

Transcranial stimulation of the human primary motor cortices



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Abstract

The primary aim of this thesis is to investigate the physiological effects of transcranial direct current stimulation (tDCS) as applied to the primary motor cortex (M1). This research was largely motivated by the need to understand the basic physiological changes of tDCS, in order to evaluate its use as a potential tool in recovery after stroke, as well as its more general applicability as a tool to modulate plasticity. The experiments in this thesis assess the ability of tDCS to modulate the primary motor cortex in healthy controls.

The effects of tDCS on cortical GABA and motor resting state functional connectivity were measured with magnetic resonance spectroscopy (MRS) and resting functional MRI (fMRI). Anodal stimulation reduced GABA concentration and increased functional connectivity in the stimulated M1. Testing these changes within the same individuals demonstrated that the magnitude of changes do not correlate across subjects. Novel evidence on the timecourse of GABA change demonstrated that the reduction in GABA is most prominent in the 30-minute period after stimulation.

To determine whether the tDCS-induced modulations in inhibition is restricted to the stimulated hemisphere or whether inhibitory changes could be observed in the non-stimulated M1, or in the interhemispheric connections between the M1s, transcranial magnetic stimulation (TMS) was used to measure intracortical inhibition in each M1 and interhemispheric inhibition and facilitation in the contralateral M1. There were no polarity-specific effects on intracortical inhibition within either M1, and no changes in interhemispheric excitability from the stimulated to non-stimulated M1.

Development of a two-voxel MRS method at ultra high field (7 Tesla) allowed for concurrent measurements of cortical neurotransmitters from both M1s with excellent spectral quality and GABA quantification. This method was used to demonstrate the timecourse of tDCS-induced changes in neurochemicals concurrently from both M1s. Anodal stimulation reduced GABA in both the anode-targeted and non-stimulated M1. Cathodal stimulation decreased GABA and glutamate in the non-stimulated M1, with no concurrent changes in the cathode-targeted M1. Bilateral stimulation reduced glutamate in both M1 with no change in GABA.

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Abbreviations

AMT	Active Motor Threshold
ANOVA	Analysis of Variance
BA	Brodmann Area
BCM	Bienenstock-Cooper-Munro
BOLD	Blood Oxygen Dependent Level
CRLB	Cramér-Rao Lower Bound
CSDE	Chemical Shift Displacement Error
CST	Corticospinal Tract
DSU	Dynamic Shim Updating
EEG	Electroencephalogram
EMG	Electromyograph
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FSL	FMRIB Software Library
FWHM	Full Width at Half Maximum
GABA	γ -Amino Butyric Acid
GAD	Glutamic Acid Decarboxylase
Gln	Glutamine
Glu	Glutamate
Glx	Composite measure of glutamate and glutamine
ICA	Independent Component Analysis
IHI	Inter-Hemispheric Inhibition
IHF	Inter-Hemispheric Facilitation
LASER	Localised by Adiabatic Selective Refocusing

LFP	Local Field Potential
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAA	N-acetyl aspartate
NIBS	Non-Invasive Brain Stimulation
NMDA	N-methyl-D-aspartic acid
OVS	Outer Volume Suppression
PMC	Premotor Cortex
PRESS	Point Resolved Spectroscopy
RF	Radio Frequency
RMT	Resting Motor Threshold
ROI	Region of Interest
RSN	Resting State Network
RT	Reaction Time
SAR	Specific Absorption Rate
SEM	Standard Error of the Mean
SICI	Short Interval Intracortical Inhibition
SMA	Supplementary Motor Area
SNR	Signal-to-Noise Ratio
tDCS	Transcranial Direct Current Stimulation
TBS	Theta Burst Stimulation
TE	Echo Time
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TR	Repetition Time
UHF	Ultra High Field
VOI	Volume of Interest

Chapter 1

Plasticity in the motor system

This introductory chapter provides an overview of the functional and anatomical organisation of the motor system and demonstrates the role of inhibition in human motor cortical plasticity. Particular attention is given to the primary motor cortex - the area of interest in the experimental chapters in this thesis.

1.1 The motor system

Voluntary movements are actions organised around the performance of a purposeful task. Unlike reflexes, where the relationships between response and stimulus are stereotyped and automatic, responses to the same stimulus during voluntary movements can vary depending on the behavioural task, and can improve with experience and learning. The motor areas of the cerebral cortex that produce such movements are subdivided into a primary motor area and several premotor areas. Each area contains a population of neurons that project from the cortex to the brain stem and spinal cord.

Studies in the early twentieth century used electrical stimulation to identify ‘motor maps’, which reflect specific motor behaviours that were correlated with anatomical and clinical observations on the effects of local lesions ([Penfield and Boldrey, 1937](#), [Zilles and Amunts, 2010](#)). The primary motor cortex (M1), located on the precentral gyrus (Brodmann’s area 4), was demonstrated to be the area in which the lowest intensity stimulation elicited movements. With greater intensities, movements can also be generated by direct electrical stimulation of the premotor cortex (PMC; Brodmann’s area 6),

which is further segregated into dorsal (PMd) and ventral (PMv) components, located anterior to the precentral gyrus on the lateral and medial surfaces of the cortex. Another important area of the motor cortex is the supplementary motor area (SMA), which lies in the medial parts of area 6. See Figure 1.1.A for an illustration of the anatomical locations of the major cortical motor regions.

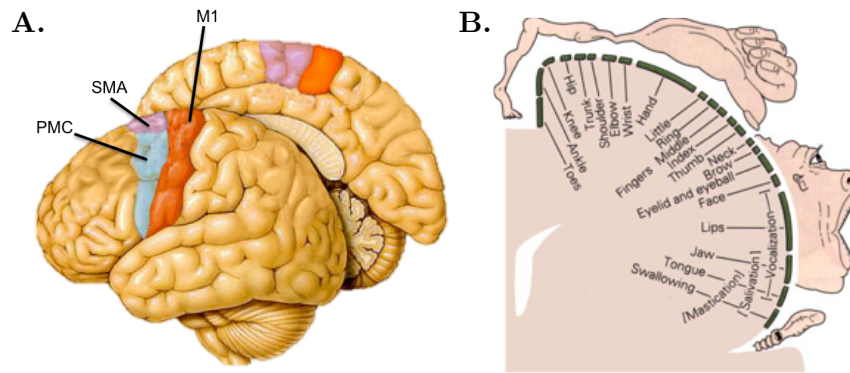


FIGURE 1.1: **Cortical motor regions and the motor homunculus.** **A.** Major cortical motor regions. Illustrates regions of the Primary motor cortex (M1), supplementary motor areas (SMA), and premotor cortex (PMC). Figure adapted from <http://thebrain.mcgill.ca>. **B.** Penfield's motor homunculus. Illustration of outputs from the motor cortex, where the amount of cortical surface dedicated to a part of the body is related to the degree of motor control exercised in that part. Figure adapted from (Kandel et al., 2012).

M1 mediates voluntary movements - it contains neurons that project directly to the spinal cord to activate somatic motor neurons. All premotor areas also project to both M1 and the spinal cord, although there are fewer projections from premotor areas to the spinal cord than from M1. Each premotor area contributes to different aspects of motor planning that are conveyed to M1 for implementation (Richter et al., 1997, Rizzolatti et al., 1998).

As M1 is intimately associated with the motor system of the spinal cord, and is the final site in the cortex for processing motor commands, it is the primary area of interest in the experimental chapters of this thesis. It should be noted, however, that the discussions throughout do not exclude the involvement of other motor areas in the effects described. Three main characteristics of the motor cortex relevant to this thesis are presented in this chapter: i) the anatomical organisation of M1, ii) that M1 has plastic functional organisation in adult humans, iii) and that inhibitory influences are important in plasticity induction.

1.1.1 Anatomical organisation of the primary motor cortex

The cerebral cortex is organised into layers (Figure 1.1.A). The number and organisation of the layers vary throughout the cortex. The precentral gyrus (M1) has prominent output layers, such as layer V, but almost no internal granule cell layer (layer IV), and is thus histologically agranular. The lack of prominence of a layer IV can be understood in relation to its connections with the thalamus. Layer IV is the main target of sensory information from the thalamus, and as the motor cortex is primarily an output region it receives little sensory information directly from the thalamus.

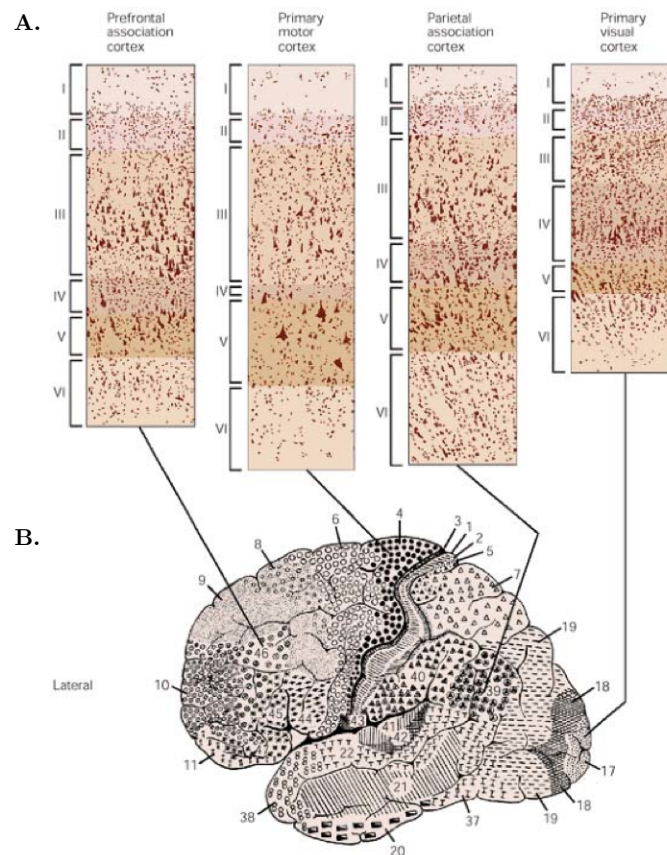


FIGURE 1.2: Layers of the primary motor cortex. A. Layers of the primary motor cortex. Layer I (molecular layer): Acellular, occupied by dendrites of the cells located deeper in the cortex and axons that travel through or form connections in this layer. Layer II (external granule layer): comprised mainly of small spherical granule cells. Layer III (pyramidal layer): contains a variety of cell types, many of which are pyramidally shaped. Layer IV (internal granule layer): similar to layer II, made up primarily of granule cells. Layer V (internal pyramidal layer): contains mainly of pyramidally shaped cells that are typically larger than those in layer III. Layer VI (multiform layer): heterogeneous layer that blends into the white matter and forms the deep limit of the cortex and carries axons to and from the cortex. **B.** Brodmann's cytoarchitectonic map of the human cortex. Lateral view, depicting the cytoarchitectural and functionally distinct regions known as Brodmann areas (BA). BA4 indicates the primary motor cortex, part of the precentral gyrus, bound by the precentral sulcus anteriorly, and the central sulcus posteriorly. Figure from (Kandel et al., 2012).

There are two major neuronal cell types in the cerebral cortex: projection neurons and interneurons. Projection neurons typically have pyramidal shaped cell bodies, and are located mainly in layers III, V and VI and use the excitatory amino acid glutamate as their primary transmitter. Local interneurons are located in all layers and make up about 20-25% of the neurons in the cortex. They use the inhibitory neurotransmitter γ -amino-butyric acid (GABA) as their primary transmitter (Buzsáki et al., 2007, Markram et al., 2004).

Anatomically, the precentral gyrus is bound by the precentral sulcus anteriorly, and the central sulcus posteriorly (Figure 1.2.B; BA4). The mapping of motor responses along the precentral gyrus demonstrates an orderly arrangement of areas responsible for control of different parts of the body. Larger representations exist for areas that are used in tasks requiring the greatest precision and finest control, such as the fingers, hands, and face (Penfield and Boldrey, 1937). This is known as the motor homunculus (Figure 1.1.B). Imaging studies have shown that within the precentral gyrus, a characteristic knob-like structure, known as the ‘precentral knob’, can be identified as a reliable landmark that most often contained motor hand function (Yousry et al., 1997). This is seen as an omega shape in the axial plane and a hook like shape in the sagittal plane (Figure 1.3).

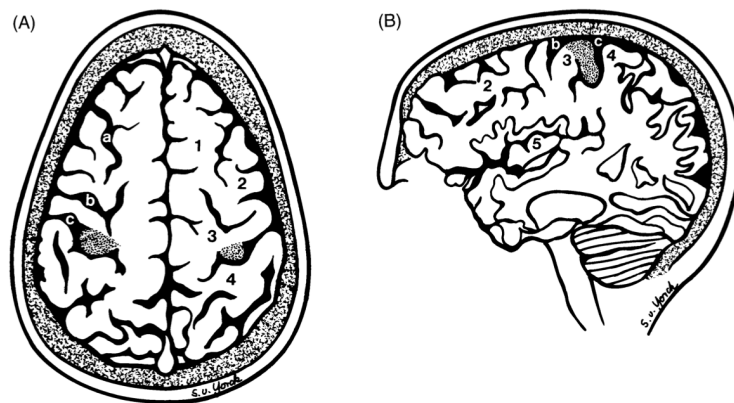


FIGURE 1.3: **Precentral hand knob.** **A.** Axial plane. **B.** Sagittal plane. The knob is a protrusion of the precentral gyrus (3) into the central sulcus (c). It is located posterior to the intersection of the superior frontal sulcus (a) that divides the superior (1) from the middle (2) frontal gyrus, and the precentral sulcus (b). In the sagittal plane, at the level of the posterior part of the insula (5), the knob has the shape of a posteriorly directed hook facing the postcentral gyrus (4). Figure from (Yousry et al., 1997).

More contemporary neuroimaging methods support the notion of a topographically segregated organisation for the motor cortex (Meier et al., 2008, Rijntjes et al., 1999), but suggest that each subdivision is not merely a map of the body but is a topographical representation of functionally relevant activity patterns (Mattay and Weinberger, 1999, Rizzolatti et al., 1998, Sanes and Donoghue, 2000). Thus, the somatotopic organisation of the motor cortex is not fixed but can be altered during motor learning and following injury. M1 executes voluntary movements and adapts them to new conditions and the flexibility of the organisation of this network provides the physical basis for plasticity (discussed in section 1.2).

1.1.2 Role of the Corpus Callosum

Anatomically, the corpus callosum is the largest white matter structure in the brain that connects the two cerebral hemispheres. Functionally, it plays a critical role in the transfer of motor information. Ferbert and colleagues (1992), were the first to study extensively the interhemispheric interactions between the two human primary motor cortices using paired-pulse transcranial magnetic stimulation (TMS). In this study, the authors demonstrated that a conditioning stimulus over one M1, had an effect on the size of the motor evoked potential (MEP) elicited in the opposite M1 (see Chapter 2 for methods). This investigation allowed for the direct measurement of transcallosal influences of each M1 on its homologue in the opposite hemisphere, which was determined to be predominantly inhibitory. Evidence that these inhibitory effects were mediated through transcallosal pathways was further supported by studies in patients with agenesis of the corpus callosum (Meyer et al., 1995). It is postulated that the transcallosal glutamatergic pathways, linking with pyramidal tract neurons through GABAergic interneurons, are responsible for the interhemispheric inhibitory effects measured (Chen, 2004, Reis et al., 2008b).

Changes in interhemispheric inhibition (IHI) between the M1s are involved in the control of unimanual skilled finger movements as well as in the acquisition and transfer of motor skills (van der Knaap and van der Ham, 2011). In stroke patients, movement of the paretic hand is accompanied by an abnormal movement-related IHI from the contralesional to the ipsilesional M1, and the magnitude of this abnormality correlates with the degree of impairment (Murase et al., 2004). Interventional strategies,

such as transcranial direct current stimulation (tDCS), that aim to rebalance this IHI by facilitating excitability in the ipsilesional M1 or down-regulating excitability in the contralesional M1 have resulted in some improvements in motor function ([Hummel and Cohen, 2006](#)). The ability to assess transcallosal interhemispheric interactions between the M1s in humans has provided a mechanistic insight into the physiological processes underlying motor control and allowed for hypothesis-driven strategies in developing interventions (discussed in section 1.3.4). However, as different forms of IHI have been measured ([Di Lazzaro et al., 1999](#)), the specific mechanisms mediating these effects are still incompletely understood.

1.1.3 Role of the ipsilateral motor cortex

M1 primarily has contralateral influence over movements, such that the left M1 controls the right side of the body, and vice versa. However, there is substantial communication between the left and right M1 ([Ferber et al., 1992](#), [Gerloff et al., 1998](#)). M1 is capable of exerting control of motor responses in both the contralateral and ipsilateral hands. A degree of hemispheric asymmetry has been observed, with greater ipsilateral activation of the left hemisphere in both left- and right-handed subjects, suggesting a possible left cerebral dominance for motor control ([Kim et al., 1993](#), [Rose et al., 2012](#), [Verstynen, 2004](#)). It has been postulated that ipsilateral activation could be a function of the degree of motoric familiarity or automaticity ([Hummel et al., 2003](#)). Activity during simple movements of the dominant hand is generally limited to the contralateral hemisphere, whereas learning or performance of complex motor tasks with the non-dominant hand can evoke activation of the ipsilateral M1 ([Kobayashi, 2003](#), [Verstynen, 2004](#)). However, the method by which ipsilateral motor pathways are engaged in voluntary motor behaviour and the neurophysiological significance of this activation is still poorly understood.

In addition to transcallosal pathways mediating ipsilateral modulation ([Ferber et al., 1992](#)), [Gerloff and colleagues \(1998\)](#) assessed whether modulation could also be achieved through subcortical routes. Electrical stimulation of the pyramidal tract at the level of the pyramidal decussation demonstrated that inhibitory influences of the human M1 on ipsilateral hand muscles is to an extent mediated sub-cortically and not entirely through transcallosal connections ([Gerloff et al., 1998](#)). These findings suggest that the inhibitory

interactions between the two M1s are relayed at multiple levels along the neuroaxis, and may become important in the case of lesions. An increasing number of studies suggest that ipsilateral motor pathways play a role in the recovery of motor function after injury (Bradnam et al., 2013, Brus-Ramer et al., 2007). Indeed, studies in stroke patients indicate that ipsilateral motor control is functionally relevant in recovery of function after injury (Johansen-Berg et al., 2002, Ward and Cohen, 2004) (discussed in section 1.3.4).

Observations of abnormal interhemispheric influences and the possible role of the ipsilesional motor cortex in stroke patients have raised the hypothesis that purposeful modulation of excitability with non-invasive brain stimulation (NIBS) techniques may contribute to improvements in motor function (section 1.3.4.1). In Chapter 5 we use TMS to investigate whether intracortical inhibitory circuits within M1 and interhemispheric excitability can be modulated by tDCS (method discussed in Chapter 2).

1.2 Concepts of plasticity

Plasticity can be defined as the reorganization of brain connectivity through experience. It allows the brain to learn and remember patterns in the sensory world, to refine movements from learning, and contributes to the restoration of function after damage. This fundamental property is the principal process responsible for recovery of function after injury such as stroke (Chen et al., 2002), and understanding the cellular and synaptic mechanisms of plasticity, and how non-invasive brain stimulation (NIBS) methods can be used to study or even manipulate these processes is of major interest (Rothwell, 2010).

1.2.1 Hebbian synaptic plasticity

In humans, plasticity occurs largely through modification of the strength of synaptic connections. In Hebb's seminal work (1949), synaptic plasticity was described as:

“When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells, such that A's efficiency, as one of the cells firing B, is increased” (Hebb, 2002).

1.2.2 LTP and LTD

Long Term Potentiation (LTP) and Long Term Depression (LTD) are two phenomena that implement Hebbian synaptic plasticity and can explain the major aspects of cortical plasticity. Both are NMDA receptor dependent, and depend on the changes in GABAergic and glutamatergic transmission at the level of the synapse. LTP and LTD are not unitary phenomena and their mechanisms will vary depending on the synapses and circuits in which they operate ([Malenka and Bear, 2004](#)).

Evidence from several sources has demonstrated that M1 contains both the substrate and the mechanism to implement plasticity ([Sanes and Donoghue, 2000](#)). The system of horizontal connections that span M1 is a likely candidate substrate for plasticity in this region ([Jacobs and Donoghue, 1991](#), [Rockland, 1993](#)). Horizontal fibers arising from the pyramidal neurons allow for the co-activation of adjacent cortical columns as well as modulating inhibitory activity via the activation of GABAergic interneurons. For example, facial nerve lesions in rats resulted in M1 reorganisation at sites having strong horizontal connections between the whisker and forelimb areas, but not in M1 areas that have sparse connections between the forelimb and whisker regions ([Huntley, 1997](#)). This finding demonstrated the pattern of horizontal connections in M1 and suggests that reorganisation could reflect the synaptic plasticity of these fibers.

This dynamic modulation to the functional architecture of M1, through changes in the efficacy of horizontal connections, would require a more stable form of synaptic modification such as LTP- and LTD-like processes. In humans, local blockade of GABA-related cortical inhibition resulted in short-term changes in synaptic efficacy involving NMDA receptor activation and induction of LTP-like mechanisms ([Ziemann et al., 1998](#)). LTP of horizontal pathways alone fails unless inhibition is transiently reduced during, but not after stimulation ([Hess et al., 1996](#), [Hess and Donoghue, 1994](#)). This finding suggests that the mechanisms involved are dependent on the removal of local inhibition ([Jacobs and Donoghue, 1991](#)).

M1 is at the center of where functional reorganisation of motor representations occurs and contains both the substrate (horizontal connection system) and the mechanism (LTP/LTD) for reorganisation. The synaptic efficacy of the interconnections among M1 neurons can be continuously adjusted, presumably as a consequence of their exposure

to the appropriate activity patterns that are likely to be strongly activity-dependent (Sanes and Donoghue, 2000).

LTP and LTD produce associative changes in the strength of individual synaptic connections, however, such plasticity is prone to instability. For example, once a synaptic input is potentiated, it becomes easier for the pre-synaptic neuron to depolarise the post-synaptic neuron and make it fire. This promotes further increases the net excitatory synaptic drive to that post-synaptic neuron, and thus, makes it easier for other inputs to depolarise that neuron (Feldman, 2009). To harness the ability of Hebbian based mechanisms to selectively modify synaptic connectivity; there must also be a mechanism that stabilises the mean cortical activity in response to the changing input levels.

1.2.3 Homeostatic plasticity

Homeostatic plasticity is a distinct form of plasticity that drives compensatory changes to restore cortical activity to a set point level (Turrigiano and Nelson, 2000). Unlike Hebbian plasticity, where associative changes result in progressive modifications of network properties, homeostatic mechanisms promote network stability.

The most influential model of homeostatic plasticity was described by Bienenstock, Cooper, and Munro (1982), and known as the Bienenstock-Cooper-Munro (BCM) model. To ensure that the activity of cortical networks stay within a useful dynamic range, the model incorporates a sliding modification threshold (θ_m), which requires both LTP-like increases and LTD-like decreases in synaptic strength in order to maintain selectivity and stability within the network. The modification threshold is a dynamic entity, and can be raised or lowered based on the recent history of post-synaptic cell firing.

Although most research has focused on excitatory glutamatergic synapses for cortical plasticity (Lee et al., 2010, Turrigiano and Nelson, 2000), accumulating evidence suggests that GABAergic circuits show especially strong homeostatic regulation in vivo. A recent study using theta-burst stimulation (TBS), a TMS protocol designed to induce LTP- or LTD-like effects through the application of groups of repeated pulses, demonstrated that priming with TBS resulted in homeostatic regulation of GABA_A intracortical inhibitory circuits in M1 (Murakami et al., 2012). This finding suggests that inhibitory

influences may serve to regulate excitatory and inhibitory synapse strength and intrinsic excitability in the human motor cortex.

Such evidence in humans are still very limited, however, will be necessary for the understanding of the role of inhibitory processes in homeostatic plasticity. In particular, how these interactions function in response to motor learning or plasticity-induction protocols that modulate the brain environment, such as tDCS, will have important implications in the way these methods are applied in therapy.

1.3 Inhibitory influences

There is significant interest amongst both the clinical and neuroscience communities in investigating the role of inhibitory processes in normal brain function and how this can help us understand pathophysiology in disease. Tools to facilitate the direct investigation of GABAergic circuits in humans have only recently become available and our understanding of its role in cortical function is still in its infancy.

Since the pioneering work of Rothman and colleagues (1993), it is now possible to measure the main inhibitory neurotransmitter GABA in humans using magnetic resonance spectroscopy (MRS). MRS allows for the accurate quantification of endogenous metabolites within a localised region (or voxel) of tissue. A full description of MRS is presented in Chapter 3. The ability to measure GABAergic inhibition in vivo has sparked interest into investigating inter-individual differences in the role of GABA in behaviour and the effects on GABA concentrations following plasticity-induction paradigms. The following sections will focus primarily on evidence from human MRS studies to summarise what has been learned about the role of inhibitory influences in inter-individual differences in behaviour and motor plasticity, before discussing the clinical implications of exogenous GABA modulation in recovery of function after stroke.

1.3.1 GABA metabolism

Within GABAergic neurons, GABA is primarily produced from glutamate via the enzyme glutamic acid decarboxylase (GAD) and is metabolised to succinic acid semialdehyde in the astrocytes by GABA transaminase (GABA-T) (Petroff and Rothman, 1998).

GABA is mainly broken down in GABAergic neurons and in some astrocytes, although the astrocytic route is less metabolically significant than neuronal degradation. It is estimated that only around 20% of GABA released at synaptic terminals is taken up by the surrounding astrocytes (Schousboe, 2003). See Figure 1.4 for an illustration of GABA metabolism.

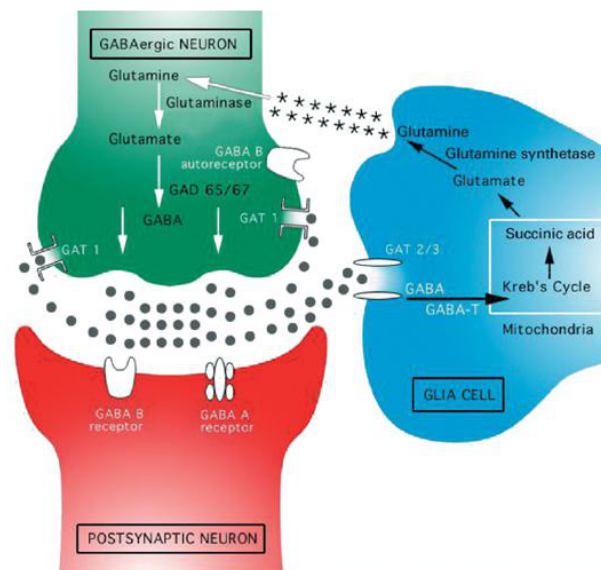


FIGURE 1.4: **GABA metabolism.** Illustration of GABA metabolism and uptake in the human brain. Glutamate is the precursor of free GABA in GABAergic terminals and comes from two different sources: the Krebs' cycle in glia cells and glutamine in nerve terminals. The enzyme glutamic acid decarboxylase (GAD) forms GABA from glutamate. After being released into the synapses, GABA is inactivated by reuptake, which is mediated by GABA transporters (GAT) into presynaptic terminals or into glia cells where it is metabolised by GABA transaminase (GABA-T). Figure from (Brambilla et al., 2003).

Two molecular forms of GAD (65 kDa and 67 kDa) exist and these are thought to regulate the two major pools of GABA - the cytoplasmic pool found throughout the neuron and the vesicular pool found in their presynaptic boutons (Martin and Barke, 1998). Cytoplasmic GABA is primarily produced by the tonically active GAD₆₇ and is found almost evenly distributed throughout the neuron. It is thought to be the rate-limiting step in GABA production (Chattopadhyaya et al., 2007) and has an activity-dependent role in GABA synthesis such that decreased network activity leads to decreased expression of GAD₆₇, which in turn leads to lower GABA levels (Lau and Murthy, 2012). Vesicular GABA on the other hand, is found in high concentrations within the presynaptic boutons and is regulated by the phasically active GAD₆₅, playing a key role in inhibitory synaptic neurotransmission (Martin and Rinvall, 1993).

Two main sub-types of receptors mediate the synaptic effects of GABA within the cortex - fast acting ionotropic GABA_A receptors that gate chloride ions and slow acting GABA_B metabotropic receptors that activate potassium channels (Schousboe and Waagepetersen, 2007). GABA_A receptors located at the GABA_A synapse mediate the fast inhibitory neurotransmission in the central nervous system (CNS). GABA also acts at non-synaptic GABA_A receptors, producing the so-called extrasynaptic ‘GABAergic tone’ (Wagner et al., 2007b). GABA_B receptors are found throughout the CNS. Synaptically released GABA can activate pre-synaptic GABA_B receptors on GABA neurons to reduce GABA release, or pre-synaptically on local glutamatergic neurons to reduce glutamate release (Gassmann and Bettler, 2012). In general, GABA_B receptors inhibit neuronal activity by local shunting and generate slow inhibitory potentials that hyperpolarise the membrane.

1.3.2 Inter-individual differences in GABA

Considerable differences exist between individuals both in the way the brain functions normally and how it recovers from injury. Recent studies using MRS have demonstrated that significant differences occur in the neural basis of basic cognitive functions such as motor behaviour. Differences in GABA levels have been related both to better and worse performance across a range of tasks and brain regions.

Sumner and colleagues (2010) reported that individual differences in baseline GABA predicted the ability to rapidly resolve competitive action decisions, here measured in the form of saccades to visual targets presented with distractors, such that people with higher GABA levels in the frontal eye fields were less influenced by the presence of distractors. Similarly, differences in baseline GABA concentration in the SMA predicted subjects’ ability to suppress subliminal motor activations evoked by visual priming (Boy et al., 2010). The SMA is a region previously associated with automatic motor control and the authors demonstrated that subjects with naturally higher levels of inhibition within the SMA were more likely to have less suppression of the initial subliminal motor activation evoked by early visual priming than those with lower GABA levels. Another study quantified GABA levels within the sensorimotor cortex and demonstrated that subjects with higher levels of GABA were worse at performing a tactile discrimination

task (Puts et al., 2011).

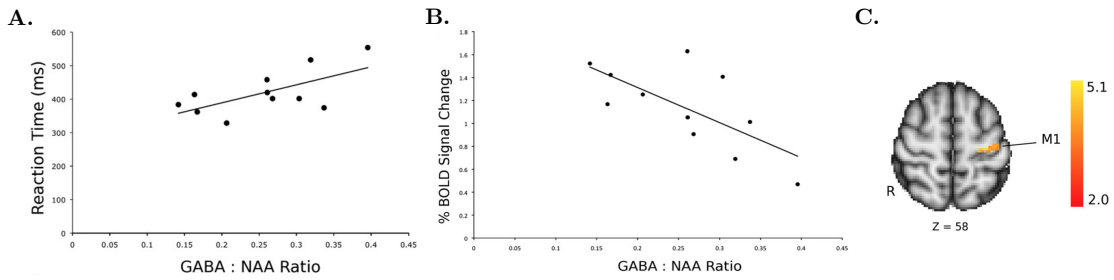


FIGURE 1.5: Baseline GABA in M1 and reaction times. **A.** Significant positive correlation between baseline GABA:NAA within the M1 voxel and the reaction times during random (non learning) blocks ($r=0.6$, $p=.03$). **B.** Negative correlation between baseline GABA:NAA and mean BOLD signal change in response to the motor boxcar regressor within a left M1 ROI ($r=-0.69$, $p=.01$). **C.** The left primary sensorimotor cortex showed a significant negative correlation between the BOLD signal change in response to the motor boxcar and baseline GABA:NAA. Figure adapted from (Stagg et al., 2011a).

With less complex behaviours, particularly those involving the primary motor and visual regions, there is an inverse relationship between GABA levels and behaviour. We've previously demonstrated that subjects with higher baseline GABA levels had slower reaction times (Figure 1.5.A) and also smaller blood oxygen level-dependent (BOLD) signal change in response to a visually-cued motor task (Figure 1.5.B) (Stagg et al., 2011a). This relationship was observed only in M1 and not in a control voxel placed on the visual cortex, suggesting a specific regional and task-relevant relationship (Figure 1.5.C). Similar findings have been demonstrated in the primary visual cortex (Muthukumaraswamy et al., 2009), where baseline GABA concentration were both inversely correlated with the magnitude of the BOLD response induced by a simple visual gating stimulus and positively correlated with the gamma oscillation frequency as measured by magnetoencephalography (MEG). These findings suggest that functional neuroimaging metrics may be dependent on the excitation and inhibition balance in an individual's cortex, here measured by resting GABA levels.

In all these studies, GABA differences were found in the voxel of interest related to the task, but not in a control voxel located in regions unrelated to the task. This highlights the regional specificity of baseline levels of GABA. Thus, in interpreting MRS studies it is important to remember that the GABA levels are measured from an a priori defined area of interest. The measurements cannot be used to infer global relationships but rather reflect regionally specific correlations. This places extra importance on a strong

a priori hypothesis for targeting specific brain regions in experimental designs. Studies measuring GABA from a less defined area are likely to be more difficult to interpret.

It is still unclear why natural differences in baseline GABA concentration occur, and what factors determine their regional specificity. Physiologically, these differences could reflect natural variation in the density of GABA interneurons in a given area, the number of synapses per neuron, or the concentration of GABA per synapse. Methodological limitations currently prevent the direct pinpointing of the cellular mechanisms involved, but it is likely that a combination of all these factors are responsible for the high degree of variability in GABA levels between subjects. Nevertheless, studies assessing intra-subject and inter-subject variability in GABA have demonstrated good reproducibility and stability at short-term and long-term intervals ([Bogner et al., 2010](#), [Near et al., 2014](#)). These findings indicate that MRS has good potential to be used in clinical and scientific settings to detect even small alterations in brain GABA.

In light of this, it is unclear how local inhibitory processing functions as part of a wider network. Recently, we have demonstrated a relationship between inhibitory activity within M1 and the strength in functional connectivity within the wider motor network, such that lower GABA levels were associated with greater functional connectivity in healthy subjects ([Stagg et al., 2014](#)). A similar finding has been demonstrated between the posteromedial cortex and connectivity in the default mode network as assessed by functional MRI ([Kapogiannis et al., 2013](#)). An increase in multimodal imaging studies such as these will have the potential to probe different aspects of how the MRS-derived measures of GABA relate to measures from other imaging modalities. For example, MRS-derived GABA concentrations have been related to MEG-derived motor cortex gamma oscillations ([Gaetz et al., 2011](#)), supporting findings from animal studies ([Roopun et al., 2006](#), [Yamawaki et al., 2008](#)).

Harnessing the strengths of different imaging methods is a powerful approach to understanding brain function as it allows measurements of metabolites, neuronal currents, and haemodynamic activity in vivo without the need for contrast agents or neuroactive compounds. In this way, the normal functioning of the brain can be assessed, as well as comparisons to be made before and after behavioural interventions. Combining this with techniques such as TMS and tDCS that can probe the responsiveness of the GABA system can help answer important questions on the role of inhibitory processes

in normal brain function and how this can help us understand pathophysiology in disease. In Chapter 4 we address the question of how tDCS affects the GABA system in M1 and how this relates to motor resting state functional connectivity within the same individuals.

1.3.3 GABAergic changes in plasticity induction

Despite the established role of GABA in cortical plasticity ([Griffen and Maffei, 2014](#), [Rockland, 1993](#)), studies investigating the changes in GABA after behavioural interventions in humans are still limited. In healthy people, GABA has been shown to be involved in motor plasticity induced in a variety of different ways. A decrease in M1 GABA has been demonstrated in response to a forearm ischemic nerve block ([Levy et al., 2002](#)), an intervention known to increase cortical excitability and induce short-term reorganisation of M1 ([Brasil-Neto et al., 1993](#), [Ziemann et al., 1998](#)). Decreases in GABA levels within M1 have also been demonstrated during learning of a visuo-motor tracking task but not during random movements or when subjects were at rest ([Floyer-Lea, 2006](#)).

In addition to endogenous modulation of plasticity within M1 (e.g. via motor learning or acute de-afferentation), a few studies have examined the response of M1 to exogenous plasticity-induction paradigms using transcranial stimulation approaches. For example, Stagg and colleagues (2009) investigated the effects of continuous TBS on MRS-assessed GABA levels within M1. Continuous TBS is a TMS protocol previously shown to decrease cortical excitability and induce LTD-like plasticity ([Huang et al., 2005](#)). In line with these findings, continuous TBS led to an increase in GABA levels within M1 but not at a control voxel ([Stagg et al., 2009b](#)).

Another way in which the GABA system can be probed is through the use of tDCS, a technique that involves passing a small electric current (1 - 2mA) through the brain for up to 20 minutes via two large electrodes placed on the scalp (method discussed in Chapter 2). The effects of tDCS are dependent on the direction of current - anodal stimulation increases excitability in the cortical region of interest, whereas cathodal stimulation decreases cortical excitability ([Nitsche and Paulus, 2000, 2001](#)).

Anodal tDCS is thought to induce LTP-like plasticity within the stimulated cortex ([Stagg and Nitsche, 2011](#)), and has been shown to decrease GABA levels within M1

(Stagg et al., 2011a, 2009a). In order to test the responsiveness of the GABAergic system in M1, and how this is related to motor learning, we performed a study that measured M1 GABA levels before and after anodal tDCS (1 mA, 10 minutes). On a different day, we measured the BOLD changes when subjects performed an explicit, visually cued sequence-learning task. We demonstrated that the degree of tDCS-induced GABA decrease is related to the degree of learning in a motor task, such that subjects with the greatest drop in anodal tDCS-induced GABA also had the greatest drop in their reaction times during a motor task (Figure 1.6.A). Thus, subjects who were better learners were also the most responsive to anodal stimulation. The study also demonstrated that the degree of GABA decrease within M1 in response to anodal tDCS further correlated with the learning-related changes in BOLD activity (Figure 1.6.B) specifically within the hand region of M1 (Figure 1.6.C).

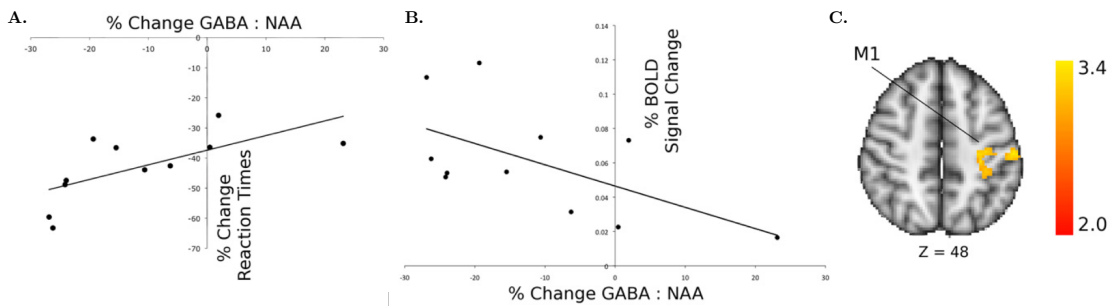


FIGURE 1.6: **Changes in M1 GABA with anodal tDCS.** **A.** Significant positive correlation between change in GABA:NAA due to anodal tDCS and change in reaction times due to learning ($r=0.65$, $p=0.03$). **B.** Negative correlation between change in GABA:NAA due to anodal tDCS and the learning-related change in fMRI activity in the left M1 region of interest (arbitrary units; $r=20.59$, $p=0.05$, uncorrected). **C.** One cluster in left primary motor cortex showed a negative correlation between learning-related change in fMRI activity and change in GABA:NAA ratios due to anodal tDCS.

Figure adapted from (Stagg et al., 2011a).

The relationship between the responsiveness of the GABAergic system and the ability of a subject to learn a task, as well as the anatomical specificity of this relationship, strongly suggest that learning processes and GABA modulation are closely linked in humans. These findings are also consistent with the hypothesis from the animal literature that a decrease in GABA may be necessary for motor cortical plasticity to occur (Jacobs and Donoghue, 1991, Trepel and Racine, 2000). However, it is important to note that the studies reported here by their nature measure correlations between GABA and the behavioural intervention and cannot demonstrate causality.

1.3.4 Implications for exogenous GABA modulation in stroke

The above evidence that anodal tDCS can decrease GABA levels within the stimulated cortex and that this decrease is related to the degree of plasticity, raise the possibility that enhancement of the brain environment to promote plasticity could be a potential target for therapeutic interventions following damage to the human cortex. For example, there is increasing evidence on the beneficial effects of tDCS as an adjunct therapy in stroke rehabilitation ([Adeyemo et al., 2012](#), [Butler et al., 2013](#)).

Stroke is the main cause of permanent disability in Europe and the USA ([Go et al., 2014](#), [Kolominsky-Rabas et al., 2001](#)). Although most stroke survivors achieve some spontaneous recovery, there are no treatments specifically designed to restore function through repair of damaged tissue ([Dobkin, 2004](#)). Current best practice in stroke management is to maximise functional ability through extensive physiotherapy, leaving the majority of patients chronically unable to use their affected hand or arm for simple activities of daily living ([Veerbeek et al., 2011](#)).

Research has directed attention to the use of NIBS techniques such as tDCS, with the hypothesis that if recovery depends on plasticity, interventions that increase plasticity should help improve recovery. However, the physiological processes underlying these approaches are still poorly understood. In Chapter 7 we address the question of how commonly used tDCS protocols modulate the brain environment in both primary motor cortices of healthy controls. Although this thesis does not directly address stroke, it is hoped that by first understanding the basic changes of tDCS in healthy controls, it can better inform hypotheses on how these interventions might be applied in the context of stroke. The following sections briefly present the theoretical basis that form the hypotheses underpinning the use of tDCS in recovery after stroke.

1.3.4.1 Models of functional recovery in stroke

Neuronal organisation after a stroke continues for several weeks after the initial injury and involves brain regions distant to the affected site ([Grefkes and Ward, 2014](#), [Ward et al., 2003](#)). Currently, there are two main models that describe functional recovery in stroke: the adaptive plasticity and interhemispheric competition model. Although the

physiological observations for the two models are possibly contradictory, the commonality for both sets of hypotheses is that interhemispheric influences from the contralesional to the ipsilesional motor regions are systematically more abnormal in patients with more impaired clinical motor function. The correct interpretation and functional significance of these observations remain unclear.

In the adaptive plasticity model, residual networks substitute the functions lost by the damaged cortex, presumably via the intact hemisphere or premotor areas. Evidence has demonstrated that there are shifts in the somatotopic representation in primary areas (Calautti et al., 2003, Pineiro et al., 2001) and increased activity in secondary motor areas in both the affected (Fridman, 2004) and unaffected (Johansen-Berg et al., 2002) hemispheres. The extent of this activity is positively correlated to the degree of functional impairment (Bestmann et al., 2010). One interpretation of this is that patients with poorer outcome will have greater damage to the projections from M1 to the spinal cord, and thus, use secondary motor areas to compensate.

The interhemispheric competition model suggests the opposite, whereby damage to one hemisphere in stroke disrupts the natural balance of inhibition between the hemispheres. Imaging studies have demonstrated widespread changes in patterns of activation during simple movements of the paretic hand (Cicinelli et al., 2003, Liepert et al., 2000) and have demonstrated that the contralesional M1 exerts an abnormally high degree of interhemispheric inhibitory drive towards the ipsilesional M1 (Grefkes et al., 2008, Murase et al., 2004). These findings have raised the hypothesis that purposeful modulation of excitability in motor regions of the intact and affected hemisphere may contribute to improvements in motor function (Hummel and Cohen, 2006).

1.3.4.2 Therapeutic strategy

The use of NIBS methods can be a powerful way to modulate human brain function. One popular strategy to influence function in the paretic hand is to up-regulate excitability in the ipsilesional cortex, or down-regulate excitability in the intact cortex (Figure 1.7).

We previously tested whether increasing excitability in ipsilesional M1 with anodal tDCS, or decreasing excitability in contralesional M1 with cathodal tDCS, are associated

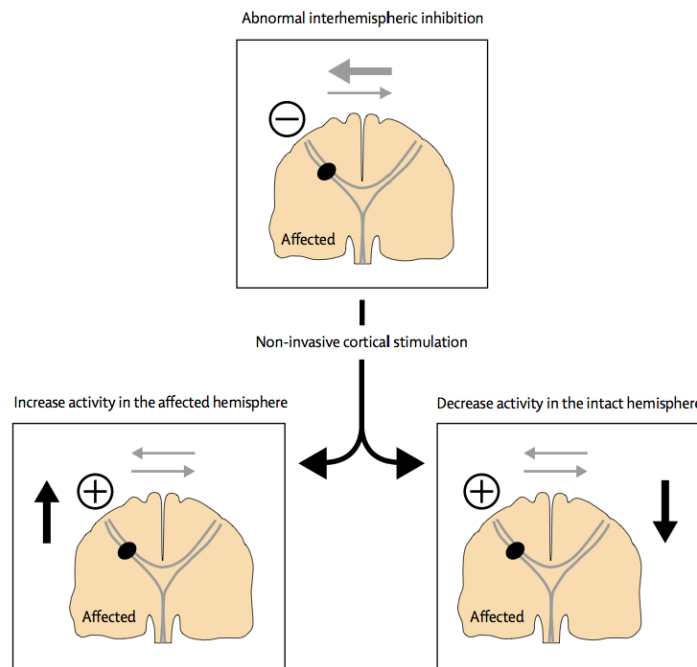


FIGURE 1.7: **Targets for intervention strategy in stroke.** Movements of the paretic hand are associated with unbalanced interhemispheric inhibition targeting the ipsilesional cortex in patients with stroke. A possible intervention strategy is to normalise this pattern by increasing excitability of the ipsilesional hemisphere, and decreasing excitability of the contralesional hemisphere. Figure from (Hummel and Cohen, 2006).

with behavioural improvements in chronic stroke patients (Stagg et al., 2012). Significant improvements (5-10%) in reaction times of the paretic hand were demonstrated with anodal stimulation (Figure 1.8.A), and this was associated with movement-related cortical activity in the stimulated M1 (Figure 1.8.B). Cathodal stimulation only led to a functional improvement when compared to the sham control condition. Interestingly, more bilateral movement-related activation patterns are demonstrated with cathodal stimulation compared to anodal stimulation in this study.

In general, studies reporting performance improvements in stroke with tDCS application have been moderate in magnitude (Adeyemo et al., 2012). In the clinic, the ability to estimate the potential for recovery of motor function will be important in improving rehabilitation planning and outcomes (Stinear, 2010). For example, we have previously demonstrated that resting GABA levels in the ipsilesional M1 of chronic stroke patients predicted their behavioural gains from anodal tDCS (O'Shea et al., 2014). Assessing the motor system's capacity for functional reorganisation in response to adjunct therapies such as tDCS may give better insights into more optimised applications to improve therapeutic potential. Critically, it is unclear whether the hemispheric 're-balancing'

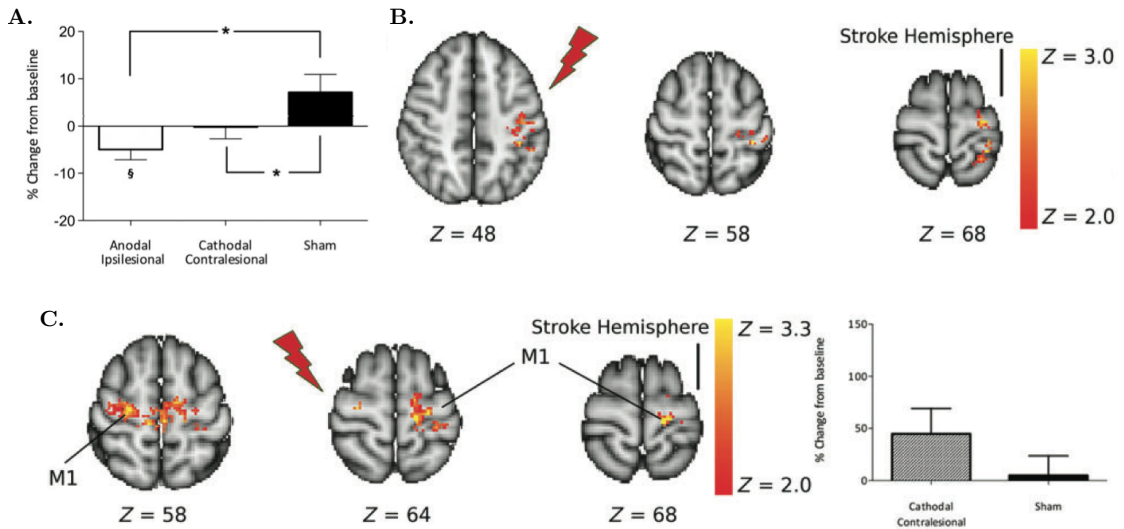


FIGURE 1.8: **Cortical activation changes underlying tDCS-induced behavioural gains in chronic stroke.** **A.** Behavioural data. Anodal stimulation to the ipsilesional M1 led to a significant decrease in response times. **B.** Areas of significant correlation between the change in motor-related activation after anodal stimulation and change in response times after anodal stimulation. **C.** Areas of increased motor-related activity in response to the reaction time task after cathodal stimulation compared with sham. [i.e. $(\text{Cathodal}_{\text{post}} - \text{Cathodal}_{\text{pre}}) - (\text{Sham}_{\text{post}} - \text{Sham}_{\text{pre}})$]. Figures adapted from (Stagg et al., 2012).

approach is appropriate for all patients. The heterogeneity in the stroke population, such as symptom severity, lesion size and location, residual anatomy, and inter-individual variability, can make these predictions difficult to discern. This is further complicated by the lack of understanding of the basic mechanisms underlying tDCS.

It is clear that better understanding of the basic physiological changes during tDCS is essential to evaluate the clinical applicability of this approach, and to determine its general applicability as a tool to modulate plasticity. This forms the motivations of the experimental chapters in this thesis. As the heterogeneity in the stroke population make predictions difficult to discern, it is hoped that by investigating the changes by which tDCS causes its effects in healthy controls, a better picture of the underlying physiological effects can be understood.

1.4 Summary

The primary aim of this thesis is to investigate the physiological effects of transcranial direct current stimulation as applied to the primary motor cortex. This chapter has provided an overview of the human motor system, and discussed the role of inhibition

in human motor cortical plasticity and how this can be applied to potential therapeutic strategies in stroke. Chapter 2 describes the transcranial stimulation methods used and Chapter 3 presents the imaging methods used to measure these changes.

The first experimental chapter investigates the effects of anodal tDCS on GABAergic inhibition and resting state functional connectivity within the stimulated M1 using MRS (Chapter 4). It aims to demonstrate the timecourse of GABA change and assess the effects during the stimulation period, which to date, has never been measured. Chapter 5 uses TMS to investigate intracortical and interhemispheric excitability with anodal and cathodal tDCS in order to determine whether tDCS has an effect on interhemispheric inhibitory influences in the non-stimulated M1. In Chapter 6 we develop a novel spectroscopy sequence at ultra-high field in order to accurately quantify GABA concurrently from both primary motor cortices. This method is used in Chapter 7 to investigate the metabolite changes with anodal, cathodal, and bilateral tDCS concurrently from both primary motor cortices. Novel findings on the timecourse of GABA change with tDCS are presented.

The thesis concludes with a summary of the key findings in Chapter 8 and future directions are discussed in the context of motor learning and recovery of function after stroke.

Chapter 2

Methods for stimulating the primary motor cortices

Non-invasive brain stimulation techniques are powerful tools able to modulate and measure cortical activity in the human primary motor cortices. Transcranial direct current stimulation (tDCS) is used in Chapters 4, 5, and 7 to modulate underlying cortical activity, and in particular, GABAergic inhibition. Transcranial magnetic stimulation (TMS) is used in Chapter 5 to measure the changes in cortical excitability as modulated by tDCS. In this chapter the methods used to stimulate the primary motor cortices are discussed.

2.1 Transcranial Magnetic Stimulation (TMS)

Merton and Morton (1980) first showed that it was possible, albeit somewhat painfully, to stimulate the motor areas of the human brain electrically through the intact scalp (transcranial electrical stimulation, TES). This method was later developed by Barker and colleagues (1985) who showed that using a pulsed magnetic field (transcranial magnetic stimulation, TMS), it was possible to directly stimulate the human motor cortex with minimal discomfort to the subject (Barker et al., 1985).

Transcranial magnetic stimulation (TMS) follows the fundamental principles of electromagnetic induction, where a magnetic field is generated via a brief current flowing through a coil of wire that is placed above the scalp. This induces a transient magnetic

field that can reach up to around 2 Tesla and lasts for about 100 μ s ([Hallett, 2007](#)). The changing magnetic field induces a flow of electric current in the underlying brain tissue, which lowers the potential of neuronal membranes and initiates a depolarization ([Hallett, 2000](#)). The probability of a neuron depolarizing in response to a TMS pulse depends on: a) the initial state of the neuronal membrane potential, b) the orientation of the neuron relative to the induced current, and c) the type of neuron ([Fitzpatrick and Rothman, 2000](#)).

2.1.1 Motor Evoked Potentials (MEPs)

When TMS is applied to the primary motor cortex it can elicit a peripheral response and its effects can be measured via electromyographic (EMG) recordings of stimulated muscles. The first dorsal interosseous (FDI) muscle is typically used when stimulating the motor cortex due to its cortical representation within M1. The EMG activity resulting from a TMS pulse is termed a motor-evoked potential (MEP). The size of the MEP corresponds to the level of excitation within descending motor tracts and is used as the physiological marker of corticospinal conduction. MEPs can be a sensitive measure to quantify excitability changes within these motor tracts, however, the size of an MEP can vary substantially in size and shape from one stimulus to the next, even if the stimulus parameters are kept constant ([Kiers et al., 1993](#)). The basic physiological mechanisms that may influence MEP size are: a) the number of recruited motor neurons in the spinal cord, b) the number of motor neurons discharging more than once to the stimulus, and c) the synchronisation of the TMS-induced motor neuron discharges ([Wassermann et al., 2008](#)).

MEPs consist of multiple components: a short latency direct wave (D-waves), and several longer latency indirect waves (I-waves). The D-wave reflects the direct depolarisation of the initial axon segment of the corticospinal neuron, and can be elicited by high intensity TMS or TES ([Reis et al., 2008b](#)). Most TMS protocols influence I-waves, which occur sequentially in approximately 1.5 ms intervals after the initial D-wave. Longer latencies reflect the increasing distances from the initial corticospinal axon, such that I₁-waves synapse directly onto the axon, whereas I₂-waves and later waves may involve polysynaptic circuits ([Reis et al., 2008b](#)).

2.1.2 Types of Stimulation

Various TMS paradigms can be used to evaluate inhibitory and excitatory circuits in the cortex. This can help localize brain functions both spatially and temporally, and are useful for understanding changes in brain physiology. The TMS protocols described in this thesis were chosen for the purposes of evaluating inhibitory circuits within M1 and interhemispheric interactions between the primary motor cortices. The protocols used are described in the following sections and a summary is shown in Table 2.1.

TABLE 2.1: **Summary of TMS measures**

TMS Measure	Pulse type	Neuronal population stimulated	Physiological significance
Motor Threshold (MT)	Single	Pyramidal neurons of the corticospinal tract	Excitability of a central core of excitatory interneurons and corticospinal neurons
Short Intracortical Inhibition (SICI)	Paired ISI 2.5ms	GABA _A interneurons	Measures suppression of intracortical excitability
Short Interhemispheric Inhibition (IHI ₁₀)	Paired ISI 10 ms	GABA _B interneurons in the target hemisphere	Measures suppression of inter-cortical excitability
Long Interhemispheric Inhibition (IHI ₄₀)	Paired ISI 40 ms	GABA _B interneurons in the target hemisphere	Measures suppression of inter-cortical excitability
Interhemispheric Facilitation (IHF)	Paired ISI 4 ms	Transcallosal facilitatory connections	Measures enhancement of inter-cortical excitability

2.1.2.1 Motor Thresholds

The excitability of the motor cortex can be assessed by measuring the motor threshold (MT), which reflects the activity of a spatially localised group of neurons. MT can vary widely across individuals, but is relatively stable within individuals over time (Wassermann et al., 2008). Thus, to produce comparable stimulation parameters across individuals, in order to make comparisons on the effects of an intervention, experimenters set the TMS intensity used as a proportion of each individual's MT by which an MEP of a certain amplitude can be achieved. MTs determined at the beginning of an experiment can be tested again after an intervention to assess the changes in excitability due to that intervention.

The resting motor threshold (RMT) is defined as the minimum stimulus intensity that produces a 0.5 mV MEP in 5 out of 10 consecutive stimuli during muscle relaxation (Rossini and Rossi, 2007). The 1mV RMT is defined as the intensity required to elicit

an MEP of 1mV on average during muscle relaxation (Rossini et al., 1994). The active motor threshold (AMT) is defined as the minimum stimulus intensity needed to produce a 1-2 mV response during isometric contraction at about 20% of maximum voluntary strength (Rossini and Rossi, 2007). To achieve this, subjects are asked to lightly pinch the index finger and thumb together and given feedback until the desired EMG activity is achieved.

2.1.2.2 Intracortical Inhibition

The ability to measure cortical inhibition is based on the principle that TMS can stimulate cortical inhibitory and excitatory interneurons, as well as the corticospinal output neurons (Rothwell, 1997). Within the primary motor cortex, GABAergic activity can be measured using paired-pulse TMS (ppTMS) approaches, which involve delivering two magnetic stimuli in close sequence to the same cortical region through a single stimulation coil.

Short-interval cortical inhibition (SICI) occurs at an interstimulus interval (ISI) between 2 to 4 ms and is thought to reflect GABA_A mediated inhibition (Kujirai et al., 1993). In this protocol, the subthreshold conditioning stimulus (CS) is set at an intensity low enough to only excite cortical interneurons but not the pyramidal neurons. This then modulates the subsequent suprathreshold test stimulus (TS) due to the influence of inhibitory GABAergic interneurons, giving a smaller magnitude of the resulting MEP. Increasing the ISI to 50-200 ms with a suprathreshold conditioning stimulus results in long intracortical inhibition (LICI) and is thought to reflect GABA_B activity (Valls-Solé et al., 1992).

In the motor cortex, a decrease in SICI (i.e. larger MEP amplitude) could mean that the cortical representation of the intended muscle has been made less prone to inhibition. Maximal inhibition of SICI is produced at ISIs of 1 and 2.5 ms (Fisher et al., 2002). SICI at an ISI of 2.5 ms reflects the activity of GABA_A interneurons (Fisher et al., 2002, Roshan et al., 2003). SICI at an ISI of 1 ms has been postulated to reflect extrasynaptic GABAergic tone (Stagg et al., 2011b), however, it is less clear exactly what this inhibition reflects. A SICI protocol using an ISI of 2.5 ms was used in Chapter 5 to assess intracortical inhibition within the motor cortex.

2.1.2.3 Interhemispheric Interactions (M1-M1)

Transcallosal projections between the two M1 hand areas are known to exist in animals (Jenny, 1979) and humans (Hanajima et al., 2001). Interactions between two homologous M1s can be investigated by applying a CS over the M1 of one hemisphere and measuring the EMG responses evoked from a TS over the opposite hemisphere with a TMS coil over each M1 hand area (Ferbert et al., 1992). Transcallosal projections are most commonly thought to be inhibitory, but facilitatory interactions also occur.

Interhemispheric inhibition (IHI) occurs over a wide range of ISIs (6-50 ms). IHI elicited at relatively short ISI (e.g. 10 ms; IHI_{10}) is thought to be mediated by different mechanisms than those elicited at longer intervals (e.g. 40 ms; IHI_{40}), but both are dependent on GABA_B-mediated neurotransmission in the target hemisphere (Kukaswadia et al., 2005). In terms of the cell populations involved, it is likely that the transcallosal projections are themselves glutamatergic, synapsing onto local inhibitory circuits within the target hemisphere (Reis et al., 2008b).

Lee and colleagues (2007) examined the effects of intracortical circuits on IHI and cortical output neurons by comparing IHI_{10} and IHI_{40} alone and when elicited in the presence of SICI or LICI. IHI_{10} , IHI_{40} , and corticospinal output were suppressed in the presence of SICI or LICI to a similar degree. These findings suggest that SICI and LICI produce relatively global inhibition, and affect the transcallosal and descending corticospinal circuits in a similar manner. It also raises the possibility that a shared population could convey each form of inhibition across the corpus callosum, with different target populations being influenced (Reis et al., 2008b).

Interhemispheric facilitation (IHF) can be elicited over a narrow range of ISIs (Ferbert et al., 1992) using specific stimulation parameters (Hanajima et al., 2001). Hanajima and colleagues (2001) showed that IHF occurred only when the delivered TS induced a current in an antero-posterior (AP) direction, suggesting that it activates the pyramidal tract via interneurons in the motor cortex consistent with I₃-wave activation. In contrast, medially directed currents activate the pyramidal tract neurons directly. To elicit facilitation, an ISI of 4-5 ms is required, which implies that the facilitatory interaction occurs approximately 10 ms after the CS, when allowing for the time taken to generate an I₃-wave in the target hemisphere (Hanajima et al., 2001). It has been shown in cats that the hand motor area has facilitatory connections with the homotopic area in the

contralateral M1, which was surrounded by a larger area of more powerful inhibition (surround inhibition)([Asanuma and Okuda, 1962](#)), and it is likely that IHF represents the activation of these facilitatory connections, whereas IHI represents this surround inhibition. However, this has yet to be definitively proven.

2.1.3 Methodological Limitations

Although TMS is a powerful tool for investigating inhibitory cortical circuits, the TMS protocols mentioned above are not entirely specific. Anatomical candidates for the various protocols exist; however, there is currently not enough evidence to confidently assign particular neuron types to them.

Excitability to the TMS pulse is likely to be highly correlated with neuronal activity, but the two may not necessarily be equivalent. For example, when a neuron is partially depolarised but has not reached firing threshold, it may appear to have increased in activity in response to the TMS pulse while not actually be physiologically active ([Stinear and Byblow, 2003](#)). Similar limitations apply to BOLD fMRI studies (see Chapter 3.4). In addition, interactions are separated into excitatory and inhibitory circuits for simplicity, but these are likely to overlap to an extent, and thus it should be kept in mind that what is being measured represents a net effect ([Reis et al., 2008b](#)). For example, interhemispheric interactions are thought to consist of excitatory transcallosal projections onto both inhibitory and excitatory local circuits within the target M1 ([Lee et al., 2007](#)).

Lastly, different behavioural states studied with TMS could condition fundamentally different cortical interactions ([Pascual-Leone et al., 1998](#)). Thus, care should be taken when making generalising conclusions on the role of inhibitory and excitatory processes beyond the behaviours specifically tested. Clarifying these interactions can give better insight into the neurophysiological basis of these stimulation paradigms.

2.1.4 Safety of TMS

Delivery of single- or paired-pulses of TMS does not appear to have any significant long-term effects in terms of changes in cortical excitability. Most of the safety concerns pertaining to the use of TMS are related to repetitive TMS (rTMS), which can produce

excitability effects that last beyond the stimulation period and have been associated with potential adverse effects such as seizures, headaches, and memory impairments (Wassermann, 1998). In general, care should be taken with medications that alter depolarisation thresholds, such as anti-depressants and anti-epileptics, and in particular drugs working on the GABAergic system, which could lengthen the refractory period immediately after the TMS pulse (Fitzpatrick and Rothman, 2000). The known side effects linked with single- and paired-pulse TMS are summarised in Table 2.2.

TABLE 2.2: **Potential side effects of TMS measures**

Side Effect	Single-Pulse TMS	Paired-Pulse TMS
Seizure induction	Rare	Not reported
Transient acute hypomania induction	No	No
Syncope	Possible, but not related to direct brain effect	Possible, but not related to direct brain effect
Transient headache, local pain, neck pain, toothache, paresthesia	Possible	Likely possible, but not reported
Transient hearing changes	Possible	Likely possible, but not reported
Transient cognitive/neuropsychological changes	Not reported	Not reported
Burns from scalp electrodes	No	No
Induced currents in electrical circuits	Theoretically possible	Theoretically possible
Structural brain changes	Not reported	Not reported
Histotoxicity	No	No
Other biological transient effects	Not reported	Not reported

The participants in this thesis are young, healthy controls that participated in single- and paired-pulse TMS only, and thus, no significant potential adverse effects are expected. Despite the minimal risks to participants, in line with current best practise guidelines (Rossi et al., 2009), participants who have had any risk factors for a decreased seizure threshold, such as a family or personal history of epilepsy, seizures, febrile convulsions, or medications that are thought to modulate the cortical excitability of the GABAergic system, were excluded from participating in any of the studies in the experimental chapters of this thesis.

2.2 Transcranial Direct Current Stimulation (tDCS)

Transcranial direct current stimulation (tDCS) is a non-invasive technique capable of modulating cortical activity in humans. Unlike other non-invasive brain stimulation (NIBS) techniques such as TMS and TES, the weak static fields induced during tDCS do not induce the rapid depolarization required to produce action potentials in neuronal membranes. Instead, weak direct current (DC) induces current flow sufficiently large enough to modify spontaneous neuronal excitability by a tonic depolarization or hyperpolarization of the resting membrane potential (Creutzfeldt et al., 1962, Purpura and McMurtry, 1965). The modifications to membrane polarity in the underlying brain tissue depend on the current density applied, which determines the induced electrical field strength, and this is defined by both the current strength and electrode size (Purpura and McMurtry, 1965). Thus, the efficacy of tDCS protocols are dependent on an interaction between: a) electrode size, b) stimulation duration, and c) current strength (Nitsche et al., 2008). It is important to note, however, that the effects of increasing current density are not linear, such that increasing stimulation periods or current amplitude can result in the reversal of the cortical excitability effects expected with shorter or less intense stimulation protocols (Batsikadze et al., 2013).

2.2.1 Practical Applications

The basic components of a tDCS setup consist of a battery operated DC stimulator and a pair of electrodes (Figure 2.1). The current is delivered by a stimulator to the brain via a pair of surface conductive rubber electrodes typically sized between 25 and 35 cm². The anode is defined as the positively charged electrode, and cathode as the negatively charged electrode. Saline soaked sponges or electrode cream can be used to place the electrodes on the head. Electrode cream can help reduce resistance and improve homogeneity of the electrical field under the electrodes. This is the preferable medium when doing concurrent tDCS in the scanner as saline sponges can often dry out during the scan duration, increasing subject discomfort. The stimulator produces a constant DC current in the order of 1 to 2 mA, and can be programmed to deliver current for a specified amount of time, typically between 10 to 20 minutes in most experimental setups.



FIGURE 2.1: **Components of a tDCS kit.** **A.** Battery operated DC stimulator. **B.** High Chloride Electrolyte-Gel for the conducting medium between the scalp and electrodes for tDCS in the scanner. **C.** Rubber electrodes and connecting wires.

To determine the ideal location for the stimulating electrode, studies have used anatomical landmarks (Stagg et al., 2011a) or functional localisation via TMS (Nitsche and Paulus, 2000). Neural elements involved in motor hand function can be located in a characteristic knob-like structure on a segment of the precentral gyrus using MRI (Yousry et al., 1997). In the intact scalp, stimulation of the hand representation within M1 is positioned approximately at C3 (left M1) or C4 (right M1), measured at 20% of the inter-aural distance, or approximately 5 cm lateral to Cz using the 10-20 electroencephalogram (EEG) system (Vines et al., 2006). The correspondence between C3 and C4 to the primary motor cortices has been confirmed by neuroimaging studies (Herwig et al., 2003, Okamoto et al., 2004). Throughout this thesis, the electrodes are placed 5 cm lateral to Cz to stimulate M1. This site has been commonly used to successfully stimulate the M1 (O'Shea et al., 2014, Stagg et al., 2011a).

2.2.2 tDCS Electrode Montages

Four tDCS conditions are described in this thesis: anodal, cathodal, bilateral, and sham stimulation. To achieve the intended electrical stimulation effects, the overall montage is important, which includes the position of the electrodes and the applied polarity. The electrode that is placed over the area of interest is commonly referred to as the 'stimulating' or 'active' electrode, and the second electrode as the 'reference' electrode. Nitsche and colleagues (2000) determined that for stimulation of the M1, the most effective montage was when the reference electrode was placed over the orbitofrontal cortex

contralateral to the treated motor cortex. An illustration of the electrode placement for the ‘real’ (anodal, cathodal, and bilateral) stimulation conditions is shown in Figure 2.2.

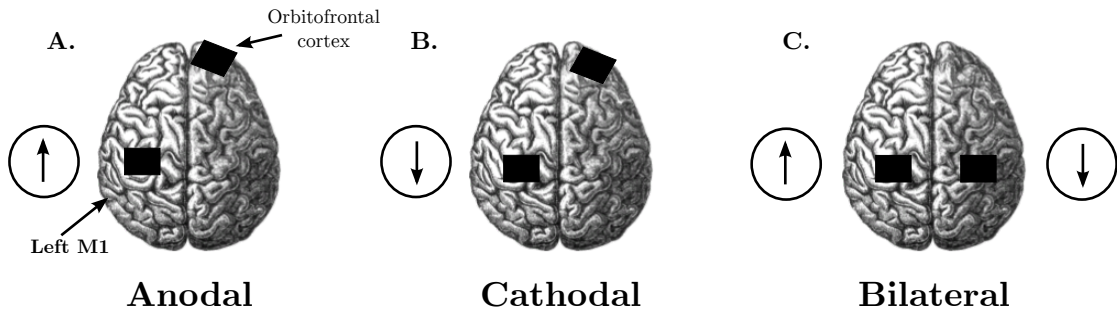


FIGURE 2.2: **Schematic of tDCS electrode placement.** A. Anodal tDCS. B. Cathodal tDCS. C. Bilateral tDCS. Arrows indicate the proposed excitability direction, upwards arrow indicate an increase in motor cortical excitability, whereas a downwards arrow indicate a decrease in motor cortical excitability.

During anodal stimulation the current flows from the stimulating electrode, placed in the area of interest, to the reference electrode. Conversely, during cathodal stimulation the current flows towards the area of interest. MEPs in the contralateral hand muscles are facilitated by anodal tDCS applied to M1 and suppressed by cathodal tDCS (Nitsche and Paulus, 2000). Thus, anodal stimulation increases cortical excitability and cathodal stimulation decreases it. Bilateral tDCS delivers both anodal and cathodal M1 tDCS simultaneously via an M1-M1 montage. During these real stimulation conditions, the current is typically ramped up and down for 10 seconds at the beginning and end of the application, respectively. This helps prevent electrical transients that can result in noticeable cutaneous sensations (itching or tingling) and retinal phosphenes (Poreisz et al., 2007).

Compared with TMS, it is easier to have double-blinded and placebo sham-controlled conditions using tDCS (Russo et al., 2013). During sham stimulation, the DC stimulator is switched off after the initial ramp-up period. With the exception of an itching or tingling sensation usually during the ramp-up period, subjects rarely experience any other sensations (Gandiga et al., 2006), and only 17% of subjects claim to feel a difference between real and sham stimulation when directly asked (Kessler et al., 2012, Poreisz et al., 2007). Although blinding may not always be effective, it is unlikely that participants would actively differentiate between stimulation conditions to significantly impact their performance. However, this could be tested directly by asking subjects in

which condition they felt they had received after each session (see for example, [Waters-Metenier et al. 2014](#)). As the experimental chapters in this thesis investigated the effects of tDCS when subjects were at rest, sham tDCS provided an effective control.

2.2.3 Physiological basis of tDCS

In animals, transcranial application of weak DC to the brain has been shown to induce excitability diminutions or enhancements in the motor cortex with long-lasting functional after effects ([Bindman et al., 1964](#), [Gartside, 1968](#)). Priori and colleagues ([1998](#)) were the first to show that a weak current (0.5 mA through a 25 cm² electrode) could modulate excitability of the human motor cortex with little or no pain, as measured by changes in the amplitude of the MEP by TMS.

Since then, Nitsche and colleagues ([2005](#), [2004a](#), [2003b](#), [2000](#), [2001](#)) have demonstrated in a series of experiments the neurophysiological effects of tDCS. The polarity specific effects were demonstrated from measurements of MEPs in the contralateral hand muscles, which are facilitated by anodal tDCS and suppressed by cathodal tDCS to M1 (Figure 2.3).

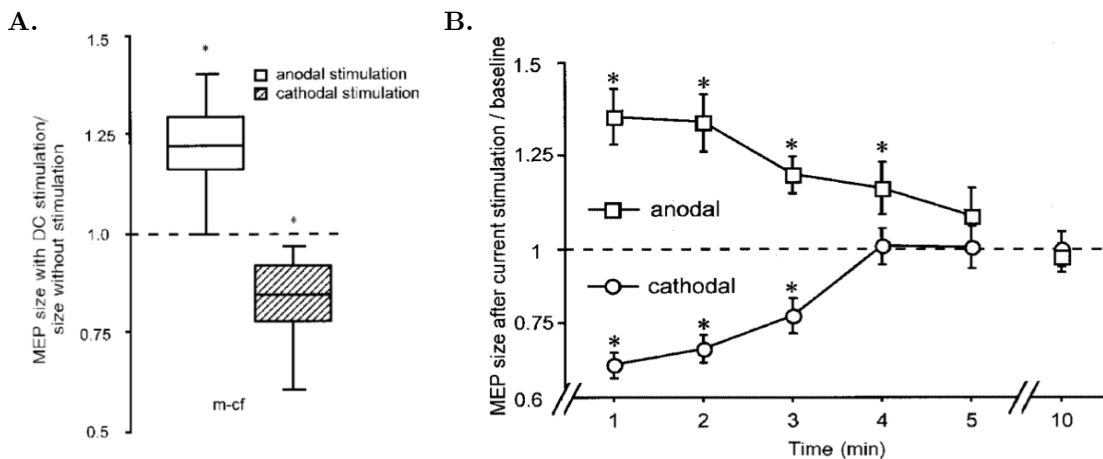


FIGURE 2.3: Polarity specific effects of tDCS. **A.** Changes in cortical excitability as measured by TMS from the abductor digiti minimi (ADM) muscle. MEP amplitudes increased with anodal and decreased with cathodal stimulation. Asterisks indicate significant differences between values with and without stimulation ($p < .05$). **B.** Time course of polarity-specific changes in motor cortex excitability. MEP amplitudes returned to baseline within 5 minutes. Asterisks indicate significant differences in MEP amplitudes after stimulation and at baseline ($p < .05$). Figures adapted from ([Nitsche and Paulus, 2000](#)).

These changes persist beyond the stimulation period and can be stable for up to an hour if tDCS is applied for 9 minutes or more (Figure 2.4) ([Nitsche et al., 2003b](#), [Nitsche and](#)

Paulus, 2001). Pharmacological manipulations probed these effects further to determine the ion channels and receptors involved. In healthy human subjects, cortical excitability shifts induced during tDCS depend on membrane polarisation that modulate the conductance of sodium and calcium channels, and the after-effects of tDCS are thought to be NMDA receptor dependent (Nitsche, 2003). Administration of Lorazepam, a GABA_A receptor agonist, selectively modulates the after effects elicited by anodal tDCS, resulting in a delayed, then prolonged excitability elevation (Nitsche et al., 2004a). Interestingly, during tDCS, neither anodal or cathodal stimulation affect measures of SICI, suggesting that tDCS may not strongly affect GABA_A interneurons during the stimulation period (Nitsche, 2005). Cathodal stimulation however, does decrease ICF, suggesting that it exerts its effects on excitatory glutamatergic interneurons, possibly in addition to GABAergic interneurons (Nitsche, 2005).

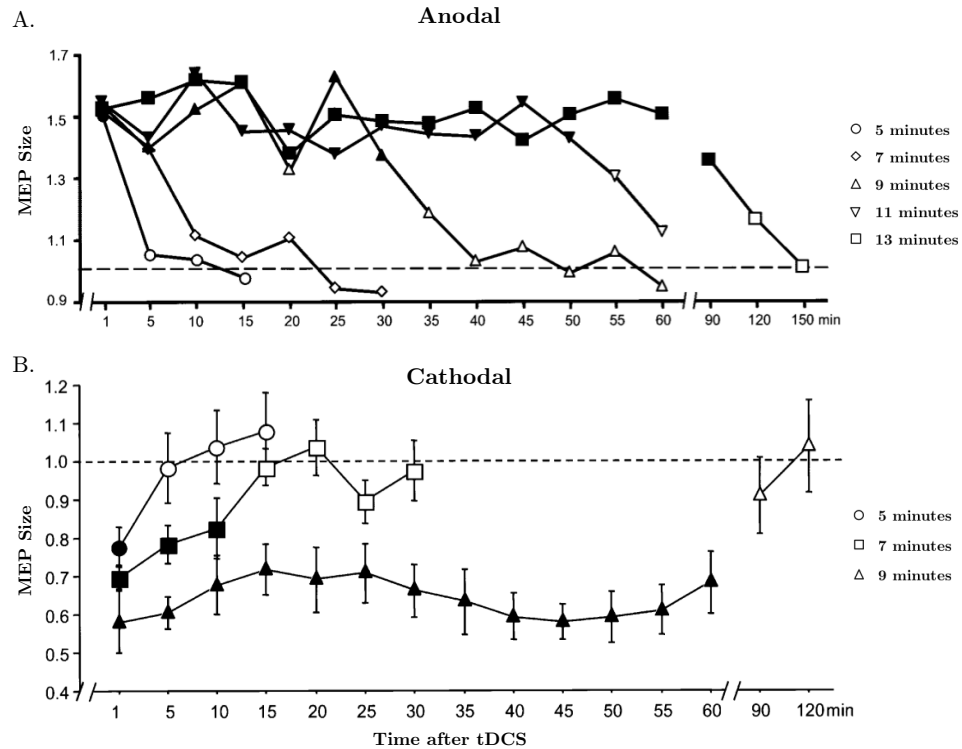


FIGURE 2.4: **Long-term effects of tDCS.** **A.** After-effects of anodal tDCS on motor cortical excitability after 5 to 13 minutes of 1 mA stimulation. After 5- and 7-minutes of stimulation, MEP amplitudes return to baseline within the first five minutes, whereas longer stimulation periods (9 to 13 min) the after-effects remain for a maximum duration of 90 minutes after the end of stimulation. Figure adapted from (Nitsche and Paulus, 2001). **B.** After-effects of cathodal tDCS. Similar to the anodal condition, after 5- and 7-minutes of stimulation the MEP amplitudes return to baseline within the first five minutes, while 9-minutes of stimulation results in after-effects remaining up to a maximum of 60 minutes after the end of stimulation. Figure adapted from (Nitsche et al., 2003b). For both A and B, filled symbols indicate the significant differences between MEP amplitudes after stimulation and baseline.

A characteristic pattern of recovery in stroke patients is the increased activation of ipsilateral motor areas during movement of the paretic hand (Johansen-Berg et al., 2002), and greater bilateral patterns of activation in poorly-recovered patients (Ward and Cohen, 2004). The re-balancing of transcallosal interhemispheric inhibition (IHI) is an intervention strategy that could explain the beneficial behavioural effects of tDCS when used in conjunction with therapy (Figure 1.7) (Hummel and Cohen, 2006, Ward and Cohen, 2004). Anodal stimulation increases excitability in the ipsilesional M1 (Hummel, 2005), whereas cathodal stimulation is thought to reduce the maladaptive IHI from contralesional to ipsilesional M1 (Duque et al., 2005, Murase et al., 2004). A large body of evidence has demonstrated the beneficial effects of anodal tDCS (Butler et al., 2013), however, evidence concerning the effects of cathodal stimulation to the contralesional M1 is more variable with some studies reporting moderate to no improvements in motor performance (Fusco et al., 2014, Stagg et al., 2012).

TABLE 2.3: **Summary of studies with bilateral stimulation in healthy controls.**
Abbreviations used: M1, primary motor cortex; RTs, reaction times; Δ MEP, change in motor evoked potential; FC, functional connectivity.

Study	Subjects	Montage	Main findings
Vines et al. (2008)	N=16 Right-handed Healthy	20 min, 1mA Anode-Right M1, Cathode-Left M1	Finger sequence task: better performance with bilateral > unilateral > sham
Mordillo-Mateos et al. (2012)	N=15 Right-handed Healthy	5 min, 2mA Anode and Cathode reversed.	Similar effects of bilateral with unilateral tDCS on the cathode side. Less robust on the anode side for bilateral tDCS compared to unilateral anodal stimulation.
Karok and Witney (2013)	N=12 Right-handed Healthy	10 min, 1.5mA Anode-Right M1, Cathode-Left M1	Reduced RTs by up to 30%; MEPs reduced by 12% in left M1 and increased by 69% in right M1.
Kidgell et al. (2013)	N=11 Right-handed Healthy	12 min, 1mA Anode-Left M1, Cathode-Right M1	No difference between bilateral and unilateral, but both reduced SICI and improved motor function on Purdue pegboard test more than sham.
Sehm et al. (2013)	N=12 Right-handed Healthy	20 min, 1mA Anode-Right M1, Cathode-Left M1	Both bi- and unilateral decreased inter-hemispheric FC during stimulation; Only bilateral increased intracortical FC within right M1 after stimulation.
Waters-Metenier et al. (2014)	N=52 Right-handed Healthy	25 min, 2mA Anode-Right M1, Cathode-Left M1	Bilateral tDCS increased learning of motor synergies - improvements in both speed and synchrony. Effects were non-specific and effector independent (generalised to untrained hand).
O'Shea et al. (2014)	N=13 Right-handed (12) Healthy	20 min, 1mA Anode-Left M1 Cathode-Right M1	Percentage Δ MEP - increased with anodal; decreased from the contralateral left hand and increased from the ipsilateral right hand with cathodal; no changes with bilateral.

It has been postulated that simultaneous application of cathodal tDCS over one motor cortex, and anodal tDCS over the other motor cortex via a bilateral M1-M1 montage could have additive effects greater than unilateral stimulation. However, despite this logic, only a handful of studies to date have investigated the effects of bilateral stimulation on the primary motor cortices, demonstrating changes in healthy controls on MEP size (O'Shea et al., 2014), learning of motor synergies (Waters-Metenier et al., 2014), motor performance (Karok and Witney, 2013, Kidgell et al., 2013, Vines et al., 2008), functional connectivity as measured by resting BOLD fMRI (Sehm et al., 2012), and motor recovery in stroke patients (Fusco et al., 2013, Lefebvre et al., 2013, Lindenberg et al., 2010, Mahmoudi et al., 2011, O'Shea et al., 2014), with heterogeneous findings. A summary of the main evidence on bilateral stimulation is shown on Table 2.3 and 2.4.

TABLE 2.4: **Summary of studies with bilateral stimulation in stroke patients.**
Abbreviations used: FM, Fugl-Meyer; WMFT, Wolf Motor Function Test; JTT, Jebsen-Taylor Test; RT, reaction time

Study	Subjects	Montage	Main findings
Lindenberg et al. (2010)	N=20 Chronic stroke	30 min, 1.5mA Anode-Ipsilesional Cathode-Contralesional	Improvements of 21% in FM and 19% in WMFT in bilateral condition compared to sham.
Mahmoudi et al. (2011)	N=10 Chronic stroke	20 min, 1mA Anode-Ipsilesional Cathode-Contralesional	Bilateral > Anodal > Cathodal > Sham tDCS on performance in the JTT (main outcome measure).
Lefebvre et al. (2013)	N=18 Chronic stroke	30 min, 1mA Anode-Ipsilesional Cathode-Contralesional	Bilateral tDCS enhanced the amount and speed of online motor skill learning compared to sham in all patients.
Fusco et al. (2013)	N=9 Subacute stroke	15 min, 1.5mA Anode-Ipsilesional Cathode-Contralesional	N=3 in each group. Sham and real tDCS counterbalanced over two consecutive days. Anodal improved manual dexterity. Cathodal no effect in force grip. Bilateral not effective.
O'Shea et al. (2014)	N=13 Chronic stroke	20 min, 1mA Anode-Ipsilesional Cathode-Contralesional	Anodal and cathodal tDCS significantly improved RT relative to sham. Bilateral did not change RT.

The assumption that bilateral tDCS induces opposing physiological effects on M1 in a simple summative fashion was directly tested by our group (O'Shea et al., 2014). In contrast to anodal and cathodal stimulation, bilateral stimulation did not change MEPs in the healthy brain, nor did it improve simple reaction times with the paretic hand in chronic stroke patients. Importantly, the bilateral montage did not combine in a simple summative fashion the constituent effects of the anode and cathode on MEPs in the healthy volunteers (Figure 2.5). Although significant changes in MEPs were measured

from either hand with the bilateral montage, correlational analysis did indicate that the bilateral montage stimulated both M1s in the expected direction, but the effects were weak and variable.

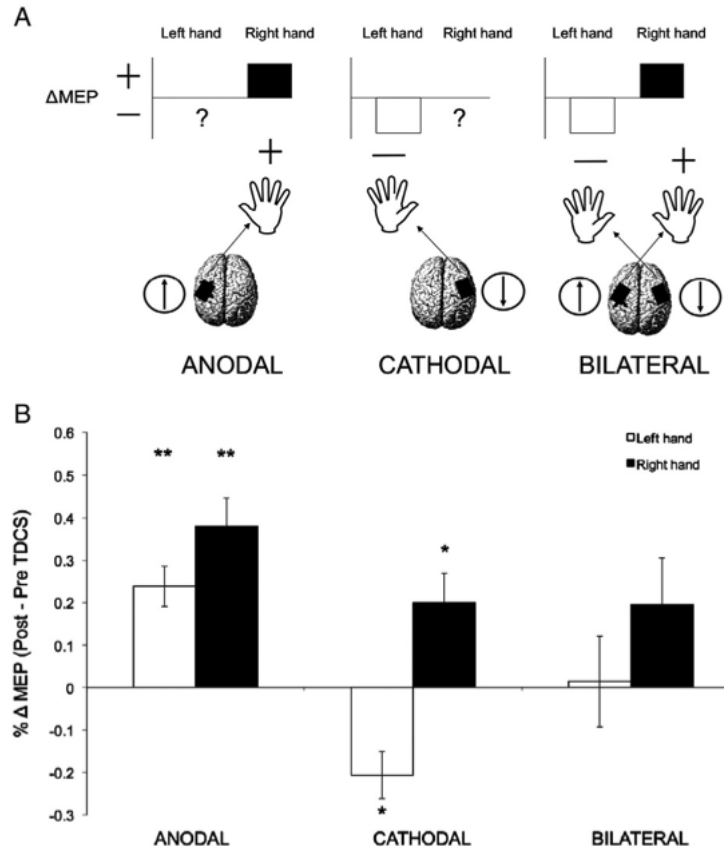


FIGURE 2.5: Comparative effects of Anodal, Cathodal, and Bilateral tDCS on motor evoked potentials (MEPs) in healthy volunteers. A. Hypothesis on the effect of each montage on MEPs. Figure illustrates the hypothesis that the constituent effects of the anode and cathode on the contralateral hand would sum together in the bilateral M1-M1 montage. **B.** Observed percentage change in the amplitude of MEPs from each hand with each tDCS montage. Anodal tDCS induced a correlated increase in MEPs from both hands. Cathodal tDCS decreased MEPs from the stimulated hemisphere and increased MEPs from the non-stimulated hemisphere, but these two effects were not correlated. Contrary to the hypothesis, the effects of bilateral tDCS were not summative, with no significant changes in MEPs from either hand. Asterisks represent significant mean changes (* $p < .05$, ** $p < .001$). Figure from (O'Shea et al., 2014).

2.2.4 Mapping the current

Unlike TMS, tDCS has limited the spatial focality due to the large electrode size. Measuring the path of current flow through the human brain in order to determine the current distribution and where the current is exerting its effects is difficult. Our current

knowledge concerning the spatial distribution of the tDCS current relies on mathematical modelling and indirect observations from animal and human studies.

Using a spherical head model, Miranda and colleagues (2006) measured the magnitude and direction of the current density of different electrode montages and found the calculated values to be comparable to those used in in vitro studies on modulation of neuronal activity (Francis et al., 2003). When the anode was placed over M1 and cathode over the right eyebrow, only 59% of the 2 mA current applied into the anode penetrated into the brain (Figure 2.6). According to this model, the current density in the brain was calculated to be in the order of 0.01 mA/cm² during a typical tDCS session (Miranda et al., 2006). In most published studies, the current density delivered is approximated to be in the range of 0.029 to 0.08 mA/cm² (Nitsche et al., 2008). These values are much lower than those with magnetic stimulation of the motor cortex with current density thresholds reaching 0.25 mA/cm² (Kowalski et al., 2002).

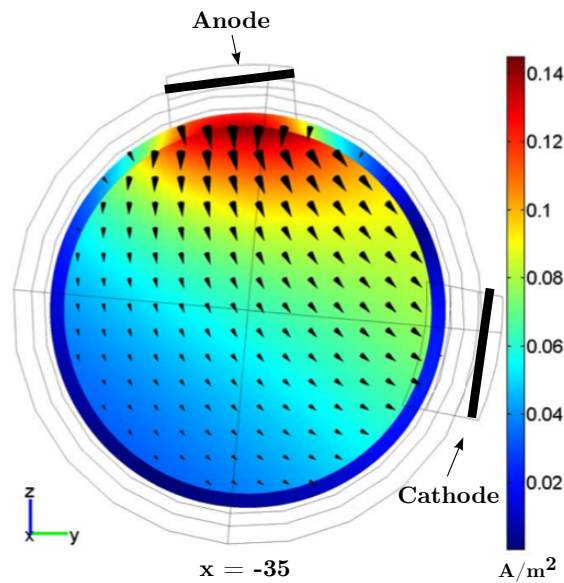


FIGURE 2.6: Spatial distribution of current density within a spherical head model. The spherical head model was positioned in MNI space and the magnitude and direction of the current density vector calculated. The stimulating current was 2 mA, electrode size 5 x 5 cm. The Anode was placed with its center radially over left M1 and cathode placed above the right eyebrow with its center 155mm anterior and 40mm lateral to Cz. Image shows the current density in the brain and skull in the sagittal plane ($x=-35$ mm), which passes through M1 and the anode, but not through the cathode. The magnitude of current density was 0.110 A/m² at M1, whereas the maximum current density in the brain in this plane was 0.145 A/m² at a position approximately 1.2 cm anterior to the electrode's center. Only 59% of the current injected into the anode penetrated into the brain. Adapted from (Miranda et al., 2006).

It is difficult to draw conclusions from spherical models as a real brain is a heterogeneous conductor and has a much more complex geometry. For example, the presence of a CSF

layer and of the dura mater were not taken into account in the spherical model, which would normally shield the brain due to shunting effects and its insulating properties (Rush and Driscoll, 1968). This would presumably lower the approximation of current density reaching the brain. The combination of these factors will have a significant impact on the current distribution between individuals, and thus, the extent to which the resting membrane potential is depolarised or hyperpolarised.

In addition, the relatively large electrode size limits the spatial resolution of the tDCS current, and adjacent cortical areas are stimulated as well as the intended site. To increase the focality of the tDCS current, studies on the motor cortex have shown excitability modifications with smaller electrodes (Boros et al., 2008, Nitsche et al., 2007). Corticospinal excitability was modulated to a similar extent to larger electrodes (Boros et al., 2008). However, the effects on intracortical inhibition and facilitation were diminished and the variability of the effects increased when the electrode size was smaller (3.5 cm² active electrode, 35 cm² reference electrode) (Boros et al., 2008). These differences could be due to greater levels of shunting of current in the scalp with smaller electrodes (Wagner et al., 2007a) and current orientation. More importantly, it also suggests that the modulatory effects of tDCS are driven by activity modulation of interconnected areas rather than by focal stimulation.

2.2.5 Safety and Ethical considerations

Extensive animal and human evidence, as well as theoretical knowledge, indicate that currently used tDCS protocols are safe (Nitsche et al., 2008, 2003a). The most commonly reported subject discomfort is a tingling under the electrodes (Brunoni et al., 2011, Poreisz et al., 2007), presumably due to electrolysis on the skin or direct electrical stimulation of the cutaneous nerves. Detailed studies assessing the safety of tDCS with currently used protocols have shown i) no evidence of neuronal damage as measured by serum neuron-specific enolase, a sensitive marker of neuronal damage (Nitsche and Paulus, 2001), ii) no edema or alterations of the blood-brain barrier or cerebral tissue as assessed by contrast-enhanced and diffusion-weighted MRI (Nitsche et al., 2004b), iii) no pathological waveforms as assessed by EEG or worsening of neurophysiological measures (Iyer et al., 2005), and iv) no heating effects under the electrode (Nitsche and Paulus, 2000).

tDCS is an attractive tool for use in the clinical setting as well as for manipulating brain activity in healthy humans as it is relatively safe and inexpensive. However, the increased accessibility of tDCS is accompanied by several ethical considerations and risks. In addition to applications in the clinic, concerns have been raised for its premature use in the developing brain ([Kadosh et al., 2012](#)), military and security services ([Sehm and Ragert, 2013](#)), and do-it-yourself public use ([Fitz and Reiner, 2013](#)). As the method is still in its infancy, tDCS devices are not currently covered under existing regulatory frameworks either in the USA ([FDA, 2012](#)) or in the European Union ([EuropeanCommission, 2012](#)). Shaping regulatory policy will be dependent on the development of robust safety standards and proper understanding of the underlying neurobiological mechanisms of tDCS on both the healthy and injured human brain.

Chapter 3

Methods for imaging the primary motor cortices

This chapter describes the main magnetic resonance techniques used in this thesis: magnetic resonance spectroscopy at 3 T and 7 T, and resting state functional connectivity imaging. The methods, advantages, and technical challenges are discussed.

3.1 Magnetic Resonance Imaging (MRI)

The origins of magnetic resonance imaging (MRI) started in 1946 when Felix Bloch proposed a set of equations to model the dynamics of the magnetisation produced by properties of the atomic nucleus in a magnetic field. The Bloch equations are still the basic equations used to understand the magnitude of the MRI signal. MRI involves imaging of the proton, the positively charged spinning nucleus of hydrogen atoms (^1H) that are abundant in tissues containing water, proteins, lipids, and other macromolecules.

3.1.1 Magnetization

Because of its spin and charge, the proton has a magnetic moment that can exhibit resonance behaviour in a magnetic field. This magnetic resonance effect, or precession, is governed by the Larmor equation - a linear relationship between the resultant resonance

frequency (ω_0) in megahertz and the magnetic field (B_0) experienced by the nucleus in Tesla:

$$\omega_0 = \gamma \times B_0$$

The relative scaling γ is the gyromagnetic ratio in megahertz per Tesla (MHz/T), and is a nucleus-specific constant. Other atoms such as phosphorus (^{31}P) and carbon (^{13}C) also have magnetic moments. However, because hydrogen's nucleus contains no neutrons, it has a gyromagnetic ratio (42.57 MHz/T) much higher than, for example, phosphorous (10.71 MHz/T) and carbon (17.25 MHz/T). In addition, hydrogen atoms are also present at high concentrations in the brain, making it the best candidate for an MR-sensitive isotope for imaging of the brain.

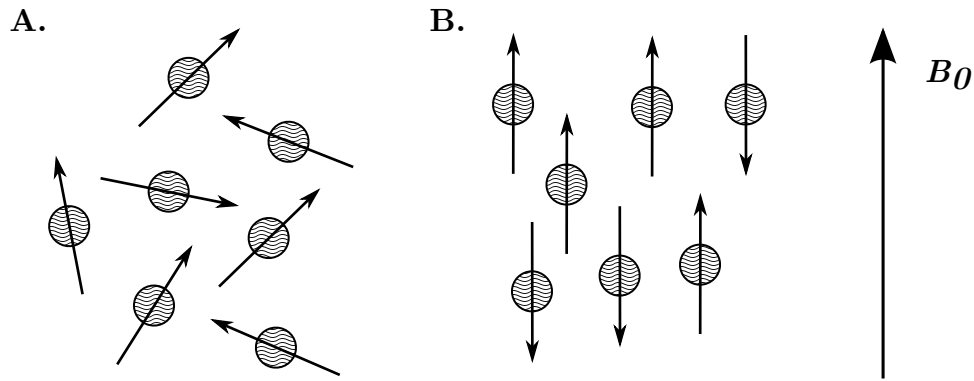


FIGURE 3.1: **Spins of atoms.** **A.** Randomly oriented protons in their natural environment. **B.** Protons aligning to an external magnetic field (B_0)

In their natural environment, atoms will spin around their own axes (Figure 3.1.A). In a magnetic field, these magnetic moments are polarised. Most protons will align themselves parallel to B_0 in a low-energy state, and some will align anti-parallel to B_0 in a high-energy state (Figure 3.1.B). The balance between the parallel and anti-parallel nuclei is termed the net magnetization (M_0). This can be visualised as a vector, with a set of axes called x, y, and z. The z-axis is always pointing to the direction of the main magnetic field (B_0), with x and y at right angles to the z-axis (Figure 3.2). At higher magnetic fields, the energy required to align anti-parallel to B_0 is greater, resulting in a larger net magnetization.

3.1.2 Excitation

Producing M_0 does not produce any measurable signal as a constant magnetic field produces no currents. According to Faraday's laws of induction, if a conductive coil is placed in a moving magnetic field, a current is produced in that coil. Thus, if M_0 is tipped away from B_0 to the x-y plane by using a radiofrequency (RF) pulse, coils along the x- and y- axis will show oscillating signals as the magnetic flux of the dipole sweeps across the coils. For the coil along the z-axis, the flux is constant so no current is induced (Figure 3.2). As a result of this excitation, the hydrogen nucleus absorbs the energy of the pulse.

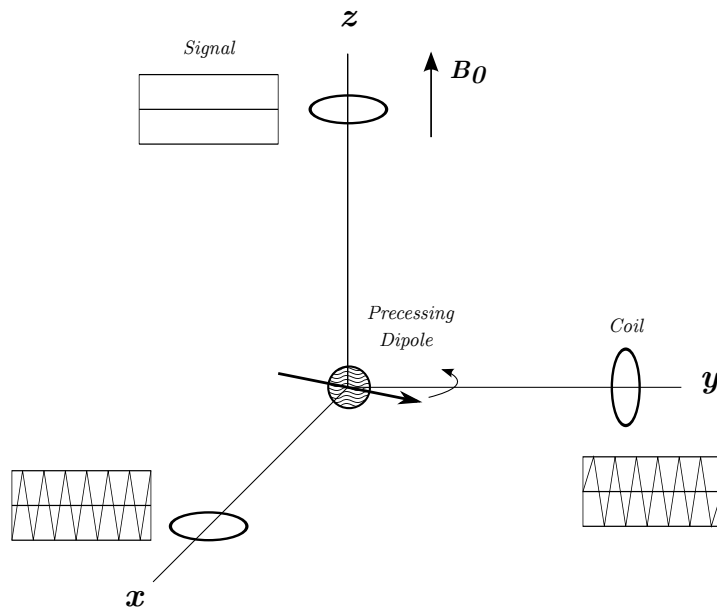


FIGURE 3.2: **Induction signal in coils.** For a dipole rotating in the x-y plane after a 90° RF pulse, coils along the x- and y-axis show oscillating signals as the magnetic flux of the dipole sweeps across the coils. For the coil along the z-axis, the flux is constant, and so no current is induced.

The RF pulse results in an oscillating magnetic field B_1 perpendicular to B_0 , and this oscillates at the proton precession frequency as determined by the Larmor equation. The B_0 field is the constant, homogenous magnetic field that is used to polarize spins, and creates magnetization. The B_1 field is the RF energy field that is applied perpendicular to the B_0 to perturb the magnetization in some manner. Most RF pulses flip the net magnetization 0° to 180° , and this parameter, called the flip angle, can be modified to determine the amount of degrees the net magnetization is rotated. After this excitation, the protons subsequently release the absorbed energy and 'relax' back to their original alignment with B_0 .

3.1.3 Relaxation

During the relaxation process, the amount of magnetization in the longitudinal plane gradually increases (recovery), while the magnetization in the transverse plane decreases (decay). This process is termed the free induction decay (FID), where ‘free’ relates to free precession, ‘induction’ is the process where a varying magnetic field produces a current in a coil, and ‘decay’ indicates the loss of signal. The rate at which this occurs is determined by the T_1 and T_2 relaxation times (Figure 3.3).

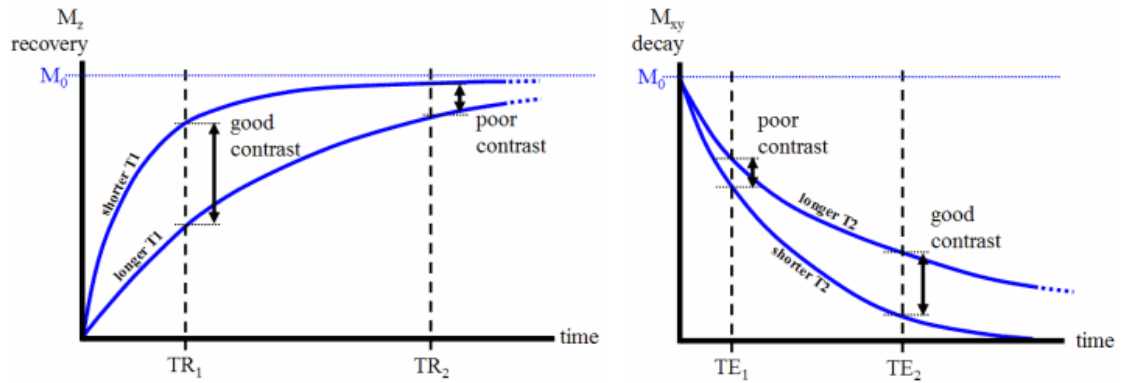


FIGURE 3.3: **The MR signal.** The signal increases with longer TR in a way that depends on T_1 and decreases with increasing TE in a way that depends on T_2 . Maximum T_1 -weighted contrast between tissues occur when the TR is approximately equal to T_1 . Maximum T_2 -weighted contrast occur when the TE is approximately equal to T_2 .

T_1 relaxation, or the spin-lattice relaxation, is the time constant defining the rate of recovery of magnetisation along the longitudinal (z) axis following an RF pulse (i.e. the net magnetisation rotates back to align itself with the z-axis). Not all protons within a particular volume will relax back to the longitudinal axis in the same way. For example, hydrogen atoms may be bound very tightly in fat tissue and less tightly in water, such that the tightly bound protons will release their energy much quicker to their surroundings (i.e. shorter T_1). Thus, each tissue type within the brain will relax at different rates, and this gives the contrast between tissue types seen in MRI (Figure 3.4).

T_2 relaxation, or the spin-spin relaxation, is the time constant defining the irreversible loss of magnetisation, and therefore signal, in the transverse (xy) plane following excitation. In tissue, T_2 happens much faster than T_1 , often by an order of magnitude. Loss of T_1 and T_2 relaxation are two independent processes, which happen simultaneously.

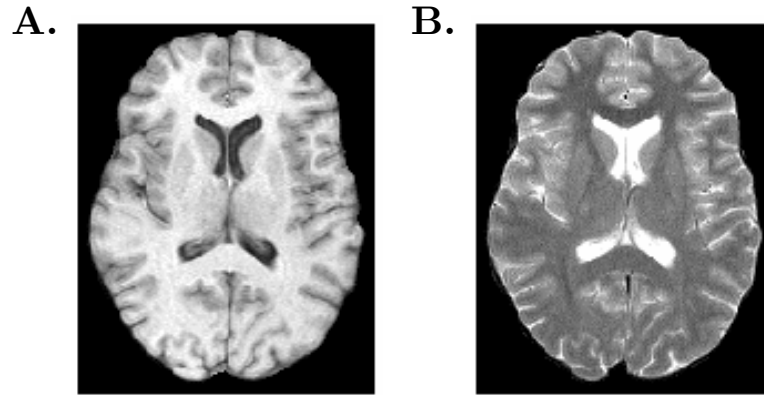


FIGURE 3.4: **MRI contrast.** **A.** T_1 -weighted image. Fat appears bright due to its high signal and water appears dark due to its low signal. **B.** T_2 -weighted image. Fat appears dark and water appears bright. Figure adapted from <https://rcbi.rochester.edu>.

T_2^* relaxation is the decay of the FID following an RF pulse. In physiological tissue, the transverse relaxation is more rapid due to local field inhomogeneities. T_2^* effects describe the faster decay of T_2 relaxation due to local magnetic field inhomogeneity and chemical shifts. In the brain, the size of these inhomogeneities depends upon its physiological state and in particular, the composition of the local blood supply.

3.1.4 Acquisition

Pulse sequences are a sequence of events required to acquire MR images, which are made up of RF pulses, periods of recovery that generate the MR image, and the signal readout. Two important components are the repetition time (TR) and echo time (TE). The TR is the time between repeated excitations of a given component of the magnetization. The TE is the time between the excitation of magnetization and the acquisition of signal. These components are illustrated in Figure 3.5.

If the amplitude of B_1 is modified, the frequency selectivity of the pulse, and therefore the slice profile, can be improved. The slice profile is the response of spins through the thickness of the slice, such that the most desirable is a perfect rectangle as this suggests that the slice has a well-defined thickness and a sharp edge. In practice this cannot be achieved, but by allowing B_1 to vary in frequency as well as amplitude, such as with an adiabatic RF pulse, the slice profile approaches a rectangle, albeit increasing the duration of the RF pulse (Tannús and Garwood, 1997).

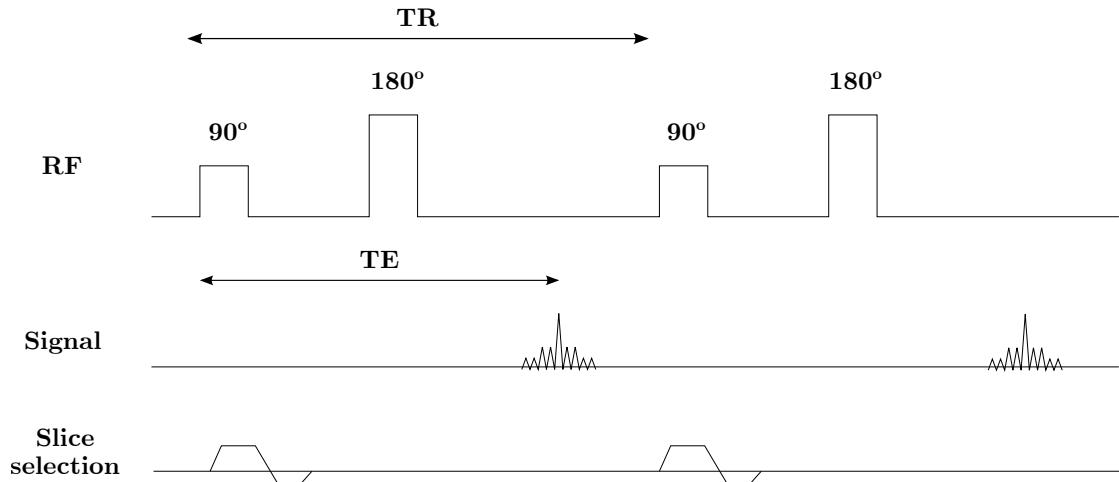


FIGURE 3.5: **Components of a pulse sequence.** Abbreviations used: RF, radiofrequency; TR, repetition time; TE, echo time.

3.1.5 Safety

Unlike other imaging techniques, MRI does not involve the use of ionising radiation and is relatively safe. Concerns on the potential biological effects of MRI primarily arise from i) strong static (B_0) magnetic fields, and ii) time-varying (gradient) fields, and iii) radiofrequency (B_1) fields ([Durbidge, 2011](#)).

Extensive safety guidelines have been developed to prevent the physical hazards of MRI. The main hazard is ferromagnetic materials such as iron, which can be strongly magnetised when placed in a magnetic field and experience a force that can turn the object into a dangerous projectile, in addition to severely distorting the image. Thus, ferromagnetic materials are always excluded from the vicinity of the MR system and participants are screened carefully to ensure that they have no ferromagnetic materials in their body ([Shellock and Crues, 2004](#)).

During MR procedures, mild sensory effects from static B_0 fields have been reported, such as vertigo, nausea, and taste sensations, and are dose-dependent with increasing field strength ([Schenck, 2000](#)). The main biological effect of time-varying magnetic fields generated by the gradients is peripheral nerve stimulation. These effects can cause some discomfort but are not thought to be harmful. To date, in the absence of foreign ferromagnetic bodies, there has been no replicated scientific study showing a health hazard associated with cumulative exposure to magnetic fields ([Schenck, 2000](#)).

The main effect of RF exposure is tissue heating. Arguably this is the greatest cause for concern as it is under the control of the MR operator and the particular pulse sequence design. However, the amount of heating is typically restricted to less than 1°C by monitoring and limiting the specific absorption rate (SAR) - the rate at which energy is absorbed by the body when exposed to an RF field. This risk is increased when wires or electrodes, such as those used to connect to stimulation devices, are placed in the scanner with the participant. In these instances, care is taken to prevent loops from forming in the wires, and that the wires are suitably protected and are not in contact with the participant.

3.2 Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance spectroscopy (MRS) allows the quantification of endogenous metabolites from a localised region (or voxel) of tissue. The MRS signal is derived from differences in the molecular structure of metabolites. If the MR signal from the voxel of interest is plotted as a function of its frequency, characteristic patterns of peaks, relating to individual molecular structure, can be seen (Figure 3.6). The magnitude of these peaks is related to the concentration of the neurochemicals from which they arise. The spectrum can thus be used to quantify individual neurochemicals within the volume of interest (VOI).

3.2.1 Basic principles of *in vivo* spectroscopy

The goal of *in vivo* MRS is to quantify biochemical substances non-invasively. The amplitude of the signal from an MRS acquisition, and the resultant peaks in the reconstructed spectrum, are directly related to the number of resonating nuclei and amount of the substance measured. Two factors determine the field variations in the frequency shifts of nuclei: the chemical shift and J-coupling.

The chemical shift refers to the changes in MR frequency due to the chemical environment surrounding the nucleus. When the nucleus is part of a chemical compound, the chemical environment around the atomic nucleus results in a shielding of B_0 by the surrounding electrons. The effect of this electronic shielding is field dependent, and thus, so are the resultant frequency shifts of the molecules in Hertz. If the resonance frequency

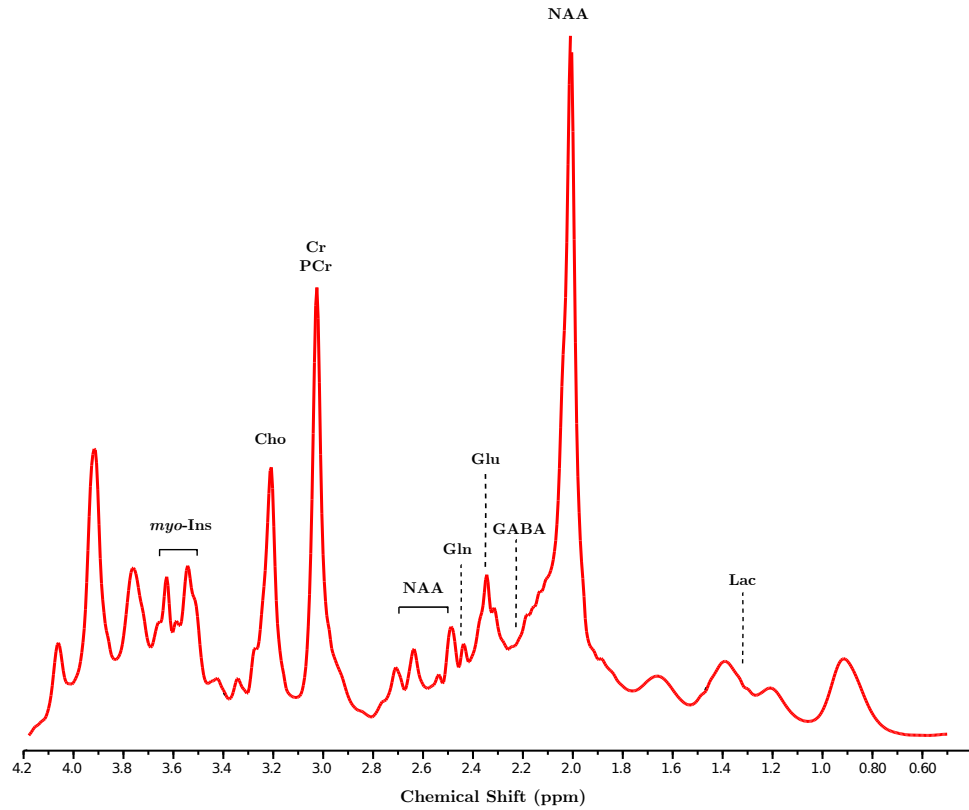


FIGURE 3.6: **Example spectra.** An LCModel output of a representative spectra from a single subject at 7 Tesla.

is calculated relative to a reference frequency, the derived chemical shift becomes independent of the applied B_0 , which facilitates comparison of spectra at different B_0 field strengths (Stagg and Rothman, 2014). ^1H chemical shifts *in vivo* are observed in the order of parts per million (ppm).

In addition to the surrounding chemical environment, the spins of neighbouring nuclei can also affect the electronic shielding of the nucleus. J-coupling provides information on the connectivity of molecules and describes the interaction between two nuclear spins that is mediated through the chemical bonds that connect them. Both the chemical shift and J-coupling rely on the molecule's chemical and geometric composition - it is these attributes that make up the 'spectroscopic fingerprint' of the biochemical, and form the basis of the MRS signal.

3.2.2 Quantification of spectra

The experimentally measured spectrum acquired is the combined information from all observable metabolites, which need to be reconstructed to quantify the individual

metabolite concentrations (Figure 3.7). Practically, this is made difficult due to the broad linewidths and limited spectral dispersion with *in vivo* data - the spread of the observed resonance peaks of most metabolites of interest are in the range of 1 - 4 ppm (Govindaraju et al., 2000). Furthermore, due to J-coupling, most resonances are multiplets (i.e. resonances seen as multiple peaks due to indirect spin-spin coupling), and the resonance lines often overlap. Given the significant spectral overlap between the metabolites, most quantification methods aim to reproduce the overall spectrum by using a scaled linear combination of the potential individual metabolite contributions (Provencher, 2001).

There are two main approaches for referencing the quantification of spectra: external and internal reference methods. The external reference method applies identical settings of an *in vivo* experiment on an *in vitro* solution that contains metabolites of known concentrations. However, as the reference signal comes from a different spatial region to the VOI, the intensity is affected by inhomogeneities in the RF field and therefore requires additional scans to map and correct for the RF field (B_1) inhomogeneity. Practically, internal reference methods are more often used, which normalise the signals to a known or assumed constant concentration within the same scan. The most commonly used reference signal is the total creatine (Cr+PCr) made up of creatine (Cr) and phosphocreatine (PCr), the concentration of which is relatively stable under normal physiological conditions (Pouwels and Frahm, 1998). Other signals often used are *N*-acetylaspartate (NAA) or water. However, NAA can be reduced in certain pathologies (Bruhn et al., 1989, Kantarci et al., 2004), and the theoretical risk of oedema with DC stimulation make referencing to water unsuitable for the experiments in this thesis. Thus, the total creatine is used as the reference compound.

Quantification errors are typically reported as Cramér-Rao lower bounds (CRLB), which describe the lowest possible standard deviations on the variance of unbiased parameter estimates that can be obtained from the data (Cavassila et al., 2001). However, it is possible for the CRLB value to be low even if the data quality is poor or the concentration estimates are unreliable, and thus, an additional quality control metric such as the linewidth and SNR should also be used (Kreis, 2004). Voxel homogeneity is measured by the linewidth (the full-width at half-maximum; FWHM) of the water resonance, measured in Hertz. The experiments in this thesis use the combination of visual inspection

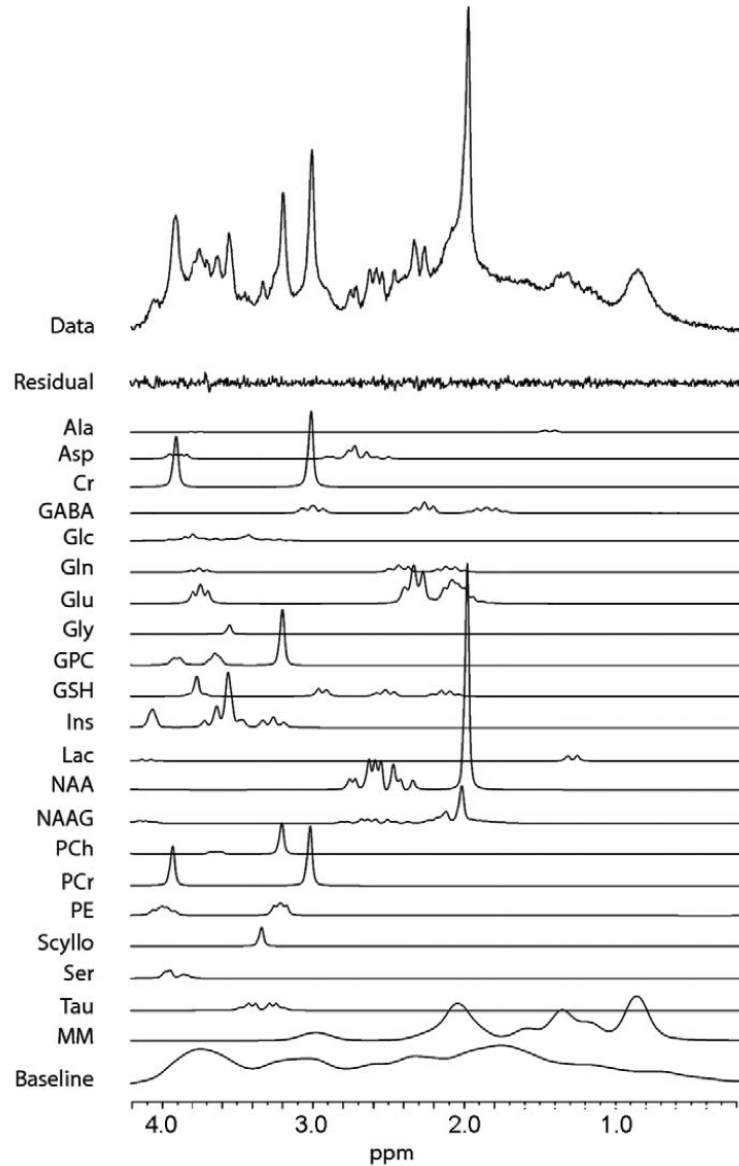


FIGURE 3.7: **Components of a spectra.** A typical model spectra (LCModel) is shown on the top row. The residual signal, and individual fits for all metabolites detected are shown in the following rows. Metabolites with lower concentrations in the brain, such as GABA, are reflected by the relatively small amplitude. Adapted from (Stagg et al., 2011b).

of the spectra, CRLB, linewidths, and SNR to determine spectral quality and as criteria to exclude spectra of poor quality.

Analysis of spectra requires prior knowledge of the ‘basis spectra’ (i.e. the basis-set), which gives information on the spectral fingerprint of the potentially detected metabolites, and is typically derived experimentally by measuring metabolites in solution. The basis spectra is specific for the particular MRS experiment as it relies on the B_0 field strength and details of the sequence, such as the RF pulses used, gradient scheme, and

sequence timing, all of which determine the exact magnitude of the spectral metabolite signals.

In addition to ^1H -MRS, other MR-visible isotopes have also been shown to give valuable information on tissue physiology and biochemistry. For example, phosphorus (^{31}P) MRS allows the quantification of components of energy metabolism (ATP, ADP, and phosphocreatine), and has been used to study bioenergetics deficits in disease (Befroy and Shulman, 2011). Intermediates of the Krebs (TCA) cycle can be studied using carbon (^{13}C) MRS, giving important information on metabolic fluxes (Shulman et al., 1979). However, these isotopes require specialised hardware and MRS methods. The spectroscopy methods used in this thesis will only concentrate on ^1H -MRS at both 3 T and 7 T.

3.2.3 Water suppression

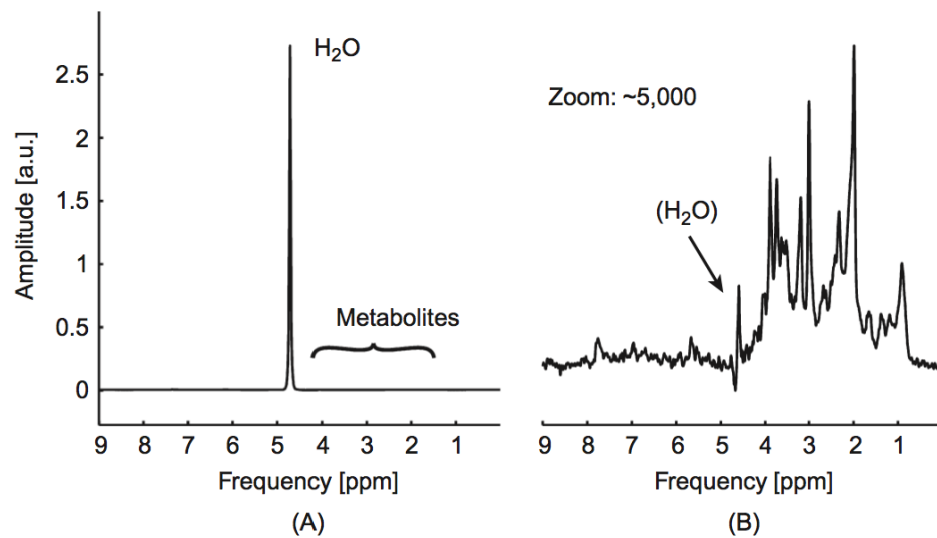


FIGURE 3.8: **Water Suppression.** **A.** Unsuppressed spectra showing the water signal. **B.** Water suppressed spectra revealing the metabolites of interest. Figure from (Stagg and Rothman, 2014).

In the healthy human brain, water concentrations are estimated to be 83% for gray matter, and 70% for white matter (McIlwain and Bachelard, 1985). In contrast, concentrations of detectable brain metabolites are 10^3 to 10^4 times smaller. Water protons in tissue *in vivo* resonate at 4.65 ppm. If unsuppressed, the large water peak overlaps almost all relevant metabolite peaks and distorts the spectral baseline, making quantification of metabolites impossible. Here, differences in the chemical shifts of water

and brain metabolites are used to selectively excite the water resonance by a frequency-selective RF pulse (Figure 3.8.A). The water signal is then minimized by phase-spoiling gradients, while the metabolite signals remain unaffected (Figure 3.8.B).

3.2.4 Outer volume suppression

Lipids and fat resonances that originate from the cranial bone have signal strengths 100 to 1000 times stronger than the detectable brain metabolites. This can distort the baseline of spectra, or obstruct metabolites. Practically, when measuring from the primary motor cortex, it is important to place the voxel without the inclusion of any dura mater that surrounds the brain, in order to minimize contamination by extra-cranial lipids. Lipids resonate at 1.3 ppm, and this signal can be suppressed using outer volume suppression (OVS). Typically, OVS uses a frequency-selective RF pulse in the presence of a magnetic field gradient to excite the unwanted magnetisation from outside the VOI before it is removed by phase-spoiling gradients.

3.2.5 The neurochemical profile detected by MRS

Although a number of brain metabolites can be identified with ^1H -MRS (Govindaraju et al., 2000), the number of substances that can be reliably detected *in vivo* are in the 15-20 range or below (Emir et al., 2011, Tkáč et al., 2009). Generally, metabolites that exist in a concentration above $0.5\mu\text{mol/g}$ can be detected *in vivo* with ^1H -NMR spectroscopy (Duarte et al., 2012). The neurochemical profile detected is region specific (Emir et al., 2011, Pouwels and Frahm, 1998), reflects functional states (Mangia et al., 2007), and can be affected by pathological conditions (Lei et al., 2009).

As discussed in Chapter 1.3, GABA plays an important role in human cortical plasticity (Bachtiar and Stagg, 2014), and thus, it is the primary metabolite of interest of this thesis. Other metabolites of interest include: Glutamate, *myo*-inositol, and Creatine. Studies have demonstrated higher concentrations of these metabolites in grey matter than in white matter, with no differences between the hemispheres (Pouwels and Frahm, 1998). The concentration ranges of the main metabolites detected in the normal adult human brain measured from solution are shown in Table 3.1.

TABLE 3.1: **Concentration ranges for metabolites in a normal adult human brain.** Human average range in mmol L⁻¹. * Brain tissue density = 1.05 g mL⁻¹.
Table adapted from (de Graaf, 2008).

	Abbreviations	Range *
Ascorbic acid	Asc	0.5 - 1.5
Aspartate	Asp	1.0 - 2.0
γ -aminobutyric acid	GABA	1.0 - 2.0
Creatine (total)	Cr	4.5 - 10.5
Glucose	Glc	1.0 - 2.0
Glutamate	Glu	3.0 - 6.0
Glutamine	Gln	6.0 - 12.5
Glutathione	GSH	1.5 - 3.0
<i>myo</i> -inositol	mI	4.0 - 9.0
<i>scyllo</i> -inositol	sI	0.2 - 0.5
Lactate	Lac	0.2 - 1.0
N-acetylaspartate	NAA	7.5 - 17.0
N-acetylaspartylglutamate	NAAG	0.5 - 2.5
Phosphocreatine	PCr	3.0 - 5.5
Phosphorylcholine	PCho	0.2 - 1.0
Taurine	Tau	2.0 - 6.0

Glutamate is the primary excitatory neurotransmitter in the cortex. At the synapse, neuronal glutamate typically has a focused excitatory effect. In contrast, extrasynaptic glutamate can originate through diffusion from the neuronal synapse but can also be released from astrocytes (Vizi et al., 2010). Once released into the extrasynaptic space, glutamate can have multiple effects on different systems, such as modulating blood flow and plays a role in neuronal synchronisation (Rodriguez et al., 2013). At lower field strengths (< 4 T), with standard sequences, glutamate is usually reported as Glx, a composite marker made up of the glutamate and glutamine complex. At higher field strengths (> 4 T), the increased separation of peaks allows glutamate and glutamine to be dissociated.

The primary function of glutamine is in recycling glutamate that has been released during neurotransmission. Glutamine is synthesised from glutamate by glutamine synthetase in glia and converted back to glutamate in neurons by glutaminase, completing the glutamate-glutamine cycle (Shen, 2013). As glutamate is a precursor to GABA,

the glutamate-glutamine cycle is also relevant to the GABA transmitter system. (see Chapter 1.3).

Inositol has several isomers, and two of the main contributors to the MRS signal are *myo*-inositol and *scyllo*-inositol. *Myo*-inositol has been reported to be increased in several neurological disorders such as dementia (Kantarci et al., 2004), Rett syndrome (Horská et al., 2009), and multiple sclerosis (Bitsch et al., 1999). *In vitro* studies have measured higher *myo*-inositol levels in cultured astrocytes compared to neurons, and it is presumed to be a marker of gliosis (Brand et al., 1993, Glanville et al., 1989). However, in the brain, *myo*-inositol levels do not always correlate with other molecular markers of gliosis (Kim et al., 2005). One study measured the changes in brain *myo*-inositol with anodal tDCS to the right frontal lobe and found a significant increase of *myo*-inositol content at 30 minutes after stimulation offset (Rango et al., 2008). However, the small sample size, use of a non-standard tDCS montage, and lack of controls make these findings difficult to interpret.

The total creatine signal as measured by MRS is made up of creatine and phosphocreatine (Cr + PCr). Creatine has a relatively homogeneous distribution throughout the cortex, but is more concentrated in grey matter (6.4 - 7.0 mM) than in white matter (5.5 - 5.7 mM) (Pouwels and Frahm, 1998). It can be thought of as a putative neuronal marker as its concentrations are assumed to be relatively stable in the brain under normal and many pathologic states (Stagg and Rothman, 2014).

3.2.6 Measuring GABA *in vivo*

In the human brain, quantifying GABA is less straightforward than for some other neurochemicals due to its low concentration and the overlap between its signal and those of other molecules found in higher concentrations such as creatine and NAA. Since the pioneering work of Rothman and colleagues (1993), it is now possible to measure GABA *in vivo* in the human brain using MRS. Spectral editing methods are a common way to reduce signal overlap in order to reveal the GABA signals at lower field strengths (< 4 T). One of the most widely applied editing methods to measure GABA is the MEscher-Garwood-Point RESolved Spectroscopy (MEGA-PRESS) sequence (Mescher et al., 1998). The MEGA-PRESS sequence is used at 3 T in Chapter 4 of this thesis.

A limitation of this approach, as with all editing methods, is its reliance upon the subtraction of signals to remove strong overlapping frequencies from the spectrum in order to reveal GABA (Puts and Edden, 2012). This can be sensitive to any experimental noise such as subject movement or instrumental factors, which can then obscure the edited GABA signal (Waddell et al., 2007). Furthermore, some protein containing macromolecules (Behar et al., 1994) resonate at a frequency coupled to a resonance near the GABA peak. Thus, at lower field strengths, the edited GABA peak can include contributions from those macromolecules. However, the variation of macromolecules is likely to be low between subjects when compared with the variation of GABA, and therefore it is unlikely that this contamination provides a practical difficulty when interpreting MRS results (Kreis et al., 2005).

One important limitation of measuring GABA using MRS is that it quantifies the total signal arising from a particular neurochemical within the voxel of interest. In the case of GABA, this means it is not possible to differentiate between different pools of GABA. It is therefore unclear how the measured GABA concentration relates to local synaptic activity, or the differentiation between vesicular or cytoplasmic pools of GABA.

In a recent study, it was demonstrated that there was no significant relationship between MRS-assessed GABA concentration and TMS measures of GABA_A and GABA_B synaptic activity (Stagg et al., 2011b) (see section 2.1 for more details). However, a significant relationship was observed between MRS-assessed GABA and a TMS measure proposed to reflect extrasynaptic GABAergic tone (Stagg et al., 2011b). It is important to note, that this relationship was demonstrated at baseline, and thus, it cannot be inferred from this result that the changes in GABA levels with plasticity induction protocols are due solely to changes in extrasynaptic GABAergic tone. Rather, it is likely that induced changes in MRS-assessed GABA levels are due to modulation of both synaptic and extrasynaptic activity.

3.3 Ultra High Field MRS

Many of the limitations of quantifying GABA *in vivo* in the human brain can be improved with increasing magnetic field strength above 4 Tesla (Tkáč et al., 2009). There are many advantages to MRS at ultra high field (UHF) including i) increased SNR,

ii) increased chemical shift dispersion, and iii) better spectral resolution from far less contamination of the spectrum and improved editing of macromolecular contributions (Otazo et al., 2006). Increased precision of metabolite quantification and detection of weakly represented metabolites can be gained at 7 T (Deelchand et al., 2014), with a 14% increase in spectral resolution at 7 T relative to 4 T (Tkáč et al., 2009).

The gains with increasing field strength are highly dependent on the experimental conditions, the region of interest, and to an extent individual subject differences, and are therefore difficult to determine precisely. There are two main advantages of high field MRS relevant to this thesis. The first is to exploit the increased SNR to measure from more than one voxel in the brain, which gives a unique ability for concurrent quantification of metabolites from two voxels of interest (Lemke et al., 2013). The second advantage is the improved quantification of the primary metabolite of interest, GABA, without the use of editing methods (Gruetter et al., 1998). In Chapter 6, we develop and assess a novel two-voxel spectroscopy sequence to quantify GABA concurrently from both human primary motor cortices.

The gains in quantification precision with increasing field strength, however, are not without several limiting factors. The main technical complexities include increased B_0 shimming and RF power requirements (Deelchand et al., 2010, Di Costanzo et al., 2003), which is discussed in turn below.

3.3.1 B_0 inhomogeneity and shimming

As magnetic field inhomogeneities can have a dramatic effect on the linewidths of the spectra, the sensitivity improvements gained at UHF would not be possible unless the field within the measured VOI is homogeneous enough. The spectral linewidth, which is a function of B_0 over the VOI, is arguably the most critical parameter affecting the precision and accuracy of metabolite quantification. At UHF, the increased magnetic susceptibility results in shorter T_2^* , and further broadening of the linewidths (Blamire et al., 1996).

Shimming is the process of optimizing B_0 homogeneity over the region of interest. This is particularly important in spectroscopy as it directly affects the linewidths of the peaks

and affects SNR. In single-voxel spectroscopy, localised B_0 shimming can improve homogeneity within the VOI selected, but can lead to variability of B_0 outside of the VOI (Blamire et al., 1996). Thus, when measuring from more than a single voxel it is necessary to compensate for these field differences with proper shimming of each individual VOI. Dynamic shim updating (DSU) can be used to compensate for these field differences. In this method, shim corrections are updated in real time almost instantaneously between slice acquisitions to give the optimum shim for each VOI (Blamire et al., 1996).

However, it should be noted that even with perfect B_0 shimming, the spectral linewidth gets broader at UHF due to the shorter T_2 relaxation times and increased tissue-dependent susceptibility effects caused by paramagnetic species in close proximity to the VOI. For example, deoxygenation of haemoglobin produces deoxyhaemoglobin, a significantly more paramagnetic species of iron, which disturbs the local magnetic field and leads to large magnetic susceptibility effects, which results in a broadening of the spectral linewidth. Typical linewidths are in the range of 10 Hz but will increase with increasing B_0 (Tkáč et al., 2009).

3.3.2 RF considerations

For both MR imaging and spectroscopy, it is also desirable to have a homogeneous RF (B_1) field that covers the entire VOI. Standard volume RF coils are typically used with conventional MR scanners and provide sufficiently homogenous RF fields. However, volume coils require increased RF power due to the larger amount of volume to cover. A risk to this increased power is the corresponding increase in RF energy that is absorbed by the tissue and then converted to heat. As the rate of this energy absorption, the SAR, needs to be restricted in humans ($< 1^\circ\text{C}$ increase), the design of the RF pulse and pulse sequence becomes important. Furthermore, with increasing B_0 , RF wavelengths are shortened, and thus, the RF field generated by standard volume coil varies over space. This non-uniformity is named the ‘dielectric resonance’, and can complicate imaging and spectroscopy at UHF (Ibrahim et al., 2001).

Critically, with increasing field strength, spectral dispersion becomes larger and this makes it difficult to achieve sufficient pulse bandwidth without exceeding SAR limits. One method to ameliorate this in spectroscopy is with the use of broad-band adiabatic RF pulses, which vary the frequency and amplitude of the RF pulse so the slice profile

approaches a rectangle. In this way, adiabatic pulses are able to generate homogenous excitation even when B_1 is extremely inhomogeneous (Tannús and Garwood, 1997). Adiabatic pulses are a feature of the semi-Localised by Adiabatic Selective Refocusing (sLASER) sequence (Öz and Tkáč, 2010) used in Chapters 6 and 7.

The excitation profile of RF pulses can affect the chemical shift displacement error (CSDE). The CSDE is an artefact that reflects differences in the spatial encoding of metabolites based on the particular metabolic chemical shift. As signals with different chemical shifts experience different slice selections, the combination of the bandwidth, RF pulse, the chemical shift of the metabolite of interest, and the strength of the slice selective gradient, all determine the exact slice selection for every signal. In spectroscopy, the CSDE leads to metabolite contributions that originate from partially differing spatial positions, and thus, pulse sequence designs will aim to minimise this error (Scheenen et al., 2008). This is particularly important with increased field strengths, as this will increase spectral dispersion, and so too the CSDE.

3.3.3 Dielectric Pads

With standard volume coils, higher B_1 signal can be seen in the centre of the human head compared with voxels in the periphery (Webb and Collins, 2010) (Figure 3.9). B_1 inhomogeneity at high fields can also result in increased spectral linewidth within areas of low signal intensity. These regions of low signal intensity can make already difficult to measure metabolites such as GABA undetectable under regular scan conditions. To date, none of the available methods can perfectly correct for this non-uniformity. Several methods have been proposed, however, require significant changes and optimization to hardware (Avdievich et al., 2009, Webb and Collins, 2010).

Recent developments have made use of high permittivity dielectric pads, which have been shown to improve B_1 sensitivity relatively locally within the VOI, without the need for changes to hardware (Teeuwisse et al., 2012, 2011). Dielectric materials can be thought of as an electrical insulator - a poor conductor of electricity, but an efficient supporter of magnetic fields. When a dielectric material is placed in an electrical field, electric charges do not flow through the material as they do with a conductor. Thus, they can be an effective method to improve image quality in regions with low RF transmit efficiency (Teeuwisse et al., 2012).

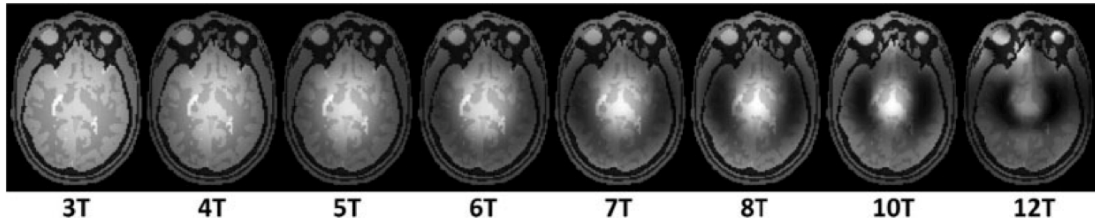


FIGURE 3.9: **Field inhomogeneity.** Simulated images as a function of field strength using a volume coil with ideal current distribution in the rungs. The signal is calculated as the product of the transmit and receive fields. With increasing field strength, higher B_1 is demonstrated in the centre of the human head (e.g. putamen, substantia nigra and caudate) compared with voxels in the periphery (e.g. frontal, occipital and motor cortices). Figure from (Webb and Collins, 2010).

Nevertheless, the patterns of improvement can vary depending on the shape of the pads and geometry of the participant's head (See Chapter 6). The fluidity of the pads and deformation under mechanical stress can be a practical drawback as the pressure of the head could locally lead to alterations in the thickness of the pads. Improvements in pad design, such as partitioning the dielectric material into smaller compartments could minimise these effects. As solutions such as this are increasingly being developed for many of the field-dependent technical limitations, high field MRS will see greater neuroscience applications in the future.

3.4 Resting State Functional MRI

Determining a baseline state is fundamental to understanding complex systems and how this system can be modulated. Over the past decade attention has turned to the existence of spontaneous activity as measured by functional MRI (fMRI) (Raichle et al., 2001), which exhibit spatial patterns that resemble discrete cortical functional networks (Beckmann et al., 2005). These patterns can be grouped into resting state networks (RSNs) and are thought to reflect the intrinsic properties of functional brain organisation that complement both the underlying structural connectivity of the brain (Zhang et al., 2010) and the functional anatomy of the regions related to task performance (Fox et al., 2005, Mennes et al., 2010), albeit with its own limitations in its interpretation (Buckner et al., 2013). Figure 3.10 illustrates the eight most commonly reported RSNs (Beckmann et al., 2005). Of particular interest to this thesis is the sensorimotor system RSN, which include activations in the pre- and post-central gyri, and the SMA (Figure 3.10.d). This pattern of activation corresponds closely to that seen in bimanual motor tasks (Biswal

et al., 1995) and are robust across individuals and imaging modalities (De Luca et al., 2006).

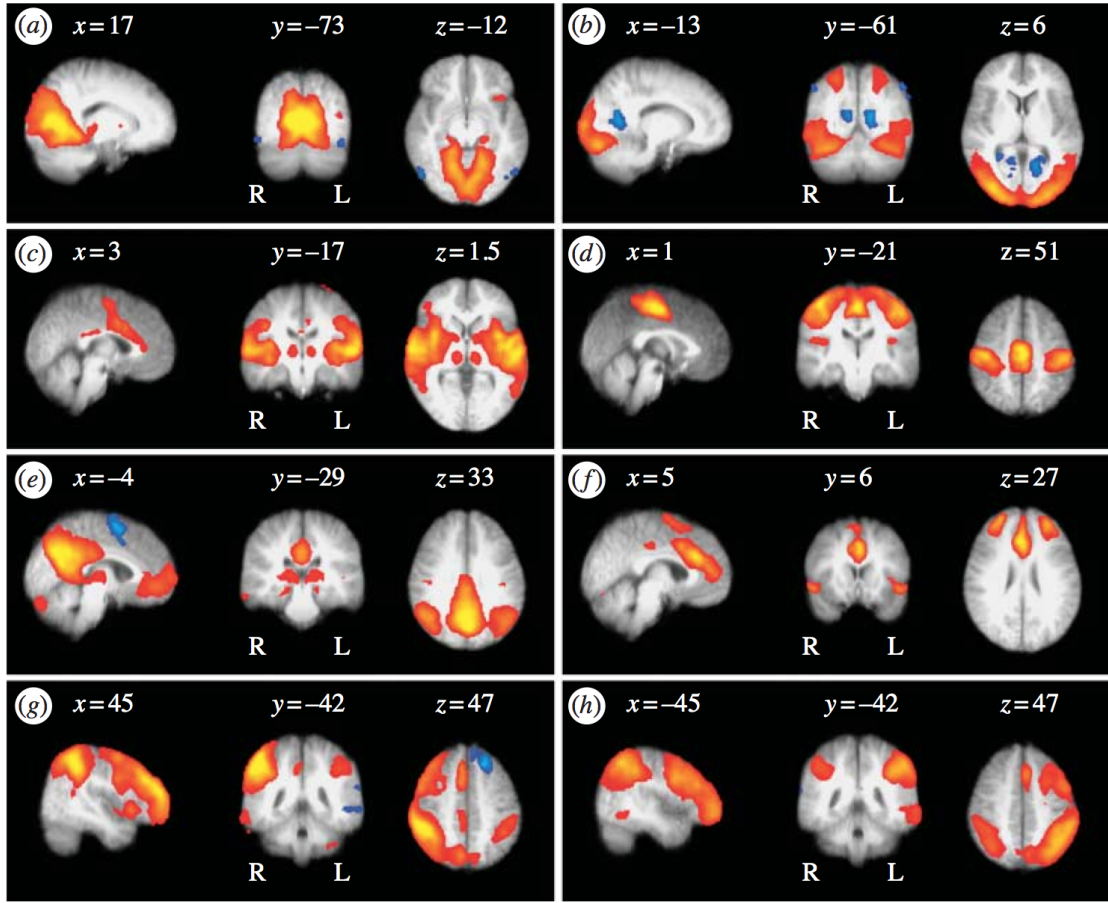


FIGURE 3.10: **Resting state networks.** Images of identified resting state networks. (a) medial visual cortical areas, (b) lateral visual cortical areas, (c) auditory system, (d) sensorimotor system, (e) default mode network (f) executive control (g) right dorsal visual stream, (h) left dorsal visual stream. Figure from (Beckmann et al., 2005).

3.4.1 The BOLD signal

The blood-oxygen-level-dependent (BOLD) contrast is the mechanism by which blood flow levels can be used as a marker of neural activity that can be related to the measured T_2^* MR signal. In the brain, the size of local field inhomogeneities depends on its physiological state and local blood supply. The BOLD contrast mechanism alters the T_2^* effects through changes in the relative concentration of oxygenated and deoxygenated blood. Unlike oxygenated haemoglobin (Hb), deoxyhaemoglobin (dHb) is an iron-containing molecule and is paramagnetic, such that it is attracted by an externally applied magnetic field and will align in the direction of the applied magnetic field, and thus, influence the MR signal (Logothetis, 2003).

The underlying physiology of the BOLD signal is not completely understood (Khader et al., 2008, Logothetis and Wandell, 2004, Raichle and Mintun, 2006). The main conclusion from this large body of work show that the electrical correlates of the fMRI BOLD signal is best related to local field potentials (LFPs) (Lauritzen, 2001, Thomsen et al., 2004). LFPs are complex signals arising from electrical fields recorded from microelectrodes in the brain. They are thought to reflect the weighted average of input signals on the dendrites and cell bodies of neurons in the vicinity of the electrode. Electrical brain activity recorded from the scalp with electroencephalogram (EEG) constitutes of a summation of a population of LFPs, conventionally described in terms of their band-limited frequency components (delta, 1-4Hz; theta, 4-8Hz; alpha, 8-12Hz; beta, 12-24Hz; and gamma, > 24Hz). Within LFP activity, slow cortical potentials (SCPs; < 4Hz) exhibit frequencies similar to that of spontaneous BOLD fluctuations (Birbaumer et al., 1990, He et al., 2008). SCPs and their BOLD counterpart reflect spatially coherent, rhythmic variations that likely represent inherent fluctuations in cortical excitability (Fox et al., 2007, Lauritzen et al., 2012).

Activity-induced changes in blood flow are often thought of as an increase in oxygen consumption (Buxton et al., 2004). However, studies have shown that the increase in oxygen consumption is much less than the increase in blood flow and glucose consumption (Mintun et al., 2001, 2002). Current evidence suggests that changes in blood flow and metabolism are related to the synaptic activity of glutamate and its recycling through astrocytes (Raichle and Mintun, 2006). An increase in glycolysis results from the uptake of glutamate into astrocytes from excitatory synapses (Magistretti et al., 1999), and it has been suggested that astrocytes may be the site for a blood flow-sensing mechanism (Kasischke et al., 2004) that is responsible in mediating the blood flow responses critical to the genesis of the BOLD signal (Takano et al., 2006). These observations are further supported as astrocytes are the sole repository of glycogen in the brain (Brown, 2004) and are uniquely involved in the coordination of the metabolic and circulatory requirements that are associated with changes in brain function and blood flow (Magistretti et al., 1999).

Interestingly, in contrast to glutamate, the exposure of GABA to astrocytes does not result in a burst of glycolysis (Chatton et al., 2003). These findings could suggest that a BOLD-based change in fMRI signal would not be associated with a change in

GABA-mediated inhibition. However, the interpretation of blood flow and its relationship to oxygen and glucose metabolism, with regards to GABAergic neurotransmission is still unclear. Studies that can show changes clearly attributable to GABAergic neurotransmission, coupled with quantitative measurements of the relevant variables of the BOLD signal, such as cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMRO₂), will be important in distinguishing these effects. A recent attempt to establish this suggests that the dynamic contribution of excitation and inhibition to the control of metabolic and haemodynamic responses is non-linear and cannot be simply assigned to a negative or positive BOLD signal alone ([Lauritzen et al., 2012](#)).

3.4.2 Analysing spontaneous BOLD data

The aim of fMRI studies of spontaneous activity is to limit any changes in sensory input and prevent subjects from making responses or performing cognitive tasks. Typically, BOLD fMRI is acquired during continuous resting-state conditions where participants are asked to lie still and either fixate on a cross hair presented on a screen or lie awake with their eyes closed.

An important consideration for analysing spontaneous activity is the potential contamination by artefacts, such as scanner instability or non-neuronal physiological fluctuations. For example, spontaneous BOLD fluctuations have been observed in a water phantom ([Zarahn et al., 1997](#)), and fluctuations in cardiac ([Wise et al., 2004](#)) or respiratory ([Birn et al., 2006](#)) activity can account for a significant fraction of the variability in spontaneous BOLD. Several methods have been developed to tackle this problem. One strategy is to measure physiological parameters during BOLD acquisition and then remove this signal from the data using linear regressions ([Birn et al., 2006](#)). Alternatively, potential noise sources can be isolated from the BOLD data itself, by regressing out the signals that are common to all voxels ([Macey et al., 2004](#)) or signals from regions with a high degree of physiological artefact relative to neuronal activity such as the ventricles and white matter ([Zarahn et al., 1997](#)). More data driven approaches such as independent component analysis (ICA), use algorithms to decompose the data into components and thereby automatically isolating the sources of noise ([Griffanti et al., 2014](#)).

The main techniques for identifying spatial patterns in BOLD activity include i) seed-based analysis, ii) cluster-based analysis, and iii) independent component analysis. The

simplest, seed-based analysis, extracts the BOLD time course from a region of interest ('seed' region) and determines the temporal correlation between the seed and the time course from all other brain voxels. Although simple and relatively easy to interpret, this approach relies heavily on an a priori definition of a seed region and does not allow multiple systems to be studied simultaneously. In cluster-based analysis, the hierarchical clustering of seed regions still requires a priori definitions, however, time courses are extracted from many seed regions and a correlation matrix is constructed. A clustering algorithm determines which regions are more closely related, and which are more distantly related. This approach allows for a topological map to be generated and can be useful for visualising connections.

Alternatively, ICA does not require an a priori definition of seed regions ([McKeown et al., 1998](#)). Instead, the entire BOLD data set is analysed and composed into statistically independent components. In task-based fMRI studies, a general linear model (GLM) analysis is commonly used to model the behaviour tested into a design matrix. This is similar to the time course matrix in ICA, except that instead of having to specify an a priori design matrix and then estimating effect size parameters in the GLM, ICA estimates both the matrix and the effect sizes simultaneously from the data. In this way, statistically independent source signals can be derived from a set of random variables ([Beckmann, 2012](#)). Each component is associated with a spatial map, such that some maps reflect neuro-anatomical systems and others reflect noise components. The advantages of an ICA approach are that it is data driven and automatically isolates sources of noise. Given its powerful model-free, data driven approach, ICA is used in the analysis of resting state BOLD data in Chapter 4.

3.4.3 Independent component analysis

ICA can be applied to resting state fMRI data to estimate inherent spatial patterns of functional connectivity ([Biswal et al., 1995](#), [Fox and Raichle, 2007](#)). These spatial patterns, or functional RSNs, characterize baseline activity of the human brain in the absence of an externally stimulated neuronal activity. To generate RSNs via ICA, the Multivariate Exploratory Linear Optimised Decomposition into Independent Components (MELODIC) tool, part of the FMRIB software library (FSL) can be used ([Beckmann and Smith, 2004](#)). To apply the ICA decomposition (Figure 3.11), the entire

4-dimensional data set is first rearranged into a 2D matrix by arranging all the voxels for each time point into a single row. This data set is then decomposed into two new matrices containing: i) a time course of an underlying signal in each column, and ii) a spatial component map in each row. The associated time course describes how each of the underlying effects contributes to the measured data at each point in time, and represents the ‘signal signature’ of the data (Beckmann, 2012).

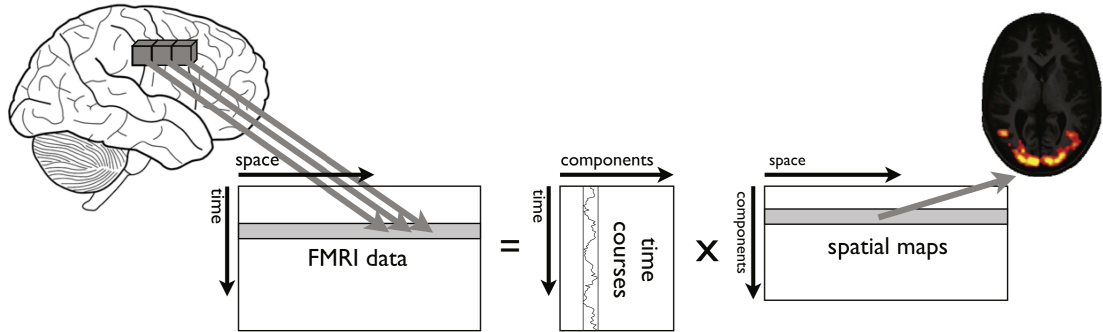


FIGURE 3.11: **Independent Component Analysis (ICA)**. Schematic of the data representation and spatial decomposition performed by ICA or BOLD fMRI data. Figure from (Beckmann, 2012).

Typically, the data set is decomposed into 25 components, a value derived from an estimation in an initial analysis of the population using model order approximations (Beckmann and Smith, 2004). From this, the RSNs of interest are selected using spatial correlations against a set of previously defined maps (Beckmann et al., 2005). Next, the dual regression approach is used to identify the subject-specific temporal dynamics and the associated spatial maps within each individual’s data set (Filippini et al., 2009, Stagg et al., 2014). Here, the group-defined RSNs are used as a regressor in a separate GLM analysis to extract individual time series of the BOLD signal from each subject. These individual timeseries are then regressed into the same dataset to extract the subject-specific spatial maps. The component maps are collated into a single 4D file, with the fourth dimension being each subject’s particular component map. The dual regression approach is a powerful method to quantify connectivity within an entire RSN, while controlling for the influence of other RSNs (Smith et al., 2014).

By measuring the intensity, or the magnitude of spontaneous BOLD fluctuation within a particular node of a RSN of interest, its strength in functional connectivity, or the coherence of that particular area can also be measured. However, how the magnitude of spontaneous BOLD fluctuations should be interpreted is ambiguous. For example,

the relationship between the magnitude of spontaneous BOLD to its regional metabolic parameters, such as glucose or oxygen consumption are still unclear.

3.4.4 Limitations

The concept of functional connectivity borrows complex methodologies and frameworks from physics, mathematics, and engineering, and apply these concepts to the analysis of physiological activity ([Fingelkurts et al., 2005](#)). This makes it difficult to interpret, as ultimately it is an indirect, relative measure of fluctuations described as representative markers of latent neural activity. In other words, the regional variations in hemodynamic activity may not necessarily reflect direct causal interactions between them. Despite the difficulties in interpretation, it is becoming clear that spontaneous BOLD patterns are an intrinsic property of the brain as opposed to the result of unconstrained mental activity ([Fox et al., 2007](#)). Due to the relative ease by which resting data can be collected, resting functional connectivity MRI is an attractive tool for studying the inherent activity of the brain at rest. However, it should be combined with other physiological data to confirm and interpret its findings.

Chapter 4

Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation

This first experimental chapter investigated the effects of anodal tDCS on M1 GABA and resting state functional connectivity, and, for the first time, test the relationship between the two measures. Novel evidence on the timecourse of GABA change from anodal tDCS is presented, and highlights that the decrease in GABA is most prominent after the stimulation period. In line with previous studies, baseline GABA levels were negatively correlated with the strength of functional connectivity within the resting motor network. However, although we confirm the previously reported findings that anodal tDCS reduces GABA concentration and increases functional connectivity in the stimulated motor cortex, these changes are not correlated, which suggests they may be driven by distinct underlying mechanisms.

4.1 Introduction

Brain stimulation techniques such as transcranial direct current stimulation (tDCS) show particular promise as methods to non-invasively modulate activity of the underlying

cortex. Anodal tDCS, when applied to the primary motor cortex (M1) has been shown to increase cortical excitability and induce LTP-like plasticity (Nitsche and Paulus, 2000, Stagg and Nitsche, 2011). Dependent on stimulation parameters, application of anodal tDCS to M1 can improve motor performance and motor learning behaviour in healthy controls (Nitsche et al., 2003c, Reis et al., 2008a, Stagg et al., 2011c) and after stroke (Adeyemo et al., 2012, Stagg et al., 2012). If applied over multiple days, the beneficial behavioural effects can outlast the stimulation period by months (Reis et al., 2009), strongly highlighting its potential as an adjunct therapy in stroke. However, despite this promise, the utility of tDCS is limited as the neurobiological mechanisms underlying these beneficial changes are unclear. Here we investigate the effects of anodal tDCS on the neurochemicals within the stimulated cortex, and further, how this affects how M1 interacts with the rest of the motor network.

Resting state fMRI is sensitive to spontaneous, low-frequency fluctuations in the BOLD signal that are thought to reflect the brain's intrinsic functional architecture (Fox and Raichle, 2007). The degree of connectivity within the motor network has been demonstrated to be modulated in a number of clinical conditions (Westlake and Nagarajan, 2011, Woodward et al., 2014) and after plasticity-inducing interventions such as motor learning (Albert et al., 2009). tDCS has also been shown to have significant effects on network-level functional connectivity at rest (Amadi et al., 2014, Peña-Gómez et al., 2012, Sehm et al., 2012). Recently, the degree of connectivity within these functional networks has been demonstrated to be negatively correlated with GABA levels within a major network node (Kapogiannis et al., 2013, Stagg et al., 2014).

Anodal tDCS has previously been shown to decrease M1 GABA levels (Stagg et al., 2011a, 2009a), with changes lasting at least 20 minutes after stimulation has ceased (Stagg et al., 2009a). In addition, we have demonstrated that anodal tDCS to the left M1 increased the strength in network-level functional connectivity across the motor network, as indexed by resting state fMRI (Stagg et al., 2014). These results suggest that anodal tDCS may exert its beneficial behavioural effects via modulation of local GABA levels, and hence widespread connectivity changes.

In this study we aimed to extend these findings in a number of important ways. We wished to determine the changes in M1 GABA during tDCS, which to date, has not been measured. Additionally, we aimed to determine the duration of the previously reported

GABA decrease after stimulation, which will have important implications for the design of future clinical studies. We hypothesised that, as previously reported anodal tDCS would decrease M1 GABA and increase functional connectivity in the motor RSN. We also wished to directly test the relationship between the effects of tDCS on GABA and functional connectivity in the motor resting state network (RSN).

4.2 Methods

4.2.1 Participants

Twelve healthy participants (4 male; aged 22-28 years, mean 24 years) gave their informed consent to participate in this study in accordance with ethical approval from the East London Research Ethics Committee (Ref: 10/H0703/50). All participants were right-handed as assessed by the Edinburgh Handedness Inventory ([Oldfield, 1971](#)).

4.2.2 Experimental design

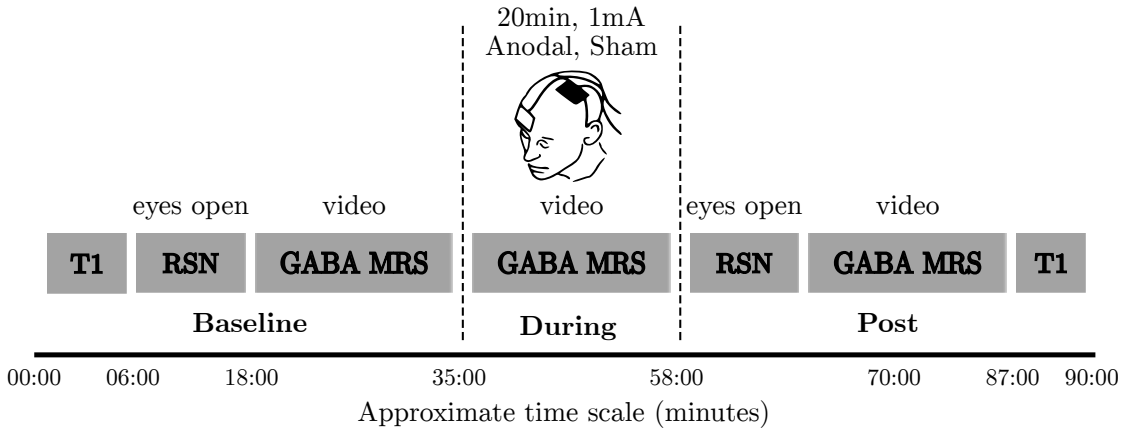


FIGURE 4.1: **Experimental design.** All subjects participated in two experimental sessions separated by at least 1 week. Subjects received anodal or sham tDCS in a counterbalanced order. GABA was measured at three time points (baseline, during, post) and resting state connectivity was measured at two time points (baseline, post). Timeline shows an estimate of the length of the scans in minutes. Abbreviations used: T1, T₁-weighted structural scan; RSN, resting state scan; GABA MRS, GABA-edited spectroscopy.

All subjects participated in two testing sessions with either anodal or sham tDCS. The sessions were separated by at least one week and the order of the sessions was counterbalanced across the group. In each experimental session, GABA measurements were

acquired at three time points, at baseline, during, and post-tDCS. Measures of resting state connectivity were acquired at baseline and after post-tDCS (Figure 4.1).

4.2.3 Transcranial direct current stimulation (tDCS)

A DC-Stimulator (Magstim, Ltd; UK) delivered a 1mA current to the brain via electrodes measuring 5 x 7 cm (EasyCap, GmbH; Germany). One electrode was centred over the left M1 and positioned 5 cm lateral to mid-pre-central position (Cz). The reference electrode was placed over the contralateral supraorbital ridge. High Chloride Electrolyte-Gel (EasyCap, GmbH; Germany) was used as the conducting medium between the scalp and electrodes. The electrodes contained 5 k Ω resistors and extension leads connected the stimulator, which was located outside of the magnetic field, to the subject positioned in the scanner. For anodal stimulation, the current was ramped up over 10 seconds and was then held at 1mA for 20 minutes, before being ramped down over 10 seconds. For sham stimulation the DC-stimulator was ramped up for 10 seconds then switched off, as previously described (Stagg et al., 2011a).

4.2.4 Magnetic resonance acquisition

All data were acquired on a 3T Siemens Verio scanner. T₁ weighted images (MPRAGE, 192x1mm axial slices, TR/TE=2040/5ms, flip angle=8°, FOV 200x181) were used to place a 2x2x2cm voxel of interest over the left precentral knob, a known landmark for the hand motor representation (Yousry et al., 1997).

Edited GABA spectra were acquired using the MEGA-PRESS sequence (TR/TE=2000/68ms, 144 averages, TA=4:56 blocks) with 20ms double-banded Gaussian inversion pulses for simultaneous spectral editing and water suppression (Mescher et al., 1998). The water suppression band was set to a frequency of 4.7 ppm, and an editing band alternated between 1.9 ppm (edit on) and 7.5 ppm (edit off) in even and odd acquisitions. Spectra were acquired in blocks of 4.56 minutes. Three blocks were acquired at baseline, four blocks during tDCS, and three blocks post-tDCS.

A multiband 2mm isotropic echo-planar imaging (EPI) acquisition (TR/TE=1300/40ms, FOV=212x212mm, bandwidth=1814Hz/Px, multi-band acceleration factor 6, voxel dimension=2mm isotropic, whole brain, acquisition time=6:42min for a total of 300 volumes) (Feinberg et al., 2010, Moeller et al., 2010) was performed before and immediately after tDCS. Subjects fixated on a cross-hair image presented centrally on the screen during resting fMRI acquisition and watched a nature video at all other times.

4.2.5 Magnetic resonance spectroscopy (MRS) analysis

MRS data pre-processing was performed using in-house scripts, including removal of motion-corrupted averages, frequency drift and zero and first order phase corrections. To increase signal to noise, data from subsequent blocks within each time period were then averaged together, resulting in GABA measurements from 9:52 minute acquisitions for the following time points: Baseline1, Baseline2, During1, During2, During3, Post1 and Post2.

MRS analysis was performed on the combined spectra using jMRUI v2.2 (<http://www.mrui.uab.es/mrui/>). As is standard, first, the residual water signal was removed using a Hankel Lanczos singular value decomposition (HLSVD) filter. Second, creatine and NAA linewidths were obtained from the first non-edited acquisition for each time point using AMARES - a non-linear least square fitting algorithm (Vanhamme et al., 2001). The creatine linewidth was used to constrain the linewidths of the GABA and glutamate/glutamine (Glx) resonances from the edited spectra. Both GABA and Glx resonances were fitted with 2 Gaussian peaks. A single Gaussian curve was fitted to the NAA resonance and was constrained to the linewidth of NAA in the non-edited spectrum. Spectra with an NAA linewidth of greater than 10Hz were excluded from further analysis. Two spectra from different sessions in the same subject were excluded in this way.

FMRIB's Automated Segmentation Tool (FAST), part of the FMRIB software library (www.fmrib.ox.ac.uk/fsl), calculated the relative quantities of grey matter and white matter within the voxel of interest on the T₁-weighted structural scan as previously reported (Stagg et al., 2011a). The amplitude of GABA peaks were corrected for the proportion of grey matter volume within the voxel (divided by $[GM]/([GM]+[WM]+[CSF])$), and creatine concentrations were corrected for the proportion of total brain tissue volume

within the voxel (divided by $(([GM] + [WM]) / ([GM] + [WM] + [CSF]))$). All neurotransmitter concentrations reported are given as a ratio to creatine (GABA:Cr). Mean GABA:Cr values for each time point were normalised to the average of the baseline values for that condition, and henceforth are referred to as GABA levels for simplicity.

4.2.6 Resting state functional connectivity analysis

Analysis of resting state fMRI data was carried out using an independent component analysis (ICA) approach as implemented in MELODIC (Beckmann and Smith, 2004) and tools from the FMRIB Software Library (FSL; www.fmrib.ox.ac.uk/fsl). Standard pre-processing steps were applied, which included motion correction, brain extraction, spatial smoothing using a Gaussian kernel of full-width at half-maximum (FWHM) of 2mm, and high-pass temporal filtering equivalent to 100s. fMRI volumes were registered to the individual's structural scan using Boundary Based Registration (BBR) (Greve and Fischl, 2009) and then to standard space images using FMRIB's Nonlinear Image Registration Tool (FNIRT). Pre-processed functional data containing 300 time points for each subject for each time point were temporally concatenated across subjects to create a single 4D data set.

A dual regression technique was used to allow for between-subject voxel-wise comparisons of resting functional connectivity as previously described (Filippini et al., 2009, Stagg et al., 2014). To identify large-scale patterns of functional connectivity in the population of subjects, the concatenated 4D data set was decomposed using ICA into 25 components (Stagg et al., 2014). The motor RSN was identified manually by eye and then confirmed by using spatial cross-correlations against a previously defined motor map (Beckmann et al., 2005). A dual regression approach was then used to identify subject-specific temporal dynamics and associated spatial maps. This involved the following steps: for each subject the group-average set of spatial maps was regressed into the subject's 4D space-time dataset. This generated a set of subject-specific time series for each group-level spatial map. Regressing these time series into the same 4D dataset resulted in a set of subject-specific spatial maps, one for each group-level spatial map. Nonparametric permutation testing (using 5000 permutations) as implemented by randomise within FSL (Nichols and Holmes, 2002) was used to investigate statistically significant differences between the anodal and sham conditions at the two time points.

Additionally, we performed region of interest (ROI) analyses, to investigate changes in resting state connectivity with the motor RSN within specific ROIs. To investigate the changes specific to the stimulated M1, a binarised mask of the left hemisphere motor network was used to identify the voxel with the maximum BOLD signal, this voxel was then dilated to create an 8x8x8mm spherical mask, which included the hand knob (Yousry et al., 1997). In addition, individual subject masks of the MRS voxel were created to quantify functional connectivity within the voxel of interest where the GABA quantification was measured. The mean measures of motor RSN functional connectivity within the ROIs were then extracted for each subject at each time point and condition.

4.2.7 Statistical analysis

Statistical analyses on the data were conducted using SPSS (Version 22, IBM). Repeated Measures ANOVA (RM ANOVA) were run with one factor of stimulation type (anodal, sham), and one factor of time (Baseline1, Baseline2, During1, During2, During3, Post1, Post2). Post hoc Student's *t*-tests (paired samples, two-tailed) were performed as appropriate.

4.3 Results

4.3.1 Baseline GABA levels and M1 functional connectivity are inversely correlated

To test the relationship between GABA levels and the strength of functional connectivity with the motor RSN within M1 at baseline, a partial correlation was computed between the combined baseline values across the two stimulation conditions, controlling for individual subjects. We found a significant negative correlation between GABA levels and the strength in motor functional connectivity within the MRS voxel ($R = -.62$, $p = .002$, bootstrap 95% confidence interval, $R = -.80$ to $-.36$; Figure 4.2), but not within the whole motor network ($R = -.23$, $p = .29$, bootstrap 95% confidence interval, $R = -.69$ to $.40$). Post hoc power analysis indicated that the statistical power for this correlation was 0.98 (alpha level $p < .05$, effect size of .71) (G*Power; Erdfelder et al. 1996).

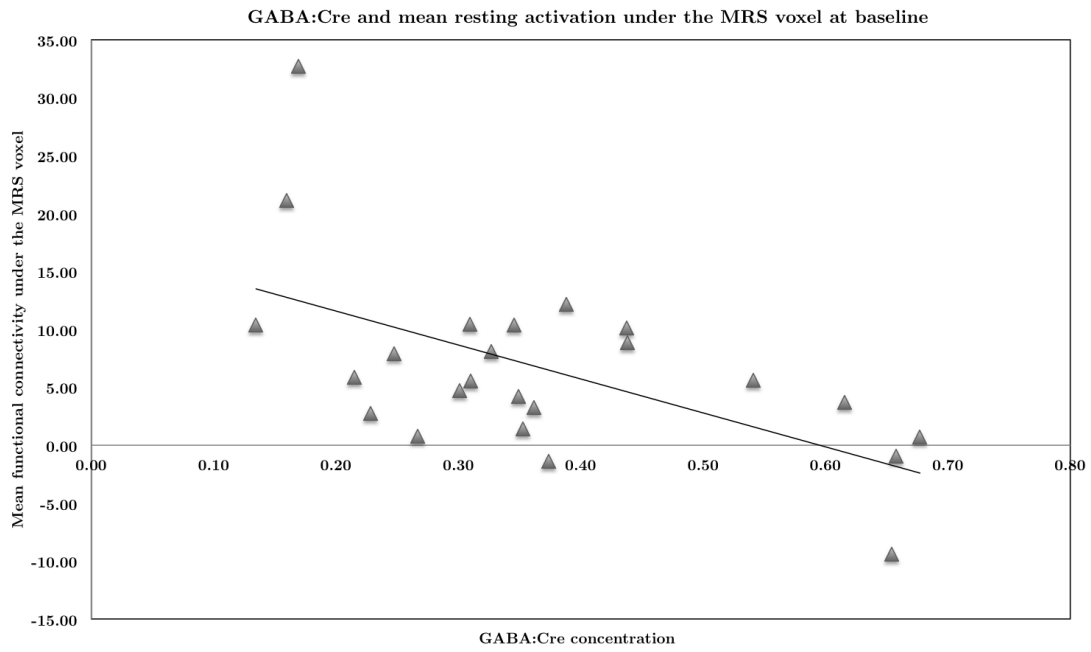


FIGURE 4.2: **Negative correlation between GABA and motor functional connectivity at baseline.** Participants with higher GABA concentration had lower strength in motor network functional connectivity within the same region at baseline. (** $R=-.62$, $p<.01$)

4.3.2 Anodal tDCS decreases GABA levels within M1

We then went on to investigate the time course of any changes in GABA induced by tDCS, using a RM ANOVA with one factor of stimulation and one factor of time. There was no significant main effect of stimulation ($F_{(1,11)}=3.22$, $p=.10$) or time ($F_{(6,66)}=.96$, $p=.46$). However, as expected a priori, there was a significant stimulation by time interaction ($F_{(6,66)}=2.29$, $p<.05$; Figure 4.3).

To investigate this interaction, we performed planned post-hoc t -tests between the two stimulation conditions at each time point. This revealed significantly lower GABA after Anodal tDCS compared with Sham at the Post1 and Post2 timepoints (paired t -test, 2-tailed, Post1: $t_{(11)}=-4.14$, $p<.01$; Post2: $t_{(11)}=-2.86$, $p=.02$). There were no differences in GABA levels at the three time points during anodal stimulation compared with sham (paired t -test, 2-tailed, During1: $t_{(11)}=.31$, $p=.76$, During2: $t_{(11)}=-.50$, $p=.63$; During3: $t_{(11)}=-.85$, $p=.41$).

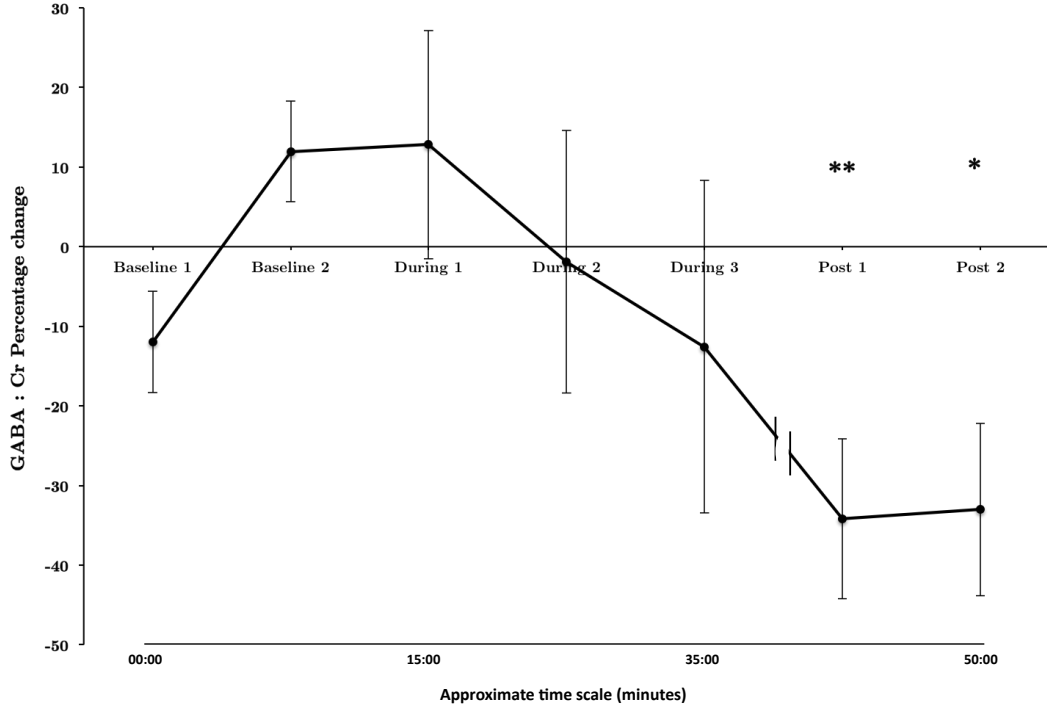


FIGURE 4.3: **GABA Timecourse.** GABA timecourse with anodal tDCS relative to the sham condition. The decrease in GABA levels build up during anodal stimulation with the most prominent decrease approximately 10-15 minutes after the stimulation period. Break in the lines indicate a time gap when approximately 7 minutes of resting state acquisition immediately after tDCS stimulation. Timescale show the approximate time from the beginning of the scan session. Error bars indicate SEM. (** $t_{(11)} = -4.14$, $p < .01$; * $t_{(11)} = -2.86$, $p = .02$)

4.3.3 Anodal tDCS increases functional connectivity within M1

To explore the tDCS-induced changes in the strength in motor network functional connectivity, the mean BOLD signal under an 8x8x8mm left M1 ROI was calculated for each subject, condition, and time point. A RM ANOVA with factors of stimulation (Anodal, Sham), and time (Baseline, Post) demonstrated no significant main effect of stimulation condition ($F_{(1,11)} = .20$, $p = .66$) nor time ($F_{(1,11)} = 2.78$, $p = .12$), but a significant stimulation by time interaction ($F_{(1,11)} = 6.39$, $p = .03$; Figure 4.4).

Further exploration with paired samples t -tests showed a significant increase in the strength of functional connectivity in the motor network within the left M1 ROI after anodal stimulation (2-tailed, $t_{(11)} = -2.45$, $p = .03$) but not in the sham condition (2-tailed, $t_{(11)} = 0.07$, $p = .95$).

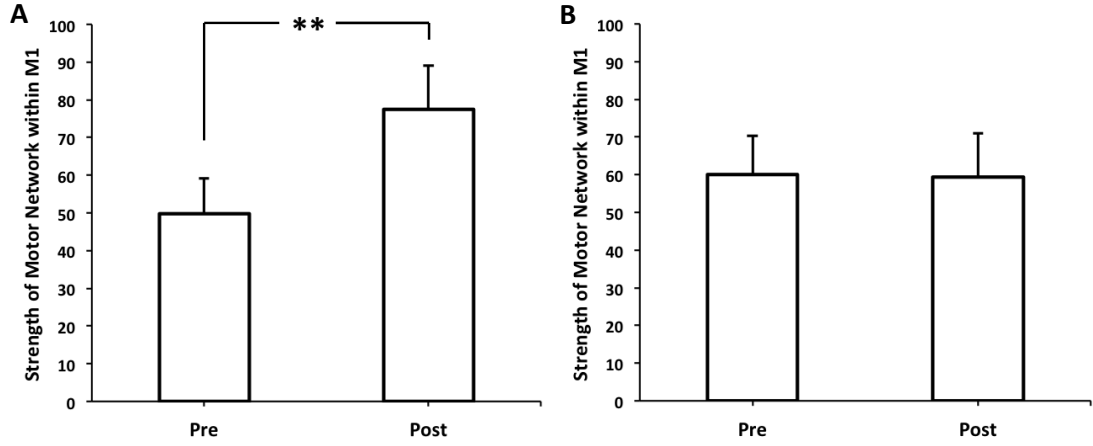


FIGURE 4.4: **Motor network functional connectivity.** **A.** Anodal tDCS applied to M1, significantly increased functional connectivity within M1 of the motor network (** $t_{(11)}=-2.45$, $p=.03$). **B.** There were no differences in the sham condition ($t_{(11)}=.07$, $p=.95$). Values indicate mean BOLD activation within the motor network under an 8x8x8mm ROI centered over the left primary motor cortex. Error bars indicate SEM.

4.3.4 Greater functional connectivity in the stimulated primary motor cortex after anodal stimulation

Given the significant effect detected in the ROI analysis described above, we performed voxel-wise analysis to determine the spatial distribution of increases in functional connectivity following anodal tDCS.

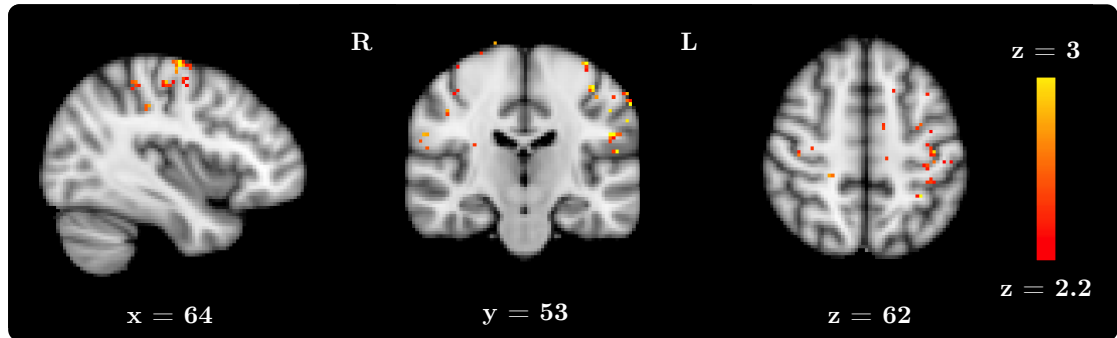


FIGURE 4.5: **Spatial distribution of in the motor network with anodal stimulation.** For exploratory purposes the thresholded raw t -stat images shown here indicate a cluster in M1 showing greater functional connectivity in the motor network with anodal tDCS more than sham. Thresholded raw t -stat image ($t_{(11)}=1.80$, $p>.05$). No significant effects were found with multiple comparisons analysis.

A voxel-wise analysis contrasting increases in functional connectivity following anodal tDCS compared with sham [$(\text{Anodal}_{\text{post}} > \text{Anodal}_{\text{pre}}) > (\text{Sham}_{\text{post}} > \text{Sham}_{\text{pre}})$] indicated a trend towards a greater increase in motor network connectivity following anodal stimulation compared to sham within the motor network, and particularly within the

stimulated (left) motor cortex (uncorrected voxelwise $p > .05$; Figure 4.5). However, no significant effects were found after correction for multiple comparisons. No effects (at uncorrected or corrected thresholds) were found for the opposite contrast $[(\text{Sham}_{\text{post}} > \text{Sham}_{\text{pre}}) > (\text{Anodal}_{\text{post}} > \text{Anodal}_{\text{pre}})]$.

4.3.5 No relationship between change in GABA levels due to tDCS and change in functional connectivity

Finally, we wished to investigate whether the relationship between M1 GABA levels and M1 functional connectivity observed at baseline (Figure 4.2) held after modulation by tDCS. There was no relationship between the tDCS-induced decrease in GABA levels [post average - baseline average] and the tDCS-induced increase in functional connectivity [post - baseline] within either the MRS voxel or within the motor network as a whole in the anodal condition (Pearson's correlation, 2-tailed, MRS voxel: $R = .18$, $p = .56$, bootstrap 95% confidence interval, $R = -.54$ to $.71$; and motor network: $R = -.09$, $p = .78$, bootstrap 95% confidence interval, $R = -.76$ to $.52$). There was no significant relationship between GABA levels post-tDCS [average of Post1 and Post2] and functional connectivity within the MRS voxel ($R = -.44$, $p = .15$, bootstrap 95% confidence interval, $R = -.90$ to $.15$), though this relationship was not significantly different from the relationship found between GABA and functional connectivity at baseline (Baseline: $R = -.62$, $p < .01$; Post: $R = -.27$, $p = .22$; Fisher's r-to-z transformation: $Z = -.96$, $p = .34$, 2-tailed).

4.4 Discussion

We confirm previously reported findings that GABA levels within M1 negatively correlate with the functional connectivity of the motor network. We also replicate previous findings that anodal tDCS reduces GABA concentration, and increases functional connectivity in the stimulated cortex. However, we show for the first time that the magnitude of these changes do not correlate across subjects, suggesting that they may be driven by distinct underlying mechanisms. We also provide novel evidence on the timecourse of GABA change with anodal tDCS and demonstrate that the previously described reduction in GABA is most prominent in the 30-minute period after stimulation.

4.4.1 Effects of anodal tDCS on GABA

As predicted we saw a significant decrease in GABA levels after anodal tDCS with effects persisting for at least 30 minutes following stimulation. These results extend previous findings (Stagg et al., 2009a), which had only tested up to 20 minutes post stimulation and did not measure during stimulation. However, as GABA values remained depressed at 30 minutes, and we could not practically continue beyond this timepoint due to the length of the scans, it is still unclear how long it would take for the values to fully return to baseline after the stimulation period.

By recording MRS data during tDCS we were also able to track GABA levels in the stimulated M1 during the stimulation period. The relative tDCS-induced decrease in GABA build up over time such that it was most prominent in the post period after the stimulation had ceased. These findings build on prior work (Stagg et al., 2009a), and here, the after-effects of tDCS were also explored. We show for the first time that cortical M1 GABA levels remain depressed for at least 30 minutes after 20 minutes of stimulation. This is in line with physiological data that have shown up to 90 minutes of excitability elevations with just 13 minutes of tDCS stimulation (Nitsche and Paulus, 2001).

The GABA levels reported in this study are proposed to be a surrogate marker for the level of physiological inhibition within a given area. Neurotransmitters such as GABA and glutamate are continuously recycled between neurons and astrocytes via re-uptake mechanisms from extracellular space (Hyder et al., 2006). However, the spatial resolution of MRS cannot determine which compartment the measured neurotransmitters occupy, but instead reflects the total concentration within a region that is neurophysiologically relevant.

4.4.2 Effects of anodal tDCS on functional connectivity

As we had hypothesized, there was a significant increase in the strength of motor network connectivity within the M1 ROI following anodal tDCS, consistent with previous reports (Stagg et al., 2014). These findings suggest that tDCS is capable of modulating the slow ($>1\text{Hz}$) intrinsic fluctuations in haemodynamics as measured by fMRI and changes can be seen regionally at the site of stimulation.

These results are in line with other studies on the effect of tDCS on functional connectivity using different analysis methods. For example, Polania and colleagues (2011) used a graph theory approach, and demonstrated an increase in functional coupling between premotor and superior parietal areas with the sensorimotor cortex.

Here, we only assessed resting fMRI before and after tDCS and therefore do not have evidence on the effects during tDCS. Sehm and colleagues (2012) measured functional connectivity during 20 minutes of anodal tDCS to the right M1. Using ICA-based analysis, they did not find an effect of tDCS on functional connectivity within the primary sensorimotor cortex. This lack of change during stimulation could parallel that of our GABA results, suggesting that changes in both functional connectivity and GABA levels are most prominent after stimulation with below threshold online changes.

4.4.3 Relationship between GABA and functional connectivity changes

This study is the first to acquire both GABA and resting fMRI before and after tDCS within the same individuals. In this way we were able to test for relationships between these measures both at baseline and in response to tDCS. At baseline, in line with previous studies (Kapogiannis et al., 2013, Stagg et al., 2014), we demonstrated a significant negative correlation between GABA and strength in motor functional connectivity within the MRS voxel. Subjects with higher resting GABA levels had less motor network strength within the MRS voxel. However, there were no significant relationships found between the change in GABA levels and change in functional connectivity measures.

This lack of a relationship suggests that changes in these modalities due to tDCS could be driven by distinct underlying mechanisms, or alternatively, that the spatial and temporal resolution of both techniques are currently unable to dissociate between the relevant variables of the BOLD signal and the GABA signal being measured. Transmission of GABA and glutamate in extracellular fluid play an important role in modulating regional microcircuits and the haemodynamic responses within a given region (Logothetis, 2008). M1 is a key node in the resting motor network and it is possible that regional neurotransmitter concentrations within M1 could predict either the functional connectivity across the motor network or within the M1 node. Better experimental paradigms that can clearly attribute GABAergic neurotransmission to the relevant variables of the

BOLD signal could be useful for a clearer understanding of the relationship between the two measures.

The use of magnetoencephalography (MEG) could add further insight into these relationships. At rest, neuronal network oscillations in the beta (15-30 Hz) frequency range, thought to be under the direct control of GABAergic modulation can be measured spontaneously in M1 ([Hall et al., 2011](#)). This could provide an alternative measure of inhibition and its relationship to network level motor connectivity, which will be important to reconcile in future investigations.

4.5 Conclusions

In summary, we demonstrated that the decrease in M1 GABA levels with anodal tDCS is most prominent in the 30 minute period after stimulation has ceased. Mapping the temporal pattern of inhibitory influences within a localized area can have important implications in determining the optimal time window for combining tDCS with motor learning and performance. Examining these interactions more closely could address the question of when tDCS should be applied during therapy after stroke, which will be important in optimising its utility as an adjunct therapy in stroke rehabilitation.

Chapter 5

The effects of tDCS on intracortical and interhemispheric excitability of the primary motor cortices

The previous chapter demonstrated the effects of anodal tDCS on GABA inhibition and resting state functional connectivity in the stimulated primary motor cortex. However, it is unclear whether this modulation of inhibition is restricted to the stimulated hemisphere or whether inhibitory changes are also observed in the non-stimulated primary motor cortex, or indeed in the inter-hemispheric connections between the primary motor cortices. In this chapter, paired pulse TMS was used to measure tDCS-induced changes in intracortical inhibition in the M1 of each hemisphere separately, as well as measuring the changes in interhemispheric inhibition and facilitation.

5.1 Introduction

Application of transcranial direct current stimulation (tDCS) has been shown to induce excitability changes that are dependent on the polarity of the current. When a 1mA

current is applied, the amplitude of motor evoked potentials (MEPs) in the contralateral hand are increased by anodal stimulation of the primary motor cortex (M1), and decreased by cathodal stimulation (Nitsche and Paulus, 2000). The vast majority of studies in humans have investigated the effects of tDCS on corticospinal circuits. Only a few studies have evaluated the effects of anodal stimulation on local intracortical inhibitory circuits (Antal et al., 2010, Hummel, 2005, Nitsche, 2005), and only two have evaluated the effects of cathodal stimulation (Di Lazzaro et al., 2012, Nitsche, 2005).

In the stimulated M1, a decrease in intracortical inhibition has been reported with anodal stimulation (Antal et al., 2010, Hummel, 2005, Nitsche, 2005), and an increase with cathodal stimulation (1mA, 13 minutes) (Nitsche, 2005). However, with a longer stimulation duration (1mA, 20 minutes), no changes in intracortical inhibition were demonstrated with cathodal tDCS in either M1 (Di Lazzaro et al., 2012). In the above studies, none have directly compared the effects of both stimulation conditions within the same individuals, and apart from Di Lazzaro et al. 2012, none have measured the effects of tDCS on intracortical inhibition from the contralateral (non-stimulated) M1.

However, data from a number of studies in healthy controls and chronic stroke patients suggest that it is likely that the modulatory effects produced by tDCS may not be limited to the targeted cortical area but may also occur at distant, interconnected sites (Lang et al., 2004, 2005, Xerri et al., 2014). TMS studies have demonstrated that local intracortical circuits within M1 can modulate interhemispheric inhibitory circuits that both project to, and receive callosal volleys from, the contralateral M1 (Avanzino et al., 2007, Lee et al., 2007). To date, only two studies have measured the effects of tDCS on interhemispheric connections between the M1s using ppTMS (Lang et al., 2004, Tazoe et al., 2014), with heterogeneous findings.

Lang and colleagues (2004) measured the duration of the ipsilateral silent period (iSP), a TMS protocol thought to be a marker of transcallosal motor inhibition (Meyer et al., 1995), and demonstrated that anodal stimulation (1mA, 10 minutes) to the left M1, increased transcallosal inhibition from right to left M1, but did not affect transcallosal inhibition from left to right M1. The lack of effects from the stimulated left M1 contradicts the hypothesis that tDCS to the stimulated cortex influences the contralateral M1 through modulation of transcallosal pathways. Subsequently, Tazoe and colleagues (2014) demonstrated that higher intensity and longer stimulation of anodal tDCS (1.5

mA, 15 minutes) increased IHI from the stimulated to the non-stimulated M1 with no change in IHI from the non-stimulated to the stimulated cortex, whereas cathodal tDCS suppressed IHI from both directions. The IHI protocol (ISI of 10 ms) used in this study is thought to be dependent on GABA_B-mediated neurotransmission in the target hemisphere (Kukawadia et al., 2005). In contrast to the findings of Lang and colleagues (2004), these findings suggest that tDCS can produce effects on the interhemispheric interactions between the motor cortices, and that these effects can be polarity specific.

It has been demonstrated that IHI and iSP do not represent the same phenomenon (Chen et al., 2003), and that iSP may not be a suitable marker for transcallosal motor inhibition (Jung et al., 2006, Jung and Ziemann, 2006). The discrepancy in the above studies could also be due to the differences in current strength and duration, as the threshold for eliciting transcallosal inhibition is higher than the resting motor threshold for MEPs (Tazoe et al., 2013, Tsutsumi et al., 2012), it could be that for the effects on callosal projections to be detected, it may require a relatively high intensity and long duration of tDCS, as used by Tazoe et al. 2014.

The aim of the present study was to investigate the effects of tDCS on intracortical and interhemispheric inhibition. We chose to test a tDCS protocol using 2mA as an increasing number of behavioural, cognitive, and clinical studies have started to use this higher intensity under the implicit assumption that higher intensities will enhance the efficacy of stimulation (Ferrucci and Priori, 2014, Fregni et al., 2006). Here, we used single- and paired-pulse TMS protocols to test the effects of tDCS on local inhibitory circuits from both the stimulated (anode- or cathode-targeted M1) and non-stimulated (contralateral) M1. In order to test for any interhemispheric interactions from the anode- or cathode-targeted M1 to the non-stimulated M1, we measured interhemispheric inhibition and facilitation to the non-stimulated M1, thus, measuring changes in MEPs generated from the non-stimulated M1. The following TMS parameters were evaluated at baseline and immediately after 20 minutes of 2mA anodal, cathodal, and sham tDCS: (1) resting and active motor thresholds; (2) intracortical inhibition from both M1; and (3) interhemispheric inhibition and facilitation to the non-stimulated M1.

We hypothesised that, in the anode- and cathode-targeted M1, anodal tDCS would reduce intracortical inhibition, and cathodal stimulation would have the opposite effect, based on previous findings (Nitsche, 2005). As we wanted to test for inhibitory influences

from the anode- or cathode-targeted M1 to the non-stimulated M1, we would expect that increasing excitability in the anode-targeted M1 would lead to increased IHI to the non-stimulated M1, and decreasing excitability in the cathode-targeted M1 would lead to decreased IHI to the non-stimulated M1, corresponding to previous findings (Tazoe et al., 2014).

5.2 Methods

5.2.1 Participants

Twelve healthy participants (5 male; aged 19-23 years, mean 21 years) gave their informed consent to participate in this study in accordance with ethical approval from the East London Research Ethics Committee (Ref: 10/H0703/50). All were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971).

5.2.2 Experimental Design

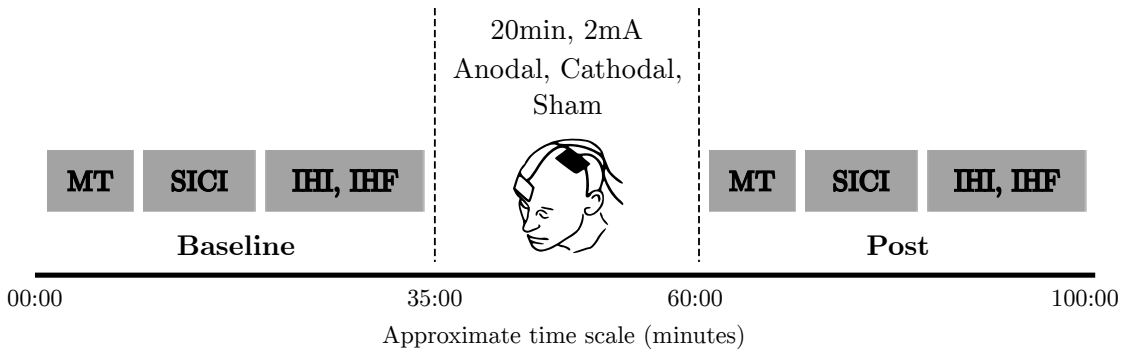


FIGURE 5.1: Experimental Design. All subjects participated in three experimental sessions separated by at least 1 week. Subjects received anodal, cathodal, or sham tDCS (20 minutes, 2mA) in a counterbalanced order. Motor thresholds, SICI, IHI₁₀, IHI₄₀, and IHF were measured at two time points before (baseline) and after (post) tDCS stimulation. Timeline shown is an estimate of the length of each session in minutes. Abbreviations used: MT, motor threshold; SICI, short intra-cortical inhibition; IHI, inter-hemispheric inhibition; IHF, inter-hemispheric facilitation.

All subjects participated in three testing sessions with anodal, cathodal, or sham tDCS. The sessions were separated by at least one week and the order of the sessions was counterbalanced across the group. In each experimental session, intracortical inhibition was measured from each M1, and interhemispheric inhibition and facilitation to the

non-stimulated (right) M1 were measured with TMS before (baseline) and immediately after tDCS stimulation (post) (Figure 5.1).

5.2.3 Transcranial Direct Current Stimulation (tDCS)

A DC-Stimulator (Magstim, Ltd; UK) delivered a 2mA (0.057 mA/cm^2) current to the brain via electrodes measuring $5 \times 7 \text{ cm}$ (Easycap, GmbH; Germany) encased in saline-soaked sponges and fixed to the head with rubber bands. One electrode was centred over the left M1 and positioned 5 cm lateral to mid-pre-central position (Cz). The reference electrode was placed over the contralateral supraorbital ridge. During anodal and cathodal stimulation, the current was ramped up over 10 seconds and was then held at 2mA for 20 minutes, before being ramped down over 10 seconds. For sham stimulation the DC-stimulator was ramped up for 10 seconds then switched off, as previously described (Stagg et al., 2011a).

5.2.4 TMS measures

TMS was performed using a monophasic Magstim BiStim stimulator (Magstim Company, Dyfed, UK), connected to 70mm figure-of-eight coils. The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline. The optimal scalp position was localised for the left and right M1 (referred to as the motor hotspot) to elicit MEPs in the contralateral first dorsal interosseous (FDI) muscle. Surface electromyography (EMG) was recorded from both hands from the FDI and abductor digiti minimi (ADM) muscle using neonatal ECG electrodes in a belly-tendon montage with a ground electrode over the styloid process of the ulna. Responses were sampled, amplified, and filtered using a CED 1902 amplifier, a CED micro1401 A/D converter (Cambridge Electronic Design, Cambridge, UK) and a computer running Signal (Version 3.07). Signals were sampled at 5000 Hz with 50 Hz notch filtering and band-pass filtered between 10 and 1000 Hz (O'Shea et al., 2014).

5.2.5 Motor threshold (1mV MT and aMT)

The resting motor threshold (1mV MT) and active motor threshold (AMT) were determined for both M1s separately. The 1mV MT was defined as the minimum stimulus

intensity that produced a mean 1mV amplitude MEP in trials in the relaxed FDI muscle. AMT was defined as the minimum stimulus intensity needed to produce a 1mV response in at least 5 out of 10 consecutive stimuli during isometric contraction of the FDI at about 20% of maximum voluntary strength. Subjects were asked to lightly pinch the index finger and thumb together and were given auditory feedback until the desired EMG activity was achieved. As the TMS intensity was adjusted to achieve a baseline MEP of 1mV, after each tDCS protocol, if needed, this was re-adjusted to compensate for effects of global excitability changes on the test-pulse amplitude (Nitsche, 2005).

5.2.6 Short interval intracortical inhibition (SICI)

A paired pulse SICI protocol using an inter-stimulus interval (ISI) of 2.5 ms was used to assess intracortical inhibition within each M1 (Kujirai et al., 1993). Two magnetic stimuli were applied through the same stimulating coil over the motor cortex to investigate the effect of a sub-threshold conditioning stimulus (70% of AMT) on the test stimulus (at 1mV MT). Twenty paired pulses were delivered in a pseudo-randomised order interleaved with twenty single pulses (at 1mV MT) to each M1, giving a total of 40 test stimuli. The order of stimulating the left and right M1 was counterbalanced between subjects, but remained constant within session.

5.2.7 Interhemispheric excitability measures

Interhemispheric excitability was tested using two 70mm figure-of-eight coils held over each motor hotspot (Ferbert et al., 1992). A conditioning stimulus (at 1mV MT) was given at ISIs of 4, 10, and 40 ms to the left M1 before a test stimulus (at 1mV MT) was delivered to the contralateral right M1. Short latency interhemispheric inhibition at ISI of 10 ms (IHI_{10}), long latency interhemispheric inhibition at ISI of 40 ms (IHI_{40}) and interhemispheric facilitation (IHF) at ISI of 4ms, were recorded. Twenty pulses of each protocol were delivered in a pseudo-randomised order interleaved with forty single pulses, giving a total of 100 test stimuli. As we wanted to test for inhibitory influences from the anode- or cathode-affected M1 to the non-stimulated M1, the conditioning stimulus was always given to the left M1 and the test stimulus to the right M1. EMG activity was recorded from the FDI and ADM of the left hand to determine the effects on the non-stimulated M1.

5.2.8 Statistical analysis

Peak-to-peak MEP amplitudes were calculated. For the paired pulse protocols the amplitude of the conditioned MEP was expressed as a ratio to the amplitude of the unconditioned test MEP. For each TMS block, significant outliers were detected using Grubb's test (<http://graphpad.com/quickcalcs/Grubbs1.cfm>) and removed as previously described (Stagg et al., 2011b). Then, for each TMS block and stimulation type separately, the mean amplitude of the MEPs were calculated and values ± 2 standard deviations of the group mean were removed from further analysis. Values were compared between conditions using Repeated Measures ANOVA (RM ANOVA) and followed up by post-hoc *t*-tests.

5.3 Results

5.3.1 Baseline values are comparable across conditions

5.3.1.1 Motor Thresholds

To test for any differences at baseline between the stimulation conditions for the motor thresholds, a separate RM ANOVA was performed for 1mV MT and AMT with one factor of stimulation and one factor of hemisphere. There were no significant main effects of stimulation, hemisphere, or interaction at baseline for 1mV MT or AMT (all $p > .1$). Mean thresholds are shown in Table 5.1.

TABLE 5.1: Mean motor thresholds $\pm$ SD

		Anodal	Cathodal	Sham
Stimulated M1	1mV MT	50.6 $\pm$ 9.6	46.6 $\pm$ 6.6	49.1 $\pm$ 5.6
	AMT	35.7 $\pm$ 6.3	33.3 $\pm$ 5.4	34.1 $\pm$ 5.5
Non-Stimulated M1	1mV MT	49.7 $\pm$ 5.2	48.3 $\pm$ 5.5	51.8 $\pm$ 7.2
	AMT	35.2 $\pm$ 5.2	35.0 $\pm$ 6.2	35.4 $\pm$ 4.7

5.3.1.2 Paired-pulse measures

To test whether the basic expected phenomena of inhibition or facilitation were observed for each of the paired pulse protocols, one-sample t -tests were performed on the mean baseline values for each protocol separately. The SICI protocol led to a significant inhibition of MEP amplitudes (pooled data from both M1, $t_{(19)}=-3.05$, $p<.01$). In terms of the interhemispheric interactions, the IHF protocol led to a significant facilitation of MEPs ($t_{(11)}=3.7$, $p<.01$) and the IHI₄₀ protocol to a significant inhibition of MEPs ($t_{(11)}=-6.93$, $p<.001$). However, the IHI₁₀ protocol had no significant effect on MEP amplitudes ($t_{(11)}=1.27$, $p=.23$) and therefore these data were excluded from all further analyses.

5.3.2 Effects of tDCS on local inhibition, and interhemispheric inhibition and facilitation

For each ppTMS measure separately, a RM ANOVA was performed with one factor of stimulation (anodal, cathodal, sham) and one factor of time (baseline, post). As we wanted to determine whether there was an effect of stimulation on the levels of excitability over time, the main result of interest is the interaction between stimulation and time. We then tested each real stimulation condition (anodal, cathodal) separately against the sham condition. Finally, we performed exploratory paired-samples t -tests for each stimulation condition to determine whether there were any changes in excitability following each stimulation condition for each measure.

5.3.2.1 Basic effects of tDCS on excitability

To test whether the expected effects of tDCS on excitability could be measured, a main RM ANOVA was performed with factors of stimulation, hemisphere, and time. There was a significant main effect of time ($F_{(1,11)}=20.46$, $p<.001$) with a decrease in 1mV MT, but no significant main effects of hemisphere, stimulation, or interaction ($p>.1$).

Despite the absence of a stimulation by time interaction in the ANOVA, we ran further exploratory paired-samples t -tests to test for changes in excitability following each stimulation condition. As would be predicted, we found a significant decrease in percentage intensity of the 1mV MT in the anode-targeted M1 after anodal stimulation

(2-tailed, $t_{(11)}=2.91$, $p=.01$). However, there was also a significant decrease in the cathode-targeted M1 with cathodal stimulation (2-tailed, $t_{(11)}=3.55$, $p<.01$). There were no changes in 1mV MT over time in the left M1 for the sham condition (2-tailed, $t_{(11)}=1.43$, $p=.18$). In the non-stimulated M1 there was a significant decrease in 1mV MT with anodal stimulation but not with cathodal stimulation (paired t -test, 2-tailed, anodal: $t_{(11)}=2.31$, $p=.04$), and cathodal: $t_{(11)}=1.17$, $p=.27$). However, there was also significant decrease in the sham condition (2-tailed, $t_{(11)}=2.26$, $p=.05$).

5.3.2.2 Intracortical inhibition

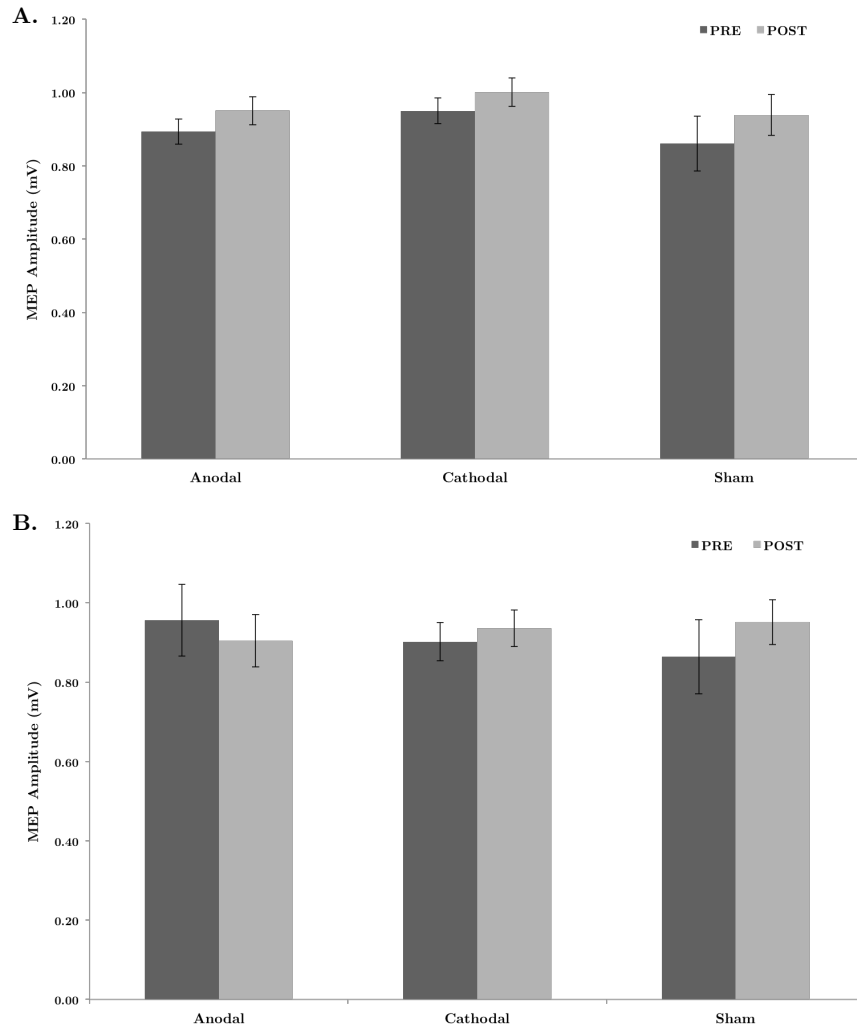


FIGURE 5.2: **Intracortical inhibition.** Effects of tDCS on local inhibition to **A.** stimulated left M1 and **B.** non-stimulated right M1. Error bars indicate SEM.

To test whether tDCS modulated intracortical inhibition in each M1, we performed a RM ANOVA with a main factor of stimulation type and a main factor of time for each

M1 separately. There were no significant main effects of stimulation or time, or stimulation by time interaction in either hemisphere ($p > .1$). There were also no significant effects when testing either of the real stimulation conditions separately against the sham condition ($p > .1$).

Further exploration of the data with paired samples t -tests indicated a trend towards less inhibition after cathodal stimulation in the stimulated M1 (paired sample, 2-tailed, $t_{(11)} = -1.95$, $p = .077$). There were no other effects in the other conditions (Figure 5.2).

5.3.2.3 Interhemispheric interactions

To investigate whether tDCS had any effects on the contralateral M1, we performed RM ANOVA with main factors of stimulation, condition, and time for IHI₄₀ and IHF. No significant main effects or interaction were demonstrated ($p > .1$). Testing each real stimulation condition against the sham condition also did not demonstrate any changes in the non-stimulated M1 with tDCS ($p > .1$). Further exploration with paired samples t -tests for each stimulation condition showed no differences in interhemispheric inhibition or facilitation to the non-stimulated M1 as measured by IHI₄₀ and IHF respectively ($p > .1$).

5.4 Discussion

This study aimed to investigate the effects of anodal and cathodal tDCS on intracortical inhibition in each M1 and interhemispheric inhibition and facilitation to the non-stimulated M1 within the same individual. We found no significant effects of stimulation on intracortical inhibition in either M1, and no changes in interhemispheric inhibition or facilitation to the non-stimulated M1. Although there was a decrease in 1mV MT stimulus intensity in the anode-targeted M1 with anodal tDCS, as might be expected, the non-specific effects between the conditions (as demonstrated by a main effect of time in the RM ANOVA) is due to a general decrease in the 1mV MT observed over time. The participants were asked to sit still for a relatively lengthy period of time (approximately 1.5 to 2 hours) and it is possible that the non-specific effects could be due to drowsiness or fatigue. Several studies have reported a decline in surface EMG and MEPs with fatigue (Kalmar and Cafarelli, 2004, Zijdwind and Kernell, 1994).

The lack of significant differences between stimulation conditions in this study could be a fault of the method and design or may reflect a true absence of physiological effects. The demonstration that most of the TMS protocols used here successfully produced the expected, previously reported (Ferber et al., 1992, Kujirai et al., 1993), basic effects of inhibition or facilitation of MEPs at baseline provides some reassurance that the experimental set-up used was sound. There are several possible reasons why no physiological changes were measured between the stimulation conditions. It is possible that the particular combination of the tDCS and TMS parameters used in this study may have rendered the effects to be underpowered, too variable to detect, or not present at all.

The results of this particular study may not translate to all varieties of tDCS or TMS protocols. To measure intracortical inhibition, we chose an ISI of 2.5 ms to elicit SICI as it has been previously demonstrated that maximal inhibition of SICI could be produced at this ISI (Fisher et al., 2002), and that it reflects the activity of GABA_A interneurons (Roshan et al., 2003) (see Chapter 2.1.2). However, previous studies reporting changes in SICI with anodal tDCS have used ISIs of 2 and 4 ms (Antal et al., 2010), and 3 ms (Hummel, 2005, Nitsche, 2005); with further variations in the parameters used for determining the conditioning stimulus. Nitsche and colleagues (2005) reported an increase in SICI with cathodal stimulation (13 minutes, 1mA) only at ISIs of 2 and 5 ms, whereas Di Lazzaro and colleagues (2012) could not find any changes in SICI with ISIs of 2 and 3 ms. It is evident that not all ISIs are modulated by tDCS in the SICI protocol to the same extent (Nitsche, 2005), making it difficult to reconcile findings from these studies.

In the non-stimulated M1, we did not demonstrate any changes in intracortical inhibition or interhemispheric inhibition or facilitation from the stimulated M1. These findings are in agreement with previous reports that also did not demonstrate any changes to the non-stimulated M1 with tDCS (Di Lazzaro et al., 2012, Lang et al., 2004). Polarity specific modulations by tDCS have been demonstrated using short interhemispheric inhibition (IHI₁₀) (Tazoe et al., 2014). However, as the basic effects with this protocol were not achieved in this study, we could not draw any conclusions from the changes. Lang and colleagues (2004) postulated that the intensity of tDCS used in their experiment (1mA, 10 minutes) might have been too weak to modulate transcallosal activity to be measured in the contralateral M1. However, the lack of any effect on the contralateral

excitability in the current study, even with the increased current strength and duration, suggests that tDCS may influence populations of cortical neurons that are unspecific to the available TMS protocols.

In addition, the use of 2mA tDCS in this study may complicate the interpretation of the findings as most previous studies have used 1mA. Since the time of data collection and study design, it has been shown that excitability effects at 2mA are non-linear, resulting in cortical excitability enhancement with both anodal and cathodal tDCS (2mA, 20 minutes) ([Batsikadze et al., 2013](#)). The present study is in line with these findings as we observed both a decrease in the 1mV MT stimulus intensity and SICI after cathodal stimulation, indicating a trend towards excitability enhancement in the cathode-targeted M1. The reasons for the lack of polarity-specific effects with 2mA tDCS are still unclear, but might involve complex phenomena such as the spread of effective current to the connected brain regions at higher intensities, or the involvement of homeostatic mechanisms. Consistent with the study by Batsikadze and colleagues ([2013](#)), we applied 20 minutes of 2mA tDCS. However, a recent study applying only 10 minutes of 2mA of stimulation, did not demonstrate significant excitability enhancements with cathodal tDCS, albeit trending towards facilitation ([Wiethoff et al., 2014](#)). These findings suggest that there may also be duration-dependent effects in the efficacy of the stimulation protocols. The abolition of the polarity-specific effects with 2mA tDCS, and the variability in its efficacy with different stimulation durations should be investigated further in future studies.

Although the number of studies reporting the effects of tDCS has increased, very few have directly measured the variability of the effects that are produced. Recently, it has been shown that only approximately 50% of individuals (n=53) had a facilitatory effect to both anodal and cathodal tDCS (2mA, 10 minutes), and the remainder only had a poor or absent response to tDCS ([Wiethoff et al., 2014](#)). These findings suggest that there is considerable variability in inter-individual responses to tDCS in healthy controls, and by this logic, if only half of an individual cohort of participants are ‘responders’ to tDCS, much larger sample sizes would be required to have sufficient power to detect the differences with confidence. The sham controlled, within subjects design in this study does control for inter-subject variation, however, it would not exclude the possibility that the study may have been underpowered.

On a more general point, TMS is assumed to activate neurons superficial to output layer

V (Wassermann et al., 2008), and although evidence has demonstrated activations in intracortical (Kujirai et al., 1993) and interhemispheric (Ferbert et al., 1992) sites for stimulation, it does not exclude the possibility that the different types of stimulation could activate complex sets of cortical and subcortical circuits (Gerloff et al., 1998). Whether the combination of both DC stimulation and multiple TMS protocols used in this study may have resulted in complex inhibitory and excitatory interactions remains unclear.

5.5 Conclusions

In conclusion, we found no significant effects of tDCS on intracortical inhibition in either M1, and no changes in interhemispheric inhibition or facilitation to the contralateral M1. We demonstrated that most of the TMS protocols used in this study successfully produced the basic effects of inhibition and facilitation at baseline. However, the heterogeneity in methodology used in the literature make the observations difficult to reconcile. The current methods suffer from the lack of precise knowledge of which neuronal populations and their related connections are activated by TMS. Imaging studies may be an attractive alternative to determine the gross changes in GABAergic inhibition from DC stimulation, and exclude the possibility of complex interactions from the TMS pulse, albeit with the caveat that MRS is still not able to determine the exact neuronal populations involved in mediating the effects of DC stimulation.

Chapter 6

Concurrent measurement of brain metabolites in both human primary motor cortices: a two-voxel 7 T MRS study

This chapter is a pilot study to assess the robustness of the two-voxel MRS method in quantifying neurochemicals in the human primary motor cortices. We show for the first time excellent spectral quality from concurrent measurement of metabolites from both human primary motor cortices using a standard volume coil at 7 Tesla. We also demonstrate effective use of barium titanate dielectric pads for optimised B_1 efficiency for GABA quantification. The validation of the method in this chapter provides the basis for Chapter 7.

6.1 Introduction

Much of the work investigating the neurobiological mechanism of tDCS to the primary motor cortex (M1) has focused on the effects within the stimulated cortex. Very few

studies have looked at the neurochemical changes occurring in the non-stimulated hemisphere. This gap in the literature is largely due to methodological limitations, particularly the difficulties involved in measuring changes in brain neurochemicals concurrently from both primary motor cortices within a single session.

MRS is a powerful tool that allows non-invasive, in vivo quantification of neurochemicals in the human brain. However, as discussed in more detail in Chapter 3, there are several challenges to the technique at ultra high field (UHF). In designing GABA MRS studies there is a need to balance several factors such as maximising signal to noise (SNR) and measurement precision with minimizing experiment time to increase subject comfort and therefore reduce motion artefacts to achieve better data quality. At UHF, the gains from increased SNR (Öz and Tkáč, 2010, Ugurbil et al., 2003) and spectral dispersion (Koch et al., 2007, Tkáč et al., 2009) make it possible to build upon existing acquisition methods to measure from multiple volumes of interest (VOI).

However, the gains afforded by UHF-MRS are not without inherent challenges such as increased B_0 shimming and RF power requirements (Deelchand et al., 2014, Oldfield, 1971). With increasing field strength, there is stronger B_0 inhomogeneity, and broader spectral linewidths due to the shorter T_2 relaxation times (Deelchand et al., 2014). At standard field strengths (< 4 T), field-map based shimming techniques are able to moderate this B_0 inhomogeneity to a relatively good degree (Koch et al., 2009). However at UHF, none of the methods currently available can perfectly correct for this non-uniformity. In single-voxel spectroscopy, localised B_0 shimming can improve homogeneity within the VOI selected, but can lead to variability of B_0 outside of the VOI (Blamire et al., 1996). These differences in B_0 inhomogeneity make it necessary to design studies with a direct focus on the VOI and use an optimised shim set for that specific VOI. Dynamic shim updating (DSU) corrections can be used to compensate for this as it can be updated in real time for each VOI to almost instantaneously switch between slice acquisitions, and thus, give an optimum shim for each VOI (Blamire et al., 1996).

At UHF, the power required to generate B_1 increases quadratically with the static magnetic field (B_0) strength (Vaughan et al., 2001). With standard volume head coils, the available RF power is insufficient to generate conventional 90° or 180° RF pulses with bandwidths that are large enough to minimize the chemical shift displacement errors

(CSDE) in spectroscopy (Tkáč and Gruetter, 2005). This is made worse by increased inhomogeneity of the transmit magnetic field (B_1) (Van de Moortele et al., 2005). In addition, standard volume coils display higher B_1 in the centre of the human head (e.g. putamen, substantia nigra and caudate) compared with voxels in the periphery (e.g. frontal, occipital and motor cortices) (Figure 3.9) (Van de Moortele et al., 2005, Webb and Collins, 2010). For spectroscopy, this reduced B_1 in the primary motor cortices can be particularly problematic for acquiring difficult to measure neurochemicals such as GABA.

Methods that have been developed for UHF include innovative RF pulse designs (Öz and Tkáč, 2010, Tkáč et al., 2001), and modifications to the conventional volume head coils to improve B_1 (Boer et al., 2012, 2011). However, these options are costly and require significant optimisation and development (Koch et al., 2009). Recently, the use of dielectric material suspensions have been shown to be a relatively cost-effective method for localised improvements to B_1 inhomogeneity at 7 T (Snaar et al., 2010). This method allows for local B_1 manipulations, without the need for increased local RF energy deposition or hardware modifications (Schaller et al., 2014, Teeuwisse et al., 2011).

Here, we tested a novel approach to acquire spectroscopy data concurrently from two VOI in the brain. By interleaving the acquisition between two VOI placed over each M1, virtually simultaneous measurements of neurochemicals can be acquired within a given time period. This allows for the concurrent measurement of neurochemicals from both the stimulated and non-stimulated hemisphere, and gives the unique opportunity to map the pattern of changes with tDCS.

The two-voxel sequence developed by spectroscopists within the physics group at FMRIB is an adaptation of the single-voxel semi-LASER (sLASER) sequence (Öz and Tkáč, 2010). The sLASER method was chosen as it has been shown to be robust against B_1 inhomogeneities and results in low CSDE at UHF. The two-voxel method discussed here extends the single-voxel sequence by incorporating DSU to acquire signal from two-voxels in a single TR (Koch et al., 2007). The DSU protocol is necessary as it optimises the shims to the particular area of interest. In addition, the method includes dynamic flip angle adjustment for better localisation, outer volume suppression, and water suppression in each VOI. Critically, this method allows acquisition of high spectral

quality from multiple voxels without the need for specialised hardware (Lemke et al., 2014).

In this study we aimed to demonstrate the reliability of the two-voxel sequence in quantifying neurochemicals concurrently within both human primary motor cortices. We also assessed the use of dielectric barium titanate pads to focus the B_1 to suit our VOI to make GABA quantification possible. We optimised the acquisition methods for the two VOI and assessed the robustness of the spectral quality of data acquired with the two-voxel and single-voxel acquisitions for each subject.

6.2 Methods

6.2.1 Participants

Six subjects (5 male; aged 22-28 years, mean 25 years) participated. All gave informed consent in accordance to an institutionally agreed technical development protocol. Four subjects were right-handed and two were left-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971).

6.2.2 Experimental design

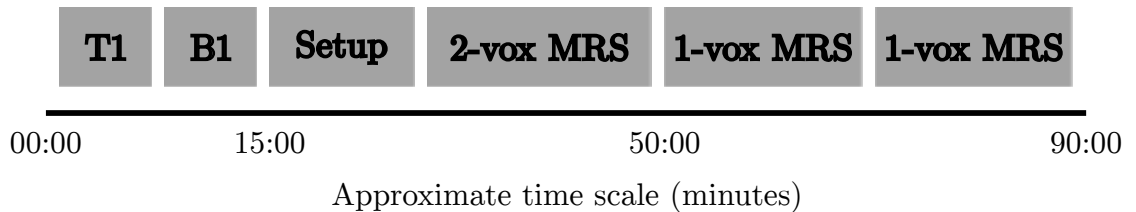


FIGURE 6.1: **Experimental design.** All subjects participated in one experimental session. Metabolites were measured using the two-voxel and single-voxel method in a counterbalanced order between the 6 participants. Timeline shown is an estimate of the length of the scans in minutes. Abbreviations used: T1, T_1 -weighted structural scan; B1, B_1 map; MRS, spectroscopy acquisition.

All subjects participated in one testing session. Neurochemical measurements were acquired using the two-voxel (Lemke et al., 2013) and single-voxel semi-LASER sequence (Öz and Tkáč, 2010). In order to control for any order effects during acquisition, the order of acquisition of the single-voxels (left and right), and which VOI was acquired

first during the two-voxel sequence, was counterbalanced between subjects. The two-voxel sequence was always acquired before the single-voxels. Subjects watched a nature documentary for the whole duration of the experiment (Figure 6.1).

6.2.3 Barium titanate dielectric pads

To optimise B_1 efficiency for the VOI (left and right M1), four different sizes of $BaTiO_3$ dielectric pads were tested. Barium titanate powder ($BaTiO_3$, $<3\ \mu\text{m}$, 99%; Sigma-Aldrich, UK) was suspended in deuterium oxide (D_2O , 99.9 atom %; Sigma-Aldrich, UK) in a mass-to-mass ratio of 4:1 to create a thick paste. This suspension was then sealed into bags of sizes a) 11 x 11 cm, b) 30 x 4 cm, c) 24 x 5 cm, and d) two of 12 x 5 cm (Figure 6.2). Each bag was filled to a thickness of 5mm and double sealed to detect any leakage of the suspension.

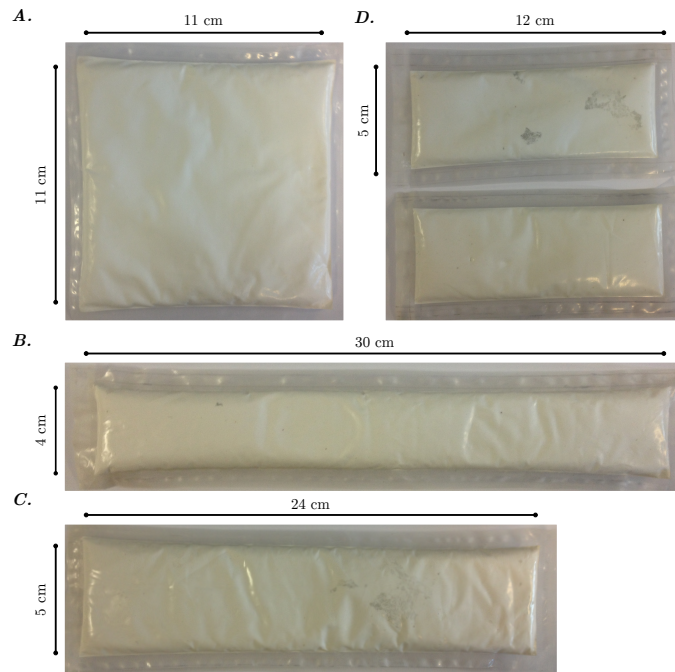


FIGURE 6.2: **Barium titanate pads.** Barium titanate suspensions in sealed bags of sizes **A.** 11 x 11 cm, **B.** 30 x 4 cm, **C.** 24 x 5 cm, and **D.** two of 12 x 5 cm. Each bag was made to a thickness of 5mm.

In an initial pilot, B_1 maps were acquired to determine the effect of the dielectric material to assess which pad would be best for the VOI (Figure 6.3). Optimum B_1 signal to both the primary motor cortices was observed using a single square pad sized 11 x 11 cm placed on the top of the head (Figure 6.3.b). As shown on the sagittal view in Figure 6.3.b, better patterns of B_1 efficiency were seen when using one square pad (11 x 11 cm) centred

on the top of the head, which best highlighted the regions of interest in comparison to the other pads tested and better than with no pad. This pad was further evaluated to be more consistent than using two square pads placed on each side of the head (Figure 6.3.c), which caused an uneven displacement of B_1 efficiency and a less symmetrical pattern (see axial view) between the two sides over the VOI. Although better signal can be seen towards the temporal regions, the asymmetry was highly variable between individuals depending on their head geometry and thus, using two pads was determined to cause more variability between the measurements over the left and right M1 voxels. We therefore used one 11 x 11 cm pad placed on the top of the head for the rest of the experiments. See Chapter 3 for a discussion on dielectric pad design.

6.2.4 Magnetic resonance acquisition

All data were acquired on a 7T Siemens MR system with a 32-channel receive array head-coil (Nova Medical, USA). A barium titanate-based dielectric pad with linear dimensions of 11 x 11cm and 5mm thick was positioned on the top of the head to increase B_1 efficiency in the VOI. B_1 maps were acquired using the actual flip angle imaging (AFI) sequence (FOV 240x240, $TR_{1/2}=6/30$ ms, $TE=2.58$ ms, slice thickness=2.5 mm, non-selective flip angle 60°). When necessary, the dielectric pad was re-positioned and the B_1 map repeated to achieve the best B_1 efficiency in both VOI.

T1 weighted images (MPRAGE, 192x1 mm axial slices, $TR/TE=2200/2.82$ ms, flip angle= 7° , FOV 192x100) were used to place a 2x2x2cm voxel of interest over both the left and right precentral knob, a known landmark for hand motor representation (Yousry et al., 1997). The two voxels were on each end of an 80 mm column, and thus, were 40 mm apart. This width was determined during piloting to provide the optimal balance for sufficient SNR and spectral quality, whilst still keeping the VOI in the primary motor cortices (Figure 6.4).

Spectra were measured using semi-localisation by adiabatic selective refocusing (sLASER) (Öz and Tkáč, 2010) pulse sequence ($TR/TE=7000/30$ ms) with VAPOR (Tkáč et al., 1999) water suppression (pulse duration = 35 ms) and 8 outer volume suppression (pulse duration = 4.6 ms). Signal was acquired from the two voxels in an interleaving fashion alternating between each voxel (64 transients each) (Lemke et al., 2013).

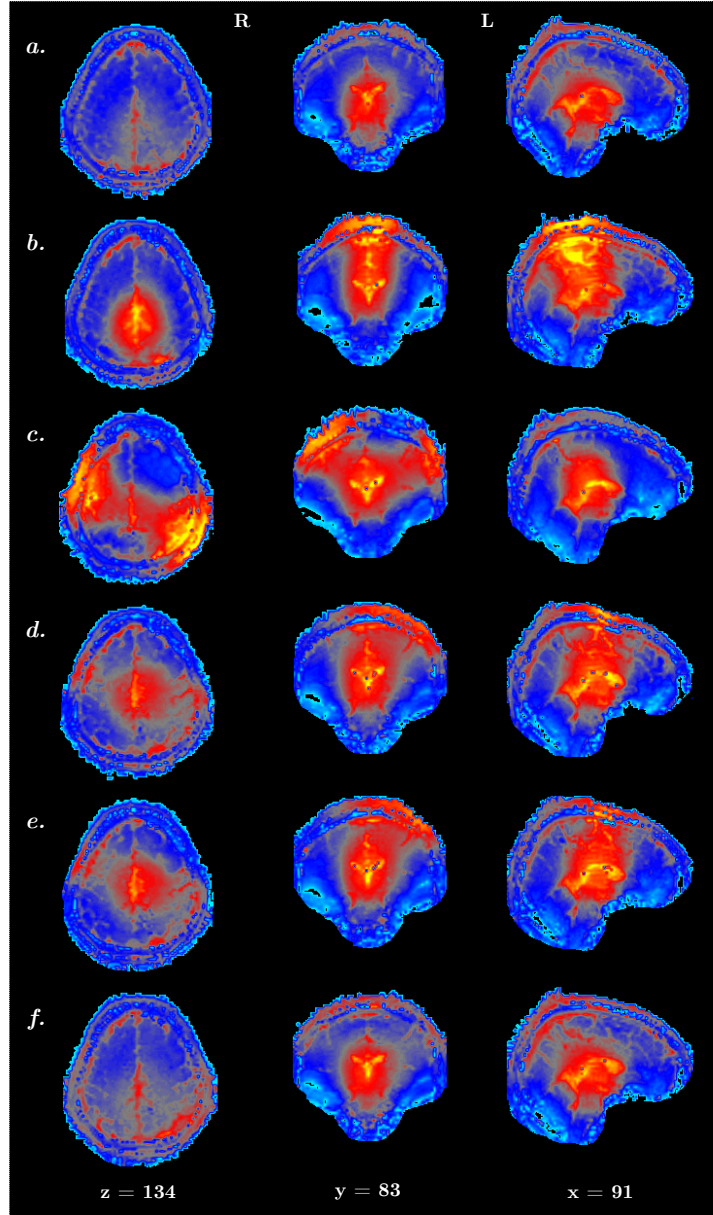


FIGURE 6.3: **Effect of dielectric pads on B_1 inhomogeneity.** B_1 maps with *a.* no pad, *b.* 11 x 11 cm pad centred on the top of the head, *c.* two of 11 x 11 cm pad one on each side of the head, *d.* 30 x 4 cm pad centred across the head, *e.* 24 x 5 cm pad centred across the head, *f.* two of 12 x 5 cm pad placed approximately over each motor cortex region. All images are shown at the same intensity.

6.2.5 Magnetic resonance analysis

Data were corrected using in house scripts on the unsuppressed water signal from the same VOI for reconstruction, eddy current correction, and zero-order phasing of array coil spectra. Any residual water signal was removed using Hankel-Lanczos singular value decomposition (HLSVD) (Cabanès et al., 2001). Metabolites were quantified using

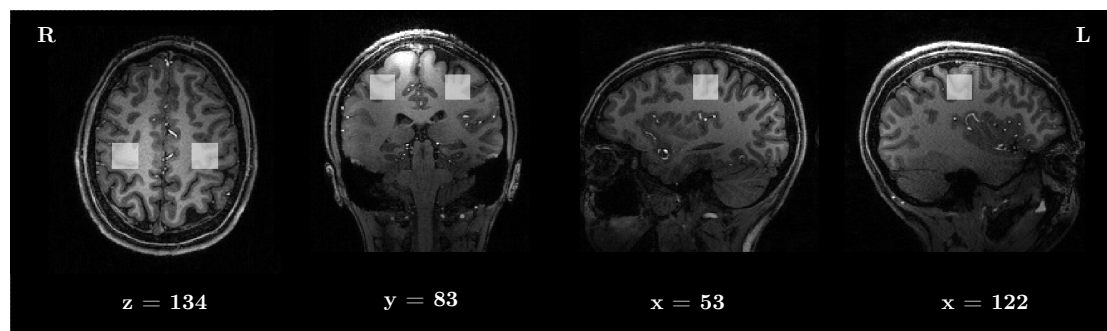


FIGURE 6.4: **Bilateral localisation of the voxels of interest.** Voxel placement from a representative subject to show bilateral localisation of both primary motor cortices. For all participants, voxel placement was determined by the same investigator. All voxels were visually inspected for each individual structural image for localisation of the hand knob.

LCModel analysis (Provencher, 2001). The model spectra were generated based on previously reported chemical shifts and coupling constants (Govindaraju et al., 2000, Tkác, 2008) by the VeSPA Project (Versatile, Simulation, Pulses and Analysis) (Soher et al., 2011), and includes in vivo macromolecule spectra. LCModel analysis was performed on all spectra within the chemical shift range 0.5 to 4.2 ppm. Metabolite concentrations were obtained relative to an unsuppressed water spectrum acquired from the same VOI assuming a water content of 71% (Whittall et al., 1997). The following criteria were applied for excluding spectra with poor quality: (i) Cramér-Rao lower bounds (CRLB) of GABA > 50%, (ii) water linewidths at full width at half maximum (FWHM) > 15 Hz, and (iii) SNR > 10. All spectra in this study met these standards.

6.3 Results

6.3.1 High spectral quality was achieved using both the single-voxel and two-voxel methods

Spectral quality for the single-voxel and two-voxel methods for both the left and right hemispheres are shown in Figure 6.5. High spectral quality with good SNR and resolution were consistently obtained from the two VOI from all 6 subjects. The spectral quality was comparable between the two methods.

In all spectra, there was minimal residual water signal, confirming effective water suppression, and minimal signals from outside voxels, confirming effective outer volume

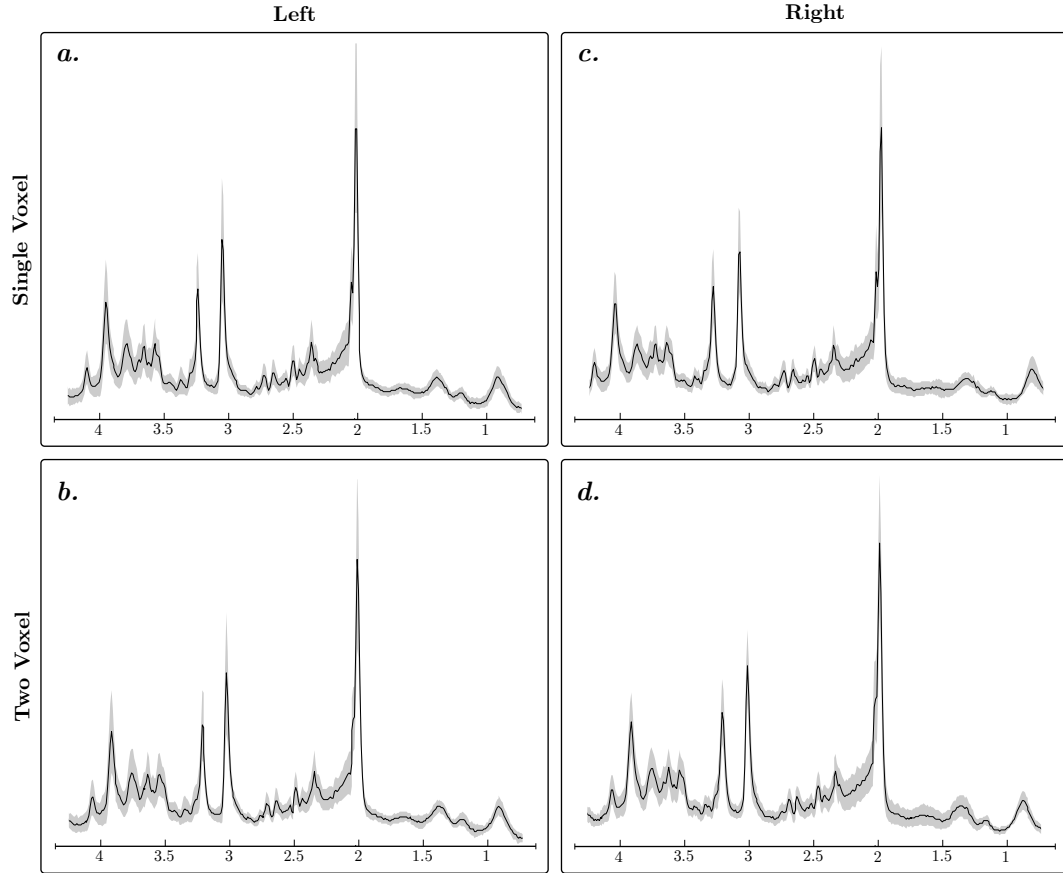


FIGURE 6.5: **Spectral quality with two-voxel and one-voxel methods.** LCModel output of spectra averaged from 6 participants for each method and hemisphere. Shaded grey indicates the standard deviation from the mean.

suppression. In addition, all of the resulting spectra had good separation of peaks and a flat baseline. For example, good separation of Glutamate and Glutamine was achieved in all spectra. The basic components of a high quality spectra is illustrated in Figure 6.6.

Dynamic B_0 shimming resulted in consistent water linewidths (FWHM) ranging from 12 Hz to 16 Hz with both single-voxel and two-voxel acquisition methods, in agreement with those previously reported (Otazo et al., 2006, Tkáč et al., 2009), and were not significantly different between the two acquisition methods (paired t -test, 2-tailed, Left hemisphere: $t_{(5)}=-.30$, $p=.78$, and Right hemisphere: $t_{(5)}=.37$, $p=.73$) (Table 6.1).

TABLE 6.1: **Water signal linewidths.** Mean FWHM (Hz) $\pm$ standard deviation for both voxels of interest and acquisition methods.

	Left M1	Right M1
Single-voxel	14.0 ± 1.2	13.7 ± 0.6
Two-voxel	14.2 ± 0.6	13.6 ± 0.8

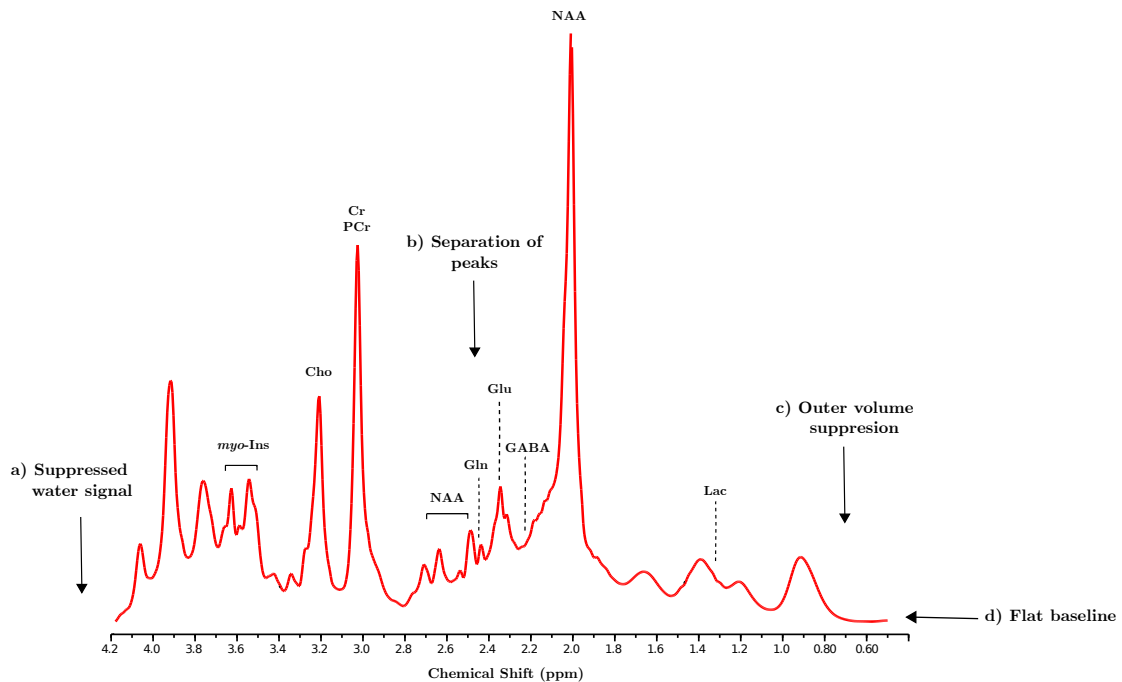


FIGURE 6.6: **Components of a high quality spectra.** LCModel output of a representative spectra from a single subject to illustrate **a)** minimal residual water signal by effective water suppression **a)** water residuals at 4.8 ppm not shown here and has been eliminated from the section of spectra with the metabolites of interest, **b)** minimal signals from outside voxels by effective outer volume suppression, **c)** good separation of peaks - for example, separation of Glutamate and Glutamine, and **d)** a flat baseline.

6.3.2 Neurochemical profile is consistent with the biochemical literature

High spectral quality allowed for the quantification of 18 metabolites (Figure 6.7) whose estimated concentrations were consistent with the biochemical literature (Emir et al., 2011). In the case of a high correlation between two metabolites measured, the sums of the metabolites are shown instead (PCho+GPC, Cr+PCr, NAA+NAAG, Glu+Gln, Glc+Tau). Cramér-Rao lower bound (CRLB) values were not significantly different between the two acquisition methods for all of the metabolites (paired *t*-test, $p > .05$), suggesting that data quality was of equal quality in both hemispheres.

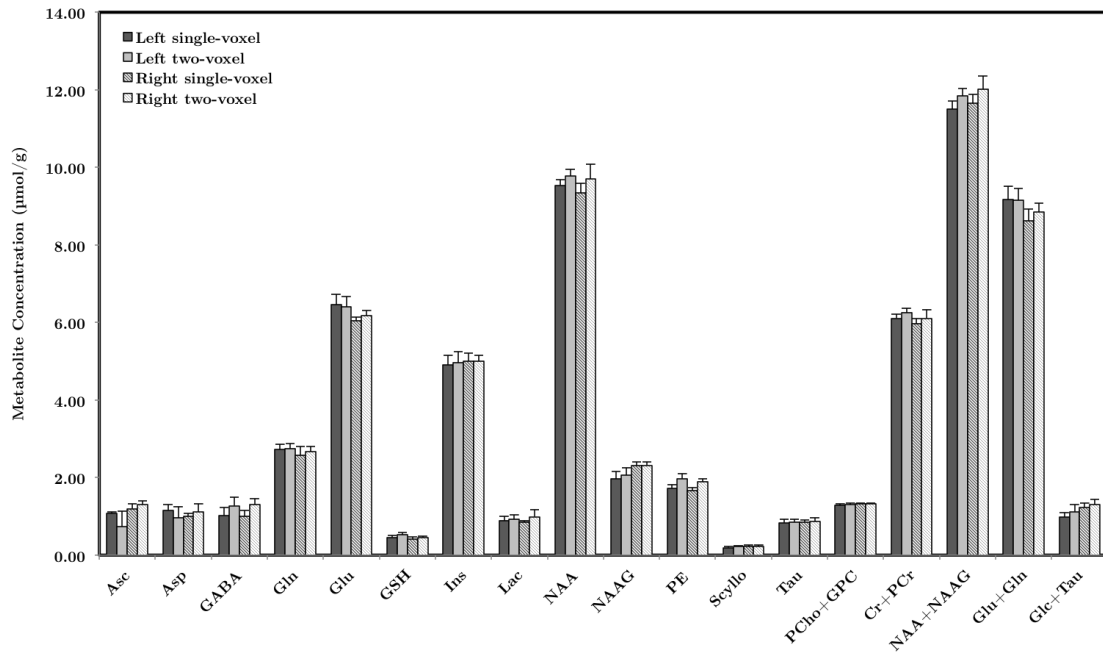


FIGURE 6.7: **Metabolite concentration.** Neurochemical profile from both voxels of interest and acquisition methods as determined by LCModel fitting. Error bars indicate SEM. Abbreviations used: Asc, Ascorbic acid; Asp, Aspartate; GABA, gamma-Aminobutyric acid; Gln, Glutamine; Glu, Glutamate; GSH, Glutathione; Ins, myo-Inositol; Lac, Lactate; NAA, N-Acetyl aspartate; NAAG, N-Acetyl aspartatyl glutamate; PE, Phosphatidylethanolamine; Scyllo, Scyllo-inositol; Tau, Taurine; PCho, Phosphocholine; GPC, Glycerophosphorylcholine; Cr, Creatine; PCr, Phosphocreatine.

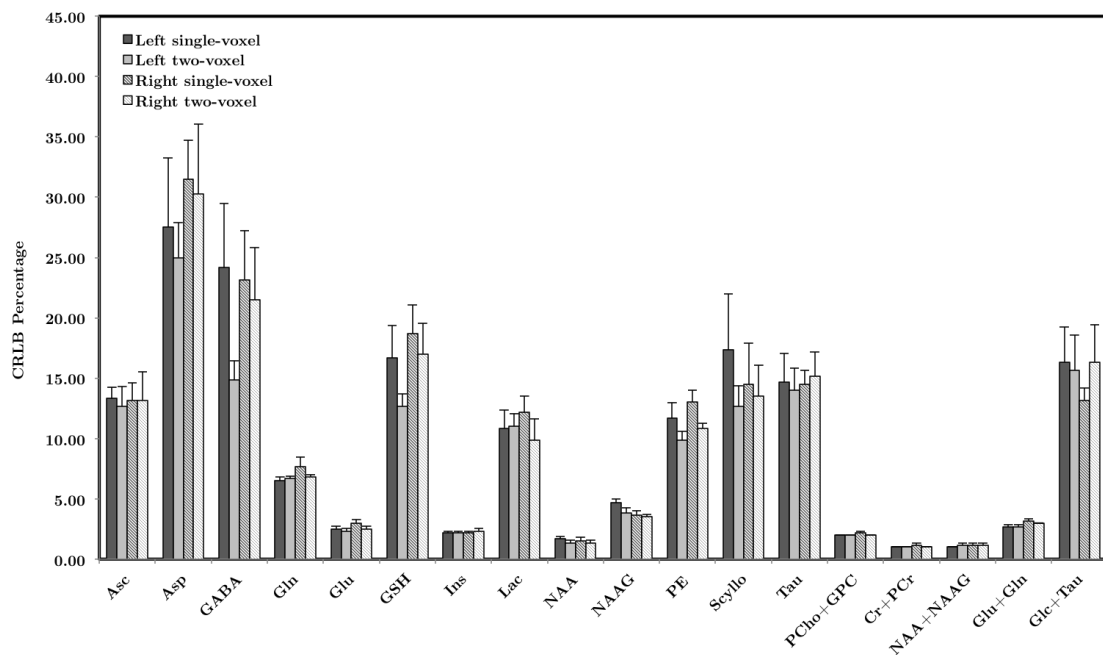


FIGURE 6.8: **Cramér-Rao Lower Bound (CRLB) percentage.** Corresponding CRLBs to the metabolites shown in Figure 6.7 from both voxels of interest and acquisition methods as determined by LCModel fitting. Error bars indicate SEM.

6.4 Discussion

This study was performed to test the robustness and reliability of the two-voxel acquisition method in quantifying metabolites concurrently from both primary motor cortices. We demonstrated for the first time high spectral quality from concurrent measurement of metabolites from both human primary motor cortices using a standard volume coil at 7 Tesla. A total of 18 metabolites could be quantified from these spectra and the neurochemical profile is consistent with the biochemical literature ([Emir et al., 2011](#)). We validated the two-voxel sLASER method in reliably quantifying metabolites from our VOI with spectra comparable with the single-voxel acquisitions. We also showed effective use of barium titanate dielectric pads for optimised B_1 efficiency for GABA quantification from both human motor cortices at UHF.

The spectral quality achieved in this study with both acquisition methods showed high SNR, efficient water suppression, and a distortion free baseline. This allowed for consistent and reliable quantification of 18 metabolites with LCModel. Notably, the CRLB percentage, a measure of quantification precision, was below 10% for the dominant metabolites (e.g. NAA, Cr+PCr, Glu) and below 50% for all other metabolites. These values are comparable with those previously reported at 7 T in the motor cortex ([Marjańska et al., 2012](#)). In addition, the high reproducibility in the water linewidths also suggests that the DSU protocol gave good shimming of B_0 inhomogeneity in the VOI. The DSU protocol optimises shims to the specific VOI, and thus, any changes in water linewidth in each VOI would suggest that the shims were no longer optimised for that area. Here we show good consistency in water linewidths within a narrow range.

RF behaviour in the human head becomes complex at UHF. With standard volume coils, higher signal intensity can be seen in the centre with a weak periphery, resulting in B_1 degradation in the motor cortex ([Van de Moortele et al., 2005](#), [Vaughan et al., 2001](#)) (for example see [Figure 3.9](#)). Here, we have demonstrated that with proper placement of the dielectric pads, sufficient B_1 for GABA quantification in both primary motor cortices can be achieved.

There are some practical considerations for the use of this acquisition method in humans. One main limitation is the relatively lengthy set-up period (approximately 20-25 minutes) for the two-voxel acquisition. This time includes the correct placement of the

dielectric pad, B_1 mapping, localisation of the VOI, and setting up the DSU protocol. Once the shim currents have been optimised in the set-up period, one spectral average from both VOI can then be acquired in approximately 11-14 minutes. In addition, the use of the adiabatic pulse in the sLASER sequence takes longer and requires more RF power than standard 180° pulses used at lower field strengths. Thus, more energy needs to be deposited into the person and results in a higher rate at which energy is absorbed by the body when exposed to an RF field, also known as the specific absorption rate (SAR). Due to SAR considerations from the adiabatic pulse, a long TR (7 seconds) is required, which further increases acquisition time.

6.5 Conclusions

In conclusion, this chapter presented data that validated a novel method for acquiring high quality spectra at 7 T in two-voxels of interest within a single session without the need for specialised hardware. Effective use of barium titanate dielectric pads was optimised for B_1 efficiency for GABA quantification in both human primary motor cortices. The two-voxel acquisition method and dielectric pad assessment presented here provides the foundation for the experiment presented in Chapter 7.

Chapter 7

Modulation of cortical neurotransmitters by transcranial direct current stimulation of the human primary motor cortices

The work presented in this chapter is a 7 Tesla MRS study that investigated changes in cortical neurotransmitters induced by transcranial direct current stimulation, in which measurements were obtained concurrently from both primary motor cortices. Novel evidence on the timecourse of GABA, glutamate, and myo-inositol changes induced by anodal, cathodal, and bilateral stimulation within the same subjects are presented. By measuring the timecourse of neurochemicals in this way we were able to directly test tDCS-induced physiological effects within the same individuals. Anodal stimulation reduced GABA in both the anode-targeted and non-stimulated M1. Cathodal stimulation decreased GABA and glutamate in the non-stimulated M1 with no concurrent changes in the cathode-targeted M1. A reduction in glutamate was demonstrated in both M1s during bilateral stimulation with no change in GABA.

7.1 Introduction

Transcranial direct current stimulation (tDCS) is a promising tool to non-invasively modulate activity of the underlying cortex. When applied to the primary motor cortex (M1), tDCS has polarity-dependent effects. Anodal tDCS increases cortical excitability and is thought to induce LTP-like plasticity, whereas cathodal tDCS diminishes cortical excitability (Nitsche and Paulus, 2000, Stagg and Nitsche, 2011). Dependent on the stimulation parameters, tDCS to M1 can improve motor performance and motor learning in both healthy controls (Reis et al., 2008a, Stagg et al., 2011a) and after stroke (Adeyemo et al., 2012, Stagg et al., 2012), suggesting its potential as a tool to promote plasticity. However, while tDCS-induced changes in cortical excitability have been related to modulations of the underlying cortical activity, less is known about the specific neurochemicals that might mediate these effects.

Similar to the induction of cortical plasticity in animals (Froc et al., 2000, Trepel and Racine, 2000), pharmacological studies in humans suggest that polarity-specific changes with DC stimulation depend on both glutamatergic and GABAergic modulation (Nitsche, 2003, Nitsche et al., 2004a). TMS studies that measure motor evoked potentials (MEPs), a marker of corticospinal excitability, have demonstrated that tDCS-induced excitability changes in MEPs are NMDA-receptor dependent (Liebetanz et al., 2002, Nitsche, 2003), a finding supported by animal studies (Fritsch et al., 2010).

Direct evidence on tDCS-induced local changes in human cortical neurotransmitters within M1 have come from studies using magnetic resonance spectroscopy (MRS). Anodal tDCS has been shown to decrease M1 GABA levels, with changes that last at least 20 minutes after stimulation has ceased (Stagg et al., 2011a, 2009a). Cathodal tDCS reduces Glx concentration, a composite marker of glutamate and glutamine, with a highly correlated decrease in GABA (Stagg et al., 2009a) (Figure 7.1.A). In one study, anodal tDCS to the right frontal lobe has been shown to significantly increase *myo*-inositol content at 30 minutes after stimulation onset (Rango et al., 2008). However, the physiological significance of this change is unclear.

An increasingly popular stimulation montage, commonly referred to as bilateral stimulation, suggests that simultaneous application of both anodal and cathodal tDCS over each primary motor cortex in an M1-M1 montage, could produce greater benefits than

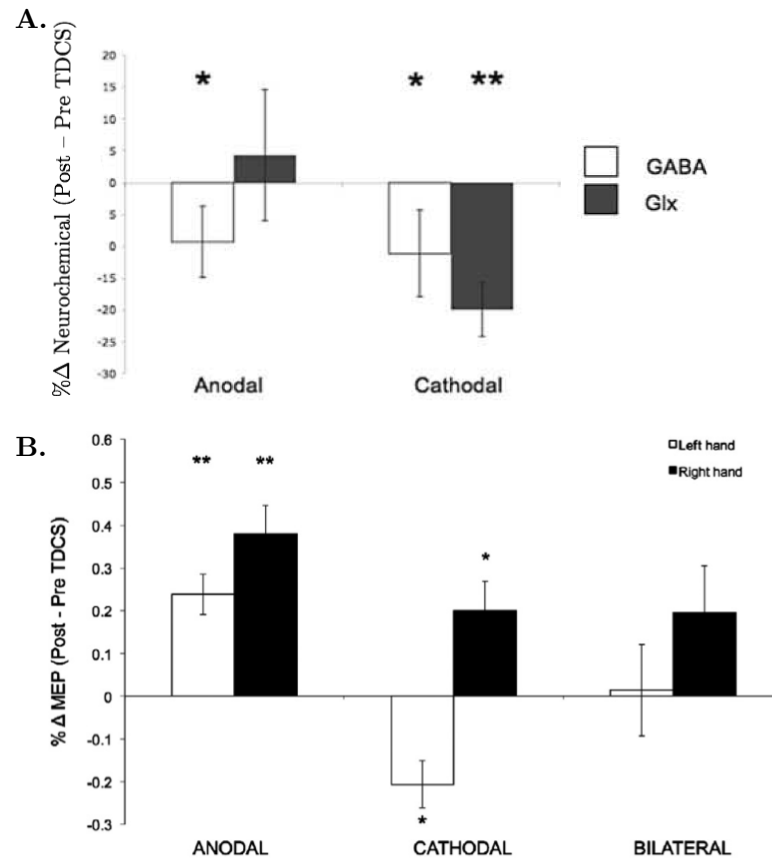


FIGURE 7.1: **M1 changes with tDCS.** **A.** MRS study assessing the change in neurochemicals in the stimulated M1 after anodal and cathodal tDCS. Figure adapted from (Stagg et al., 2009a). **B.** Effects of anodal, cathodal, and bilateral tDCS on MEPs in the contralateral and ipsilateral hands (O'Shea et al., 2014).

its constituent unilateral tDCS interventions (Karok and Witney, 2013, Vines et al., 2008). This is particularly relevant in the context of stroke as it is hypothesised that simultaneously increasing ipsilesional M1 and decreasing contralesional M1 excitability could have summative effects (Lindenberg et al., 2010, Mahmoudi et al., 2011). However, evidence on the efficacy of bilateral stimulation is heterogeneous (see Chapter 2.2.3) and the local cortical changes in M1 neurotransmitters that underly bilateral stimulation have not been tested.

The effects of anodal and cathodal tDCS on contralateral hand excitability are well-established, however, the effects on the ipsilateral hand are not routinely investigated. It is possible that such ipsilateral effects could complicate the interpretation of bilateral stimulation. A neurophysiological study by our group directly comparing anodal, cathodal, and bilateral tDCS demonstrated that bilateral tDCS does not have an effect

on reaction times in stroke patients, nor does it induce physiological effects in a simple summative fashion in healthy controls (O'Shea et al., 2014). In this study, MEPs were measured from both hands of healthy controls after anodal, cathodal, and bilateral stimulation (1mA, 10 minutes) (Figure 7.1.B). As would be expected, anodal stimulation increased and cathodal stimulation decreased MEPs from the contralateral hand. Interestingly, both anodal and cathodal stimulation increased MEP amplitudes in the ipsilateral hand. These findings suggest that both unilateral anodal and cathodal tDCS had effects on MEPs in both hands (O'Shea et al., 2014). However, there were no significant effects on cortical excitability in either hand after bilateral tDCS. Correlational analyses between the bilateral condition and unilateral anodal and cathodal stimulation indicated that each electrode in the bilateral condition stimulated the contralateral MEPs in the expected direction, but the effects were weak and highly variable. There are a number of reasons why the bilateral montage may be less efficacious than the unilateral montages, such as current shunting across the scalp, complex interhemispheric interactions, or effects from hemispheric dominance or handedness.

Ultra high field spectroscopy, as validated in Chapter 6, provides a novel method that allows us to a) directly measure changes in cortical neurotransmitters from M1, b) measure changes during the stimulation period, c) determine the timecourse of tDCS-induced changes to different cortical neurotransmitters, and d) assess the changes occurring concurrently to the non-stimulated M1. Here, we used this method to investigate the neurochemical changes induced by anodal, cathodal, and bilateral DC stimulation in both primary motor cortices of the same individuals.

We aimed to extend the findings from the previous MRS studies of tDCS in a number of important ways. We wished to determine the changes in M1 GABA during cathodal and bilateral tDCS, as well as replicate the previously reported effects of a GABA decrease following anodal stimulation (Chapter 4 of this thesis, Stagg et al. 2011a, 2009a). We also aimed to determine the timecourse and duration of any tDCS-induced changes to cortical neurotransmitters. Additionally, as GABA is only one possible neurochemical system that may be affected by DC current, we aimed to determine the effects of the three stimulation montages on glutamate and *myo*-inositol, both of which have previously been reported to be modulated by tDCS (Rango et al., 2008, Stagg et al., 2009a). Finally, we wished to determine the concurrent changes in neurochemicals in the non-stimulated M1, which to date, have not been measured. To our knowledge, this is the first study to give

evidence on the timecourses of GABA, glutamate, and *myo*-inositol change concurrently in both M1s with anodal, cathodal, and bilateral stimulation in the same individuals.

It is hypothesised that, in the stimulated M1, anodal tDCS would decrease GABA concentration and cathodal tDCS would decrease both GABA and glutamate concentration, as previously reported (Stagg et al., 2009a). Corresponding to the reported MEP changes in our previous study (O’Shea et al., 2014), we would expect a similar decrease in GABA in the non-stimulated M1 with both anodal and cathodal stimulation.

7.2 Methods

7.2.1 Participants

Twelve healthy participants (2 male; aged 20-32 years, mean 25 years) gave their informed consent to participate in this study in accordance with ethical approval from the East London Research Ethics Committee (Ref: 10/H0703/50). All were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971).

7.2.2 Experimental design

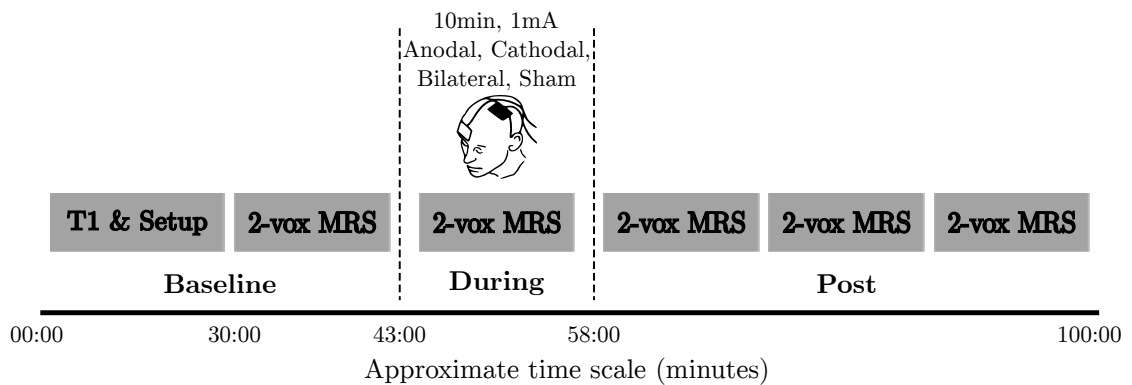


FIGURE 7.2: **Experimental Design.** All subjects participated in four experimental sessions separated by at least 1 week. Subjects received anodal, cathodal, bilateral, or sham tDCS in a counterbalanced order. GABA was measured at five time points (Baseline, During, Post1, Post1, Post3) for both the left and right M1. Timeline shown is an estimate of the length of the scans in minutes. Abbreviations used: T1, T₁-weighted structural scan; MRS, magnetic resonance spectroscopy acquisition.

All subjects participated in four testing sessions with either sham, anodal, cathodal, or bilateral tDCS (Figure 7.2). Two subjects did not complete the bilateral condition

due to personal time constraints. All sessions were separated by at least one week and the order of the sessions was counterbalanced across the group. In each experimental session, measurements of neurochemicals were acquired at five time points: at baseline, during tDCS, and at three timepoints post-tDCS (Post1, Post1, Post3). A total of 460 spectra were acquired (5 timepoints from each hemisphere for 4 sessions per subject for 10 subjects and for 3 sessions per subject for 2 subjects). Subjects watched a nature documentary for the entire duration of the experiment.

7.2.3 Transcranial direct current stimulation (tDCS)

A DC-Stimulator (Magstim, Ltd; UK) delivered a 1mA current to the brain via electrodes measuring 5 x 7 cm (Easycap, GmbH; Germany). The active electrode was centred over the left M1, positioned 5 cm lateral to Cz. For the anodal, cathodal, and sham conditions, the reference electrode was placed over the contralateral supraorbital ridge. For the bilateral condition, the anode was placed over the left M1 and the cathode over the right M1, and each electrode was positioned 5 cm lateral to Cz. Figure 7.3 illustrates the three electrode montages.

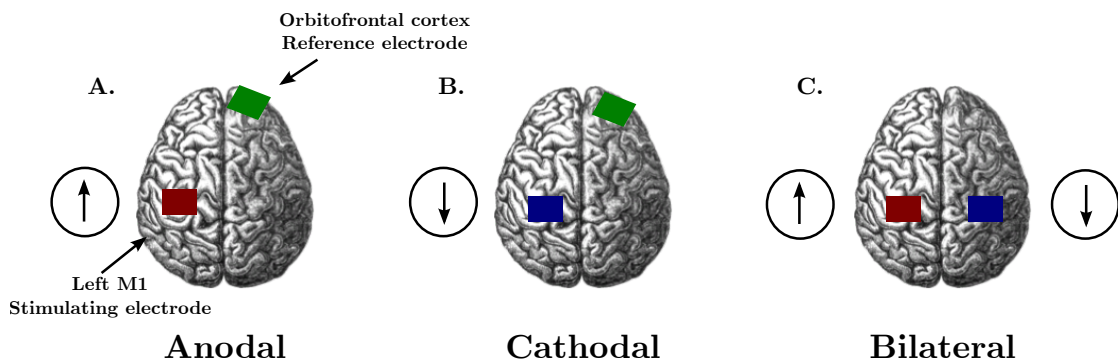


FIGURE 7.3: Placement of electrodes for the three stimulation conditions. **A.** Anodal tDCS: anode over the left M1 and the reference electrode over the orbitofrontal cortex. **B.** Cathodal tDCS: cathode over the left M1 and the reference electrode (green) over the orbitofrontal cortex. **C.** Bilateral tDCS: anode over the left M1 and cathode over the right M1. In all protocols, the M1 electrode was positioned 5 cm lateral to Cz. Colours: Red - Anode, Blue - Cathode, Green - Reference electrodes.

High Chloride Electrolyte-Gel (Easycap, GmbH; Germany) was used as the conducting medium between the scalp and electrodes. The electrodes contained 5 k Ω resistors and extension leads connected the stimulator, which was located outside of the magnetic field, to the subject positioned in the scanner. For the real stimulation conditions (anodal, cathodal, bilateral) the current was ramped up over 10 seconds and was then held at 1mA

for 10 minutes, before being ramped down over 10 seconds. For sham stimulation the DC-stimulator was ramped up for 10 seconds then switched off, as previously described (Stagg et al., 2011a).

7.2.4 Magnetic resonance acquisition

All data were acquired on a 7T Siemens MR system as reported in Chapter 6. A barium titanate-based dielectric pad with linear dimensions of 11 x 11cm and 5mm thick was positioned on the top of the head to increase B_1 efficiency within the volume of interest (VOI). B_1 maps were acquired using the actual flip angle imaging (AFI) sequence (FOV 240x240, $TR/2=6/30$ ms, $TE=2.58$ ms, slice thickness=2.5mm, non-selective flip angle 60°). When necessary, the dielectric pad was re-positioned and the B_1 map repeated to achieve the best B_1 efficiency in the VOI.

T_1 weighted images (MPRAGE, 192x1mm axial slices, $TR/TE=2200/2.82$ ms, flip - angle= 7° , FOV 192x100) were used to place a 2x2x2cm voxel of interest over the left and right precentral knob, a known landmark for hand motor representation (Yousry et al., 1997). The two voxels were on each end of a 80mm column, and thus, were 40mm apart. This width was tested to be the optimal balance for sufficient SNR and spectral quality.

Spectra were measured using semi-localisation by adiabatic selective refocusing (sLASER) (Öz and Tkáč, 2010) pulse sequence ($TR/TE=7000/30$ ms) with VAPOR (Tkáč et al., 1999) water suppression (pulse duration = 35ms) and 8 outer volume suppression (pulse duration = 4.6ms). Signal was acquired from the two voxels in an interleaving fashion alternating between each voxel (64 transients each) (Lemke et al., 2013).

7.2.5 Magnetic resonance analysis

Data were corrected using in house scripts on the unsuppressed water signal from the same VOI for reconstruction, eddy current correction, and zero-order phasing of array coil spectra. Any residual water signal was removed using Hankel-Lanczos singular value decomposition (HLSVD) (Cabanès et al., 2001). Neurochemicals were quantified using LCModel analysis (Provencher, 2001). The following criteria were applied for excluding spectra with poor quality: (i) Cramér-Rao lower bounds (CRLB) $> 50\%$, (ii)

Water linewidths at full width at half maximum (FWHM) > 15 Hz, and (iii) SNRs > 10 . As an additional check each spectra from the LC Model output were inspected visually to determine the quality of the peaks at the GABA, glutamate, and *myo*-inositol resonances. Of the 460 spectra acquired, 15 spectra from 5 subjects were excluded for the GABA measurements. Two spectra from 1 subject were excluded for the glutamate and *myo*-inositol measurements.

7.2.6 Statistical analysis

Statistical analyses on the data were conducted using SPSS (Version 22, IBM). All neurotransmitter concentrations reported are given as a ratio to the total creatine (Cr+PCr), and henceforth are referred to as GABA, glutamate, and *myo*-inositol levels for simplicity. To control for any between-session differences, the absolute change in neurochemical concentrations from the baseline value and each time point after were calculated for GABA, glutamate, and *myo*-inositol for both the left and right M1. For each neurochemical, a main Repeated Measures ANOVA (RM ANOVA) was run with one factor of stimulation (anodal, cathodal, bilateral, sham), and one factor of time (during, post1, post2, post3). Subsequently, a RM ANOVA was performed to test each real stimulation condition (anodal, cathodal, bilateral) separately against the sham condition, with one factor of stimulation and one factor of time. In each case, the main result of interest is a main effect of stimulation.

7.3 Results

7.3.1 Spectral quality

Excellent spectral quality was achieved from both M1 voxels. All resulting spectra had good separation of peaks and a flat baseline (Figure 7.4). Consistent linewidths was achieved in both the left and right VOI (FWHM in Hz $\pm$ SD, Left M1: 12.6 ± 1.5 , and Right M1: 12.8 ± 1.4), and were not significantly different between the left and right (paired *t*-test, 2-tailed, $t_{(45)}=-1.71$, $p>.05$).

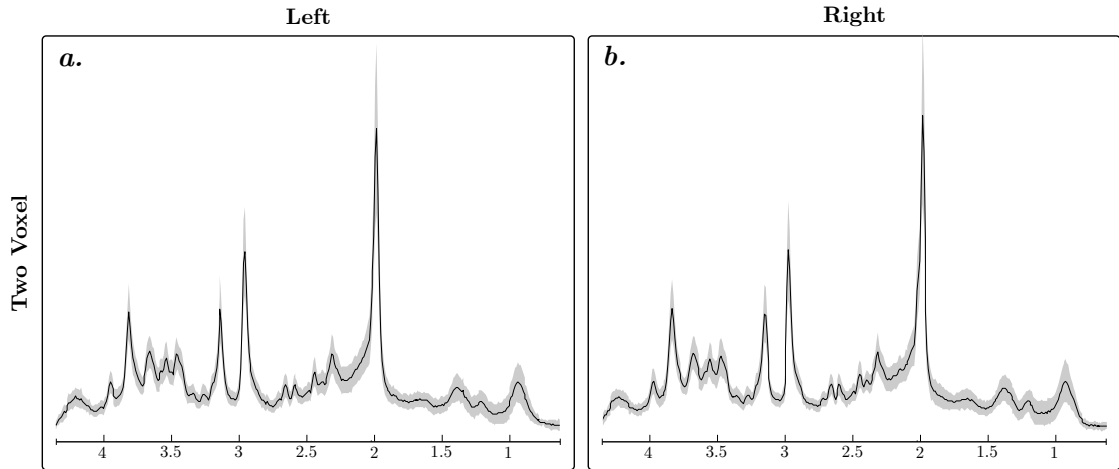


FIGURE 7.4: **Spectral quality.** LCMoDel output of spectra averaged from 12 participants. Shaded grey indicates the standard deviation from the mean.

7.3.2 No differences at baseline between conditions

As the neurochemicals were referenced to total creatine (Cr+PCr), we first wanted to test for any changes in creatine over time. A RM ANOVA with one factor of stimulation and one factor of time showed no main effects of stimulation or time, or stimulation by time interaction for either M1 (all $p > .05$). Thus, we were confident that creatine was a stable reference metabolite. All further analyses are carried out using neurochemical concentrations referenced to the total creatine.

To test for any differences at baseline between the stimulation conditions a RM ANOVA was performed with one factor of stimulation and one factor of hemisphere for each metabolite separately. There were no significant main effects of stimulation at baseline for GABA, glutamate, or *myo*-inositol (all $p > .1$). There was however, a significant main effect of hemisphere at baseline for GABA ($F_{(1,7)}=121.26$, $p < .001$), glutamate ($F_{(1,9)}=82.76$, $p < .001$), and *myo*-inositol ($F_{(1,9)}=5.07$, $p = .05$). These results reflect consistently higher values for all metabolites for the right compared to the left hemisphere. In order to control for any possible, systematic baseline differences between the left and right M1s, we calculated absolute change in neurochemical concentrations from baseline and those values are used in all further analyses.

7.3.3 Sham condition

RM ANOVA was performed on the data from the sham session with factors of time and hemisphere for each metabolite separately. There were no significant main effects of time or hemisphere, or time by hemisphere interaction in the sham condition for either glutamate or *myo*-inositol (all $p > .1$). However, there was a significant main effect of time for GABA ($F_{(3,27)} = 3.01$ $p = .05$), but no significant main effects of hemisphere or time by hemisphere interaction.

7.3.4 Effects of stimulation on metabolites for each hemisphere

In each condition, the absolute change for each time point from the baseline value were calculated for GABA, glutamate, and *myo*-inositol. For each neurochemical and hemisphere separately, a main RM ANOVA was performed on the absolute change at each time point with one factor of stimulation (anodal, cathodal, bilateral and sham) and one factor of time (during, post1, post2, post3). There was a significant main effect of stimulation in both the stimulated and non-stimulated M1 for GABA (main effect of stimulation, stimulated M1: $F_{(3,15)} = 3.17$, $p = .05$, and non-stimulated M1: $F_{(3,24)} = 4.19$, $p = .02$). There was no significant main effect of stimulation in either M1 for glutamate or *myo*-inositol (all $p > .1$).

We went on to investigate these effects further as follows. First, we tested the changes in neurochemicals for each real stimulation condition separately against the sham session by performing RM ANOVAs (sham and anodal, sham and cathodal, sham and bilateral) with one factor of stimulation and one factor of time (during, post1, post2, post3). In each case, the main result of interest is the main effect of stimulation. Second, we performed Pearson's correlations to determine the relationship between GABA and glutamate within and between M1s at each timepoint. Finally, we tested the relationship between the left and right M1 for the change in each neurochemical for each stimulation condition. These findings are presented by stimulation type below, to give an overall picture of the effects of each stimulation montage on each neurochemical of interest in the primary motor cortices.

For ease of visualisation, and as we were interested in the change occurring with real stimulation compared to the sham condition, all timecourse data are presented as the percentage change from baseline corrected to the sham condition (Figures 7.5 to 7.6).

7.3.4.1 Effects of anodal stimulation

As expected, there were significantly lower GABA levels after the anodal condition compared with sham. A RM ANOVA with one factor of stimulation and one factor of time demonstrated a significant main effect of stimulation in both the anode-targeted and non-stimulated M1 for GABA (main effect of stimulation, anode-targeted M1: $F_{(1,8)}=8.93$, $p=.02$, and non-stimulated M1: $F_{(1,11)}=7.90$, $p=.02$). At later post-stimulation timepoints, the decrease in the anode-targeted M1 GABA was correlated with the concurrent decrease in GABA in the non-stimulated M1 (Pearson's correlation, 2-tailed, Post2: $R=.66$, $p=.02$, and Post3: $R=.64$, $p=.03$).

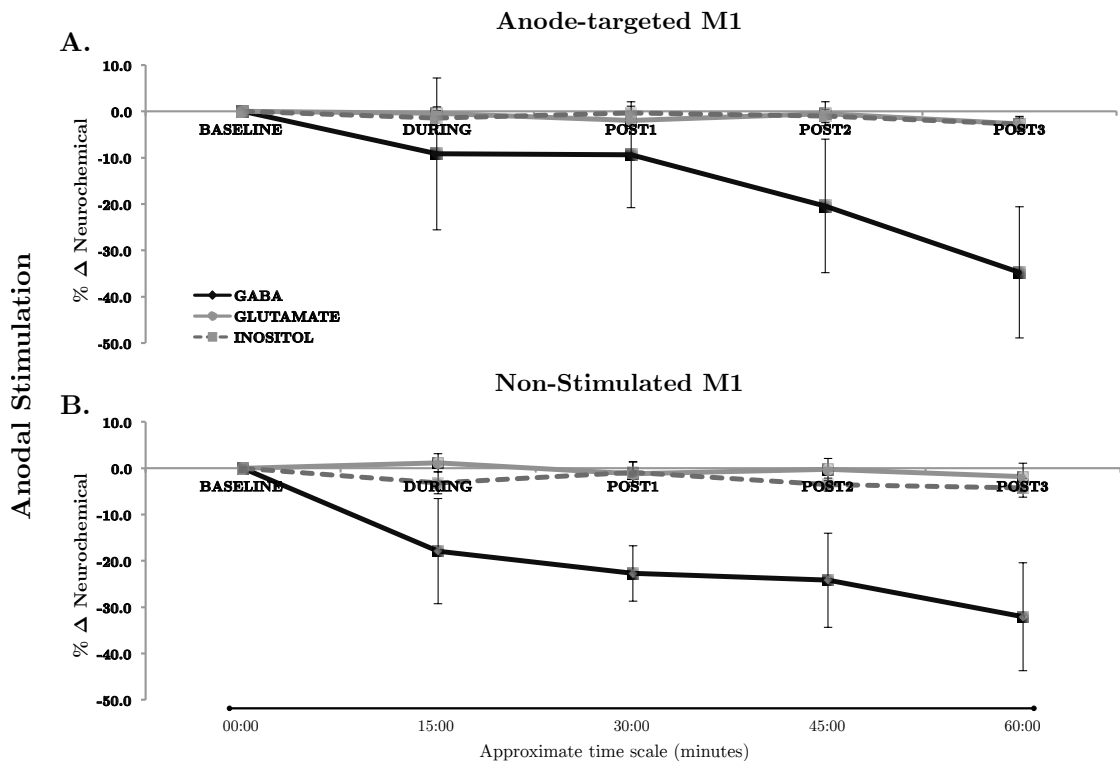


FIGURE 7.5: **Effects of anodal stimulation on cortical neurotransmitters in M1.** Mean percentage change in neurochemicals from baseline corrected to the sham condition for **A.** Anode-targeted M1. **B.** Non-stimulated M1. Error bars indicate SEM.

No significant effects of stimulation were observed in either M1 for glutamate or *myo*-inositol (all $p>.05$). Within the anode-targeted M1, the decrease in GABA was correlated with a corresponding decrease in glutamate in the early timepoints (Pearson's

correlation, 2-tailed, During: $R=.81$, $p<.001$, and Post1: $R=.62$, $p=.03$). There was no relationship between GABA and glutamate within the non-stimulated M1.

7.3.4.2 Effects of cathodal stimulation

In the cathodal condition, a RM ANOVA with one factor of stimulation and one factor of time demonstrated no significant main effect of stimulation on GABA or glutamate levels within the cathode-targeted M1 ($p>.1$). However, a decrease in both GABA and glutamate was seen in the non-stimulated M1 (RM ANOVA, main effect of stimulation GABA: $F_{(1,9)}=13.11$, $p<.01$, and glutamate: $F_{(1,11)}=4.98$, $p<.05$). No significant main effect of stimulation was observed in *myo*-inositol levels in either M1 ($p>.05$). In addition, there were no relationships between stimulation-induced changes in GABA and glutamate, or between stimulation-induced changes in the left and right M1 for either neurochemical at any of the timepoints.

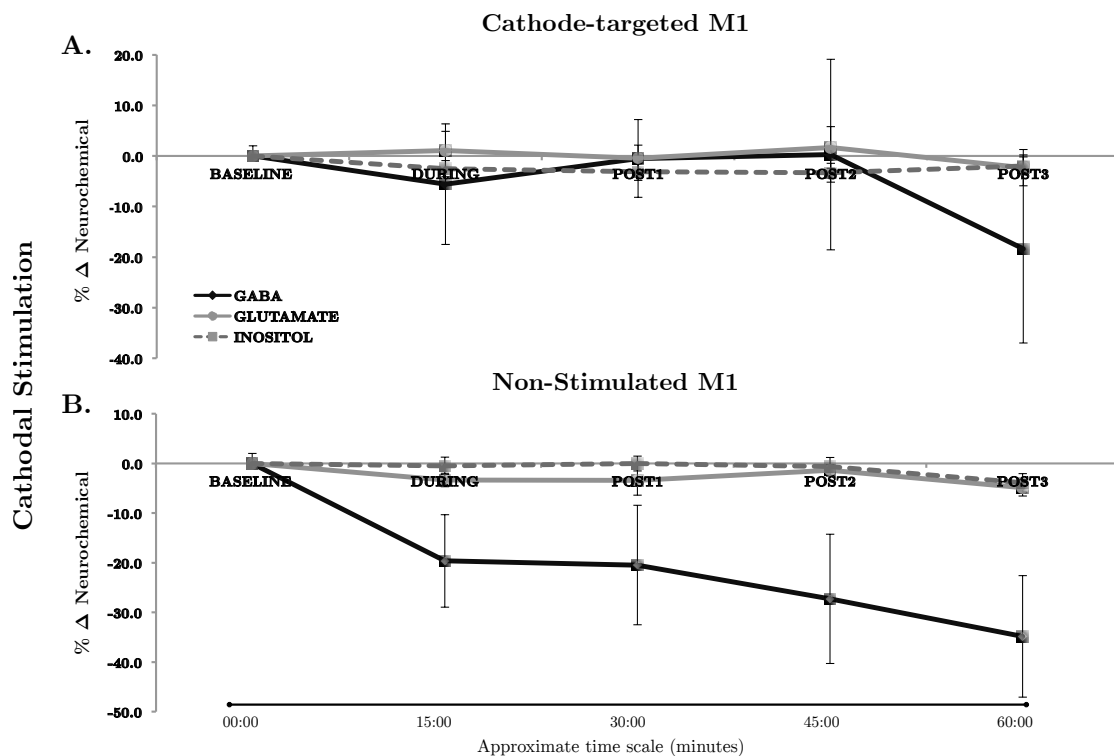


FIGURE 7.6: Effects of cathodal stimulation on cortical neurotransmitters in M1. Mean percentage change in neurochemicals from baseline corrected to the sham condition for **A.** Cathode-targeted M1. **B.** Non-stimulated M1. Error bars indicate SEM.

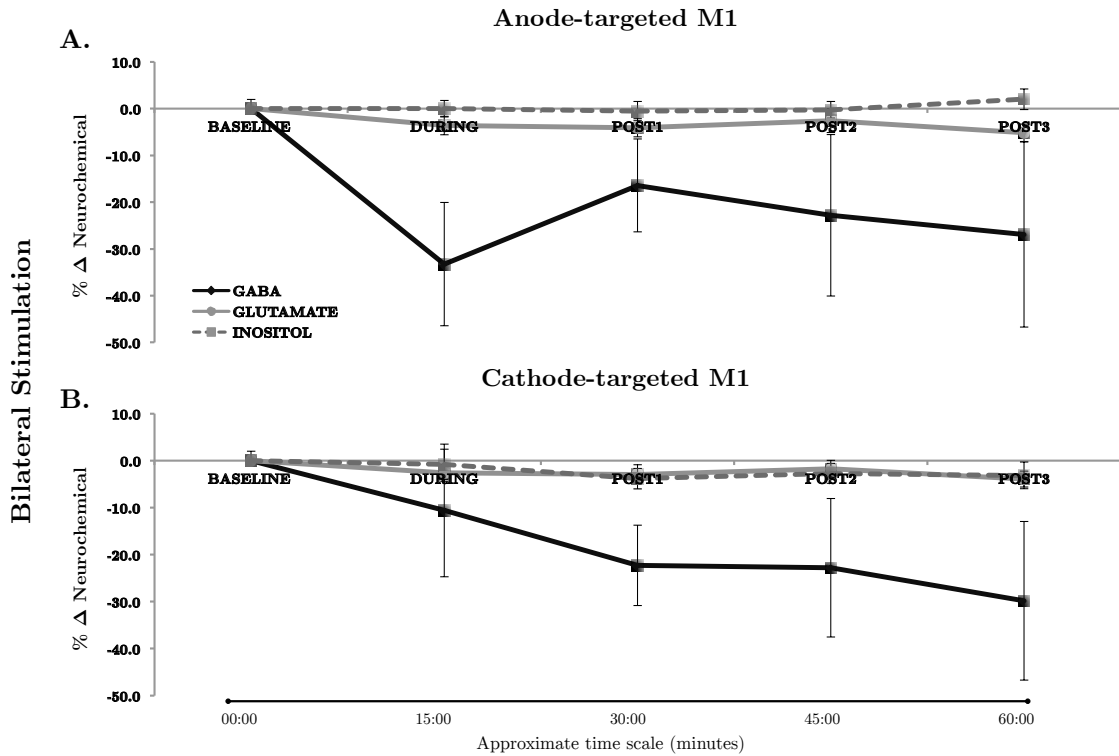


FIGURE 7.7: Effects of bilateral stimulation on cortical neurotransmitters in M1. Mean percentage change in neurochemicals from baseline corrected to the sham condition for **A.** Anode-targeted M1. **B.** Cathode-targeted M1. Error bars indicate SEM.

7.3.4.3 Effects of bilateral stimulation

In the bilateral condition, a RM ANOVA with one factor of stimulation and one factor of time demonstrated no significant main effect of stimulation in the anode-targeted M1 for GABA concentration, with a trend towards a decrease in the cathode-targeted M1 (main effect of stimulation, anode-targeted M1: $F_{(1,7)}=1.60$, $p=.25$, and cathode-targeted M1: $F_{(1,9)}=4.47$, $p=.06$). The effects of the bilateral condition on glutamate levels were significantly different from the sham condition for both the anode- and cathode-targeted M1 (RM ANOVA, main effect of stimulation, anode-targeted M1: $F_{(1,9)}=6.46$, $p=.03$, and cathode-targeted M1: $F_{(1,9)}=5.21$, $p<.05$). No significant main effect of stimulation was observed in *myo*-inositol levels in either M1 ($p>.1$).

Although no significant main effects of stimulation were demonstrated for GABA, we wished to determine whether the pattern of changes in the bilateral condition could be predicted from the responses in the unilateral anodal and cathodal conditions. Linear regression analysis was performed on the overall change (average of post stimulation timepoints) for each condition to investigate the possible predictive relationship

between i) Unilateral anode-targeted M1 and Bilateral anode-targeted M1, and ii) Unilateral cathode-targeted M1 and Bilateral cathode-targeted M1. The change in GABA levels in the unilateral anode-targeted M1 predicted the change in the bilateral anode-targeted M1 (adjusted R square=.40; $F_{(1,8)}=6.90$, $p=.03$; $\beta=-.68$). However, the unilateral cathode-targeted M1 did not predict the change in GABA in the bilateral cathode-targeted M1 ($p>.1$).

7.4 Discussion

This study aimed to characterise the timecourse of neurochemical changes induced by anodal, cathodal, and bilateral tDCS in both primary motor cortices of the same individuals. We replicated previously reported findings and demonstrated that anodal tDCS reduced GABA concentration in the anode-targeted M1. We showed for the first time that this decrease was related to a concurrent decrease in GABA in the non-stimulated M1. In the cathodal condition, there was a significant decrease in both GABA and glutamate in the non-stimulated M1, with no significant changes in the cathode-targeted M1. There was a decrease in glutamate concentration in both M1s with bilateral tDCS and no change in GABA levels.

Below, the effects of each stimulation condition on M1 GABA and glutamate concentration are considered in turn. In each case, the findings of the current study are related to a previous MRS study assessing changes in neurochemicals in the stimulated hemisphere after unilateral anodal and cathodal montages ([Stagg et al., 2009a](#)) and a previous neurophysiological study assessing the effects of anodal, cathodal, and bilateral montages on MEPs in the contralateral and ipsilateral hands ([O'Shea et al., 2014](#)). The current findings are related to the findings of [O'Shea et al. 2014](#) as this study measured both contralateral and ipsilateral MEPs in the same subjects and directly compared anodal, cathodal, and bilateral tDCS in healthy controls. The direction of findings in the previous studies and the findings from the present study are summarised in [Figure 7.8](#). The figure does not take into account the magnitude of changes with each stimulation condition and only illustrates the direction of change.

	Anode-targeted M1	Non-stimulated M1	Cathode-targeted M1	Non-stimulated M1	Anode-targeted M1	Cathode-targeted M1
A. Stagg et al. (2009) % Δ GABA	↓	n/a	↓	n/a	n/a	n/a
B. O'Shea et al. (2014) % Δ MEP	↑	↑	↓	↑	(↑)	(↓)
C. Hypothesis for Δ GABA	↓	↓	↓↑	↓	(↓)	(↑)
D. Δ GABA	↓	↓	—	↓	—	—
Δ GLU	—	—	—	↓	↓	↓
	ANODAL		CATHODAL		BILATERAL	

FIGURE 7.8: **Summary of tDCS effects on M1 neurochemicals.** **A.** Direction of GABA change with anodal and cathodal tDCS measured from the stimulated M1 based on the findings of [Stagg et al. 2009a](#). **B.** Direction of MEP change for each stimulation condition based on the findings of [O'Shea et al. 2014](#). The arrows indicate the corresponding M1 that produced the MEPs from the contralateral and ipsilateral hands. **C.** Predicted change in GABA for each stimulation condition based on the previous results shown in A and B. **D.** Direction of tDCS-induced M1 GABA and glutamate change measured in this study. Dashed lines indicate no significant changes measured. Arrows in brackets indicate a trend towards significance.

No changes in *myo*-inositol were measured in any of the tDCS conditions. One study has reported a significant increase in *myo*-inositol content at 30 minutes after anodal stimulation offset in the right frontal lobe ([Rango et al., 2008](#)). The physiological basis of how DC current might affect *myo*-inositol levels, and if it would affect *myo*-inositol levels in M1, are still unclear, and the differences in brain regions and stimulation montage to the previous study make these findings difficult to reconcile. Here, we found no measurable effects of anodal, cathodal, or bilateral tDCS on M1 *myo*-inositol levels.

7.4.1 Effects of anodal stimulation on M1 neurochemicals

We demonstrated a GABA decrease in the anode-targeted M1 with anodal stimulation, which persisted for at least 30 minutes after stimulation has ceased. These findings correspond to the previously reported MRS-assessed decrease in M1 GABA ([Stagg et al., 2011a, 2009a](#)) and the timecourse of GABA change reported in Chapter 4. We demonstrate for the first time a GABA decrease in the non-stimulated M1 that was correlated

with the decrease in the stimulated M1 at the later timepoints (Post2, Post3). These findings correspond to the previously reported neurophysiological effects of anodal stimulation, where MEP amplitude increased in both contralateral and ipsilateral hands in healthy controls at rest (O'Shea et al., 2014) (Figure 7.1.B).

Although the behavioural effects of tDCS were not investigated here, previous studies in healthy controls have shown similar reductions in M1 GABA following motor learning (Floyer-Lea, 2006), and have demonstrated that the degree of motor learning is related to the degree of anodal tDCS-induced GABA decrease (Stagg et al., 2011a). In both these studies, GABA was measured from the left M1 - contralateral to the hand performing the task and below the anodal electrode, respectively. The magnitude of anodal tDCS-induced GABA decrease in M1 has been demonstrated to predict individual differences on a robotic force adaptation and de-adaptation task, such that those with a greater decrease in GABA also had reduced reaching errors during adaptation (motor learning) and increased reaching errors during de-adaptation (motor memory) (Kim et al., 2014).

The behavioural relevance of the GABA decrease in the non-stimulated M1 observed in this study is unclear and should be investigated in future studies. For example, measuring the degree of anodal tDCS-induced GABA decrease in each M1 during performance of a unimanual and bimanual task, or during learning of a complex motor task, may give insight into how tDCS augments motor learning and the responses between the hemispheres. It has recently been demonstrated that bilateral tDCS led to more synchronized execution during learning of motor synergies using a task that required the production of difficult muscular activation patterns (Waters-Metenier et al., 2014). Whether anodal tDCS might yield greater or less efficacy in such tasks by concurrently decreasing GABA in both M1 should be investigated in future studies.

In the context of stroke, it could be hypothesised that the greater behavioural improvement reported in patients with chronic stroke after anodal stimulation compared with cathodal or bilateral stimulation (O'Shea et al., 2014, Stagg et al., 2012) might be a consequence of increased cortical facilitation of both hemispheres with anodal tDCS. Recruitment of contralesional premotor areas has been shown to be functionally relevant to recovery in more impaired patients after stroke (Johansen-Berg et al., 2002). Thus, if anodal tDCS decreases inhibition in both hemispheres, it is possible that the

improvements in behaviour observed with this montage are a consequence of a facilitation in these distant areas (via a decrease in GABAergic inhibition) in addition to the facilitation of the anode-targeted ipsilesional M1. This would support the adaptive plasticity model of recovery after stroke (see Chapter 1.3.4), whereby the efficacy of anodal tDCS might be explained by augmentation of distant inter-connected networks.

To date, investigations into the contralesional hemisphere is often neglected in measurements of tDCS-induced effects in stroke, primarily due to methodological limitations in being able to measure GABA from both M1s simultaneously. This has made conclusions on the role of the ipsilateral motor cortex difficult to discern. The current methods may be an attractive way to help clarify the role of tDCS-induced GABA modulations in motor learning and recovery after stroke and should be probed further in future studies.

7.4.2 Effects of cathodal stimulation on M1 neurochemicals

There were no significant changes in neurochemicals in the cathode-targeted M1 with cathodal stimulation. Instead, we observed a decrease in GABA and glutamate in the non-stimulated M1 (Figure 7.8.D).

Using the same stimulation parameters to the present study, Stagg and colleagues (2009a) reported a significant decrease in both M1 GABA and Glx (glutamate and glutamine complex) in the cathode-targeted M1. The direction of the GABA change in that study is in disagreement with the neurophysiological effects of cathodal tDCS, which has been reliably shown to decrease excitability (Nitsche and Paulus, 2000). It was postulated that the unexpected decrease in GABA might be due to the close biochemical relationship between GABA and Glx. Measuring the neurophysiological effects of cathodal tDCS, O'Shea et al. 2014 demonstrated a decrease in MEPs from the hand contralateral to the cathode-affected M1, and an increase MEPs from the non-stimulated M1 (Figure 7.8.B). It should be highlighted that in the O'Shea et al. 2014 study the stimulating electrode was placed over the right M1 of healthy controls in order to mimic the placement of electrodes for a left-hemispheric stroke. Here, we wanted to compare the effects of stimulation montages on metabolites, and thus, the cathodal electrode was placed over the left M1, in the same position as the anodal electrode in the anodal condition.

Corresponding to the two previous studies, we might expect M1 GABA levels to either decrease (Stagg et al., 2009a) or increase (if the excitability effects are due to GABA alone) (O'Shea et al., 2014) in the cathode-targeted M1, and decrease in the non-stimulated M1 (O'Shea et al., 2014) (Figure 7.8.C). By contrast, we find no change in the cathode-affected M1 and instead, observed a decrease in GABA and glutamate in the non-stimulated M1. Here, the GABA decrease in the non-stimulated M1 is in agreement with O'Shea et al. 2014, albeit with differences in the side of the non-stimulated M1 (Left M1 in our study, Right M1 in the O'Shea et al. study). Both studies investigated healthy right-handed participants.

It is possible that complex hemispheric dominance or asymmetry may explain the disagreement between the findings in the cathode-affected M1. To explore the possible effects of these factors, future studies could measure the differences between cathodal stimulation over the left M1 and compare this to stimulation over the right M1 within the same individual. Previous studies have demonstrated hemispheric specialisation amongst right-handers, indicating subtle asymmetry in motor cortical inhibitory tone as measured by TMS (Ilić et al., 2004), and findings indicate that the left M1 may respond more robustly than the right M1 to anodal stimulation (Schambra et al., 2011). Whether the same might be true for cathodal stimulation is unclear.

In general, the above findings suggest that the pattern of effects with cathodal stimulation is less straightforward when compared to anodal stimulation. There are a number of reasons that could explain the heterogeneity in the effects of cathodal stimulation. One possible reason could be the path of current flow through the human brain with the cathodal montage. During cathodal stimulation, the current flows towards the cathode placed over M1 from the reference electrode over the orbitofrontal cortex and thus needs to pass through the frontal sinuses in order to reach M1, which could potentially render it more susceptible to shunting effects (Rush and Driscoll, 1968) resulting in a more disperse distribution of the current. Modelling studies mapping the path of current distribution with tDCS are currently limited (see Chapter 2.2.4 for a discussion) and further investigations are needed to determine the efficacy of the cathodal montage.

7.4.3 Effects of bilateral stimulation on M1 neurochemicals

There were no significant changes in GABA with bilateral stimulation. However, we demonstrated a decrease in glutamate concentration in both the anode-affected and cathode-affected M1.

Using the same stimulation parameters to this study, [O'Shea et al. 2014](#) demonstrated no significant neurophysiological effects with bilateral stimulation. However, further analysis indicated that the percentage change in MEPs in the bilateral data could be explained by the response of each constituent electrode, suggesting that the bilateral montage did stimulate both M1s in the expected direction but the overall effects were weak and variable. In this study, we did not have strong predictions concerning the effects of bilateral stimulation on M1 neurotransmitters other than a hypothesis derived from combining the effects of unilateral tDCS of the anode-affected and cathode-affected M1. Although we measured no significant effects of tDCS on GABA levels, when we tested the data to determine whether the responses in the bilateral condition could be determined by the responses in the unilateral anodal and cathodal conditions, we demonstrated that this was true for the anode-targeted M1 but not for the cathode-targeted M1.

Bilateral tDCS has been shown to have non-specific, generalised effects on the trained and untrained hand during learning of motor synergies ([Waters-Metenier et al., 2014](#)). It was postulated that the bihemispheric current may be modulating the communication between motor cortices during training, however, the mechanisms underlying the improvements observed in this study were unclear. Specifically how the bihemispheric current exerts its effects on each M1, and on the interactions between the two M1s will need to be tested in future studies. In this study, we demonstrated that the bilateral montage decreases glutamate concentration in both M1, which may suggest that the lack of changes shown in previous studies, and the heterogeneity in the bilateral stimulation literature, could be a consequence of complex interactions involving glutamate between the hemispheres. These interactions will need to be probed further in future investigations.

7.4.4 Caveats and limitations

We found significantly higher neurotransmitter levels for the right M1 compared to the left M1. However, it is unclear why these discrepancies exist. The spectra are of high quality and have good linewidths that were not significantly different between the left and right VOI. Given the high quality of the spectra, the hemispheric asymmetry in concentration estimates are unlikely to reflect a hardware anomaly. Neurotransmitter asymmetries have been reported in the post-mortem human brain, indicating a right-side bias for GABA in M1 ([Glick et al., 1982](#)). As our primary hypotheses concerned the change in neurochemical concentration over time, these systematic baseline differences between hemispheres are unlikely to influence the main results.

In plotting the data we had noticed a gradual upward drift in GABA in the sham condition over time. As the participants were asked to lie still in the scanner for a relatively lengthy period of time, it is possible that this could arise from non-specific effects such as drowsiness. Reports from previous studies have shown a similar upward drift over time with GABA levels in the sham condition ([Stagg et al., 2009a](#)). The cause for this trend could be tested further, for instance, by using EEG measures of drowsiness. However, as all stimulation conditions are contrasted to sham, and the order of sessions was counterbalanced, we do not expect this upward drift to cause problems for the interpretation of the main results.

7.5 Conclusions

This study characterised the neurochemical changes underlying anodal, cathodal, and bilateral stimulation in both human primary motor cortices of healthy controls at rest. The timecourse and direction of changes in M1 neurochemicals for each stimulation montage were presented. We postulated that the montage specific modulations in neurotransmitter levels may be a means by which tDCS modulates the brain environment to promote the behavioural changes reported with DC stimulation. It is hoped that the present findings could help formulate future hypotheses for the purposeful modulation of the brain environment with tDCS. Further work is needed to reconcile these findings with the timecourse of GABA and glutamate change during motor learning and in recovery after stroke.

On a general note, the neurochemical levels reported here as measured by spectroscopy are a surrogate marker for the level of physiological inhibition or excitation within a given area. Although GABA and glutamate were treated separately in the discussions of the results, it should be noted that glutamate is a precursor to GABA, and both are continuously recycled between neurons and astrocytes via re-uptake mechanisms from extracellular space ([Hyder et al., 2006](#), [Shen, 2013](#)). Given the limited spatial and temporal resolution of MRS, the reported measurements of cortical neurotransmitters in this study can only reflect the gross total concentrations within a region that is neurophysiologically relevant. Given the constraints of the technique, specific conclusions on the exact role of each neurochemical cannot be inferred.

Chapter 8

Conclusions and future directions

The experimental chapters of this thesis investigated the physiological effects of transcranial direct current stimulation (tDCS). This chapter summarises the key findings and draws overall conclusions from them. Suggestions for future research are discussed.

8.1 Thesis overview

The aim of this thesis was to investigate the physiological effects of transcranial direct current stimulation (tDCS) as applied to the primary motor cortex (M1). This line of research was largely motivated by the need to understand the basic physiological changes of tDCS in order to evaluate its use as a potential tool in recovery after stroke, as well as its more general applicability as a tool to modulate plasticity. The experimental chapters of this thesis were therefore designed with the following aims:

- To determine the effects of tDCS on GABA levels within the stimulated M1 and resting state functional connectivity, and test the relationship between the two measures (Chapter 4).
- To investigate whether tDCS-induced cortical modulations in inhibition are restricted to the stimulated hemisphere or whether changes can be observed in the non-stimulated M1 (Chapter 5).

- To develop and assess the validity of a novel MR spectroscopy method in quantifying neurochemicals concurrently from both primary motor cortices (Chapter 6).
- To investigate the effects of tDCS on cortical neurotransmitters measured concurrently from both primary motor cortices (Chapter 7).

The key findings of these studies, and suggestions for future research, are summarised and discussed in the following sections.

8.1.1 Summary of key findings

The experimental chapters of this thesis used tDCS to modulate the M1 and employed TMS, functional MR imaging and MR spectroscopy at both 3 T and 7 T to measure the changes.

Chapter 4 presented results on the effects of anodal stimulation on GABA and motor resting functional connectivity in the anode-targeted M1. We confirmed previous findings that anodal tDCS reduces GABA concentration and increases functional connectivity in the anode-targeted M1. By testing the changes in the same individuals, we demonstrated for the first time that the magnitude of changes do not correlate across subjects, suggesting that they may be driven by distinct underlying mechanisms. Novel evidence on the timecourse of M1 GABA change was presented, which demonstrated that the GABA decrease was most prominent after the stimulation period (as opposed to during stimulation) and was present for up to 30 minutes after stimulation has ceased.

Chapter 5 investigated whether the tDCS-induced modulation of inhibition was restricted to the stimulated hemisphere or whether inhibitory changes could be observed in the non-stimulated M1, or in the interhemispheric connections between the M1s. No significant effects of tDCS on intracortical inhibition were demonstrated within either M1, and no changes in interhemispheric inhibition or facilitation were observed from the stimulated to non-stimulated M1. The lack of significant differences between stimulation conditions in this study could be a fault of the method and design, or may reflect a true absence of physiological effects. We reported trends that at 2 mA current intensity, there was excitability enhancement in the cathodal condition, in agreement with previous studies (Batsikadze et al., 2013, Wiethoff et al., 2014).

It was postulated that the use of MRS was an attractive alternative to determine the gross changes in tDCS-induced inhibition, and exclude the possibility of complex interactions from the TMS pulse, albeit with the caveat that MRS is not able to determine the exact neuronal populations involved in mediating the effects of the DC stimulation.

In Chapter 6, we developed and assessed the robustness of a novel two-voxel MRS method at 7 Tesla for quantifying a number of neurochemicals from both primary motor cortices simultaneously. Effective use of barium titanate dielectric pads was demonstrated for optimised B_1 efficiency in both M1s for GABA quantification.

Chapter 7 investigated changes in cortical neurotransmitters induced by tDCS, in which measurements were obtained concurrently from both M1s using the method validated in Chapter 6. By measuring the timecourse of neurochemicals in this way, we were able to directly test the tDCS-induced physiological effects in both M1s within the same individuals across a range of polarities. Anodal stimulation reduced GABA in both M1s. Cathodal stimulation decreased GABA and glutamate in the non-stimulated M1, with no concurrent changes in the cathode-targeted M1. Bilateral stimulation reduced glutamate in both M1 with no change in GABA.

In the above studies, it was demonstrated that tDCS is capable of modulating neurochemicals in the primary motor cortices. Extending the findings from previous literature, the timecourse of GABA change with anodal tDCS was measured, and for the first time, the changes occurring during the stimulation period were demonstrated. Novel evidence on the timecourses for glutamate and GABA change, across a range of polarities, were examined in both M1s concurrently within the same individuals. In this way we were able to exclude between-subject differences and directly compare how each stimulation montage affected neurochemicals in both M1s, within individuals. This approach allowed for a more thorough examination than previous studies in testing the basic physiological effects of tDCS. Evidence on the timecourse of changes in neurochemicals will have important implications in determining the possible timing-dependent effects of tDCS application. It is hoped that this will lead to more meaningful, hypothesis driven, and more robust protocols in the application of tDCS.

In this thesis, the M1 was the primary area of interest, however, the discussions of the effects described cannot exclude the involvement of other inter-connected motor areas. It should also be noted that, given the constraints of the techniques used, specific

conclusions on the exact role of each neurochemical cannot be inferred. Instead, the findings from MRS reflect the changes in the gross total concentrations within a region that is neurophysiologically relevant. The present evidence demonstrated the physiological effects of tDCS in healthy controls at rest across a range of polarities, and testing how these effects relates to behavioural changes will be the next challenge for future investigations.

8.2 Future directions

The above evidence suggests that the montage specific modulations in neurochemicals may be a way by which tDCS modulates the brain environment to promote the behavioural changes with DC stimulation reported in the literature. In particular, it was demonstrated that tDCS induced modulations of GABAergic processes, which have been shown to be important in plasticity induction in humans. However, the evidence presented in this thesis did not measure behavioural changes. In the following sections, the present findings are related to motor learning and recovery after stroke. Suggestions for future research and the key areas stemming from the findings of this thesis are presented.

8.2.1 Purposeful modulation of GABA in motor learning

Previous MRS studies in healthy controls have shown reductions in M1 GABA following motor learning ([Floyer-Lea, 2006](#)), and have demonstrated that the degree of motor learning can be related to the degree of GABA decrease induced by anodal stimulation ([Kim et al., 2014](#), [Stagg et al., 2011a](#)).

In Chapter 4, we demonstrated that the GABA decrease is most prominent in the 30-minute period after stimulation has ceased, as opposed to during the stimulation period. Future investigations should determine the critical time period when it would be optimal to combine DC stimulation with motor learning. For example, if both motor learning and anodal tDCS decreases M1 GABA, would application of anodal tDCS during a motor task lead to a further decrease of GABA than when subjects are at rest, or would mechanisms of homeostatic plasticity prevent further reduction? Application of tDCS prior to motor learning has been shown to impair performance ([Stagg et al., 2011c](#)), suggesting a role for regulatory mechanisms. Examining these interactions more closely

will have implications on how tDCS is used in the clinical context and address the question of when tDCS should be applied during therapy after stroke.

In Chapter 7, we demonstrated a concurrent decrease in GABA within the non-stimulated M1 in the anodal condition, which was supported by the findings of a previous neurophysiological study measuring changes in MEPs (O'Shea et al., 2014). The behavioural relevance of this concurrent decrease is unclear and should be tested in future investigations. For example, measuring the degree of anodal tDCS-induced decrease in each M1 during performance of a unimanual or bimanual task, could give insight into how tDCS augments motor learning behaviour in each M1 and the responses between the hemispheres.

Probing the responsiveness of the GABA system in healthy humans could lead to interesting insights into the capacity of the motor system for plasticity, and whether tDCS is a tool that could promote these effects. Of particular importance would be to combine different neuroimaging methods to support a particular conclusion. For example, if it is hypothesised that anodal tDCS would improve bimanual motor performance via a reduction of inhibition in both M1 (as measured by MRS), it would be expected that this might correlate with the BOLD measures of bimanual performance, such that the degree of M1 GABA decrease would correlate with the degree of M1 BOLD signal change during synchronised bimanual movements from both hands.

8.2.2 Purposeful modulation of GABA in stroke recovery

In Chapter 7, it was demonstrated that anodal stimulation induced a concurrent GABA decrease in both M1s. It could be hypothesised from these findings that the greater efficacy reported with anodal stimulation more than cathodal or bilateral stimulation (Fusco et al., 2013, O'Shea et al., 2014, Stagg et al., 2012), might be a consequence of increased facilitation of distant intact areas, which have been reported to be functionally relevant to recovery in more impaired patients after stroke (Johansen-Berg et al., 2002). Improvements in hand motor function with anodal tDCS have been assumed to be a consequence of the increased excitability in the ipsilesional M1 as studies have largely neglected to measure the possible changes occurring to the contralesional M1. Future investigations should determine whether the behavioural improvements in stroke with anodal tDCS are mediated via: a) cortical facilitation of the ipsilesional M1 (as

previously assumed), b) facilitation of the contralesional M1 through interhemispheric interactions or via increased activity of the premotor areas in the intact hemisphere, or c) the sum of decreasing inhibition in both the ipsilesional and contralesional M1.

As discussed in the introduction (section 1.3.4), the hypothesised strategy for using tDCS in recovery after stroke is largely based on the interhemispheric competition model (Hummel and Cohen, 2006), which is to ‘re-balance’ the abnormal interhemispheric influences that are assumed to be maladaptive. Critically, it is unclear whether this approach is appropriate for all patients. It would be expected that there would be stronger evidence for the use of bilateral stimulation if the re-balancing approach was accurate, which is not the case. Furthermore, the correct interpretation and functional significance of these abnormal changes, which have been argued to represent both adaptive and maladaptive responses, are still unclear. This is further hampered by the limited knowledge on the physiological significance of using NIBS tools in the context of stroke.

Although MRS cannot directly determine the interhemispheric interactions between the two M1s and can only measure gross changes in neurochemicals, it may be able to assess the inter-individual differences in the motor system’s capacity for modulation with tDCS (O’Shea et al., 2014, Stinear, 2010). Estimating the individual patients’ potential for recovery of motor function could lead to more targeted applications of tDCS, leading to hypothesis driven predictions of which stimulation montage is appropriate for which type of patients, which will have significant implications in improving rehabilitation planning and outcomes. Examining these interactions further will be an important avenue for future investigations.

8.2.3 Consensus in stimulation parameters

In Chapter 5, the variability in the physiological outcomes of different stimulation parameters and the difficulties in capturing the possible physiological effects are highlighted. The use of 2 mA current may have complicated the interpretation of the findings as since the time of data collection it was demonstrated that excitability effects are non-linear with increasing current strength (Batsikadze et al., 2013, Wiethoff et al., 2014). The variability in the tDCS literature generated over the past decade has demonstrated that there is a lack of a consensus on the parameters of its basic applications.

Only recently have studies begun to direct attention to the variability in inter-individual responses to tDCS in healthy controls (Wiethoff et al., 2014). Although it is generally acknowledged that responses to NIBS protocols are variable, very few have directly tested the variability of the responses that are produced. In one study it was demonstrated that approximately 50% of individuals (n=53) are poor ‘responders’ to tDCS (Wiethoff et al., 2014). These findings highlight the need to develop more robust protocols and determine the individual factors that results in the variability in responsiveness of DC stimulation. This would have strong implications on the future use of tDCS and a consensus will need to be reached in order to both evaluate the potential of this tool and also to apply it in a meaningful way.

The ability to measure and draw conclusions to a particular question is, to an extent, limited by the tools that are available to probe these processes. In vivo work in humans are restricted to non-invasive techniques such as neuroimaging or NIBS methods. It may be that by improving our currently available methods, such as that demonstrated in Chapter 6, different perspectives of processes could be determined. Developments of tools in neuroscience are not without its difficulties, however, will be a worthwhile challenge for future investigations.

8.3 General conclusions

In conclusion, this thesis demonstrated that modulation of neurotransmitter levels, and in particular changes to inhibition, are a possible way by which tDCS may mediate its physiological effects. The changes and direction of the modulations to neurochemicals are dependent on the particular montage and stimulation parameters used, which should be considered carefully when designing future investigations. It is hoped that the present findings will help formulate future hypotheses for purposeful modulation with tDCS in order to achieve the intended behavioural outcomes, and lead to more meaningful approaches in its application.

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