cortex. This is because of the relative ease by which reliable indicators of TMS effects can be obtained by measuring motor evoked potentials (MEPs). MEPs are cortically driven muscle contractions (induced by stimulating the motor cortex) that can be

measured using electromyography (EMG) from electrodes attached to a target muscle. MEPs provide a non-invasive quantifiable measure of induced activity, without the need for specially designed cognitive tasks or invasive procedures. Although this approach can be used to gain insights about various facets of motor cortex excitability (discussed in depth in 1.2.3 and 1.2.4), it does not allow for more detailed and direct information about neuronal responses. Therefore, in order to discuss the biological underpinning of TMS it is necessary to first discuss research using single cell recording. In this method small electrodes are placed directly into epidural or subdural space in the spinal cord, from which electrical impulses traveling down the fibres can be recorded. This procedure is invasive and much less accessible than MEP recording, although it does offer some critical insights into the effects of TMS delivered to the motor cortex.

It should be noted that the relationship between single cell recordings and MEPs are not straight forward. This is because even a focal TMS pulse can excite many corticospinal neurones (CSNs) within the stimulated area, and of those excited only a small proportion may be destined to influence the motor neurone pool of the muscle from which the MEP response is being recorded via EMG (Di Lazzaro et al., 2008). Nevertheless, MEPs are a well-established and popular research tool for exploring cortical excitability which have been greatly informed by research carried out with single cell recordings.

2.1.2 Biological underpinnings

Early studies conducted in the 1930s and 1950s using animals such as cats and non-human primates, revealed that when a single electrical stimulus was applied directly to the motor cortex a number of high frequency waves were detectable via electrodes placed in the medullary pyramid or the dorsolateral surface of the cervical spinal cord (Adrian & Moruzzi, 1939; Patton & Amassian, 1954). Two types of waves were distinguished following a single stimulation which were termed direct waves (D-wave) and indirect waves (I-wave) (Patton & Amassian, 1954). The D-wave appeared to stem directly from excitation of the fast conducting corticospinal neurones (CSN), whereas the I-waves were found to

be largely dependent on the integrity of the cortical grey matter and were thought to originate indirectly via trans-synaptic activation of the CSN (Patton & Amassian, 1954). These early findings in animals have proved to be robust, and have since been found in human participants and in response to transcranial stimulations.

Note:

The terms Pyramidal Tract Neurone and Cortico Spinal Neurones are both used by researchers to describe biological underpinnings.

The terms are somewhat interchangeable as the majority of PTNs project onto the corticospinal tract (Di Lazzaro et al., 2008).

Some of the first studies investigating the effects of modern electrical stimulation in humans were conducted with patients under anaesthetic during surgery. These studies revealed that a strong electrical currents (up to 750v) delivered by anodal transcranial electrical stimulation (TES), or induced via monophasic transcranial magnetic stimulation (TMS), evoked a series of transcending waves down the corticospinal tract which resembled D and I-waves. Although both stimulation types resulted in recordable volleys, the properties of the waves differed between them. In particular Berardelli et al. (1990) found that the initial waves generated by TMS were of slightly longer latency and smaller amplitude than those generated by TES. Similar effects have been found from epidural recordings in non-anaesthetised humans (Di Lazzaro, Oliviero, et al., 1998; Nakamura et al., 1996), however, the findings have not always been consistent. For example, Burke et al. (1993) reported that the threshold for D-waves produced using TMS was lower than for I-waves. This has not been found in subsequent studies and may be due to confounding effects of anaesthesia (Di Lazzaro, Oliviero, et al., 1998). Overall these studies provide strong evidence that various forms of transcranial electrical stimulation can successfully influence the motor cortex, leading to distinctive patterns of activity that can be measured further down the spinal cord. The different patterns of the descending volleys suggest differences in how the underlying tissue responds to the electrical field. However, the complex nature of these interactions means that even within a single stimulation type such as TMS, there are variations in the patterns of

response. For TMS these effects seem to be particularly dependent on factors such as intensity of stimulation and direction of current flow.

Further research into the effects of TMS gave rise to the characterization of different I-waves evoked from a single monophasic pulse, resulting in the categorization of early and late I-waves. These appear to be influenced by the intensity of the TMS pulse, and suggest that the threshold for activation and consequently the mechanisms of action differ. Low TMS intensities using a posterior to anterior (P-A) current flow result in a single descending volley termed the I1-wave, which is thought to be produced by indirect activation of corticospinal cells (via activation of monosynaptic cortical-cortical connections projecting onto CSNs). At higher TMS intensities, later volleys known as late I-waves are measurable; it has been suggested that these arise from complex mechanisms which result in repetitive discharge to pyramidal tract neurones (PTNs) (Di Lazzaro, Profice, et al., 2012). Manipulations of the inhibitory neurotransmitter GABA using the benzodiazepine Lorazepam found that the drug did not affect threshold levels of stimulation, or the I1-wave, but caused pronounced suppression of later I-waves (Di Lazzaro et al., 2000). This has been interpreted as further evidence to suggest that these late I-waves are generated by networks which are presynaptic to CSN (Di Lazzaro et al., 2008). If the TMS intensity is increased even further, this can result in the production of D-waves. These do not appear to be altered by experimentally manipulated changes in cortical excitability and are therefore thought to be generated by the direct activation of corticospinal axons some way away from the cell body (Di Lazzaro, Profice, et al., 2012; Di Lazzaro et al., 2008).

Another insight gleaned from experiments using direct recording is that the effects of stimulation are influenced by the direction in which the current is traveling. Nakamura et al. (1996) found that orientating the TMS coil in a way which induced posterior to anterior (P-A) current flow using a monophasic pulse resulted in preferential production of I-waves. Conversely orientating the coil to induce lateral-medial (L-M) current flow resulted in preferential production of D-

waves. A similar effect was found by Kaneko et al. (1996), who suggested that P-A stimulation preferentially stimulates the corticospinal tract trans-synaptically resulting in I-waves, whereas L-M current flow preferentially influences the corticospinal tract via non-synaptic activity which results in D-waves. Similar effects have also been found using lower TMS intensities (Di Lazzaro, Oliviero, et al., 1998).

The findings relating to coil orientation suggest that different neuronal populations have different selective sensitivities to the direction of current flow. Variation in cortical folding and orientation of neurones is likely to be one of a number of factors which causes individual variability reported in a number of studies (Berardelli et al., 1990; Burke et al., 1993; Di Lazzaro et al., 2001; Houlden, Schwartz, Tator, Ashby, & MacKay, 1999).

The complex nature of the interactions between the induced current and the underlying cortical tissue make it incredibly difficult to pinpoint exact mechanisms of TMS, and there are still aspects which are not well understood. Nevertheless, single cell recordings have provided valuable insights and highlight the importance of careful consideration of factors such as TMS intensity and coil angle in experimental use.

Key points:

- TES and TMS can induce descending corticospinal volleys in subtly different ways.
- TMS intensity influences responses. Lower intensities produce I1 waves associated with indirect activation of CSNs via monosynaptic corticocortical connections. Higher intensities produce late I waves which are associated with repetitive discharge to CSNs via presynaptic networks.
- Coil orientation can critically influence the effect of the TMS pulse. In particular, P-A current flow preferentially produces I-waves. Whereas L-M currents preferentially produce D-waves.
- The effects of a single TMS pulse on the human motor cortex are highly complex and relate to properties of the induced current and the structural arrangement of neural circuits.

2.1.3 Single pulse measures

Single pulse TMS involves the application of a TMS pulse in isolation. Although a number of pulses may be applied, the temporal gap between them is typically thought to be too long to induce any interactive effects; therefore, the pulses can be seen as independent from each other. There are various methods in which single pulse TMS can be used to assess cortical excitability. Of particular interest are motor threshold and input-output curves, both of which involve measuring MEPs.

MEPs can vary in both size and shape, even in response to identical stimulation (Ellaway et al., 1998). As a result, the measurement of IO curves, motor threshold (MT) and other techniques typically involve the measurement of multiple MEPs in response to the same stimulation. These responses are then averaged to give an estimate of MEP amplitude in response to given parameters. The variability of MEPs is likely to stem from a combination of factors, including,

the number of recruited motor neurones in the spinal cord, the number of motor neurones discharging more than once to a stimuli, and the synchronization of the TMS-induced motor neurone discharges (Rosler & Magistris, 2008). As these effects are particularly difficult to predict or account for, the use of averaged MEP amplitude in response to stimulation has become standard practice and is used in the measurement of all the following methods.

Motor threshold (MT)

A critical advantage of applying TMS to the motor cortex is the ability to individualize the intensities used; this can be achieved by calibrating intensities to what is known as an individual's motor threshold (MT). MT is defined as the amount of stimulation needed (expressed as a percentage of maximal stimulator output) to reliably generate a MEP of a predefined magnitude (typically 50-100 μV). This can be measured in a muscle at rest (resting motor threshold RMT) or during a slight contraction (active motor threshold AMT) (Devanne et al., 1997). AMT is typically lower than RMT and also results in a shorter delay between pulse delivery and MEP production (Hess et al., 1987). MT is lower in intrinsic hand muscles and increases in proximal muscles occurring in the lower limbs, arms and trunk (Chen et al., 1998).

MT (measured using a standard monophasic P-A TMS pulse) is thought to largely reflect the effects of the I1-wave (Hallett, 2007), and is considered to be predominantly reflective of indirect activation of corticospinal neurones (CSNs) (via activation of monosynaptic cortical-cortical connections) (Di Lazzaro, Profice, et al., 2012). Therefore, MT is thought to reflect the excitability of the main corticospinal projections to the target muscle with the lowest excitation threshold (Hallett, 2007).

Pharmacological studies have found that voltage-gated sodium channel blockers such as carbamazepine increase MT (Menzler et al., 2014; Ziemann, Lonnecker, et al., 1996). Voltage-gated sodium channels are known to be critical in regulating the excitability of axons, therefore these results provide further

support that MT reflects the excitability of cortical-cortical fibre axons excited by TMS (Ziemann, 2013). Glutamatergic mechanisms may also be important. Ketamine, which is a NMDA receptor antagonist and indirectly increases glutamate via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Du et al., 2007), has been found to decrease motor threshold (Di Lazzaro et al., 2003). This may suggest that MT also reflects fast ionotropic glutamatergic neurotransmission via the synapses connecting these fibres to corticospinal neurones (Ziemann, 2013).

MTs are known to be highly variable between, but not within individuals (Mills & Nithi, 1997). They also appear to have a genetic component, as the MTs of siblings have been found to be highly correlated (Wassermann, 2002). This correlation may reflect the heritability of factors such as skull density or potentially even intrinsic neuronal properties. A strong predictor of MT is scalp to cortex distance (Cukic et al., 2009; Herbsman et al., 2009; Kozel et al., 2000; McConnell et al., 2001; Stokes et al., 2005). It has been estimated that for every 1mm away from the TMS coil it is necessary to increase the stimulator output by approximately 3% to induce the equivalent amount of stimulation within the motor cortex (Stokes et al., 2005). The orientation of white matter fibres has also been found to be predictive of MT (Herbsman et al., 2009). Taken together scalp-cortex distance and white matter fibre orientation in the corticospinal tract have been estimated to predict up to 82% of the between subjects variance in MT (Herbsman et al., 2009). Some studies have reported a correlation with age suggesting that aging increases MT (Rossini et al., 1992; Smith et al., 2009), however, this appears to be somewhat unreliable as other studies have found no association (Herbsman et al., 2009; McConnell et al., 2001; Wassermann, 2002). The effects of handedness may also contribute to variation in MT, however, as with age, the evidence is mixed. Some studies report that MT is significantly lower in response to stimulation of the dominant hemisphere (Macdonell et al., 1991; Triggs et al., 1994), but this has not always been found to be the case (Civardi et al., 2000; Rossini et al., 1992; van der Kamp et al., 1996). Interestingly even when MT was not found to be significantly different, the stimulated area

which could produce an MEP has been found to be larger in the dominant hemisphere (Triggs et al., 1999).

Input output (IO) curves

Another common method of assessing cortical excitability is to calculate input output (IO) curves (also sometimes known as recruitment curves or stimulus response curves). IO curves are measured by recording MEP amplitudes evoked from various intensities of TMS; typically, these intensities are individualized for each participant using percentages of their MT (see Figure 2.3 for example).

Compared with MT, IO curves can measure activity from neurones which are spatially further away from the centre of activation. They can be used as an index of excitability within a wider region of the cortex and are thought to reflect the strength of corticospinal projections (Chen, 2000). Evidence from a range of pharmacological studies suggests that there are important differences between the mechanisms for MT and those which mediate MEPs evoked from higher stimulation intensities (Ziemann, 2013). As IO curves measure a range of intensities they encompass different physiological processes at different parts of the curve.

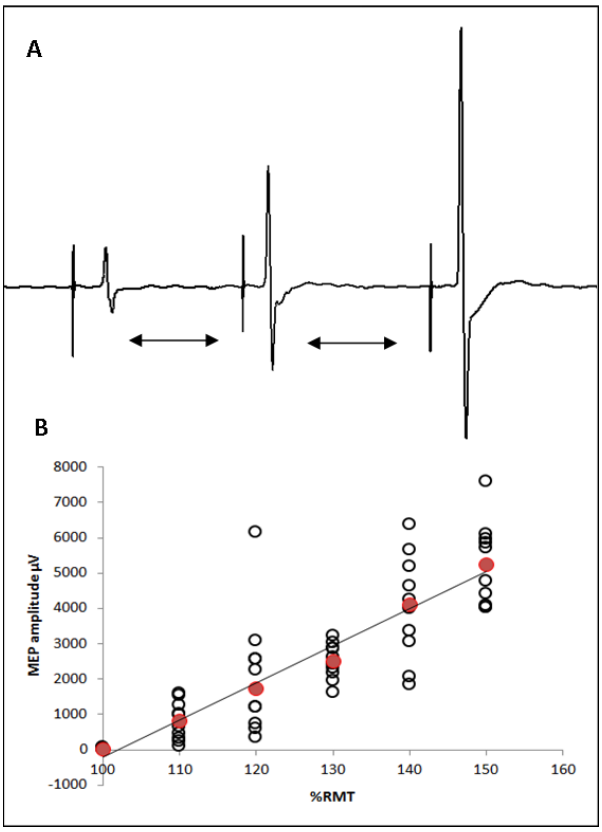


Figure 2.3 A. *Example of EMG recording showing increasing MEP amplitude resultant of increased TMS intensity. Arrows indicate passing of time. B. IO curve data with slope fitted to median values for each intensity (red circles).*

The IO curve contains two distinctive components: the bias level (also known as the threshold) and the gain/slope (Devanne et al., 1997). In addition to this, IO curves may also show a plateau, which is characteristic of their sigmoidal shape in which MEP amplitude increases steeply close to MT and then eventually plateaus at higher intensities (Devanne et al., 1997; Hess et al., 1987). As previously noted, low intensities of TMS (delivered with a P-A current flow) appear to elicit a single I-wave, whereas stronger intensities result in the production of additional late I-waves (Di Lazzaro et al., 2008). It has been suggested that these later I-waves are likely to occur through the activation of corticospinal interneurons via excitatory interneurons which are controlled by many neurotransmitters (see (Ziemann, 2013) for review). In addition to this the higher TMS intensities have also been found to elicit D-waves resulting from direct activation of the corticospinal tract (Di Lazzaro et al., 2001). As a result of

the different underlying mechanisms the slope and intercept of IO curve should be examined separately.

Unlike MT there is evidence that IO curves may be effected by participant age. Pitcher et al (2002) found that although the maximum amplitude of MEPs in older and younger subjects was similar, it took higher stimulator intensities to achieve this in the older participants. This is an important factor to consider when conducting studies with a large age range.

When measuring IO curves it is standard protocol to measure each intensity multiple times in order to address variability within the MEP response. This can be done by measuring the response to stimulation in a ramped (blocked trials of ascending or descending intensity) or in a randomized fashion. The method chosen may have a significant effect on the data collected, particularly when short inter-trial-intervals (ITIs) such as 5 seconds (s) are used. Moller et al. (2009) found that measuring IO curves using increasing intensity strengths resulted in shallower curves than when stimulation was decreased; the effect of randomized pulses resulted in a curve between the other two. This was found for 5s ITIs but not when 20s was used. Pearce et al. (2013) also found no significant differences between ramped and randomized pulses when ITI was set at 6-20s. Therefore, when short ITIs are used, the order of stimulus intensity should be randomized.

2.1.4 Paired pulse measures

Another method of assessing cortical excitability using TMS involves the use of paired pulse protocols. These techniques use a conditioning-test paradigm in which the first pulse modulates the second. The two pulses are typically delivered to the same location with a set Inter-Stimulus-Interval (ISI) between them, the duration of which determines whether the effects are excitatory or inhibitory (see Figure 2.4). Short ISIs of 1-5 milliseconds (ms) typically lead to an increase in inhibition, whereas longer ISIs of 8-30ms have been found to lead to facilitation (O'Shea & Walsh, 2007). Interestingly, increasing the ISI to 50-200ms has also been found to lead to inhibition, however, for these long ISIs to become inhibitory it is necessary to use a different conditioning stimulus intensity than used for 1-5ms protocols. Some notable paired pulse techniques are short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and long interval intracortical inhibition (LICI).

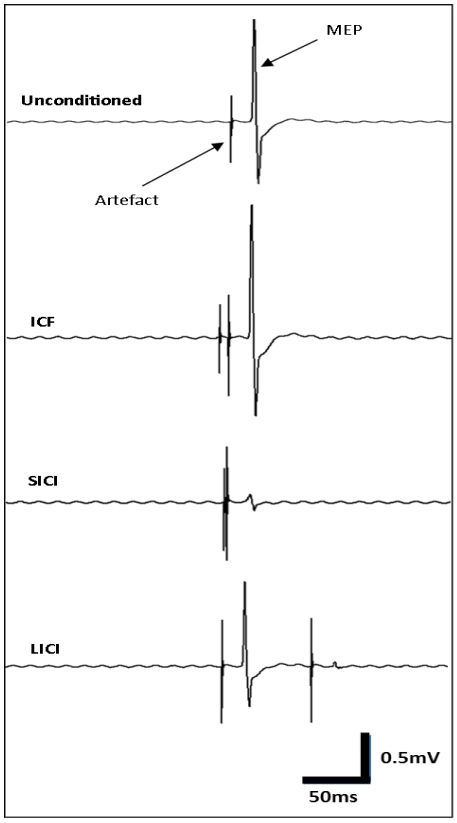


Figure 2.4. *Example EMG readout for various TMS measures.*

Short interval intracortical inhibition (SICI)

One of the most established paired pulse techniques is short interval intracortical inhibition (SICI), which involves the application of a sub-threshold conditioning stimulation (CS) followed by a supra-threshold test stimulation (TS) delivered through the same coil after an ISI of 1-5ms (Hanajima & Ugawa, 2008). SICI was first documented by Kujirai et al. (1993) in the human motor cortex, and has since been studied extensively in both healthy subjects (Garry & Thomson, 2009; Roshan et al., 2003) and patient populations (Cantello et al., 2002;

Daskalakis et al., 2002; Gilbert et al., 2011; Ridding et al., 1995; Ziemann et al., 1997). The subthreshold conditioning stimuli used in SICI is not thought to influence excitability in the spinal cord, as these intensities do not appear to produce recognisable epidural volleys when tested alone (Di Lazzaro, Restuccia, et al., 1998). At present it is widely thought that the conditioning pulse causes a short lasting inhibitory postsynaptic potential in corticospinal neurones via the activation of a low-threshold cortical inhibitory circuit. The engagement of this circuit is then thought to inhibit the action potentials generated in the same pool of corticospinal neurones in response to the test stimulus (see Ziemann (2013) for a review).

Animal models and pharmacological studies have suggested that the effects of SICI are strongly mediated by activity linked to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), in particular GABA-A receptors appear to be heavily implicated (Hanajima & Ugawa, 2008). For example, a number of pharmacological studies have found that administration of benzodiazepines (thought to positively modulate GABA-A receptors) increases levels of SICI (see Ziemann (2013) for a review). In addition the exploration of the effects of other drug manipulations on SICI suggest that the effect is more related to the receptor subtypes a2- and a3-GABA-A rather than a1-GABA-A receptors (Ziemann, 2013). GABA-B receptors have also been implicated, for example, the GABA-B receptor agonist baclofen has been found to significantly decrease the effect (McDonnell et al., 2006). The effects of GABA-B modulation have been linked to activation of presynaptic GABA-B receptors which are effective in reducing GABA release. It has been proposed that it is this process that influences SICI (McDonnell et al., 2006).

Interestingly the effects of SICI may be non-uniform. Although SICI is noted to occur using ISIs of 1-5ms, SICI resulting from 1ms ISIs appears to have different properties than those occurring at later time points (Cengiz et al., 2013; Fisher et al., 2002; Roshan et al., 2003). Initially it was proposed that the effects of 1ms ISIs may be due to refractory periods of cortical-cortical axons (Fisher et

al., 2002), however, this was cast into doubt by later research which found that levels of inhibition were increased when higher TS were used (Roshan et al., 2003). Roshan et al. (2003) argued that if refractory periods were responsible then increasing the intensity of the TS would lead to a reduction in inhibition. This is because TS intensities at increasing strengths would lead to the recruitment of non-refractory interneurons in addition to those in refractory states caused by the CS. Based on this, conclusions were made that 1ms SICI cannot be explained by axonal refractory periods alone, and that synaptic inhibition may also contribute (Roshan et al., 2003). These findings have been supported by some (for example, Vucic et al. (2009)), however, there are others who favour the refractory period theory as an explanation of their findings. For example, a study by Stagg et al. (2011) found a correlation between levels of GABA measured using magnetic resonance spectroscopy (MRS) and the amount of inhibition that was found using a 1ms SICI protocol. No correlations were found between MRS measured GABA and SICI measured using a 2.5ms ISI. The authors speculated that MRS may measure extra-synaptic, tonic GABA, which has the effect of depolarizing neuronal axons (via increases in chloride ions) leading to the lengthening of the refractory periods. This may seem counter-intuitive at first as increased tonic GABA would typically be expected to cause hyperpolarization of the cell membrane, however, the authors argue that differences between the concentrations of chloride ions between the cell body and axon can account for these results. Research using transcranial Direct Current Stimulation (tDCS) by Cengiz et al. (2013) also supports a distinction between the mechanisms of early and late SICI. The effects of tDCS were found to influence the early component of SICI in the opposite direction to that of SICI measures with ISIs of 2.5-5s. However, although both Stagg et al. (2011) and Cengiz et al. (2013) suggest refractory periods as possible mechanisms of 1ms SICI, neither are able to test this directly. Therefore, the origins of the response still remain somewhat elusive.

Intracortical facilitation (ICF)

ICF is measured using a similar condition-test protocol to SICI, but with longer intervals of 7-20ms between the two pulses (Kujirai et al., 1993). Although the mechanisms of ICF are less well understood, there is a general consensus that ICF tests the excitability of an excitatory neuronal motor network which is distinctive to the SICI network (Ziemann, 2013). Differences have been found between the threshold and optimal coil angle for the two measures (Ziemann, Rothwell, et al., 1996). In addition to this, the profile of effects of various drugs on ICF are very different to the pattern seen for SICI (Ziemann, 2013). Both N-methyl-D-aspartate (NMDA) receptor antagonists and manipulations that positively modulate GABA-A receptors have been found to reduce ICF (Ziemann, 2013). This suggests that ICF is in part modulated via processes that involve glutamatergic and GABAergic mechanisms. Positive modulators of norepinephrine have been found to consistently enhance ICF, which suggest a strong modulating effect (for review see Ziemann, 2013).

Long Interval Intracortical Inhibition (LICI)

Long interval intracortical inhibition (LICI) is a paired pulse protocol which, like SICI, leads to inhibition of the size of MEPs evoked from a test pulse. LICI can be measured using two supra-threshold pulses separated by 50-200ms (Claus et al., 1992; Di Lazzaro et al., 2002; Valls-Sole et al., 1992), and is thought to be mediated by GABA-B receptors. Direct evidence for this comes from the findings that the GABA-B receptor agonist baclofen has been found to strongly increase the effects of LICI (McDonnell et al., 2006). Although baclofen has also been found to effect SICI (McDonnell et al., 2006), the two mechanisms are distinct. In particular SICI is thought to be strongly mediated by GABA-A receptors and is influenced by the administration of the GABA-A agonist lorazepam (Di Lazzaro et al., 2006; Teo et al., 2009) whereas LICI is not affected by this manipulation (Teo et al., 2009). In addition, increasing the test pulse strength has been found to increase SICI effects but reduce those of LICI (Sanger et al., 2001). Increasing the intensity of the test stimuli is thought to result in the recruitment of neurones

spatially further away from the site of action and/or with higher thresholds. The evidence suggests that these neurones are more sensitive to SICI than LICI protocols and, therefore, provide evidence that the effects are mediated by distinct cell populations.

While LICI is reported to occur using ISIs of 50-200ms, it appears that the exact ISI used exerts a subtle influence over the effect. Di Lazzaro et al. (2002) found that LICI occurring at ISIs of 100 and 150ms caused a reduction in MEP amplitude and also supressed I2 and later waves, which are thought to be of cortical origin. Within the same participants, ISIs of 50ms were also found to reduce MEP amplitudes, however, the amplitude of late I-waves was increased. This suggests that the inhibitory effects of 50ms LICI may occur at subcortical locations such as the spinal cord. As a result of this finding much subsequent research has used ISIs of 100ms and longer to measure LICI.

2.2 Magnetic Resonance Spectroscopy

Proton MRS (^{1}H MRS) is a non-invasive neuroimaging technique which can be used to quantify levels of metabolites within a predefined location. Unlike TMS the MRS technique allows neurotransmitters such as GABA and glutamate to be measured in various brain regions and is not restricted to the motor cortex. This is done through the placement of a small Volume of Interest (VOI), for example a 2*2*2cm voxel cube, within a predefined location during scanning. Levels of metabolites within this location are then measured by detecting radiofrequency signals that arise from hydrogen nuclear spins within the tissue. The signals produced by different metabolites have chemically specific frequencies caused by the chemical environment of the hydrogen spins (Puts & Edden, 2012). Once acquired, the resulting MRS signals can be separated into a spectrum known as the chemical shift, from which it is possible to quantify levels of metabolites present in the voxel.

Although MRS has proven to be a useful research tool, it is not clear exactly where the signals it detects are produced. For example, GABA can be present intracellularly within both neurones and glia, in addition to being present in extracellular locations. Furthermore, GABA is part of a complex metabolic cycle and is largely synthesised from glutamine via glutamate, therefore at any one time point only a fraction of MRS-GABA will be neurotransmitter (Rae, 2014). This is a complex and important issue. In the instance of the neurotransmitter GABA, these different pools can lead to subtly different processes known as tonic and phasic inhibition. GABA present at the synapse is associated with phasic inhibition. This involves the release of GABA within synaptic vesicles which then diffuses across the synaptic cleft to occupy postsynaptic GABA receptors. This process is very quick as the receptors have a low-affinity meaning the molecules only occupy the cleft for a very brief period of time, resulting in a brief postsynaptic conductance change (Brickley & Mody, 2012). Extra synaptic ambient levels of GABA are associated with tonic inhibition, which occurs via extra synaptic GABA acting on related extra synaptic receptors. These receptors have a high-affinity and, therefore binding here can result in persistent conductance and longer lasting inhibition (Brickley & Mody, 2012).

As previously noted, MRS measures the total concentration of neurochemicals within a predefined area, in doing so it is not possible to distinguish separate functional pools, however, it is possible to examine correlations between other measures such as TMS to help understand the origin of the signal. Tremblay et al. (2013) found SICI and LICI were not significantly associated with MRS measured levels of GABA; this was also found by Stagg et al. (2011), albeit with subtly different parameters. These findings suggest that the GABA measured by MRS is not predominantly of synaptic origin. Stagg et al. (2011) identified a significant relationship between MRS measured GABA and SICI measures using a 1ms ISI. The mechanisms by which 1ms SICI works are still somewhat elusive, however, it is possible that it may reflect refractory periods occurring at the axon, which may suggest an involvement of tonic GABA levels. On the basis of this, Stagg et al. (2011) have suggested that MRS measured GABA

reflects extra-synaptic inhibitory tone. This should be viewed with caution as the finding is highly speculative and yet to be replicated. Stagg et al. (2011) also found that the slope of IO curves was significantly correlated with measures of MRS glutamate, which suggests this reflects pre-synaptic levels of glutamate. However, IO curves were also correlated with MRS measured GABA, whereby higher levels of GABA were associated with steeper slope. This is counter intuitive as steeper slopes are indicative of higher cortical excitability. The authors speculate that this relationship is probably driven through the close biochemical relationship between the two neurotransmitters as the majority of GABA is metabolised from glutamate. A further complication is the fact that it is not always possible to separate out individual metabolites. For example, the ability to separate glutamate and glutamine is dependent on properties relating to the scanner and also the scanning sequence as a result a number of studies report a composite measure known as glx. These factors should be considered when reviewing MRS findings across studies.

2.3 Transcranial Direct Current Stimulation

As outlined in Chapter 1, tDCS is a non-invasive stimulation technique, which has the ability to effectively modulate cortical excitability. The underlying mechanisms of tDCS have been explored using a range of different methods including animal models, TMS and MRS. Key findings are discussed below.

2.3.1 Insights from animal research

As previously mentioned in Chapter 1, findings from early animal work have provided a fundamental starting point for understanding the influence of electrical stimulation in the human brain. With regard to tDCS this research has been particularly important for understanding polarity specific effects. Two notable studies which address this were conducted by Bindman et al. (1964) and Creutzfeldt et al. (1962) using direct cellular recordings from anesthetised animals. Using these techniques, it was found that spontaneous neuronal discharges were increased by surface positive current and decreased by surface

negative current. Interestingly the polarity specific effect did not appear to be true for all neurones. At deeper levels of the cortex (more than 3mm deep) Creutzfeldt et al. (1962) found positive currents had an inhibitory effect and vice versa for negative currents. In addition to this, research by Purpura and McMurtry (1965) revealed that both non-pyramidal tract neurones and pyramidal tract neurones were sensitive to different current densities, with non-pyramidal tract neurones being stimulated at lower total charge than pyramidal neurones (which were not influenced by charge densities of 40-80μV/mm). These findings suggest that interneuron populations may be more sensitive to the low intensities of stimulation typically applied during tDCS (1/2mA) than pyramidal tract neurones.

These findings have important implications for studies in humans. They indicate that the orientation of neurones relative to the electrical field is likely to be critical to the response. They also highlight that tDCS may stimulate different populations of neurones and that the effects may differ depending on the intensity of the stimulation used and the depth of the neurones stimulated. Another important finding from the animal literature is that the effects of electrical stimulation are present both during and after current application (Bindman et al., 1964; Purpura & McMurtry, 1965). These findings have since been explored in humans and are typically referred to as 'online' and 'offline' effects. The so called offline effects of tDCS may be dependent on long term depression (LTD) and long term potentiation (LTP) type plasticity, whereby synaptic connections are strengthened or weakened in response to previous activity. Some direct evidence for this is provided by Fritsch et al. (2010) who found evidence of LTP-like plasticity in an in vitro mouse study, where an anodal stimulation was passed through a slice preparation of the motor cortex. This was found to be dependent on the neurotrophic brain-derived neurotrophic factor (BDNF), which is implicated in synaptic plasticity and is thought to be dependent on NMDA receptor activation (Fritsch et al., 2010).

With the exception of a handful of invasive studies (for example, Dymond et al. (1975)), tDCS research in humans has been carried out using non-invasive methods with lower intensities than those used in the animal research. The current is applied to the scalp which differs from many early animal studies (Bindman et al., 1964; Creutzfeldt et al., 1962; Purpura & McMurtry, 1965) in which the current was applied directly to the surface of the cortex. Applying the current to the scalp results in a less focal stimulation as the current flow can become distorted by a number of factors including the conductivity of the different tissue types it passes through (Sadleir et al., 2010). This is important to consider when comparing between animal and human studies. Further insights into the mechanisms underlying tDCS effects in humans come from studies using pharmacological manipulations, TMS and brain imaging techniques such as MRS. These studies have revealed differences between online and offline effects and will therefore be discussed separately.

2.3.2 Insights from pharmacology, TMS and MRS

Online effects of tDCS

Findings from pharmacological and TMS research suggest that the online effects of tDCS are largely mediated by changes in membrane potential rather than changes in synaptic plasticity (Stagg & Nitsche, 2011). Nitsche, Fricke, et al. (2003) found that when calcium-gated ion channels were blocked using Flunazine (FLU) anodal stimulation did not change cortical excitability. The blocking of sodium-gated ion channels using Carbamazepine (CBZ) also prevented any effects of online anodal stimulation. Interestingly the online effects of cathodal stimulation were not influenced by FLU or CBZ; this could be due to cathodal stimulation hyperpolarizing the neuronal membrane which would make it insensitive to the effects of both FLU and CBZ (Nitsche, Fricke, et al., 2003). Evidence that online anodal tDCS does not influence glutamatergic or GABAergic interneurons comes from the finding that the TMS measures of ICF (a measure of glutamatergic interneurons) and SICI (a measure of GABAergic interneurons) are not significantly influenced by the stimulation (Nitsche et al.,

2005). Blocking of glutamatergic NMDA receptors using dextromethorphan (DMO) also did not influence the effects (Nitsche, Fricke, et al., 2003). Interestingly the effects of cathodal stimulation appear to be subtly different to those of anodal. Although blocking of NMDA receptors using DMO did not influence effects (Nitsche, Fricke, et al., 2003), online cathodal stimulation has been found to reduce the effects of ICF and also to alter the slope of IO curves (Nitsche et al., 2005). The changes in ICF appear to suggest an involvement of glutamatergic interneurons. However, the changes in IO curves provide further support that the effect occurs by modulating the membrane potential of glutamatergic interneurons rather than synaptic activity, because changes in IO curves are typically thought to represent changes in interneuronal activity (Stagg, 2014).

Offline effects of tDCS

Residual offline effects of tDCS are commonly found after stimulation, and have been known to last from a few minutes to a number of hours. The strength and duration of these effects appears to be dependent on the duration and intensity of the stimulation used, however, for the most part the mechanisms underlying these effects appears to be similar.

The after-effects of anodal tDCS are thought to reflect changes in synaptic strength and appear to be dependent on membrane depolarization. Pharmacological blocking of calcium and sodium channels using CBZ and FLU prevented the occurrence of any observable alterations in cortical excitability (Nitsche, Fricke, et al., 2003). Evidence from TMS studies (see Table 2.1) suggests that offline anodal effects are likely to involve widespread interneurons, as they have been found to influence IO curves but not motor thresholds (Nitsche et al., 2005). It also seems likely that the effects involve synaptic modulation and, in particular, GABAergic and glutamatergic systems may be influenced. The effects of SICI have generally been reported to reduce following anodal stimulation (Batsikadze et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005), which strongly

suggests the involvement of GABA-A receptors. In addition, studies using MRS measured levels of GABA have reported a significant reduction in the stimulated area following anodal stimulation (Kim et al., 2014; Stagg et al., 2009). ICF has also been found to increase after anodal tDCS (Batsikadze et al., 2013; Nitsche et al., 2005) and a trend towards increase in Glx (a composite of glutamate and glutamine) has been reported in an MRS study following stimulation (Stagg et al., 2009). In addition, pharmacological blocking of N-methyl-D-aspartate (NMDA)-receptors (which are glutamate sensitive) using DMO has been found to prevent after-effects (Liebetanz et al., 2002; Nitsche, Fricke, et al., 2003). The involvement of NMDA receptor activity is particularly interesting as these receptors have known involvement in cortical neoplastic mechanisms like LPT and LTD (Bennett, 2000). Pharmacological manipulations have also revealed that the after-effects may be influenced by neuromodulators such as norepinephrine, dopamine and serotonin. In particular, the effects of dopaminergic systems may have an important role due to their involvement in synaptic plasticity (see Stagg (2014) for review).

The after-effects of cathodal stimulation appear to be less dependent on membrane polarization than those of anodal. Pharmacologically blocking of sodium and calcium channels using CBZ and CBU does not appear to influence the effects (Nitsche, Fricke, et al., 2003). However, it is possible that this is due to measurement issues (as discussed for online effects) and, therefore, it is not yet possible to conclude that membrane polarization changes are not at least in part involved. As with anodal stimulation there is evidence that a wide pool of interneurons may be involved in the effect. This evidence comes from the finding that although motor thresholds are not influenced, the slope of IO curves has been found to decrease (Nitsche et al., 2005) as has the size of MEPs evoked from TMS intestines which previously resulted in 1mV MEP amplitudes(Furubayashi et al., 2008; Nitsche & Paulus, 2000). Cathodal after-effects also appear to be in part dependent on the modulation of glutamatergic synapses. ICF was significantly reduced following stimulation (Nitsche et al.,

2005) and blocking of NMDA receptors using DMO has been reported to abolish after-effects (Nitsche, Fricke, et al., 2003). Evidence from MRS work also supports the role of glutamate; in a study by Stagg et al. (2009) glutamate concentration was found to reduce following cathodal stimulation. Taken together the evidence seems to support a strong role of the modulation of glutamatergic activity, however, the results of pharmacology studies have not always been consistent and some attempts to modify glutamate have failed to impact on after-effects (see Stagg (2014)). Evidence for the involvement of GABAergic mechanisms are also somewhat mixed between the different methods. TMS research using SICI has found that SICI appears to be increased following cathodal stimulations of less than 2mA (Batsikadze et al., 2013; Nitsche et al., 2005). A reduction in GABA concentration has also been reported using MRS (Stagg et al., 2009), however, the evidence from pharmacology studies is more mixed. The variations found between the different methods may be due to their different sensitivities and the possibility that they are measuring/influencing neurotransmitters at different sites (such as synaptic and extra synaptic levels). In addition, some of the complex findings from pharmacological studies may relate to the close association which exists between glutamate and GABA (Stagg & Nitsche, 2011).

Overall the mechanisms underlying the effects of anodal tDCS both online and offline appear to be clearer than those of cathodal stimulation. Nevertheless, we are coming closer to understanding how the effects truly work which can only be beneficial in understanding their future uses, particularly as therapeutic interventions.

Table 2.1. Notable *TMS investigations of tDCS effects.*

TMS Measure	Protocol	Mechanisms	Anodal after effects	Cathodal after effects
Resting motor threshold (RMT)	Intensity needed to induce an MEP of a predefined amplitude (typically 50-100μV).	Corticospinal tract (CSP) neurones and closely associated intracortical neurones. Neuronal membrane excitability (Ziemann, Rothwell, et al., 1996).	Not effected (Batsikadze et al., 2013; Nitsche et al., 2005; Quartarone et al., 2005; Scelzo et al., 2011)	Generally not effected (Batsikadze et al., 2013; Di Lazzaro, Manganelli, et al., 2012; Nitsche et al., 2005; Quartarone et al., 2005). However one study did find a difference in individuals with schizophrenia and matched controls (Hasan et al., 2012).
Single pulse MEPs (at 1mV or over)	Single pulse TMS, multiple pulses at the same intensity.	CSP neurones and intracortical inter-neurones over a wider area. Neuronal membrane excitability but also GABAergic involvement (Boroojerdi et al., 2001), particularly at higher intensities.	With a few exceptions (Priori et al., 1998), generally found to increase MEP amplitude (Batsikadze et al., 2013; Furubayashi et al., 2008; Kidgell et al., 2013; Nitsche & Paulus, 2000)	With a few exceptions (Priori et al., 1998), generally found to decrease MEP amplitude (Furubayashi et al., 2008; Nitsche & Paulus, 2000)when intensities of less than 2mA are used (Batsikadze et al., 2013). No effects found by Strube et al. (2016).
Input- output curve (IO curve)	Single TMS pulses delivered at increasing intensities.	See single pulse MEPs.	Slope increase (Nitsche et al., 2005), although not always found (Batsikadze et al., 2013; Strube et al., 2016).	Slope decrease reported (Nitsche et al., 2005) although not always found to be significant (Batsikadze et al., 2013).
Short intracortical inhibition 1ms (SICI)	Paired pulse technique. First pulse sub threshold, second supra. ISIs of 1ms.	Unclear, potentially refractory periods (Fisher et al., 2002) and/or synaptic inhibition (Roshan et al., 2003).	Offline effects unknown. Increase in inhibition found during stimulation (Cengiz et al., 2013).	Offline effects unknown. Decrease in inhibition found during stimulation (Cengiz et al., 2013).
Short intracortical inhibition (SICI)	Paired pulse technique. First pulse sub threshold, second supra. ISIs of 1.5-5ms.	GABAA ergic interneurones (Ziemann, 2013)	Reduce inhibition reported (Batsikadze et al., 2013; Cengiz et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005)	Increased inhibition reported (Nitsche et al., 2005), opposite effects reported when 2mA stimulation used (Batsikadze et al., 2013)

Long intracortical inhibition (LICI)	Paired pulse technique, both pulses supra threshold. ISI of 50-200ms.	GABAB receptors (McDonnell et al., 2006).	No significant effects reported (Antal et al., 2010)	
Intracortical facilitation (ICF)	Paired pulse technique. First pulse sub threshold, second supra. ISIs of 7-20ms.	Glutamatergic and potentially GABAergic interneurons (Liepert et al., 1997; Ziemann, Lonnecker, et al., 1996) .	Increased facilitation (Batsikadze et al., 2013; Nitsche et al., 2005)	Decreased facilitation (Nitsche et al., 2005), opposite effects reported with 2mA stimulation (Batsikadze et al., 2013).

Chapter 3: Exploring the temporal effects of tDCS

Key words: *transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), resting motor threshold (RMT), input output curve (IO curve).*

3.1 Introduction

As discussed in the previous chapter, much of the past research into the effects of tDCS suggests the existence of a well-established pattern of responses, whereby anodal currents increase and cathodal currents decrease excitability. It is also established that tDCS has both online and offline effects which are mediated by partially distinct mechanisms. Online effects are thought to arise from changes occurring at the neuronal membrane, whereas the offline effects appear to occur primarily through changes in synaptic plasticity (Stagg & Nitsche, 2011).

The duration of the offline effect is thought to be dependent on the duration and intensity of the stimulation used, for example, intensities of 1mA appear to induce longer lasting effects than those occurring following intensities of 0.8mA and below (Nitsche & Paulus, 2000). On the basis of these findings it is tempting to assume that stronger intensities applied for longer durations will lead to more prolonged effects; however, this assumes that the effects follow a linear stimulus-response type pattern. Research by Batsikadze et al. (2013) highlights that this is not always the case. Batsikadze et al. (2013) found that although 20minutes of 1mA cathodal stimulation applied to the motor cortex caused a reduction in cortical excitability, 2mA stimulation caused an unexpected increase. This reversal of effects at higher intensities was not found for anodal stimulation which appears to be more stable. Interestingly the reversal following cathodal tDCS only became significant 90 minutes after stimulation ended. This is a critical finding as it suggests that the effects of tDCS are at least partially non-linear; it also highlights that the optimal time for measuring change may not

always be immediately following stimulation. The findings have added importance as 2mA intensities have become increasingly popular in behavioural, cognitive and clinical studies (Batsikadze et al., 2013; Nitsche & Paulus, 2011). If these findings can be replicated, it would strongly suggest that caution is warranted when using cathodal stimulation in therapeutic contexts, particularly if 2mA is applied with the aim of reducing cortical excitability. At the time of writing, the findings of Batsikadze et al. (2013) have yet to be replicated. One notable study conducted by Wiethoff et al. (2014) found that, overall, 2mA of cathodal stimulation had no discernible effect on cortical excitability. However, methodological differences between the two studies may account for the discrepancy. In particular Wiethoff et al. (2014) used a stimulation duration of 10 minutes and only measured effects immediately after stimulation, therefore any effects occurring at later time points would have been missed.

The mechanisms underlying the effects of 2mA cathodal stimulation are unknown. However, similar instances of non-linear effects have been reported as a result of increased intensity using stimulation protocols such as theta burst TMS (Doeltgen & Ridding, 2011), transcranial random noise stimulation (tRNS) and transcranial alternating current stimulation (tACS) (V. Moliadze et al., 2012). It is speculated by Batsikadze et al. (2013) that these reversal effects may relate to the amount of calcium influx caused by the respective stimulations. This would stand to reason for offline tDCS effects, as these are known to be somewhat dependent on calcium channel mechanisms (Nitsche, Fricke, et al., 2003). These changes in calcium channel mechanisms could relate to different populations and parts of neurones being stimulated. In early animal studies Purpura and McMurtry (1965) found that non pyramidal tract neurones and pyramidal tract neurones were sensitive to different current densities. Therefore, there is a possibility that increasing stimulation intensity leads to stimulation of different parts of the neurone present at different depths, or even different types of neurone, and that this results in distinctive response patterns.

A further possible explanation for the reversal of cathodal effects following 20 minutes of stimulation at 2mA relates to the engagement of homeostatic type processes. This was also argued by Batsikadze et al. (2013) as a potential reason for the delayed response following 2mA of stimulation. Homeostasis refers to the properties of a system which allow it to remain stable within certain limits. These regulatory mechanisms have been proposed to maintain neuronal activity within a useful and effective range, and have been identified as playing an important role in stabilizing properties of neuronal circuits (Siebner et al., 2004). Compelling evidence for the influence of these mechanisms in the human motor cortex comes from studies in which two periods of non-invasive stimulation are applied in close temporal proximity to each other (Fricke et al., 2011; Monte-Silva et al., 2010; Siebner et al., 2004). In these studies, TMS or tDCS is used to pre-condition the motor cortex; shortly following this a second period of stimulation is given and changes in cortical excitability are measured. If homeostatic mechanisms are not engaged, an additive effect may be expected in which two periods of inhibitory or excitatory stimulation simply lead to more inhibition/ excitation. However, this does not seem to be the case; in fact in all three of these studies a reversal of the initial effect was found. It is possible that the reversal of the expected effects of cathodal stimulation found by Batsikadze et al. (2013) relates to these mechanisms.

If homeostatic mechanisms play an important role in moderating the effects of tDCS, baseline excitability is likely to be an important factor. This is supported by the finding that baseline MEP amplitudes and responses to 2mA anodal tDCS have been found to be significantly correlated (Wiethoff et al., 2014). Wiethoff et al. (2014) also found that the relationship between baseline and 2mA cathodal stimulation approached significance. However, a sham condition was not included which makes it difficult to draw strong conclusions from these findings.

The following experiment was conducted to investigate changes in cortical excitability caused by anodal, cathodal or sham stimulation applied at 1mA (experiment 1) or 2mA (experiment 2) for 20 minutes to the motor cortex. The effects of stimulation were explored at various time points for 90 minutes. The study aimed to add to the original single pulse TMS findings of Batsikadze et al. (2013); in particular the finding that 2mA cathodal increased cortical excitability whereas 1mA cathodal decreased it. In order to assess changes in cortical excitability IO curves were measured. Batsikadze et al. (2013) found significant effects using SI 1mV measures but failed to find any significant change in IO curves. However, it is possible that this is due to the fact that they only measured IO curves immediately after stimulation. In this study the decision not to measure SI 1mV was made as IO curves typically encompass effects similar to those measured by SI 1mV in addition to capturing change in excitability within a wider population of neurones. This is because IO curves measure change in MEP amplitudes in response to a range of stimulator intensities, whereas SI 1mV only measures responses to one.

3.2 Method

3.2.1 Participants

A total of 22 healthy participants who did not smoke were recruited, of these 11 completed both experiment 1 and experiment 2 (Table 3.1). Participants were right-handed as measured by an adapted, shortened version (M. S. Cohen, 2008) of the Edinburgh handedness inventory (Oldfield 1971). All were free from CNS active medication and had no counter indications to TMS (Rossi et al., 2009). Participants gave informed written consent prior to the study and received financial compensation for participation. All experimental procedures were approved by the University of Nottingham Research Ethics Committee.

Table 3.1. *Subject characteristics. Data are presented as mean ± SD; N= number of participants; F= Female; M=Male; RMT= Resting Motor Threshold.*

Experimental session	N	Sex (M/F)	Age	rMT
Experiment 1				
1mA anodal	18	9M, 9F	18-41 years (22 ± 5.4)	41.1 ± 8.3
1mA cathodal	18	9M, 9F	18-41 years (22 ± 5.4)	40.7±7.8
Sham	18	9M, 9F	18-41 years (22 ± 5.4)	41.3±8.2
Experiment 2				
2mA anodal	15	8M, 7F	18-26 years (20±2)	42.3±8
2 mA cathodal	15	8M, 7F	18-26 years (20±2)	42.9±7.9
Sham	15	8M, 7F	18-26 years (20±2)	42.5±8.3

3.2.2 Design

In Experiment 1 a within-subjects design was used to investigate changes in cortical excitability over a period of time following anodal, cathodal or sham stimulation applied at 1mA intensity to the motor cortex. Time of measurement (0, 30, 60 and 90 minutes) and tDCS condition (anodal, cathodal or sham) acted as independent variables. Change in MEP amplitude as measured by IO curves served as the dependent measure.

Experiment 2 closely followed the design of experiment 1, however, a stronger tDCS intensity of 2mA was used.

3.2.3 tDCS of the motor cortex

tDCS was delivered via a NeuroConn DC- stimulator (GmbH, Ilmenau, Germany) with a maximum stimulation output of 4.5mA. Stimulation was applied using surface sponge electrodes each measuring 35 cm^2 to the area representing the first dorsal interosseous (FDI) muscle in the hand (identified using TMS) and to the contralateral right orbit. The electrodes were soaked in saline solution of up to 154mM to increase conductance whilst attempting to limit participant discomfort (Dundas et al., 2007). The current was run between the electrodes for a total of 20 minutes at 1 mA (experiment 1) or 2 mA (experiment 2). The current was ramped up over 8 seconds in the 1mA condition, followed by an 8 second ramp down period. The ramp up/ ramp down periods were increased to 15 seconds in the 2mA condition for participant comfort. The intensity under the

electrodes correspond to current densities of 0.028 mA cm² in the 1mA condition (1mA/35cm²) and 0.057 mA cm² in the 2mA condition (2mA/35cm²).

Sham stimulation mimicked the timings used by Batsikadze et al. (2013); 20 second ramp up, 30 seconds of stimulation and a 10 second ramp down period. The current was ramped up to 1mA in both experiments because 1mA has previously been found to create reliable sham effects in participants (Ambrus et al., 2012), and was, therefore, considered sufficient. To limit any potential effects of sham polarity, this was counterbalanced, hence for half of the participants a cathodal electrode arrangement was used, whereas for the other half an anodal arrangement was utilized. Subjects participated in all three conditions (anodal, cathodal and sham stimulations), with a minimum of a week separating active stimulations. The order of experimental session was counterbalanced within each experiment.

3.2.4 TMS measurements and EMG recording

TMS stimulation was delivered using a Magstim 200 (Magstim, Whiteland, Dyfed, UK) with a figure of 8 magnetic coil (diameter of one winding 70mm). The coil was held tangentially to the scalp and positioned 45° from the midline. The optimal location for stimulation ('hot spot') of the contralateral FDI was defined as the location over the left motor cortex which when stimulated consistently resulted in the largest MEP. This was marked lightly with adhesive tape to inform tDCS electrode placement.

MEPs were recorded using disposable, Ag-AgCl surface electrodes attached to the right FDI muscle in a belly tendon montage. Alcohol wipes were used to prepare the skin prior to application of the electrodes. The signals were amplified and bandpass filtered (10Hz- 2kHz, sampling rate 5kHz) and digitalized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position on a table directly in front of them. Resting motor threshold (RMT) was determined as the lowest

intensity needed to yield an MEP response of >100μV in the relaxed FDI muscle in a minimum of 5 of 10 trials.

A neural navigation system (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used to track coil position in relation to the participants head and the location of the identified hotspot. This was done using a template which was constructed from a consenting individuals anatomical brain scan. Individuals head were registered to this template as individual anatomical scans were not available. A chin rest was used during stimulation to maintain the position of the participant's head. Participants were informed that they could take breaks if necessary and move if uncomfortable.

IO curves were measured using TMS intensities of 95, 100, 105, 110, 115, 120, 125 and 130% RMT. Each block was presented 10 times; hence participants experienced 80 TMS pulses during a single IO curve measurement. The order of the stimuli was randomized (using Matlab) as opposed to using a 'ramped' procedure (whereby stimuli are measured in steps from high to low intensity). This was chosen to avoid any potential order effects, however this precaution may have been unwarranted as the effects of these different methods have since been found to show no statistical differences (Pearce et al., 2013). Each TMS pulse was separated by an inter stimulus interval (ISI) of 5 seconds.

3.2.5 Procedure

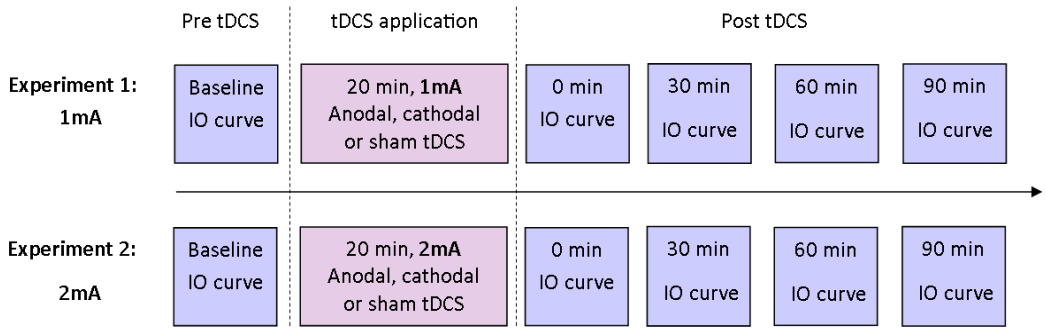


Figure 3.1. *Experimental protocol for each testing session. Arrow demonstrates the passage of time over an experimental testing session.*

Experiment 1: 1mA tDCS

After gaining informed consent, participants were seated in a comfortable chair with their head positioned in a chin rest and their right hand placed in a relaxed position in front of them. The location of the participant's head was then registered to a template using the Brainsight system (Rogue research Inc., Montreal Quebec, Canada), and disposable electrodes were attached to the hand. Following this, the hotspot for FDI stimulation was identified using TMS, which was then mapped onto the template brain to aid coil localization and marked lightly on the scalp with mildly adhesive tape. RMT was then determined and baseline IO curves recorded. 1mA anodal, cathodal or sham tDCS was then applied to the identified hotspot for 20 minutes, which was ramped up for 8 seconds at the start of stimulation and down for 8 seconds following the end of stimulation. Participants were blind to the stimulation polarity, however due to constraints on resources the researcher was not. IO curves were then re-measured following tDCS; on average this occurred 6.30 ± 3.05 minutes after the end of stimulation due to re-localization of the TMS coil and removal of the tDCS electrodes. As this was the first time point collected this is hereafter referred to as time point 0 or the time immediately following stimulation. IO curves were also measured at 30, 60 and 90 minute intervals post

tDCS (see Figure 3.1). RMT was kept constant and not adjusted over the course of a testing session.

Participants were asked to get a good night's sleep before each experimental session and watched films during the experiment in an attempt to reduce fatigue and maintain alertness. This method was used as fatigue and its subsequent effects on alertness have previously been found to increase cortical excitability (De Gennaro et al., 2007; Huber et al., 2013) resulting in increases in RMT and both low and high threshold measures using TMS. The order of experimental conditions was counterbalanced between participants to avoid potential order effects. The time of testing was held constant whenever possible for each participant to limit any potential influences of the time of day and circadian rhythms, as these may influence cortical excitability (Huber et al., 2013).

Experiment 2: 2mA tDCS

The experimental protocol for experiment 2 was identical to experiment 1, with the exception that 2mA of intensity was used and that the ramp up/ ramp down times were increased to 15 seconds. Sham protocol remained the same as in experiment 1. The first IO measure started on average 6.34 ±2.39 minutes after tDCS.

3.2.6 Analysis and statistics

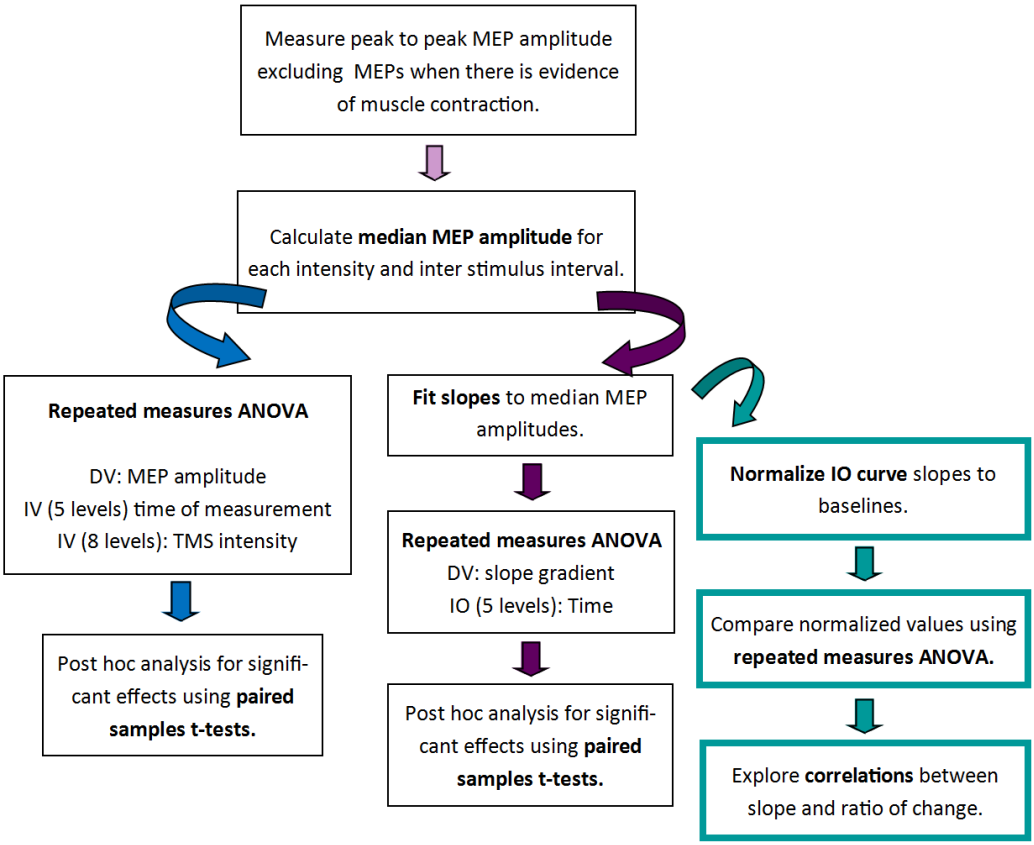


Figure 3.2. *Schematic of data analysis with key aspects highlighted.*

MEP data was analysed by measuring peak-to-peak amplitudes using an in-house Matlab script. Any trials in which there was evidence of pre-contraction of the FDI muscle in the period prior to the TMS pulse were excluded as this is known to inflate the MEP response. For each individual, the data was further processed by calculating the median MEP amplitude for each of the 8 intensities measured during each IO curve. Median rather than mean was used in an attempt to limit the effect of outliers.

For completeness the resultant data was analysed using three methodologies (see Figure 3.2). Only one method will be discussed in depth here, however, further information regarding the other statistical analyses performed is available in Appendix.i, Appendix.ii and Appendix.iii. These analyses were all initially carried out for each polarity respectively (anodal, cathodal, sham) to allow for in-depth exploration of effects within a given testing session.

However, further analysis in which polarity is also entered into the model is included for completeness, as this allows more direct comparisons between the measures to be made.

Analysis of tDCS effects on RMT

The potential effects of tDCS on RMT were explored using paired samples t-tests to compare any changes in MEP amplitudes evoked by the 100%RMT condition. The stimulator intensity used to measure RMT was held constant over the course of each testing session, therefore, change in MEP amplitude can be used as a proxy to suggest a change in threshold.

Analysis of tDCS effects on IO curve slope

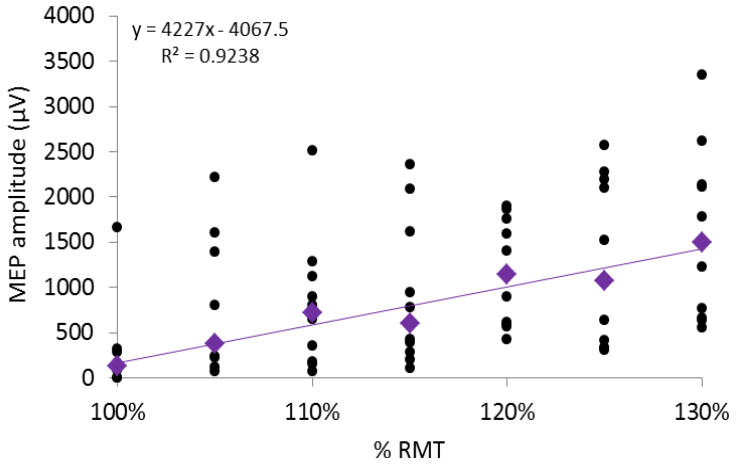


Figure 3.3. *Example IO curve slope fit. Black circles indicate individual MEP amplitudes, purple diamonds show median values for each intensity.*

The data was analysed by fitting a linear trend of best fit to the median MEP values of intensities from 100-130% RMT. 95% RMT was not included because for many participants this was subthreshold and resulted in no measurable MEPs (See Figure 3.3 for example IO curve). Linear fitting was deemed appropriate based on visual inspection of the data which showed no clear evidence of high or low end slope plateau. Average fitting for Experiment 1 was good (mean R^2=0.86) as was fitting for Experiment 2 (mean R^2= 0.86).

By using slope fits the need to include intensity as a variable is removed. This makes it possible to more easily explore the data further by normalizing

slope values to their respective baselines to provide an indication of the magnitude and direction of change in IO curve slope following stimulation. This approach was used to address naturally occurring differences in baseline slopes between individuals. Without exploring the data using this method, differences in baselines could skew the results as a seemingly small increase in slope can involve a larger proportion of change for some individuals than others. Normalized values were entered into repeated measures ANOVAs to assess any effects of time. This was done separately for each polarity and intensity.

To explore the potential effects of baseline cortical excitability on the magnitude and direction of change following stimulation, correlational analyses were conducted in which baseline slope was correlated with the normalized slope values (post/pre) at each time point for each condition.

3.3 Results

3.3.1 Effects of tDCS on RMT

Experiment 1: 1mA

RMT was held constant throughout each testing session, therefore, change in MEP amplitudes evoked by TMS pulses at RMT can be used as a proxy for change. As any significant change in MEP amplitudes would suggest that a change in RMT had occurred. Paired sampled t-tests revealed no significant differences in MEP amplitude at RMT between baseline and measures taken after 1mA anodal tDCS (all p> .7). No significant differences from baseline were found following cathodal stimulation (all p>.07) or sham stimulation (all p>.13).

Experiment 2: 2mA

MEP amplitudes at RMT were not significantly altered from baseline immediately, 30 and 60 minutes following anodal stimulation (all p>0.34). A significant difference was found between baseline and 90 minutes (t(15)=2.23, p=.04), however, this did not survive correction for multiple comparisons. No significant differences were found following cathodal stimulation (all p>0.16) and there were no significant changes immediately, 30 and 90 minutes following sham stimulation (all p>0.13). A significant difference was found between baseline and 60 minutes post sham stimulation (t(15)=-2.63, p=0.02) although this effect did not survive correction for multiple comparisons.

3.3.2 Effects of tDCS on IO curve slope

Repeated measures ANOVAs were used to assess change in IO curve slope across the different time points using ratio data *(post tDCS/ baseline)*. This was done to control for subtle variations in baseline between conditions. 1m*A* cathodal and sham conditions violated the assumption of sphericity, therefore, Greenhouse-Geisser correction was applied.

Experiment 1: 1mA

Time of measurement (baseline, 0, 30, 60 , 90 minutes) did not significantly influence IO curve slope for the 1mA anodal *F(4,68)*=.21, *p*=.93, the 1mA cathodal *F(2.3,38.45)*=.1.43, *p*=.25, or the 1mA sham conditions *F(4,44.47)*=.21, *p*=.07. Average IO curves can be seen in Figure 3.5.

Experiment 2: 2mA

The 2mA anodal condition was significantly influenced by time of measurement *F*(4,56)=3.55, *p*=.01. Exploration of this effect using paired sample t-tests revealed that this effect arose from differences between baseline and 30 minutes post stimulation *t*(14)=2.4, *p*=.03 measures. The difference between baseline and 60minutes post stimulation was also significant *t*(14)=2.23, *p*=.04. However, neither effect survived correction for multiple comparisons using the Bonferroni method. There was no significant effect of time of measurement for the 2mA cathodal *F*(4,56)=.62, *p*=.65 or sham condition *F*(4,56)= 1.03, *p*=.4. Averaged IO curves can be seen in Figure 3.6.

Change in slope over the different time points can be seen in Figure 3.4. This illustrates the individual variability within conditions even when the effects are found to be significant at group level.

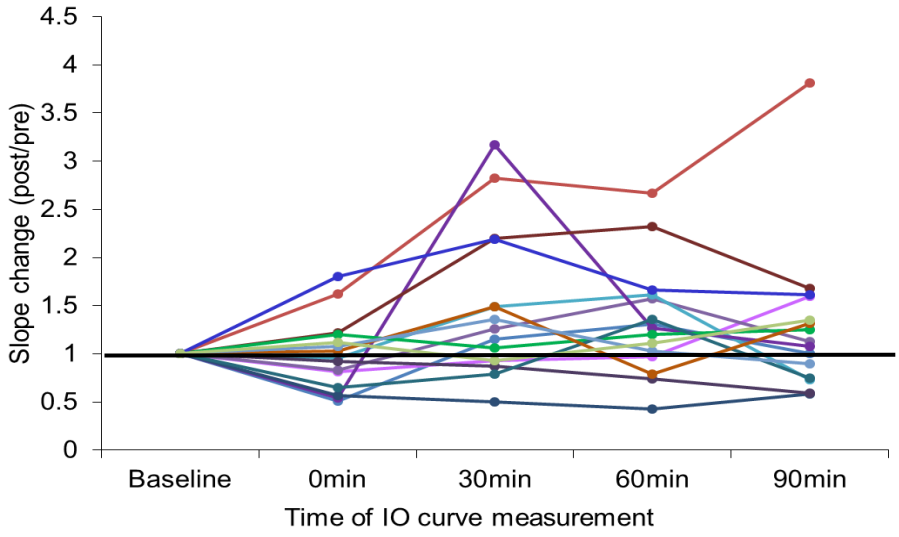


Figure 3.4. *Ratio values showing change in slope following 2mA anodal stimulation. Each colour represents an individual participant's data set. Black line indicates no change from baseline.*

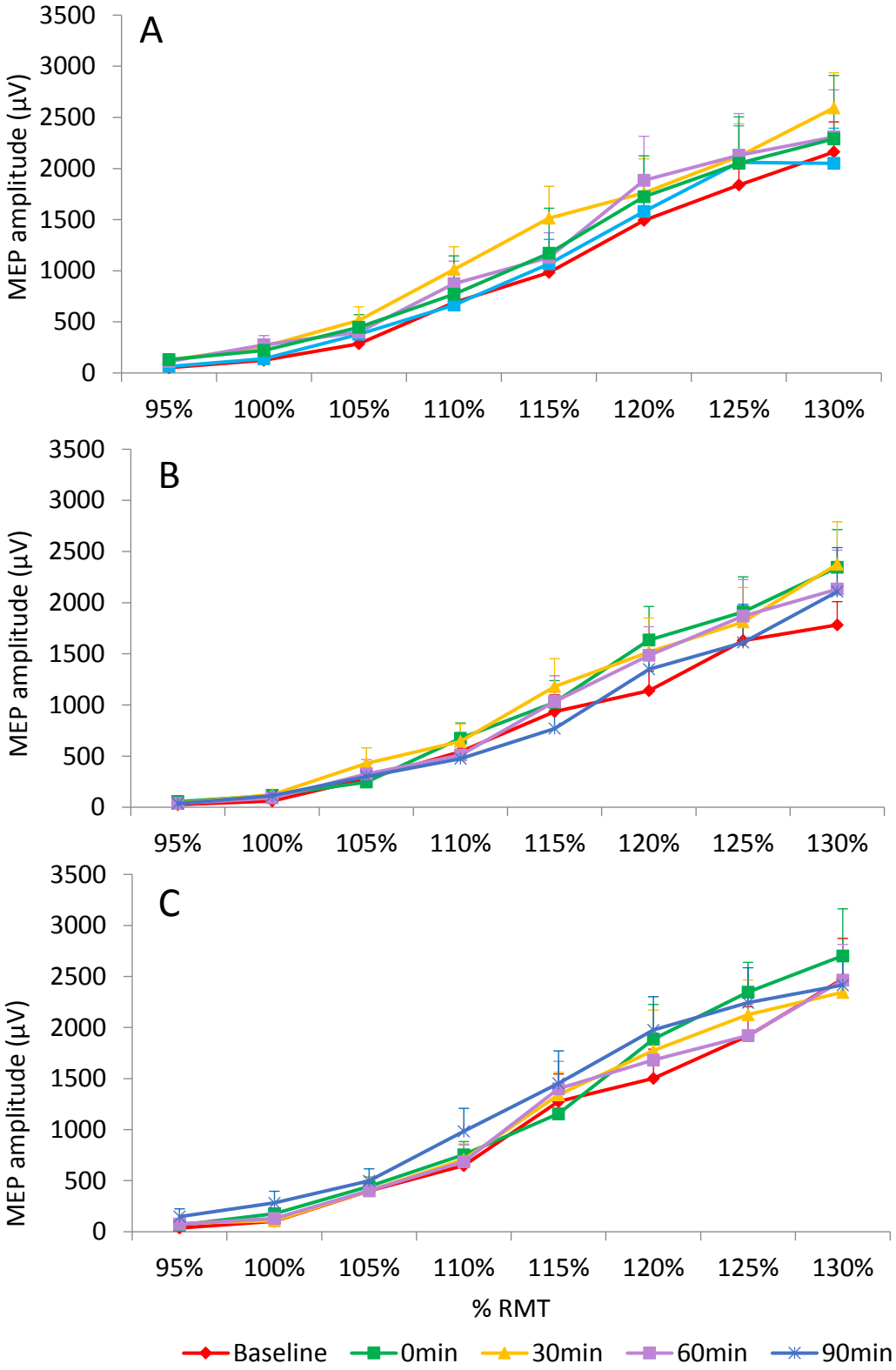


Figure 3.5. *Mean and SEM of average MEP amplitudes at each given TMS intensity pre and post stimulation for* ***A: 1mA Anodal, B: 1mA Cathodal, C: 1mA Sham.***

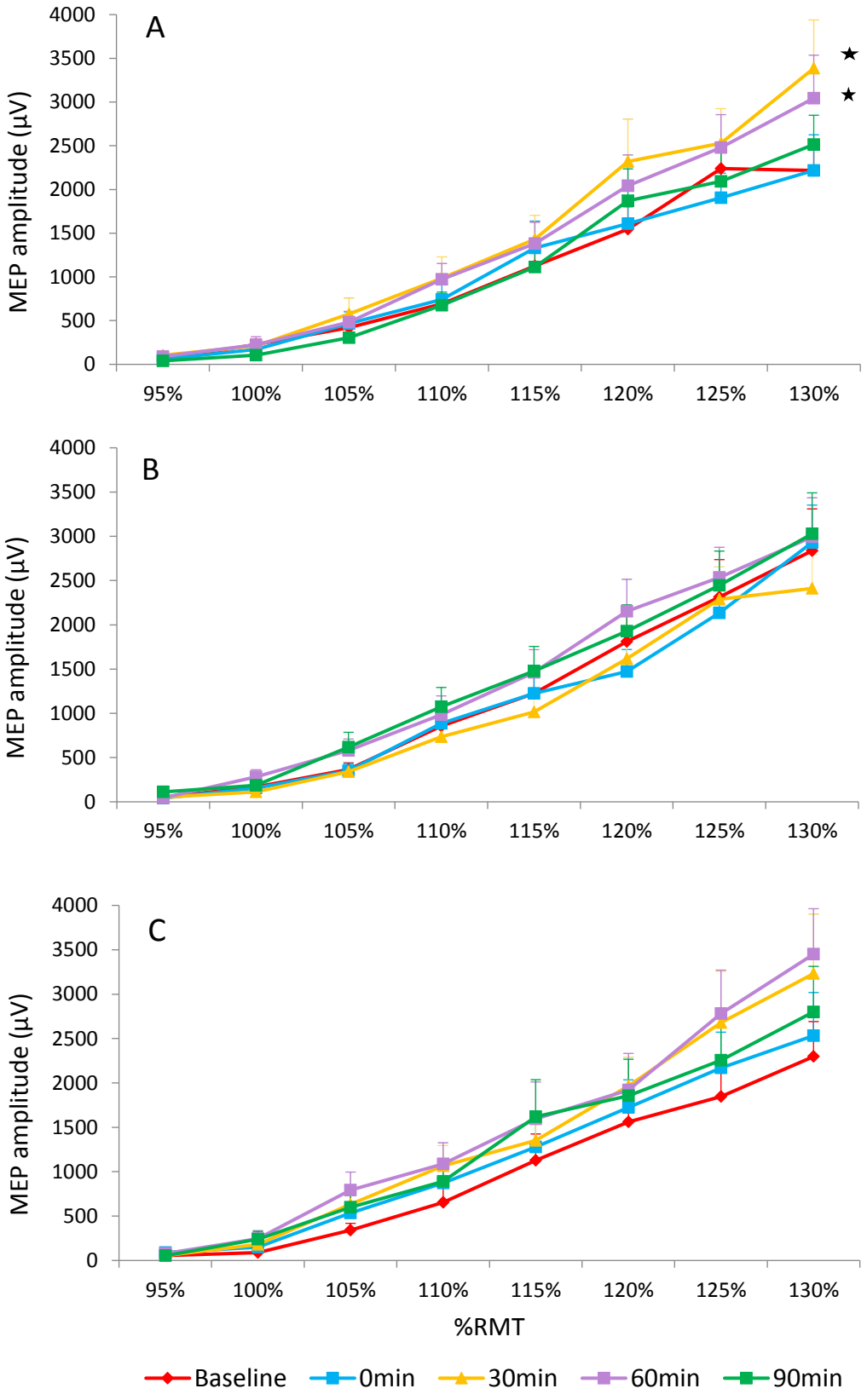


Figure 3.6. *Mean and SEM of average MEP amplitudes at each given TMS intensity pre and post stimulation for* ***A: 2mA Anodal, B: 2mA Cathodal, C: 2mA Sham.*** ★ *Indicated significant difference from baseline*.

3.3.3 Correlational analysis: baseline relationships with slope change

The results of Pearson's correlation analysis are shown without correction for multiple comparisons. However, in the case of correlation coefficients reaching conventional statistical significance (p=<0.05) the Bonferroni correction was applied and is discussed. Figure 3.7 and Figure 3.8 show spider plot diagrams of Pearson's correlation coefficient for slope change at each time point and respective baseline; filled circles indicate correlations that reach conventional levels of statistical significance (p=< .05).

Experiment 1: 1mA

Baseline slope was not related to slope change following 1mA anodal tDCS at any time point (all p > 0.44) as is shown in Figure 3.7A. Change in slope following 1mA cathodal stimulation (Figure 3.7B) was not found to be related to baseline at any time point (all p>0.36). Baseline slope was associated with slope change following sham stimulation (Figure 3.7 C) immediately following stimulation (r=-0.48, p=0.04), post 30 minutes (r=-0.55, p=0.02), post 60 minutes (r=-0.55, p=0.02) and post 90 minutes (r=-0.62, p=0.00). However, when the Bonferroni correction for multiple comparisons is applied only the correlation between baseline and 90 minute follow up remained statistically significant (r=-0.62, p=0.02).

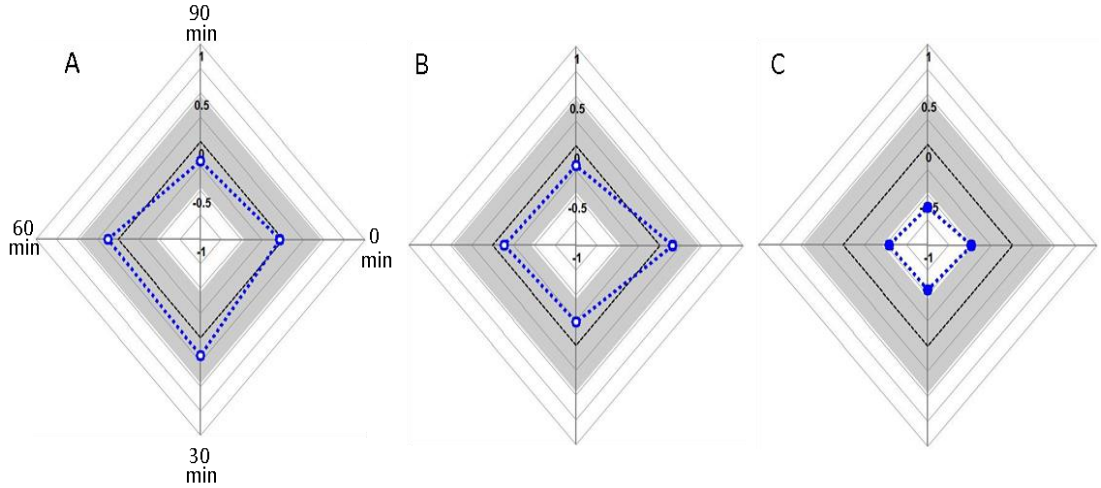


Figure 3.7. *Uncorrected Pearson's correlation coefficients of baseline slope with slope change at the four time points for A: 1mA anodal; B: 1mA cathodal and C: 1mA sham condition. Filled circles indicate significant correlations (p=<0.05).*

Experiment 2: 2mA

Baseline slope was not related to slope change following 2mA anodal tDCS at any time point (all p>0.16) as shown in Figure 3.8 A. However, baseline slope was correlated with change in slope immediately following 2mA cathodal stimulation (r=-0.57, p=0.03), marginally correlated with slope 30 minutes after stimulation (r=-0.57, p=0.05) and correlated with slope measures 60 minutes following stimulation (r=-0.55, p=0.03). Baseline was not correlated with slope measures 90 minutes following stimulation (r=-0.47, p=0.08), see Figure 3.8 B. Baseline slope was also not found to be significantly correlated with slope following sham stimulation at any time point (all p=>0.11), see Figure 3.8 C.

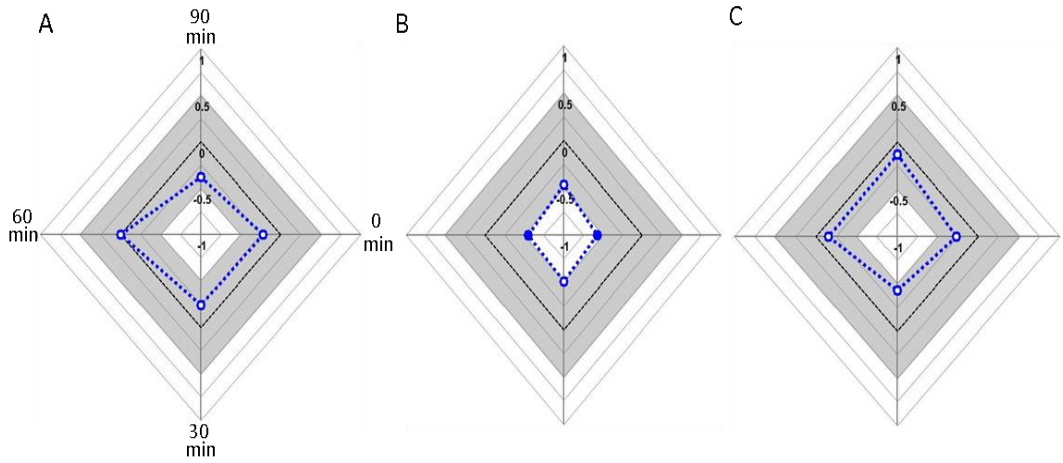


Figure 3.8. *Uncorrected Pearson's correlation coefficients of baseline slope with slope change at the four time points for A: 2mA anodal; B: 2mA cathodal and C: 2mA sham conditions. Filled circles indicate significant results (p=<0.05).*

3.3.4 Effects of tDCS type and time on IO curve slope

Experiment 1: 1mA

In order to compare the effects of the different polarities more directly, additional repeated measures ANOVAs were calculated using the slope ratio data from all conditions. This analysis was also conducted using raw slope values, details of which can be found in Appendix.iii. Slope change was entered as the dependent variable. Polarity (3 levels) and time of measurement (5 levels) served

as independent factors. The Greenhouse-Geisser correction was used to correct for any significant violations of sphericity.

No significant effects of tDCS polarity were found in the analysis of the 1mA data (Table 3.2). There was no significant interaction between time and polarity; however, a significant main effect of time was revealed $F(4,56)= 2.73$, $p=.04$. Post hoc tests using the Bonferroni correction revealed a single significant difference between slope change from baseline at the 0 minute measurement ($p=.02$), which showed a general increase across all tDCS measures at this time point. This can be seen in part in Figure 3.5 (in which baseline as indicated in red, is always below 0min measure indicated in green).

Table 3.2. *Results of repeated measures ANOVAs calculated for 1mA data in which the effects of time, and polarity on IO curve slope are explored*

Factor	df	F	P	η_p^2
Polarity	2,28	1.08	.35	.06
Time	4,56	2.73	.04*	.14
Polarity*Time	4.44,75.44	.63	.66	.04

Experiment 2: 2mA

No significant effects of polarity were found in the analysis of 2mA data (Table 3.3). The interaction was also not found to be significant, however, as in the 1mA condition there was a significant main effect of time, $F(4,56)=3.24$, $p=.02$. Post hoc Bonferroni tests revealed a significant difference between baseline and slope change 60 minutes after stimulation ($p=.02$). Inspection of the data shown in Figure 3.6 suggests that this effect is primarily driven by the anodal and sham conditions.

Table 3.3. *Results of repeated measures ANOVAs calculated for 2mA data in which the effects of time, and polarity on IO curve slope are explored.*

Factor	df	F	P	η_p^2
Polarity	2,28	.184	.833	.013
Time	4,56	3.239	.018*	.188
Polarity*Time	8,112	.939	.487	.063

3.4 Discussion

Changes in cortical excitability following <u>1mA tDCS</u>

In line with previous studies there was no evidence of either 1mA anodal, cathodal or sham conditions significantly effecting RMT (Batsikadze et al., 2013; Nitsche et al., 2005; Quartarone et al., 2005; Scelzo et al., 2011).

Extensive analysis of change in IO curve slope following 1mA tDCS failed to reveal any significant differences from baseline for either anodal, cathodal or sham conditions. This was true of measurements taken immediately following stimulation and at each subsequent time point measured. The lack of an effect is somewhat at odds with previous literature (Nitsche et al., 2005), as the IO curve measures in this study show no evidence of significantly increased cortical excitability following anodal stimulation, nor decrease following cathodal stimulation. In addition to this there was no evidence that anodal, cathodal and sham stimulations influenced IO curves in distinct ways. If anything there was a tendency towards a slight increase in IO curve slope over the course of each experimental session regardless of tDCS type.

Correlational analysis revealed that change in IO curve slope following sham stimulation was significantly correlated with baseline at all time points. However, only the relationship between baseline and 90 minutes post stimulation survived the adjustment for multiple comparisons. Baseline slope

was not found to significantly correlate with change in IO curves for either anodal or cathodal stimulation.

Changes in cortical excitability following 2mA tDCS

2mA cathodal tDCS was not found to have any significant influence on RMT, however, a significant difference was identified between RMT measured at baseline and the 90 minutes following anodal stimulation. This finding is somewhat novel and not supported by previous literature (Batsikadze et al., 2013), however, the effect did not survive correction for multiple comparisons, and may be due to methodological issues/natural fluctuation over time rather than an effect of tDCS. This view is partially supported by the finding of a significant difference between baseline and 60minute follow up in the sham condition, although this also failed to survive the correction for multiple comparisons. It is possible that the use of a slightly higher threshold criteria (>100μV rather than 50-100 μV) may also have contributed to these effects.

Analysis of the 2mA anodal data revealed a significant increase in IO curve slope from baseline at 30 and 60 minutes following stimulation; however, neither of these effects were strong enough to survive the multiple comparison correction. 2mA cathodal and sham stimulation failed to have any significant effect on slope at any time point. It is theoretically possible that significant cathodal differences may have been observed at a later time point, as effects were previously found up to 120 minutes post stimulation (Batsikadze et al., 2013). However, this seems unlikely as the 2mA anodal effects found in the present experiment were actually found earlier (30 at 60 minutes post stimulation) than those found by Batsikadze et al. (2013) who reported significant effects only after 60 minutes. It is unclear why 2mA anodal effects would show an earlier time course, while cathodal effects would be more delayed within the same sample of participants.

In depth analysis of the data suggests that the effects on IO curve slope following 2mA anodal stimulation are largely driven by changes occurring in

MEPs evoked from the strongest 130%RMT pulses. This is apparent upon visual inspection of the data and also in analysis in which the effects on individual TMS intensities were explored (discussed in Appendix.i and Appendix.ii). This may relate to the total amount of neurones stimulated by each TMS intensity and the focality of tDCS stimulation. The amount of current reaching the cortical surface as a result of tDCS is thought to be varied and widespread (Datta et al., 2012; Miranda et al., 2006), particularly when standard rectangular electrodes are used (Datta et al., 2012). As a result, higher stimulation intensities, such as 130% RMT (which recruits neurones more widely than lower intensities), may be more able to capture changes induced by tDCS. Although it is possible that in the present study higher intensities may have revealed more effects, it should be noted that previous studies have successfully identified changes in MEP amplitudes using SI 1mV intensities (Batsikadze et al., 2013; Furubayashi et al., 2008; Kidgell et al., 2013; Nitsche & Paulus, 2000). In the current data set this would generally reflect responses to TMS intensities from 115% RMT and upwards, yet significant differences are only apparent at 130%.

Although the independent analyses revealed some support that anodal tDCS, but not sham or cathodal stimulation, significantly altered IO curve slope, no significant differences between polarities were found in the group analysis. This suggests that the effect is somewhat weak and contrasts with many studies which have reported significant differences between MEP amplitudes following different polarities of tDCS and sham conditions (Batsikadze et al., 2013; Fricke et al., 2011; Nitsche et al., 2007; Nitsche & Paulus, 2000). It is unclear why the findings using IO curve would be so different to those measuring MEPs from a single TMS intensity, nevertheless it is worth noting that despite many studies using SI 1mv only a few have used IO curves. Of those studies using IO curves one reported a significant change depending on stimulation type (Nitsche et al., 2005) however another failed to replicate this (Batsikadze et al., 2013).

The potential impact of baseline cortical excitability on the amount and direction of subsequent change following stimulation was also explored for the

2mA condition. This was done by calculating correlational values between baseline slope values and ratios of change at each time point. Interestingly, although in the 1mA condition baseline slope was correlated with changes following sham stimulation, the same was not found in the 2mA data set. Here neither baseline slope for sham nor anodal stimulation were significantly related to subsequent change. For the 2mA cathodal condition, baseline slope was significantly related to change at 0 and 90 minutes following stimulation, however, neither of these correlations survived correction for multiple comparisons. These results differ to those found in the sham condition for experiment 1, hence making clear interpretation difficult.

The results of the correlations also appear to differ from a recent experiment conducted by Wiethoff et al. (2014) in which baseline MEP amplitude correlated with changes following 2mA anodal tDCS. Wiethoff et al. (2014) also identified a trend between baseline and change following 2mA cathodal stimulation, a finding which is weakly implicated at specific time points in the current data set. A number of methodological differences between the studies may account for the lack of effects in this study, in particular, the sample size which was much larger (n=53) in the study by Wiethoff et al. (2014), and differences between the TMS protocols used.

A final point of interest is the finding that even in the 2mA anodal condition, in which there is some evidence of change in cortical excitability, there is substantial variability between participants. At the time of starting this study (in 2013) there was little published research exploring this issue, however, since then a number of articles have been published which show that the effects of tDCS are not as clear cut and polarity specific as initially believed. In particular, the study by Wiethoff et al. (2014) revealed that in a large sample of 53 participants, 50% showed only minor or no response to stimulation. In addition, the direction of responses was mixed, with some participants showing increased excitability in response to both anodal and cathodal stimulation (2mA) and others showing increase following anodal and decrease following cathodal. This

variability is an interesting issue and warrants further exploration if tDCS is to fulfil its potential as both a research tool and therapeutic technique.

Limitations

As with most, the design of this study is not perfect and it cannot be ruled out that methodological issues may have contributed to the findings. In particular, TMS coil placement and the ability of participants to watch films during the study may have contributed.

Although neural navigation software was used throughout the experiment, this involved registering the participants to a template head, as individual anatomical scans were not available. Although useful this process is not exact, and therefore there is a possibility that the TMS coil was not always localized in exactly the same area each time. As a result, this could influence MEP amplitudes and thereby add error into the measure.

Another potential source of variability within the experiment was differences in participant mood and arousal level as a result of the films they chose to watch. Although horror and comedy films were excluded (to limit participants' movements from laughter/ startle responses) participants were broadly allowed to choose what to watch. Previous research has found that viewing highly emotive static picture stimuli (both pleasant and unpleasant) increases MEP amplitudes in comparison to neutral stimuli (Coombes et al., 2009; Hajcak et al., 2007). It is, therefore, possible that some variability within the data could relate to the material participants were viewing, although the effects of such viewing material are likely to differ from the static, high valence images used in previous studies. In addition, it is not clear if the effects of fatigue, boredom and natural changes in emotional state would have had an impact had there not been something for participants to watch and engage in.

Conclusions

With the exception of the 2mA anodal condition, the study largely failed to replicate previous findings. Unlike past research, 1mA anodal tDCS did not significantly increase cortical excitability, nor did 1mA cathodal stimulation decrease it. The study also failed to replicate the increase in cortical excitability found by Batsikadze et al. (2013) following 2mA cathodal stimulation. As the methods used are not identical to previous published works it cannot be ruled out that methodological issues contributed to the lack of significant effects. However, thorough inspection of the data revealed high variability with regard to the direction and magnitude of change seen for each individual. This is even apparent when the effects are significant at a group level (see **Figure 3.4**). It is, therefore, suggested that natural variability occurring between participants may also have contributed to the lack of effects. This issue is explored in depth in the next chapter.

Chapter 4: Exploring intra and inter-subject reliability and stability in response to tDCS

Some of this work has been previously reported in: Dyke, K., Kim, S., Jackson, G. M., & Jackson, S. R. (2016). Intra-Subject Consistency and Reliability of Response Following 2 mA Transcranial Direct Current Stimulation. *Brain Stimulation* 9(6), 819-825.

Key words: *transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), resting motor threshold (RMT), input output curve (IO curve), short interval intracortical inhibition (SICI), long intracortical inhibition (LICI), intracortical facilitation (ICF), intra-subject variability, inter-subject variability.*

4.1 Introduction

Chapter 3 reported studies in which the effects of various tDCS parameters were explored using single pulse TMS. These studies failed to replicate previous findings of polarity specific effects following 1mA stimulation (Nitsche et al., 2005), and also were unable to replicate the reversal of cathodal effects when delivered at 2mA (Batsikadze et al., 2013). The only significant effect observed was the finding that 2mA anodal stimulation significantly increased cortical excitability 30 and 60 minutes following tDCS application.

In tDCS studies a number of factors are known to influence outcomes, including the intensity and duration of stimulation used (Batsikadze et al., 2013; Monte-Silva et al., 2013), the measures used to quantify effects (Horvath, 2014; Jacobson et al., 2012) and the electrode montage used (Miranda et al., 2006; Nitsche & Paulus, 2000). Although these factors are important, they cannot explain the variability found within studies. For example, Wiethoff et al. (2014) found substantial inter-subject variability in response to both 2mA anodal and cathodal stimulation. The studies discussed in Chapter 3 also show high variability between individuals, even when the group level effects are significant.

This issue of variability between participants in response to stimulation is not unique to tDCS. Variability has also been reported in response to other non-invasive brain stimulation methods such as Paired Associative Stimulation (PAS) and intermittent Theta Burst Stimulation (iTBS) (Fratello et al., 2006; Hamada et al., 2013; Lopez-Alonso et al., 2015; Muller-Dahlhaus et al., 2008). Given individual differences in anatomy, including skull shape, thickness and density, and additional factors such as baseline neuronal states, it is perhaps not surprising that these techniques do not yield identical results across individuals. However, developing an understanding of the factors that may predict this variability is a critical step in increasing the reliability and usefulness of such techniques for use within both research and therapeutic contexts.

To date, a number of inter-subject factors have been identified as influencing tDCS including: anatomical structure (Bikson et al., 2012; Datta et al., 2012), age (Fujiyama et al., 2014; V Moliadze et al., 2014), and potentially even genetic profile (for review see Li et al. (2015)). These factors can be useful in explaining differences occurring between participants, however, less is known about the reliability of the effects within individuals. A few notable studies have explored these issues for anodal stimulation at 1mA (Horvath et al., 2016; Lopez-Alonso et al., 2015) and at 0.5mA (Chew et al., 2015). Interestingly, although 1mA anodal tDCS was found to have a reasonable level of inter-subject reliability (Lopez-Alonso et al., 2015) the same was not true of 0.5mA (Chew et al., 2015). Furthermore, even when reliability has been explored using the same intensity the results are conflicting. Horvath et al. (2016) found that reliability across sessions was poor, which contrasts with the findings of Lopez-Alonso et al. (2015). It is possible that the differences between the studies reflect the different methodologies used. A few notable and potentially influential differences include electrode size, duration between testing sessions, duration of stimulation, use of neural-navigation and the amount of sessions tested. Less research exists exploring the reliability of cathodal stimulation, with the notable exception of a study at 1mA by Horvath et al. (2016) in which consistency was found to be poor.

Of the three studies which explored intra-subject consistency, only one reported the effects of tDCS on paired pulse TMS measures. These measures can provide more specific information about the biological underpinnings of tDCS effects. For example, while MEP amplitudes evoked from relatively high TMS intensities may reflect global excitability (combination of inhibitory and facilitatory outputs) measures such as short interval intracortical inhibition (SICI) can give specific information about the engagement of GABAergic mechanisms, in particular effects occurring at GABA-A receptor sites. Interestingly, the one study which did explore the effects of SICI failed to replicate previous findings in which SICI is reduced following anodal stimulation (Batsikadze et al., 2013; Cengiz et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005). Lopez-Alonso et al. (2015) found that 1mA anodal tDCS did not significantly alter SICI. However, the amount of change in this measure was found to have some consistency between two testing sessions when measured 6 minutes but not 46 minutes following anodal stimulation.

As previously discussed, one of the parameters known to influence the effects of tDCS is the intensity at which it is applied. At present, the maximum used in humans is often 2mA, and although considered safe (Fregni et al., 2014), the effects at this intensity are less well explored than those resulting from 1mA applications. However, the use of 2mA intensities in both therapeutic and research contexts is not uncommon, and for many, the idea of using a 'stronger dose' of stimulation may be appealing. In fact, the assumption that a stronger intensity may yield stronger changes in cortical excitability is tentatively supported by the findings in Chapter 3, in which it was possible to see some change in excitability following 2mA but not 1mA anodal stimulation.

This study aimed to explore inter and intra-subject variability using a repeated measures design in which participants experienced multiple sessions of 2mA tDCS applied to the motor cortex (M1)for 20 minutes. Changes in cortical excitability were assessed using the following single and paired pulse TMS measures: IO curves, resting motor thresholds (RMT), short interval intracortical

inhibition (SICI), intracortical facilitation (ICF) and long interval intracortical inhibition (LICI). The TMS parameters used in the study were informed by the previous work in Chapter 3 and also by a specially conducted pilot study.

4.2 Pilot

The aim of the pilot study was to identify the optimal paired pulse parameters which reliably showed inhibition/ facilitation without producing ceiling effects. As a result, the optimal parameters were not always the ones showing the largest change in MEP as this would have limited the ability to see the influence of tDCS on those measures.

The three main factors influencing the effects of paired pulse protocols are the inter stimulus interval (ISI), the conditioning stimulus (CS) and the test stimulus (TS). Overall there is strong consensus that when a subthreshold CS is applied before a suprathreshold TS the result will be increased inhibition if the ISI was 1-5ms, and increased facilitation with ISIs of 8-30ms (O'Shea & Walsh, 2007). Choosing the optimal CS and TS parameters to elicit these effects is difficult due to the wide range of intensities which could be used and methodological factors such as the use of Resting (RMT) or Active Motor Thresholds (AMT).

Much of the previous literature utilizing SICI and ICF protocols has used suprathreshold TS of SI 1mV or 120%RMT. Although the intensity of the TS is known to influence the magnitude of SICI effects (Garry & Thomson, 2009; Ilic et al., 2002), intensities that yield MEPs of 1mV (SI 1mV) or are delivered at 120% RMT are commonly used; and there is evidence to support that these intensities are optimal. For example, Garry and Thomson (2009) found that CS intensities of 110-120%RMT yield the largest inhibitory effects when ISIs of 1-5ms were used. It was beyond the scope of this pilot study to explore both the effects of TS and CS on the different protocols, therefore, it was decided that the TS would remain fixed at SI 1mV and that the optimal CS intensity would be explored. SI 1mV was used rather than 120% RMT in line with previous key studies investigating the

effects of tDCS on paired pulse protocols (Batsikadze et al., 2013; Nitsche et al., 2005).

Previous research with SICI has revealed that when 3ms ISIs are used the relationship between CS intensity and the inhibitory effects appears to form a U-shaped pattern (Kossev et al., 2003; Peurala et al., 2008), with CS intensities of 60-75% RMT reportedly producing the largest inhibitory effects (Kossev et al., 2003). CS intensities ranging from 70-80% RMT have been traditionally used (for example: (Kujirai et al., 1993; Roshan et al., 2003), however, it is not easy to find a general consensus as some researchers prefer to use a percentage of active rather than resting threshold (for example, Batsikadze et al. (2013)). With regard to SICI occurring using ISIs at and around 1ms, there is less information available. However, there is some evidence that the optimal CS intensities for this distinct measure are lower than those used in later SICI (Fisher et al., 2002; Vucic et al., 2009). In this pilot study the effects of CS intensity on 1ms and 3ms SICI were explored. Percentages of RMT were used throughout.

This pilot study also explored the effects of CS intensity on ICF measurements using ISIs of 10, 12 and 15ms. Previous research suggests that CS intensities of 70% AMT are effective when measuring ICF with ISIs of 10 and 15ms (Batsikadze et al., 2013; Nitsche et al., 2005); but also that CS intensities of 70, 80 and 85% RMT are effective when 13ms ISIs are used (Kossev et al., 2003).

The final paired pulse measure explored in the pilot study was LICI. The effects of LICI are thought to be reliably produced by ISIs of 100ms (McDonnell et al., 2006; Sanger et al., 2001), and can be found using a range of suprathreshold CS intensities (Sanger et al., 2001). Unlike in SICI and ICF protocols the intensities used for both CS and TS in LICI can be identical as both are required to be suprathreshold. In this pilot the effects of various suprathreshold CS intensities were explored using an ISI of 100ms and a TS set at SI 1mV. Few studies have reported investigating the effects of tDCS using this measure, and when this has been done it has been with patient populations (Antal et al., 2010) which may differ to healthy controls. LICI effects can reach ceiling level rapidly, therefore, a

key aim of this pilot was to identify CS intensities which would avoid this problem.

Pilot Method

Participants

Five healthy participants (2 male, 3 female) with a mean age of 26 years (range 25-27) took part in this study. Participants were right handed and free of any medications or counter indications to TMS.

Design

The pilot experiment was designed to select the optimal parameters for paired pulse measures. This was done by examining the effects of a range of TMS protocols on MEP amplitudes. Participants completed all conditions with the measures for ICF 12 and 15 being completed in a secondary testing session.

TMS delivery and recording of MEPs

TMS was delivered using Magstim 200 stimulators connected via a Bistim module (Magstim, Whiteland, Dyfed, UK) through a figure of eight magnetic coil (diameter of one winding 70mm). The coil was held tangentially to the scalp and positioned 45° from the midline. The optimal location for stimulation ('hot spot') of the contralateral FDI muscle was defined as the location over the left motor cortex which when stimulated, consistently resulted in the largest MEP. To keep the location of the coil stable the neural navigation software Brainsight (Rogue Research Inc., Montreal Quebec, Canada) was used. Anatomical brain scans were not available for the participants, therefore, the location was mapped to a template head (constructed from a consenting individuals anatomical scan), which was registered to anatomical landmarks on each participant. This method allowed coil placement with reasonable accuracy although it was compromised slightly by the use of a template. Consequently, the coil location was also marked in pen onto a swimming cap which the participants wore throughout the study. During stimulation participants' head positions were kept stable with the aid of a chin rest. The TMS coil was held in place using a Manfrotto mechanical arm

(Vitec Group, Italy). Participants were asked to remain as still as possible during testing, but were offered frequent breaks to stretch and adjust their position.

MEPs were recorded using disposable, Ag-AgCl surface electrodes attached to the right FDI muscle in a belly tendon montage. The signals were amplified and bandpass filtered (10 Hz- 2kHz, sampling rate 5kHz) and digitized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position throughout testing.

All trials were triggered using an in-house software program (written using Matlab: Mathworks, MA, USA). Paired pulse and single pulse trials were intermixed to limit any effects of presentation order from occurring.

TMS parameters

Resting motor threshold (RMT)

Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP with a peak-to-peak amplitude of >50μV in the relaxed FDI muscle, in a minimum of 5 of 10 trials.

Stimulus intensity needed for 1mV MEP amplitude (SI 1mV)

A 1mV (SI 1mv) threshold was also determined by calculating the lowest intensity needed to evoke an MEP of 1mV in 5 of 10 consecutive trials.

Short Interval Intracortical Inhibition (SICI)

Two ISIs were tested, 1ms and 3ms. The conditioning stimulus (CS) was delivered at one of nine intensities ranging from 40-80% RMT in 5% increments. The test stimulus (TS) was delivered at SI 1mV. Each condition was tested 10 times, hence, 90 trials were used to test 1ms ISI and an additional 90 trials were used to test 3ms ISI.

Intracortical Facilitation (ICF)

ICF was tested using ISIs of 10, 12 and 15ms. Conditioning pulses were delivered at one of nine intensities between 40% and 80% of the individual's

RMT in 5% increments. Each condition was tested 10 times and 90 trials were used in total to study each ISI.

Long Interval Intracortical Inhibition (LICI)

A single ISI of 100ms was investigated using one of seven conditioning pulses ranging from 100-130% RMT in 5% increments. The test pulse was SI 1mV. Each condition was repeated 10 times, hence a total of 90 trials were used to measure LICI.

Unconditioned trials

A total of 60 trials were measured at SI 1mV in the first experimental session. A further 30 unconditioned trials were measured in the second session in which ICF was measured with ISIs of 12 and 15ms.

Procedure

After gaining informed consent, participants were seated in a comfortable chair with their head positioned in a chin rest and their right hand placed in a relaxed position in front of them. The location of the participant's head was then registered to a computation template using the Brainsight system (Rogue Research Inc., Montreal Quebec, Canada), and disposable electrodes were attached to the hand. The hotspot for FDI stimulation was then identified using TMS, which was then mapped onto the template brain to aid coil localization and marked lightly onto the swimming cap that participants wore throughout. RMT and SI 1mV were then determined. The various paired pulse measures were then collected in a randomized order. Exactly the same procedure was followed for the second session of testing in which ICF with 12 and 15ms ISIs was collected in addition to 30 unconditioned trials.

Analysis and Statistics

Peak-to-peak amplitude of MEPs evoked by the TS were measured using in-house software (programed using Matlab, Mathworks, MA, USA). Any trials in which there was evidence of pre-contraction of the FDI muscle were excluded. Median MEP sizes were calculated for each CS at each respective ISI. The resulting values were then normalized to the respective median of unconditioned trials to create a ratio.

Repeated measures ANOVAs were used to identify any significant effects of CS intensity on MEP amplitude for each TMS measure (1ms SICI, 3ms SICI, 10ms ICF, 12ms ICF, 15ms ICF and 100ms LICI). CS intensity served as a within subjects independent factor whereas ratio of change in MEP amplitude (CS/TS) served as the dependent factor. Any significant effects were explored further using one sample t-tests in which normalized values were compared to a value of no change (1). Data are not corrected for multiple comparisons and should therefore be interpreted with a degree of caution.

Pilot Results

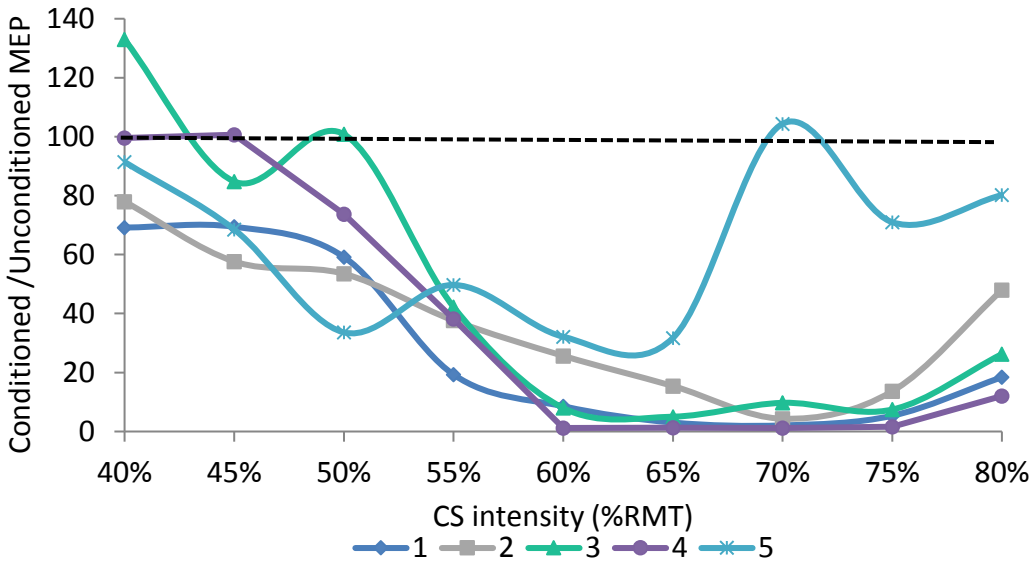


Figure 4.1. *Normalized values showing median change in MEP amplitude for 1ms SICI protocol. Black dotted line indicates no change in amplitude from unconditioned pulses. Each colour represents an individual participant.*

A repeated measures ANOVA revealed that CS intensity significantly influenced MEP amplitude in the **1ms SICI** condition $F(8,32) = 8.51$, $p = 0.00$. One sample T-tests revealed significant inhibitory effects at CS intensities of 45% RMT $t(4) = -3.18$, $p = .03$; 50%; 50% RMT $t(4) = -3.20$, $p = .03$; 55% RMT, $t(4) = -12.48$, $p = .00$; 60% RMT, $t(4) = -14.47$, $p = .00$; 65% RMT $t(4) = -15.65$, $p = .00$; 70%r RMT $t(4) = --3.77$, $p = .02$, 75% RMT $t(4) = -6.2$, $p = .00$ and 80%RMT $t(4) = 5.09$, $p = .01$. Data is shown in Figure 4.1.

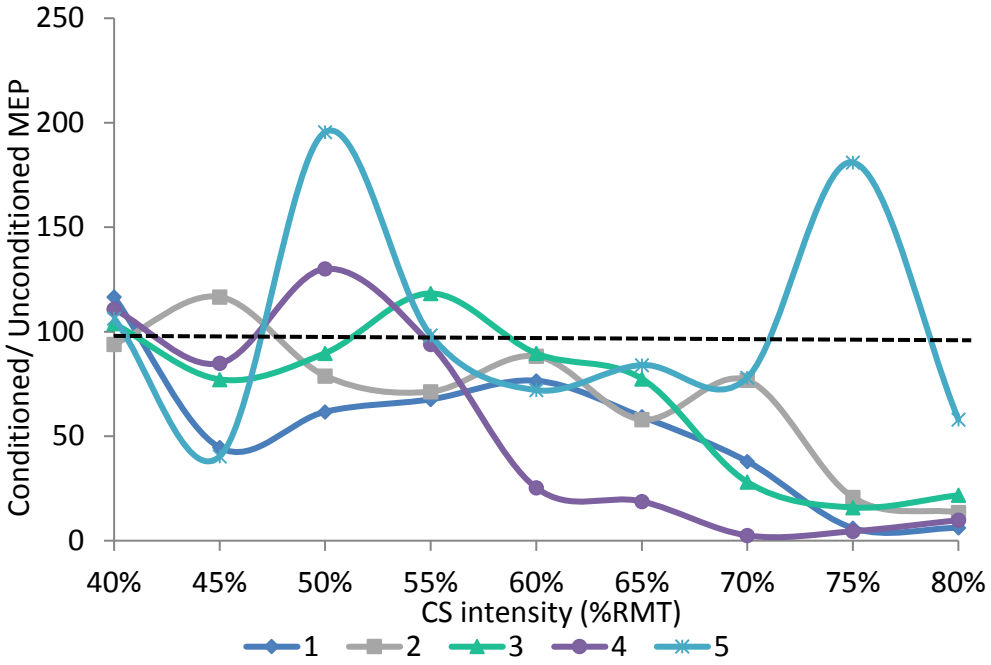


Figure 4.2. *Normalized values showing median change in MEP amplitude for the 3ms SICI protocol. Black dotted line indicates no change from unconditioned pulses. Each colour represents an individual participant.*

A separate repeated measures ANOVA revealed that CS has a significant impact on the data in the **3ms SICI** condition $F(8,32) = -3.74$, $p = .00$. One sample t-tests revealed that a CS intensity of 65% RMT yielded a significant reduction in MEP size $t(4) = -3.56$, $p = .02$, as did 70% RMT $t(4) = -3.8$, $p = .02$ and 80% RMT $t(4) = -8.31$, $p = .00$. Relevant data are shown in Figure 4.2.

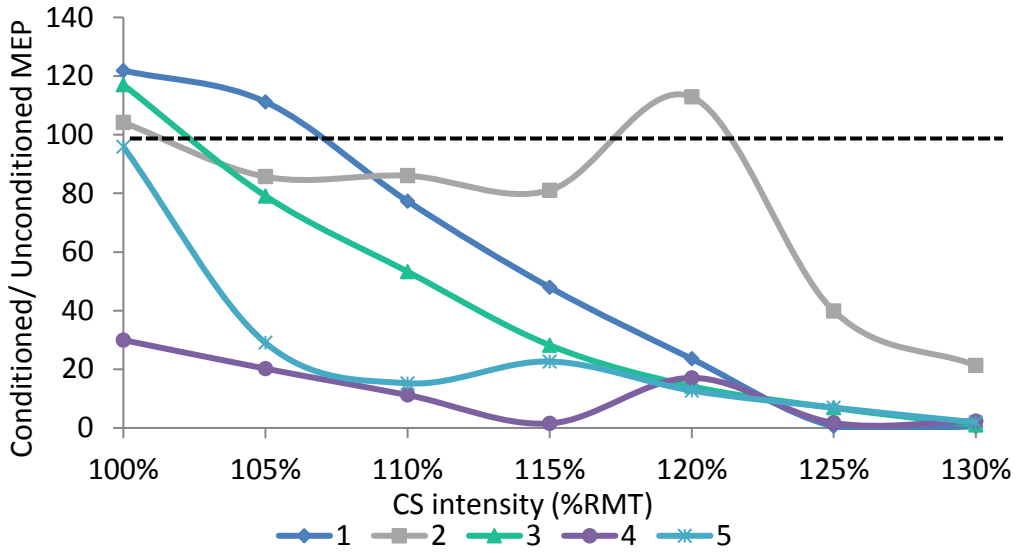


Figure 4.3 *Normalized values showing median change in MEP for 100ms LICI protocol. Black dotted line indicates no change from unconditioned pulses. Each colour represents an individual participant*.

CS intensity was found to have a significant effect on MEPs in the **100ms LICI** condition $F(6,24) = 9.689$, $p = .000$ (Figure 4.3). One sample t-tests revealed significant differences between unconditioned and conditioned MEP sizes at CS intensities of 110% RMT $t(4) = -3.33$, $p = .029$; 115% RMT $t(4) = 4.75$, $p = .009$; 120%RMT $t(4) = 3.31$, $p = .030$; 125%RMT $t(4) = 12.18$, $p = .000$; and 130%RMT(4)=23.69, $p = .000$.

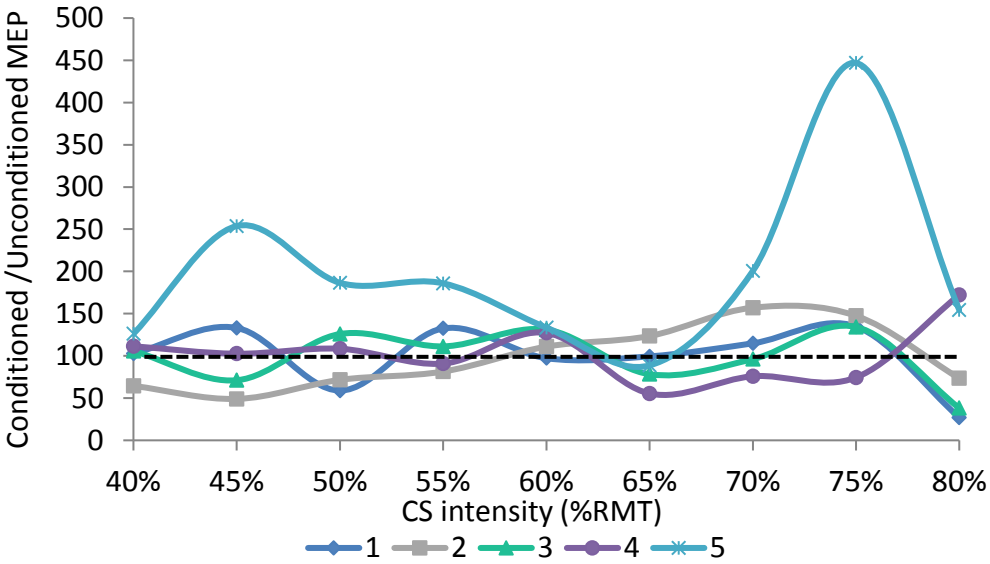


Figure 4.4. *Normalized values showing median change in MEP amplitude for 10ms ICF protocol. Black dotted line indicates no change from unconditioned pulses. Each colour represents an individual participant*.

No significant differences were found between MEP amplitudes evoked from different CS intensities in the **10ms ICF** condition F(8,32) =.881, *p*=.4 (Figure 4.4). Further explorative analysis using one sample t-tests revealed a significant facilitator effect of CS intensity of 60% RMT *t*(4)=2.895, *p*=.044. No other significant effects were identified.

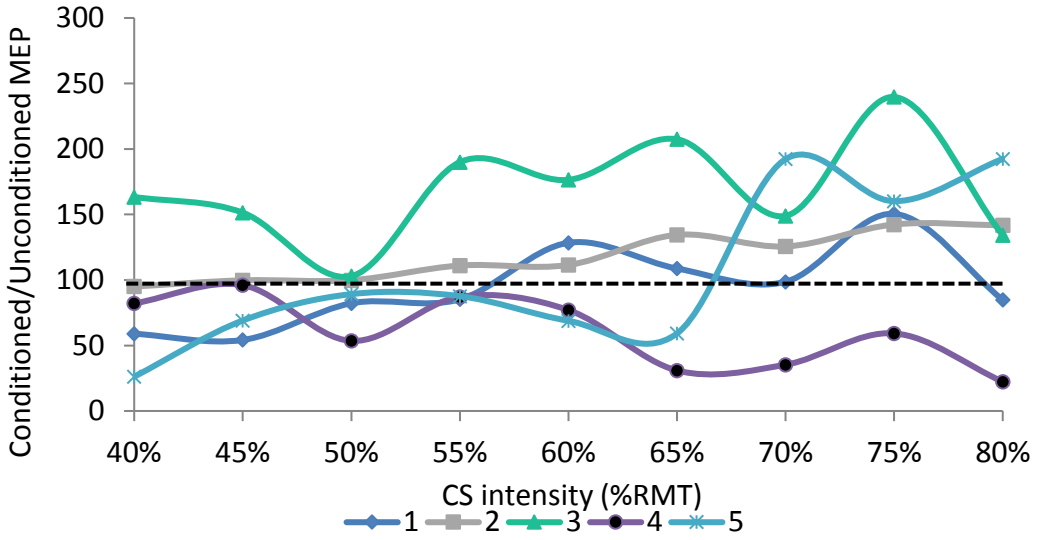


Figure 4.5. *Normalized values showing median change in MEP amplitude for 12ms ICF protocol. Black dotted line indicates no change from unconditioned pulses. Each colour represents an individual participant*.

12ms ICF was not found to be significantly influenced by CS intensity *F*(8,32)=1.504, p=0.195 (Figure 4.5).

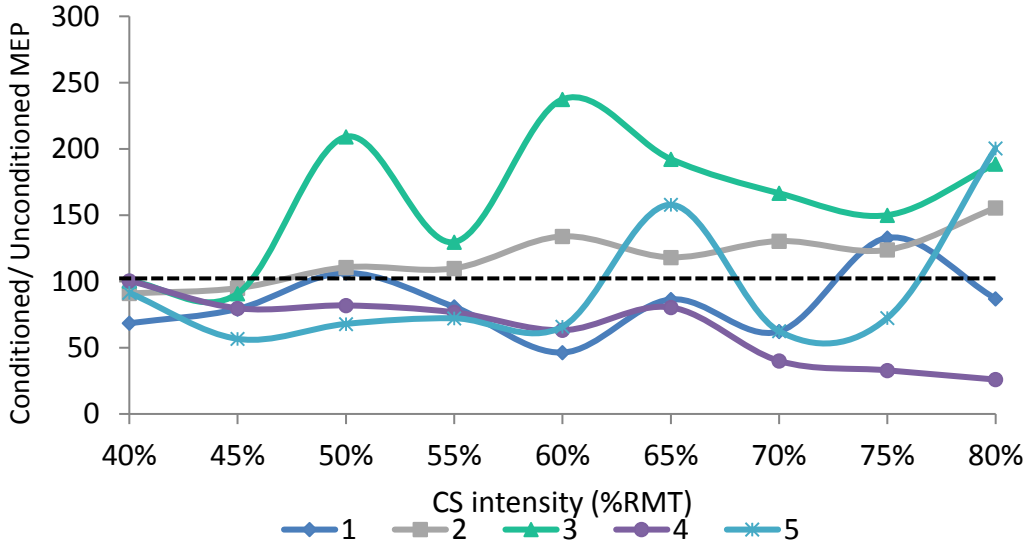


Figure 4.6 *Normalized values showing median change in MEP amplitude for 15ms ICF protocol. Black dotted line indicates no change from unconditioned pulses. Each colour represents an individual participant.*

15ms ICF was also not found to be significantly influenced by CS $F(8,32)=1.18$, $p=0.34$ (Figure 4.6).

Discussion of pilot data

A wide range of CS intensities were found to lead to significant inhibition of MEPs for 1ms SICI and 100ms LICI. The range of CS intensities capable of leading to significant inhibition evoked from 3ms SICI was slightly narrower, and required higher CS intensities than the effects of 1ms SICI. The finding that 1ms SICI requires lower CS intensities to be effective is in agreement with previous findings (Fisher et al., 2002; Vucic et al., 2009). Although the sample size is very small this could be seen as further evidence to suggest that the mechanisms underlying 1ms and 3ms SICI effects differ.

Unlike Kossev et al. (2003) 3ms SICI did not show a U-shaped pattern of response in the pilot study. This is probably due to methodological differences between the experiments. In particular, the TS used in this experiment was 1mV whereas in the previous studies 120% RMT was used. In addition to this, Kossev et al. (2003) measured CS intensities of up to 85% RMT which is closer to threshold than the 80% used here. It seems likely that if a wider range of CS intensities were measured a U-shaped pattern would have emerged; this is almost apparent in the 1ms SICI condition.

ICF measured using ISIs of 10, 12 and 15ms were not found to lead to significant facilitation. This was unexpected as these ISIs have previously been found to be effective (Kujirai et al., 1993; Ziemann, Rothwell, et al., 1996) and have been used within tDCS literature to demonstrate alterations in cortical excitability following stimulation (Batsikadze et al., 2013; Nitsche et al., 2005). Batsikadze et al. (2013) found that 2mA anodal and cathodal tDCS significantly increased facilitation using 10ms ICF whereas 15ms ICF was unaffected. As this pilot study revealed no clear differences between the ISIs tested, 10ms ICF was selected to be used in the main study based on this literature. Visual inspection of graphical data presented by Batsikadze et al. (2013) shows only a small effect

of ICF at baseline, yet clear changes following tDCS are visible therefore even if ICF does not reveal powerful effects at baseline it may still be possible to measure tDCS induced changes.

The pilot study was designed to select the optimal parameters for use in subsequent research into the effects of tDCS. In order to avoid confounds of ceiling effects the optimal parameters for SICI and LICI cannot be those which show absolute inhibition of MEP responses. On this basis CS intensities of 40, 45, 50 and 55% RMT were used to measure 1ms SICI; intensities of 60, 65, 70 and 75% RMT were used to measure 3ms SICI and CS intensities of 65, 70 and 75% RMT were used to measure 10ms ICF. In order to reduce the amount of total pulses used in the experiment only one CS intensity of 115% was used to measure LICI.

4.3 Method

4.3.1 Participants

Ten participants were enrolled in the study, of whom 7 were female. The mean age was 24 ± 4 years. The anodal and cathodal stimulation conditions were completed by all ten participants. The sham stimulation condition was completed by all but one participants (F, 23.4years) who was unable to complete the sham condition due to relocating. All participants were healthy, free from medication and counter indications to TMS. Participants were deemed right-handed using an adapted, shortened, version of the Edinburgh Handedness Inventory (Oldfield, 1971).

4.3.2 Design

A within subjects design was utilized to explore the stability of tDCS effects across a number of sessions. All participants completed anodal and cathodal tDCS conditions; nine also completed the sham condition. The study was initially designed to investigate the stability of 2mA anodal tDCS but was extended to include cathodal and sham conditions. As a result participants

always completed the anodal session first followed by completion of sham and cathodal conditions, the order of which was counterbalanced between participants. On average the study took 6 months for participants to complete all 10 testing sessions. Anodal and cathodal sessions were separated by a minimum of one month (mean separation period 5 months). The participants were blind to the experimental condition, however, for practical reasons the researcher was not. Change in MEP amplitude following tDCS or sham stimulation served as the dependent measure.

4.3.3 tDCS of the motor cortex

tDCS was delivered using a NeuroConn DC- stimulator (GmbH, Ilmenau, Germany) with a maximum stimulation output of 4.5mA. Stimulation was delivered using saline soaked surface sponge electrodes, each measuring 35 cm^2, to the area representing the first dorsal interosseous (FDI) muscle for the right hand (identified using TMS). The reference electrode was located over the contralateral right orbit. For anodal and cathodal stimulation, the current was ramped up to 2mA over 15 seconds, held constant for 20 minutes and then ramped down over a further 15 seconds. By contrast, in the sham condition the current was ramped up to 2mA over a period of 15 seconds, sustained at this intensity for 30 seconds and then ramped down over a further period of 15 seconds. Electrodes were removed during TMS.

4.3.4 TMS measurements and EMG recording

TMS was delivered using a BiStim TMS system (Magstim, Whiteland, Dyfed, UK) with a figure of 8 coil (diameter of one winding 70mm). The coil was held tangentially to the scalp and positioned 45° from the midline resulting in a posterior to anterior current flow. Neural navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used in conjunction with each participant's anatomical brain scan to aid accurate coil placement over the left motor cortex. The coil was moved in small increments within the anatomical target to locate the optimal stimulation site ('hot spot'), which was identified as the location which when stimulated produced the largest MEP amplitude. The

optimal location was tracked using the software and was also marked onto a swimming cap which the participant wore during stimulation. Participants were asked to remain as still as possible during testing with the aid of a chin rest, but were offered frequent breaks to stretch and adjust their position. The coil was held stable over the hot spot using a Manfrotto mechanical arm (Vitec Group, Italy) and adjusted as necessary.

MEPs were recorded using disposable Ag-AgCl surface electrodes attached to the right FDI muscle in a belly tendon montage. The signals were amplified, bandpass filtered (10Hz- 2kHz, sampling rate 5kHz), and digitized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position throughout testing.

Threshold determination

Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP with a peak-to-peak amplitude of >50µV in the relaxed FDI muscle in a minimum of 5 of 10 trials. A 1mV (SI 1mv) threshold was also determined by calculating the lowest intensity needed to evoke an MEP of 1mV in 5 of 10 consecutive trials.

Input Output curves

IO curves were measured using TMS intensities set at 100, 110, 120, 130, 140 and 150% of RMT. Ten pulses at each of the 6 intensities were delivered in a randomized order with 5 seconds occurring between each pulse.

Intracortical inhibition and facilitation

1ms SICI

Subthreshold conditioning stimuli (CS) were delivered at one of four intensities (45, 50, 55, 60% RMT) which were followed after a 1ms interval by a supra threshold test stimulus (TS) of S1 1mV delivered to the same location within M1. Ten trials were measures for each CS- TS pairing.

3ms SICI

Subthreshold CS was delivered at 60, 65, 70 or 75% RMT prior to a suprathreshold TS at S1 1mV that was delivered after an interval of 3ms. Ten trials were measured for each CS- TS pairing.

LICI

A single ISI of 100ms was adopted between a suprathreshold CS of 115% RMT and a TS that was also delivered at 115% RMT. A total of 20 trials were measured.

ICF

One ISI of 10ms was tested. Conditioning pulses were delivered at one of three subthreshold intensities at 65, 70 or 75% RMT and followed by a suprathreshold TS of S1 1mV after 10ms. Each CS-TS pairing was measured 10 times.

Unconditioned trials

A total of 30 unconditioned trials were measured at SI 1mV.

4.3.5 Experimental procedures

During the first testing session the hot spot for FDI stimulation was identified with the aid of an anatomical target displayed using neuro-navigation software. This location was then used to guide coil placement for subsequent sessions, and was only subject to minor adjustments. Following hot spot identification, both RMT and S1 1mV were measured. IO curves and paired pulse measures were then taken, the order of which was counterbalanced within and between subjects. Following the TMS measures of baseline cortical excitability the tDCS electrodes were placed on the scalp and stimulation was applied for 20 minutes. Immediately after tDCS stimulation the electrodes were removed, the TMS coil was replaced, and TMS coil location and thresholds (both RMT and SI 1mv) were checked. If necessary small adjustments to thresholds were made prior to measuring IO curves and paired pulse measures the second time. The

same procedure was completed four times for cathodal and anodal conditions; and twice for the sham condition. Each testing session was separated by a minimum of 3 and a maximum of 4 days, and where possible the time of testing was kept constant within subjects. To maintain relatively constant levels of alertness and arousal throughout testing, subjects watched wildlife documentaries throughout the testing sessions.

4.3.6 Data analysis

Peak-to-peak MEP amplitudes were estimated using in-house Matlab software (Mathworks, MA, USA). All trials in the 500ms period prior to MEP were carefully visually inspected and any trials in which there was evidence of pre-contraction of the FDI muscle were excluded.

IO curve measurements were estimated by calculating the median intra-individual MEP amplitudes for each TMS intensity value (i.e. 100-150% of RMT); linear fits were then applied to the resultant values (mean $R^2 = 0.89$). For alternative slope fitting please see Appendix.iv. Median values were calculated rather than the mean in order to limit the effect of non-standard distribution of individual data. For one participant 150% RMT could not be tested. Slopes were therefore fitted to the available values (i.e. 100-140% RMT).

Paired pulse data was analysed by normalizing median MEP amplitudes evoked from conditioned trials to the respective median of unconditioned trials. Intra-subject median inhibition or facilitation across the various CS intensities was then calculated for each protocol. This method was chosen because average slope fits were poor and highly variable across participants (for example the mean R^2 value for baseline measures in the 3ms SICI anodal condition was R^2=0.63, with a range of R^2=0.12- 0.99).

Repeated measures ANOVAS were calculated for each TMS measure (IO curve slope, 1ms SICI, 3ms SICI, LICI and ICF) with time of testing (pre/post) and experimental session (1-4) entered as within-subject independent factors. Mauchley's test of sphericity was performed and corrections were made using

the Greenhouse-Geisser correction when necessary. *A priori* assessment of baseline threshold differences were carried out using Students *t* tests (paired samples, two tailed, *p <0.05).*

In order to investigate intra-subject reliability of tDCS induced changes, ratios of IO curve slope change were calculated by dividing each individual's post-tDCS IO slope by their baseline (pre tDCS) slope for each session. Ratios of change in the paired pulse measures were also calculated in the same manner. Intra-class correlation coefficient (ICC (2,1)) analysis was then used to explore the reliability of tDCS induced changes over the four sessions for anodal, cathodal and sham data. ICC results are reported based upon Lahey et al. (1983), whereby ICC values of <0.4 are considered to indicate poor intra-class reliability, values >0.4 and <0.59 are fair, values >0.6 and <0.74 are good, and values >0.74 are excellent. Negative ICC values are taken to indicate a lack of reliability within the measure.

4.4 Results

Paired sample t-tests confirmed that baseline values for RMT did not significantly differ between sessions ($p > 0.05$) for anodal (all $t(9) < 1.63$, all $p > 0.14$), cathodal (all $t(9) < 1.13$, all $p > 0.29$) or sham conditions ($t(8)=.610$, $p=0.56$). These details can be seen in Table 4.1.

Paired samples t-tests also confirmed that RMT did not significantly alter from baseline for any session following anodal stimulation (all $p>0.168$), cathodal stimulation (all $p>0.343$) or sham (all $p>0.347$).

Table 4.1. M*ean ± standard deviation of RMT for each condition and testing session.*

	Anodal RMT	Cathodal RMT	Sham RMT
Session 1	45.8 ± 4.6	45.8 ± 4.3	45.6 ± 5.8
Session 2	46 ± 4.4	45.9 ± 4.8	45.8 ±5.8
Session 3	46.2 ± 4.7	46 ± 5.0	Na
Session 4	46.3 ± 4.6	47.1 ± 5.0	Na

4.4.1 Analysis of group effects

Input Output curve slope

For the **anodal** condition a repeated-measures ANOVA revealed a significant main effect of time of stimulation (i.e., Pre vs. Post tDCS), $F(1,9)=5.232$, $p = 0.048$. There was no significant effect of testing session $F(3,27)=2.5$, $p=0.08$, and no significant interaction between these two factors $F(3,27)=0.58$, $p=0.63$. Figure 4.7 shows mean MEP amplitudes at each TMS intensity before and after anodal stimulation.

For the **cathodal** condition a separate repeated-measures ANOVA was calculated. The ANOVA revealed no significant main effects of time of stimulation $F(1,9)=3.491$, $p=0.095$ or of testing session $F(3,27)=0.03$, $p=0.99$, and the interaction between these two factors was not significant $F(3,27)$ 0.53, $p=0.67$. Figure 4.8 shows mean MEP amplitudes at each TMS intensity before and after cathodal stimulation.

For the **sham** condition a repeated-measures ANOVA revealed no significant main effect of time of stimulation, $F(1,8)=.218$, $p=.653$. There was no significant effect of testing session $F(1,8)=.424$, $p=0.533$, and no significant interaction between these two factors $F(1,8)=0.186$, $p=0.678$. Figure 4.9 shows mean MEP amplitudes at each TMS intensity before and after sham stimulation

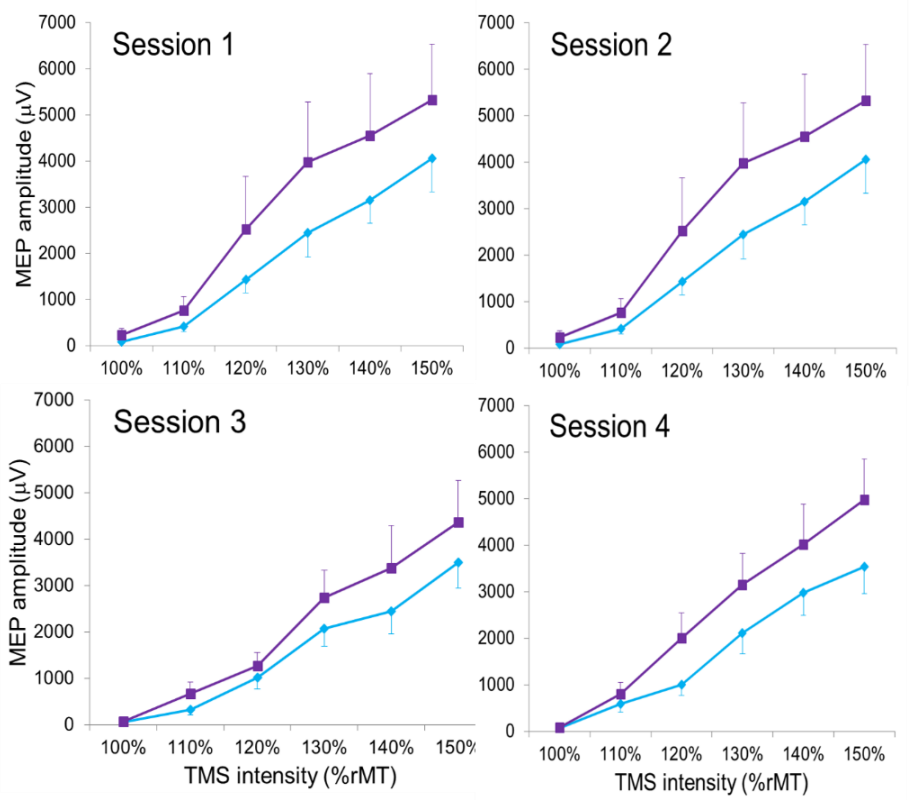


Figure 4.7. *Mean & SEM MEP amplitude pre and post* ***anodal*** *tDCS.*

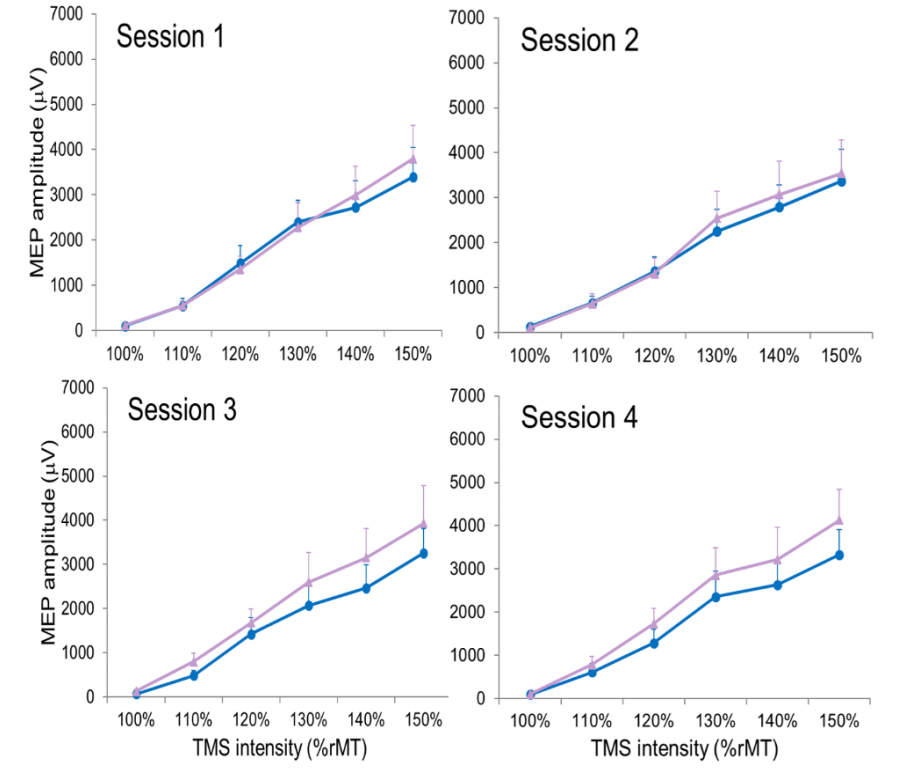


Figure 4.8. *Mean & SEM MEP amplitude pre and post* ***cathodal*** *tDCS.*

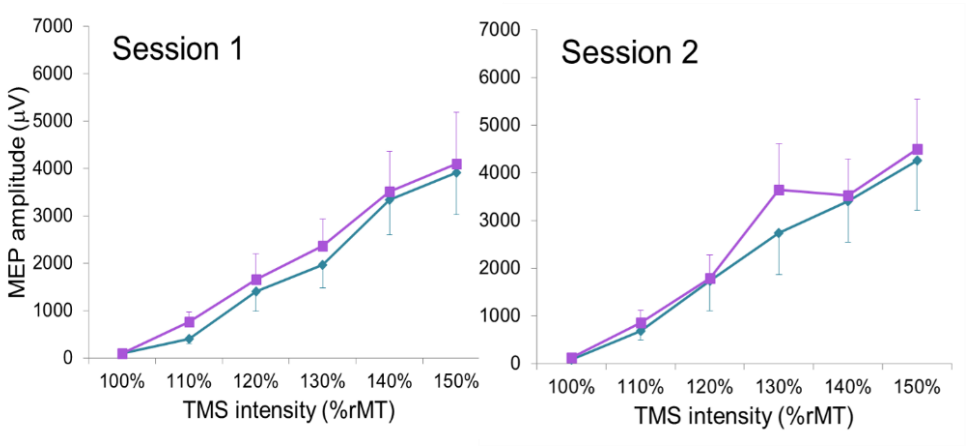


Figure 4.9. *Mean & SEM MEP amplitude pre and post* ***sham*** *tDCS.*

Intracortical facilitation

Anodal tDCS was not found to significantly alter the amount of ICF (Figure 4.10 A). Full results can be seen in Table 4.2.

Repeated measures ANOVA revealed no significant main effect of **cathodal** tDCS on ICF, nor a significant main effect of time (pre/post). However, a significant interaction was revealed. Further exploration using paired samples t-tests revealed a significant difference between pre (*M*=104.1, *SD*=37.0) and post (*M*=133.8, *SD=48.6)* measures in session 4 *t*(9)=-3.01, *p*=0.015. Relevant data can be seen in Figure 4.10 B and Table 4.3.

ICF was not significantly altered in the sham condition on either of the two testing sessions (Figure 4.10C). See Table 4.4 for full results.

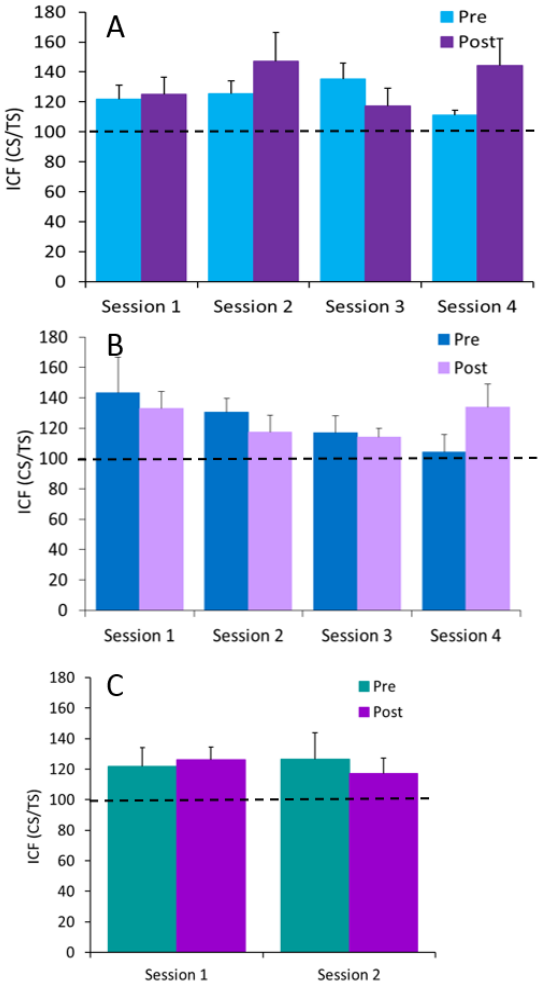


Figure 4.10. *Mean and SEM change in ICF pre/post anodal (A), cathodal (B) or sham (C) stimulation.*

Short interval intracortical inhibition (1 and 3ms)

1ms SICI was not significantly altered by **anodal** tDCS (Figure 4.11 A), no significant main effects or interactions were found. Contrasting with the findings of 1ms SICI, the ANOVA run using the 3ms SICI data (Figure 4.11 B) revealed a significant main effect of measurement time (pre/post) $F(1,9)=7.803$, $p=0.021$. No main effect of session was revealed $F(3,27)=.687$, $p=.568$, however a significant interaction between the two factors was observed $F(3,27)=3.727$, $p=0.023$. Paired sample t-tests revealed a trend towards a reduction in inhibition after tDCS for sessions 1, 2 and 4, however, this difference was only found to be significant for session 4, *t(9)=-4.425, p=.002.* A significant difference between baseline and post anodal inhibition was also found for session 3, however, this showed the opposite pattern of results $t(9)=3.590$, $p= .006$. Further exploration of the data suggests that the results for session three should be interpreted with caution. This is because during this session the average MEP amplitude of the 1mV test pulse differed significantly between baseline (*M*=0.84, *SD=0.76)* and post measures (*M*=1.33, *SD=0.62)* for this session ($t(9)=-2.419$, $p=.039$). TS intensity has been found to significantly influence inhibition caused by SICI (Garry & Thomson, 2009), with lower TS intensities found to yield less inhibition. Therefore, it should be considered that differences between TS intensities in session 3 could be masking any true effects of anodal tDCS.

1ms SICI was not significantly altered by **cathodal** tDCS (Figure 4.11 C). The repeated measures ANOVA revealed no significant main effects nor a significant interaction. 3ms SICI was also not significantly altered (Figure 4.11 D). Full results are shown in Table 4.3 Similarly, the effects of **sham** stimulation also did not significantly alter 1ms or 3ms SICI (Figure 4.11 E, F). See Table 4.4 for full results.

Long interval intracortical inhibition

LICI was found to reach ceiling level and totally abolish the MEP response in 7 of 10 participants during baseline anodal sessions (Figure 4.12). Consequently, the results of LICI were not further analysed as this would make it impossible to identify any significant decreases. As a result of the findings during the anodal testing session the measure was dropped from cathodal and sham conditions.

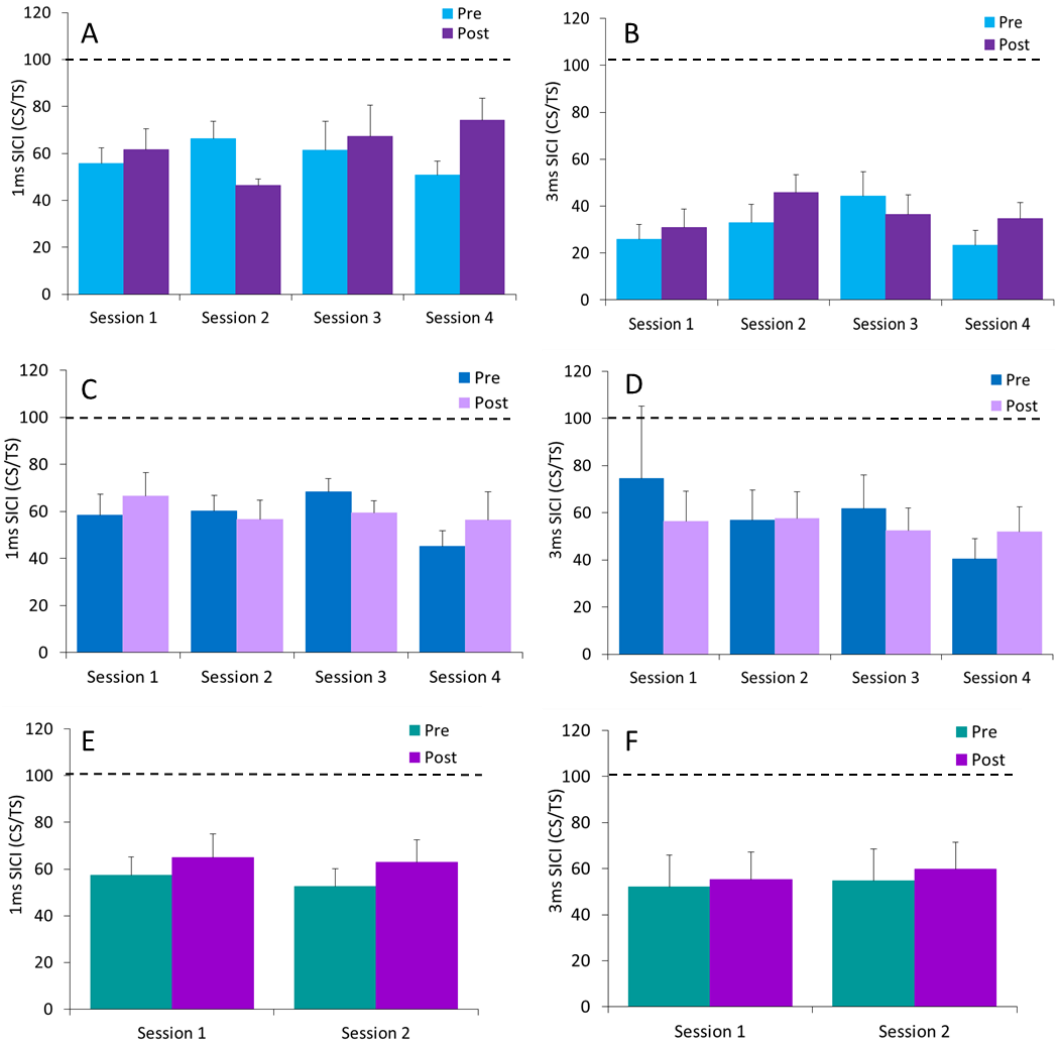


Figure 4.11. *Mean & SEM levels of inhibition for 1ms SICI pre/post anodal (A), cathodal (C) or sham (E) stimulation. Pre/post level of inhibition for 3ms SICI pre/post anodal (B), cathodal (D) or sham (F) stimulation.*

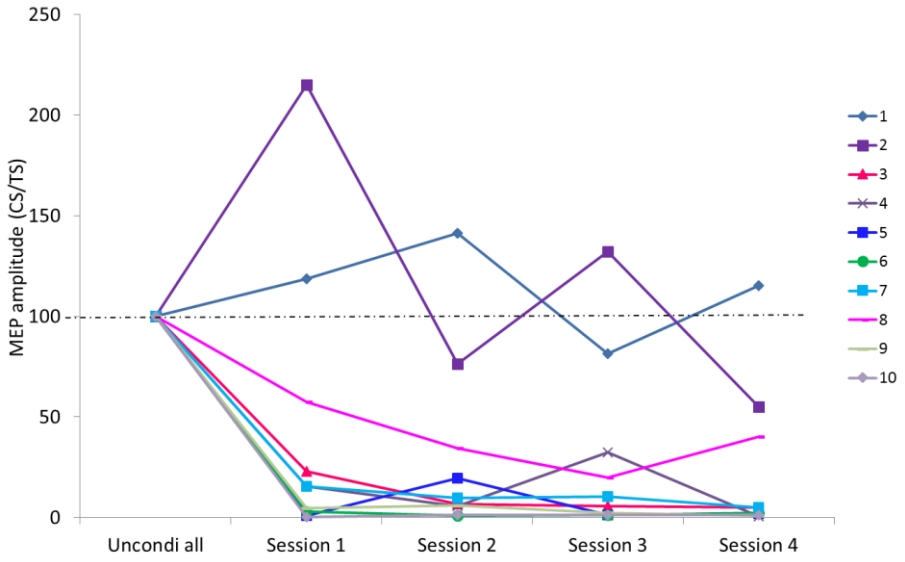


Figure 4.12. *Average inhibition caused by LICI for each participant over each session of pre anodal stimulation. Each colour represents an individual participant. Dashed line illustrates no change from unconditioned stimulus elicited by TS alone (uncondi all).*

Table 4.2. *Summary of Repeated measures ANOVA results for* ***anodal*** *sessions.*

Measurement	Factor	d.f	*F*	*P*	η_p^2
IO curve slope	Session	3	2.505	.08	.218
	Time	1	5.232	.048*	.368
	Session*Time	3	.583	.631	.061
1ms SICI	Session	1.5	.190	.902	.021
	Time	1	1.897	.202	.174
	Session*Time	3	2.038	.132	.185
3ms SICI	Session	3	.687	.568	.071
	Time	1	7.803	.021*	.464
	Session*Time	3	3.727	.023*	.293
ICF	Session	3	.761	.526	.078
	Time	1	.770	.403	.079
	Session*Time	1.6	.2.321	.142	.205

Table 4.3. *Summary of Repeated measures ANOVA results for **cathodal** sessions.*

Measurement	Factor	d.f	*F*	*P*	η_p^2
IO curve slope	Session	3	.030	.993	.003
	Time	1	3.491	.095	.279
	Session*Time	3	.525	.669	.055
1ms SICI	Session	3	1.751	.180	.163
	Time	1	.346	.571	.037
	Session*Time	3	1.679	.195	.157
3ms SICI	Session	1.3	1.140	.326	.112
	Time	1	.284	.607	.031
	Session*Time	1.3	1.577	.218	.149
ICF	Session	3	1.274	.302	.124
	Time	1	.011	.919	.001
	Session*Time	3	3.295	.036*	.268

Table 4.4. *Summary of Repeated measures ANOVA results for **sham** sessions.*

Measurement	Factor	d.f	*F*	*P*	η_p^2
IO curve slope	Session	1	.424	.533	.050
	Time	1	.218	.653	.027
	Session*Time	1	.186	.678	.023
1ms SICI	Session	1	1.643	.236	.170
	Time	1	2.366	.163	.228
	Session*Time	1	.058	.815	.007
3ms SICI	Session	1	.254	.628	.031
	Time	1	.990	.349	.110
	Session*Time	1	.045	.837	.006
ICF	Session	1	.043	.841	.005
	Time	1	.079	.786	.010
	Session*Time	1	1.33	.282	.143

4.4.2 Analysis of inter-subject reliability

To investigate the intra-subject reliability of the effect of anodal, cathodal and sham tDCS on the slope of each participant's IO curve, an intra-class correlation coefficient (ICC) analysis was conducted, based upon the ratio of post-tDCS and pre-tDCS slopes, separately for each stimulation. For anodal stimulation, the change in IO curve slope was found to be poorly related across the four separate testing sessions, *ICC(2,1)* = 0.28 . Relevant data are presented in Figure 4.13A. When the same analyses were conducted for cathodal tDCS the ICC analysis revealed poor interclass reliability, *ICC(2,1)* = 0.04, Relevant data are presented in Figure 4.13B . ICC analysis of the sham condition revealed fair interclass reliability across the two testing sessions *ICC*(2,1)=.44. Relevant data are shown in Figure 4.13C.

To allow for more accurate comparisons to the sham condition ICC was also calculated using the first two sessions for the 9 participants who completed all conditions. For anodal stimulation this revealed a poor reliability *ICC*(2,1)=0.1, poor reliability was also found for the cathodal condition ICC(2,1)=0.35.

Change in 1ms SICI was not reliable across the different anodal testing sessions *ICC*(2,1)=.02. Nor was it reliable across cathodal sessions *ICC*(2,1)=-0.15; or sham sessions *ICC*(2,1)=-0.21. Change in 3ms SICI was also found to be unreliable across the different anodal testing sessions *ICC*(2,1)=0.33; cathodal sessions *ICC*(2,1)=0.23 and sham sessions *ICC*(2,1)=0.06. ICC analysis of the ICF data revealed no reliable changes across sessions following anodal stimulation *ICC*(2,1)=0.37; cathodal stimulation *ICC*(2,1)=0.10; or sham *ICC*(2,1)=0.01.

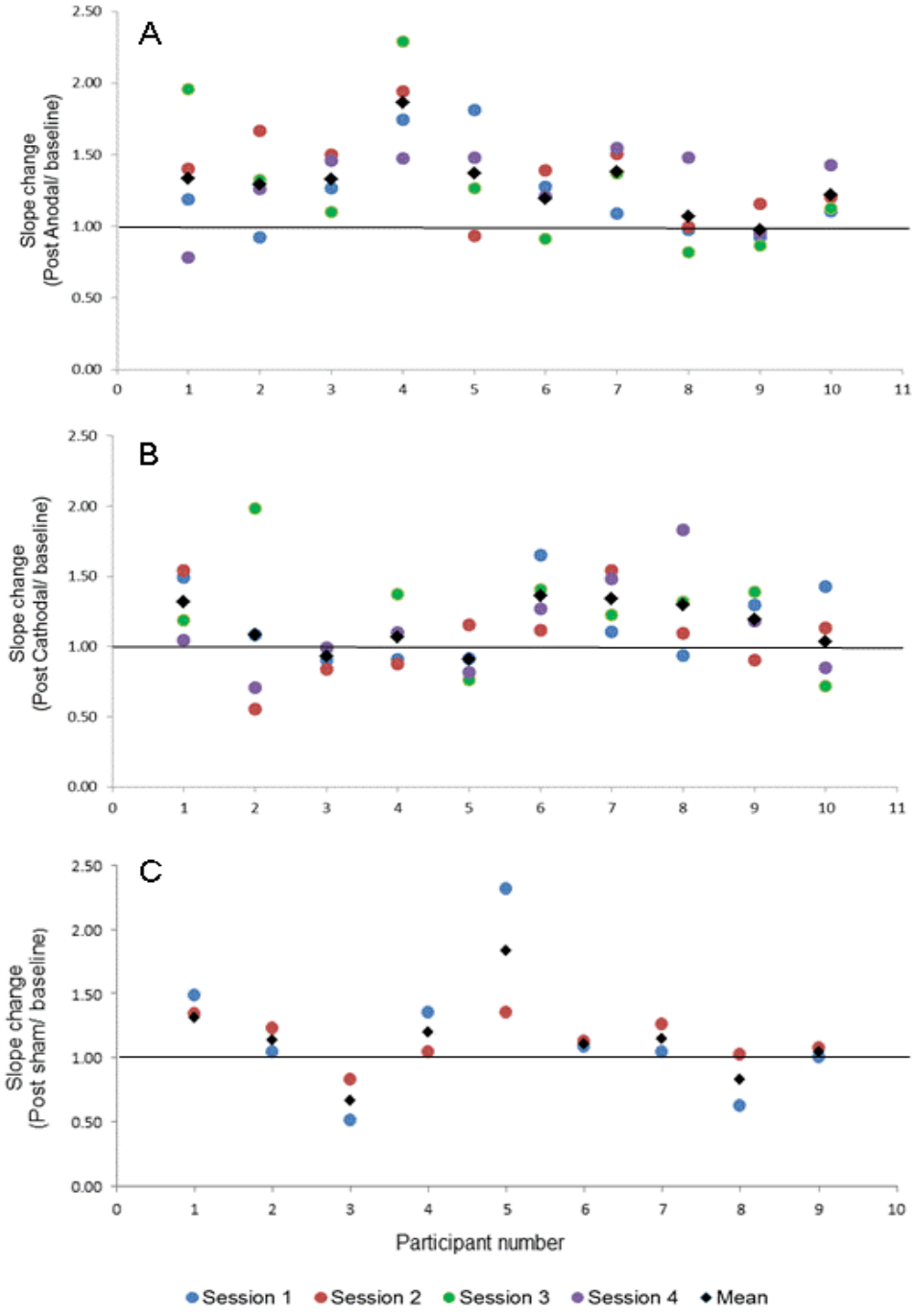


Figure 4.13. *Amount of change in IO curve slope (pre/post) for each participant following (A) anodal, (B) cathodal and (C) sham stimulations. Each coloured data point represents a single session, black diamonds indicate mean change. Horizontal line indicated no change from baseline.*

4.5 Discussion

This study investigated the reliability and consistency of the effects of 2mA tDCS by assessing changes induced in a number of single and paired pulse TMS measures over repeated testing sessions. The effects of anodal, cathodal and sham stimulations were assessed at group level and also individually across the multiple testing sessions. For clarity, the results of the single pulse (IO curve) and paired pulse measures (SICI, ICF, LICI) will be discussed separately.

tDCS effects on single pulse (IO curve) measures of cortical excitability

The results demonstrate a group level increase in IO curve slope following anodal tDCS, but no significant change in this measure following cathodal or sham stimulation. IO curves are thought to reflect the strength of cortico-spinal projections (Chen, 2000) and the balance of inhibitory and facilitatory processes. Therefore, the finding of increased IO curve slopes following anodal tDCS is a good indication of increased cortical excitability overall, and a finding which has also been reported by others (Nitsche & Paulus, 2000; Nitsche et al., 2005). Interestingly, although this effect was signficant at a group level, individual analysis of the data for each testing session revealed variability both within and between subjects in response to the stimulation, with ICC anlysis suggesting the effect to be of poor reliability within individuals. The effects of cathodal tDCS were also found to show poor reliability across sessions, whereas the sham condition revealed moderate stability.

It should be noted that the above findings and conclusions are restricted to the effects occurring within a 30 minute period after 2mA tDCS stimulation was applied to the motor cortex for 20 minutes. It is possible that the lack of effects in the cathodal condition are resultant of the time the measurements were taken. This is because previous research (Batsikadze et al., 2013) has reported a significant change in excitability which only became apparent after 90 minutes. Batsikadze et al. (2013) inferred change in cortical excitability by a change in MEP amplitude from a single intensity rather than in an IO curve

measurement, therefore, timing is not the only difference between their study and this one. However, the differences between the measures used are not necessarily problematic. This is because IO curves measure the change in MEP amplitude over a range of TMS intensities and would, therefore, encompass those measured using a single intensity such as that used by Batsikadze et al. (2013).

The results reported by Batsikadze et al. (2013) are counter to previous studies reporting the effects of cathodal tDCS on motor excitability insofar as they reported that cortical excitability, as indexed by MEP amplitude, was increased following 2mA cathodal tDCS whereas previous studies of cathodal tDCS at lower stimulation intensities had reported that cathodal tDCS decreases cortical excitability (Nitsche, Nitsche, et al., 2003; Nitsche & Paulus, 2001). Although non-significant, inspection of Figure 4.8 suggests that the findings are broadly consistent with the findings of Batsikadze et al. (2013), in that many participants showed an *increase* rather than a decrease in motor excitability and this effect was close (p=0.08) to conventional statistical significance thresholds. However, this effect was variable across participants and more importantly it was variable within participants, as indicated by ICC analysis. It is possible that a longer follow-up period using TMS would have revealed significant effects and furthermore that these effects may have become more stable and consistent following a sizeable delay such as 90-120 minutes. However, for practical reasons this was not tested and, therefore, this can only be speculation.

The finding that the effects of 2mA anodal stimulation were not reliable within individuals despite significant group level effects is particularly interesting as it suggests that group level analysis may hide substantial variability which occurs both between and within subjects. Previous evidence regarding stability of anodal effects has been mixed. The results of this study are more in line with the recent findings of Horvath et al. (2016) than those of Lopez-Alonso et al. (2015). However, as previously noted there are methodological differences between the studies which may in part contribute to the findings. In particular,

the study by Lopez-Alonso et al. (2015) was conducted with sessions separated by 6-12 months and only two sessions were compared, although it should be noted that this work was conducted with a larger sample. Our finding of moderate stability following sham stimulation is also in agreement with previous work (Horvath et al., 2016).

Throughout the study efforts were made to maintain stability across testing sessions (e.g. by the use of neuro-navigation software). However, one factor which may have varied across the sessions was the exact timing of when the TMS measurements were taken. This was due to three factors: 1) Delays due to set-up issues such as removing tDCS electrodes and re-registering the participants head using neuro-navigation software. 2) TMS delays such as identifying the optimal stimulation location and if necessary re-calculate RMT and SI1mV. 3) Counterbalancing of TMS measures (pp/ IO) which was done to allow for better detection of group level effects. It is difficult to say how much of the variability seen in the data reflects these timing issues. However, it should be noted that when a large amount of TMS measures are collected (as in this study) it takes time, therefore even if data collection starts exactly as tDCS finished this may be acquired over a period of 10-20 minutes. When fewer TMS measurements are taken it becomes possible to measure more discrete time points, this was done by both Horvath et al. (2016) and Lopez-Alonso et al. (2015) who measured every 10 minutes following tDCS. Using this method and examining change in a single MEP amplitude Horvath et al. (2016) found the effects of 1mA anodal tDCS to be unreliable over a 30 minute follow up period. Contrarily Lopez-Alonso et al. (2015) found the effects to be of fair reliability in the 0-30 minute period following stimulation, but not after 30-60 minutes. These findings are based on ICC analysis with binned data, yet it is worth noting that when individual time points are studied (such as 10, 15 and 20 minutes) the ICC values drop in the study by Lopez-Alonso et al. (2015) to revealed poorer reliability in most instances. Based on these findings the variability found in the present results seems unlikely to be due to timing issues alone.

tDCS effects on paired pulse (SICI, ICF) measures of cortical excitability

2mA **anodal** tDCS was not found to significantly alter 3ms SICI. Although Lopez-Alonso et al. (2015) also failed to show a significant change in SICI (using a 2ms ISI), this is counter to previous work which has reported reductions during anodal stimulation (Cengiz et al., 2013), and also following stimulation applied at 0.8mA (Kidgell et al., 2013); 1mA (Batsikadze et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005) and 2mA (Batsikadze et al., 2013).

SICI is considered to be primarily dependent on GABA-A synaptic activity (Ziemann et al., 2015), and therefore many have argued that a reduction in SICI indicates that the effects of anodal tDCS are at least in part resultant of synaptic changes and alterations in levels of GABA. Our results do not provide clear support for this; however, neither do they strongly refute them, as in three of the four sessions there was a trend for SICI to be reduced.

ICC analysis of 3ms SICI effects showed that the influence of **anodal** stimulation on this measure was unreliable across sessions. This conflicts with the findings of Lopez-Alonso et al. (2015) whose ICC analysis revealed 'fair' reliability. As previously discussed there are a number of differences between the two studies which may have contributed to the different findings, including differences in the intensity and duration of tDCS used, differences in sample sizes and differences between SICI measurements.

Less is currently known about 1ms SICI with regards to its origin. However, it has been found that the effects of anodal tDCS (both 1 and 2mA) on 1ms SICI appear to be the reverse of those occurring when SICI is measured using ISIs of 3ms (Cengiz et al., 2013). The study by Cengiz et al., (2013) was conducted during stimulation, and therefore is not directly comparable to the offline results seen here. However, it is worth noting that in this offline study there is no clear evidence that 1ms and 3ms SICI were influenced differently by anodal tDCS.

Cathodal tDCS also failed to influence either of the SICI measurements. The effects of 2mA cathodal stimulation are far less explored than those of 1mA;

and although there is evidence that 1mA cathodal stimulation increases 3ms SICI (Nitsche et al., 2005); the effects of cathodal stimulation are known to be more difficult to produce and have been found to show a more variable pattern of responses (Wiethoff et al; Horvath et al 2016). The study by Batsikadze et al. (2013), revealed a significant reduction in SICI following cathodal stimulation which became apparent only 60 and 90 minutes after tDCS. Interestingly the significant change in SICI was only found for ISIs of 5ms but not in the 2 or 3ms conditions. This highlights further complexities in the study of tDCS effects using TMS. Particularly as there is no clear reason to believe that the underlying mechanisms of 3 and 5ms SICI to be different (although 2ms may be closer to the seeming distinct processes underlying 1ms SICI effects).

Neither **anodal** nor **sham** stimulation significantly altered ICF measures. This is counter to previous research which has reported increases in ICF following anodal stimulation (Batsikadze et al., 2013; Nitsche et al., 2005). The finding that 2mA cathodal stimulation significantly increased ICF on a single session is similar to the increase reported by Batsikadze et al. (2013); however it's unclear why this difference was only apparent on the fourth session.

Limitations:

The counterbalancing of the conditions tested in this experiment was not optimal, and ideally the experimenter would be blind to the stimulation used. Unfortunately, for practical reasons this was not feasible. However, experimental controls were in place (such as the use of neuro-navigation systems to guide TMS coil placement) to offset these limitations.

A further potential limitation of the study is the sample size. Although small sample sizes are common within tDCS research and this sample size was large enough to reveal a significant group effect following anodal stimulation, a larger sample may have provided more robust results. Recent work has revealed surprisingly low rates of responses to tDCS even for anodal stimulation which is thought to be more reliable than cathodal. For example, in a study of 45

participants Lopez-Alonso et al. (2015) estimated 60-64% of participants responded to anodal stimulation with an overall increase in cortical excitability. Another study by Wiethoff et al. (2014) estimated that approximately 50% of the 53 participants they tested had minor or no response to tDCS.

Conclusions:

In summary, we investigated the reliability and consistency of the effects of 2mA anodal and cathodal tDCS on motor excitability by examining how a number of TMS measures were influenced by tDCS. We found that anodal stimulation significantly increased the slope of TMS IO curves and that this effect was consistent at a group level across repeated testing sessions. Using ICC analysis these effects were not found to be reliably consistent within individuals. Sham stimulation failed to significantly influence IO curve slope, however the effects of this intervention were found to be moderately reliable within subjects. The results of the paired pulse stimulations revealed no clear tDCS induced changes for any measures, with the exception of an increase in ICF following cathodal stimulation in a single session.

As previously discussed, variability with non-invasive brain stimulation techniques is not uncommon. However, in order to develop techniques such as tDCS into more powerful methods in both research and therapeutic contexts, understanding the sources of this are likely to be critical. In particular furthering our understanding regarding the reliability and consistency of these effects may allow us to better identify responders and non-responders to particular paradigms. The present research suggests that at least some of the variability comes from non-fixed factors (opposed to more stable factors such as anatomy), as variability was apparent within participants. This makes the task of identifying features causing variability more complex, and it is unlikely that all will be identified. Nevertheless, research identifying key sources could be particularly useful in furthering the development of tDCS as an effective treatment alternative to conventional approaches.

Chapter 5: Can cathodal tDCS reduce tics in Tourette's syndrome?

Key words: Gilles de la Tourette's syndrome (GTS), *transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), resting motor threshold (RMT), input output curve (IO curve), Yale global tic severity score (YGTSS).*

5.1 Introduction

As previously discussed in Chapter 1, Gilles de la Tourette's syndrome (GTS) is a childhood onset disorder, characterized by the presence of motor and phonic tics which are present for a minimum of 1 year (Leckman, 2002). Tics can vary in their complexity, intensity and frequency. They can also vary over time and from person to person, for example, one individual with GTS may experience only 'simple' tics such as eye blinking and sniffing, whereas another may experience more 'complex' tics such as imitating actions or repeating words. Tics are thought to wax and wane over both long and short periods of time. Tics are often at their worst in children aged 10-12 years and follow a time course in which approximately three-quarters of children will see their tics largely diminish by the time they are adults (Bloch & Leckman, 2009). Over a shorter time period stress and fatigue may exacerbate tics (Hoekstra et al., 2004; Lin et al., 2007), whereas engaging in enjoyable activities have been reported to reduce them.

Although tics and reactions to them can vary from person to person, the quality of life in adults with GTS has been found to be strongly related to GTS symptomology (Jalenques et al., 2012). In both adults and children, tics have been found to influence various aspects of life including social, occupational/academic and psychological well-being (Conelea et al., 2011; Conelea et al., 2013). Tics may also put a physical strain on the body, for example sudden tics may be painful, tissue damage may occur from repetitive tics and injury secondary to a tic may occur (-such as by striking an object). Tics may even

result in stress fractures and musculoskeletal damage (Fusco et al., 2006; Moon et al., 1998). Sadly, painful tics are not uncommon. A recent large scale study found that 60% of adults with GTS reported having at least one tic which caused pain or physical damage (Conelea et al., 2013); for children a similar large scale study found rates to be 64% (Conelea et al., 2011). Consequently, developing treatments which reduce tics are critical in improving quality of life for individuals with GTS.

At present, two of the most common treatments for GTS are medication and behavioural interventions. The most popular behavioural intervention is 'habit reversal training' which is a form of cognitive behavioural therapy involving two stages. The first stage is 'awareness training', during which the individual learns to focus on any sensory phenomena they experience prior to a tic. These sensations as often referred to as premonitory urges and can involve unpleasant sensations such as feelings of pressure which are temporarily relived or reduced following a tic (Woods et al, 2005). The second stage is known as 'competing response training', and involves learning to perform a voluntary action that is incompatible with the tic whenever an urge to tic occurs. This voluntary action is intended to help manage the urge to perform a tic in a way that differs from tic suppression (Piacentini et al., 2010). There is evidence that habit reversal training can work well to reduce tics (Piacentini et al., 2010; Yates et al., 2016), however, access to this type of treatment is limited by the amount of trained professionals able to deliver it. The treatment is also time consuming and may be too large a commitment for families, particularly if a long commute is necessary to visit specialists.

At present the main alternative to behavioural therapy is medication and although few medications have received formal FDA approval for the treatment of GTS, a number of different drugs can be prescribed by clinicians, often based on their personal experience (Kurlan, 2014). Some of the main drugs prescribed for treatment of GTS are antipsychotics such as Haloperidol; dopamine depletors such as Tetrabenazine, and alpha-agonists such as Clonidine (Kurlan, 2014).

Although these treatments will work for some, others may not respond, or find that the side effects outweigh the benefits. For example, antipsychotics are often prescribed but have been associated with sedation, depression, increased appetite and Parkinsonism (Kurlan, 2014). Tardive dyskinesia has also been reported as a side effect of the antipsychotic Aripiprazole (Pena et al., 2011). Due to the known side effects of this class of drug, clinicians may prescribe other medications first, including alpha-2 adrenergic drugs such as clonidine, and drugs which deplete dopamine such as Tetrabenazine (Kurlan, 2014). Although Clondine has been found to outperform placebo in the treatment of tics (Leckman et al., 1991) and Tetrabenazine has also been found to be helpful (Porta et al., 2008), neither are without side effects. Clondine has been associated with sedation, headaches, dizziness and irritability (Kurlan, 2014); and sedation, depression, insomnia and restlessness are reported side effects of Tetrabenazine (Jimenez-Shahed & Jankovic, 2013).

Although there are clear merits to behavioural therapies and pharmacological interventions, the disadvantages of these treatments cannot be ignored. There is a clear need to develop alternatives which are both convenient and free of side effect. These alternatives may come in the form of non-invasive brain stimulation (NIBS) techniques.

The approach of using NIBS to treat symptoms of GTS is rather different from the approaches used in behavioural therapies and pharmacology. One key difference is that NIBS techniques allow a specific brain region to be targeted, unlike behavioural interventions which are less direct or pharmacological interventions which may involve influencing the level of a neurotransmitter throughout the whole brain.

As discussed in Chapter 1, the neurological underpinnings of GTS are not yet fully understood. Although advances in neuroimaging techniques have implicated an array of different brain regions, many of which support the idea of dysfunction in cortico-striato-thalamo-cortical networks (Greene et al., 2015; Mink, 2006), the results are not always consistent and can be contradictory. One

area of the brain in which the evidence for alterations is somewhat more consistent is the supplementary motor area (SMA). This area of the brain has extensive connections to areas relating to motor control and cognitive processing (Picard & Strick, 2001) and has been linked to the genesis of tics in a number of multi-modal studies. fMRI blood oxygen level dependent (BOLD) signal in the SMA has been found to increase proceeding the occurrence of a tic (Bohlhalter et al., 2006). In addition to this, evidence from a positron emission tomography (PET) study found increased SMA activity in individuals with GTS (Eidelberg et al., 1997). Magnetic encephalography (MEG) has also been used and implicated the SMA as an important area in GTS. Based on the findings of a MEG study involving simple motor movements, Franzkowiak et al. (2012) suggested that the hyper-activity within M1 in GTS is caused by increases in the functional interaction between SMA and this area.

In addition to findings from fMRI, PET and MEG, stimulation studies in individuals with GTS and neurologically typical participants provide further support for the involvement of the SMA in GTS. A recent study by Finis et al. (2013) demonstrated that it was possible to induce tic like behaviours (similar to echolalia) in neurologically typical individuals by stimulating the SMA with 5Hz rTMS. As 5Hz rTMS protocols are known to lead to temporary increases in excitability (Pascual-Leone et al., 1994), this provides support for the theory that elevated SMA excitability can contribute to the genesis of tics.

As outlined in Chapter 1, using NIBS to reduce excitability within the SMA could be an effective method of reducing tics in individuals with GTS. Following promising results of an open label study in GTS and OCD (Mantovani et al., 2006); Mantovani et al. (2007) conducted a further study in which it was found that inhibitory 1Hz rTMS applied to the SMA for 10 days reduced tic symptoms in two individuals for up to 4 months after treatment. Kwon et al. (2011) also found that 10 sessions of 1Hz rTMS reduced tic symptoms in 10 children/adolescents (ages 11-14) over the course of a 12 week follow-up. A similar finding was reported by Le et al. (2013) who found that 20 sessions of 1Hz rTMS to the SMA reduced tic

symptoms, and that these effects lasted up to 6 months in some of the 25 participants tested.

Studies with tDCS have also shown promise in reducing tics. Carvalho et al. (2015) identified significant changes in tics which were still present at 6 months following 10 sessions of cathodal tDCS applied to the SMA. Finally, Mrakic-Sposta et al. (2008) found tics significantly reduced following 5 days of tDCS applied to the left motor cortex. These effects were found to be greater than in a sham condition. Although the previous work has shown promise, with the exception of the work by Mrakic-Sposta et al. (2008) the studies have not been sham controlled and the majority have been conducted using rTMS. One of the key advantages of tDCS stimulation is that it has potential for home use due to its portability and comparably cheap price. It is also associated with a much lower risk of major side effects than rTMS. Therefore, there is a need for more studies investigating its potential in addition to a need for further sham controlled studies using rTMS.

In this study, the effects of a single session of cathodal/sham tDCS on tic symptoms was investigated. Many previous studies, including most discussed above, have assessed changes in tic severity using self-report based measures and semi-structure interviews such as the Yale Global Tic Severity Scale (YTSSG). In this study tics were assessed using short video recordings of participants. This method has a number of advantages, including the ability for trained individuals to objectively assess tics whilst being blind to the condition. It is also relatively quick and is not restricted by the participant's ability to describe their experiences of tics. Furthermore, it is less likely to be subject to self-reporting biases as a result of experiencing an intervention.

In addition to video recordings, TMS was also used in an effort to explore how tDCS may be influencing cortical excitability. This was done by recording changes in MEP amplitudes at M1. Unlike the previous studies reported in Chapters 3 and 4, tDCS was not applied at the same location, therefore, any effects observed may suggest an indirect change resulting from the influence the SMA may exert over M1.

5.2 Method

5.2.1 Participants

A total of ten participants with a diagnosis of Tourette's syndrome (*N*=9) or Chronic tic disorder (*N*=1) were recruited. The mean age of participants was 22.8 years (range 16-33 years); five were male and five were female. Participants were recruited through a UK charity, Tourettes Action, and through a local NHS clinic. Some participants had diagnoses of additional co-occurring disorders and some were taking medications (see Table 5.1 for details).

Table 5.1. *Participant demographics*

ID	Sex (M/F)	Age	Tic diagnosis	Co-occurring diagnoses	Medication
1	M	23.3	GTS	N/A	Clonidine
2	M	16.1	GTS	Anxiety	Clonidine, Aripiprazole, Sertraline
3	M	20.5	GTS	ADHD	Pentasa (*not CNS active*)
4	F	20.5	GTS	N/A	N/A
5	F	18.4	GTS	OCD, dyscalculia, depression	Citalopram (20mg)
6	F	32.2	GTS	N/A	N/A
7	F	33.3	GTS	ADHD	Concerta, Fluoxetine
8	M	20.3	GTS	N/A	Clonidine (175mg)
9	M	20.5	GTS	ADHD	Methylphenidate hydrochloride
10	F	23.1	CTD	N/A	N/A

5.2.2 Design

A within subjects design was used to explore the effects of sham and cathodal tDCS on tic expression and motor cortical excitability. The independent variable was tDCS which had two levels (sham/ cathodal), the dependent variables were the amount of change in TMS measures (IO curve and SI 1mv) and also the change in tic expression measured via short video clips. Each experimental session was separated by a minimum of one week to avoid any potential carry over effects. The order in which participant's experienced stimulation (sham or cathodal) was counterbalanced across participants.

5.2.3 tDCS of the supplementary motor area (SMA)

tDCS was delivered via a NeuroConn DC- stimulator (GmbH, Ilmenau, Germany) with a maximum stimulation output of 4.5mA. Stimulation was applied using surface sponge electrodes measuring 35 cm^2. The 'active' electrode was placed on the area of the scalp thought to be directly above SMA in a way which afforded bilateral stimulation. This location was identified in accordance with previous studies (Enticott et al., 2012; Finis et al., 2013; Mantovani et al., 2007) in which the 10-20 EEG system was used to identify the site which is 15% of the distance between nasion and inion anterior of the CZ location. The reference electrode was placed on the right hand side of the participant's forehead. In the cathodal condition a 1mA current was run between the two electrodes for 20 minutes, this was ramped up for 15 seconds at the start of the stimulation and ramped down over 15 seconds at the end. In the sham condition the current was also ramped up and down over a 15 second period, although it was only held constant at 1mA for 30 seconds. This resulted in a maximum current density of 0.028 mA cm^2 (1mA/35cm^2) in both conditions. Participants were blind to the experimental condition, however for practical reasons the researcher was not.

5.2.4 TMS measurement and EMG recording

TMS was delivered using a Magstim 200 (Magstim, Whiteland, Dyfed, UK) with a figure-of-8 magnetic coil (diameter of one winding 70mm). The coil was

held tangentially to the scalp and positioned 45° from the midline. The optimal location for stimulation ('hot spot') of the contralateral FDI was defined as the location over the left motor cortex which when stimulated consistently resulted in the largest MEP.

MEPs were recorded using disposable, Ag-AgCl surface electrodes attached to the right FDI muscle in a belly tendon montage. Alcohol wipes were used to prepare the skin prior to application of the electrodes. The signals were amplified and bandpass filtered (10Hz- 2kHz, sampling rate 5kHz) then digitalized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position on a table directly in front of them. Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP response of 50-100µV in the relaxed FDI muscle, in a minimum of 5 of 10 trials.

A neural navigation system (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used to track coil position in relation to the participant's head and the location of the identified hotspot. This was done using a template as individual anatomical scans were not available. A chin rest was used during stimulation to maintain the position of the participant's head. Participants were informed that they could take breaks if necessary and move if uncomfortable.

IO curve measurement

IO curves were measured using TMS intensities of 100, 110, 120, 130, 140 and 150% RMT. The order of the stimuli was randomized, controlled and triggered via a software program (Matlab, The Mathworks, MA, USA). Each intensity was tested a total of 10 times and each TMS pulse was separated by an inter-stimulus interval (ISI) of 5 seconds (S). There was a pause every 10 pulses in which the coil position was checked carefully and participant comfort was also assessed.

SI 1mV measurement

A 1mV threshold was identified as the intensity needed to yield an MEP of approximately 1mV when the coil was located over the hot spot. A total of 20 pulses were delivered to this area with an ISI of 5s separating each individual pulse.

TMS thresholds (RMT, SI 1mV) were not adjusted following tDCS, thereby allowing for identification of any changes in threshold through change in MEP amplitudes following stimulation.

5.2.5 Video recording

Video recordings lasting 8 minutes were collected both before and after tDCS. During this time participants were instructed to not supress their tics, to sit and relax and to try to stay awake. The researcher waited outside the room throughout recording.

5.2.6 Yale Global tic severity scale

The Yale global tic severity scale (YGTSS; (Leckman et al., 1989)) was used to rate the number, frequency, intensity, complexity and interference of motor and phonic tics at baseline. The scale was also used to provide an overall estimate of impairment (by summing motor + phonic + impairments scores). The YGTSS is a common measurement instrument within GTS research and has been found to have good psychometric properties, including internal consistency, convergent validity and association with clinician rating of impairment (Leckman et al., 1989; Storch et al., 2005).

The YGTSS was administered by one of two experienced researchers; this was held constant for both sessions (sham/ cathodal). For baseline YGTSS score in each condition see Table 5.3 and Table 5.4.

Table 5.2. *Participant demographics YGTSS, sham.*

Sham			Tic Severity			Pattern of current tics	
Participant number	Sex (M/F)	Age (y/m)	Global	Motor	Phonic	Clear presence of complex tics (Y/N)	Characteristic motor/ phonic tics (upper body only)
1	M	23.3	29	9	17	Yes	*Motor*: eyebrow raising, mouth movement, head nodding/jerks, shoulder shrug, facial grimace. *Phonic*: grunting, throat clearing.
2	M	16.1	66	17	9	Yes	*Motor:* eye blink, eyebrow raising, mouth/jaw movement, head jerks/movement, shoulder shrugging, facial grimace. *Phonic:* throat clearing, grunting, 'hm' & 'mm' sounds, occasional words.
3	M	20.5	48	18	10	Yes	*Motor*: eye blink, eyebrow raising, mouth/jaw movement, head jerk, head nodding, shoulder shrug, trunk movement, facial grimace. *Phonic*: throat clearing, sniffing, sounds with lips.
4	F	20.5	27	18	0	Yes	*Motor:* eye movement, head jerk/movement, shoulder movement (sometimes related to arm/hand movements). *Phonic*: none
5	F	18.4	24	11	14	Yes	*Motor*: eye blink, eye movement, eyes squeezed shut, nose movement, head jerk/movement, facial grimace. *Phonic*: coughing, throat clearing, whistling, 'ch', 'ff', 'th' and 'sh' sounds, chirping sounds, blowing.
6	F	32.2	7	6	0	Yes	*Motor:* eye blink, nose movement, mouth movement, facial grimace. *Phonic:* none.
7	F	33.3	52	19	18	Yes	*Motor*: eye blink, eye movement, eyebrow raise, nose movement, mouth movement, facial grimace. *Phonic*: throat clearing, coughing, clicking with tongue, echolalia, paralalia, a few words.
8	M	20.3	60	16	9	No	*Motor:* eye blink, eye movement, nose movement, mouth movement, trunk. *Phonic:* throat clearing, sniffing, grunting.
9	M	20.5	39	17	12	No	*Motor:* eye blink, head jerk/ movement, shoulder movement. *Phonic:* throat clearing, sniffing.
10	F	23.1	59	16	0	Yes	*Motor:* eye blink, head jerk/movement, mouth, shoulder shrug, trunk movement. *Phonic:* none.
Average *Global*: 41.1 ± 19.06; Average *Motor*: 14.7 ± 4.42; Average *Vocal*: 8.9 ± 6.85							

Table 5.4. *Participant demographics YGTSS, cathodal.*

Cathodal			Tic Severity			Pattern of current tics	
Participant number	Sex (M/F)	Age (y/m)	Global	Motor	Phonic	Clear presence of complex tics (Y/N)	Characteristic motor/ phonic tics (upper body only)
1	M	23.3	31	8	19	Yes	*Motor:* eye blink, eyebrows raise, mouth movement, head nodding/jerk, shoulder shrug, facial grimace. *Phonic:* throat clearing, coprolalia.
2	M	16.1	60	16	14	Yes	*Motor:* eye blink, mouth/jaw movement, head jerk/movement, abdominal tensing, shoulder shrugging, facial grimace. *Phonic:* throat clearing, grunting, mm & ch sounds, occasional words.
3	M	20.5	55	22	13	Yes	*Motor:* eye blink, squeeze eyes shut, eye movement, eyebrow raise, mouth movement, head jerks/ movement, shoulder shrug. *Phonic:* throat clear, sniff, sounds with lips.
4	F	20.5	35	17	0	Yes	*Motor:* eye movement, head jerk/movement, shoulder movement (sometimes related to arm & hand gestures). *Phonic:* none.
5	F	18.4	39	15	14	Yes	*Motor:* eye blink, eye movement, eyes squeezed shut, nose movement, head jerk/movement, facial grimace. *Phonic:* sniffing, whistling, chirp sounds, syllables (ch, th), spitting sound.
6	F	32.2	9	7	0	Yes	*Motor:* nose movement, mouth movement, abdominal tensing, facial grimace. *Phonic:* none.
7	F	33.3	47	18	16	Yes	*Motor*: eye blink, eye movement, eyebrows raise, nose movements, mouth movement, facial grimace. *Phonic:* throat clearing, coughing, clicking with tongue, echolalia.
8	M	20.3	60	16	9	No	*Motor:* eye blink, eye movement, nose movement, abdominal tensing. *Phonic:* throat clearing, grunting.
9	M	20.5	35	18	12	No	*Motor:* eye blink, eyebrow raise, eye movements, head jerks/ movement, shoulder movement. *Phonic:* throat clearing, sniffing.
10	F	23.1	55	15	0	Yes	Motor: eye blink, mouth movements, head jerk/movement, shoulder shrugging, trunk movement. *Phonic:* none.
Average *Global*: 41.9 ± 17.2; Average *Motor*: 15.0 ± 4.4; Average *Vocal*: 9.7± 7.17							

5.2.7 Procedure

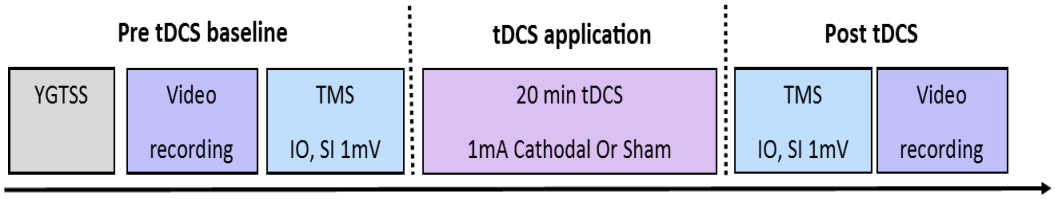


Figure 5.1. *Schematic of experimental procedure.*

After gaining informed consent the YGTSS was administered by the primary investigator (KD) or an experienced research nurse (JF). This took the form of a semi-structured interview in which participants were asked about the tics they had experienced within the last week. Tics noticed by the researcher but not reported by the participant were included in the total score. On average this took 15-30 minutes to complete.

Following completion of YGTSS the participant was moved to sit directly in front of a camera and an 8-minute video recording was taken with the researcher and any other individuals (such as parents) outside of the room.

After the video recordings the participant was seated in a comfortable chair with their head positioned on a chin rest and their right hand and forearm placed in a relaxed position on a table directly in front of them. The location of the participant's head was then registered to a template (constructed from a consenting individuals anatomical brain scan) using the Brain sight system (Rogue Research Inc., Montreal Quebec, Canada), and disposable electrodes were attached to the hand. Following this, the hotspot for FDI stimulation was identified using TMS, which was then mapped onto the template brain to aid coil localization. RMT was then defined before the measurement of IO curves. Following this SI 1mV threshold was measured and data was collected from 20 pulses delivered at this intensity.

After the first session of TMS the head tracker was removed and the researcher measured the approximate location of the SMA on the scalp using the

method previously described. This was marked in pen to aid placement of the tDCS electrode. The saline soaked sponge covered tDCS electrodes were then placed over this mark and over the right side of the forehead. These were attached using a rubber band and elasticated bandage. Participants then remained seated during 20 minutes of sham or active stimulation. After tDCS stimulation the electrodes were removed, the headband tracker was replaced and re-registration was performed using the neural navigation software. The hotspot was then checked using the previously sampled location; at times this required minor adjustments due to registration issues. IO curves and SI 1mV measures were then taken using the same intensities as in the pre tDCS condition. Throughout the TMS and tDCS protocols participants were able to watch wildlife documentaries. This was done in an attempt to maintain levels of arousal and attention during stimulation.

After the second session of TMS, another 8-minute video recording was made with the participant alone in the room. Following this the participants were thanked and received financial compensation for their time. The whole procedure was completed twice for each participant in a counter-balanced fashion with at least one week separating each session.

5.2.8 Data analysis

Tic coding procedure

Prior to tic coding the videos were anonymised, therefore, coders scored all videos while blind to the experimental condition. A list of potential tics was generated to aid tic identification using the tic type subscale of the YGTSS. Videos were played using VLC media player. The advanced tools options were used to allow videos to be slowed down and played frame by frame. Whenever possible a continuous 5-minute segment was sampled from the 8 minute videos. This sample was taken from the 2-minute point onwards to allow participants to relax and become familiar with the situation. For two data sets it was only possible to score 3 minutes of data due to camera failure and the participant falling asleep.

In addition to this it was not possible to analyse a continuous 5-minute segment for a further three data sets. For each video segment in each condition, motor and phonic tics were counted and averaged per minute resulting in a score of tics per minute (TPM). These TPM scores were then averaged to give the mean tic rate for each video clip in accordance with previous protocols (Himle et al., 2006; Nixon et al., 2014).

Each video recording was also scored using the Modified Rush Video Scale (Goetz et al., 1999). The scale has five components which are as follows: number of body areas, motor/phonic tic frequency (scored as TPM) and motor/phonic tic severity, each of which have been found to correlate well with comparable items on the YGTSS (Goetz et al., 1999). The total impairment score, calculated from the Rush by summing the five measured components, has also been found to correlate with the 'overall impairment rating' on YGTSS (Goetz et al., 1999). Each component on the Rush is typically scored on a scale of 0-4, however, for the purposes of this study it was only possible to score 0-3 on the body areas component. This is because tics were only counted from the upper body and face, meaning the maximal amount of body areas was 5, which corresponds with a rating of 3 on the scale. As a result, the maximal score possible on the Rush in this study was 29 rather than 30. The scores from each minute segment were combined to calculate the mean Rush score for each video clip.

By using the Rush scale it is possible to get an overall impression of an individual's tics over a short period of time, including the severity of them. This is not recognised in TPM analysis, therefore, there is merit in studying the two side-by-side.

Assessment of inter-rater reliability

The 5-minute video segments were first analysed by the primary investigator (KD) who then trained two secondary coders (ER and KF). Training was conducted using 1-minute video segments taken from the start of recordings

(these were not included in the later analysis). Once the coders were familiar with the distinct tics of each participant they were given 2 minute segments to rate. Each coder (KF and ER) scored half of the participants resulting in 40% of the total data being reviewed twice. As previous studies have used a sample of 24% (Himle et al., 2006; Nixon et al., 2014) this was considered more than adequate.

Inter-rater agreement for total tic counts was assessed using the frequency-within-interval method (Sharenow et al., 1989). In accordance with previously established protocols (Himle et al., 2006), each of the 2-minute video clips were divided into 12 consecutive 10 second intervals. The amount of tics counted in each of these intervals was compared with the corresponding amount of tics counted by the primary coder (KD) by dividing the lower number of tics observed by the higher number and then multiplying these values by 100. The scores were then averaged across all 12 segments to calculate the percentage agreement for each video. Using this method, the median agreement between the primary (KD) and secondary (ER) coders was found to be 74% (range: 50-94%) for motor tics and 96% for phonic tics (range: 55-100%). Agreement between the primary coder (KD) and the other secondary coder (KF) was 73% (range: 50-100%) for motor and 96% for phonic tics (range: 67-100%).

Inter-rater agreement was also checked for the Rush scores. The Rush protocol was designed to be used in longer time segments, therefore, the scores for each of the two minutes were compared by dividing the lower score by the higher score and then calculating average agreement across the two as opposed to calculating agreement in 10 second intervals. This revealed 85% agreement between coders KD and KF (range: 71-96%), and 86% agreement between coders KD and EF (range: 67-100%).

Intra-class correlation co-efficient (ICC) analysis was also calculated to assess agreement (for full details see Appendix.v). This showed varying degrees of agreement for the different measures/subscales, however, overall reliability of measurement for total impairment on the Rush was deemed excellent between

primary and secondary coders (KD-KF reliability: ICC(2,1)=.92; KD-ER reliability: ICC(2,1)=.81). ICC also revealed excellent reliability for total tic score using the average TPM method between the primary coder (KD) and the secondary coder (KF), ICC(2,1)=.97 and fair reliability between primary coder (KD) and secondary coder (ER), ICC(2,1)=.59.

Based on the inter-rater agreements established, analysis of the full data set was conducted using the scores of the primary coder.

Input-Output curves and SI 1mV

Peak-to-peak MEP amplitudes were estimated using in-house Matlab software (Mathworks, MA, USA). All trials in the 500ms period prior to MEP were carefully visually inspected and any trials in which there was evidence of pre-contraction of the FDI muscle were excluded.

IO curve measurements were estimated by calculating the median intra-individual MEP amplitudes for each TMS intensity value (i.e., 100-150% of RMT). Linear fits were then applied to the resultant values (mean $R^2 = 0.87$). For alternative slope fitting details and analysis please see Appendix.vi. Median values were calculated rather than the mean in order to limit the effect of outliers.

5.3 Results

Video monitoring of tics: tics per minute

A repeated measures ANOVA was calculated to explore any significant differences in **total tic score** (average TPM) following cathodal or sham stimulation. This revealed no significant main effect of tDCS type $F(1,9)=2.25$, $p=.17$; no significant main effects of time $F(1,9)=2.28$, $p= .65$ and no significant interaction $F(1,9)=.21$, $p=.65$. Change in average TPM scores pre/post sham

stimulation can be seen in Figure 5.2, whereas Figure 5.3 shows change pre/post cathodal stimulation.

Additional repeated measures ANOVAs were calculated to assess any significant differences in motor tics following stimulation. This revealed no significant main effects of tDCS on **motor tics** $F(1,9)=1.51$, $p=.25$; no significant main effects of time of testing (pre/post) $F(1,9)=.97$, $p=.35$, and no significant interaction between the two $F(1,9)=1.44$, $p=.26$. tDCS also failed to have a significant main effect on **phonic tics** $F(1,9)=3.33$, $p=.10$; there were no significant effects of time $F(1,9)=.77$, $p=.08$, and no significant interactionbetween the two $F(1,9)=.75$, $p=.41$.

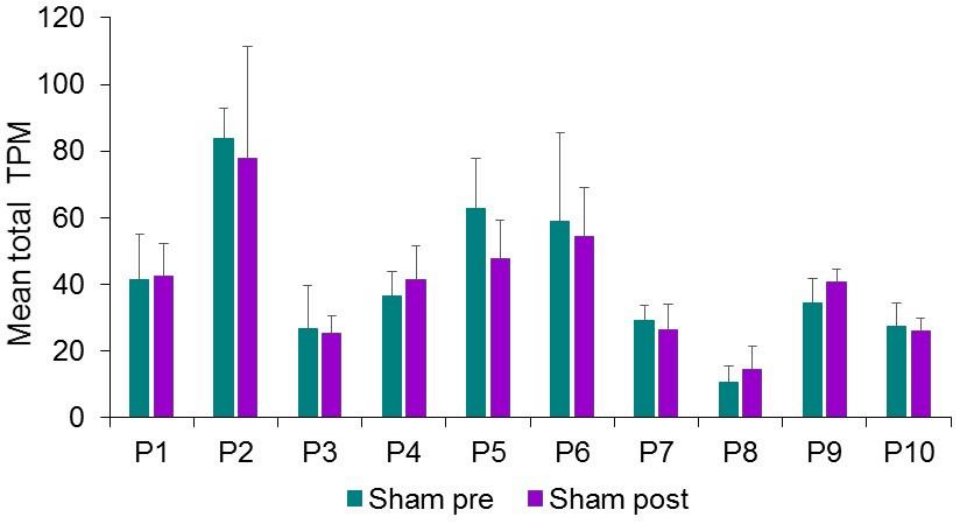


Figure 5.2. *Mean ± SD total tics per minute (TPM) for videos taken before and after **sham** stimulation.*

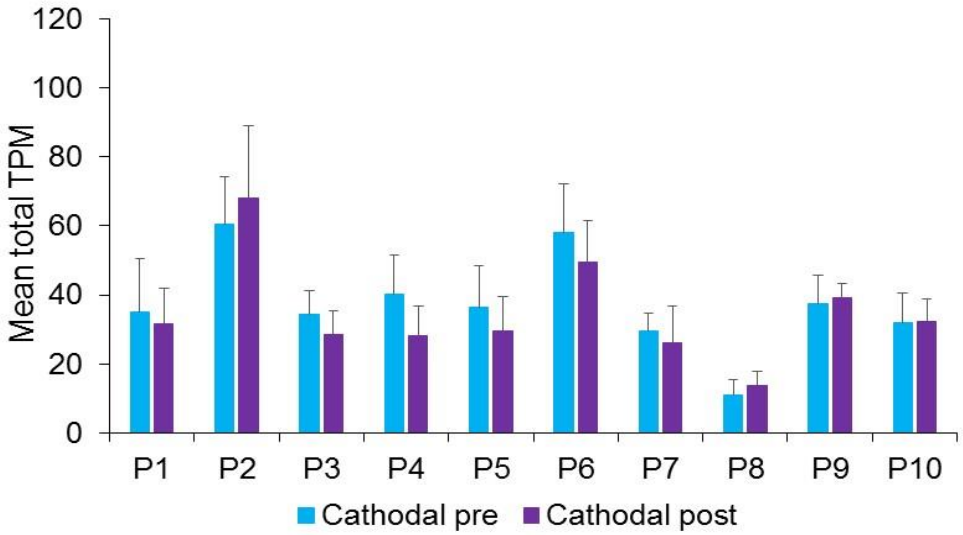


Figure 5.3. *Mean SD total tics per minute (TPM) from videos taken before and after **cathodal** stimulation.*

Video monitoring of tics: Rush

A repeated measures ANOVA calculated using the **total impairment score** (body areas + tic frequency + tic severity) from the Rush revealed a significant main effect for tDCS type (cathodal/sham) F(1,9)=6.7 p=.03. However, the main effect of time (pre/post) was not found to be significant $F(1,9)=1.25$, $p=.29$; and there was no significant interaction between the two factors $F(1,9)=.01$, p=.93. Paired samples t-tests (two tailed) revealed no significant baseline differences between the sham and cathodal conditions $t(9)= 1.65$, $p=.134$. However, when comparing post tDCS tic severity scores, there was a significant difference between post sham ($M=9.66$, $SD=3.29$) and post cathodal ($M=8.8$, $SD=3$) conditions $t(9)=2.35$, $p=.04$. It should be noted that on average there was a small reduction in scores in both sham and cathodal conditions, however in both cases this difference was small. Average change in total impairment scores can be seen for each individual in Figure 5.4 and Figure 5.5. Analysis of all sub-components of the Rush can be seen in Appendix.vii.

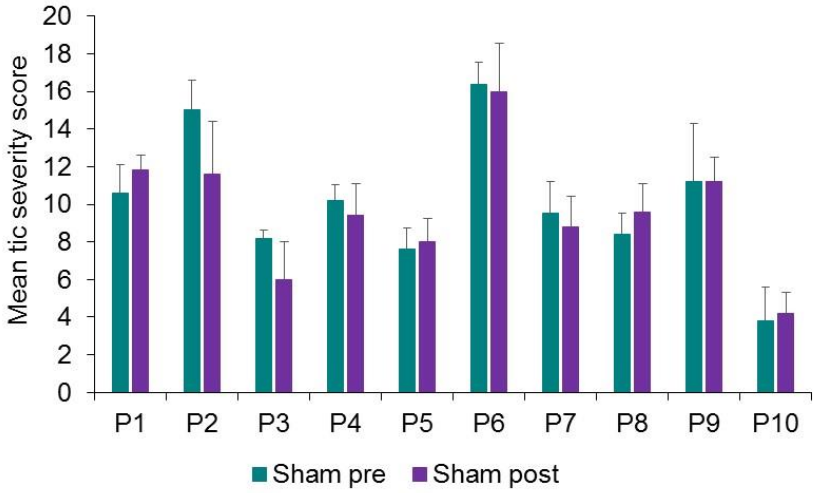


Figure 5.4.*Mean ± SD tic severity score using Rush scale before and after sham stimulation.*

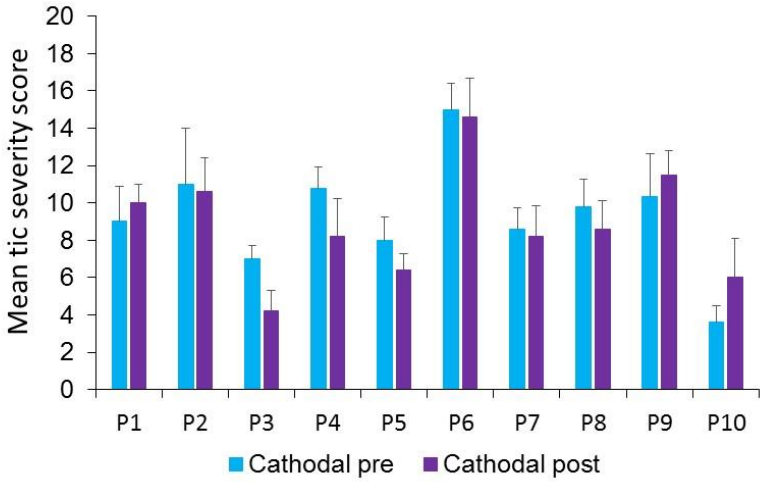


Figure 5.5. *Mean ± SD tic severity score using Rush scale before and after cathodal stimulation.*

Transcranial magnetic stimulation: IO curve

Paired samples t-tests revealed no significant differences between IO curve slopes measured in the pre sham (*M*=65.53, *SD*=30.71) and pre cathodal (*M*=67.65, *SD*=46.14) conditions *t*(9)=-.17, *p*= .87. A repeated measures ANOVA was calculated in which time (pre/ post) and tDCS type (sham/ cathodal) served as independent factors. IO curve slope was entered as the dependent variable. The analysis revealed no significant main effects of tDCS type *F*(1,9)=.11, *p*=0.75 or time *F*(1,9)=1.16, *p*= .31 and no significant interaction between these two

factors $F(1,9)=.03, p=.87$. Data showing average IO curve plots for each condition seen in Figure 5.6.

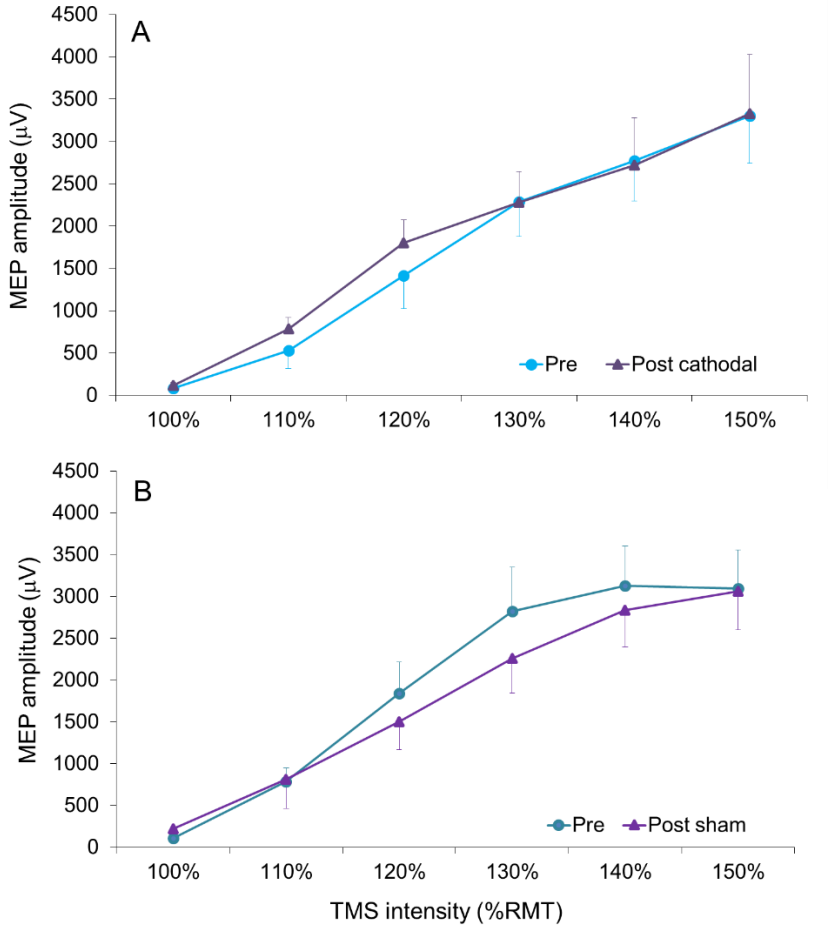


Figure 5.6. *Mean ± SEM IO curve slots. A: before and after cathodal tDCS, B: before and after sham tDCS.*

Transcranial magnetic stimulation: SI 1mV data

Paired samples t-tests revealed no significant difference between baseline MEP amplitude evoked in the SI 1mV condition in the cathodal (*M*=1302.1, *SD*=278.4) and sham conditions (*M*=1467.53, *SD*=514.7), *t*(9)=-.89, *p*=.397. Repeated measures ANOVA revealed no significant effects of tDCS type *F*(1,9)=.67, *p*=0.43; no significant effect of time *F*(1,9)=2.60, *p*= .14 and no significant interaction between the two factors *F*(1,9)=.11, *p*=.75. See Figure 5.7 for average change in SI 1mV measures.

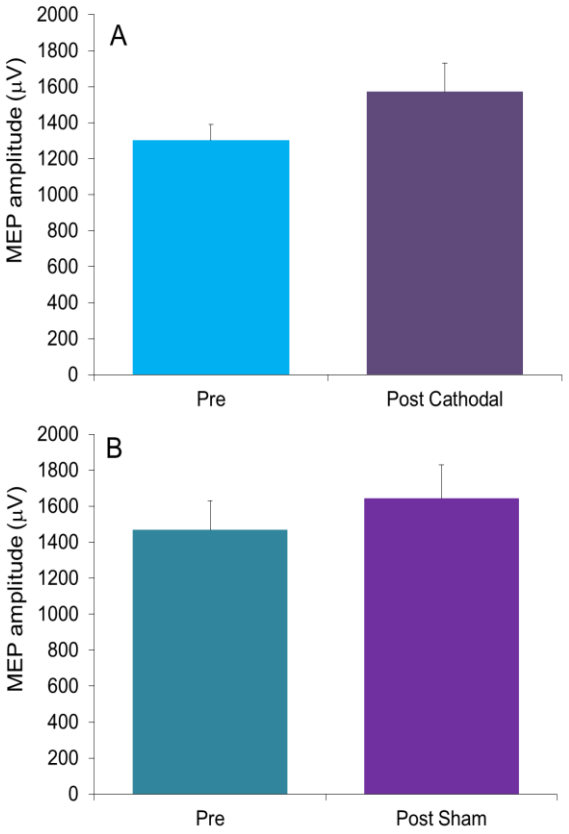


Figure 5.7. *Mean SEM MEP amplitude evoked from SI 1mV pulse A: Pre/ post cathodal stimulation and B: pre/post sham stimulation.*

5.4 Discussion

Effects of cathodal tDCS on tics

The effects of applying 1mA cathodal or sham stimulation to the SMA for 20 minutes were explored. Average scores of TPM were compared before and after cathodal and sham stimulation. The analysis revealed no significant effect of tDCS type, no effect of time (pre/post) and no interaction between the two factors. Analysis of the video data using the Rush total impairment score (in which both tic frequency and severity are taken into account) revealed no significant main effects of time and no significant interaction. However, there was a significant main effect of tDCS type. Further exploration with paired

sample t-tests revealed that although there were no significant differences at baseline between sham and cathodal conditions, there was a significant difference between them in the post stimulation measure. However, this effect and the differences between the two measures was small, and without a significant interaction must be treated with caution. Further analysis with the sub-component of the Rush scale failed to reveal any further significant differences.

Although there was no strong evidence of change in tic frequency or severity here, it should be noted that there are a number of important differences between this study and those which have previously reported cathodal stimulation to be effective (Carvalho et al., 2015; Mrakic-Sposta et al., 2008). In particular, the effects of cathodal stimulation were only explored immediately following a single application. To my knowledge there are no other papers investigating the immediate effects of cathodal tDCS on tics, however, there is evidence suggesting that more prolonged stimulations may lead to stronger effects. In their single case study, Carvalho et al. (2015) reported a significant 21% reduction in the YGTSS (tic severity + deficit) following 5 sessions of stimulation; this increased to 41% following 10 sessions. Mrakic-Sposta et al. (2008) also reported an increase in effect as time went on, with the effects of 5 days of cathodal stimulation applied to the motor cortex being significantly stronger than those occurring on the 4 previous days.

In some ways the lack of an effect immediately after stimulation is surprising, as numerous behavioural and physiological studies have suggested that tDCS can exert an influence both during, and for a number of hours after stimulation. However, it is possible that influencing a phenomenon such as tics in GTS is far more complex than simply changing excitability within M1 or increasing performance on simple motor based tasks. The mechanisms underlying the effects of tDCS within M1 are still not fully understood, and the underlying effects of longer courses of stimulation and at different locations remain even more enigmatic. However, it has been speculated that the more

long-term effects caused by tDCS may depend on modulations of the strength of underlying synaptic connections (Stagg & Nitsche, 2011). This may explain why findings of accumulative effects of tDCS stimulation in neurologically typical individuals revealed sustained increases in cortical excitability with increased testing session, rather than showing increased sensitivity to stimulation at each session (Galvez et al., 2013).

It is theoretically possible that if repeated testing sessions were used to build up accumulative effects then changes in tics would have been revealed even with this small sample size. However, at present, this study serves as the first of its kind to investigate the immediate effects of cathodal tDCS applied to the SMA.

Effects of cathodal tDCS applied to the SMA on cortical excitability at M1

Although the main focus of this study was exploration of cathodal tDCS on tics, changes in MEPs measured from M1 were also explored in an attempt to provide physiological evidence of change in cortical excitability induced by cathodal stimulation. It may seem counter-intuitive to measure MEPs from the hand area of M1 when cathodal stimulation was applied to the SMA, however, these areas are known to be highly connected and, therefore, influencing excitability of the SMA may impact on M1. Evidence to support this comes from dual site TMS experiments in which applying a conditioning pulse to the SMA has been found to influence MEP amplitudes measured from the M1 just milliseconds afterwards (Arai et al., 2012; Civardi et al., 2001; Oliveri et al., 2003). These studies clearly demonstrate connectivity between the regions and how causing a brief interference within the SMA can impact on the output of M1.

Although no studies appear to have investigated changes in MEP amplitude after tDCS stimulation of the SMA, this has been explored using rTMS. Matsunaga et al. (2005) found that 750 pulses of 5Hz rTMS to the SMA increased MEP amplitudes for up to 10 minutes, which strongly suggest that changes of excitability induced by NIBS within the SMA can be measured by recording MEPs

at M1. Therefore, it is not clear why significant differences were not found in this present study between the sham and cathodal conditions for either IO curves of SI 1mV.

Timing of measurements seems unlikely to be an issue, as all TMS measurements were repeated within 5-15 minutes of tDCS ending which should still be well within the period of expected after-effects. It is possible that the stimulation parameters used (1mA cathodal tDCS for 20 minutes) may have been inadequate to induce significant changes in excitability in this participant sample. Although a number of studies have demonstrated significant decreases in cortical excitability following similar applications of cathodal stimulation (see Table 2.1), the effects produced appear to be weaker on average when compared to those of anodal stimulation (Horvath et al., 2015) and physiological changes following cathodal stimulation are not always found (for example Horvath et al. (2016), Kim et al. (2014)). In addition to this, the majority of evidence of physiological change induced by tDCS has all been measured from M1 and it is possible that the effects at the SMA may differ. It is also possible that the electrode montage used in this study was not optimal for stimulating SMA.

Summary and limitations

The effects of 20-minute stimulation of the SMA using 1mA cathodal/sham tDCS were explored. The average number of tics per minute was not significantly altered by either stimulation. However, a significant difference was observed between the post sham and post cathodal scores for the Rush measure of total tic impairment, despite no significant differences at baseline. Although this finding is somewhat promising, it should be treated with extreme caution as the difference was small. Measures of cortical excitability (IO curve, SI 1mV) failed to be influenced by either stimulation.

The findings suggest that a single session of cathodal tDCS has no clear, immediate effects on tics. As discussed, it may be that a single session is not

enough to induce any measurable behavioural changes in such a complex phenomenon, and that repeated stimulations may be necessary. In order to fully test the effectiveness of cathodal tDCS in reducing tics, a sham controlled study over a number of days is necessary. Arguably, this should involve a range of measures such as video recording, self-report and physiological measures in order to identify objective, subjective and physiological markers of change.

Chapter 6: Case study report - Can repeated applications of cathodal tDCS help to reduce tics in Tourette's syndrome?

Key words: Gilles de la Tourette's syndrome (GTS), *transcranial direct current stimulation (tDCS), supplementary motor area (SMA), Yale global tic severity score (YGTSS),* premonitory urge to tic scale (PUTS), false discovery rate (FDR), field of view (FOV), repetition time (TR), echo time (ET) tics per minute (TMS).

6.1 Introduction

As discussed in Chapter 5, there is promising evidence that NIBS techniques could be effective in reducing tics in individuals with GTS. However, despite some positive findings, the quantity of research is sparse, particularly with regard to tDCS. With the exception of two small scale studies (Carvalho et al., 2015; Mrakic-Sposta et al., 2008) there is little published work and, therefore, little consensus regarding what works and how these effects manifest. In my own work (presented in Chapter 5), I failed to find any clear evidence that a single session of cathodal tDCS was any different to sham stimulation. It is possible that a single session of stimulation is too weak an intervention to have any measureable impact, whereas repeated sessions may be more effective. In support of this, substantially larger reductions in tics have been reported after 10 days of stimulation when compared with four (Carvalho et al., 2015), and the effects of five days of stimulation have been found to be larger than those measured on any of the previous four days (Mrakic-Sposta et al., 2008).

Although the two studies by Mrakic-Sposta et al. (2008) and Carvalho et al. (2015) both suggest that tDCS could be a useful tool in the reduction of tics, there are important methodological differences between the two. Mrakic-Sposta et al. (2008) conducted a sham controlled study to compare the effects of cathodal/sham tDCS in two individuals with GTS. Effects on tics were measured using video recordings and the Yale global tic severity scale (YGTSS), a visual

analogue scale (VAS) for wellness was also used. A significant reduction in tic frequency (measured from video data) was found in the cathodal but not the sham condition, this was also apparent in scores on the YGTSS and ratings of 'quietness' on the VAS. These findings provide support for the effectiveness of cathodal tDCS in reducing tics, however, the measures tell us nothing about the biological underpinnings of these changes. This could be particularly important in understanding any long term effects, and was explored in more depth by Carvalho et al. (2015). In a single case study Carvalho et al. (2015) found that ten sessions of consecutive cathodal stimulation applied to the SMA reduced scores on the YGTSS, hence suggesting a reduction in perceived tics. Importantly, a change in resting state fMRI was also seen between scans taken before and after the ten days of stimulation. In particular, activity within the left precentral region and left cerebellum of the sensorimotor resting state network was reduced. The sensorimotor region has repeatedly been found to show increased activity in individuals with GTS (Rickards, 2009) as has the cerebellum (Bohlhalter et al., 2006; Pourfar et al., 2011) and animal work has suggested that spiking activity occurs in these regions immediately before the production of tic like behaviours (McCairn et al., 2013). Given these findings, it may be that cathodal tDCS can reduce tics by downregulating these areas.

In the following case-study I aimed to build upon the previous work by Carvalho et al. (2015) and Mrakic-Sposta et al. (2008) by using a sham controlled, multi modal design to explore the effects of cathodal tDCS on tics. Video recordings were used to obtain an objective measure of tics and their severity, questionnaires recorded the participants' own views and MRI scanning was used to identify any substantial neuroanatomical changes.

Unlike previous studies, stimulation was applied in the participants own home with remote supervision. The reasons for doing this were two fold. Firstly, for tDCS to fulfil its potential as a viable alternative to pharmacology or other forms of stimulation, it is necessary that it works in day-to-day life. Secondly, this method caused the least disturbance to the normal routine of the participant

and their family. Although two well cited advantages of tDCS are its portability and relative ease of use, very few studies have been conducted outside of highly controlled laboratory settings. In one notable exception, Kasschau et al. (2016) found that patients with muscular sclerosis were able to cope well with remote supervision of tDCS application and that both tolerability of stimulation and protocol adherence were good. If tDCS is to be developed and marketed as a useful therapeutic technique, it is critical that it can be flexible. Therefore, what is relinquished in experimental control in home use studies is gained in valuable experience and knowledge of the techniques potential for treatment in day-to-day life.

6.2 Method

6.2.1 Participant details

The participant was a 16-year-old male who had received a formal diagnosis of Tourette's syndrome at the age of 8 years. The participant had no additional diagnoses, however, elevated levels of anxiety had previously been an issue, for which the participant took the SSRI Sertraline (100mg). Some obsessive compulsive behaviours were also reported, although there was no formal diagnosis of OCD. In addition to Sertraline the participant was also on a stable medication regime using both Clondine (200mg) and Aripiprazole (10mg).

6.2.2 Design

Phase 1

A schematic of all experimental procedures is summarised in *Figure 6.1*. During the first part of this case study, the participant and one of their parental guardians visited the University of Nottingham where baseline MRI scans and questionnaire measures (described below in 6.2.5) were collected. During this visit, the participant and their guardian were trained by the primary researcher (KD) to safely and effectively use the pre-programed tDCS machine. They then returned home with detailed instructions and the equipment needed to start the first phase of this home use study.

Over 10 consecutive days, the participant received sham stimulation within the comfort of his own home. Application of the stimulation was monitored remotely by the primary researcher (KD). With the exception of a few issues on the first day, application and use of tDCS went well. During the stimulation period, ten-minute video segments of the participant passively watching the television were recorded every other day. The first video clip was assessed to give guidance regarding camera positioning and lighting to ensure that tics could clearly be seen. After this, the videos were not seen by the primary researcher (KD) so that they could later be coded with the researcher blind to the condition.

Four days after the final sham stimulation the participant returned to the University of Nottingham to repeat the questionnaire measures and to be scanned. The participant then returned home to complete a further 10 days of consecutive 1mA cathodal stimulation and video recordings every other day. Five days after the last application of tDCS the participant returned to the University of Nottingham for follow up questionnaires and scanning. An additional follow up was completed approximately one month later.

Phase 2

After reviewing the findings of the first study, it was decided that a second phase of the experiment would be run in which a stronger intensity of tDCS would be used (Figure 6.1B). Two months elapsed between the final 1mA cathodal stimulation and the new data collection. As sham stimulation appeared to have a limited effect on tics, the effects of a sham protocol were not measured again in phase 2. Instead a more extensive baseline was measured in which home video recordings were included in addition to scans and questionnaire measures. Following the 10-day baseline, a further 10 days of consecutive stimulation were completed, this time using 1.5mA cathodal stimulation. During this time, videos were also recorded every other day. A final follow up including scanning and questionnaire measures was taken five days after the last stimulation. The participant was always blind to the experimental

condition, and all data were analysed with details of experimental condition removed.

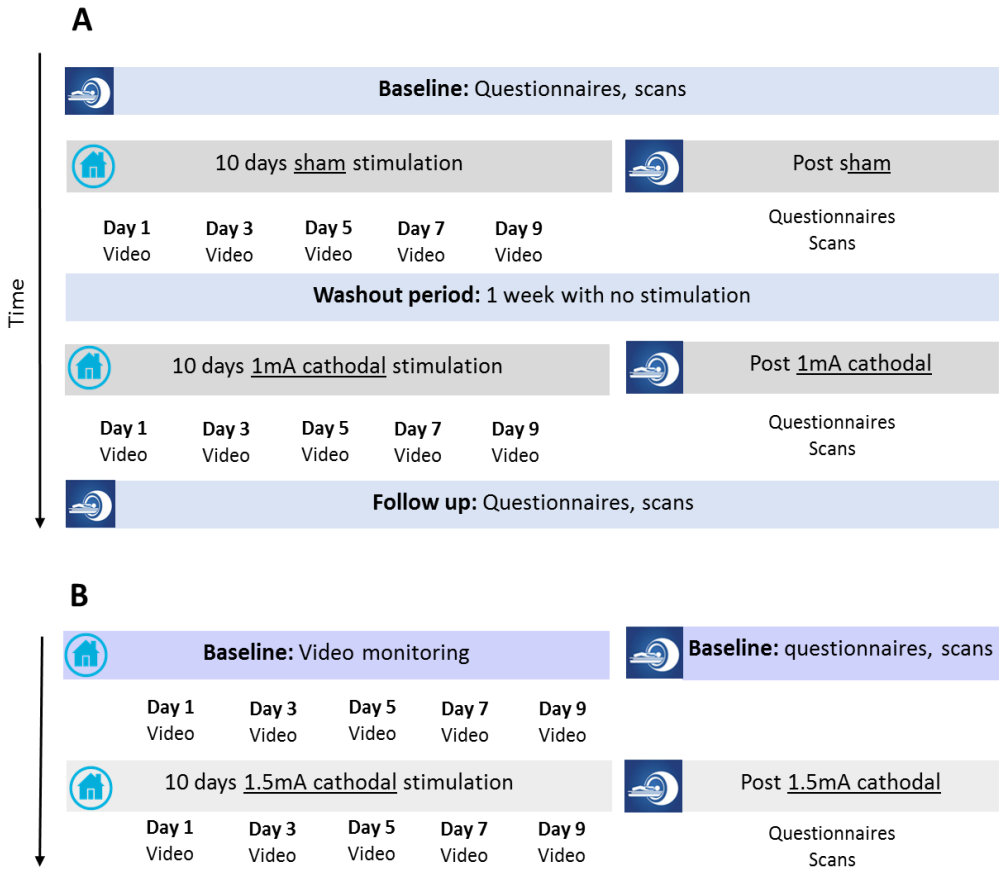


Figure 6.1. *Schematic showing the study design.* ***A****. shows the initial baseline, sham, 1mA cathodal and 1 month follow up conditions which were tested.* ***B****. shows the second phase of the study in which 1.5mA cathodal stimulation was tested against a new baseline. Two months elapsed between the final 1mA cathodal stimulation and the measurement of the second baseline.*

6.2.3 tDCS of the supplementary motor area (SMA)

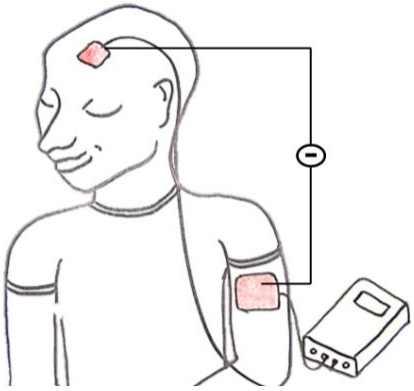


Figure 6.2. *Schematic of electrode placement with the smaller electrode placed over the SMA, and the larger one placed over the upper deltoid muscle of the arm.*

tDCS was delivered via a NeuroConn DC stimulator mobile (GmbH, Ilmenau, Germany). This was programed in advance by the researcher (KD) to deliver one of three options (sham, 1mA, 1.5mA). The stimulator was set so that it could only be used once within a 22 hour period and the settings could not be changed by anyone other than the researcher. In the first session, the stimulator was set to deliver a 20-minute sham protocol, in which the current was ramped up for 15s, held constant at 1mA for 30s and then ramped down over a further 15s period. For the second part of the initial study the machine was programed to deliver 1mA stimulation for 20 minutes with a ramp up and ramp down time of 15s at the start and end. In phase 2 of the study the stimulator intensity was increased to 1.5mA and kept constant for 20 minutes; the ramp up and down time were also set to 15s. The current was run between two saline soaked sponge covered electrodes, the smallest of which measured (25cm^2) and was placed approximately over the SMA. This location was identified in accordance with previous studies (Enticott et al., 2012; Finis et al., 2013; Mantovani et al., 2007), in which the 10-20 EEG system was used to identify the site which is 15% of the distance between the nasion and inion, anterior of the CZ location. The 'reference' electrode measured 100cm^2 and was placed on the upper deltoid muscle on the participant's right arm (Figure 6.2). The participant and their guardian were trained to apply the stimulation during the baseline session. Over the first few sessions the electrode placement was monitored remotely via photographs showing electrode montage. Communication and support were provided throughout the studies duration.

6.2.4 Video recordings

10-minute video clips were recorded on alternative days during stimulation periods. The video clips were taken by the participant's guardian while the participant passively watched television in their family home. These video clips were collected with the aim of generating naturalistic data in which day-to day tic levels could be recorded. Unfortunately, this resulted in some variation in the timings for both videos and stimulation, and a miscommunication resulted in the videos being recorded before stimulation in phase 1 (sham and 1mA cathodal conditions) of the study. In the sham condition the videos were taken on average 39 minutes prior to stimulation (SD= 50 minutes) at 17.28pm (earliest 15:51pm, latest 19:41pm). In the 1mA cathodal condition the average duration between video and stimulation was 32minutes (SD=26 minutes) and the videos were taken on average at 17:46pm (earliest 16:08 pm, latest 19:33pm) which is similar to those recorded during sham.

During the second phase of the study (no stimulation baseline and 1.5mA cathodal) the videos were consistently recorded after stimulation. However, the average timing of video recordings was far later in the 1.5mA condition. At baseline videos were taken on average at 18:22pm (earliest 16:45pm, latest 22:27pm) whereas this was 21.30pm in the 1.5mA cathodal condition (earliest: 19:14pm, latest 22:43pm). In the 1.5mA condition the duration between video and stimulation was 147 minutes on average (SD=125 minutes). These timing differences should be kept in mind when considering the results and interpretations of this measure.

6.2.5 Questionnaire measures

All questionnaire measures were administered by an experienced professional (JF) who was blind to the experimental condition. The measures used were as follows:

Yale Global tic severity scale (YGTSS)

The Yale global tic severity scale (YGTSS; (Leckman et al., 1989)) was used to rate the number, frequency, intensity, complexity and interference of motor and phonic tics at the various time points. The YGTSS was selected as it has good psychometric properties including internal consistency, convergent validity and association with clinician rating of impairment (Leckman et al., 1989; Storch et al., 2005).

Premonitory urge to tic scale (PUTS)

The Premonitory Urge for Tics Scale (PUTS; Woods et al. (2005)) was used to measure the experience of sensation prior to the onset of a tic. PUTS is a self-report measure which contains 10 items designed to assess different sensory properties of urges and their relationships to tics. The measure has been found to have good internal consistency, test-retest reliability and construct validity among 11-16 year olds (Woods et al., 2005).

6.2.6 Scanning protocol (anatomical, resting state FMRI)

All scanning procedures took place at the Sir Peter Mansfield Imaging Centre (SPMIC), University of Nottingham using a 3T Philips Achieva system (Philips Healthcare, Best, Netherlands) and a 32-channel SENSE radio frequency head coil. Following thorough explanation of the scanning procedure, the participant was placed supine and head-first into the scanner. Head motion was minimized by inserting foam pads between the participant's head and the coil. The participant wore ear plugs and also large headphones for hearing protection; the headphones also provided additional padding around the head, thereby helping to reduce potential head motion.

T1- weighted anatomical scans were acquired using the magnetization-prepared rapid gradient echo (MPRAGE) sequence (180 continuous axial slices with $1mm^3$ isotropic resolution, field of view (FOV)= 256x256 mm, matrix size = 256x224, repetition time (TR)= 8.6ms, echo time (TE)= 4.0ms). Following this T2*

weighted resting-state functional MRI (rs-fMRI) images were acquired using gradient-echo echo-planar imaging (GE-EPI) sequence (TR=2000ms, TE=35ms, FOV= 208x208 mm, matrix size 64x64 with 34 slices, 320 dynamics with voxel size = 3.25mm^2).

6.2.7 TMS data collection

In the original design of this study it was planned that TMS measures of global cortical excitability (IO curve), intracortical inhibition (SICI) and facilitation (ICF) would be recorded during each visit to the University of Nottingham. TMS was included in the design to supplement the other techniques, and to give insight into any potential changes in GABAergic activity. However, although this was achieved in the first baseline session, it was not possible to obtain full data sets in two subsequent time points due to participant discomfort (headache and desire to tic). As a result, the measure was dropped from the experiment and not analysed further due to a lack of data.

6.2.8 Tic coding procedure (analysis of video data)

Prior to tic coding the videos were anonymised, thereby allowing coders to score the videos while blind to the experimental condition. A list of potential tics was generated to aid tic identification using the tic type subscale of the YGTSS. Videos were played using VLC media player and the advanced tools options were used to allow videos to be slowed down and played frame by frame. For phase one of the experiment (sham vs. 1mA cathodal) the first video from each condition was dropped from the analysis due to poor quality in the first sham session. The 10-minute video clips were analysed in whole by the primary coder (KD) in the first phase of the experiment. However, statistical analysis revealed no differences between scores calculated from 10 vs. 5 minute segments, therefore only 5 minutes of video were scored for the second phase (baseline vs. 1.5mA). For each video segment, motor and phonic tics were counted and averaged per minute resulting in a score of tics per minute (TPM). In

accordance with previous protocols (Himle et al., 2006; Nixon et al., 2014) these TPM scores were then averaged to give the mean tic rate for each video clip.

Each video recording was also scored using the modified Rush video scale (Goetz et al., 1999), resulting in scores for motor/phonic tic severity, motor/phonic tic frequency, number of body areas and a total impairment score calculated from the sum of the others. Each component on the Rush is typically scored on a scale of 0-4, however, for the purposes of this study it was only possible to score 0-3 on the body areas component. This was because tics were only counted from the upper body and face meaning the maximal amount of body areas was 5, which corresponds with a rating of 3 on the scale. As a result, the maximum score possible on the Rush in this study was 29 rather than 30. The scores from each minute segment were combined to calculate the mean Rush score for each video clip.

Assessment of inter-rater reliability

In the first phase (sham vs 1mA cathodal) of the study, continuous 2 minute segments from each video were selected at random and scored by a trained secondary coder (KF), resulting in 25% of the total video duration being scored twice. As previous studies have used a sample of 24% (Himle et al., 2006; Nixon et al., 2014) this was considered more than adequate. For the second phase of the experiment (baseline vs 1.5mA cathodal) 1.5 minutes of data were cross-checked (30% duration of the 5 minute videos analysed). Using this method, the median agreement between the two coders for phase one of the experiment was 69%; this was 77% for the second phase.

6.2.9 rs-fMRI data pre-processing

The rs-fMRI data was pre-processed using the Statistical Parametric Mapping toolbox (SPM v8; www.fil.ion.ac.uk/spm) for Matlab (Mathworks, MA, USA). The structural and functional images were manually reoriented so that the origin was set to the anterior-commissure posterior-commissure (AC-PC) plane. The functional scans were then corrected for head motion using the six rigid

body motion parameters and by realigning to the first volume in the scanning sequence. In order to account for potential head movements between the anatomical and resting state scans the mean functional image was then co-registered to the respective structural scan. Following these processing steps, both the structural image and functional images were normalized to the Montreal Neurological Institute (MNI) template. Finally, the normalized functional images were smoothed using an 8mm full-width half-maximum (FWHM) Gaussian kernel. Throughout the pre-processing steps, data was visually inspected to ensure quality. The data were further pre-processed using ART-based scrubbing for functional outlier detection using the functional connectivity SPM toolbox Conn v.15d (Whitfield-Gabrieli & Nieto-Castanon, 2012). Scrubbing is a censoring technique proposed by Power et al. (2012) which makes it possible to identify and address subtle movement artefacts the data imaging data. This method identified a total of 7 time points during the sham scan, 9 during the active scan, 2 during the phase2 baseline scan and 5 during the final scan which was measured after the 1.5mA cathodal condition. No time points were identified as outliers in the phase 1 baseline condition, nor in the follow up condition.

Analysis of resting state connectivity

Following the initial pre-processing steps the SPM toolbox Conn v.15d (Whitfield-Gabrieli & Nieto-Castanon, 2012) was used to assess connectivity between different areas at rest. This was achieved by entering each individual scan into the first level analysis as a separate condition, with the respective motion parameters (identified previously using the six rigid body motion parameters) and outliers (detected using ART-based scrubbing) entered as covariates in order to guard against spurious correlations which have been known to arise due to subject motion (Power et al., 2012). The steps applied during this analysis included linear de-trending and de-meaning followed by regression of nuisance parameter signals (entered as co-variates) from white matter, CSF and their first temporal derivatives. Functional rs-fMRI images were

then band-pass filtered ($0.009 < f < 0.001$) in order to remove physiological fluctuations occurring due to respiratory and cardiac noise. Following these steps, a general linear model (GLM) for connectivity was estimated for each scan and these correlations were transformed using r-z-transformation. A total of 132 regions of interest (ROI) were identified based on the default atlas available in CONN which combines the cortical and subcortical regions of the FSL Harvard-Oxford atlas with cerebral areas from the automated anatomical labelling (AAL) atlas. It was not always possible to generate z-scores for all ROIs; in particular, in 4 of the 6 scans connectivity to some areas of the cerebellum was not calculated (3-4 regions per scan of a possible 18 identified as cerebellum by the atlas). This was due to issues during the planning of the scan which resulted in this area being cropped from the FOV. These data points were therefore removed from further analysis. In addition to this, if the sum of the z-scores for a particular ROI was smaller than 0.01 and larger than -0.01 these ROIs were removed from further analysis. This only effected the values obtained in the second baseline scan and resulted in the removal of additional ROIs within the cerebellum.

Statistical analysis of rs-fMRI: average connectivity

Paired sample t-tests were used to compare **average differences** in functional connectivity between the different scans. This was done separately for negative and positive correlations based on regions identified from the **active condition**. Only half of the correlation matrix was used in order to avoid duplicates. The following steps were applied:

1. Any ROIs showing positive connectivity values (z-score >0) in the **active condition** were identified.
2. The z-scores identified for these ROIs were then compared between the different scans. For example, to assess differences in the averaged functional connectivity between sham and baseline scans the z-scores in the predefined ROIs (from the Active scan) were compared using paired samples t-tests.

3. The same procedure was completed to test for changes in ROIs which showed negative connectivity in the **active condition** (z-score <0).

The same steps were used for the second phase of the experiment, however, the scan acquired after 1.5mA cathodal (active2) was used in steps 1 and 2 rather than the scan acquired after 1mA stimulation (active).

Statistical analysis of rs-fMRI: Identifying differences in functional connectivity across ROIs

The data were analysed further to identify statistically significant differences in functional connectivity between ROIs across the different scans. Paired sample t-tests were used to compare the connectivity of each ROI in the **sham** condition to **baseline**. The same procedure was also carried out to compare the connectivity of ROIs in the **Active** condition to those in the **baseline** condition and between the **active and sham** conditions. Paired samples t-tests were also used to assess differences between ROIs in the **baseline2 and active2** conditions. The resulting *p* values from these t-tests were corrected using the false discovery rate (FDR).

Statistical analysis of rs-fMRI: identifying ROI pairs which predict change

Using the data acquired from phase 1 of the experiment it is possible to explore how any changes from baseline in the active condition may predict the change from baseline seen in the Post condition. This was explored using two different methods. The first method involved using a customised script in Matlab (Mathworks, MA, USA) to calculate a series of linear regression analyses for the purpose of identifying any significant ROI pairs in the **active - baseline** condition which significantly predicted change in connectivity in the **post - baseline** condition. The false discovery rate was used to correct for multiple comparisons. To determine if the levels of variance explained by these ROI pairs was significantly different to that which would be expected from non-intervention (**sham-baseline**) a paired samples t-test was used to compare differences between the obtained R^2 values.

Using the second analysis method the correlation coefficients between **sham and post**, and between **active and post** were measured. Corrections for multiple comparisons were addressed using false discovery rate. A z-test was then used to test whether the correlations obtained for the **active-baseline** condition were significantly different from those obtained in the **sham- baseline** condition.

6.3 Results

6.3.1 Tic counts measure from video data

Phase 1

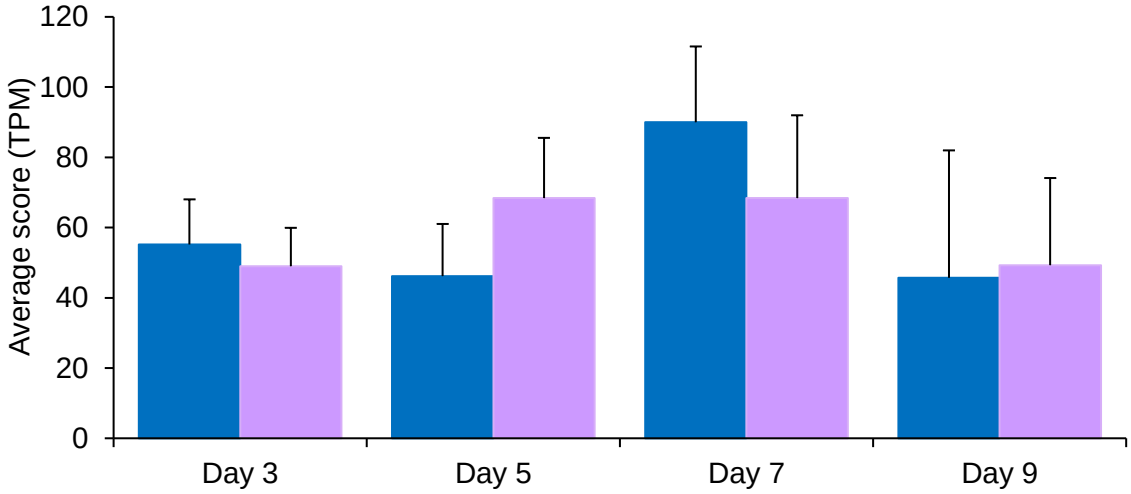


Figure 6.3. *Mean and SD of tic per minute counted from ten-minute video segments recorded on alternate days during sham and 1mA cathodal (active) conditions.*

There were no clear differences in total tics per minute between the sham and cathodal conditions. There was also no clear pattern of results when the video data was analysed using the Rush scale. Average tics per minute are shown in Figure 6.3.

Phase 2

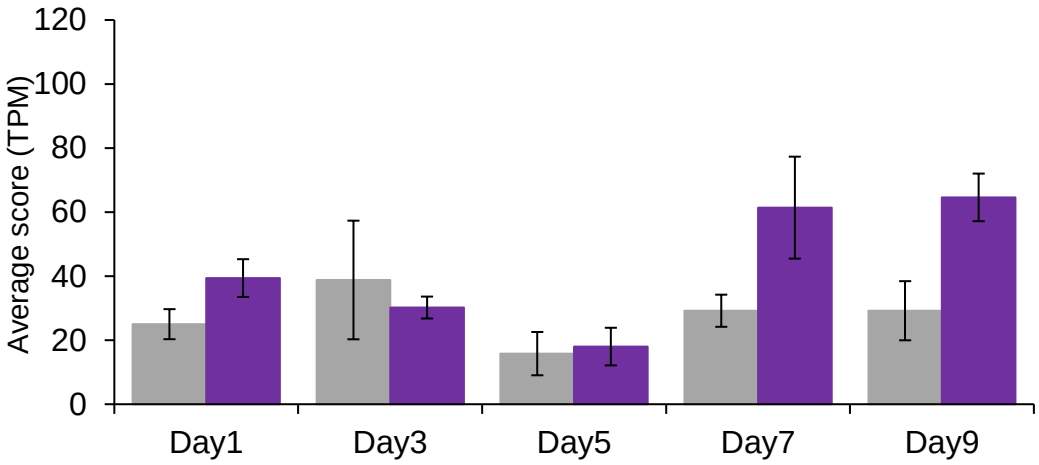


Figure 6.4. *Mean and sd of tic per minute counted from video segments collected during baseline2 which was taken after 2 months with no stimulation, and after 10 days of 1.5mA cathodal stimulation (active2).*

There was no clear difference between the number of tics counted per minute in the sham and 1.5mA cathodal tDCS. Rush scores were also not clearly influenced by the stimulation. Average scores from each video are shown in **Figure 6.4**.

6.3.2 Scores on questionnaire measures

Phase 1

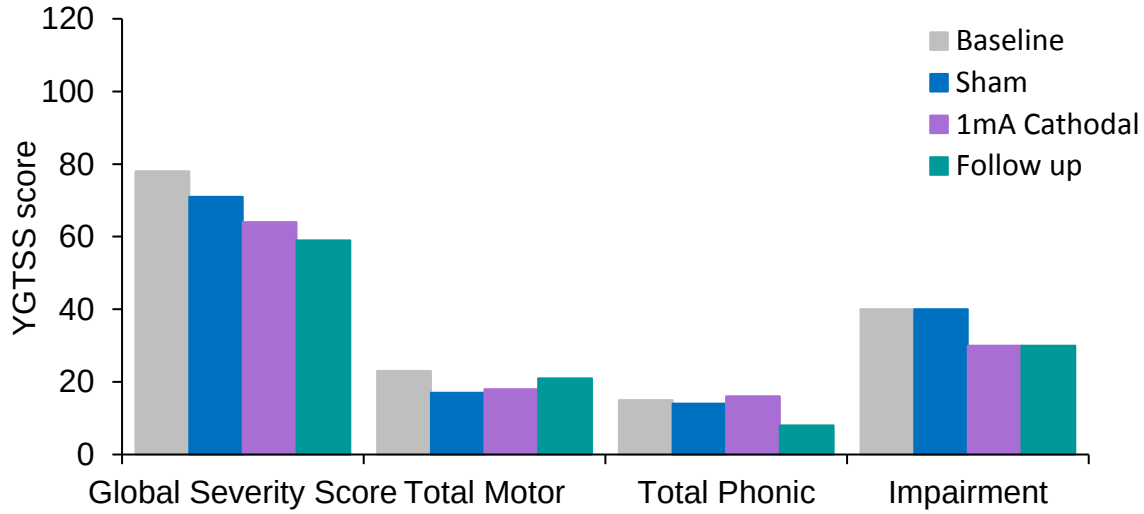


Figure 6.5. *YGTSS score on the various subcomponents measured at baseline, after 10 days of sham stimulation, after 10 days of 1mA cathodal stimulation (active) and in a 1 month follow up (post) condition.*

There was a gradual decrease in global severity score on the YGTSS in which the final follow-up measure was 24% smaller than the value given at baseline (Figure 6.5). Examination of the subscales for total motor and total vocal (sum of the number, frequency, intensity, complexity and interference components) revealed no clear pattern of tic reduction following 1mA cathodal stimulation. The effects on the global severity score appear to be primarily driven by changes in impairment ratings.

Scores on the PUTS also gradually declined over the course of the study. Scores in the sham condition showed a 21.7% decrease from baseline, which enlarged to a 34.8% decrease after cathodal and a 39.1% decrease in the follow-up measure.

Phase 2

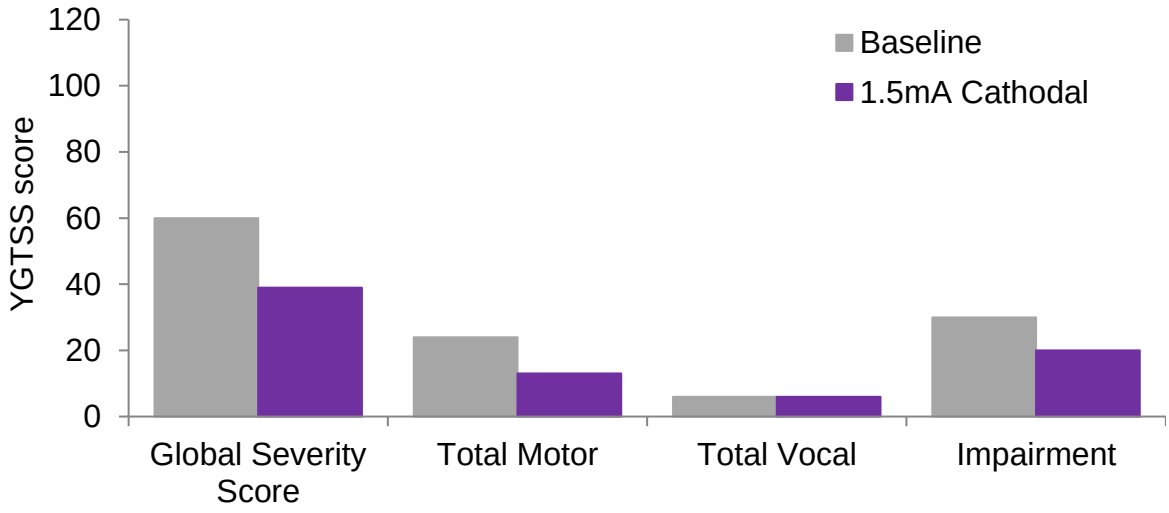


Figure 6.6. *YGTSS score on the various subcomponents measured during a second baseline (baseline2) taken over 2 month after any active stimulation took place and after 10 days of 1.5mA cathodal stimulation (active2).*

Scores on the YGTSS global severity score decreased 35% from baseline after 1.5mA cathodal stimulation. Scores on the total motor tic component reduced by 45.8%, and impairment rating decreased by 33.3% (Figure 6.6). There was no change in the total vocal tic score. Scores on the PUTS reduced by 14.3% after 1.5mA cathodal tDCS.

6.3.3 Resting state fMRI

Phase 1: average connectivity

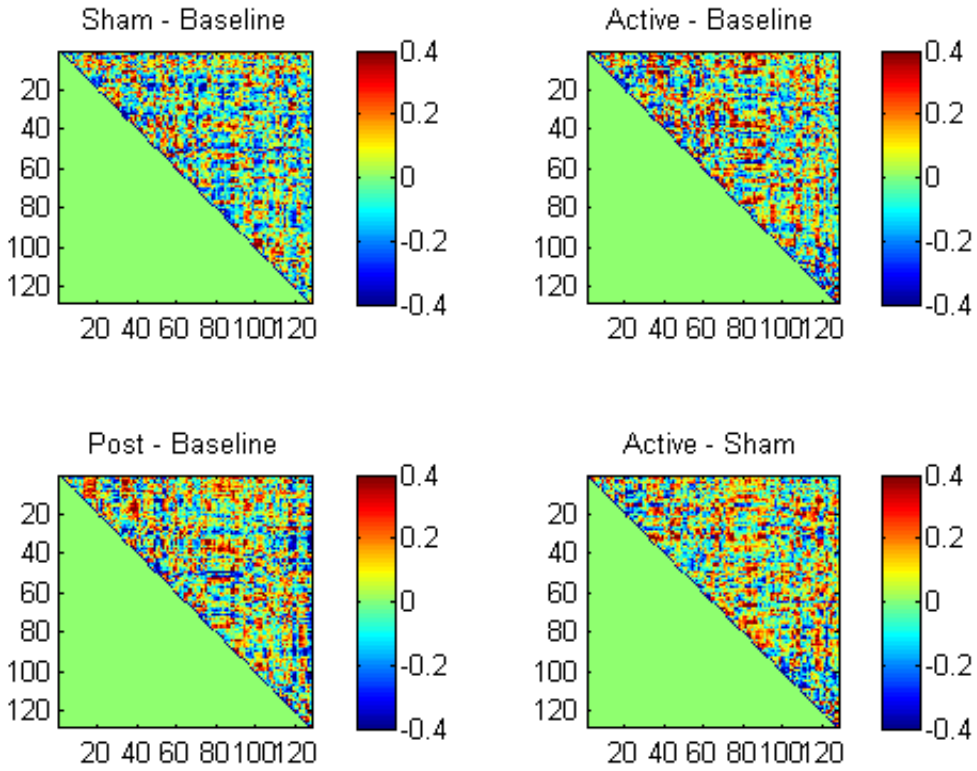


Figure 6.7. *Plots showing changes in functional connectivity from baseline for sham stimulation (top left), active stimulation (top right) and post stimulation (bottom left). Bottom right shows differences in functional connectivity following active stimulation compared to sham).*

A total of 128 ROI pairs remained in the analysis after the removal of regions for which there was insufficient data (defined as ROIs in which the sum across all possible pairings was <0.01 and >-0.01). This data was then analysed using the methods described above in 6.2.9. Paired sample t-tests revealed that the average amount of positive connectivity between ROIs was significantly larger in the scan taken after 1mA cathodal stimulation (**active**) compared to the **baseline** $t(4849)=31.04$, $p= <.0001$. There was also significantly stronger positive connectivity overall in the 1 month follow up condition (**post**) in comparison to **baseline** $t(4849)=7.68$, $p=<.0001$. Positive connectivity was actually lower in the

sham condition than at **baseline**, however, this effect was notably smaller than the other comparisons $t(4849)=-3.01$, $p=.0027$. Finally and most importantly, there was a clear difference between the scans taken following **sham** and **active** stimulation $t(4849)=35.91$, $p= <.001$, with the active scan revealing significantly more positive connectivity.

Separate paired sample t-tests were calculated to explore differences between areas of negative connectivity. These revealed that the **active** scan had significantly stronger levels of negative connectivity than the **baseline** scan $t(3277)=31.93$, $p= <.0001$. The scan taken following **sham** stimulation also had significantly stronger negative functional connectivity than the **baseline** condition $t(3277)=5.71$, $p= <.0001$. No significant differences were found between the **baseline** and follow up (**post**) scan $t(3277)=1.28$, $p= .20$. The strength of the negatively connected ROIs was stronger after 1mA cathodal stimulation (**active**) compared to connectivity following **sham** stimulation $t(3277)=27.01$, $p= <.0001$. The contrasts between the different scans can be seen in **Figure 6.7**.

Phase 1: Identifying statistical differences in functional connectivity across ROIs

In order to further investigate differences between ROIs, paired sample t-tests were used to compare the connectivity of each ROI to all other ROIs between **baseline and sham** scans, **baseline and active** scans, and between **sham and active** scans. The resulting *p* values were corrected for multiple comparisons using FDR.

The analysis revealed a total of 41 ROIs which showed significant differences ($p <0.05$, FDR corrected) in connectivity between the **baseline and sham** scans, including one region of the cerebellum and both the left and right accumbens. When a more conservative alpha level was used ($p<0.01$, FDR corrected), a total of 23 ROIs were identified including areas within the precentral gyrus, inferior frontal gyrus (IFG), superior temporal gyrus (STG) and inferior temporal gyrus (ITG). Differences in the left accumbens also remained

statistically significant. A full list of ROIs can be found in Appendix.Viii.

Comparison of ROIs in the **active** scan compared to the **baseline** scan revealed 42 ROIs which showed statistically significant differences in connectivity (p<0.05, FDR corrected), including 5 regions within the cerebellum and the right accumbens. A total of 16 differences between ROIs were identified when a more stringent criteria was applied (p<0.01, FDR corrected), including two regions of the cerebellum, regions in the IFG, ITG and supramarginal gyrus (SMG). A full list of ROIs can be found in Appendix.ix.

A number of the ROIs identified as statistically different from **baseline** in the **sham** condition, were also found to be statistically different from **baseline** in the **active** condition (12 in total). However, when the scans from the **active** and **sham** conditions were compared directly, a large number of differences were also found. Using a conservative threshold of p=<0.01 (FDR corrected) a total of 51 ROIs were found to be statistically different (A full list of ROIs can be found in Appendix.x. Of these differences, some of the largest (p<0.001) were seen between the right post central gyrus, the left posterior division of the superior temporal gyrus (STG), the left posterior division of the IFG, the right postcentral gyrus, 3 regions of the lateral occipital cortex, left/right central and parietal operculum cortex and the right planum temporale. There was also a strong statistical difference (p<0.001) between both left and right regions of the supplementary motor area (SMA).

Phase 1: Identifying ROI pairs which predict change

First it was necessary to identify if any of the ROI pairs which were different in the active condition compared to baseline (active – baseline) were significantly different to the relative amount of change between the two scans (active – baseline). In order to assess this the following steps were followed:

1: Mean and standard deviation of change in the **active – baseline** condition were calculated.

2: Scores from the **active** condition were standardized relative to those derived from step 1.

3: ROI pairs with change scores greater or less than 1.96 STD from the mean were identified.

This analysis revealed a large number of ROI- pairs that can be seen in Figure **6.8**. These ROIs were used in the subsequent analysis described below.

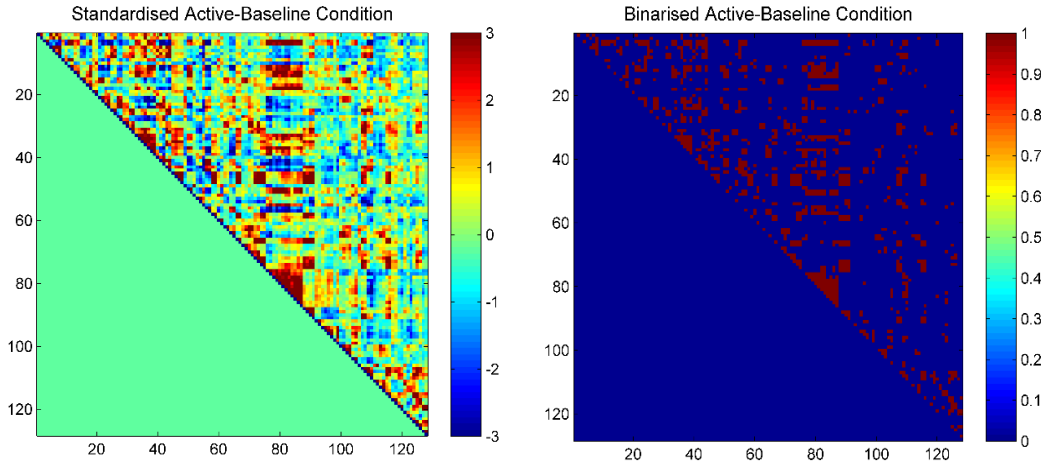


Figure 6.8. *Left image: shows the change in connectivity strength (active-baseline) expressed as standard scores. Right image: shows a binarised image identifying those ROI pairs with standard scores greater than 1.96 or less than -1.96.*

Two methods were used to identify ROIs which predict change following **active** stimulation in the follow up condition (**post**). The first involved a series of linear regression analyses which were conducted to identify any significant ROI pairs in the **active-baseline** condition (identified above) which predict change in connectivity in the **post-baseline** condition (corrected for multiple comparisons using false discovery rate (FDR)). This analysis identified a number of ROIs. In order to test that the variance explained by this condition significantly differed to that which would be expected in a non-intervention case the results obtained were tested against those found when the same analysis was conducted for the **sham-baseline** condition. This was done using a paired sample t-test comparing the R^2 values obtained from the **active-baseline** condition (*M*=0.52, *SD*= 0.12) to

those obtained using Sham-Baseline condition as predictors (*M*=0.47, *SD*= 0.19). The t-test revealed that the two are statistically different *t*(125)=-4.23, *p*=<.001. This suggests that changes in connectivity found in the **post** condition are more reliably predicted by changes from **baseline** in the **active** than **sham** conditions.

Using the second analysis method, the correlation coefficients between ROI pairs in the **sham** and **post** conditions and between **active** and **post** conditions were measured. Corrections for multiple comparisons were addressed using false discovery rate. A z-test was then used to determine whether the correlations obtained for the **active/baseline** condition were significantly different from those obtained in the **sham/ baseline** condition. A number of ROI pairs were identified by this analysis.

A total of 25 ROIs were identified by both analyses out of a possible 128. Of these 8 are of particular interest (right caudate, right accumbens and 6 regions within the cerebellum). All regions identified by both analyses can be seen in Appendix.xii.

Phase 2: average connectivity

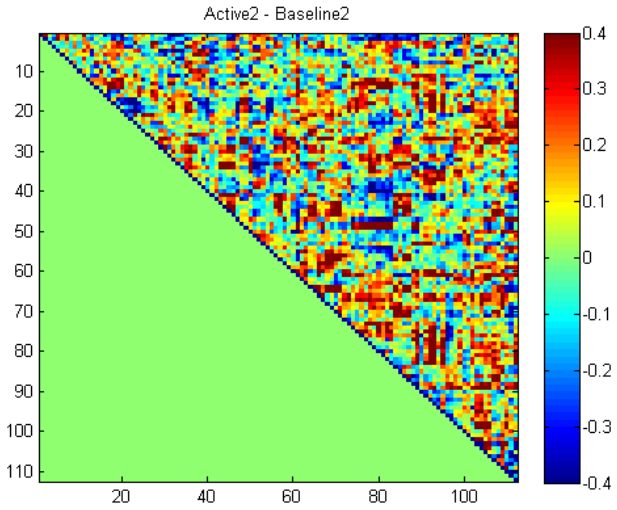


Figure 6.9. *Plot showing changes in functional connectivity for baseline2 for active2. Whereby active2 is the scan taken shortly after the completion of 10 sessions of 1.5mA stimulation.*

A total of 112 ROI pairs remained in the analysis after the removal of regions for which there was insufficient data (defined as ROIs in which the sum of z scores across all possible pairings was <0.01 and >-0.01). Paired sample t-tests revealed that the average amount of positive connectivity between ROIs was significantly larger in the scan taken after 1.5mA cathodal stimulation (**active2**) compared to the second baseline scan (**baseline2**) $t(3619)=77.75$, $p=$ <.0001. Interestingly the **active2** condition also showed significantly stronger negative connectivity than **baseline2** $t(2595)=-64.13$, $p=<.0001$. The data can be seen in Figure 6.9.

Phase 2: Identifying statistical differences in functional connectivity across ROIs

Paired sample t-tests were used to compare the connectivity of each ROI to all other ROIs in the **baseline2** and **active2** scans. The resulting *p* values were corrected for multiple comparisons using FDR. This analysis revealed a total of 47 ROIs in which connectivity to other ROIs was significantly different between the two conditions (Appendix.xi).

6.4 Discussion

Previous research (Carvalho et al., 2015; Mrakic-Sposta et al., 2008) has found that multiple sessions of cathodal tDCS can have a strong and significant impact on the number of tics experienced by individuals with GTS. This study aimed to extend and build upon the previous work. The main findings of the experiment are summarized below.

Findings from the questionnaire data

Questionnaire data provide a unique insight into the participants own feelings towards premonitory urges and tics and are, therefore, a valuable tool for assessing tDCS as an intervention. In phase 1 (baseline, sham, active, post conditions) scores on the global tic severity component of the YGTSS decreased over the course of the study (Figure 6.5). The global tic severity component reduced from baseline to active measures and remained reduced in the 1 month follow up. Scores on the PUTS also decreased from baseline as the study

progressed. These results could suggest that both tics and the urge to tic reduced following tDCS intervention, and that this persisted even one month afterwards. However, it should be noted that these reductions in scores from baseline were present in both the sham and 1mA active conditions. Furthermore, the reductions in YGTSS scores were primarily influenced by changes in the ratings given for the impairment subsection of the scale. While this is an important component of the YGTSS, the findings of previous studies (Carvalho et al., 2015; Mrakic-Sposta et al., 2008) suggest that a change in the motor or vocal component would have also been expected. As a result, although the findings in phase 1 hint at an effect of tDCS, it is not possible to rule out a placebo effect or a natural reduction in tics over time.

In phase two of the experiment there was a substantial drop in values from the baseline condition to those collected after 10 sessions of 1.5mA cathodal stimulation (Figure 6.6) Unlike in phase 1, the scores on the YGTSS decreased in the motor, impairment and global severity aspects of the questionnaire. These reductions were also far larger than those seen in phase 1 of the experiment. In particular, global severity score was reduced by 35% after stimulation, whereas in phase 1 of the experiment this reduction was 24%. Scores on the PUTS also decreased, although in this measure the reduction was smaller than in phase 1. It is likely that this reflects the differences between the baseline scores as in phase 1 these were far larger (PUTS score: 23 of a maximum score of 36) than the baseline2 (PUTS score: 14).

Although it could be argued that the reductions in the YGTSS scores seen in this study reflect a natural, gradual decline in tic severity due to the participants age, this is not supported by the data. This is because there are no clear differences between the YGTSS scores in the Post and Baseline2 conditions. If a natural decline were occurring independent of any intervention, then it would be expected that the scores in Baseline2 would be lower, as these were measured 2 months after the Post condition. The scores on the PUTS were also identical between Post and Baseline2 measures which suggests that intervention

(or at least perception of an intervention) influenced the results opposed to a natural decline.

Taken together, the results of the questionnaire measures suggest that the participant perceived a reduction in tic related symptoms over the course of the study. There is tentative evidence to suggest that the 1.5mA cathodal tDCS intervention had an impact on tics and urge to tic that went far beyond the effects of the 1mA intervention.

Findings from video data

There were no apparent differences in the amount of tics executed during videos taken in the sham condition compared to those recorded in the 1mA cathodal condition (Figure 6.3). Somewhat surprisingly, there were also no clear differences between the amount of tics counted during the 1.5mA cathodal condition and the second baseline measure (**Figure 6.4**). These findings are at odds with the results from the questionnaire data, particularly for phase 2 of the experiment. They also conflict with previous research conducted by Mrakic-Sposta et al. (2008).

Mrakic-Sposta et al. (2008) found that the amount of motor and vocal tics exhibited by their two participants reduced substantially by the end of 5 days of cathodal stimulation; critically this was not found after sham stimulation. Furthermore, unlike the findings of this study, the drop in tics counted during the videos also corresponded with a drop in tic symptoms reported on the YGTSS. There are a number of differences between the studies regarding the application of tDCS, however, it seems unlikely that these would fully account for the differences. For example, although Mrakic-Sposta et al. (2008) applied 2mA cathodal stimulation above the left M1 and the current study stimulated the SMA at 1 or 1.5mA, the optimal stimulation parameters are likely to differ from individual to individual. Furthermore, stimulating the SMA has previously been found to be effective in reducing tics (Carvalho et al., 2015; Kwon et al., 2011; Le et al., 2013; Mantovani et al., 2007; Mantovani et al., 2006) and weaker

intensities such as 1mA have been found to influence cortical excitability (Batsikadze et al., 2013; Furubayashi et al., 2008; Nitsche & Paulus, 2000). It seems more likely that the discrepancy in findings (particularly with the results of the 1.5mA intervention) relate to methodological issues regarding data collection which may have undermined the quality of the data, hence making it difficult to see any real effects.

Due to the prolonged nature of this study, compromises were made in order to limit the impact on day-to-day living for the participant and his family. It was decided that videos should only be taken every other day and that the timings of these could be slightly flexible to fit in with their normal schedule. Unfortunately, this led to more variability than initially expected. It is possible that differences between the timings of when the videos were taken could have impacted on the findings, especially in phase 2 of the experiment. This is because on average the videos were taken much earlier (approx. 18.30pm) during the baseline2 condition than during the active2 condition (approx. 21.30 pm). The participant and his parents reported that his tics typically became worse later into the evening, as a result any differences between the conditions may have been masked. This may explain the discrepancy between the video and the questionnaire data, particularly in phase 2.

Findings from the imaging data

Analysis of the overall patterns of connectivity between the different scans revealed statistically significant changes from baseline in the active and sham conditions and also partial differences (only for positive connectivity) between baseline and the follow up (post) scans. Although the sham condition was found to be statistically different to baseline in both analyses (exploring negative and positive connectivity), this was always smaller than the difference between baseline and active. Furthermore, when sham and active conditions were directly compared they were found to be significantly different. Analysis of phase 2 of the experiment also revealed statistically significant differences between baseline2 and active2 scans. These results are

encouraging, however, additional analysis was required in order to study these findings in more depth.

The data was further analysed to identify differences between the connectivity of specific ROIs. In **phase 1** of the experiment statistically significant differences between a variety of ROIs were found when baseline and sham conditions were compared. A large number of ROIs were also found to have significantly altered connectivity between the baseline and active scans. Although some of the same regions were identified in the sham/baseline and active/baseline comparisons, there were also a large number of differences which were further highlighted when these conditions were directly compared. Interestingly, the total number of ROIs found to be different between the baseline/sham conditions was similar to the number found for baseline/active conditions. This was somewhat surprising, as it was initially expected that more change from baseline would be observed in the active condition. Furthermore, the results are particularly difficult to interpret as the lack of clear effects on the motor/vocal component of the YGTSS and from the video data make it impossible to interpret what the consequences of these changes in connectivity may be.

Although in their current form it is not possible to rule out that the findings in phase 1 of the experiment were in part due to extraneous factors (see conclusions/ limitations) it is worth noting that there were changes in a number of ROIs in the cerebellum in the baseline/active comparison which were not found in the baseline/sham comparison. Furthermore, there were statistically significant differences between these areas when the two scans were directly compared. A total of 5 regions within the cerebellum were found to have statistically significant differences in connectivity ($p<.05$), of which 3 (cerebellum 3 left, 3 right and 4 5 right in the atlas) had significantly lower connectivity in the active than in the sham scan when tested at $p<.01$.

Differences within the cerebellum are notable because in a previous case study Carvalho et al. (2015) found that activity within left regions decreased after

10 sessions of cathodal tDCS. Furthermore, the cerebellum has been implicated in a number of GTS studies using a variety of experimental methods. For example, in an event related fMRI study the cerebellum was found to be more active during the beginning of a tic action (Bohlhalter et al., 2006) and in an animal model of GTS increased neuronal spiking in the region has also been associated with tic onset (McCairn et al., 2013). Studies using PET have found increased metabolic activity within the region at rest (Pourfar et al., 2011) and altered GABA-A receptor binding bilaterally (Lerner et al., 2012). Structural differences have also been noted, with individuals with GTS having bilateral reductions in cerebral hemispheres when compared to controls (Tobe et al., 2010).

Unfortunately, in this experiment a large proportion of the cerebellum was not captured by the scans in baseline2 and active2 conditions, therefore changes in these regions could not be assessed for phase 2 of the study. A number of ROIs were identified as having significantly altered levels of connectivity between these two scans. However, although a clear reduction in tics was seen on the YGTSS between baseline2 and active2, the finding of widespread change in connectivity in the sham-baseline condition makes it difficult to draw any strong conclusions regarding how change in specific ROIs may relate to changes in tics following stimulation. Consequently, this is not speculated here, although a full list of identified ROIS can be found in Appendix.xi.

In **phase 1** of the experiment it was possible to assess how change in the active condition may predict the change from baseline seen in the post condition. Both the analyses used implicated the cerebellum, with a total of 6 regions being identified as significant predictors. Scores on the global impairment scale of YGTSS remained stable between the active and post scans, and the PUTS score actually dropped slightly over this time. Taken together it may be that changes within the cerebellum had a sustained effect on some aspects relating to the participant's perception of their own tics and feelings of

premonitory urge. However, as there were also changes in the questionnaire measures between baseline and sham conditions and little effect on the motor/vocal component of the YGTSS, it is not possible to conclude that these changes were necessarily the result of 1mA cathodal tDCS or even linked to changes in tics.

In addition to the cerebellum, a number of regions were implicated as significant predictors. Of particular interest were changes in the right Caudate nucleus and the right accumbens. Findings within the caudate are particularly notable for a number of reasons. Firstly, the caudate is known to receive inputs from the SMA (Vergani et al., 2014), secondly, the caudate has repeatedly been implicated as both structurally and functionally different in individuals with GTS when compared to control subjects. In particular, the caudate has been reported to be smaller in children and adolescents with GTS (Makki et al., 2008; Peterson et al., 2003), and the volume of the caudate in childhood has been found to be predictive of symptom severity in adults with GTS (Bloch et al., 2005), with smaller caudate volumes in childhood (<14 years old) correlating with higher scores on the YGTSS years later in life (>16 years old). Furthermore, in a study of 13 adults with GTS, Wang et al. (2011) found that activity within the caudate was reduced in GTS individuals during spontaneous tics in comparison with self-paced tic-like- movements in 22 healthy controls, hence implicating the region in tic production. The caudate is thought to be important in exerting top-down control over motor pathways, therefore when taken together with research in GTS it seems logical that changes in this region may contribute to changes in symptomology. However, although the caudate was implicated in this analysis, it is important to note that the connectivity of this region was not found to be significantly altered when the active or sham conditions were compared to baseline. Thus while the findings suggest that the caudate is an interesting ROI to examine in future work, strong conclusions about this area cannot be drawn from this data.

A final notable finding from the imaging data was that changes in the connectivity of the right accumbens following sessions of 1mA tDCS predicted change from baseline in the follow-up condition (post). Changes in this region are interesting as the area has previously been found to have altered structural connectivity in adults with GTS (Neuner et al., 2011). Furthermore, there is evidence that deep brain stimulation of the accumbens may help with symptoms of obsessive compulsive disorder (OCD) (Denys et al., 2010), a disorder which commonly co-occurs with GTS (Bitsko et al., 2014).However, in the present study it is not possible to draw strong conclusions about changes in the accumbens and changes in tics as a result of 1mA cathodal stimulation. This is due to the finding that resting state connectivity in the accumbens was significantly different between the baseline and sham scans, in addition to being different when active and baseline scans were compared (see Appendix ix and x). Nevertheless, the finding that change in the **active – baseline** condition was predictive of **post-baseline** condition and that during this time the scores on the global severity rating of the YGTSS remained stable while scores on PUTS dropped could implicate this region as linked with the participants own experiences of their tics.

Although no strong conclusions can be drawn from the imaging data, the findings may play an important role in identifying the physiological basis of change in tics for future studies in which larger sample sizes and full counterbalancing of conditions could be used.

Evaluation of home use stimulation

To my knowledge this study is the first to trial home use stimulation for the treatment of GTS and one of a very small number of studies to use tDCS outside of the laboratory. Despite a few teething issues with electrode placement at the start, overall application of tDCS went well. Compliance was very high, and all of the 30 advised stimulations were attempted (10 for sham, 1mA cathodal and 1.5mA cathodal respectively). Of these, only one was not fully completed due to an electrode moving after a head tic which caused the machine to abort stimulation for that day. Rates of compliance in larger studies

have also shown that tDCS application at home is feasible. Kasschau et al. (2016) found that compliance was high in a group of 20 participants with muscular sclerosis (MS) with 98% completing at least 8 of the 10 advised sessions. Kasschau et al. (2016) also found that of 192 supervised treatment sessions none required discontinuations and no adverse events were reported. In this study the participant did report some feelings of mild nausea and headache following stimulation. However, these were reported in both the sham and cathodal conditions and were reported by the participant and their family to be tolerable.

The main challenge of the home use approach in this study was not the application of tDCS, but the accurate data collection of video clips. Although video recordings in naturalistic settings provide a rich data set which can give insight into the frequency, intensity and type of tics experienced, this type of data comes with some inherit variability, and it proved difficult to obtain good quality video recordings using this method.

Conclusions and limitations

Overall the study provides some support that tDCS may be a useful tool in reducing tics in individuals with GTS, however, there are some important limitations which must be considered. Firstly, the study consisted of a single participant, and while efforts were made to include a sham condition it remains a possibility that the order in which the different sessions were tested may have had an impact. Furthermore, the participant was a 16 year old and it is known that tics may wax and wane with age and that many adolescents will experience a remission in symptom severity as they approach adulthood (Bloch & Leckman, 2009). Despite this, a natural decline in tics over the course of the study seems an unlikely explanation for the effects seen after 1.5mA cathodal stimulation, due to the finding that scores on the PUTS and YGTSS did not decrease during the two month interval between Post and Baseline2 data collection. Nevertheless, without additional participants and counter balancing it is not possible to rule out the possibility that random fluctuation in tic symptoms unrelated to tDCS may have contributed to the data.

Although the application of tDCS within the participant's home was relatively unproblematic, this was not true of the video recordings. A number of issues were present which may have compromised the quality of the data and could account for the discrepancy between scores on the YGTSS and tic count; in particular, in phase 2 of the experiment in which a decrease in motor tics was reported yet not evidenced in the video data.

The findings of the resting state fMRI analysis are somewhat difficult to interpret, particularly as significant differences were identified in the sham condition in addition to after the 1mA cathodal and 1.5mA cathodal sessions. Furthermore, a surprisingly large number of ROIs were found to show significant alterations in connectivity between the different scans. Although some of this may be resultant of the intervention/perceived intervention in the sham condition, it is also possible that a number of additional uncontrolled variables contributed to these findings. In particular, although the participant was instructed to keep his eyes shut it was not possible to check this. Furthermore, it was apparent that during some scans the participant may have fallen asleep. Previous work has demonstrated that having the eyes open or closed can have an impact on resting state data (McAvoy et al., 2008; Yan et al., 2009) as can being asleep or awake (Tagliazucchi & Laufs, 2014). In larger scale studies this may be less problematic, however, in a single case design such as in this study the impact is likely be more apparent.

It is also possible that some of the differences found between the scans may reflect differences in the participant's level of tic suppression/ tic enactment during the scan. Previous work using fMRI has identified distinct patterns of activity occurring shortly before tic onset and during tic execution (Bohlhalter et al., 2006), and differences have also been found between patterns of activity during tics compared with voluntary motor actions (Bohlhalter et al., 2006; Hampson et al., 2009; Wang et al., 2011). These studies have implicated a number of regions particularly within the motor pathway including the sensorimotor cortex, putamen, palladium, substantial nigra (Wang et al., 2011),

the SMA (Bohlhalter et al., 2006; Hampson et al., 2009) and cerebellum (Bohlhalter et al., 2006). In this study some of these regions were found to be significantly different across the scans, however, as no information about tic occurrence or suppression was collected during the scan it is not possible to decipher if these differences were related to tic occurrence or if they were more stable. This is a consideration for future research in order to determine if the effects seen using techniques such as resting state fMRI reflect stable changes or the consequences of differences in the level of tics an individual experiences while in the scanner. It may also be beneficial to use other methods to assess change, including any change in white matter connectivity.

To conclude, this study provides some support that the home application of tDCS may be useful in reducing tics in Tourette's syndrome. The evidence from the YGTSS suggests that 1.5mA cathodal stimulation was more effective than 1mA, this was also reflected by the participants' own sentiments after receiving this intervention. This research adds to that of Carvalho et al. (2015) and Mrakic-Sposta et al. (2008) and highlights the need for larger, counterbalanced, sham controlled studies to fully evaluate the techniques' potential.

Chapter 7: What are we measuring with magnetic resonance spectroscopy and why is this important?

Some of this work has previously been presented in: Dyke, K., Pépés, S, E., Chen, C., Kim, S., Sigurdsson, H. P., Draper, A., Husain, M., Nachev, P., Gowland, P, A., Morris, P. G., & Jackson, S. R. (2017). Comparing GABA-dependent physiological measures of inhibition with proton magnetic resonance spectroscopy measurement of GABA using ultra-high-field MRI. Neuroimage, 152, 360-370.

Key words: *transcranial magnetic stimulation (TMS), resting motor threshold (RMT), input output curve (IO curve), short interval intracortical inhibition (SICI), long intracortical inhibition (LICI), intracortical facilitation (ICF), magnetic resonance spectroscopy (MRS),* gamma-aminobutyric acid (GABA), glutamate (Glu) glutamine (Gln).

MRS data collection was completed by Sophia Pépés with whom co-authorship of this study is shared.

7.1 Introduction

Although the exact mechanisms underlying Tourette's Syndrome (GTS) remain partially unclear, there is a large amount of convergent evidence to suggest that GTS is characterised by altered GABA function. GABA is the primary inhibitory neurotransmitter in the human brain, and has been estimated to be present at 20-50% of all synapses. GABA plays a critical role in the regulation of cortical excitability, and is of great importance during brain maturation (Ben-Ari et al., 2012). Dysregulation of GABA has been reported in a number of neurodevelopmental disorders including autism spectrum disorder (Hussman, 2001; Pizzarelli & Cherubini, 2011) , epilepsy (Treiman, 2001), Tourette's syndrome (Draper et al., 2014) and schizophrenia (Gonzalez-Burgos et al., 2010). As a result, GABA is a major target for drug development and interventions using NIBS.

The evidence for dysregulation of GABAergic systems in GTS is convergent across multiple methods, and consistent with the view that GTS is specifically linked to alterations in GABA signalling and the operation of inhibitory brain

circuits. It is generally acknowledged that cortical-striatal-thalamic-cortical circuits (CSTC) are dysfunctional in GTS. As a result of this dysfunction, subsets of striatal neurons are thought to become active in inappropriate contexts. This is thought to result in disinhibition of thalamo-cortical projections (Albin & Mink, 2006) and hyper-excitability of limbic and motor regions of the brain, both of which are thought to result in the occurrence of tics (Bohlhalter et al., 2006; Orth, 2009; Tinaz et al., 2015). Consistent with these findings, post-mortem studies have found significant reductions in the amount of GABAergic interneurons within the striatum in individuals with GTS (Kalanithi et al., 2005; Kataoka et al., 2010). In addition to this, positron emission tomography (PET) has revealed a reduction in GABA-A receptor binding sites in the striatum, thalamus and insular cortex (Lerner et al., 2012). Evidence from paired pulse TMS studies also suggests abnormalities in GABA levels. Individuals with GTS have been found to have lower levels of SICI responses (Gilbert et al., 2005; Orth et al., 2005; Ziemann et al., 1997) which suggests a reduction of GABA-A receptor mediated inhibition within the motor cortex. Furthermore, studies using animal models have suggested a direct link between GABA and tic generation. For example, injecting different parts of the striatum with a GABA antagonist has been found to induce motor and phonic tic like behaviours in monkey and rat models (Bronfeld et al., 2013).

A recent study using ultra-high-field strength (7 Tesla) magnetic resonance spectroscopy (MRS) found significantly elevated levels of GABA within the supplementary motor area (SMA) in individuals with GTS when compared with closely matched control subjects (Draper et al., 2014). Although this finding may seem counter intuitive at first, Draper et al. (2014) argued that this seemingly paradoxical increase may be a compensatory, adaptive mechanism, through which individuals with GTS develop increased control over motor outputs. Draper et al. (2014) also found that levels of MRS-GABA within the SMA were correlated with tic severity, which suggest that this compensatory mechanism may arise due to the gain of motor outputs being altered as a result of increased GABA levels within the SMA. Interestingly, Draper et al. (2014) failed to find any

significant alterations in GABA levels within the motor cortex. This is somewhat surprising, as a number of previous studies using SICI have suggested a reduction of GABAergic activity within this brain region (Gilbert et al., 2005; Orth et al., 2005; Ziemann et al., 1997). However, it is possible that the discrepancy between these results at M1 and the seemingly paradoxical elevation of GABA at the SMA, relate to differences between the two techniques.

It has been speculated that what is measured in MRS may reflect extracellular levels of GABA which are linked to 'tonic' GABA-ergic inhibition (Rae, 2014). Whereas, TMS measures of SICI are thought to primarily reflect the operation of low threshold, transiently activated, cortical GABA interneurons (Ziemann, Lonnecker, et al., 1996; Ziemann, Rothwell, et al., 1996). If the two techniques are measuring from distinct pools of GABA, it would be unsurprising to find a decrease in one and increase in another, particularly in the context of syndromes such as GTS whereby an adaptive compensatory response may be apparent.

Enhancing our understanding into what we are measuring with MRS is important to better comprehend the causes and consequences of disorders such as Tourette's syndrome. In addition to this, enhancing knowledge about MRS measures could also help to develop our understanding of the effects of NIBS techniques.

Past research with TMS (Batsikadze et al., 2013; Cengiz et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005) and MRS (Stagg et al., 2009) has suggested that tDCS can influence levels of GABA. This could be particularly important with respect to using these techniques therapeutically, as long-term potentiation (LTP) type plasticity within the neocortex is thought to be critically dependent on the modulation of this neurotransmitter (Trepel & Racine, 2000). However, the effects of tDCS on GABA levels may not be direct, particularly for cathodal stimulation. This is supported by the finding that administration of the GABA-A receptor agonist Lorazepam did not significantly alter the after-effects of cathodal stimulation (as measures by TMS), although it did significantly reduce

anodal tDCS after-effects (Nitsche et al., 2004). This finding has led to speculation that the observed after-effects of cathodal stimulation may be due to the tight coupling between GABA and Glutamate (Stagg & Nitsche, 2011). As GABA is synthesised from glutamate, and glutamate level has been shown to change following cathodal stimulation (Nitsche et al., 2005; Stagg et al., 2009) this seems plausible. Testing this further using a multi-modal approach is likely to answer this question, but in order for this to be truly effective it is important that questions such as exactly where is the MRS-GABA signal coming from are answered.

Two recent studies have sought to address the relationship between TMS and MRS measures of GABA and glutamate/glutamine within the sensorimotor cortex (Stagg et al., 2011; Tremblay et al., 2013). Both studies acquired TMS and MRS measures using conventional field strengths (3 Tesla) within the same individuals. Tremblay et al. (2013) failed to find any significant correlations between any TMS measures (including 3ms SICI) and MRS measured GABA levels. However, Stagg et al. (2011) reported that although MRS-GABA concentrations within motor cortex (M1) were uncorrelated with 2.5ms SICI, there was a significant correlation with the slope of the 1ms SICI curve. As discussed in chapter 2, the physiological mechanisms underlying 1ms SICI effects are thought to be distinct from those occurring at later ISIs, and although these mechanisms are not yet fully understood, it has been speculated that the effects may reflect axonal refractory periods (Fisher et al., 2002). In line with this, Stagg et al. (2011) discuss the possibility that the relationship suggests that MRS measures extra synaptic, tonic GABA which may relate to the duration of the refractory periods of neuronal axons. Although a plausible explanation, the refractory periods theory of 1ms SICI is not held by all, and some argue that synaptic involvement in the effect is also likely (Vucic et al., 2009). In addition to this, although Tremblay et al. (2013) also failed to identify correlations between MRS measured GABA and later SICI (3ms SICI), they did not measure 1ms SICI, hence the results of Stagg et al. (2011), have yet to be replicated.

The Stagg and Tremblay studies also differ in the associations that they reported for other TMS/MRS metabolite pairings, partially due to the use of different TMS measurements. Thus, while Stagg et al. (2011) reported that a general measure of cortical excitability - the slope of the TMS recruitment (IO) curve - was significantly associated with MRS glutamate concentrations within M1, Tremblay et al. (2013) reported a significant positive correlation between Glx (a composite measure of glutamate and glutamine) and the duration of the cortical silent period (CSP). Furthermore, in addition to differing on which particular TMS measures to relate to MRS measures of motor cortical excitability and physiological inhibition, the above studies also differed in that one study used a GABA-edited MRS sequence (Tremblay et al., 2013) while the other (Stagg et al., 2011) did not. This may have had important implications for the measurements obtained, particularly as both studies were conducted at conventional MR field strengths.

The advantages of ultra-high field (7 Tesla) are the increased signal-to-noise ratio (SNR) obtained and greater chemical shift dispersion (Tkác et al., 2009). The increased SNR improves the detection sensitivity and efficiency of metabolites, especially those with low concentration such as GABA. Greater chemical shift dispersion increases the separation of signals with similar resonance frequencies, allowing a more accurate identification and quantification of each metabolite. For instance, due to spectral overlapping the differentiation of GABA, Glu and Gln signals are difficult in ^{1}H spectra at field strengths of 3 T or less (Puts & Edden, 2012), and Glx (a composite measure of Glu + Gln) is reported instead. By contrast, GABA, Glu and Gln become separable at field strengths of 7 T or above. Also, GABA-edited (J-difference editing) sequences (Mescher et al., 1998; Near et al., 2013) rely on subtraction to remove overlapping signals from the spectrum. This technique is therefore particularly susceptible to motion-related errors that are less of an issue for non-edited MRS sequences (e.g., STEAM) that can be utilised at 7 T (Bhattacharyya et al., 2007; Bogner et al., 2014).

In this study, we investigated how TMS and MRS measures relate by directly comparing a wide range of TMS measurements of motor cortical excitability (including TMS recruitment curves and paired-pulse measures of intracortical facilitation [ICF]) and physiological inhibition (including both 1ms and 3ms SICI) with MRS measures of GABA, Glu and Gln acquired at ultra-high-field (7 Tesla) using a non-edited STEAM sequence.

In addition to investigating correlations of TMS and MRS measured at baseline, the effects of a pharmacological manipulation of GABA using the drug gabapentin (GBP) were explored. Gabapentin (1-(aminomethyl)cyclohexaneacetic acid (Neurontin®)) is a safe and widely prescribed drug typically used to treat conditions such as epilepsy and neuralgia. It was originally synthesised as a GABA analogue, however, animal experiments revealed that the drug failed to show direct GABAergic receptor action (Ziemann et al., 2015). The exact mechanisms of action for GBP are yet to be fully understood, however, there is evidence that GBP may increase GABA synthesis and turnover, decrease glutamate release and block $\alpha 2\sigma$ voltage gated calcium channel sub-units (VGCC) (Ziemann et al., 2015).

Although GBP does not appear to interact directly with GABA-A or GABA-B receptors (Goa & Sorkin, 1993; Sills, 2006; Taylor et al., 1998), and does not appear to be a GABA-A (Kondo et al., 1991) or GABA-B agonist (Lanneau et al., 2001), it has been found to influence both TMS and MRS measures associated with GABA. Of particular interest are the findings of two TMS studies in which 800mg and 1200mg doses of GBP were found to increase SICI and decrease ICF, while not influencing motor thresholds (Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996). As MT is known to be effected by voltage-gated sodium channel blockers such as carbamazepine (Menzler et al., 2014; Ziemann, Lonnecker, et al., 1996) but is not influenced by GBP, this suggests that calcium channel inactivation is unlikely to fully explain the effects. It has been proposed that GBP's effect on SICI could be a result of increased GABA synthesis (Ziemann et al., 2015). Unfortunately, neither TMS study was placebo controlled, and there

appears to have been no subsequent attempts to establish the effects of GBP using TMS.

Evidence from MRS studies supports the hypothesis that GBP influences GABA synthesis, and the drug has repeatedly been found to cause elevated levels of GABA in vivo in the human brain. Three notable studies have been documented, the first of which was conducted by Petroff et al. (1996). They found elevated levels of GABA in patients with epilepsy who were medicated with GBP compared with individuals who were taking medications such as carbamazepine. Interestingly the effects appeared to be dose specific, with those taking higher doses showing higher levels of GABA. In a more recent 7T MRS study with healthy participants, Cai et al. (2012) found that a single 900mg dose of GBP significantly elevated levels of GABA within the visual cortex. This elevation was far greater than that seen in the control condition and therefore, the effects cannot be accounted for by natural GABA fluctuations over time. Finally, Kuzniecky et al. (2002) studied the effects of acute and chronic doses of GBP in healthy participants using MRS at a field strength of 4.2 Tesla. A single acute dose of the drug (adjusted to participant's weight (17mg/kg)), caused significant increases in GABA measures 6 but not 3 hours after ingestion. Significantly elevated levels of GABA were also measurable after a 4 week course of the drug, although not after 2 weeks.

It appears that no placebo controlled trials have been conducted in which the effects of a single dose of GBP are studied using both TMS and MRS from the same individuals. Therefore, the aims of this study were two fold. Firstly, to investigate the relationship between MRS and TMS measures of GABA, Glu and Gln, and secondly, to explore the effects a single dose of GBP may have on both of these measures.

7.2 Method

7.2.1 Participants

29 healthy right-handed adults (age range 19-27) participated in the study. All participants were free from neurological or psychiatric illness and any

contra-indications for MR scanning or TMS. Of the 29 participants recruited, two were subsequently excluded from the analysis of baseline MRS/TMS measures: one due to poor quality TMS data (insufficient test pulse intensity) and one due to poor quality MRS data. Details of the 27 participants included in the data analyses for baseline correlations are contained in Table 7.1.

Table 7.1. *Participant demographics for baseline analysis. Data are presented as mean value ± sd. RMT = mean resting motor threshold. SI 1mV = mean stimulator output required to produce a MEP with an amplitude of 1mV. † Percentage of maximal stimulator intensity.*

N	Sex (M/F)	Age	RMT †	S1 1MV †
27	13/14	23.1 ± 2.4	45.7 ±6	55.3±6.8

Of the 29 participants tested 14 were assigned to the placebo condition and 15 were assigned to the GBP condition. Due to the exclusion of one data set for poor quality TMS in the placebo group, and one data set from the GBP group due to poor quality MRS data, there were slight variations between sample sizes. Participant demographics for the different conditions can be seen in Table 7.2.

Table 7.2. *Participant demographics for analysis of drug manipulation data. Data are presented as mean value ± sd.*

	N	Sex (m/f)	Age	BMI
MRS placebo	14	8F, 6M	22.3 ± 2.4	21.8±3.6
MRS gabapentin	14	7F, 7M	23.6 ± 2.4	21.9 ± 2.6
TMS placebo	13	7F, 6M	23 ± 2.5	22.1 ± 3.7
TMS gabapentin	15	7F, 8M	23.6 ± 2.4	21.9 ± 2.6

7.2.2 MR acquisition

All MR data were acquired using an ultra-high field 7T Philips Achieva system (Philips Healthcare, Best, Netherlands) with a 32-channel radio frequency head coil at the Sir Peter Mansfield Imaging Centre (SPMIC), University of

Nottingham. Participants were placed supine and head-first into the scanner. Foam pads were inserted between the participant's head and the coil to minimise and control head movement; a pair of prism glasses was provided to allow participants to view a screen outside the magnet bore.

At the start of each imaging session ^{1}H image localiser and B0 maps were acquired, followed by BOLD-fMRI T_2^*-weighted images, which were acquired to guide placement of the left motor cortex (M1) spectroscopy voxel in the following MRS scans. The BOLD-fMRI used a single shot EPI sequence (TR/TE=1999/25ms, FOV=208×192 mm3, matrix=112×112, 30 slices, slice thickness=4mm, no slice gap, 160 dynamics). During the fMRI scan, eight blocks of bimanual finger-to-thumb opposition tapping were performed in a blocked-trial paradigm as follows. The words 'TAP' and 'REST' were alternately displayed for 8s and 32s, respectively. Participants were asked to tap their thumbs to each finger with both hands simultaneously and continuously during the 'TAP' phase and to rest (withhold movement) during the 'REST' phase. Maximum activation of the left M1 was found by analysing the BOLD response on-line using the Philips IViewBOLD software.

T_1-weighted anatomical images were then acquired with a MPRAGE sequence (TR/TE/TI=7.3/3.4/998ms, FA=8^{o}, FOV=224×224×120 mm^3, isotropic resolution=1mm^3) for tissue segmentation. Anatomical landmarks from these images were also used to assist in the placement of the left M1 voxel for MRS.

In vivo ^{1}H MRS data were acquired from a voxel of interest (VOI=20×20×20mm^3) placed over the hand area in the left M1 (Figure 7.1A) using a STEAM sequence (TE/TM/TR=17/17/2000ms, sample size=4096, spectral bandwidth=4000Hz, phase cycling=8, 288 averages, 9.6mins). Water suppression was performed using multiply optimised insensitive suppression train (MOIST) (Murdoch, 1993). Prior to this, a non-suppressed water reference spectrum (16 averages) from the same VOI was acquired for eddy current correction and quantification. B0 shimming of the VOI was performed automatically by the

Philips pencil beam (PB) algorithm (Gruetter, 1993) in order to increase B0 field homogeneity.

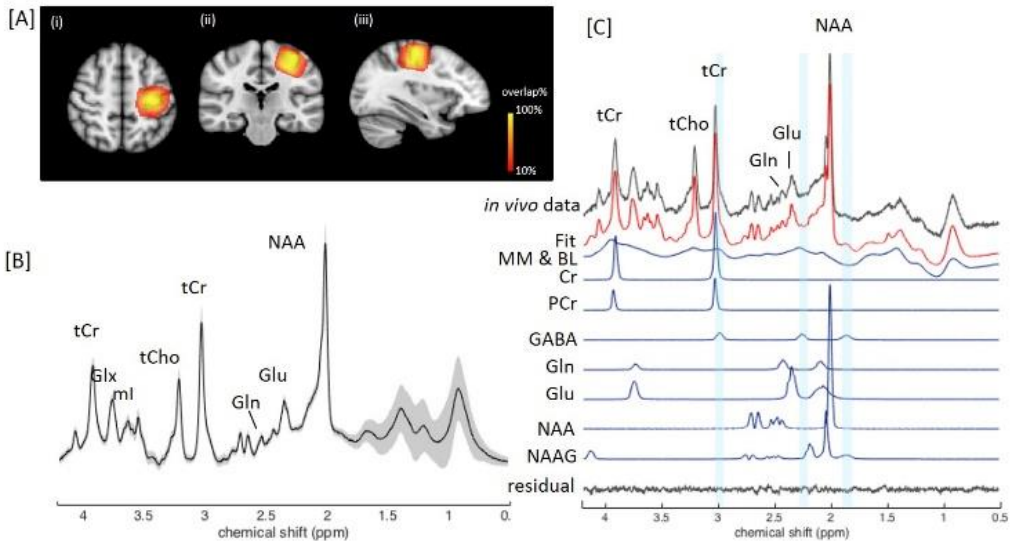


Figure 7.1 *[A] Position of the voxel of interest (VOI=20×20×20mm3) located over the left-hand area of M1 shown in (i) sagittal, (ii) axial (iii) and coronal views. [B] Standard deviations (shaded area) overlying the group mean in vivo spectrum acquired from the VOI obtained with STEAM sequence (TS/M=17/17ms) at 7T are shown (N=27). [C] A representative in vivo spectrum obtained from the M1 VOI is shown, together with its LCModel fot. Residual and fitted signals for metabolites of interest and macromolecules (MM) and baseline (BL) are shown.*

7.2.3 TMS measurements and EMG recording

MR scanning sessions were performed before TMS measurements were obtained. On average the time in between the final MR scan and the commencement of the collection of TMS measurements was 30 minutes.

TMS was delivered using a Magstim Bistim system (Magstim, Whiteland, Dyfed, UK) with a figure-of-eight magnetic coil (70mm diameter of each winding). The coil was held tangentially to the scalp and positioned 45° from the midline, resulting in a posterior to anterior current flow. Neuro-navigation software (Brainsight, Rogue Research Inc., Montreal Quebec, Canada) was used in conjunction with individual T_1-weighted anatomical images (acquired using the

MPRAGE sequence outlined above) to aid coil placement over the hand area of the left motor cortex. All TMS measures were obtained from the motor hot spot identified for the First Dorsal Interosseous (FDI) muscle. This was defined as the location that consistently yielded the largest MEP amplitudes for the FDI. Participants were required to remain still during testing, and head movements were minimised with the aid of a chin rest. Participants were offered frequent breaks to stretch and adjust their position and neuro-navigation was used to accurately reposition the coil over the motor hot spot on each occasion. The coil was held stable over the hot-spot using a Manfrotto mechanical arm (Vitec Group, Italy) and adjusted when necessary.

Motor evoked potentials (MEPs) were recorded using disposable Ag-AgCl surface electrodes attached to the right FDI muscle in a belly-tendon montage. The signals were amplified and bandpass filtered (10 Hz- 2kHz, sampling rate 5kHz) then digitalized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position throughout testing.

All trials were controlled using an in-house program (written using Matlab: Mathworks, MA, USA), with an inter-trial interval of 5s occurring between each trial for all measures. Intracortical inhibition and facilitation were investigated using a range of TMS paired pulse protocols, with a range of inter-stimulus intervals (ISIs) including 1, 3, 10 and 12ms (Kujirai et al., 1993). A 100ms ISI inhibitory protocol was also measured (Claus et al., 1992; Valls-Sole et al., 1992). All paired pulse measures and unconditioned trials were randomized and presented within the same session. Input output curves were always calculated prior to paired pulse measures.

Threshold determination

Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP with a peak-to-peak amplitude of >50μV in the relaxed

FDI muscle in a minimum of 5 of 10 trials. A 1mV (SI 1mV) threshold was also determined by calculating the lowest intensity needed to evoke an MEP of 1mV in 5 of 10 consecutive trials.

Input output curves

TMS intensities at 100, 110, 120, 130, 140 and 150% of RMT were used. 10 pulses at each of the 6 intensities were delivered in a randomized order.

Unconditioned trials

A total of 30 unconditioned trials were measured at SI 1mV.

Short interval intracortical inhibition (SICI)

SICI was measured using 1 and 3ms ISIs. The selection of conditioning stimuli (CS) intensities was informed by a pilot study (data not shown) which revealed 1ms SICI to have a lower threshold than that of 3ms SICI. This finding confirms previous research (Fisher et al., 2002) and, therefore CS intensities of 45,50,55 and 60% RMT were used to measure 1ms SICI, whereas 60, 65, 70 and 75% RMT were used to measure 3ms SICI. Each CS was followed by a supra-threshold test stimulus (TS) of SI 1mV delivered to the same location. Ten trials were measured for each CS-TS pairing for both 1 and 3ms ISIs.

Long interval intracortical inhibition (LICI)

A single ISI of 100ms was tested using a supra-threshold CS of 110% RMT and a TS delivered at SI 1mV. A total of 20 trials were measured.

Intracortical facilitation (ICF)

10 and 12ms ISIs were measured using a CS at 75% RMT followed by a SI 1mV TS. 20 trials were measured for each ISI.

7.2.4 Experimental procedure

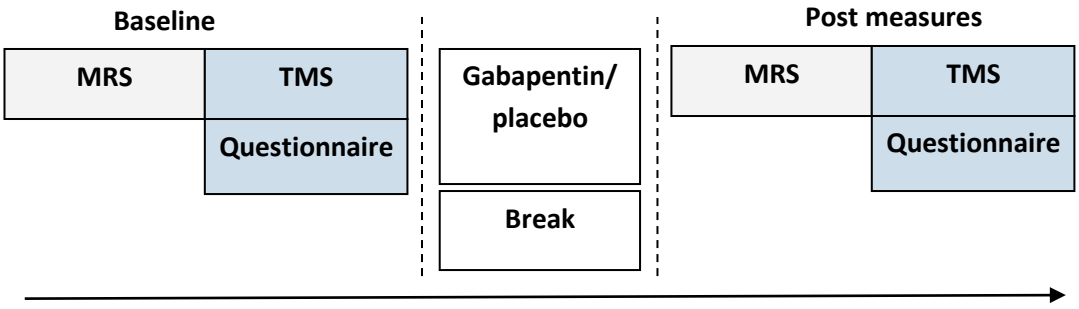


Figure 7.2. *Schematic showing study timeline.*

The testing sessions commenced at the same time each morning for all participants and lasted approximately 6 hours in total (see Figure 7.2). A 7T Philips Achieva system (Philips Healthcare, Best, Netherlands) scanner was used to collect anatomical and MRS data (using MPRAGE and STEAM sequences respectively). Approximately 30 minutes after this, TMS measures (IO curve, 1ms SICI, 3ms SICI, 10ms ICF, 12ms ICF and LICI) were collected. After completing the first set of TMS measures, participants were asked to fill out a simple bespoke questionnaire to indicate their perceived level of fatigue and tiredness on a scale of 1(absent) to 10 (severe).

Participants were then given a drink containing 900mg of GBP or with nothing added. A strong sugar free blackcurrant flavoured drink was used to mask the taste of the drug. Assignment of participants to the drug or control condition was done by a researcher (HS) who was not involved in data analysis but was present during scanning. The primary investigators (KD and SP) were kept blind to the condition until data analysis was complete. After receiving the drug or control drink, participants ate a light lunch which typically involved a sandwich, a low sugar snack such as crisps and a low sugar drink (typically water). During the break participants were closely monitored and engaged in restful activities such as watching films or reading. Participants were re-scanned on average 1.59 hours after consuming the control/ GBP containing drink. The second set of TMS measurements were then performed. On average these took

place 2.52 hours following drug/placebo intake. If necessary thresholds (RMT/ SI 1mV) were adjusted during the second measurement. Finally, participants were asked to rate their level of fatigue and tiredness again on the questionnaire. Participant comfort was closely monitored throughout the experiment.

7.2.5 Analyses of MRS data

In vivo ^{1}H spectra were fitted and quantified with the LCModel software package (Provencher, 1993). The basis set used for quantification included an experimentally acquired macromolecule spectrum and model spectra of 20 metabolites. The LCModel analysis was performed within the chemical shift range 0.5 to 4.2 ppm. Water scaling was applied using the non-suppressed water reference. The LCmodel control parameters were based on previously published parameters (Tkác et al., 2009). The absolute concentration for each cerebral metabolite are reported in institutional units. Metabolites with Cramer-Rao lower bound (CRLB) >20% were rejected from further analysis. The linewidth of in vivo spectra were 10.35±1.99 Hz. Total Cr (tCr, i.e., PCr+Cr) was used as the internal reference for quantification due to its relatively high and stable concentration in the human brain (Danielsen & Ross, 1999; Stagg & Rothman, 2014).The group mean in vivo spectrum acquired from the VOI is presented in Figure 7.1B. Any significant outliers were identified and removed using Grubbs test.

7.2.6 Analyses of TMS data

All trials were carefully visually inspected and trials in which there was evidence of pre-contraction of the FDI muscle in the period 500ms prior to an MEP were excluded. For the remaining data peak-to-peak MEP amplitudes were measured using in-house software (programmed using Matlab, The Mathworks, MA, USA). When analysing individual participant data median values were calculated to indicate average MEP amplitude in response to a particular stimulator output. Significant outliers were identified and removed from further analysis using Grubbs test.

Resting motor thresholds (RMT)

The group mean RMT was 45.7 (±6) of maximum stimulator output. The group mean for the 1mV (SI mV) threshold (i.e., the lowest intensity needed to evoke an MEP of 1mV in 5 of 10 consecutive trials) was 55.3(±6.8) %. The group mean MEP amplitude for a TMS pulse delivered at SI 1mV was 1365.3 (±556.9)mV.

TMS recruitment (IO) curves

Single pulse TMS IO curves were measured by calculating for each individual the median MEP amplitude for each given TMS intensity (100 -150% RMT). Four-parameter sigmoidal fits were then applied to the resultant values and the maximal slope and the plateau of the curve were calculated. The sigmoidal function used to fit curves to the individual datasets was:

$$\mathrm{MEP}_S = y_0 + \frac{\mathrm{MEP}_{\mathrm{MAX}}}{1 + 10^{(S_{50} - S)k}}$$

MEP_{MAX} is the maximum MEP amplitude measured, S_{50} is the TMS intensity needed to produce 50% of the maximum MEP, k is the gradient of the maximum steepness of the curve and y_0 is the minimal MEP response, which was set to 0. Two measures of interest were taken from the sigmoid curve: the maximum slope of the sigmoid (IO curve slope) and plateau of the sigmoid (IO plateau).

Paired pulse data

Paired pulse trials were analysed by calculating for each individual the median MEP amplitude for each CS intensity at each ISI. These values were then divided by the median MEP amplitude for unconditioned trials to create a ratio measure. Linear slopes were fitted to 1ms and 3ms SICI measures (Figure 7.3) and a median value was calculated across the different CS intensities to reveal the average level of inhibition. Individual median MEP values were also calculated for ICF trials; the resultant group data are presented in Figure 7.3.

Inspection of this figure clearly illustrates that while the SICI paired pulse trials (ISIs 1ms and 3ms) led to a large reduction of the MEP produced by the test stimulus, the ICF trials (ISIs 10ms and 12ms) led to a large increase in the MEP to the test stimulus. These data are consistent with the inhibitory and excitatory effects of SICI and ICF paired pulse TMS stimulation. Statistical analysis using a one-way ANOVA confirmed that there was a significant effect of stimulator intensity on MEP amplitudes for both the 1ms SICI curve ($F(3,104) = 32.58$, $p < 0.0001$) and the 3ms SICI curve ($F(3,107) = 19.23$, $p < 0.0001$). Paired sample t-tests confirmed that the values for both 10ms ICF trials ($t(26)=10.8$, $p=.00$) and 12ms ICF ($t(26)=10.63$, $p=.00$) differed significantly to unconditioned trials.

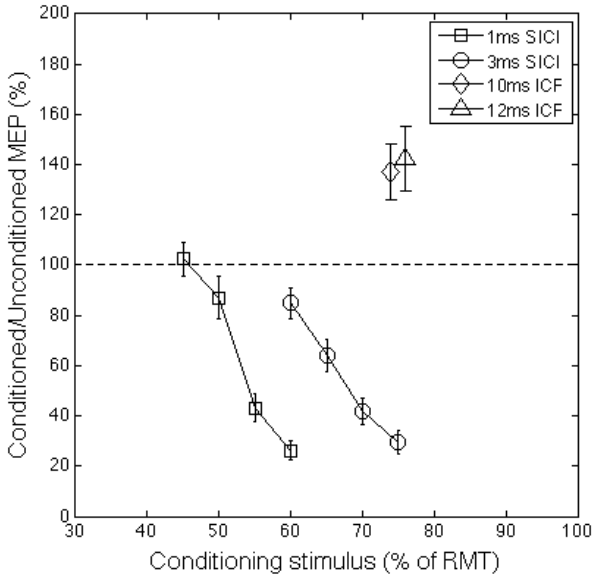


Figure 7.3. *Group mean of individual median MEP values for paired pulse SICI (1ms and 3ms ISI) and ICF (10ms and 12ms ISI) trials. Error bars represent the standard error of the mean.*

Following initial analyses, it was determined that data from LICI trials would not be analysed further. This was based upon the large percentage of participants who reached floor levels for this measure (i.e., there was a complete inhibition of the MEP in 8/27 participants). While this illustrates that the LICI paired pulse TMS procedure was highly effective, it reduces individual variability in LICI values and, therefore, makes correlation analyses less effective.

7.2.7 Correlations between MRS and TMS measures

One of the key aims of this study was to directly examine the association between TMS measures of physiological inhibition - assessed by TMS stimulation of the hand area of the motor cortex (M1) of the left hemisphere - with MRS measurement of GABA concentration – acquired from a voxel centred over the hand area of left hemisphere M1. To examine these association Pearson correlation coefficients were calculated for the entire set of TMS measures against a measure of M1 GABA concentration. For completeness correlations are reported without adjustment for multiple comparisons. Where correlation coefficients are statistically significant the adjusted statistical threshold (alpha) calculated for multiple comparisons using the Holm-Bonferroni correction method is reported separately for each neurotransmitter.

The TMS measures used in the analyses consisted of the following ten measurements for each individual: the resting motor threshold (**50-100μV**); the median MEP for a single, unconditioned, TMS stimulus delivered at that individual's RMT (**med MEP**); the stimulator output (%) required to produce an MEP of approximately 1mV (**SI 1mV**); the linear slope of the TMS IO curve (**IO curve**); the median inhibition value (%) observed for 1ms SICI trials (**1ms SICI**); the linear slope value for 1ms SICI trials (**1ms SICI slope**); the median inhibition value (%) observed for 3ms SICI trials (**3ms SICI**); the linear slope value for 3ms SICI trials (**3ms SICI slope**); the median value (%) observed for 10ms ICF trials (**10ms ICF**); and the median value (%) observed for 12ms ICF trials (**12ms ICF**).

While the primary focus of this study was to examine the association between TMS measures of physiological inhibition (e.g., SICI) and MRS measures of GABA (hereafter referred to as MRS-GABA), and also the effects of GBP on GABA. For completeness, the relationship of TMS measures with MRS measures of glutamate (Glu), glutamine (Gln), and the ratio of glutamine to glutamate were also explored.

7.2.8 Analysis of acute effects of gabapentin/placebo administration

Grubbs test was used to identify and remove outliers in the post GBP and post sham conditions. Once outliers were removed from each data set, percentage change from baseline was calculated. Independent samples t-tests were then used to test for significant differences occurring between the placebo and GBP groups for each TMS/MRS measure. Pearson's correlation analysis was also used to examine any relationships between change in MRS/TMS measures and baseline measures for each group.

7.3 Results

7.3.1 Correlations between baseline TMS and MRS measures

Association of TMS measures with GABA (GABA/tCR)

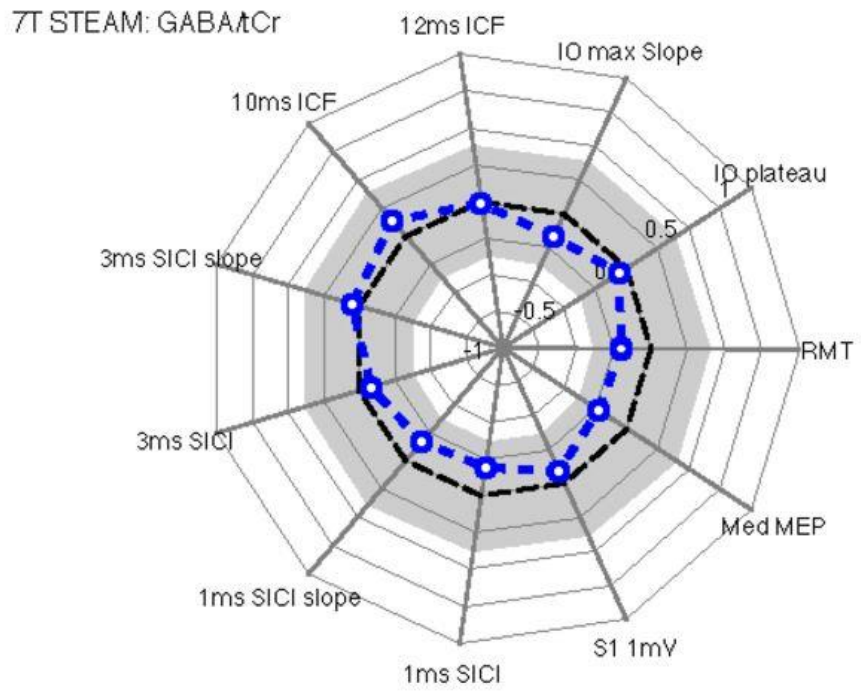


Figure 7.4 *Spider's web plot illustrating Pearson correlation coefficients for TMS measures with GABA/tCR ratios. Plot shows correlation coefficients running from 1.0 (outer ring) to -1.0 (inner ring) with the broken black line representing a correlation coefficient of 0. Open blue circles are not statistically significant (p>0.05), whereas filled blue circles represent statistically significant correlations (p<0.05).*

Figure 7.4 displays a spider plot of the Pearson correlation coefficients of the TMS measures with GABA/tCr ratio. These analyses revealed that all effects failed to reach conventional levels of statistical significance (all $p > 0.29$). In particular, the correlation coefficients for 1ms and 3ms SICI did not approach statistical significance for either median inhibition (1ms SICI: $r = -0.19$, $p=0.34$; 3ms SICI: $r = -0.08$, $p=0.68$), or slope (1ms SICI: $r = -0.17$, $p = 0.39$; 3ms SICI: $r = 0.06$, $p = 0.77$).

Association of TMS measures with glutamate (Glu/tCR)

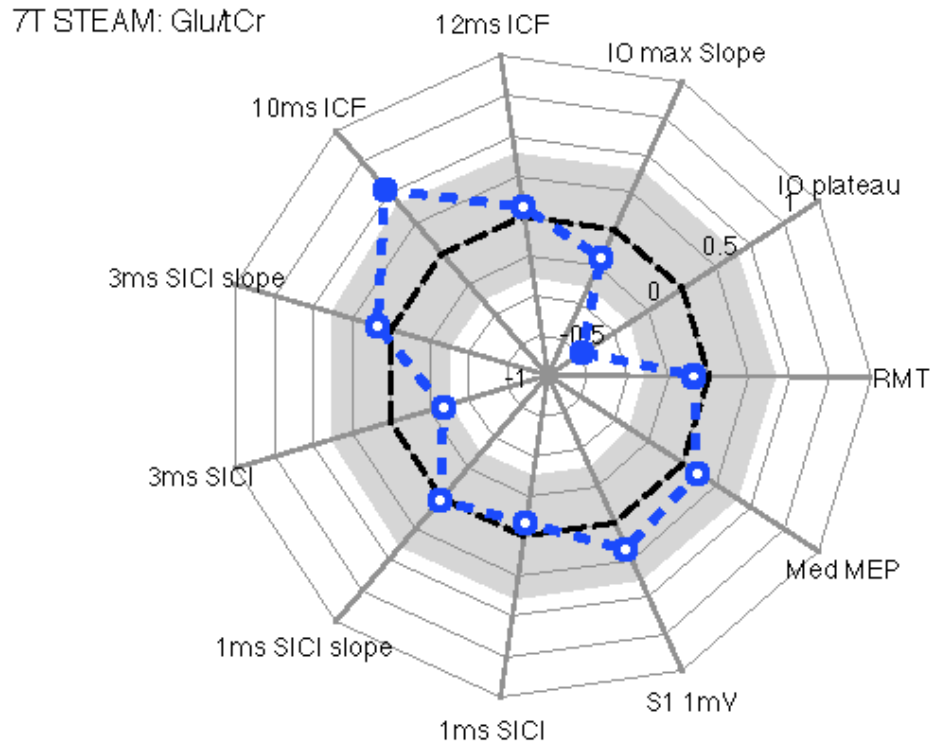


Figure 7.5. *Spider web plot illustrating Pearson correlation coefficients between individual values for each TMS measurement and glutamate concentrations (Glu/tCr ratios). Filled circled indicate significant relationships for 10ms ICF and IO plateau with Glu/Cr.*

Figure 7.5 displays a spider's web plot of the Pearson correlation coefficients of the TMS measures with Glu/tCr ratio. Similar relationships were found as expected. A significant negative relationship was found between IO plateau and Glu/tCr (r=-0.74, p<0.0001 [corrected using Holm-Bonferroni method]) but not IO maximal slope (r=-0.2, p=0.34) or RMT (r=-0.09, p=0.67). A

significant positive relationship was found between Glu/tCR and 10ms ICF (ICF 10ms: r=0.52, p<0.01), however, this did not survive Holm-Bonferroni correction (*r*=0.52, *p*=0.08). 12ms ICF was not found to have any relationship to Glu/tCR (ICF 12ms: *r*=0.06, *p*=0.78).

Association of TMS measures with glutamine (Gln/tCR)

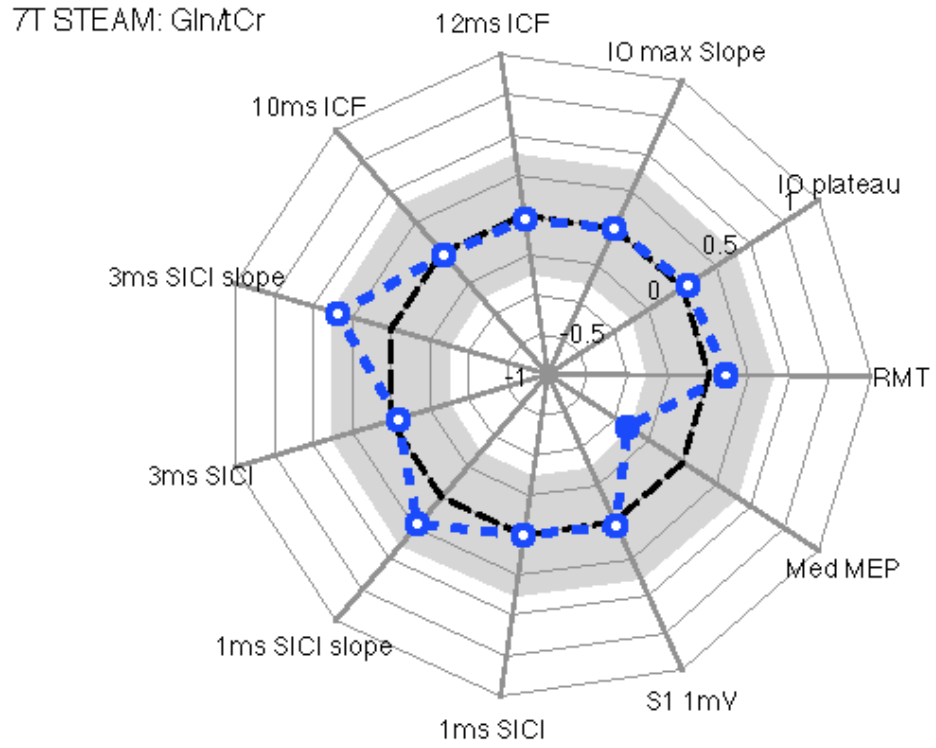


Figure 7.6. *Spider web plot illustrating Pearson correlation coefficients between individual values for each TMS measurement and glutamine concentrations (Gln/tCr ratios). Filled circled indicate significant relationships for Median MEP amplitude at RMT (Med MEP) and Gln/Cr.*

Figure 7.6 displays a spider's web plot of the Pearson correlation coefficients of the TMS measures with Gln/tCr ratio. The analysis revealed a significant correlation between the Gln/tCr ratio and the median amplitude of MEPs in response to stimulation at RMT (*r*=-0.40, *p*=0.05), although this did not survive the Holm-Bonferroni correction (*r*=-0.4, *p*=0.5). Neither RMT (*r*=0.11, *p*=0.61) nor IO maximal slope (*r*=-0.01, *p*=0.97) were significantly associated with

Gln/tCR concentration and all other correlations failed to reach statistical significance.

Multiple regression analyses

To address any potential multivariate effects, the following six MRS measures were entered as separate predictors into a multiple regression model: GABA/tCR; Glu/tCR; Gln/tCR; GABA/Glu; GABA/Gln and Gln/Glu. These predictors were then used to predict each of the TMS measurements in turn.

These analyses confirmed that the magnitude of the median MEP obtained at RMT was predicted by a combination of the Gln/Glu ratio (t=-3.43, p=0.03) and GABA/Glu ratio (t=-2.85, p=.01)(t = -3.09, p = 0.01); R^2 = 0.4, F = 7.1, p = 0.00.

The model also revealed that 10ms ICF was predicted by Glu/tCR ratio (t=-2.92, p=.01), $R^{2=}$ .27, F=8.5, p=.01. In addition to this Glu/tCR was found to significantly predict IO plateau (t=-2.92, p=.01), $R^{2=}$ .27, F=8.45, p=.01.

Bayesian Statistics

A Bayesian Hypothesis test was employed primarily to evaluate whether there was evidence in favour of the null hypothesis (H_0), i.e. that a metabolite bore no relationship with neurotransmitter function as tested by TMS. It further allowed us to quantify the strength of the relationship of any correlations found (evidence in support of the alternative hypothesis, H_1). Bayes Factors (BF_{10}) were calculated using JASP (JASP-Team, 2016); JASP uses the Bayesian correlation test proposed by Jeffreys (1961). Bayes Factors above 1 show support for H_1 whilst below 1 show support for the H_0, the magnitude of the BF_{10} shows the strength of the evidence in support of either the H_1 or H_0. For cut-off values for the different strengths of evidence please see *Table 7.3*.

In support of previous analyses, the Bayesian Hypothesis test showed there was decisive evidence that Glu/tCr is related to IO plateau (BF_{10}=1119.59) and substantial evidence that ICF 10ms is related to Glu/tCr (BF_{10}=7.05). Further,

there is substantial evidence that the majority of TMS measures do not relate to MRS measures of Glu/tCr, Gln/tCr or GABA/tCr. Most notably there is substantial evidence for no relationship between SICI 3ms, and SICI 3ms slope and GABA/tCr (BF_{01}=3.86 and 4.02 respectively). For full results of the Bayesian Hypothesis Test please refer to Table 7.4.

***Table 7.3**. Category values for BF10, adapted from Wetzels and Wagenmakers (2012)*

Bayes factor BF_{10}			Interpretation
> 100	-	∞	Decisive evidence for H_1
> 30	-	< 100	Very strong evidence for H_1
> 10	-	< 30	Strong evidence for H_1
> 3	-	< 10	Substantial evidence for H_1
> 1	-	< 3	Anecdotal evidence for H_1
	1		No evidence
> 1/3	-	< 1	Anecdotal evidence for H_0
> 1/10	-	< 1/3	Substantial evidence for H_0
> 1/30	-	< 1/10	Strong evidence for H_0
> 1/100	-	< 1/30	Very Strong evidence for H_0
0	-	< 1/100	Decisive evidence for H_0

Table 7.4. *Bayesian hypothesis test for correlations. Data presented are each BF10*

	Glu/Cr	Gln/Cr	GABA/Cr
RMT	0.28†	0.28†	0.37
MedMEP	0.28†	1.67	0.43
S1 1mV	0.35	0.24†	0.26†
SICI 1ms	0.26†	0.24†	0.37
SICI 1ms Slope	0.24†	0.42	0.34
SICI 3ms	0.88	0.24†	0.26†
SICI 3ms Slope	0.27†	1.02	0.25†
ICF 10ms	7.05*	0.25	0.3†
ICF 12ms	0.25†	0.24†	0.24†
IO Maximal Slope	0.38	0.24†	0.35
IO Plateau	1119.59***	0.25†	0.25†

*substantial evidence for H1, **strong evidence for H1, ***decisive evidence for H1, † substantial evidence for H0 BF_{10} > 1 supports H1, BF_{10}<1 supports H0, BF_{10}=1 suggests no evidence.

7.3.2 Acute effects of gabapentin/placebo on MRS measures

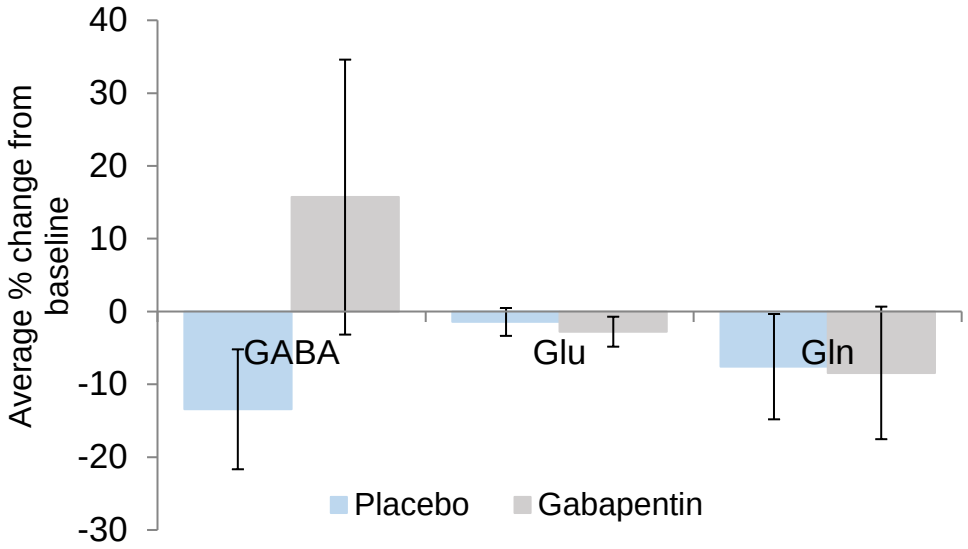


Figure 7.7. *% change from baseline for GABA, Glu and Gln measures shown as mean and SEM for placebo and GBP groups.*

GABA/tCr

Independent samples t-tests revealed no significant differences between the two groups for percentage change in GABA level, (t(26)=-1.42, p=.17). Bayesian statistics revealed anecdotal support in favour of no real differences existing between the two groups (BF_{10}=0.74). Relevant data can be seen in *Figure 7.7*.

Glu/tCr

There were no significant differences between the amount of change in Glu levels between the two groups (t(25)=.45, p=.66). Bayesian statistics provide anecdotal support in favour of there being no difference between the two (BF_{10}=0.39). Relevant data can be seen in *Figure 7.7*.

Gln/tCr

There were no statistically significant differences between the amount of change in Gln levels between the two groups (t(26)=.08, p=.93). Bayesian

statistics provide anecdotal evidence that the effects were not different (BF_{10}=0.35). Relevant data can be seen in *Figure 7.7*.

7.3.3 Effects of baseline MRS measure and subsequent change

Gabapentin condition

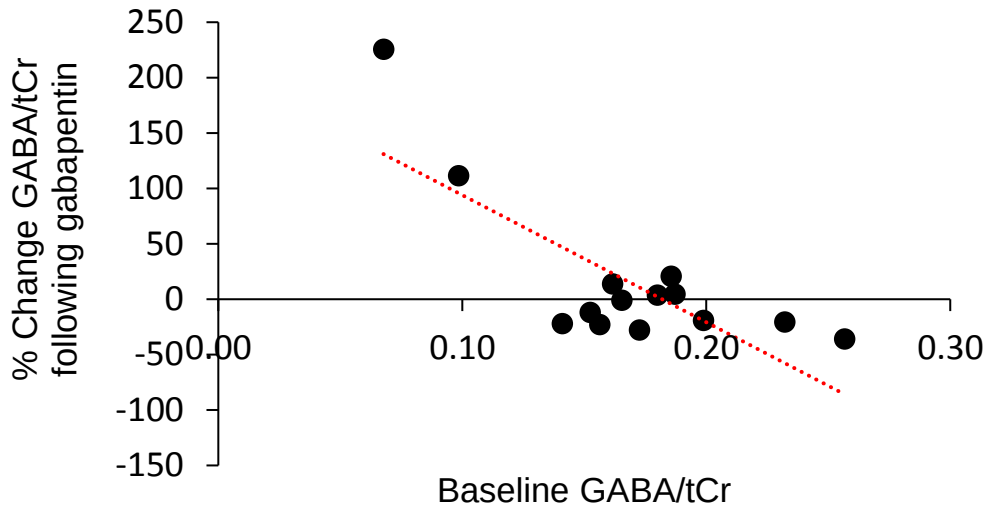


Figure 7.8. *Change in GABA level following gabapentin plotted against baseline GABA level.*

A significant relationship was found between baseline *GABA* and subsequent change in GABA level (*r*=-.78, *p*=.00). The results of the Bayesian hypothesis testing suggest very strong support that baseline GABA predicted the % change in GABA following GBP administration (BF_{10}=41.82). This relationship can be seen in *Figure 7.8*.

Baseline levels of *glutamate* were not found to significantly relate to subsequent levels of change in glutamate (*r*=-.53, *p*=.06). Bayesian hypothesis testing revealed only anecdotal support in favour of the relationship (BF_{10}=1.63).

Baseline levels of *glutamine* were not significantly related to subsequent change in glutamine measures (*r*=-.18, *p*=.55). Bayesian hypothesis testing

revealed anecdotal evidence in favour of no relationship between the two (BF_{10}=0.39).

Placebo condition

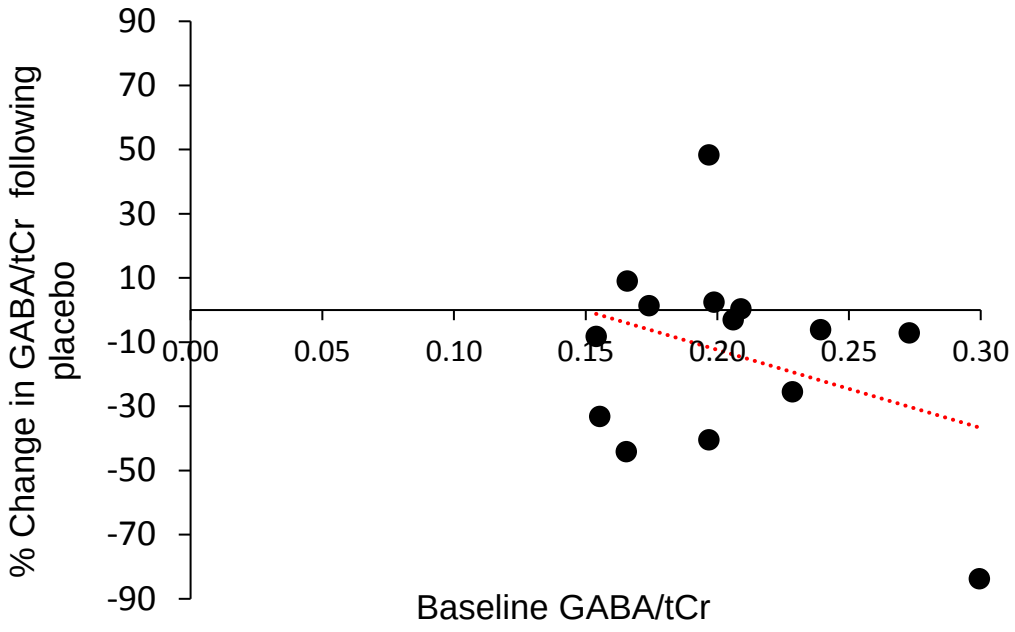


***Figure 7.9**. Change in GABA level <u>following placebo</u> plotted against baseline GABA level.*

Levels of *GABA* at baseline were not significantly related to levels of GABA following placebo (*r*=-.34, *p*=.24). The results of the Bayesian hypothesis test showed anecdotal evidence in favour of there being no relationships (BF_{10}=.63). This data can be seen in *Figure 7.9*.

No statistically significant relationship was found between baseline *glutamate* levels and subsequent change (*r*=-.421, *p*=.128). Bayesian hypothesis testing also failed to provide support for a relationship (BF_{10}= 0.948).

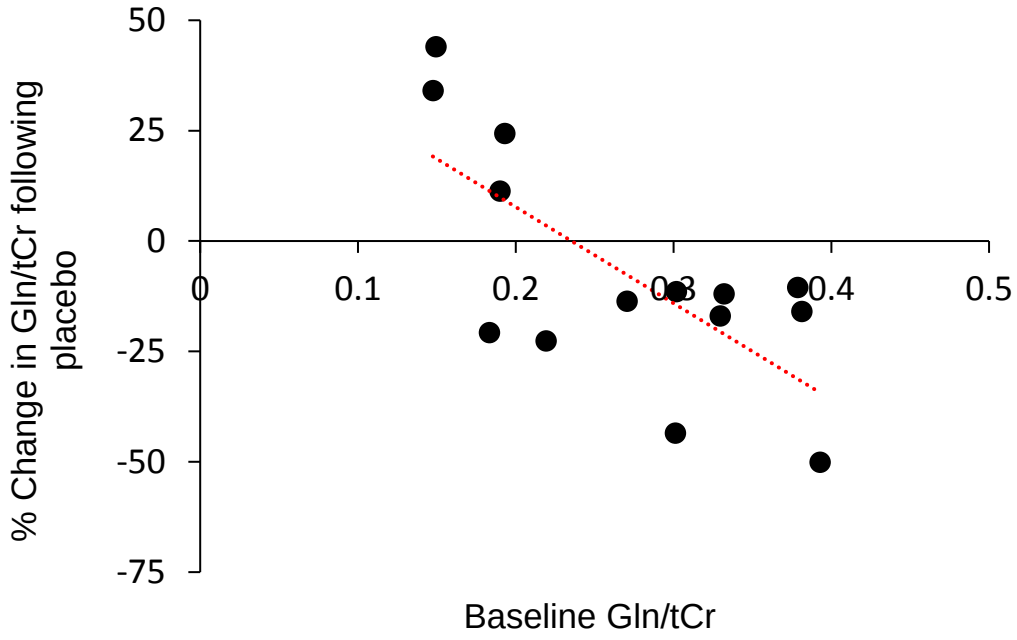


***Figure 7.10**. Change in Gln/tCr level <u>following placebo</u> plotted against baseline Gln/tCr level.*

Baseline measures of *glutamine* were found to significantly correlate with the amount of change in this measure after placebo (*r*=-.71, *p*=.004). Bayesian hypothesis testing suggests strong evidence in favour of the existence of this relationship (BF_{10}= 12.86). Relevant results can be seen in ***Figure 7.10***.

7.3.4 Acute effects of gabapentin/placebo on TMS measures

There were no significant differences between the two participant groups for any of the TMS variables, including those thought to reflect GABAergic inhibition. In particular 3ms SICI median inhibition *(t(26)=1.0, p=.33)* and 3ms SICI slope (*t*(26)=-.17, *p*=.87) were not significantly different between groups. Change from baseline in measures of 1ms SICI median inhibition (*t*(26)=-1.59, *p*=.12) and 1ms SICI slope (*t*(25)=-.32, *p=.76)* were also not significantly different.

7.3.5 Effects of baseline TMS measure and subsequent change

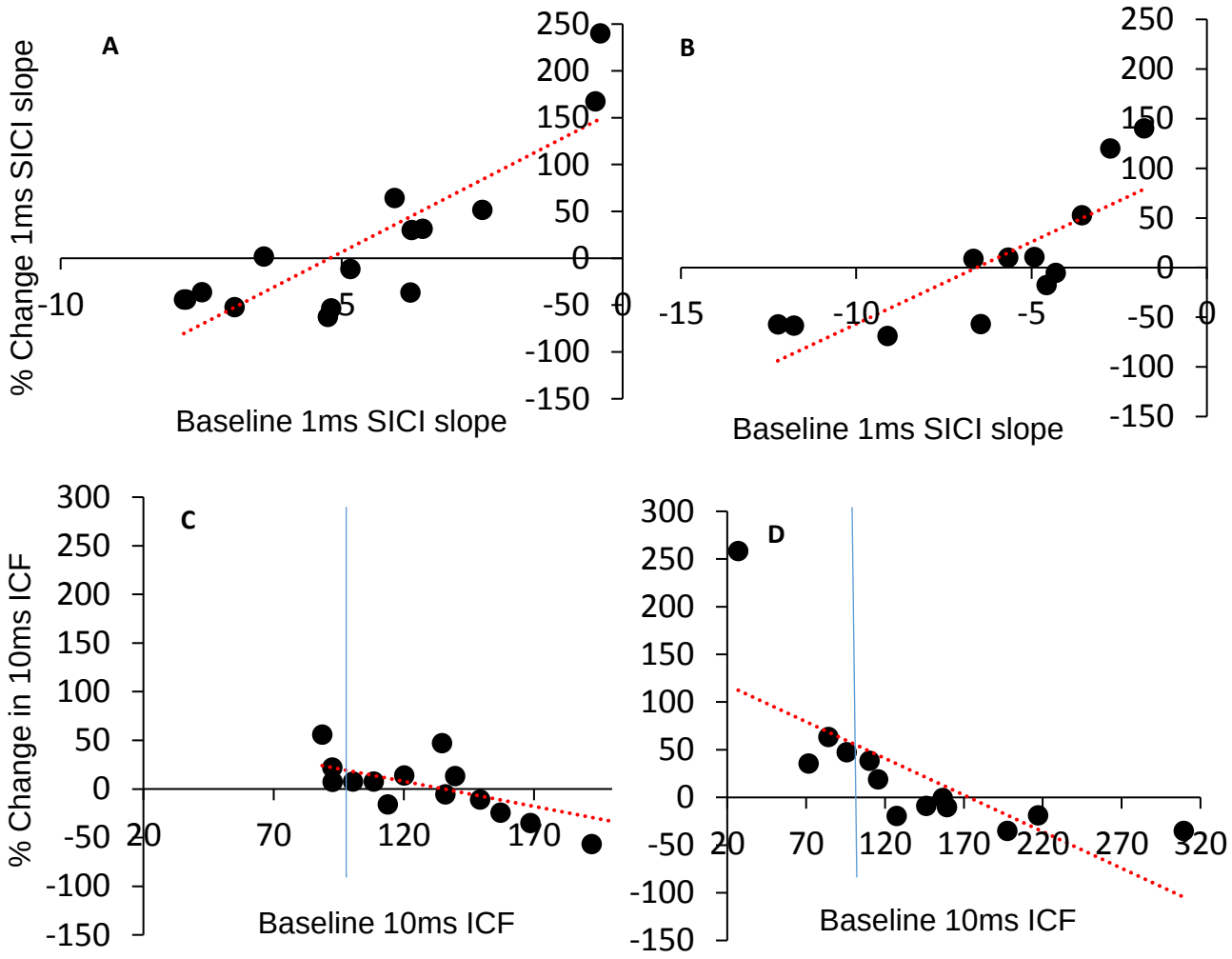


Figure 7.11. *Results of Pearson's correlational analysis between A: baseline 1ms SICI slope and % slope change following gabapentin; B: baseline 1ms SICI slope and % change following placebo; C: baseline 10ms ICF and % change following gabapentin; D: 10ms ICF baseline and % change following placebo. Anything left of the blue line in C and D indicates a lack of true ICF effect in that conditioned MEPs were not larger than those from un-conditions trial.*

Gabapentin

Baseline values were not found to significantly relate to subsequent change in 3ms SICI median (r=-.42, p=.12) nor 3ms SICI slope (r=-.021, p=.94). Bayesian hypothesis testing revealed anecdotal evidence that there was no

relationship between baseline and change for either measure (BF_{10}=0.99 and BF_{10}=.32).

Change in 1ms SICI median inhibition (*r*=-.76, *p*=.00) and 1ms SICI slope (*r*=-.85, *p*=.00) were found to negatively correlate with their respective baseline values. For the 1ms SICI median condition the model shows that as baseline median MEP amplitudes increase, the percentage change from baseline in the second measurement decreases or becomes negative. Larger median MEP amplitudes suggest lower levels of inhibition, therefore, this implies that those with initially low levels of inhibition at baseline show less change/a tendency towards increased inhibition (lower MEP amplitudes). Whereas, those who showed higher levels of inhibition at baseline (indexed by low med MEP amplitude) were more likely to show a larger percentage increase in MEP amplitude, hence indicating a shift towards lower levels of inhibition than previously measured. A similar pattern of results is true for 1ms SICI slope measures (Figure 7.11 A) in which, as baseline slope increases (larger negative values suggest steeper slope), the percentage change from baseline decreases or becomes negative. Those with low slope values at baseline (indicating less inhibition) were more likely to show increased slope values in the second testing session (indicating an increase in inhibition) whereas the opposite is true for individuals whose baseline slopes were initially high. Bayesian hypothesis testing revealed very strong evidence of the relationship between baseline and subsequent change in 1ms median inhibition (BF_{10}=40.19) and decisive support for the relationship between baseline and change for 1ms SICI slope (BF_{10}=407.5).

Baseline values from 10ms but not 12m ICF significantly correlated with subsequent change (*r*=-.69, *p*=.00). The results can be seen in Figure 7.11 C, in which it can be observed that as baseline ICF values increased the percentage change decreased or became negative. This suggests that those who showed the largest ICF effects at baseline were then more likely to show a decrease in

subsequent measures and vice versa was true for those with lower values at baseline.

Bayesian hypothesis testing revealed strong support for the existence of a negative correlation between baseline 10ms SICI and subsequent change after GBP (BF_{10}=13.38).

Placebo

The correlations between baseline TMS measures and their subsequent change are similar to those found for the GBP condition. Specifically, correlations between baseline and subsequent change were found for 1ms SICI slope and 10ms ICF but no other measures.

Change in 1ms SICI slope (*r*=-.81, *p*=.00) was significantly negatively correlated with its baseline values (Figure 7.11 B). Bayesian statistics suggest there is strong evidence in favour of the relationship (BF_{10}=30.39).

As was the case with the GBP participants, baseline 12ms ICF was not related to subsequent change in this measure, however, baseline 10ms ICF was (*r*=-.72, *p*=.00). Bayesian statistics revealed strong support for the negative correaltion (BF_{10}=11.52). Relevant data can be seen in Figure 7.11 D.

7.3.6 Questionnaire measures

Independent samples t-tests revealed no significant differences between baseline ratings of fatigue *t*(24)=1.13, *p*=2.71. Ratings of tiredness were also not significantly different between the two groups at baseline *t*(24)=1.86, *p*=.08.

A repeated measures ANOVA revealed no significant main effect of group (*F*(1,24)=.11, *p*=.75) on *fatigue* ratings. However, ratings were found to significantly increase when measured at the end of the study (*F*(1,24)=8.47,

p=.00). The interaction between time of measurement and group was not statistically significant (*f*(1,24)=2.86, p=.10).

Ratings of *tiredness* significantly increased in the second measurement (*F*(1,24)=7.21, *p*=.01). There was also a significant interaction between time and group (*F*(1,24)=6.14, *p*=.02), although the main effect of group was not significant (*F*(1,24)=.42, *p*=.52). Further exploration using paired samples t-tests revealed no significant differences between pre- and post-fatigue ratings for the placebo group (*t*(11)=-1.55, *p*=.15), but a significant difference was found for the GBP group (*t*(13)=-3.7, *p*=.00).

Correlations between percentage of change in fatigue/tiredness rating and percentage of change in MRS-GABA were also assessed using Pearson's correlation co efficient. However, no significant correlations were found.

7.4 Discussion

Imbalances in glutamatergic (excitatory) and GABA (inhibitory) signalling within key brain networks are thought to underlie many brain disorders including: schizophrenia (Gonzalez-Burgos et al., 2010), depression (Kalueff & Nutt, 2007) and 'hyperkinetic' neurodevelopmental disorders such as TS (Clarke et al., 2012; Ramamoorthi & Lin, 2011). For this reason there is considerable current interest in the use of MRS to measure in vivo concentrations of brain molecules (e.g. GABA, glutamate, glutamine) that can be correlated with brain function and dysfunction. While it is tempting to equate the GABA measured using MRS (hereafter referred to as MRS-GABA) with neurotransmitter function, and with physiological or behavioural inhibition (e.g. Dharmadhikari et al. (2015); Haag et al. (2015)) it is important to note that at any point in time only a fraction of MRS-GABA will be neurotransmitter, and that increased MRS-GABA concentrations do not necessarily mean that there is increased physiological or behavioural inhibition (Rae, 2014). Furthermore, it is currently unclear whether

MRS-GABA represents the entire pool of GABA available for measurement (i.e., metabolic, intracellular, and extracellular GABA), or as some have argued, represents instead largely extracellular, extra-synaptic, GABA that is unrelated to the synaptic transmission of GABA (Rae, 2014; Stagg, 2014).

In the current study we compared directly, in the same individuals, MRS-GABA (and other important neurometabolites such as glutamate and glutamine) measured from a voxel located in the sensorimotor cortex against TMS measures of cortical excitability and GABA-mediated physiological inhibition measured from the hand area of motor cortex. We also used a placebo controlled drug manipulation to explore the effects GBP may have on TMS and MRS measures. It is important to note that using these techniques we obtained high-quality MRS data and replicated each of the previously reported TMS effects (i.e., TMS recruitment curves, 1ms SICI and 3ms SICI curves, and 10ms and 12ms ICF effects) that we set out to examine in this study.

The main results of our study are summarised below.

1. Individual concentrations of MRS-GABA were unrelated with any TMS measurements, including TMS measures of: general cortical excitability (i.e., TMS recruitment curve slopes); GABA mediated physiological inhibition (i.e., 3ms SICI), and TMS measures thought to be dependent upon the glutamatergic NMDA receptor (i.e., 10ms ICF and 12ms ICF).

2. Individual levels of MRS-glutamate (Glu) were significantly negatively correlated with the plateau of the IO curve; a measure which is thought to reflect the balance of excitatory and inhibitory components of the corticospinal volley (Devanne et al., 1997). The relationship suggests that as levels of MRS-Glu increase the maximum MEP amplitude predicted by the model reduces. Multiple regression analysis revealed Glu/tCR to be the only significant predictor of this measure and, furthermore, Bayesian hypothesis tests provide very strong support in favour of the experimental hypothesis.

3. MRS-Glu was also found to be marginally correlated with 10ms but not 12ms ICF. The multiple regression analysis confirmed that Glu/tCR was a significant predictor of 10ms but not 12ms ICF. Bayesian hypothesis testing confirmed that there is substantial evidence in favour of the relationship.

4. There was some evidence of correlation between glutamine (Gln) concentrations and median amplitude of the MEP response to TMS stimulation delivered at RMT. However, the Bayesian hypothesis test revealed only anecdotal support in favour of the relationship. This is possibly explained by the findings of the linear regression model which reveal that median MEP amplitude was found to be predicted by both the Gln/Glu and GABA/Glu ratios, but not significantly by Gln/tCR.

5. Gabapentin did not significantly change MRS or TMS measures when compared with the placebo group. GBP also did not appear to significantly alter MRS measures of glutamate or glutamine.

6. Levels of baseline MRS-GABA were significantly correlated with percentage change in MRS-GABA approximately 2 hours after participants received GBP, but not placebo. Those with the lowest levels of MRS-GABA at baseline saw the largest increases, however, those with initially high levels of MRS-GABA showed a decrease in the second scanning session. Bayesian hypothesis testing confirmed very strong support for the relationship in the GBP condition only.

7. Baseline measures of 1ms SICI and 10ms ICF were found to be negatively correlated with subsequent change in these measures for both the GBP and placebo conditions.

MRS-GABA and physiological inhibition

Our finding, that individual concentrations of MRS-GABA are unrelated to GABA-mediated physiological inhibition, as measured by 3ms SICI, replicates previous reports for 3ms SICI (Tremblay et al., 2013) and 2.5ms SICI (Stagg et al., 2011). The physiological mechanisms that underpin both 2.5ms and 3ms SICI

effects are well established and are thought to primarily involve post-synaptic inhibition mediated through GABA-A receptors (Ziemann et al., 2015). Although SICI has also been found to be modulated by neurotransmitters such as dopamine (Gilbert et al., 2006; Korchounov et al., 2007; Ziemann, Bruns, et al., 1996), the contribution such neurotransmitters make to GABAergic neurotransmission is complex (see (Hasselmo, 1995) and beyond the scope of this study (Hasselmo, 1995). As a result, it is suggested that the lack of correlation between 3ms SICI and MRS-GABA observed in this study and others indicates that the primary source of MRS-GABA is unlikely to be that associated with GABAergic synaptic transmission, but instead most likely relates to concentrations of metabolic GABA and to levels of ambient extracellular GABA that contribute to tonic GABAergic activity and, therefore, to the GABAergic tone of a brain region (Rae, 2014; Stagg, 2014).

In a previous study, Stagg and colleagues (Stagg et al., 2011) reported a significant correlation between MRS-GABA concentrations and 1ms SICI slopes. It is important to note that the underlying physiological mechanisms for the 1ms SICI effect are thought to be distinct from those associated with longer ISIs such as the 3ms SICI effect (Cengiz et al., 2013; Fisher et al., 2002; Roshan et al., 2003), however, the exact mechanisms underlying 1ms SICI are currently unclear. Some have proposed that the effects may relate to the refractory periods of inter-neurons (Cengiz et al., 2013; Fisher et al., 2002), whereas others have argued that synaptic processes may also play a role (Roshan et al., 2003; Vucic et al., 2009).

Unfortunately, in this study the findings observed by Stagg et al. (2011) are not directly replicated and it is not fully clear why this would be. It is unlikely to be due to a lack of power, as the sample size used here was more than double that used previously (i.e., N=27 versus N=12). Similarly, it is also unlikely to be due to the efficacy of our TMS procedures as our 1ms SICI protocol was highly effective in producing effective inhibition and a 1ms SICI curve is clearly present. The main differences between the two studies were the nature of the MRS

protocol used (i.e. SPECIAL versus STEAM) and the field strength of the MR scanners (i.e., 3T versus 7T). Obviously it is difficult to conclude anything from a null effect, so at this point it is only possible to say that the previously observed relationship between 1ms SICI and MRS-GABA is not entirely reliable.

MRS-glutamate and glutamine and cortical excitability

TMS recruitment curves are thought to reflect cortical excitability more generally and the strength of cortico-spinal projections (Chen, 2000). However, different physiological processes may contribute to the TMS recruitment curve across the different stimulator intensities used, and various neuromodulators and neurotransmitters, including both GABA and Glu, may contribute to the effects observed (Ziemann, 2013). Furthermore, different aspects of the recruitment curve may relate to different mechanisms. For example, it may be that measures of the slope of the curve are distinct from measures of the plateau (Kouchtir-Devanne et al., 2012).

Stagg et al. (2011) reported a significant correlation between cortical excitability, as indexed by the slope of the TMS recruitment curve, and MRS-glutamate. This finding was not replicated in this study which in fact found quite the opposite, at least at first appearance. We found the plateau of the IO curve to be negatively correlated with Glu/tCr. We did not, however, find a relationship between IO curve and Glu/tCr. The most obvious differences between both protocols as mentioned are the differences between the sequences used to acquire MRS data, the scanner strength and the sample. Other possible explanations for the differences between these two studies may be the subtle differences between the protocols used for measuring TMS recruitment curves in each study. In this study we used a standard procedure; increasing the stimulator intensity in 10% increments proportional to an individual's resting motor threshold. By contrast, Stagg et al. (2011) used a procedure in which the percentage of the intensity needed to yield an MEP of 1mV was tracked over time. This method ensures a similar degree of neuronal recruitment across participants' mid-slope, whereas the method that we used ensures that this is

the case at 100% of RMT. However, it is important to note that despite these procedural differences, the IO curves themselves appear to be similar. In particular, the mean amplitude for MEPs in response to the highest stimulation was highly similar across both experiments (i.e., approximately 3: 3.5mV) indicating that the two methods were producing comparable effects. For this reason, we feel that the different results obtained in the two studies are unlikely to have resulted from procedural differences in TMS stimulation.

At first glance our finding appears to be somewhat unorthodox, however, it highlights that what we are measuring with MRS (and TMS) is most probably not just "excitation" vs. "inhibition". Initially a high plateau may indicate more excitation, in which case, we may expect more glutamate. This is simply not the case. Prior literature describes that the maximum plateau of a recruitment curve does not simply reflect the maximum output of the corticospinal system (Devanne et al., 1997; Houdayer et al., 2008; Kouchtir-Devanne et al., 2012). It may instead reflect the intrinsic balance of excitation and inhibition at M1 (Devanne et al., 1997). Few articles discuss the cortical origins that lead to the plateau of a recruitment curve. One experiment shows that decreases in maximum plateau across different active muscle conditions is accompanied by reductions in SICI and LICI but not in ICF (Kouchtir-Devanne et al., 2012) and we have speculated that this may relate to the functional coupling between intracortical circuits within M1. At least one study has shown that slope and plateau do not always change together and that active high frequency rTMS can reduce plateau and increase slope (Houdayer et al., 2008), thus our findings may not be entirely incompatible with Stagg's 2011 paper. Any conclusions from this finding can only be speculation on our part, particularly since to our knowledge there are no similar findings in the literature between IO plateau and glutamate (or any other neurometabolite).

A significant relationship was found between 10ms but not 12ms ICF and MRS-glutamate. The lack of a significant relationship with 12ms is consistent with the previous findings by Stagg et al (2011), however, the relationship with 10ms

ICF is novel as this was not measured in either of the previous TMS-MRS studies (Stagg et al., 2011; Tremblay et al., 2013). The mechanisms underlying ICF are not yet fully understood, however, ICF is generally thought to test the excitability of an excitatory neuronal motor network which is likely to be modulated by both glutamatergic and GABAergic mechanisms (Ziemann, 2013). ICF can be measured using ISIs of 7-20ms (Kujirai et al., 1993; Vucic et al., 2006) and although this is a relatively large range of effective ISIs, to our knowledge no clear distinction has been drawn between these parameters (unlike SICI). Therefore, although the results suggest support for the experimental hypothesis, further study and replication of this effect is warranted to draw strong conclusions about this relationship.

Finally, it was demonstrated that individual MEP amplitudes were predicted by a linear combination of the ratios of glutamine/glutamate and GABA/glutamate. Glutamate exists in several metabolic pools in the brain and these pools serve as the source of glutamate for neurotransmission. Also, there is a balanced cycling between glutamate and glutamine that is essential for the normal operation of brain functions and the levels of these neurometabolites are highly correlated with one another in the healthy brain (Rae, 2014). Specifically, glutamate removed from the synaptic cleft is converted to glutamine within astrocytes and astrocyte-derived glutamine is then used as a precursor for the synthesis of glutamate or GABA within neurons. This cycling of glutamate and glutamine between cell types in the brain is highly dynamic and is thought to account for 80% of cerebral glucose consumption (Ramadan et al., 2013). For this reason it is very likely that TMS-induced changes in cortical excitability may be indexed by subtle changes in the balance between glutamate and glutamine.

Effects of Gabapentin on TMS measures

GBP was not found to significantly alter any of the TMS measures when compared with the placebo group. This was somewhat surprising as based on the previous literature (Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996) it was

expected that GBP would significantly increase SICI, and reduce ICF. To our knowledge, this study is the first to use a double blind, sham controlled method to investigate the effects of GBP on TMS measures. This study also appears to be the first not to show a clear effect of GBP on TMS measures. It is not entirely clear why the present study failed to replicate previous findings, although it is possible that subtle differences between experimental designs may have contributed.

Power seems an unlikely reason for the lack of observable effects, as our sample was substantially larger (N=15) than the first experiment conducted by Ziemann, Lonnecker, et al. (1996) (N=6) and slightly larger than that used by Rizzo et al. (2001) (N=11). Drug dosage also seems an unlikely reason for the lack of effects, as, although this study used a 900mg dose which was lower than that use by (Ziemann, Lonnecker, et al., 1996)(1200mg) it was higher than the 800mg used by Rizzo et al. (2001).

Another possible reason for the lack of replication may be the timing between drug uptake and TMS measurement. On average the second TMS measurements were taken 3 hours after receiving GBP, however, this ranged from 2.36-3.25 hours between participants. In their study Rizzo et al. (2001) found increased SICI and decreased ICF 3 hours after participants received an 800mg dose of GBP. This may suggest, that in this study the duration for drug uptake may have been too short for some individuals. However, Ziemann, Lonnecker, et al. (1996) found significant effects of SICI and ICF after just 2 hours. Unfortunately, as different doses and timings were used in all studies it is not possible to draw strong conclusions about the lack of effects seen here. If a similar study were to be conducted again it would be interesting to measure effects over a number of time points to map the time course of GBP and its peak effects.

Pearson's correlational analysis was used to explore any impact baseline may have on subsequent change in TMS measures. Although baseline was significantly correlated with measures including 1ms SICI slope and 10ms ICF,

these correlations were present for both GBP and control groups. The pattern of results and the finding of similar correlations in both conditions strongly suggest a regression to mean type effect, and provides no further explanation for the lack of observable GBP effects on TMS measures.

Effects of GBP on MRS measures

Contrary to previous findings (Cai et al., 2012; Kuzniecky et al., 2002; Petroff et al., 1996) GBP did not significantly alter levels of MRS-GABA. As was the case with the TMS measurements it is possible that the lack of replication in this study reflects the timing of the measures. Approximately 2 hours elapsed between drug intake and scanning in this study, however, this ranged from 1.45-2.23 hours. Interestingly, although Cai et al. (2012) found significantly elevated GABA levels 2.5 hours after drug uptake, similar effects were only found 6, but not 3 hours after intake by Kuzniecky et al. (2002). Although the studies differ in a number of ways including the field strengths used to collect MRS data and the amount of gabapentin given to participants, these findings both suggests that 2 hours may have been too early to see effects.

In order to explore the potential effect baseline GABA levels may have on subsequent levels of change correlational analyses was conducted. A significant correlation was found between baseline and subsequent % change following GBP (*Figure 7.8*) but not placebo (*Figure 7.9*). The correlation between baseline and change in GABA following GBP was also reported by Cai et al. (2012). However, unlike the relationship found by Cai et al. (2012) not all participants showed a percentage increase in GABA; in fact, some of those with higher baseline GABA levels showed a percentage decrease in the second MRS measurement. It is not entirely clear why this would be so, and although speculation regarding homeostatic mechanisms or regression to mean are possible, without further testing these explanations would be superfluous.

There are a number of similarities between the present studies design and that used by Cai et al. (2012) including performing the study at 7T and using

a 900mg dose of gabapentin. However, there also remain a few key differences between the two. One potentially important difference is that in their study Cai et al. (2012) tested only male participants. The decision to do so appears to be related to previous findings that females show more variability in GABA level (Epperson et al., 2006), which can be particularly elevated during certain times of the menstrual cycle. Given the correlation between GABA level and increase following GBP (Cai et al., 2012) it could be that effects in females may be smaller if measured during such a time when GABA levels are elevated. Our sample size is too small to make strong claims about the potential differences between sexes, however, comparison of the percentage of change in GABA following GBP for males (N=7) and females (N=7) revealed no clear differences (data not shown). In addition to this the changes in MRS-GABA were found by Kuzniecky et al. (2002) whose participant sample include both males and females.

Another difference between this study and those previously conducted, is that participants were able to eat immediately following drug consumption. Although this may seem problematic, there is little evidence that gabapentin absorption is negatively influenced by food. In fact, some studies have even found that foods with higher levels of protein such as a milk based breakfast option (Gidal et al., 1996) and chocolate pudding (Gidal et al., 1998) have been found to moderately enhance absorption. Nevertheless, it is true that participants did not consume exactly the same food so it cannot be ruled out that this increased variability within the effects.

The aims of this study were two-fold. Firstly, we aimed to investigate how levels of key neurometabolites (i.e., glutamate, glutamine and GABA) relate to TMS measures of cortical excitability and physiological inhibition (i.e., TMS recruitment curves, ICF and SICI). The results of this study reveal mixed support for the previous findings thus reported in the limited number of studies which have explored this relationship. Specifically, it was found that 3ms SICI and MRS measured GABA are uncorrelated. These findings appear to be consistent with the view that the GABA concentrations measured using MRS largely represent

pools of extracellular GABA that are linked to tonic rather than phasic inhibition and thus contribute to the inhibitory tone of a brain area rather than GABAergic synaptic transmission (Rae, 2014; Stagg, 2014).

The secondary aim of the study was to explore the effects GBP may have on TMS and MRS measures using a double blind, sham controlled design. The results showed no clear effects of GBP on either TMS or MRS measures and did not replicate previous findings. Although there is no clear, singular reason why GBP did not influence our TMS and MRS measures, it is possible that the duration between drug uptake and measurement was too short for clear effects to be observed. This may be particularly true for MRS measurements.

Chapter 8: General discussion

***Key words**: transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), magnetic resonance spectroscopy (MRS), Gilles de la Tourette's syndrome, variability, reliability, therapy.*

The primary aim of this thesis was to explore the effects of tDCS and its therapeutic potential in the treatment of Tourette's syndrome. A secondary aim of the thesis was to explore what is being measured with MRS, with particular reference to the inhibitory neurotransmitter GABA.

In this chapter the key findings from each of the preceding experimental chapters will be discussed and reviewed. For clarity, they will be organized under the following titles: tDCS effects in neurologically typical individuals (Chapters 3 and 4), tDCS effects and Tourette's syndrome (Chapters 5 and 6) and exploring the origins of MRS measured GABA (Chapter 7). General conclusions and limitations of the work presented in this thesis will also be discussed.

8.1 tDCS effects in neurologically typical individuals

In Chapter 3 the time course of 1mA and 2mA tDCS effects were explored using TMS. Based on the general consensus of past literature, it was expected that 1mA anodal stimulation would produce an increase in cortical excitability (as indicated by increased MEP amplitudes), whereas, 1mA cathodal stimulation would produce a decrease. The predicted outcome of the 2mA condition was less well defined, due to more recent findings suggesting non-linear effects following 2mA cathodal stimulation (Batsikadze et al., 2013).

The initial aim of the study was to identify the optimal stimulation parameters for altering cortical excitability, with the hope that these could then be used to inform parameter selection in subsequent work. It was also hoped that the study would identify the time course of the effects and that this would

help inform when best to measure any therapeutic outcomes. Disappointingly, the results of the study failed to provide clear answers to either of these questions. 1mA stimulation was not found to significantly alter cortical excitability (as measured by TMS IO curves) in either anodal or cathodal conditions at any of the time points measured. The effects of 2mA tDCS were also disappointing, and only revealed one small effect of increased excitability 90 minutes following anodal stimulation. 2mA cathodal stimulation did not significantly increase excitability, consequently, the study by Batsikadze et al. (2013) remains the only one which has reported this effect.

What was most apparent in Chapter 3 was the large amount of variability which occurred between participants in response to stimulation, even in conditions when a significant group level effect was found. At the time of conducting this research it became apparent that others were also experiencing high levels of variability within their own data (Wiethoff et al., 2014) and that the well accepted pattern of tDCS effects was starting to be questioned.

In Chapter 4, I aimed to explore this variability using a variety of TMS measures. I was particularly interested in exploring the stability of tDCS effects within individuals, as finding intra-subject stability would suggest the possibility that anatomical features such as skull-cortex distance could be used to individualise stimulation parameters. In addition to this, if intra-subject stability were found, it may become possible to identify responders/non responders in a single pre-testing session. However, this was not to be.

Despite making improvements to the experimental design used in Chapter 3 (such as using anatomical brain scans for accurate TMS coil localization), no significant effects were found in TMS measures taken immediately after 2mA cathodal stimulation. Analysis of intra-subject reliability using interclass correlation coefficients (ICC) revealed poor inter-subject reliability across the four sessions tested. This was true of changes from baseline in IO curve, SICI and ICF measures.

The results of the 2mA anodal condition were more promising. At a group level there was a significant increase in IO curve measures, and some evidence of significant changes in 3ms SICI after stimulation. The increase in IO curves after anodal tDCS replicated previous findings (Nitsche & Paulus, 2000; Nitsche et al., 2005), however, the 3ms SICI results were less clear. Although SICI was found to decrease after anodal stimulation in the majority of the 4 sessions, significant effects between baseline and post stimulation were only found for 2 of the 4 sessions. Somewhat surprisingly, in one session the effects on 3ms SICI appeared to be reversed, revealing a significant increase after 2mA anodal stimulation, however, this can largely be accounted for by methodological issues relating to differences in test pulse intensity. Consequently, although the findings highlight the importance of vigilance when measuring SICI, the results were not in direct conflict with previous work in which SICI was reduced after anodal tDCS (Batsikadze et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005). Hence the IO curve and SICI results largely support the previously reported findings that anodal tDCS increases overall cortical excitability, which may in part be mediated by a reduction in GABA.

The findings of ICC analysis revealed that despite significant group level effects on IO curves these changes were unreliable within individuals across the different testing sessions. ICC analysis also revealed poor reliability for changes in 3ms SICI, which conflicts with the previous findings of Lopez-Alonso et al. (2015). It is possible that the variability found in this measure could reflect methodological differences, although it should also be noted that Lopez-Alonso et al. (2015) failed to find any significant changes in SICI measures.

Key findings from Chapters 3 and 4

To summarize, the findings of the experiments discussed in Chapters 3 and 4 reveal some difficulty in replicating findings of tDCS induced changes in cortical excitability. In particular, both studies failed to find evidence of significant change in excitability following cathodal stimulation. Furthermore, the

studies revealed high levels of both inter and intra subject variability, even when group effects were significant.

8.2 tDCS effects and Tourette's Syndrome

In Chapter 5 I aimed to explore the potential of tDCS to reduce tics in individuals with Tourette's syndrome. Gilles de la Tourette's syndrome (GTS) is a neurodevelopmental condition characterized by the presence of motor and phonic tics which occur for a minimum of 1 year (Leckman, 2002). These tics can be embarrassing, socially alienating and physically harmful, and can start to become apparent in children as young as 3 years old (Leckman et al., 1998). The treatment options available for individuals with GTS are limited, and while behavioural interventions such as habit reversal training (HRT) do exist, they may not be accessible to all. Consequently, many individuals with GTS take medications including forms of antipsychotics, which may have a number of undesirable side effects (Kurlan, 2014). Of those taking medications many will be children, as tics are often at their worst when individuals are 10-12 years old (Bloch & Leckman, 2009). This makes it particularly pertinent that alternative avenues of treatment are explored, one of which may be the development of non-invasive brain stimulation techniques (NIBS) such as tDCS.

Two small scale studies have reported significant reductions in tics following repeated sessions of cathodal stimulation (Carvalho et al., 2015; Mrakic-Sposta et al., 2008). These results are promising, however, the sample sizes in these studies were particularly small, and the findings limited to the effects of prolonged applications of stimulation. Therefore, despite disappointing results in healthy individuals regarding cathodal stimulation effects (Chapters 3 and 4), I chose to extend the previous findings of others using a sham controlled design in which the immediate effects of tDCS were assessed using video recordings/ tic counts and TMS.

Although I had previously failed to find significant cathodal effects, I felt that methodological differences such as participant sample, outcome measures and stimulation site may result in different findings than those reported in Chapters 3 and 4. Disappointingly this was not to be, and no clear differences were observed between the sham and cathodal conditions in any measure.

Analysis of the TMS data (both IO curves and SI1mv) revealed no significant change in cortical excitability following either sham or cathodal tDCS. These results may appear to replicate the lack of cathodal effects seen in Chapters 3 and 4, however, one important methodological difference should be considered. Unlike the previous experiments, stimulation was applied to the SMA rather than directly to M1. Although work using TMS had demonstrated that stimulation to SMA can influence MEPs derived from M1 (Arai et al., 2012; Civardi et al., 2001; Oliveri et al., 2003) it may be that tDCS effects are simply too weak to do so. Furthermore, without individualized models of current flow it is not possible to know if tDCS had any direct influence on M1. As a result, although the findings yet again failed to show reduced cortical excitability following cathodal stimulation they are not directly comparable to those discussed in Chapters 3 and 4.

The lack of change in the amount of tics counted in the video data was particularly disappointing, as this was a far more direct measure of clinical outcome. However, significant changes in such measures have only previously been reported after prolonged periods of stimulation which are repeated over a number of days (Carvalho et al., 2015; Mrakic-Sposta et al., 2008). Therefore, it is possible that a single session of tDCS may not be enough to cause any clear effects on such a complex phenomenon as tics.

In Chapter 6 an in-depth, sham controlled case study was presented in which the effects of 10 day tDCS applications (sham, 1mA and 1.5mA) were explored using a variety of measures. This study not only tested the potential of tDCS to reduce tics, but also explored the feasibility of home use stimulation.

This posed a number of challenges, in particular with regard to data collection using video recordings.

In phase 1 of the study a clear decrease in impairment scores measured using the YGTSS questionnaire was found. Although there was a decrease from baseline after 10 sessions of 1mA cathodal stimulation, there was also a decrease in the sham condition. Furthermore, the subscales of the YGTSS which relate specifically to motor and phonic tics did not show a clear reduction. The results of the 1.5mA cathodal intervention appeared to be far more successful. Scores on the YGTSS clearly reduce compared to baseline, including scores on the motor tic aspect of the measure. Following the 1.5mA cathodal intervention, the participant also reported experiencing positive effects which were far beyond those reported after sham and 1mA conditions.

The video data failed to show any clear changes from baseline in the amount of tics counted during either the 1mA or 1.5mA interventions. Although this is disappointing and does not reflect the previous findings reported by Mrakic-Sposta et al. (2008), there remains a strong possibility that this was the result of a number of methodological issues caused by the home video data collection. If true, this may explain the discrepancy between the findings from the video data and the findings from the questionnaires.

The results of the imaging data revealed widespread change in resting state connectivity, including between the baseline and post sham stimulation scans. As a result, it is difficult to interpret which changes between regions were the result of the cathodal stimulation. Nevertheless, a number of regions within the cerebellum were found to have significantly altered levels of connectivity from baseline in the scans taken shortly after 10 days of 1mA cathodal stimulation and 1 month later. These regions were not found to significantly alter in the sham condition. The cerebellum has been implicated in a number of studies exploring the neurophysiology of GTS (Bohlhalter et al., 2006; Lerner et al., 2012; McCairn et al., 2013; Pourfar et al., 2011; Tobe et al., 2010) and work by Carvalho et al. (2015) saw a decrease in this region following 10 sessions of

cathodal stimulation. This may suggest tDCS induced changes within the region, although it is clear that more comprehensive studies would be needed to confirm this. Unfortunately, the scans in phase 2 did not fully capture the cerebellum, although a number of significant differences were found between a second baseline condition and after 1.5mA tDCS applications.

Although collecting data outside of the laboratory using video recordings proved difficult, compliance with tDCS application was good and none of the 30 stimulation days were missed. With regard to side effects the participant did report mild nausea and mild headache after stimulation, however, assessment of their personal notes revealed that this was no different in the sham and active condition during phase 1. In phase 2 daily notes were not recorded, however, the participant was told to inform the experimenter of any effects that they were concerned about; nothing was reported. The successful application and remote supervision of home use tDCS echoes the findings of a recent study by Kasschau et al. (2016) which also found good compliance and adherence to treatment regimes in 20 participants when remote supervision was provided. This is particularly promising for tDCS, as it suggests that home use is a viable option.

Key findings from Chapters 5 and 6

The results in Chapter 5 suggest that a single session of 1mA cathodal tDCS applied to the SMA is not sufficient to have an effect on tics in the period shortly after stimulation. Furthermore, the stimulation was not found to have any distinct effects on cortical excitability as measured by MEPs. Although the results are somewhat discouraging, it may be that a single session of stimulation is simply not enough to influence complex behaviours such as tics.

The effects of repeated applications of cathodal tDCS were explored in Chapter 6 with some success. Although the results of the different measures were conflicting, the participant's self-report of symptoms and the results from the YGTSS suggest that for this participant repeated sessions of 1.5mA cathodal tDCS may be beneficial in reducing tics. The imaging analysis also revealed

distinctive changes in regions of the cerebellum which were present after 1mA cathodal but not sham stimulation. Although the therapeutic outcomes of the case study are less clear than previous work (Carvalho et al., 2015; Mrakic-Sposta et al., 2008) the study does successfully demonstrate how home use of tDCS under remote supervision is possible.

8.3 Exploring the origin of the MRS-GABA signal and the effects of Gabapentin

A secondary aim within this thesis was to explore exactly what is being measured using MRS, particularly with reference to GABA. MRS is a useful tool which has been used to explore the biological basis of tDCS effects (Stagg et al., 2009) and also to enhance understanding of the underlying neurobiology of GTS (Draper et al., 2014; Puts et al., 2015). However, the findings of such studies are limited by a lack of understanding regarding exactly where the MRS-GABA signal originates from.

In Chapter 7, MRS-measured GABA and other metabolites were explored using TMS. The effects of gabapentin (GBP) were also explored. In line with previous studies (Stagg et al., 2011; Tremblay et al., 2013) there was no significant relationship between baseline MRS-GABA and later phases of SICI (thought to be primarily GABA-A receptor mediated (Ziemann, 2008)). This suggests that the primary source of MRS-GABA is not dependent on GABAergic synaptic transmission, instead it is more likely to reflect ambient extracellular levels of GABA which relate to tonic GABAergic inhibition.

Unlike the findings in Stagg et al. (2011) baseline levels of MRS-GABA did not significantly correlate with 1ms SICI. The underlying mechanisms of 1ms SICI are somewhat unclear, and it is not fully apparent why this discrepancy between studies occurred. One possibility is that the use of different MRS protocols contributed, however, without directly comparing the sequences this can be nothing more than speculation.

The findings of MRS-Glu were somewhat mixed when compared with findings reported in the previous literature. Levels of MRS-Glu were found to significantly correlate with the plateau of the IO curve. However, unlike the findings of Stagg et al. (2011), no relationship was found between this measure and IO curve slope. Furthermore, although 12ms ICF was not found to be significantly related to MRS-Glu as found by Stagg et al. (2011), 10ms ICF was. As of yet no distinctions have been drawn between the mechanisms underlying 12 and 10ms ICF, however, it remains possible that subtle differences between them may exist. Further exploration and replication would be needed to confirm this finding.

Interestingly, despite in-depth exploration of the data, GBP failed to significantly alter either MRS or TMS measures. It is largely unclear why these findings reported in Chapter 7 differ to previous work exploring the effects of GBP (Cai et al., 2012; Kuzniecky et al., 2002; Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996). In the case of MRS, it could be that insufficient time was allowed between drug uptake and scanning to allow effects to be seen. However, the duration between drug administration and TMS measures was similar to Rizzo et al. (2001) and longer than Ziemann, Lonnecker, et al. (1996) ; therefore timing is an unlikely explanation for the lack of effects.

Key findings from Chapters 7

Despite using a larger sample size than Stagg et al. (2011), a relationship between 1ms SICI and MRS-GABA was not found. It is possible that this reflects differences in the scanning sequences used, nevertheless, it suggests that at best the relationship is unreliable and in need of further exploration.

Although the findings differ slightly from previous work conducted by Stagg et al. (2011) and Tremblay et al. (2013), they are largely in agreement with the previous assertion that MRS measures pools of extracellular GABA. These pools of GABA are linked to tonic rather than phasic inhibition and thus

contribute to the inhibitory tone of a brain area rather than GABAergic synaptic transmission.

In light of previous work (Cai et al., 2012; Kuzniecky et al., 2002; Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996), it was surprising to find that GBP failed to influence either TMS or MRS measures. While the lack of change in MRS may reflect a timing issue, the timings of the TMS measures reflects those used in previous work (Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996). Furthermore, as this study was sham controlled and with a far larger sample than that used in previous TMS studies (Rizzo et al., 2001; Ziemann, Lonnecker, et al., 1996), this raises questions regarding the ability of GBP to truly influence these measures. Hence raising questions about the underlying mechanisms of GBP. In order to investigate the effects of GBP in more detail it may be necessary to include a physical measure of drug uptake (such as blood samples) which could be used to verify that enough time had elapsed and a suitable dose had been delivered. If this method were applied it may be possible to not only enhance understanding into the effects of the drug, but also to explore the origin of the MRS-GABA signal – in particular if a discrepancy between TMS and MRS results were identified.

8.4 Limitations and suggestions for future research

One of the fundamental problems with tDCS is that unlike TMS there is no established method for individualising and optimising stimulation parameters. In Chapters 3, 4, 5 and 6, the selection of stimulation parameters was based upon the findings of previous studies; these parameters were then used for all participants without exception. Although this is common practise within tDCS research, it is far from ideal. It is highly possible that this issue contributes to some of the inter-subject variability found in response to tDCS, which has been reported in this thesis and in the work of others (Labruna et al., 2016; Wiethoff et al., 2014).

It is becoming increasingly important that future tDCS research addresses the cause of inter-subject variability, as this may help to identify markers which could be used to predict responses. This could be particularly helpful in identifying individuals who may benefit therapeutically. Recently, associations between sensitivity to TMS measures and response to tDCS have been identified (Labruna et al., 2016; Strube et al., 2016; Wiethoff et al., 2014), although the results are not always synonymous. For example, Wiethoff et al. (2014) found a correlation between baseline MEP amplitude (in response to SI 1mV stimulation) and tDCS induced change. Specifically, individuals with smaller MEP amplitudes were found to be more prone to show increased cortical excitability following anodal or cathodal tDCS. Whereas Strube et al. (2016) found the opposite to be true. Interestingly, a recent study by Labruna et al. (2016) found that higher sensitivity to TMS (as indexed by lower thresholds for SI1mv) was related to larger MEP enhancement following anodal but not cathodal stimulation. These finding suggest that it may be possible to use TMS to predict responses to tDCS, although it is clear that further research is needed.

Integrating computational modelling into future tDCS studies may help to optimise the selection of stimulation parameters with regard to electrode placement. It is known that differences in anatomical structure have critical effects on current flow (Bikson et al., 2012; Datta et al., 2012), and it has already been shown that subject-specific modelling can facilitate more effective use of tDCS (Datta et al., 2012). The utilization of such models is not yet commonplace in tDCS research, however, as more evidence amounts of their efficiency, they could prove a particularly useful tool with regard to the therapeutic use of the technique.

In Chapter 4, high levels of intra-subject variability were found in response to 2mA tDCS. This poses another potential issue for the technique as it suggests that causes of variability may go beyond anatomy. For example, it may be that transient changes in cortical excitability and neurotransmitter levels also influence the effect. If so, this could make identifying individuals who may

benefit from the stimulation particularly difficult. However, it may also be that the methodological issues between different testing sessions contribute to findings, hence revealing more variability than there truly is. Replication of results and the use of multimodal techniques will be important in answering these questions. Furthermore, this may help to understand why some studies such as Lopez-Alonso et al. (2015) reported acceptable levels of intra-subject stability whereas Horvath et al. (2016) and the research reported in Chapter 4 did not.

The need to develop a multi-modal approach to investigate the biological underpinnings of tDCS is highlighted by the fact that the exact method used to measure the effects is likely to influence the outcome. For example, although TMS IO curves are thought to index global cortico-spinal excitability (Abbruzzese & Trompetto, 2002; Devanne et al., 1997) which is supposedly influenced by tDCS, a number of studies have now failed to find clear effects using IO curves (Batsikadze et al., 2013; Strube et al., 2016) despite finding significant changes in MEP amplitude when using SI 1mV. It is unclear exactly why this may be, however, the amount of TMS pulses given and the intensity at which these occur are possible explanations. This may explain why 2mA anodal tDCS was not found to significantly alter IO curves immediately after stimulation in Chapter 3, but did so in Chapter 4 in which higher TMS intensities were used.

Methods such as MRS may be useful in providing a different perspective on tDCS. For example, significant alterations in MRS-GABA have previously been reported following tDCS (Kim et al., 2014; Stagg et al., 2009). Based on the findings of Chapter 7 and those reported by Stagg et al. (2011) and Tremblay et al. (2013), it seems that MRS measures pools of extracellular GABA. Hence, when combined with the wide ranging evidence that SICI is also altered by tDCS (Batsikadze et al., 2013; Cengiz et al., 2013; Kidgell et al., 2013; Nitsche et al., 2005) it can be concluded that the effects of tDCS on GABA are widespread and not restricted to change at the synapse. MRS could prove useful in exploring intra-subject variability following stimulation. For example, tDCS induced

changes in neurotransmitter levels could be measured multiple times in the same individual (as in chapter 4). The results of this could then be used to confirm or refute that the lack of intra-subject stability found in Chapters 4 and by Horvath et al. (2016) was the result of methodological issues pertaining to the TMS protocols.

To summarise, the main limitations of the work presented in this thesis are:

- Limited ability to generalize findings of Chapters 3, 4, 5 and 6. It is likely that altering tDCS parameters (intensity, duration or electrode placement) would also alter the effects found.
- Dependence on TMS alone to measure tDCS effects in Chapters 3 and 4. In Chapter 3 it is possible that changes in cortical excitability were missed due to inadequate outcome measures.
- Issues with parameter selection and the lack of individualised protocols. The necessary stimulation parameters to cause significant physiological or behavioural change are likely to differ from person to person. This is apparent in Chapter 6, in which 1.5mA stimulation appeared to be more effective than 1mA.

Future work should aim to:

- Increase understanding of factors which may cause the inter and intra-subject variability often reported in tDCS studies.
- Use multi-modal techniques to explore factors which may predict an individual's response to tDCS. This is critical to developing methods which allow stimulation parameters to be tailored to each individual. This is likely to be an important step in improving efficiency and therapeutic outcomes.
- Replicate previous findings and publish failures to replicate. This may be particularly important with regard to the effects of cathodal stimulation.

8.5 Conclusions

tDCS effects proved to be more elusive than initially expected, and over the course of this thesis it became apparent that developing tDCS as an effective therapeutic intervention may be far more complex than initially anticipated.

The findings in Chapters 3 and 4 suggest that although a number of studies have reported significant reductions in cortical excitability following cathodal stimulation (see Table 2.1), these effects are unreliable at best and can be difficult to replicate. In Chapter 4, it was found that while 2mA anodal tDCS did successfully increase cortical excitability at a group level, there was substantial inter and intra-subject variability. This finding makes important contributions to the current literature and raises some real questions regarding the use of the technique and the reporting of experimental findings.

The findings in Chapter 5 suggest that a single session of cathodal tDCS applied to the SMA has no clear effects on tics in individuals with Tourette's syndrome. This is a novel finding, and suggests that the previous reports of tic reduction following stimulation (Carvalho et al., 2015; Mrakic-Sposta et al., 2008) are the result of accumulative tDCS effects. The findings in Chapter 6 offer some support for this hypothesis, as there was evidence of a meaningful reduction in tics after 10 days of 20-minute cathodal stimulation delivered at 1.5 mA. The findings in Chapter 6 also highlight the need to optimise stimulation parameters for each individual, as the effects following 1mA were not that distinctive from the sham condition.

In Chapter 7, evidence was found which supports the previous assertion that MRS-GABA reflects extra-synaptic GABA tone (Stagg et al., 2011; Tremblay et al., 2013). The study also highlighted a number of interesting relationships between TMS and MRS measures, and raised some interesting questions regarding the ability of gabapentin to raise GABA levels in healthy participants.

To conclude, the field of non-invasive stimulation continues to grow and a substantial amount of progress has been made in recent years. The once widely held assertions of tDCS effects are recently being challenged, and it is being found that cathodal tDCS does not always reduce cortical excitability. Furthermore, recent work has focused on discussing and unpicking variability found in individual responses to the stimulation. The work presented in this thesis, contributes to this and suggest that the technique and its effects are far more variable and difficult to predict than might initially be expected. Despite this, the findings in Chapter 6 show that although tDCS effects may be variable, repeated applications of cathodal stimulation may still be effective in reducing tics in Tourette's syndrome. Furthermore, the findings highlight that application of tDCS outside of the lab is possible with remote supervision. Although more work is clearly needed, tDCS has proved that it still has therapeutic potential, and perhaps with a little more research this potential will finally be reached.

References

Abbruzzese, G., & Trompetto, C. (2002). Clinical and research methods for evaluating cortical excitability. *The Journal of Clinical Neurophysiology, 19*(4), 307-321.

Adrian, E. D., & Moruzzi, G. (1939). Impulses in the pyramidal tract. *Journal of Physiology, 97*(2), 153-199.

Albin, R. L., & Mink, J. W. (2006). Recent advances in Tourette syndrome research. *Trends in Neurosciences, 29*(3), 175-182.

Ambrus, G. G., Al-Moyed, H., Chaieb, L., Sarp, L., Antal, A., & Paulus, W. (2012). The fade-in--short stimulation--fade out approach to sham tDCS--reliable at 1 mA for naive and experienced subjects, but not investigators. *Brain Stimulation, 5*(4), 499-504.

Antal, A., Keeser, D., Priori, A., Padberg, F., & Nitsche, M. A. (2015). Conceptual and Procedural Shortcomings of the Systematic Review" Evidence That Transcranial Direct Current Stimulation (tDCS) Generates Little-to-no Reliable Neurophysiologic Effect Beyond MEP Amplitude Modulation in Healthy Human Subjects: A Systematic Review" by Horvath and Co-workers. *Brain Stimulation, 8*(4), 846-849.

Antal, A., Terney, D., Kuhnl, S., & Paulus, W. (2010). Anodal Transcranial Direct Current Stimulation of the Motor Cortex Ameliorates Chronic Pain and Reduces Short Intracortical Inhibition. *Journal of Pain and Symptom Management, 39*(5), 890-903.

Arai, N., Lu, M. K., Ugawa, Y., & Ziemann, U. (2012). Effective connectivity between human supplementary motor area and primary motor cortex: a paired-coil TMS study. *Experimental Brain Research, 220*(1), 79-87.

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet, 325*(8437), 1106-1107.

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., & Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *The Journal of Physiology, 591*(7), 1987-2000.

Ben-Ari, Y., Khalilov, I., Kahle, K. T., & Cherubini, E. (2012). The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders. *Neuroscientist, 18*(5), 467-486.

Bennett, M. R. (2000). The concept of long term potentiation of transmission at synapses. *Progress in Neurobiology, 60*(2), 109-137.

Berardelli, A., Inghilleri, M., Cruccu, G., & Manfredi, M. (1990). Descending volley after electrical and magnetic transcranial stimulation in man. *Neuroscience letters, 112*(1), 54-58.

Bhattacharyya, P. K., Lowe, M. J., & Phillips, M. D. (2007). Spectral quality control in motion-corrupted single-voxel J-difference editing scans: An interleaved navigator approach. *Magnetic Resonance in Medicine, 58*(4), 808-812.

Bikson, M., Rahman, A., & Datta, A. (2012). Computational models of transcranial direct current stimulation. *Clinical EEG Neuroscience, 43*(3), 176-183.

Bindman, L. J., Lippold, O. C. J., & Redfearn, J. W. T. (1964). The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. *The Journal of Physiology, 172*(3), 369-382.

Bitsko, R. H., Holbrook, J. R., Visser, S. N., Mink, J. W., Zinner, S. H., Ghandour, R. M., & Blumberg, S. J. (2014). A national profile of Tourette syndrome, 2011-2012. *Journal of Developmental and Behavioral Pediatrics, 35*(5), 317-322.

Bloch, M. H., & Leckman, J. F. (2009). Clinical course of Tourette syndrome. *Journal of Psychosomatic Research, 67*(6), 497-501.

Bloch, M. H., Leckman, J. F., Zhu, H., & Peterson, B. S. (2005). Caudate volumes in childhood predict symptom severity in adults with Tourette syndrome. *Neurology, 65*(8), 1253-1258.

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., & Fregni, F. (2008). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *The International Journal of Neuropsychopharmacology, 11*(2), 249-254.

Bogner, W., Gagoski, B., Hess, A. T., Bhat, H., Tisdall, M. D., van der Kouwe, A. J. W., Strasser, B., Marjanska, M., Trattnig, S., Grant, E., Rosen, B., & Andronesi, O. C. (2014). 3D GABA imaging with real-time motion correction, shim update and reacquisition of adiabatic spiral MRSI. *Neuroimage, 103*, 290-302.

Bohlhalter, S., Goldfine, A., Matteson, S., Garraux, G., Hanakawa, T., Kansaku, K., Wurzman, R., & Hallett, M. (2006). Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. *Brain, 129*(8), 2029-2037.

Boroojerdi, B., Battaglia, F., Muellbacher, W., & Cohen, L. G. (2001). Mechanisms influencing stimulus-response properties of the human corticospinal system. *Clinical Neurophysiology, 112*(5), 931-937.

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *Journal of Clinical Neurophysiology, 9*(1), 132-136.

Brickley, S. G., & Mody, I. (2012). Extrasynaptic GABA(A) Receptors: Their Function in the CNS and Implications for Disease. *Neuron, 73*(1), 23-34.

Bronfeld, M., Israelashvili, M., & Bar-Gad, I. (2013). Pharmacological animal models of Tourette syndrome. *Neuroscience and Biobehavioral Reviews, 37*(6), 1101-1119.

Brunelin, J., Mondino, M., Gassab, L., Haesebaert, F., Gaha, L., Suaud-Chagny, M. F., Saoud, M., Mechri, A., Poulet, E. (2012). Examining Transcranial Direct-Current Stimulation (tDCS) as a Treatment for Hallucinations in Schizophrenia. *American Journal of Psychiatry, 169*(7), 719-724.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Internation Journal of Neuropsychopharmacology, 14*(8), 1133-1145.

Brunoni, A. R., Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Boggio, P. S., Giacopuzzi, M., Barbieri, S., & Priori, A. (2011). Transcranial direct current stimulation (tDCS) in unipolar vs. bipolar depressive disorder. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 35*(1), 96-101.

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., Edwards, D. J., Valero-Cabre, A., Rotenberg, A., Pascual-Leone, A., Ferrucci, R., Priori, A., Boggio, P. S., & Fregni, F. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimulation, 5*(3), 175-195.

Burke, D., Hicks, R., Gandevia, S. C., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology, 470*(1), 383-393.

Cai, K., Nanga, R. P., Lamprou, L., Schinstine, C., Elliott, M., Hariharan, H., Reddy, R., & Epperson, C. N. (2012). The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T (1)H-MRS study. *Neuropsychopharmacology, 37*(13), 2764-2771.

Cantello, R., Tarletti, R., & Civardi, C. (2002). Transcranial magnetic stimulation and Parkinson's disease. *Brain Research Reviews, 38*(3), 309-327.

Carvalho, S., Gonçalves, Ó. F., Soares, J. M., Sampaio, A., Macedo, F., Fregni, F., & Leite, J. (2015). Sustained effects of a neural based intervention in a refractory case of Tourette Syndrome. *Brain Stimulation, 8*(3), 657.

Cengiz, B., Murase, N., & Rothwell, J. C. (2013). Opposite effects of weak transcranial direct current stimulation on different phases of short interval intracortical inhibition (SICI). *Experimental Brain Research, 225*(3), 321-331.

Chen, R. (2000). Studies of human motor physiology with transcranial magnetic stimulation. *Muscle & Nerve, 9*, S26-32.

Chen, R., Tam, A., Butefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., & Cohen, L. G. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *Journal of Neurophysiology, 80*(6), 2870-2881.

Chew, T., Ho, K.-A., & Loo, C. K. (2015). Inter-and Intra-individual Variability in Response to Transcranial Direct Current Stimulation (tDCS) at Varying Current Intensities. *Brain Stimulation, 8*(6), 1130-1137.

Civardi, C., Cantello, R., Asselman, P., & Rothwell, J. C. (2001). Transcranial magnetic stimulation can be used to test connections to primary motor areas from frontal and medial cortex in humans. *Neuroimage, 14*(6), 1444-1453.

Civardi, C., Cavalli, A., Naldi, P., Varrasi, C., & Cantello, R. (2000). Hemispheric asymmetries of cortico-cortical connections in human hand motor areas. *Clinical Neurophysiology, 111*(4), 624-629.

Claus, D., Weis, M., Jahnke, U., Plewe, A., & Brunholzl, C. (1992). Corticospinal conduction studied with magnetic double stimulation in the intact human. *Journal of the Neurological Sciences, 111*(2), 180-188.

Cohen, L. G., Roth, B. J., Nilsson, J., Dang, N., Panizza, M., Bandinelli, S., Friauf, W., & Hallett, M. (1990). Effects of coil design on delivery of focal magnetic stimulation. Technical considerations. *Electroencephalography and Clinical Neurophysiology, 75*(4), 350-357.

Cohen, M. S. (2008). Handedness questionnaire. Retrieved 10/10/2013, 2013, from http://www.brainmapping.org/shared/Edinburgh.php

Conelea, C. A., Woods, D. W., Zinner, S. H., Budman, C., Murphy, T., Scahill, L. D., Compton, S. N., & Walkup, J. (2011). Exploring the impact of chronic tic disorders on youth: results from the Tourette Syndrome Impact Survey. *Child Psychiatry & Human Development, 42*(2), 219-242.

Conelea, C. A., Woods, D. W., Zinner, S. H., Budman, C. L., Murphy, T. K., Scahill, L. D., Compton, S. N., & Walkup, J. T. (2013). The impact of Tourette Syndrome in adults: results from the Tourette Syndrome impact survey. *Community Mental Health Journal, 49*(1), 110-120.

Coombes, S. A., Tandonnet, C., Fujiyama, H., Janelle, C. M., Cauraugh, J. H., & Summers, J. J. (2009). Emotion and motor preparation: A transcranial magnetic stimulation study of corticospinal motor tract excitability. *Cognitive, Affective, & Behavioral Neuroscience, 9*(4), 380-388.

Creutzfeldt, O. D., Fromm, G. H., & Kapp, H. (1962). Influence of transcortical d-c currents on cortical neuronal activity. *Experimental Neurolology, 5*(6), 436-452.

Cukic, M., Kalauzi, A., Ilic, T., Miskovic, M., & Ljubisavljevic, M. (2009). The influence of coil-skull distance on transcranial magnetic stimulation motor-evoked responses. *Experimental Brain Research, 192*(1), 53-60.

Danielsen, E. R., & Ross, B. (1999). *Magnetic resonance spectroscopy diagnosis of neurological diseases*. New York: Marcel Dekker.

Daskalakis, Z. J., Christensen, B. K., Chen, R., Fitzgerald, P. B., Zipursky, R. B., & Kapur, S. (2002). Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. *Archives of General Psychiatry, 59*(4), 347-354.

Datta, A., Truong, D., Minhas, P., Parra, L. C., & Bikson, M. (2012). Inter-Individual Variation during Transcranial Direct Current Stimulation and Normalization of Dose Using MRI-Derived Computational Models. *Frontiers in Psychiatry, 3*, 91.

De Gennaro, L., Marzano, C., Veniero, D., Moroni, F., Fratello, F., Curcio, G., Ferrara, M., Ferlazzo, F., Novelli, L., Pellicclari, M. C., Bertini, M., & Rossini, P. M. (2007). Neurophysiological correlates of sleepiness: A combined TMS and EEG study. *Neuroimage, 36*(4), 1277-1287.

Denys, D., Mantione, M., Figee, M., van den Munckhof, P., Koerselman, F., Westenberg, H., Bosch, A., & Schuurman, R. (2010). Deep Brain Stimulation of the Nucleus Accumbens for Treatment-Refractory Obsessive-Compulsive Disorder. *Archives of General Psychiatry, 67*(10), 1061-1068.

Devanne, H., Lavoie, B. A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Experimental Brain Research, 114*(2), 329-338.

Di Lazzaro, V., Manganelli, F., Dileone, M., Notturno, F., Esposito, M., Capasso, M., Dubbioso, R., Pace, M., Ranieri, F., Minicuci, G., Santoro, L., & Uncini, A. (2012). The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex. *Journal of Neural Transmission, 119*(12), 1499-1506.

Di Lazzaro, V., Oliviero, A., Mazzone, P., Pilato, F., Saturno, E., Insola, A., Visocchi, M., Colosimo, C., Tonali, P. A., & Rothwell, J. C. (2002). Direct demonstration of long latency cortico-cortical inhibition in normal subjects and in a patient with vascular parkinsonism. *Clinical Neurophysiology, 113*(11), 1673-1679.

Di Lazzaro, V., Oliviero, A., Meglio, M., Cioni, B., Tamburrini, G., Tonali, P., & Rothwell, J. C. (2000). Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clinical Neurophysiology, 111*(5), 794-799.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M. A., Pilato, F., Zito, G., Dileone, M., Nicoletti, R., Pasqualetti, P., & Tonali, P. A. (2003). Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. *The Journal of Physiology, 547*(2), 485-496.

Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., Mazzone, P., Tonali, P., & Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 109*(5), 397-401.

Di Lazzaro, V., Oliviero, A., Saturno, E., Pilato, F., Insola, A., Mazzone, P., Profice, P., Tonali, P., & Rothwell, J. C. (2001). The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. *Experimental Brain Research, 138*(2), 268-273.

Di Lazzaro, V., Pilato, F., Dileone, M., Ranieri, F., Ricci, V., Profice, P., Bria, P., Tonali, P. A., & Ziemann, U. (2006). GABAA receptor subtype specific enhancement of inhibition in human motor cortex. *The Journal of Physiology, 575*(Pt 3), 721-726.

Di Lazzaro, V., Profice, P., Ranieri, F., Capone, F., Dileone, M., Oliviero, A., & Pilato, F. (2012). I-wave origin and modulation. *Brain Stimulation, 5*(4), 512-525.

Di Lazzaro, V., Restuccia, D., Oliviero, A., Profice, P., Ferrara, L., Insola, A., Mazzone, P., Tonali, P., & Rothwell, J. C. (1998). Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. *Experimental Brain Research, 119*(2), 265-268.

Di Lazzaro, V., Ziemann, U., & Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation, 1*(4), 345-362.

Doeltgen, S. H., & Ridding, M. C. (2011). Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. *Clinical Neurophysiology, 122*(7), 1411-1416.

Draper, A., Stephenson, M. C., Jackson, G. M., Pepes, S., Morgan, P. S., Morris, P. G., & Jackson, S. R. (2014). Increased GABA Contributes to Enhanced Control over Motor Excitability in Tourette Syndrome. *Current Biology, 24*(19), 2343-2347.

Du, J., Machado-Vieira, R., Maeng, S., Martinowich, K., Manji, H. K., & Zarate, C. A. (2007). Enhancing AMPA to NMDA throughput as a convergent mechanism for antidepressant action. *Drug discovery today: Therapeutic strategies, 3*(3), 519-526.

Dundas, J. E., Thickbroom, G. W., & Mastaglia, F. L. (2007). Perception of comfort during transcranial DC stimulation: Effect of NaCl solution concentration applied to sponge electrodes. *Clinical Neurophysiology, 118*(5), 1166-1170.

Dymond, A. M., Coger, R. W., & Serafetinides, E. A. (1975). Intracerebral Current Levels in Man during Electrosleep Therapy. *Biological Psychiatry, 10*(1), 101-104.

Eidelberg, D., Moeller, J. R., Antonini, A., Kazumata, K., Dhawan, V., Budman, C., & Feigin, A. (1997). The metabolic anatomy of Tourette's syndrome. *Neurology, 48*(4), 927-934.

Ellaway, P. H., Davey, N. J., Maskill, D. W., Rawlinson, S. R., Lewis, H. S., & Anissimova, N. P. (1998). Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. *Electromyography and Motor Control-Electroencephalography and Clinical Neurophysiology, 109*(2), 104-113.

Elliott, P. (2014). Electrical stimulation and the brain: An historical evaluation. In R. Cohen Kadosh (Ed.), *The Stimulated Brain* (pp. 3-33). London: Accademic Press, Elsevier.

Enticott, P. G., Rinehart, N. J., Tonge, B. J., Bradshaw, J. L., & Fitzgerald, P. B. (2012). Repetitive transcranial magnetic stimulation (rTMS) improves movement-related cortical potentials in autism spectrum disorders. *Brain Stimulation, 5*(1), 30-37.

Epperson, C. N., Gueorguieva, R., Czarkowski, K. A., Stiklus, S., Sellers, E., Krystal, J. H., Rothman, D. L., & Mason, G. F. (2006). Preliminary evidence of reduced occipital GABA concentrations in puerperal women: a 1H-MRS study. *Psychopharmacology, 186*(3), 425-433.

Finis, J., Enticott, P. G., Pollok, B., Munchau, A., Schnitzler, A., & Fitzgerald, P. B. (2013). Repetitive transcranial magnetic stimulation of the supplementary motor area induces echophenomena. *Cortex, 49*(7), 1978-1982.

Fink, M. (2001). Convulsive therapy: a review of the first 55 years. *Journal of Affect Disorders, 63*(1-3), 1-15.

Fisher, R. J., Nakamura, Y., Bestmann, S., Rothwell, J. C., & Bostock, H. (2002). Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. *Experimental Brain Research, 143*(2), 240-248.

Franzkowiak, S., Pollok, B., Biermann-Ruben, K., Sudmeyer, M., Paszek, J., Thomalla, G., Jonas, M., Orth, M., Munchau, A., & Schnitzler, A. (2012). Motor-cortical interaction in Gilles de la Tourette syndrome. *Plos One, 7*(1), e27850.

Fratello, F., Veniero, D., Curcio, G., Ferrara, M., Marzano, C., Moroni, F., Pellicciari, M. C., Bertini, M., Rossini, P. M., & De Gennaro, L. (2006). Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. *Clinical Neurophysiology, 117*(12), 2667-2674.

Fregni, F., Boggio, P. S., Nitsche, M. A., Marcolin, M. A., Rigonatti, S. P., & Pascual-Leone, A. (2006). Treatment of major depression with transcranial direct current stimulation. *Bipolar Disorders, 8*(2), 203-204.

Fregni, F., Gimenes, R., Valle, A. C., Ferreira, M. J., Rocha, R. R., Natalle, L., Bravo, R., Rigonatti, S. P., Freedman, S. D., Nitsche, M. A., Pascual-Leone, A., & Boggio, P. S. (2006). A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. *Arthritis & Rheumatism, 54*(12), 3988-3998.

Fregni, F., Nitsche, M. A., Loo, C. K., Brunoni, A. R., Marangolo, P., Leite, J., Carvalho, S., Bolognini, N., Caumo, W., Paik, N. J., Simis, M., Ueda, K., Ekhtiari, H., Luu, P., Tucker, D. M., Tyler, W. J., Brunelin, J., Datta, A., Juan, C. H., Venkatasubramanian, G., Boggio, P. S., & Bikson, M. (2014). Regulatory considerations for the clinical and research use of transcranial direct current stimulation (tDCS): Review and recommendations from an expert panel. *Clinical Research and Regulatory Affairs, 32*, 22-35.

Fricke, K., Seeber, A. A., Thirugnanasambandam, N., Paulus, W., Nitsche, M. A., & Rothwell, J. C. (2011). Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology, 105*(3), 1141-1149.

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y. Y., Cohen, L. G., & Lu, B. (2010). Direct Current Stimulation Promotes BDNF-Dependent Synaptic Plasticity: Potential Implications for Motor Learning. *Neuron, 66*(2), 198-204.

Fujiyama, H., Hyde, J., Hinder, M. R., Kim, S. J., McCormack, G. H., Vickers, J. C., & Summers, J. J. (2014). Delayed plastic responses to anodal tDCS in older adults. *Frontiers in Aging Neuroscience, 6*.

Furubayashi, T., Terao, Y., Arai, N., Okabe, S., Mochizuki, H., Hanajima, R., Hamada, M., Yugeta, A., Inomata-Terada, S., & Ugawa, Y. (2008). Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. *Experimental Brain Ressearch, 185*(2), 279-286.

Fusco, C., Bertani, G., Caricati, G., & Della Giustina, E. (2006). Stress fracture of the peroneal bone secondary to a complex tic. *Brain Development, 28*(1), 52-54.

Galvez, V., Alonzo, A., Martin, D., & Loo, C. K. (2013). Transcranial direct current stimulation treatment protocols: should stimulus intensity be constant or incremental over multiple sessions? *International Journal of Neuropsychopharmacology, 16*(1), 13-21.

Garry, M. I., & Thomson, R. H. S. (2009). The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. *Experimental Brain Research, 193*(2), 267-274.

Gidal, B. E., Maly, M. M., Budde, J., Lensmeyer, G. L., Pitterle, M. E., & Jones, J. C. (1996). Effect of a high-protein meal on gabapentin pharmacokinetics. *Epilepsy Research, 23*(1), 71-76.

Gidal, B. E., Maly, M. M., Kowalski, J. W., Rutecki, P. A., Pitterle, M. E., & Cook, D. E. (1998). Gabapentin absorption: effect of mixing with foods of varying macronutrient composition. *Annals of Pharmacotherapy, 32*(4), 405-409.

Gilbert, D. L., Isaacs, K. M., Augusta, M., Macneil, L. K., & Mostofsky, S. H. (2011). Motor cortex inhibition: a marker of ADHD behavior and motor development in children. *Neurology, 76*(7), 615-621.

Gilbert, D. L., Sallee, F. R., Zhang, J., Lipps, T. D., & Wassermann, E. M. (2005). Transcranial magnetic stimulation-evoked cortical inhibition: a consistent marker of attention-deficit/hyperactivity disorder scores in tourette syndrome. *Biological Psychiatry, 57*(12), 1597-1600.

Goa, K. L., & Sorkin, E. M. (1993). Gabapentin. A review of its pharmacological properties and clinical potential in epilepsy. *Drugs, 46*(3), 409-427.

Goetz, C. G., Pappert, E. J., Louis, E. D., Raman, R., & Leurgans, S. (1999). Advantages of a modified scoring method for the Rush Video-Based Tic Rating Scale. *Movement Disorders, 14*(3), 502-506.

Gonzalez-Burgos, G., Hashimoto, T., & Lewis, D. A. (2010). Alterations of Cortical GABA Neurons and Network Oscillations in Schizophrenia. *Current Psychiatry Reports, 12*(4), 335-344.

Greene, D. J., Schlaggar, B. L., & Black, K. J. (2015). Neuroimaging in Tourette Syndrome: Research Highlights From 2014-2015. *Current Developmental Disorders Reports, 2*(4), 300-308.

Gruetter, R. (1993). Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magnetic Resonance in Medicine, 29*(6), 804-811.

Hajcak, G., Molnar, C., George, M. S., Bolger, K., Koola, J., & Nahas, Z. (2007). Emotion facilitates action: A transcranial magnetic stimulation study of motor cortex excitability during picture viewing. *Psychophysiology, 44*(1), 91-97.

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature, 406*(6792), 147-150.

Hallett, M. (2007). Transcranial magnetic stimulation: A primer. *Neuron, 55*(2), 187-199.

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J. C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cerebral Cortex, 23*(7), 1593-1605.

Hampson, M., Tokoglu, F., King, R. A., Constable, R. T., & Leckman, J. F. (2009). Brain Areas Coactivating with Motor Cortex During Chronic Motor Tics and Intentional Movements. *Biological Psychiatry, 65*(7), 594-599.

Hanajima, R., & Ugawa, Y. (2008). Paired-pulse measures. In E. M. Wassermann, V. Walsh, C. M. Epstein, T. Paus, U. Ziemann & S. H. Lisanby (Eds.), *The Oxford handbook of Transcranial Stimulation* (pp. 103-117). Oxford: Oxford University Press.

Hasan, A., Aborowa, R., Nitsche, M. A., Marshall, L., Schmitt, A., Gruber, O., Falkai, P., & Wobrock, T. (2012). Abnormal bihemispheric responses in schizophrenia patients following cathodal transcranial direct stimulation. *European Archives of Psychiatry and Clinical Neuroscience, 262*(5), 415-423.

Herbsman, T., Forster, L., Molnar, C., Dougherty, R., Christie, D., Koola, J., Ramsey, D., Morgan, P. S., Bohning, D. E., George, M. S., & Nahas, Z. (2009). Motor threshold in transcranial magnetic stimulation: the impact of white matter fiber orientation and skull-to-cortex distance. *Human Brain Mapping, 30*(7), 2044-2055.

Hess, C. W., Mills, K. R., & Murray, N. M. F. (1987). Responses in Small Hand Muscles from Magnetic Stimulation of the Human-Brain. *Journal of Physiology-London, 388*(1), 397-419.

Himle, M. B., Chang, S., Woods, D. W., Pearlman, A., Buzzella, B., Bunaciu, L., & Piacentini, J. C. (2006). Establishing the feasibility of direct observation in the assessment of tics in children with chronic tic disorders. *Journal of Applied Behavior Analysis 39*(4), 429-440.

Hoekstra, P. J., Steenhuis, M. P., Kallenberg, C. G., & Minderaa, R. B. (2004). Association of small life events with self reports of tic severity in pediatric and adult tic disorder patients: a prospective longitudinal study. *Journal of Clinical Psychiatry, 65*(3), 426-431.

Horvath, J. C., Forte, J. D., & Carter, O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. *Neuropsychologia, 66*, 213-236.

Horvath, J. C., Vogrin, S. J., Carter, O., Cook, M. J., & Forte, J. D. (2016). Effects of a common transcranial direct current stimulation (tDCS) protocol on motor evoked potentials found to be highly variable within individuals over 9 testing sessions. *Experimental Brain Research*.

Horvath, J. C., Carter, O., & Forte, J. D. (2014). Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Frontiers in Systems Neuroscience, 8*.

Huber, R., Maki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A. G., Tononi, G., & Massimini, M. (2013). Human Cortical Excitability Increases with Time Awake. *Cerebral Cortex, 23*(2), 1-7.

Hussman, J. P. (2001). Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. *Journal of Autism and Developmental Disorders, 31*(2), 247-248.

Ilic, T. V., Meintzschel, F., Cleff, U., Ruge, D., Kessler, K. R., & Ziemann, U. (2002). Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. *The Journal of Physiology, 545*(1), 153-167.

Jacobson, L., Koslowsky, M., & Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Experimental Brain Research, 216*(1), 1-10.

Jalenques, I., Galland, F., Malet, L., Morand, D., Legrand, G., Auclair, C., Hartmann, A., Derost, P., & Durif, F. (2012). Quality of life in adults with Gilles de la Tourette Syndrome. *BMC Psychiatry, 12*, 109.

JASP-Team. (2016). JASP (Version 0.7.5.5) jasp-stats.org.

Jeffreys, H. (1961). *Theory of Probability*. Oxford, UK: Oxford University Press.

Jimenez-Shahed, J., & Jankovic, J. (2013). Tetrabenazine for treatment of chorea associated with Huntington's disease and other potential indications. *Expert Opinion on Orphan Drugs, 1*(5), 423-436.

Kadosh, R. C. (2015). Modulating and enhancing cognition using brain stimulation: Science and fiction. *Journal of Cognitive Psychology, 27*(2), 141-163.

Kalanithi, P. S., Zheng, W., Kataoka, Y., DiFiglia, M., Grantz, H., Saper, C. B., Schwartz, M. L., Leckman, J. F., & Vaccarino, F. M. (2005). Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. *Proceedings of the National Academy of Sciences of the United States of America, 102*(37), 13307-13312.

Kalueff, A. V., & Nutt, D. J. (2007). Role of GABA in anxiety and depression. *Depression & Anxiety, 24*(7), 495-517.

Kammer, T., Beck, S., Thielscher, A., Laubis-Herrmann, U., & Topka, H. (2001). Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clinical Neurophysiology, 112*(2), 250-258.

Kaneko, K., Kawai, S., Fuchigami, Y., Morita, H., & Ofuji, A. (1996). The effect of current direction induced by transcranial magnetic stimulation on the corticospinal excitability in human brain. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 101*(6), 478-482.

Kasschau, M., Reisner, J., Sherman, K., Bikson, M., Datta, A., & Charvet, L. E. (2016). Transcranial Direct Current Stimulation Is Feasible for Remotely Supervised Home Delivery in Multiple Sclerosis. *Neuromodulation: Technology at the Neural Interface.* Retrieved from doi:10.1111/ner.12430.

Kataoka, Y., Kalanithi, P. S., Grantz, H., Schwartz, M. L., Saper, C., Leckman, J. F., & Vaccarino, F. M. (2010). Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. *Journal of Comparative Neurology, 518*(3), 277-291.

Kidgell, D. J., Daly, R. M., Young, K., Lum, J., Tooley, G., Jaberzadeh, S., Zoghi, M., & Pearce, A. J. (2013). Different current intensities of anodal transcranial direct current stimulation do not differentially modulate motor cortex plasticity. *Neural Plasticity, 2013*, 603502.

Kim, S., Stephenson, M. C., Morris, P. G., & Jackson, S. R. (2014). tDCS-induced alterations in GABA concentration within primary motor cortex predict motor learning and motor memory: A 7 T magnetic resonance spectroscopy study. *Neuroimage, 99*, 237-243.

Kondo, T., Fromm, G. H., & Schmidt, B. (1991). Comparison of Gabapentin with Other Antiepileptic and Gabaergic Drugs. *Epilepsy Research, 8*(3), 226-231.

Kossev, A. R., Siggelkow, S., Dengler, R., & Rollnik, J. D. (2003). Intracortical inhibition and facilitation in paired-pulse transcranial magnetic stimulation: effect of conditioning stimulus intensity on sizes and latencies of motor evoked potentials. *Journal of clinical neurophysiology, 20*(1), 54-58.

Kozel, F. A., Nahas, Z., deBrux, C., Molloy, M., Lorberbaum, J. P., Bohning, D., Risch, S. C., & George, M. S. (2000). How coil-cortex distance relates to age, motor threshold, and antidepressant response to repetitive transcranial magnetic stimulation. *Journal of Neuropsychiatry and Clinical Neurosciences, 12*(3), 376-384.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., Wroe, S., Asselman, P., & Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *The Journal of Physiology, 471*, 501-519.

Kurlan, R. M. (2014). Treatment of Tourette syndrome. *Neurotherapeutics, 11*(1), 161-165.

Kuzniecky, R., Ho, S., Pan, J., Martin, R., Gilliam, F., Faught, E., & Hetherington, H. (2002). Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology, 58*(3), 368-372.

Kwon, H. J., Lim, W. S., Lim, M. H., Lee, S. J., Hyun, J. K., Chae, J. H., & Paik, K. C. (2011). 1-Hz low frequency repetitive transcranial magnetic stimulation in children with Tourette's syndrome. *Neuroscience Letters, 492*(1), 1-4.

Labruna, L., Jamil, A., Fresnoza, S., Batsikadze, G., Kuo, M. F., Vanderschelden, B., Ivry, R. B., & Nitsche, M. A. (2016). Efficacy of Anodal Transcranial Direct Current Stimulation is Related to Sensitivity to Transcranial Magnetic Stimulation. *Brain Stimulation, 9*(1), 8-15.

Lahey, M. A., Downey, R. G., & Saal, F. E. (1983). Intraclass Correlations - Theres More There Than Meets the Eye. *Psychological Bulletin, 93*(3), 586-595.

Lanneau, C., Green, A., Hirst, W. D., Wise, A., Brown, J. T., Donnier, E., Charles, K. J., Wood, M., Davies, C. H., & Pangalos, M. N. (2001). Gabapentin is not a GABA(B) receptor agonist. *Neuropharmacology, 41*(8), 965-975.

Le, K., Liu, L., Sun, M. L., Hu, L., & Xiao, N. (2013). Transcranial magnetic stimulation at 1 Hertz improves clinical symptoms in children with Tourette syndrome for at least 6 months. *Journal of Clinical Neuroscience, 20*(2), 257-262.

Leckman, J. F. (2002). Tourette's syndrome. *Lancet, 360*(9345), 1577-1586.

Leckman, J. F., Hardin, M. T., Riddle, M. A., Stevenson, J., Ort, S. I., & Cohen, D. J. (1991). Clonidine treatment of Gilles de la Tourette's syndrome. *Archives of General Psychiatry, 48*(4), 324-328.

Leckman, J. F., King, R., & Cohen, D. (1998). Tics and tic disorders. In J. Leckman & D. Cohen (Eds.), *Tourette's Syndrome -- Tics, Obsessions, Compulsions: Developmental Psychopathology and Clinical Care* (pp. 23-42): Wiley.

Leckman, J. F., Riddle, M. A., Hardin, M. T., Ort, S. I., Swartz, K. L., Stevenson, J., & Cohen, D. J. (1989). The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *Journal of the American Academy of Child and Adolescent Psychiatry, 28*(4), 566-573.

Lerner, A., Bagic, A., Simmons, J. M., Mari, Z., Bonne, O., Xu, B., Kazuba, D., Herscovitch, P., Carson, R. E., Murphy, D. L., Drevets, W. C., & Hallett, M. (2012). Widespread abnormality of the gamma-aminobutyric acid-ergic system in Tourette syndrome. *Brain, 135*(Pt 6), 1926-1936.

Li, L. M., Uehara, K., & Hanakawa, T. (2015). The contribution of interindividual factors to variability of response in transcranial direct current stimulation studies. *Frontiers in Cellular Neuroscience, 9*, 181.

Liebetanz, D., Koch, R., Mayenfels, S., Konig, F., Paulus, W., & Nitsche, M. A. (2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clinical Neurophysiology, 120*(6), 1161-1167.

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain, 125*(10), 2238-2247.

Liepert, J., Schwenkreis, P., Tegenthoff, M., & Malin, J. P. (1997). The glutamate antagonist riluzole suppresses intracortical facilitation. *Journal of Neural Transmission, 104*(11-12), 1207-1214.

Lin, H., Katsovich, L., Ghebremichael, M., Findley, D. B., Grantz, H., Lombroso, P. J., King, R. A., Zhang, H., & Leckman, J. F. (2007). Psychosocial stress predicts future symptom severities in children and adolescents with Tourette syndrome and/or obsessive-compulsive disorder. *Journal of Child Psychology and Psychiatry, 48*(2), 157-166.

Lopez-Alonso, V., Fernandez-Del-Olmo, M., Costantini, A., Gonzalez-Henriquez, J. J., & Cheeran, B. (2015). Intra-individual variability in the response to anodal transcranial direct current stimulation. *Clinical Neurophysiology, 126*(12), 2342-2347.

Macdonell, R. A. L., Shapiro, B. E., Chiappa, K. H., Helmers, S. L., Cros, D., Day, B. J., & Shahani, B. T. (1991). Hemispheric Threshold Differences for Motor Evoked-Potentials Produced by Magnetic Coil Stimulation. *Neurology, 41*(9), 1441-1444.

Makki, M. I., Behen, M., Bhatt, A., Wilson, B., & Chugani, H. T. (2008). Microstructural Abnormalities of Striatum and Thalamus in Children with Tourette Syndrome. *Movement Disorders, 23*(16), 2349-2356.

Mantovani, A., Leckman, J. F., Grantz, H., King, R. A., Sporn, A. L., & Lisanby, S. H. (2007). Repetitive Transcranial Magnetic Stimulation of the Supplementary Motor Area in the treatment of Tourette Syndrome: report of two cases. *Clinical Neurophysiology, 118*(10), 2314-2315.

Mantovani, A., Lisanby, S. H., Pieraccini, F., Ulivelli, M., Castrogiovanni, P., & Rossi, S. (2006). Repetitive transcranial magnetic stimulation (rTMS) in the treatment of obsessive-compulsive disorder (OCD) and Tourette's syndrome (TS). *International Journal of Neuropsychopharmacology, 9*(1), 95-100.

Matsunaga, K., Maruyama, A., Fujiwara, T., Nakanishi, R., Tsuji, S., & Rothwell, J. C. (2005). Increased corticospinal excitability after 5Hz rTMS over the human supplementary motor area. *Journal of Physiology-London, 562*(1), 295-306.

McAvoy, M., Larson-Prior, L., Nolan, T. S., Vaishnavi, S. N., Raichle, M. E., & d'Avossa, G. (2008). Resting states affect spontaneous BOLD oscillations in sensory and paralimbic cortex. *Journal of Neurophysiology, 100*(2), 922-931.

McCairn, K. W., Iriki, A., & Isoda, M. (2013). Global dysrhythmia of cerebro-basal ganglia–cerebellar networks underlies motor tics following striatal disinhibition. *The Journal of Neuroscience, 33*(2), 697-708.

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E., & George, M. S. (2001). The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: a replication in healthy adults comparing two methods of assessing the distance to cortex. *Biological Psychiatry, 49*(5), 454-459.

Mccreery, D. B., Agnew, W. F., Yuen, T. G. H., & Bullara, L. (1990). Charge-Density and Charge Per Phase as Cofactors in Neural Injury Induced by Electrical-Stimulation. *IEEE Transactions on Biomedical Engineering, 37*(10), 996-1001.

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research, 173*(1), 86-93.

Menzler, K., Hermsen, A., Balkenhol, K., Duddek, C., Bugiel, H., Bauer, S., Schorge, S., Reif, P. S., Klein, K. M., Haag, A., Oertel, W. H., Hamer, H. M., Knake, S., Trucks, H., Sander, T., & Rosenow, F. (2014). A common SCN1A splice-site polymorphism modifies the effect of carbamazepine on cortical excitability--a pharmacogenetic transcranial magnetic stimulation study. *Epilepsia, 55*(2), 362-369.

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral cortex in the intact human subject. *Nature, 285*(5762), 227.

Mescher, M., Merkle, H., Kirsch, J., Garwood, M., & Gruetter, R. (1998). Simultaneous in vivo spectral editing and water suppression. *NMR in Biomedicine, 11*(6), 266-272.

Mills, K. R., & Nithi, K. A. (1997). Corticomotor threshold to magnetic stimulation: Normal values and repeatability. *Muscle & Nerve, 20*(5), 570-576.

Mink, J. W. (2001). Basal ganglia dysfunction in Tourette's syndrome: a new hypothesis. *Pediatric Neurology, 25*(3), 190-198.

Mink, J. W. (2006). Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. In J. T. Walkup, J. W. Mink & P. J. Hollenbeck (Eds.), *Advances in Neurology* (2006/03/16 ed., Vol. 99, pp. 89-98). New York: Raven Press.

Miranda, P. C., Lomarev, M., & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clinical Neurophysiology, 117*(7), 1623-1629.

Moliadze, V., Atalay, D., Antal, A., & Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimulation, 5*(4), 505-511.

Moliadze, V., Schmanke, T., Andreas, S., Lyzhko, E., Freitag, C. M., & Siniatchkin, M. (2014). Stimulation intensities of transcranial direct current stimulation have to be adjusted in children and adolescents *Clinical Neurophysiology, 126*(7), 1392-1399.

Moller, C., Arai, N., Lucke, J., & Ziemann, U. (2009). Hysteresis effects on the input-output curve of motor evoked potentials. *Clinical Neurophysiology, 120*(5), 1003-1008.

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimulation, 6*(3), 424-432.

Monte-Silva, K., Kuo, M. F., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2010). Shaping the Optimal Repetition Interval for Cathodal Transcranial Direct Current Stimulation (tDCS). *Journal of Neurophysiology, 103*(4), 1735-1740.

Moon, B. S., Price, C. T., & Campbell, J. B. (1998). Upper extremity and rib stress fractures in a child. *Skeletal Radiology, 27*(7), 403-405.

Moreno-Duarte, I., Gebodh, N., Schestatsky, P., Guleyupoglu, B., Reato, D., Bikson, M., & Frengini, F. (2014). Transcranial electrical stimulation: Transcranial direct current stimulation (tDCS), Transcranial alternating stimulation (tACS), Transcranial paired current stimulation (tPCS), and Transcranial random noise stimulation (tRNS). In C. R. Kadosh (Ed.), *The stimulated brain: cognitive enhancement using non-invasive brain stimulation* (pp. 35-61). London, UK: Accademic Press.

Mrakic-Sposta, S., Marceglia, S., Mameli, F., Dilena, R., Tadini, L., & Priori, A. (2008). Transcranial direct current stimulation in two patients with Tourette syndrome. *Movement Disorders, 23*(15), 2259-2261.

Muller-Dahlhaus, J. F., Orekhov, Y., Liu, Y., & Ziemann, U. (2008). Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Experimental Brain Research, 187*(3), 467-475.

Murdoch, J. B., & Lampman, D. A. (1993). *Beyond WET and DRY: optimized pulses for water suppression*. Paper presented at the Society of Magnetic Resonance in Medicine, Twelfth Annual Meeting, New York.

Nakamura, H., Kitagawa, H., Kawaguchi, Y., & Tsuji, H. (1996). Direct and indirect activation of human corticospinal neurons by transcranial magnetic and electrical stimulation. *Neuroscience letters, 210*(1), 45-48.

Near, J., Evans, C. J., Puts, N. A., Barker, P. B., & Edden, R. A. (2013). J-difference editing of gamma-aminobutyric acid (GABA): simulated and experimental multiplet patterns. *Magnetic Resonance in Medicine, 70*(5), 1183-1191.

Neuner, I., Kupriyanova, Y., Stocker, T., Huang, R. W., Posnansky, O., Schneider, F., & Shah, N. J. (2011). Microstructure assessment of grey matter nuclei in adult tourette patients by diffusion tensor imaging. *Neuroscience Letters, 487*(1), 22-26.

Nitsche, M. A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2007). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of Neurophysiology, 97*(4), 3109-3117.

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., Henning, S., Tergau, F., & Paulus, W. (2003). Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. *The Journal of Physiology, 553*(1), 293-301.

Nitsche, M. A., Liebetanz, D., Schlitterlau, A., Henschke, U., Fricke, K., Frommann, K., Lang, N., Henning, S., Paulus, W., & Tergau, F. (2004). GABAergic modulation of DC stimulation-induced motor cortex excitability shifts in humans. *European Journal of Neuroscience, 19*(10), 2720-2726.

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology, 114*(4), 600-604.

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology, 527* (3), 633-639.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899-1901.

Nitsche, M. A., & Paulus, W. (2011). Transcranial direct current stimulation--update 2011. *Restorative Neurology and Neuroscience, 29*(6), 463-492.

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., Fricke, K., Liebetanz, D., Lang, N., Antal, A., Paulus, W., & Tergau, F. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of Physiology, 568*(1), 291-303.

Nixon, E., Glazebrook, C., Hollis, C., & Jackson, G. M. (2014). Reduced Tic Symptomatology in Tourette Syndrome After an Acute Bout of Exercise: An Observational Study. *Behaviour Modification, 38*(2), 235-263.

O'Shea, J., & Walsh, V. (2007). Transcranial magnetic stimulation. *Current Biology, 17*(6), R196-199.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*(1), 97-113.

Oliveri, M., Babiloni, C., Filippi, M. M., Caltagirone, C., Babiloni, F., Cicinelli, P., Traversa, R., Palmieri, M. G., & Rossini, P. M. (2003). Influence of the supplementary motor area on primary motor cortex excitability during movements triggered by neutral or emotionally unpleasant visual cues. *Experimental Brain Research, 149*(2), 214-221.

Orth, M. (2009). Transcranial magnetic stimulation in Gilles de la Tourette syndrome. *Journal of Psychosomatic Research, 67*(6), 591-598.

Orth, M., Amann, B., Robertson, M. M., & Rothwell, J. C. (2005). Excitability of motor cortex inhibitory circuits in Tourette syndrome before and after single dose nicotine. *Brain, 128*(6), 1292-1300.

Pascual-Leone, A., Valls-Sole, J., Wassermann, E. M., & Hallett, M. (1994). Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain, 117 (Pt 4)*, 847-858.

Patton, H. D., & Amassian, V. E. (1954). Single and multiple-unit analysis of cortical stage of pyramidal tract activation. *Journal of Neurophysiology, 17*(4), 345-363.

Pearce, A. J., Clark, R. A., & Kidgell, D. J. (2013). A comparison of two methods in acquiring stimulus-response curves with transcranial magnetic stimulation. *Brain Stimulation, 6*(3), 306-309.

Pena, M. S., Yaltho, T. C., & Jankovic, J. (2011). Tardive dyskinesia and other movement disorders secondary to aripiprazole. *Movement Disorders, 26*(1), 147-152.

Peterson, B. S., Thomas, P., Kane, M. J., Scahill, L., Zhang, H., Bronen, R., King, R. A., Leckman, J. F., & Staib, L. (2003). Basal Ganglia volumes in patients with Gilles de la Tourette syndrome. *Archives of General Psychiatry, 60*(4), 415-424.

Petroff, O. A. C., Rothman, D. L., Behar, K. L., Lamoureux, D., & Mattson, R. H. (1996). The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. *Annals of Neurology, 39*(1), 95-99.

Peurala, S. H., Muller-Dahlhaus, J. F., Arai, N., & Ziemann, U. (2008). Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). *Clinical Neurophysiology, 119*(10), 2291-2297.

Piacentini, J., Woods, D. W., Scahill, L., Wilhelm, S., Peterson, A. L., Chang, S., Ginsburg, G. S., Deckersbach, T., Dziura, J., Levi-Pearl, S., & Walkup, J. T. (2010). Behavior Therapy for Children With Tourette Disorder A Randomized Controlled Trial. *Jama-Journal of the American Medical Association, 303*(19), 1929-1937.

Picard, N., & Strick, P. L. (2001). Imaging the premotor areas. *Current Opinion in Neurobiology, 11*(6), 663-672.

Piccolino, M. (1998). Animal electricity and the birth of electrophysiology: the legacy of Luigi Galvani. *Brain Research Bulletin, 46*(5), 381-407.

Pizzarelli, R., & Cherubini, E. (2011). Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plasticity, 2011*, 297153.

Poreisz, C., Boros, K., Antal, A., & Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain research bulletin*, *72*(4), 208-214.

Porta, M., Sassi, M., Cavallazzi, M., Fornari, M., Brambilla, A., & Servello, D. (2008). Tourette's syndrome and role of tetrabenazine: review and personal experience. *Clinical Drug Investigation, 28*(7), 443-459.

Pourfar, M., Feigin, A., Tang, C. C., Carbon-Correll, M., Bussa, M., Budman, C., Dhawan, V., & Eidelberg, D. (2011). Abnormal metabolic brain networks in Tourette syndrome. *Neurology, 76*(11), 944-952.

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage, 59*(3), 2142-2154.

Priori, A. (2003). Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clinical Neurophysiology, 114*(4), 589-595.

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *Neuroreport, 9*(10), 2257-2260.

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine, 30*(6), 672-679.

Purpura, D. P., & McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *Journal of Neurophysiology, 28*(1), 166-185.

Puts, N. A., & Edden, R. A. (2012). In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Progress in Nuclear Magnetic Resonance Spectroscopy, 60*, 29-41.

Puts, N. A., Harris, A. D., Crocetti, D., Nettles, C., Singer, H. S., Tommerdahl, M., Edden, R. A. E., & Mostofsky, S. H. (2015). Reduced GABAergic inhibition and abnormal sensory symptoms in children with Tourette syndrome. *Journal of Neurophysiology, 114*(2), 808-817.

Quartarone, A., Rizzo, V., Bagnato, S., Morgante, F., Sant'Angelo, A., Romano, M., Crupi, D., Girlanda, P., Rothwell, J. C., & Siebner, H. R. (2005). Homeostatic-like plasticity of the primary motor hand area is impaired in focal hand dystonia. *Brain, 128*(8), 1943-1950.

Rae, C. D. (2014). A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochemical Research, 39*(1), 1-36.

Rickards, H. (2009). Functional neuroimaging in Tourette syndrome. *Journal of Psychosomatic Research, 67*(6), 575-584.

Ridding, M. C., Inzelberg, R., & Rothwell, J. C. (1995). Changes in Excitability of Motor Cortical Circuitry in Patients with Parkinsons-Disease. *Annals of Neurology, 37*(2), 181-188.

Rizzo, V., Quartarone, A., Bagnato, S., Battaglia, F., Majorana, G., & Girlanda, P. (2001). Modification of cortical excitability induced by gabapentin: a study by transcranial magnetic stimulation. *Neurological Sciences, 22*(3), 229-232.

Robertson, M. M. (2008). The prevalence and epidemiology of Gilles de la Tourette syndrome. Part 1: the epidemiological and prevalence studies. *Journal of Psychosomatic Research, 65*(5), 461-472.

Roshan, L., Paradiso, G. O., & Chen, R. (2003). Two phases of short-interval intracortical inhibition. *Experimental Brain Research, 151*(3), 330-337.

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology, 120*(12), 2008-2039.

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P. B., George, M. S., Hallett, M., Lefaucheur, J. P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M. A., Pascual-Leone, A., Paulus, W., Rossi, S., Rothwell, J. C., Siebner, H. R., Ugawa, Y., Walsh, V., & Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral

nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology, 126*(6), 1071-1107.

Rossini, P. M., Desiato, M. T., & Caramia, M. D. (1992). Age-related changes of motor evoked potentials in healthy humans: non-invasive evaluation of central and peripheral motor tracts excitability and conductivity. *Brain research, 593*(1), 14-19.

Sackeim, H. A., Prudic, J., Fuller, R., Keilp, J., Lavori, P. W., & Olfson, M. (2007). The cognitive effects of electroconvulsive therapy in community settings. *Neuropsychopharmacology, 32*(1), 244-254.

Sadleir, R. J., Vannorsdall, T. D., Schretlen, D. J., & Gordon, B. (2010). Transcranial direct current stimulation (tDCS) in a realistic head model. *Neuroimage, 51*(4), 1310-1318.

Sanger, T. D., Garg, R. R., & Chen, R. (2001). Interactions between two different inhibitory systems in the human motor cortex. *The Journal of Physiology, 530*(2), 307-317.

Scelzo, E., Giannicola, G., Rosa, M., Ciocca, M., Ardolino, G., Cogiamanian, F., Ferrucci, R., Fumagalli, M., Mameli, F., Barbieri, S., & Priori, A. (2011). Increased short latency afferent inhibition after anodal transcranial direct current stimulation. *Neuroscience Letters, 498*(2), 167-170.

Sharenow, E. L., Fuqua, R. W., & Miltenberger, R. G. (1989). The treatment of muscle tics with dissimilar competing response practice. *Journal of Applied Behavior Analysis, 22*(1), 35-42.

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., & Rothwell, J. C. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. *The Journal of Neuroscience, 24*(13), 3379-3385.

Sills, G. J. (2006). The mechanisms of action of gabapentin and pregabalin. *Current Opinion in Pharmacology, 6*(1), 108-113.

Silva, R. R., Munoz, D. M., Daniel, W., Barickman, J., & Friedhoff, A. J. (1996). Causes of haloperidol discontinuation in patients with Tourette's disorder: management and alternatives. *Journal of Clinical Psychiatry, 57*(3), 129-135.

Smith, A. E., Ridding, M. C., Higgins, R. D., Wittert, G. A., & Pitcher, J. B. (2009). Age-related changes in short-latency motor cortex inhibition. *Experimental Brain Research, 198*(4), 489-500.

Stagg, C. J. (2014). The physiological basis of brain stimulation. In R. Cohen Kadosh (Ed.), *The Stimulated Brain. Cognitive enhancement using non-invasive brain stimulation* (pp. 145-177): Elsevier .

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., Morris, P. G., Matthews, P. M., & Johansen-Berg, H. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *Journal of Neuroscience, 29*(16), 5202-5206.

Stagg, C. J., Bestmann, S., Constantinescu, A. O., Moreno, L. M., Allman, C., Mekle, R., Woolrich, M., Near, J., Johansen-Berg, H., & Rothwell, J. C. (2011). Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. *Journal of Physiology-London, 589*(23), 5845-5855.

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *The Neuroscientist, 17*(1), 37-53.

Stagg, C. J., & Rothman, D. (Eds.). (2014). *Magnetic resonance spectroscopy: tools for neuroscience research and emerging clinical applications*. London: Elsvier.

Stokes, M. G., Chambers, C. D., Gould, I. C., Henderson, T. R., Janko, N. E., Allen, N. B., & Mattingley, J. B. (2005). Simple metric for scaling motor threshold based on scalp-cortex distance: Application to studies using transcranial magnetic stimulation. *Journal of Neurophysiology, 94*(6), 4520-4527.

Storch, E. A., Murphy, T. K., Geffken, G. R., Sajid, M., Allen, P., Roberti, J. W., & Goodman, W. K. (2005). Reliability and validity of the Yale Global Tic Severity Scale. *Psychological Assessment, 17*(4), 486-491.

Strube, W., Bunse, T., Nitsche, M. A., Nikolaeva, A., Palm, U., Padberg, F., Falkai, P., & Hasan, A. (2016). Bidirectional variability in motor cortex excitability modulation following 1 mA transcranial direct current stimulation in healthy participants. *Physiological Reports, 4*(15).

Tagliazucchi, E., & Laufs, H. (2014). Decoding Wakefulness Levels from Typical fMRI Resting-State Data Reveals Reliable Drifts between Wakefulness and Sleep. *Neuron, 82*(3), 695-708.

Taylor, C. P., Gee, N. S., Su, T. Z., Kocsis, J. D., Welty, D. F., Brown, J. P., Dooley, D. J., Boden, P., & Singh, L. (1998). A summary of mechanistic hypotheses of gabapentin pharmacology. *Epilepsy Research, 29*(3), 233-249.

Tecchio, F., Cancelli, A., Cottone, C., Zito, G., Pasqualetti, P., Ghazaryan, A., Rossini, P. M., & Filippi, M. M. (2014). Multiple sclerosis fatigue relief by bilateral somatosensory cortex neuromodulation. *Journal of Neurology, 261*(8), 1552-1558.

Teo, J. T., Terranova, C., Swayne, O., Greenwood, R. J., & Rothwell, J. C. (2009). Differing effects of intracortical circuits on plasticity. *Experimental Brain Research, 193*(4), 555-563.

Tinaz, S., Malone, P., Hallett, M., & Horovitz, S. G. (2015). Role of the right dorsal anterior insula in the urge to tic in Tourette syndrome. *Movement Disorders, 30*(9), 1190-1197.

Tkác, I., Oz, G., Adriany, G., Uğurbil, K., & Gruetter, R. (2009). In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. . *Magnetic Resonance in Medicine, 62*(4), 868–879.

Tobe, R. H., Bansal, R., Xu, D., Hao, X., Liu, J., Sanchez, J., & Peterson, B. S. (2010). Cerebellar morphology in Tourette syndrome and obsessive-compulsive disorder. *Annals of Neurology, 67*(4), 479-487.

Treiman, D. M. (2001). GABAergic mechanisms in epilepsy. *Epilepsia, 42*, 8-12.

Tremblay, S., Beaule, V., Proulx, S., de Beaumont, L., Marjanska, M., Doyon, J., Pascual-Leone, A., Lassonde, M., & Theoret, H. (2013). Relationship between transcranial magnetic stimulation measures of intracortical inhibition and spectroscopy measures of GABA and glutamate plus glutamine. *Journal of Neurophysiology, 109*(5), 1343-1349.

Trepel, C., & Racine, R. J. (2000). GABAergic modulation of neocortical long-term potentiation in the freely moving rat. *Synapse, 35*(2), 120-128.

Triggs, W. J., Calvanio, R., Macdonell, R. A., Cros, D., & Chiappa, K. H. (1994). Physiological motor asymmetry in human handedness: evidence from transcranial magnetic stimulation. *Brain Research, 636*(2), 270-276.

Triggs, W. J., Subramanium, B., & Rossi, F. (1999). Hand preference and transcranial magnetic stimulation asymmetry of cortical motor representation. *Brain Research, 835*(2), 324-329.

Valls-Sole, J., Pascual-Leone, A., Wassermann, E. M., & Hallett, M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalography and Clinical Ceurophysiology/Evoked Potentials section, 85*(6), 355-364.

Van der Kamp, W., Zwinderman, A. H., Ferrari, M. D., & van Dijk, J. G. (1996). Cortical excitability and response variability of transcranial magnetic stimulation. *Journal of Clinical Neurophysiology, 13*(2), 164-171.

Vergani, F., Lacerda, L., Martino, J., Attems, J., Morris, C., Mitchell, P., Thiebaut de Schotten, M., & Dell'Acqua, F. (2014). White matter connections of the supplementary motor area in humans. *Journal of Neurology Neurosurgery and Psychiatry, 85*(12), 1377-1385.

Vucic, S., Cheah, B. C., Krishnan, A. V., Burke, D., & Kiernan, M. C. (2009). The effects of alterations in conditioning stimulus intensity on short interval intracortical inhibition. *Brain Research, 1273*, 39-47.

Wang, Z. S., Maia, T. V., Marsh, R., Colibazzi, T., Gerber, A., & Peterson, B. S. (2011). The Neural Circuits That Generate Tics in Tourette's Syndrome. *American Journal of Psychiatry, 168*(12), 1326-1337.

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clinical Neurophysiology, 113*(7), 1165-1171.

Wetzels, R., & Wagenmakers, E. J. (2012). A default Bayesian hypothesis test for correlations and partial correlations. *Psychonomic Bulletin & Review, 19*(6), 1057-1064.

Wiethoff, S., Hamada, M., & Rothwell, J. C. (2014). Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimulation, 7*(3), 468-475.

Woods, D. W., Piacentini, J., Himle, M. B., & Chang, S. (2005). Premonitory urge for tics scale (PUTS): Initial psychometric results and examination of the premonitory urge phenomenon in youths with tic disorders. *Journal of Developmental and Behavioral Pediatrics, 26*(6), 397-403.

Worbe, Y., Gerardin, E., Hartmann, A., Valabregue, R., Chupin, M., Tremblay, L., Vidailhet, M., Colliot, O., & Lehericy, S. (2010). Distinct structural changes underpin clinical phenotypes in patients with Gilles de la Tourette syndrome. *Brain, awq293*.

Worbe, Y., Malherbe, C., Hartmann, A., Pelegrini-Issac, M., Messe, A., Vidailhet, M., Lehericy, S., & Benali, H. (2012). Functional immaturity of cortico-basal ganglia networks in Gilles de la Tourette syndrome. *Brain, 135*(6), 1937-1946.

Yan, C. G., Liu, D. Q., He, Y., Zou, Q. H., Zhu, C. Z., Zuo, X. N., Long, X. Y., & Zang, Y. F. (2009). Spontaneous Brain Activity in the Default Mode Network Is Sensitive to Different Resting-State Conditions with Limited Cognitive Load. *Plos One, 4*(5).

Yates, R., Edwards, K., King, J., Luzon, O., Evangeli, M., Stark, D., McFarlane, F., Heyman, I., Ince, B., Kodric, J., & Murphy, T. (2016). Habit reversal training and educational group treatments for children with tourette syndrome: A preliminary randomised controlled trial. *Behaviour Research and Therapy, 80*, 43-50.

Yook, S. W., Park, S. H., Seo, J. H., Kim, S. J., & Ko, M. H. (2011). Suppression of seizure by cathodal transcranial direct current stimulation in an epileptic patient - a case report. *Annuals of Rehabilitation Medicine, 35*(4), 579-582.

Ziemann, U. (2008). Pharmachology of TMS measures. In E. M. Wassermann, V. Walsh, C. M. Epstein, T. Paus, U. Ziemann & S. H. Lisanby (Eds.), *The Oxford Handbook of Transcranial Stimulation* (pp. 135-151). Oxford: Oxford University Press.

Ziemann, U. (2013). Pharmaco-transcranial magnetic stimulation studies of motor excitability. In A. M. Lozano & M. Hallett (Eds.), *Brain Stimulation: Handbook of Clinical Neurology* (2013/10/12 ed., Vol. 116, pp. 387-397).

Ziemann, U., Lonnecker, S., Steinhoff, B. J., & Paulus, W. (1996). Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. *Annals of Neurology, 40*(3), 367-378.

Ziemann, U., Paulus, W., & Rothenberger, A. (1997). Decreased motor inhibition in Tourette's disorder: Evidence from transcranial magnetic stimulation. *American Journal of Psychiatry, 154*(9), 1277-1284.

Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., & Muller-Dahlhaus, F. (2015). TMS and drugs revisited 2014. *Clinical Neurophysiology, 126*(10), 1847-1868.

Ziemann, U., Rothwell, J. C., & Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *Journal of Physiology, 496*(3), 873-881.

Appendices

Appendix i: Effects of tDCS on MEPs (Ch.3)

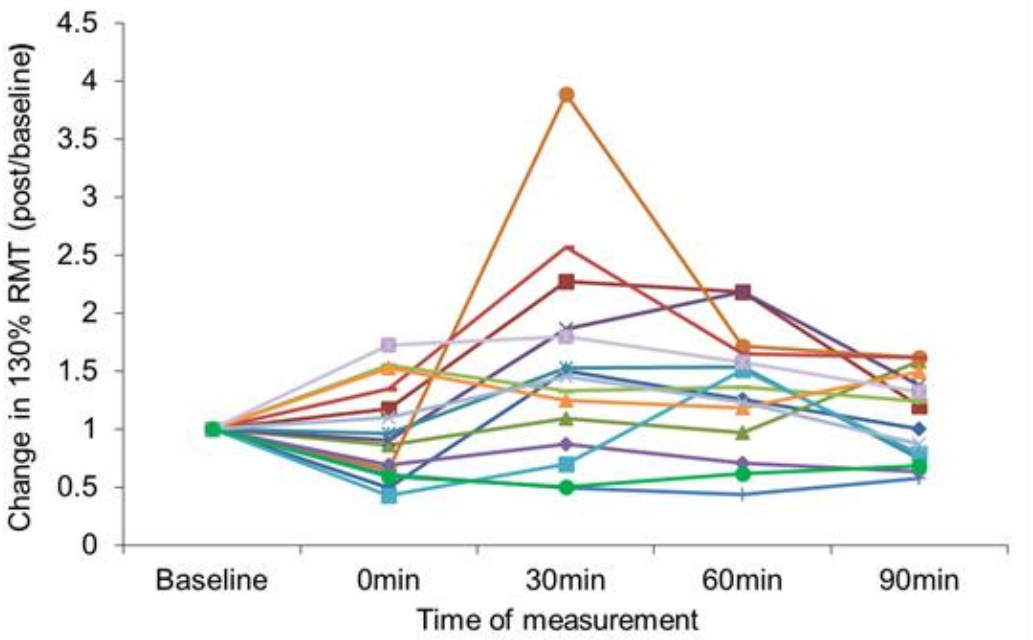
Repeated measures ANOVAs in which time and intensity served as within subject's factors with 5 and 8 levels respectively. The analysis was run separately for each tDCS parameter (anodal, cathodal or sham, at 1mA or 2mA) to allow for in-depth exploration of changes from baseline. Significant effects were further analysed using students t-tests (paired samples, two tailed, $p<0.05$). The Greenhouse-Geisser correction was used to correct for significant violations of sphericity.

Polarity	Factor	d.f	F	P	η_p^2
Anodal 1mA	Time	2.45	.88	.44	.05
	Intensity	1.27	29.6	.00*	.64
	Time*Intensity	2.91	.62	.60	.04
Cathodal 1mA	Time	2.35	1.3	.29	.07
	Intensity	1.23	35.72	.00*	.68
	Time*Intensity	4.74	1.17	.33	.06
Sham 1mA	Time	2.52	.8	.48	.05
	Intensity	1.40	40.56	.00*	.71
	Time*Intensity	5.55	.93	.48	.05
Anodal 2mA	Time	4	2.48	.05*	.15
	Intensity	1.33	40.22	.00*	.74
	Time*Intensity	5.39	2.46	.04*	.15
Cathodal 2mA	Time	4	1.34	.27	.09
	Intensity	1.37	48.01	.00*	.77
	Time*Intensity	5.2	.87	.51	.06
Sham 2mA	Time	2.08	1.69	.20	.11
	Intensity	1.31	29.95	.00*	.68
	Time*Intensity	3.35	1.37	.26	.09

Uncorrected paired samples t-tests were used to examine the significant interaction found for the **2mA anodal** condition. At 130%RMT significant differences emerged between baseline (*M*=2216.71, *SD*=968.35) and 30 minutes post stimulation (*M*=3386.85, *SD*=2138.46) *t*=-2.758, *p*=.02; and also between baseline (*M*=2216.71, *SD*=968.35) and 60 minutes (*M*=3044.53, *SD*=1902.35), *t*=-2.62, *p*=.02. No significant differences were found between baseline and 0 minutes or baseline and 90 minutes after stimulation. No significant differences were found for lower intensities.

Appendix ii: Individual variability & 2mA anodal tDCS (Ch. 3)

Although the results of the ANOVA suggest that **2mA anodal** stimulation significantly increased MEP amplitudes 30 and 60 minutes following stimulation, there was substantial variability even within this condition. Figure below shows normalized MEP values from the 130% condition for each individual following 2mA anodal stimulation.



Appendix iii: Effects of tDCS on raw IO curve slope (Ch. 3)

Raw values from linear fitting were entered in to separate repeated measures (rm) ANOVAs, in which time was also included as an independent factor. Post hoc analysis were conducted using students t-tests (paired samples, two tailed, p<0.05). When necessary the Greenhouse-Geisser correction was applied.

Rm ANOVAs revealed no significant effect of time for **1mA anodal** tDCS *F(2.17,38.92*)=.246, *p*=.801. **1mA cathodal** tDCS also failed to significantly alter slope at any time point, *F(*2.08,35.39)=1.234, *p=.30;* as did **sham** stimulation *F(4,68*)=.74, *p*=.57.

Rm ANOVA revealed a significant effect of time in the **2mA anodal** condition *F(4,56*)=4.28, *p=.00.* Uncorrected paired samples t-tests revealed a significant increase in IO curve slope at 30minutes post tDCS when compared to baseline *t*(15)= 2.37, *p*=.03. A significant increase 60minutes following tDCS was also apparent *t*(15)=2.46, *p*=.03. Time was did not significantly alter slope following **cathodal** *F*(4,56)=.26, *p*=.62 or **sham** stimulation *F*(2.05,28.67)=3.16, *p=.31.*

To allow the different measures to be compared more directly the ANOVAs were run again with tDCS polarity included as a factor. No significant differences were found between baseline slopes in the **1mA** conditions (all t=<1.89, all p>0.08). No significant effects were revealed – see table below.

Factor	df	F	P
Polarity	2,34	.64	.53
Time	2.04	1.02	.37
Polarity*Time	4.04, 68.61	.6	.67

No significant differences between baseline slopes were found between the **2mA** conditions (all t=<1.72, all p=>.28). No significant effects of polarity were found, however, there was a significant main effect of time F(4,56)=2.82, p=0.34. This appears to be driven by an increase in slope from baseline at 30minutes post stimulation (t(45)=2.29, p=0.03) and also a significant increase in slope from baseline at 60minutes (t(45)=2.48, p=0.2).

Factor	df	F	P
Polarity	1.4,19.76	.19	.75
Time	4,56	2.82	.03*
Polarity*Time	4.26,59.64	.891	.480

Appendix iv: Sigmoidal fitting with IO curve data (Ch. 4)

Four-parameter sigmoidal fits were applied to the median MEP amplitude for each TMS intensity (100-150%). From this fitting the maximal slope and the plateau of the curve were calculated. The sigmoidal function used to fit curves to the individual datasets was:

$$\mathrm{MEP}_S = y_0 + \frac{\mathrm{MEP}_{\mathrm{MAX}}}{1 + 10^{(S_{50} - S)k}}$$

In which $\mathrm{MEP}_{\mathrm{MAX}}$ is the maximum MEP amplitude measured, S_{50} is the TMS intensity needed to produce 50% of the maximum MEP, k is the gradient of the maximum steepness of the curve and y_0 is the minimal MEP response, which was set to 0.

In some instances sigmoidal fitting yielded higher R^2 values than linear fitting, however, it also produced some surprisingly high predictions when looking at estimates of plateau, and revealed a number of outliers when fitting slopes. Outliers were identified and removed using Grubbs test (alpha 0.01) prior to analysis. For Slope values this resulted in the removal of 2 data sets from anodal, 1 from cathodal and 4 from sham conditions. For slope plateau this resulted in the removal of 8 data sets from the anodal, 5 from sham and 1 from the sham condition. RmANOVAs were used to explore any significant change over time (pre/post) and across the four different sessions tested for each tDCS condition (anodal, cathodal, sham). Results for slope can be seen below.

Measurement	Factor	d.f	*F*	*P*	η_p^2
Anodal slope	Session	3	1.03	.4	.13
	Time	1	.24	.64	.03
	Session*Time	3	.92	.45	.12
Cathodal slope	Session	3	.52	.68	.06
	Time	1	.18	.68	.02
	Session*Time	3	.57	.64	.07
Sham slope	Session	1	.32	.6	.06
	Time	1	.05	.84	.01
	Session*Time	1	2.32	.19	.32
Anodal plateau	Session	3	3.34	.04*	.36
	Time	1	1.97	.21	.25
	Session*Time	1.4	1.09	.35	.15
Cathodal plateau	Session	1.36	1.72	.23	.2
	Time	1	1.54	.26	.18
	Session*Time	3	.18	.91	.03
Sham plateau	Session	1	.01	.92	.00
	Time	1	.63	.45	.08
	Session*Time	1	.45	.52	.06

Justification for use of linear opposed to sigmoidal fits

Although IO curves are reportedly sigmoidal in shape (Devanne et al., 1997; Hess et al., 1987), this is dependent on testing a large range of intenties to capture both the bias level and the top plateau after which increase in MEP amplitude slows dramatically. The parameters used in this study (100-150% RMT) are not necesserily broad enough to reveal either of these factors. Although linear fits are not prefect, they do appear to reflect the data well.

Appendix v: ICC analysis for Rush (Ch. 5)

To assess the degree of reliability between primary and secondary coders ICC analysis was conducted. The ICC results are reported based upon Lahey et al. (1983), ICC values of <0.4 are considered to indicate poor intra-class reliability, values >0.4 and <0.59 are fair, values >0.6 and <0.74 are good, and values >0.74 are excellent.

ICC analysis was first carried out using the mean **rush total score**, which was calculated by averaging the total score per minute for each of the two minute video segments. This revealed excellent reliability between the primary coder (KD) and the secondary coder (ER) ICC(2,1)=.81. The reliability between the primary coder (KD) and the other secondary coder (KF) was also found to be excellent ICC(2,1)=.92.

Further analysis on the individual components of the rush scale revealed different levels of reliability between the primary coder **(KD)** and secondary coder **(ER)** which are discussed below. As with the analysis of total score the average score across the two minute video clips was used. ICC analysis revealed fair reliability for scores on the **body areas component** ICC(2,1)=.462. It was poor for **motor tic frequency** ICC(2,1)=.35; excellent for **phonic tic frequency** ICC(2,1)=.85; good for **motor tic severity** ICC(2,1)=.68 and excellent for **phonic tic severity** ICC(2,1)=7.42.

For consistency, ICC analysis was also used to assess the agreement between the mean amount of tics per minute counted by each coder during the two minute video clips. Average tic score was assessed by calculating the mean amount of tics over the twelve 10S time segments.

ICC analysis for **average total tics** revealed fair reliability between primary coder (KD) and secondary coder (ER) ICC(2,1)=.59. Reliability between primary coder and second coder (KF) was found to be excellent ICC(2,1)=.97. ICC analysis **motor tics** was fair between KD and ER, ICC(2,1)=.47; and excellent between KD and KF, ICC(2,1)=.96. ICC analysis for **phonic tic** scores revealed excellent reliability between scores of the primary coder (KD) and the secondary coder (ER) ICC(2,1)=.88; and also excellent reliability between the primary coder and secondary coder (KF) ICC(2,1)=.96.

Appendix vi: Sigmoidal curve fitting (Ch. 5)

Four-parameter sigmoidal fits (as described in Appendix iv) were applied to the median MEP amplitude for each TMS intensity (100-150%). From this fitting the maximal slope and the plateau of the curve were calculated.

Although the slope fits were generally very good (average R^2 values = 0.95), sigmoidal fitting resulted in some significant outliers which were detected using Grubbs test (using an alpha level of 0.01). This resulted in the removal of one data set from the pre sham, and one from the post sham condition for maximal slope value. A further data set was removed for the plateau calculation form the post sham condition. This was not an issue when linear slope fits were used.

A repeated measures ANOVA was calculated in which time (pre/ post) and tDCS type (sham/ Cathodal) served as independent factors. IO curve slope was entered as the dependent variable. No significant effects were revealed, see below.

Measurement	**Factor**	**d.f**	***F***	***P***	η_p^2
Slope plateau	tDCS type	1,9	.97	.35	.097
	Time	1,9	1.0	.35	.1
	Type*Time	1,9	.99	.35	.1
Maximal slope	tDCS type	1,9	1.08	.33	.11
	Time	1,9	.34	.57	.04
	Type*Time	1,9	1.23	.3	.12

Appendix vii: Further analysis with RUSH score (Ch. 5)

Repeated measures ANOVAs were calculated for each component of the RUSH to explore any effects of tDCS (sham/cathodal) and time (pre/post).

Measurement	Factor	d.f	*F*	*P*	η_P^2
Total impairment	tDCS type	1,9	6.7	.03*	.427
	Time	1,9	1.25	.29	.12
	Type*Time	1,9	.01	.93	.00
Motor frequency	tDCS type	1,9	1.98	.19	.18
	Time	1,9	1.84	.21	.17
	Type*Time	1,9	3.67	.09	.29
Phonic frequency	tDCS type	1,9	2.53	.15	.219
	Time	1,9	.33	.58	.04
	Type*Time	1,9	1.14	.31	.11
Motor severity	tDCS type	1,9	.33	.58	.04
	Time	1,9	2.53	.15	.22
	Type*Time	1,9	1.14	.31	.11
Phonic severity	tDCS type	1,9	.28	.61	.03
	Time	1,9	.18	.68	.02
	Type*Time	1,9	.26	.62	.03

Appendix viii: ROIs in Baseline/Sham comparisons (Ch. 6)

ROIs identified as statistically different between **Baseline and Sham** scans using paired sample t-tests. R indicated right, L indicated left. **Indicates significantly difference at p=<0.01 (FDR corrected). ***Indicates significant at p=<0.001(FDR corrected), otherwise significant at p<0.05 (FDR corrected).

Atlas no.	Region
1	Frontal pole R
2	Frontal pole L **
5	Superior Frontal Gyrus R
9	Inferior Frontal Gyrus, pars triangularis Right ***
11	Inferior Frontal Gyrus, pars opercularis Right **
13	Precentral Gyrus R **
14	Precentral gyrus L ***
17	Superior Temporal Gyrus, anterior division R **
18	Superior Temporal Gyrus, anterior division L **
20	Superior Temporal Gyrus, posterior division L ***
21	Middle Temporal Gyrus, anterior division R
22	Middle Temporal Gyrus, anterior division L
24	Middle Temporal Gyrus, posterior division L
25	Middle Temporal Gyrus, temporooccipital part R
27	Inferior Temporal Gyrus, anterior division Right
30	Inferior Temporal Gyrus, posterior division L **
31	Inferior Temporal Gyrus, temporooccipital part R **
32	Inferior Temporal Gyrus, temporooccipital part L ***
33	Postcentral gyrus R **
38	Supramarginal Gyrus, anterior division L
39	Supramarginal Gyrus, posterior division R **
40	Supramarginal Gyrus, posterior division L
41	Angular gyrus right
49	Frontal Medial Cortex ***
50	Supplementary motor area R **
51	Supplementary motor area L **
56	Cingulate Gyrus, posterior division **
57	Precuneous Cortex
63	*Parahippocampal Gyrus, anterior division L*
70	Temporal Fusiform Cortex, posterior division R
71	Temporal fusiform cortex, posterior division L **
77	*Frontal Operculum Cortex Left ****
78	Central Opercular Cortex R
81	Parietal Operculum Cortex R ***
90	Occipital pole R
91	Occipital pole L
98	*Palladium R **
99	*Palladium L*
102	*Amygdala R **
104	*Accumbens R*
105	*Accumbens L **
115	*Cerebelum 6 L*

Appendix ix: ROIs in Baseline/Active comparisons (Ch. 6)

ROIs identified as statistically different between **Baseline and Active** scans using paired sample t-tests. R indicated right, L indicated left. **Indicates significantly difference at p=<0.01 (FDR corrected). ***Indicates significant at p=<0.001(FDR corrected), otherwise significant at p<0.05 (FDR corrected).

Atlas no.	Region
1	Frontal pole R **
2	Frontal pole L
5	Superior Frontal Gyrus R **
7	Middle frontal gyrus
9	Inferior Frontal Gyrus, pars triangularis R **
11	Inferior Frontal Gyrus, pars opercularis R **
12	Inferior Frontal Gyrus, pars opercularis L
21	Middle Temporal Gyrus, anterior division R
23	Middle Temporal Gyrus, posterior division R **
27	Inferior Temporal Gyrus, anterior division R **
39	Supramarginal Gyrus, posterior division R **
42	Angular gyrus L
44	Lateral Occipital Cortex, superoir division L **
45	Lateral Occipital Cortex, inferior division R
46	Lateral Occipital Cortex, inferior division L
47	Intracalcarine Cortex R
49	Frontal medial cortex
53	Paracingulate Gyrus Right **
55	Cingulate Gyrus, anterior division
56	Cingulate Gyrus, posterior division
57	Precuneous Cortex ***
59	Cuneal Cortex L
60	*Frontal Orbital Cortex R ****
61	*Frontal Orbital Cortex L*
62	*Parahippocampal Gyrus, anterior division R*
64	*Parahippocampal Gyrus, posterior division R*
65	*Parahippocampal Gyrus, posterior division L **
67	*Lingual Gyrus L*
69	*Temporal Fusiform Cortex, anterior division L*
75	*Occipital Fusiform Gyrus L*
79	Central Opercular Cortex L
80	Parietal Operculum Cortex R **
86	Planum temporale R ***
100	*Hippocampus R*
104	*Accumbens R*
106	*Brain-Stem*
108	*Cerebelum Crus1 R*
111	*Cerebelum 3 L*
113	*Cerebelum 4 5 L **
114	*Cerebelum 4 5 R **
117	*Cerebelum 7b L*
120	*Cerebelum 8 R*

Appendix x: ROIs in Active/Sham comparisons (Ch.6)

ROIs identified as statistically different between **Active and Sham** scans using paired sample t-tests. R indicated right, L indicated left. **Indicates significantly difference at p=<0.01 (FDR corrected). ***Indicates significant at p=<0.001(FDR corrected), otherwise significant at p<0.05 (FDR corrected).

Atlas no.	Region
1	Frontal pole R
3	Insular cortex R **
5	Superior Frontal Gyrus R **
10	Inferior Frontal Gyrus, pars triangularis Left
13	Precentral Gyrus R ***
14	Precentral gyrus L **
18	Superior Temporal Gyrus, anterior division L **
20	Superior Temporal Gyrus, posterior division L ***
23	Middle Temporal Gyrus, posterior division R
24	Middle Temporal Gyrus, posterior division L **
30	Inferior Temporal Gyrus, posterior division L ***
32	Inferior Temporal Gyrus, temporooccipital part L **
33	Postcentral Gyrus R ***
34	Postcentral Gyrus L
37	Supramarginal Gyrus, anterior division R
44	Lateral Occipital Cortex, superoir division L ***
45	Lateral Occipital Cortex, inferior division R ***
46	Lateral Occipital Cortex, inferior division L ***
47	Intracalcarine Cortex R
48	Intracalcarine Cortex L
50	Supplementary motor area R ***
51	Supplementary motor area L ***
55	Cingulate Gyrus, anterior division **
57	Precuneous cortex
58	Cuneal cortex R **
59	Cuneal cortex L **
60	*Frontal Orbital Cortex R ** *
62	*Parahippocampal Gyrus, anterior division R ** *
63	*Parahippocampal Gyrus, anterior division L ** *
64	*Parahippocampal Gyrus, posterior division R *** *
66	*Lingual Gyrus R *** *
67	*Lingual Gyrus L *** *
74	*Occipital Fusiform Gyrus R *** *
75	*Occipital Fusiform Gyrus L *** *
77	*Frontal Operculum Cortex L*
78	Central Opercular Cortex R ***
79	*Central Opercular Cortex L *** *
80	Parietal Operculum Cortex R ***
81	Parietal Operculum Cortex L ***
84	Heschl''s Gyrus R **
85	Heschl''s Gyrus L **
86	Planum temporale R ***
87	Planum temporale L **
88	Supracalcarine Cortex R

93	Thalamus L **
98	Palladium R **
100	Hippocampus R ***
101	Hippocampus L
102	Amygdala L **
103	Amygdala R
105	Accumbens L
106	Brain stem **
108	*Cerebelum Crus1 R*
111	*Cerebelum 3 L ** *
112	*Cerebelum 3R ** *
114	*Cerebelum 4 5 R ** *
117	*Cerebelum 7b L*

Appendix xi: ROIs in Baseline2/Active2 conditions (Ch.6)

Significant differences (p=<0.05, FDR corrected) in connectivity between **Baseline2 and Active2**. R = right, L = left. **Regions were significantly different at p=<0.01 (FDR corrected). *** p=<0.001(FDR corrected).

Atlas no.	Region
2	Frontal pole L
3	Insular cortex R
4	Insular cortex L **
8	Middle front gyrus L
12	Inferior frontal gyrus (pars opercularis) L **
13	Precentral gyrus R ***
15	Temporal pole R **
16	Temporal pole L ***
21	Middle temporal gyrus (anterior division) R
25	Middle Temporal Gyrus, temporooccipital part R **
26	Middle Temporal Gyrus, temporooccipital part L ***
29	Inferior Temporal Gyrus, posterior division R ***
30	Inferior Temporal Gyrus, posterior division L ***
31	Inferior Temporal Gyrus, temporooccipital part R **
32	Inferior Temporal Gyrus, temporooccipital part L ***
33	Postcentral gyrus R **
34	Postcentral gyrus L **
37	Supramarginal Gyrus, anterior division R **
47	Intracalcarine Cortex R **
48	Intracalcarine Cortex L
51	Juxtapositional Lobule Cortex/ SMA-L
60	*Frontal Orbital Cortex R **
61	*Frontal Orbital Cortex L ***
62	*Parahippocampal Gyrus, anterior division R **
63	Parahippocampal Gyrus, anterior division, L***
64	Parahippocampal Gyrus, posterior division R
66	Lingual gyrus R **
67	Lingual gyrus L
70	Temporal Fusiform Cortex, posterior division R ***
71	Temporal fusiform cortex, posterior division L ***
72	Temporal Occipital Fusiform Cortex R **
73	Temporal occipital fusiform cortex left **
77	Frontal operculum cortex L
78	*Central Opercular Cortex R*
79	*Central Opercular Cortex L **
80	80: Parietal Operculum Cortex R **
82	Planum Polar R **
83	Planum polar L **
85	Heschl's Gyrus L **
86	Planum temporale R
87	Planum temporale L **
92	Thalamus R
93	Thalamus L ***
94	Caudate R **
99	Pallidum L
102	Amygdala R
103	Amygdala L

Appendix xii: ROIs implicated in Active-Baseline which are predictive of connectivity in Post condition (Ch.6)

Atlas no.	Region
15	Temporal pole R
17	Superior Temporal Gyrus, anterior division R
19	Superior Temporal Gyrus, posterior division R
27	Inferior Temporal Gyrus, anterior division R
28	Inferior Temporal Gyrus, anterior division L
32	Inferior Temporal Gyrus, temporooccipital part L
54	Paracingulate Gyrus L
56	Cingulate gyrus posterior division
60	*Frontal Orbital Cortex R*
61	*Frontal Orbital Cortex L*
62	*Parahippocampal Gyrus, anterior division R*
68	Temporal Fusiform Cortex, anterior division R
78	*Central Opercular Cortex R*
79	*Central Opercular Cortex L*
94	Caudate R
98	Pallidum R
104	*Accumbens R*
109	Cerebellum Crus2 L
110	Cerebellum Crus2 R
111	Cerebellum 3 L
112	Cerebellum 3 R
119	Cerebellum 8 L
120	Cerebellum 8 R
125	Vermis 1 2
128	Vermis 6