Investigating Human Motor Learning Using Multimodal Imaging and Brain Stimulation

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Abstract

Many post-stroke motor rehabilitation programmes are developed from methods used to augment and improve motor skill learning in healthy controls. An important step towards developing effective motor rehabilitation strategies is therefore understanding the neurophysiology of healthy motor learning, and then assessing if (and if so, how) this changes after a stroke.

This thesis contains work using multimodal methods to further understanding of the neurophysiological underpinning of motor skill learning in both healthy controls and stroke survivors. Functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS) and transcranial magnetic stimulation (TMS) are used to measure brain activity and neurotransmitter concentrations, while transcranial direct current stimulation (tDCS) is used to modulate both brain activity and motor learning performance.

The first experiment, in Chapter Three, uses ultra-high field MRS to investigate healthy motor learning, and observed a reduction in GABA concentration which is specific to performance of a learning task. This finding replicates previous literature, and provides support of the important role of inhibition in normal motor learning.

In the following two Chapters, the role of the ipsilateral motor cortex is investigated in tDCS-influenced healthy motor learning. Following stroke, the ipsilateral (or contralesional) motor cortex has been shown to increase involvement in movement, but it's role is less well established. Here, cathodal tDCS to the task-dominant (left) hemisphere in healthy controls is combined with fMRI (Chapter Four) or bilateral TMS (Chapter Five).

In Chapter Four, offline cathodal tDCS prior to task performance resulted in increased ipsilateral motor cortex activity during motor task performance. In Chapter Five, the same tDCS stimulation paradigm did not find group-wide cortical excitability changes, however relationships were observed at the individual subject level between behaviour and TMS measures (baseline response time predicts left hemisphere cortical excitability change, and rate of learning correlates with right hemisphere facilitation change). These relationships may provide insight into the neurophysiological changes caused by cathodal tDCS and how these may relate to behaviour.

The final experimental chapter focuses on identification of differences in learning ability between healthy controls and stroke survivors, and presents a successful proof-of-principle study introducing a novel motor task which could be used to assess this intergroup difference. This task has been developed to overcome limitations of existing tasks which require volitional movement, and often dexterity, to perform.

Taken together, these studies strengthen our knowledge of the key role of inhibition in healthy motor learning, highlight the importance of understanding the contralesional hemisphere following stroke, and introduce a novel task which could be developed further to investigate potential differences between motor skill learning in healthy controls and stroke survivors.

This thesis is approximately 40,000 words.

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Publications

The below publication has directly arisen from the work within Chapter Three of my thesis:

- Kolasinski J*, **Hinson E. L.*,** Zand A. P. D. , Rizov A, Emir U. E. and Stagg C. J. The dynamics of cortical GABA in human motor learning *J. Physiol.* (2018).

The below publications have not arisen directly from, but are related to, work presented in this thesis:

- Johnstone A*, **Hinson E. L.*** & Stagg C. J. (2016) tDCS and Magnetic Resonance Imaging. [Book Chapter] In Transcranial Direct Current Stimulation in Neuropsychiatric Disorders: Clinical Principles and Management. Editors: Brunoni A, Nitsche M & Loo C. Springer

- Kolasinski J, Logan J. P., **Hinson E. L.**, Manners D, Zand A. P. D., Makin T. R., Emir U. E., Stagg C. J. Linking cortical architecture and perception: a mechanistic role for GABA?. Curr. Biol. 1–7 (2017).

- Johnstone, A., Levenstein, J. M., **Hinson, E. L.** & Stagg, C. J. Neurochemical changes underpinning the development of adjunct therapies in recovery after stroke: A role for GABA? J. Cereb. Blood Flow Metab. (2017).

- Frangou, P. Emir U. E., Karlaftis V. M., Nettekoven C., **Hinson E. L.**, Larcombe A., Bridge H., Stagg C. J., Kourtzi Z. Learning to optimize perceptual decisions through suppressive interactions in the human brain. Nat. Commun. 10, 474 (2019)

The below publication has been published during the course of my DPhil, but is not related to the work in this thesis:

- Nowak M, **Hinson E. L.**, Pogosyan A., Guerra A., van Ede F., Quinn A., Brown P., Stagg C. J. Driving human motor cortical oscillations leads to behaviourally relevant changes in local $GABA_A$ inhibition: a transcranial alternating current stimulation (tACS) study. J. Neurosci. 37, 0098-17 (2017)

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Abbreviations

9HPT	9 Hole Peg Tests
AFI	Actual Flip Angle Imaging
aMT	Active Motor Threshold
ANOVA	Analysis of Variance
ARAT	Action Research Arm Test
AROMA	Automatic Removal of Motion Artefacts
ATP	Adenosine Triphosphate
BBR	Boundary Based Registration
BCM	Bienenstock-Cooper Munroe (theory)
BET	Brain Extraction Tool
BOLD	Blood Oxygen Level Dependent
CLRB	Cramér-Rao Lower Bound
Cr	Creatine
CS	Conditioning Stimulus
CSF	Cerebrospinal Fluid
CSI	Chemical Shift Imaging
CST	Corticospinal Tract
DW-CRT	Density-Weighted Concentric Ring Trajectory
ECR	Extensor Carpi Radialis
ECT	Electroconvulsive Therapy
EEG	Electroencephalography
EMG	Electromyography
ESS	Epworth Sleep Scale
FA	Fractional Anisotropy
FAST	FMRIB's Automated Segmentation Tool
FCR	Flexor Carpi Radialis
FEAT	FMRIB's Expert Analysis Tool
FDA	Food and Drug Administration
FDI	First Dorsal Interosseous
FIX	FMRIB's ICA-based Xnoiseifier
FLIRT	FMRIB's Linear Image Registration Tool
fMRI	Functional Magnetic Resonance Imaging

fMRS	Functional Magnetic Resonance Spectroscopy
FNIRT	FMRIB's Non-Linear Image Registration Tool
FOV	Field of View
FTT	Force Tracker Task
FWHM	Full-Width Half-Maximum
GABA	γ-Aminobutyric Acid
Glx	Glutamate + Glutamine
GM	Grey Matter
HRF	Haemodynamic Response Function
ICA	Independent Component Analysis
ICF	Intracortical Facilitation
ISI	Interstimulus Interval
LI	Laterality Index
LICI	Long Interval Intracortical Inhibition
LMM	Linear Mixed Model
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MCFLIRT	Motion Correction FLIRT
MEGA-PRESS	MEscher-Garwood-Point RESolved Spectroscopy
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MEP	Motor Evoked Potential
MoCA	Montreal Cognitive Assesment
MPRAGE	Magnetisation Prepared RApid Gradient Echo
MR(I)	Magnetic Resonance (Imaging)
MRS	Magnetic Resonance Spectroscopy
MRSI	Magnetic Resonance Spectroscopic Imaging
MT_{1mv}	1 mV Motor Threshold
MVC	Maximum Voluntary Contraction
NAA	N-Acetylaspartate
NIBS	Non-Invasive Brain Stimulation
NMDA	N-methyl-D-aspartate
optiBET	Optimized brain extraction script for patients' brains

PAS	Paired Associative Stimulation
PCr	Phosphocreatine
PET	Positron-Emission Tomography
PMC	Premotor Cortices
ppTMS	Paired Pulse TMS
RF	Radio Frequency
rMT	Resting Motor Threshold
ROI	Region of Interest
RT	Response Time
rTMS	Repetitive Transcranial Magnetic Stimulation
SEM	Standard Error of the Mean
SD	Standard Deviation
semi-LASER	semi-Localised by Adiabatic Selective Refocusing
SICI	Short Interval Intracortical Inhibition
SMA	Supplementary Motor Areas
SNR	Signal-to-Noise Ratio
SOR	Supraorbital Ridge
spTMS	Single Pulse Transcranial Magnetic Stimulation
SRTT	Serial Reaction Time Task
SSRI	Selective Serotonin Reuptake Inhibitor
SVS	Singe Voxel Spectroscopy
TBS	Theta-Burst Stimulation
tCr	Total Creatine
tDCS	Transcranial Direct Current Stimulation
TE	Echo Time
TR	Repetition Time
TMS	Transcranial Magnetic Stimulation
TS	Test Stimulus
(UE)FM	(Upper Extremity) Fugl Meyer
VAPOR	VAriable Power and Optimised Relaxation Delay
VOI	Voxel of Interest
WM	White Matter

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Chapter 1: Introduction

The first section of this introductory chapter outlines the current literature of neuroplasticity in the motor system and the role of inhibition (and specifically the role of GABA) in human motor learning. Humans are clearly very capable producing dexterous movement and developing motor skills throughout life, and the understanding of the neurophysiology of this learning process becomes important when trying to assist relearning and rehabilitation processes after brain injury such as a stroke. The second section of this introduction discusses how researchers have developed and used methods to modulate motor learning ability, specifically via the use of transcranial direct current stimulation (tDCS). Thirdly, I discuss how we study the motor system following a stroke, and how motor rehabilitation has been affected by our growing understanding of the role of the contralesional hemisphere. The final section of this introduction introduces the aims of each chapter of this thesis.

Plasticity in the motor system

Neuroplasticity is defined as the reorganisation of neural connections as a result of experience or other external influence. This process is thought to underlie the healthy brain's ability to learn, to remember, and to develop skills. It is also thought to contribute to recovery from, and compensation for, damage sustained to the brain. By understanding the normal physiological process of neuroplasticity, and how we can exogenously influence it, we can perhaps contribute to development of rehabilitation programmes which could improve outcomes in cases of brain injury.

The motor network

A widely distributed network of bilateral cortical and subcortical areas are involved in the performance of motor tasks (Karni *et al.*, 1995; Hardwick *et al.*, 2013). These regions interact to support the learning of motor tasks. Classically, the planning of a movement is thought to be controlled by supplementary motor areas and premotor cortex (SMA and PMC) and movements are initiated within the subcortical basal ganglia nuclei. Execution of movements is mostly performed by the primary motor cortices (M1) and error correction within the cerebellum (Albus, 1971, 1989; Marr and Thach, 1991), though it is becoming clear that these regions may have more complex roles than those listed here (King *et al.*, 2019), and many phases of learning rely on changes in connectivity between these regions rather than merely the activity within them individually. Furthermore, the phases of initial and later learning are thought to be mediated by different sections of this network with initial learning dominated by the SMA, PMC and M1. In the latter stages, smaller and more subtle modifications are made between connections of the basal ganglia, cerebellum and M1 in order to consolidate and fine tune learning (Karni *et al.*, 1998).

Primary motor cortex

In his seminal work in the mid-twentieth century, Walter Penfield identified a highly organised map of body areas within the primary motor and sensory cortices of the human brain (Penfield and Rasmussen, 1950). These maps are now widely recognisable as the motor and sensory homunculi and represent a topographical map of body parts (Figure 1.1). Changes to these maps can be observed following both low and high use of particular body parts, for example chronic disuse has been shown to induce rapid change in both cortical structure (Langer *et al.*, 2012) and excitability (Facchini *et al.*, 2002) and highly skilled usage (such as observed in musicians) results in enlargement of cortical representations (Elbert *et al.*, 1995). Motor learning has been demonstrated to result in both structural and functional changes to M1 (Dayan and Cohen, 2011; Sampaio-Baptista *et al.*, 2018) including topographical reorganisation (Karni *et al.*, 1998), synaptogenesis (Kleim *et al.*, 2002) and myelination changes (Sampaio-Baptista *et al.*, 2015). In rodents, intracortical recordings have demonstrated that remapping is essential for skill acquisition, but not for memory of the same skills (Molina-Luna *et al.*, 2008). With the advance of higher temporal and spatial resolution functional MRI, it has been possible to observe alterations to perceptually relevant human somatotopy following intervention of hand usage (gluing of fingers) (Kolasinski *et al.*, 2016).

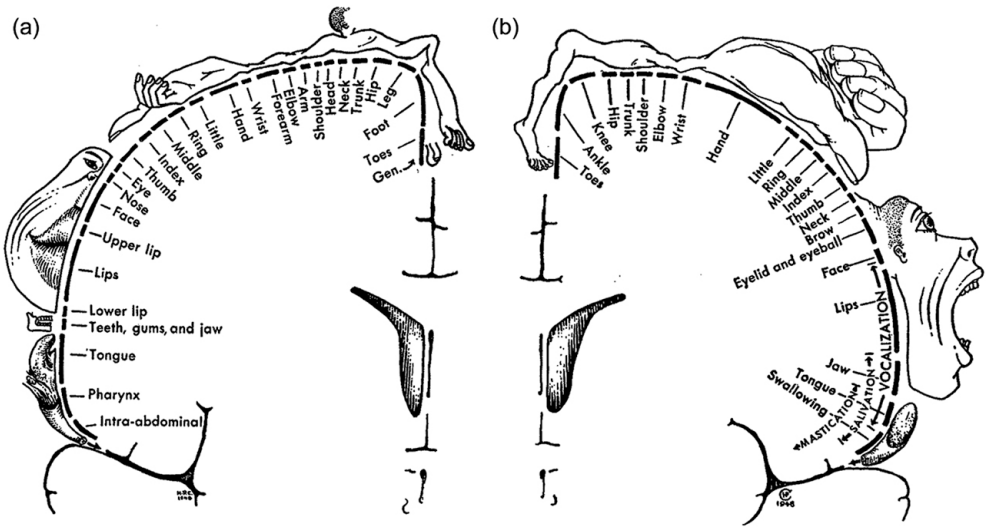

Figure 1.1: The sensory (A) and motor (B) homunculi proposed by Penfield and Rasmussen in 1950

Physiological basis of motor learning

Hebbian Synaptic Plasticity

Based on numerous studies of motor learning in animal models, synaptic plasticity has been established to occur via the strategic strengthening of synaptic connections. Donald Hebb first postulated the general mechanism underpinning synaptic plasticity in 1949, stating *"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, 1949). Hebbian Synaptic Plasticity is seen in the brain in two opposing ways, by which synaptic connections are either strengthened (Long Term Potentiation, LTP (Andersen and Lømo, 1966; Bliss and Lømo, 1973)) or weakened (Long Term Depression, LTD (Bramham and Srebro, 1987; Ito, 1989)). In animal neocortex, synaptic plasticity is implemented by similar processes to the LTP process widely studied and observed within the hippocampus, hence it is known as LTP-like plasticity. Multiple different changes have been observed in the neocortex following motor learning paradigms, including

creation of both new synapses and dendritic spines (Kleim *et al.*, 2002; Xu *et al.*, 2009; Yang, Pan and Gan, 2009; Rogerson *et al.*, 2014; Hayashi-Takagi *et al.*, 2015). This LTP-like plasticity occurs within the horizontal connections within primary motor cortex (Hess and Donoghue, 1994; Huntley, 1997). Unlike other areas of the brain, LTP-like induction within the neocortex is dependent on GABAergic interneurons (specifically a reduction in local GABAergic tone) (Hess and Donoghue, 1996; Trepel and Racine, 2000), as well as NMDA receptors (Castro-Alamancos *et al.*, 1995; Hess *et al.*, 1996). From pharmacological studies (discussed below), it is likely that the physiological basis of Non-Invasive Brain Stimulation (NIBS) methods such as Transcranial Direct Current Stimulation (tDCS) act via a similar mechanism as LTP-like plasticity, allowing for modulation of this process.

The role of inhibition in motor learning

Phasic and Tonic GABA

Inhibitory, GABAergic, neurons make up approximately 20% of neurons within the human cortex. Cortical inhibition is a crucial modulator of cortical plasticity. GABA has three main functions within the cortex: contributing to "phasic" inhibitory signalling via GABAergic receptors, "tonic" inhibition via neuromodulatory action on non-synaptic $GABA_A$ channels (Martin and Rimvall, 1993), or as a metabolite within GABAergic neurons (Martin and Rimvall, 1993).

"Phasic" inhibitory signalling within the cortex occurs via either ionotropic $GABA_A$ chloride channels or metabotropic $GABA_B$ receptors linked to potassium channel receptors. GABAergic neurotransmission is proposed to contribute to motor learning via mechanisms possibly allowing synaptic remapping, or un-masking of silent synapses (Hess, Aizenman and Donoghue, 1996; Trepel and Racine, 2000). In awake, behaving animals, LTP-like plasticity

can be blocked by administration of $GABA_A$ agonists (Hess *et al.*, 1996; Trepel and Racine, 2000; Chen *et al.*, 2015).

Quantifying changes in the GABAergic system is inherently more difficult in humans than in animal models or slice preparations and the number of studies investigating GABAergic changes in people are therefore limited. Nevertheless, methods such as Magnetic Resonance Spectroscopy (MRS) and Paired-Pulse Transcranial Magnetic Stimulation (ppTMS) do allow for investigation of the human GABAergic system with some limitations, particularly in terms of their spatial and temporal resolution (see Methods Chapter for further discussion).

In humans, M1 plasticity induction can be reduced, or even blocked by pharmacological agents such as lorazepam (a $GABA_A$ allosteric modulator) (Ziemann *et al.*, 1998) and baclofen (a $GABA_B$ agonist) (McDonnell *et al.*, 2007), however these pharmacological interventions lack regional specificity and will have side-effects which could impact a learning task (such as the mild-sedative or muscle-relaxant effects of GABA agonists). Non-pharmacological methods of influencing modulating brain activity in humans have been shown to both reduce GABA, and to impact M1 plasticity. These methods can either act to increase LTP-like plasticity (anodal tDCS: Monte-Silva *et al.*, 2013) or decrease LTD-like plasticity (continuous theta burst stimulation: Huang *et al.*, 2005).

Studying relationships with GABA in individual subjects

On an individual subject basis, activity in the GABAergic system has been found to correlate with various aspects of task performance, allowing an insight into the relationship between physiology and behaviour. In M1, lower concentrations of GABA have been correlated with faster reaction times (Stagg, Bachtiar and Johansen-Berg, 2011a). Furthermore lower

sensorimotor GABA is correlated with both higher functional connectivity in the resting motor network (Bachtiar *et al.*, 2015) and higher motor task induced functional activation (Stagg, Bachtiar and Johansen-Berg, 2011a). In SMA, individual differences in motor control can be predicted by GABA concentration, such that those participants with higher GABA performed better (Boy *et al.*, 2010). Outside of the motor network, higher GABA within the frontal eye fields has been shown to correlate with a better ability to ignore distractor stimuli (Sumner *et al.*, 2010) and in the visual cortex, higher GABA correlates with better orientation discrimination (Edden *et al.*, 2009). Together these studies strongly support a hypothesis of inhibitory GABAergic tone for maintenance of functional architecture within primary sensory cortical regions. Via a process of lateral inhibition, tonic GABA localises and modulates neuronal responses, resulting in topographical organisation. Topographical organisation is a vital component of sensory perception, and therefore there are close links between the GABAergic systems and perceptual ability.

The relationship between MRS measured GABA concentration and perceptual ability have been supported by recent studies in both the primary visual and sensorimotor regions (Edden *et al.*, 2009; Kolasinski *et al.*, 2017). In the sensorimotor cortex, participants with the best behavioural performance (in terms of highest tactile acuity) showed the most selective cortical activity during individual digit movement, which in turn was underpinned by highest concentrations of inhibitory tone (Kolasinski *et al.*, 2017). In the visual cortex, GABA has been shown to correlate with orientation discrimination performance such that those participants with highest GABA showed highest discrimination ability (Edden *et al.*, 2009).

Measuring changes in motor cortex GABA associated with plasticity induction

As well as correlations between cortical GABA and aspects of motor or perceptual ability, changes in GABA within the motor cortex have been observed over the course of plasticity inducing protocols, and during learning. Decreases in GABA were first observed following ischemic nerve block (Levy *et al.*, 2002), an intervention known to induce reorganisation of M1 and increase cortical excitability (Ziemann *et al.*, 1998). More recently, the degree of responsiveness of an individual's GABAergic system following delivery of tDCS has been shown to correlate with the degree of improvement on a motor learning task (Stagg *et al.*, 2011). When observing dynamic changes in GABA during leaning without exogenous influence by pharmacological agent or NIBS, MRS has been used to observe a learning-condition specific reduction in GABA during a visuo-motor tracking task (Floyer-Lea, 2006; see also Chapter Three). Furthermore, ppTMS measures of inhibitory neurotransmitter systems have been used to infer decreases in inhibition during the early phase of motor learning (Liepert *et al.*, 1998; Perez *et al.*, 2004; Rosenkranz *et al.*, 2007).

In more recent work, GABAergic signalling has been shown to help stabilise a learnt skill during a period of "overlearning" after a performance plateau has been reached. This increased GABAergic signalling appears to prevent interference from a second task, by stabilising the first skill (Shibata *et al.*, 2017). Additionally, it appears that the amount of GABA in the parietal cortex relates to an individual's ability to decipher relevant task-related information from noisy stimuli (such that higher GABA relates to better suppression of irrelevant information), which is important for making correct perceptual decisions (Frangou *et al.*, 2019). In the same study, greater learning related changes to GABA concentration in the visual cortex were observed to relate to better task performance (Frangou *et al.*, 2019). The careful balance of excitation and inhibition within the human brain is crucial for normal

function, and disruptions in the excitatory-inhibitory balance can be seen in several neuropsychiatric disorders including autism (Rubenstein and Merzenich, 2003) and schizophrenia (Lewis, Hashimoto and Volk, 2005). Barron and colleagues (2016) manipulated the inhibitory GABAergic system using tDCS to alter the excitatory-inhibitory balance within a target region of the temporal cortex. This disruption was used to unmask otherwise silent cortical memories, demonstrating that the balance of excitation and inhibition is a critical component of memory storage.

The studies discussed above, particularly those performed in recent years, have highlighted how the development of more advanced neuroimaging and brain stimulation methods, as well as the ability to combine these techniques, have resulted in improved temporal and spatial resolution of neurotransmitter quantification. Taken together, these advances make it more feasible to perform complex multimodal studies to attempt both replication and extension studies based on previous findings.

Modulating motor learning using brain stimulation

Transcranial Direct Current Stimulation (tDCS)

tDCS is a method of subthreshold modulatory stimulation of the brain's cortex via a continuous current. Nitsche and Paulus' original investigations into tDCS demonstrated a seemingly simple dichotomy in its effects on the cortex, such that anodal tDCS was facilitatory, and would augment or encourage underlying neuronal activity. Cathodal tDCS was demonstrated to be inhibitory to the underlying targeted cortical region, reducing cortical excitability (Nitsche and Paulus, 2000; Nitsche *et al.*, 2003). The now extensive literature using

tDCS has progressed from these initial studies within the motor cortex to target a wide range of cortical regions, and to assess effects in an ever increasing range of clinical groups.

Physiological Basis of tDCS

The neurophysiological basis of tDCS has been investigated since its first demonstration by Priori and colleagues in 1998 of its ability to alter the excitability of the human motor cortex (Priori *et al.*, 1998). Polarity specific effects of tDCS were reported by Nitsche and colleagues through the modulation of the amplitude of an motor evoked potential (MEP) in the contralateral hand muscles, such that anodal tDCS augmented MEP size, whereas cathodal tDCS reduced them (Nitsche and Paulus, 2000). These changes were demonstrated to outlast the stimulation period by a few 10s of minutes following a stimulation period of approximately 10 minutes (Nitsche and Paulus, 2001; Nitsche *et al.*, 2003).

In TMS studies, anodal tDCS has been shown to result in increased intracortical facilitation, and reduced intracortical inhibition, with the reverse effects observed following cathodal tDCS as measured using paired pulse TMS (ppTMS) (Nitsche *et al.*, 2005). MRS methods, however, have resulted in observations of GABAergic reductions following both anodal (Stagg *et al.*, 2009; Stagg, Bestmann, *et al.*, 2011; Bachtiar *et al.*, 2015; Patel *et al.*, 2019) and cathodal tDCS (Stagg *et al.*, 2009). Furthermore, MRS studies have also reported reduction in glutamate following cathodal tDCS, and a trend to the same result following anodal tDCS (Stagg *et al.*, 2009; Clark *et al.*, 2011). Together, these results demonstrate that the physiological effects of anodal and cathodal tDCS are likely based in modulation of multiple neurotransmitter systems rather than being specific to one system (Stagg and Nitsche, 2011; Stagg, Antal and Nitsche, 2018).

Pharmacological investigations of the mechanisms of tDCS

A series of pharmacological studies have attempted to elucidate the mechanisms of both anodal and cathodal tDCS, although it is very likely that the stimulation influences a number of physiological processes simultaneously. Pharmacological studies can be employed to systematically modulate neurotransmitter systems in order to alter the effects of tDCS, although their effects are not spatially specific to the same extent as NIBS techniques. There is also a confound associated with side-effects of the pharmacological agents used, which may also affect task performance (for example the mild sedative or muscle relaxant effects of some GABA agonists and the possible stimulant effects of GABA antagonists).

From the extensive pharmacological literature, it is known that the online effects of anodal tDCS are exerted via membrane depolarisation as sodium and calcium channel blockers result in reduced and abolished stimulation effects respectively (Nitsche *et al.*, 2003) (Figure 1.2A, 1.2B), whereas NMDA receptor antagonists (Nitsche *et al.*, 2003) and $GABA_A$ agonist (Nitsche *et al.*, 2004) have no effect on the online response (Figure 1.2C, 1.2D). Online cathodal tDCS effects are thought to be exerted by membrane hyperpolarisation (Purpura and McMurtry, 1965) as neither sodium or calcium channel blockers (Nitsche, Fricke, *et al.*, 2003) (Figure 1.2A, 1.2B), nor NMDA antagonists (Nitsche, Fricke, *et al.*, 2003) or $GABA_A$ agonist (Nitsche, Liebetanz, *et al.*, 2004) affect online stimulation effects (Figure 1.2C, 1.2D).

In contrast to online effects, the longer-term after-effects of both anodal and cathodal tDCS can be blocked by NMDA-receptor antagonists (Liebetanz *et al.*, 2002; Nitsche, Fricke, *et al.*, 2003) (Figure 1.2C) and augmented by NMDA agonists (Nitsche, Jaussi, *et al.*, 2004), indicating these receptors are involved in neuroplastic change resulting from stimulation. It is also evident that GABAergic inhibition is involved in the after-effects of anodal tDCS with

$GABA_A$ agonist Lorazepam reducing the anodal tDCS induced excitability increase for the first few minutes after stimulation before enhancing and prolonging the excitability elevation seen with anodal tDCS alone (Nitsche, Liebetanz, *et al.*, 2004) (Figure 1.2D). This delayed excitability elevation was suggested to be caused by remote mechanisms such as the cortico-cortical connections between primary and pre-motor areas, or the cortico-thalamic pathways from the basal ganglia to motor cortex (Michael A Nitsche, Liebetanz, *et al.*, 2004).

Other neuromodulators have been assessed pharmacologically: amphetamines can enhance anodal tDCS effects however these effects have been shown to be absent when NMDA antagonists are also present, suggesting catecholamine activity specifically modulates NMDA dependent LTP-plasticity (Nitsche *et al.*, 2004). Increasing dopaminergic tone through application of Levodopa prolongs the inhibitory effects of cathodal tDCS and reverses excitatory effects of anodal tDCS, resulting in similar inhibitory effects as seen following cathodal tDCS (Kuo, Paulus and Nitsche, 2008). Finally, selective serotonin reuptake inhibitors (SSRIs) such as Citalopram augment and prolong anodal tDCS aftereffects (Nitsche *et al.*, 2009), and have been combined with anodal tDCS as a potential therapy for motor rehabilitation post stroke (Prehn *et al.*, 2016).

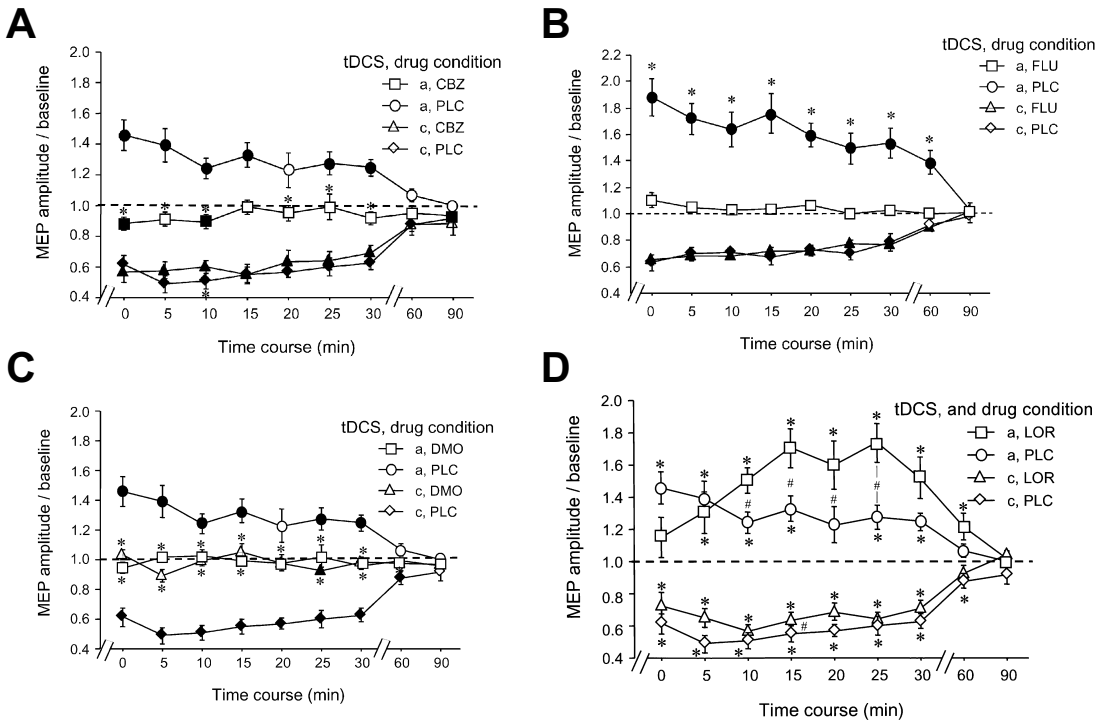

Figure 1.2: Pharmacological influence on tDCS effects:

A: Figure 2 from Nitsche, Fricke *et al.* (2003)**:** Carbamazepine (CBZ), a sodium channel blocker, reduces online anodal tDCS effects but does not alter cathodal tDCS effects. X-axis indicates online stimulation period.

B: Figure 3 from Nitsche, Fricke *et al.* (2003)**:** Flunarizine (FLU), a calcium channel blocker, abolishes the online anodal tDCS effects but does not alter cathodal tDCS effects. X-axis indicates online stimulation period.

C: Figure 4 from Nitsche, Fricke *et al.* (2003)**:** Dextromethorphane (DMO), an NMDA antagonist eliminates the offline effects of both anodal and cathodal tDCS. X-axis indicates offline period following stimulation.

D: Figure 2 from Nitsche, Liebetanz *et al.* (2004)**:** Lorazepam (LOR), a $GABA_A$ agonist initially delays but then enhances and prolongs the offline effects of anodal tDCS, but not cathodal tDCS. X-axis indicates offline period following stimulation.

Gating of motor learning by tDCS

The timing of any NIBS technique in relation to the learning period has been shown to have an important impact on behavioural and cortical excitability effects. The Bienenstock-Cooper Munroe (BCM) theory suggests that the threshold of for induction of LTP and LTD within a post-synaptic membrane is not fixed, but is variable dependent on the previous activity of

the post-synaptic neuron (Bienenstock, Cooper and Munro, 1982). By this theory, NIBS methods such as tDCS can be used to modify cortical excitability (both during the period of stimulation, and for a short period afterwards) and therefore "gate" the plasticity within that cortical region. It has, for example, been shown that short trains of repetitive TMS (rTMS) which themselves have little or no effect on cortical excitability can become inhibitory or excitatory to the same cortical region, if preceded by either anodal or cathodal tDCS respectively (Lang *et al.*, 2004; Siebner *et al.*, 2004).

The homeostatic mechanisms observed in the human brain to prevent unregulated dynamic modulation of cortical excitability mean that a stimulus or intervention which would characteristically increase cortical excitability could result in a decrease, when applied after another excitatory stimulus (Bienenstock, Cooper and Munro, 1982; Ziemann and Siebner, 2008). An example of this was shown by Amadi and colleagues (2015), who used serial anodal tDCS and motor learning to target $GABA_A$ synapses in humans. Both methods have previously been shown to target $GABA_A$ receptors and so the authors hypothesised that they would see homeostatic interactions when the two interventions were applied one after the other. The results did indeed show a slowing of learning rate when anodal tDCS was applied prior to learning (but not when the two were applied simultaneously). This reduced performance was correlated with an increase in $GABA_A$ receptor activity, as measured by ppTMS demonstrating that these receptors could be a location of interaction of tDCS and motor learning in the human brain.

Effects of tDCS on human motor learning

Methods such as tDCS have significantly advanced our ability to study the brain, allowing for investigation of links between changes in cortical excitability and behaviour. We are able to

assess how individual differences in behavioural performance can be linked to variability in both brain structure and function using MR, however by modulating cortical functioning using non-invasive brain stimulation, we can further investigate the links between brain and behaviour. Multiple studies have demonstrated improvement in motor learning when anodal tDCS is applied during practice (Nitsche *et al.*, 2003; Boggio *et al.*, 2006; Reis *et al.*, 2009; Stagg *et al.*, 2009; Stagg *et al.*, 2011). There are far fewer studies assessing effects of cathodal tDCS on motor learning, resulting in a less clear picture, with some studies showing decremental effects to learning (Stagg, Jayaram, *et al.*, 2011), and others showing no effect (Nitsche *et al.*, 2003; Reis *et al.*, 2009).

Increasingly it is becoming clear from the tDCS literature is that results are highly variable: many studies have shown null effects of tDCS (either anodal or cathodal), or mixed results with a mixture of responders and non-responders (López-Alonso *et al.*, 2014). Furthermore, recent studies have even demonstrated inconsistencies within the same subjects across multiple sessions (Chew *et al.*, 2015; Ammann *et al.*, 2017). This mixture of results could, to some extent be explained by variation in study design, however a recent subject of investigation is that of responsiveness of participants to the stimulation itself and how this might vary between individuals.

Individual differences in responsiveness to tDCS

Understanding the variability of the effects produced by tDCS is important for possible future clinical translations of this technique outside of a research setting. From the ever-growing tDCS literature, one of the dominating discussions in recent years has been the diverse and inconsistent range of outcomes resulting from tDCS interventions, including the non-reproducibility of results and the possibility of some participants being classed as "non-

responders" to the stimulation protocols. When targeting the motor cortex, inter-subject variability has been observed (López-Alonso *et al.*, 2014; Wiethoff *et al.*, 2014; Chew *et al.*, 2015; Dyke *et al.*, 2016), however the underlying causes of variability are still unknown. Suggestions have been made that these differences can be caused by the participants brain state during the period of stimulation (Amadi *et al.*, 2015), and electric field differences caused by variability in skull thickness, gyri and sulci anatomy and cerebrospinal fluid volume (Laakso *et al.*, 2019).

An additional point of discrepancy between the original tDCS research and newer findings is that of the polarity-specificity. As detailed above, Nitsche and Paulus observed a dichotomy whereby anodal tDCS produced enhancement of cortical excitability and cathodal tDCS produced reduction. Recent work from Michael Nitsche's group have extensively investigated the apparent non-linearity of cortical effects of cathodal tDCS (Samani *et al.*, 2019). In short, this work found that 1 and 3 mA cathodal tDCS resulted in significant inhibition of cortical excitability whereas 20 minutes of 2 mA cathodal tDCS produced cortical excitability increases. This work highlights the necessity for caution when using tDCS, and the need for further large scale investigations such as that by Samani and colleagues.

tDCS is by no means the only method to demonstrate interindividual differences, with similar effects observed in other cortical modulation methods, including paired associative stimulation (PAS) (Fratello *et al.*, 2006), and theta-burst stimulation (TBS) where a proportion of participants are reported to fail to respond in the "expected" way (Martin *et al.*, 2006; Goldsworthy *et al.*, 2012). Ridding and Ziemann (2010) and Krause and Cohen Kadosh (2014) both provide extensive discussion of possible factors affecting stimulation efficacy in their reviews. These factors include participant variations (age, gender, genetics and physical activity

level) and study design factors (time of day, priming, attention, voluntary muscle activity during or prior to stimulation). Furthermore, pharmacological influences, which are known to influence stimulation efficacy are discussed, although these are usually well controlled for within participant recruitment. It is important to note that these factors may interact with one another resulting in a much more complex influence on stimulation efficacy.

Studying the motor system post-stroke

Stroke is the second leading cause of death worldwide (after ischemic heart attack), and is one of the leading causes of long-term worldwide disability in adults, with more than 100,000 people having a stroke in the UK every year (The Stroke Association, 2018). There are over 1.2 million stroke survivors living in the UK at the current time (The Stroke Association, 2018). With the incidence of stroke predicted to rise over the coming years and more and more people surviving their stroke, there is an intense burden on the health service to deal with the consequences of stroke on patients, and their families.

The normal timeline of stroke recovery

The normal course of recovery following stroke begins in hospital, with acute care before rehabilitation can begin once stroke survivors are medically stable. The effects of stroke are diverse, and can affect any or all of a number of functions (such as memory and speech, increasing fatigue and apathy) and physical abilities (weakness in arms and/or legs, swallowing difficulties and balance). The vast majority of stroke survivors will require some kind of support following their stroke in order to continue with their activities of daily living (The Stroke Association, 2018).

At a recent roundtable taskforce, a number of leading researchers in stroke recovery and rehabilitation established a number of standards for the field (Bernhardt *et al.*, 2017). This included an agreed timeline of stroke recovery (Figure 1.4). Only a very small number of rehabilitation trials have taken place within the acute (<1 week) phases following stroke. During this period, neural plasticity is high, and therefore the possibilities for encouraging increased recovery are higher. However, this is when patients are most unwell, often medically unstable and therefore unable to undergo any intensive rehabilitation (Stinear *et al.*, 2013). The majority of rehabilitation studies have taken place in either the subacute or chronic phases of stroke recovery (Veerbeek *et al.*, 2014; Hayward *et al.*, 2017), when stroke survivors are more stable, and therefore able to participate. While this has obvious benefits, the critical window of high plasticity has probably been missed by the majority of these studies. Nevertheless, with an ageing population, and a growing number of stroke survivors in our population, the possibilities for continued improvement or finding strategies to enhance plasticity in the late-subacute and chronic phases of stroke are of great interest.

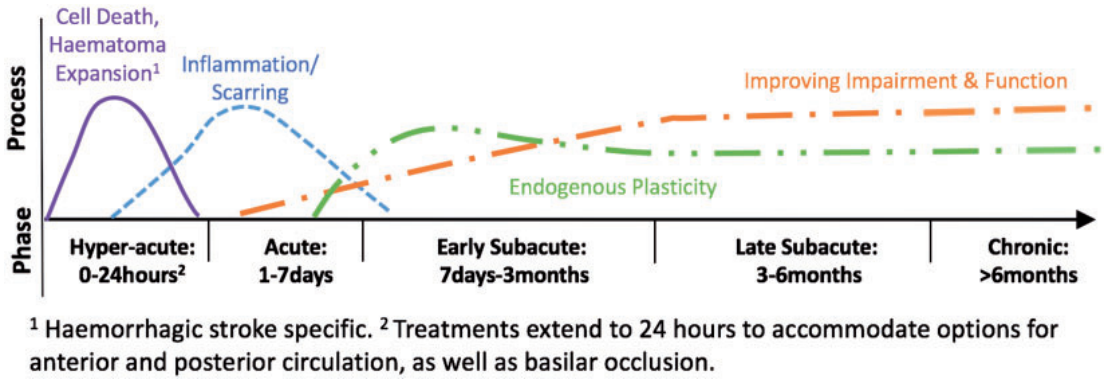

Figure 1.4: Framework for the stroke rehabilitation timetable (Taken from Bernhardt et al. (2017), Figure 1): Framework to describe definitions of critical timepoints following a stroke which link to the currently known biology of recovery post stroke developed by The Stroke Recovery and Rehabilitation Roundtable (SRRR) taskforce.

Assessing motor learning after stroke

Understanding the process of learning new motor skills in the healthy brain is a crucial step towards designing programmes to encourage re-learning and recovery of function following a stroke. By understanding motor learning within the healthy brain, we can subsequently assess how this process differs following damage to the brain such as following a stroke. On a practical note, it is much more feasible to perform numerous intensive, time consuming studies with complex details, multiple sessions and modalities in a young healthy cohort. By cherry-picking the relevant, or interesting results from these studies, we can inform a more specifically focussed study in both older adult and patient cohorts. It is well established that motor learning is a requirement of motor recovery after stroke (Krakauer, 2006) and is the basis of many rehabilitation strategies (Hubbard *et al.*, 2009), and despite this, assessments of motor learning in stroke survivors have received seemingly limited systematic investigation.

Within the literature, decrements in a number of aspects of motor learning have been reported post stroke, however it is clear that the process of motor learning is not completely abolished (Pohl *et al.*, 2001; Boyd *et al.*, 2007; Boyd, Vidoni and Wessel, 2010; Fleming, Newham and Rothwell, 2016). Compared to healthy controls, stroke survivors have been reported to demonstrate slower response times (Boyd *et al.*, 2007; Fleming *et al.*, 2016), slower speed of movement (Fleming *et al.*, 2016) and a slower rate of improvement during a learning task (Wadden *et al.*, 2017). Many studies, however, have shown a residual ability to improve performance with repeated task related practice.

By combining motor learning assessments with functional MRI, it has been observed that improved task performance following task-specific training is associated with shifts in the laterality index to reduce contralesional cortical activity (Boyd, Vidoni and Wessel, 2010). The

same was not true for non-task specific, random-sequence training, suggesting that robust reorganisation of the motor system can be achieved though task-specific training (Boyd, Vidoni and Wessel, 2010). However, it has been shown that even though a well-recovered (mild, subcortical) stroke survivor can demonstrate a matched behavioural performance to healthy controls, functional connectivity within the extended motor network remains abnormal, particularly during motor imagery (Sharma *et al.*, 2009).

Choice of task to assess motor learning after stroke

When assessing motor learning after stroke, the choice of task has the potential to cause both positive and negative impacts on potential findings. It is possible to target specific aspects of a movement or learning process through an appropriate task choice, but also possible to inadvertently limit a participant's performance if a less appropriate choice is made. The existing literature demonstrates the wide variety of available tasks, each of which target and measure different aspects of skill learning. Motor learning tasks usually assess skill (and improvement in skill) by measuring one or more of: speed, accuracy, and motor control. In addition, tasks will regularly require fine-motor movements in order to create a novel movement dynamic with scope for improvement with practice (for example pressing a specific sequence of button presses). Clear considerations when studying stroke survivor participants are the functional impairment and reduced residual abilities, as well as the likely older average age of the studied group. Evidence from healthy older adult cohorts suggests that as we age, humans demonstrate reduced fine motor skill, as well as reduced sensorimotor, perceptual and cognitive processing (for a detailed review, see Voelcker-Rehage, 2008). These effects would only be exacerbated following a stroke, making the careful consideration of motor task choice more important. Furthermore, when assessing motor learning after stroke,

tasks are chosen which reflect aspects of "real-life" motor learning in order to better relate findings to motor rehabilitation.

Within the existing literature of post-stroke motor learning, many groups have attempted to mitigate the aforementioned limitations of residual functional ability by adapting motor tasks. Examples include whole arm or hand movements rather than dexterous finger movements for sequence movement tasks (Pohl *et al.*, 2001, 2006; Boyd *et al.*, 2007; Fleming, Newham and Rothwell, 2016). Very recent work has used a isometric force tasks in both stroke survivors and healthy controls (Mooney *et al.*, 2020), finding skill improvement in both groups, with a lesser improvement in the stroke cohort when compared to age-matched controls. Isometric force tasks do not require dexterous movement, and instead measure skill acquisition via improvement in tracking accuracy and motor control.

Motor rehabilitation after stroke

When attempting to recover lost motor function post stroke, physiotherapy is the gold standard motor rehabilitation method, but it is both expensive to deliver and time consuming for both the stroke survivor and the physiotherapist working with them. Once discharged to home care, the onus is then on the stroke survivor and their carers to continue the rehabilitation process (typically either by personally paying for further physiotherapy, or by continuing their own programme at home).

All motor rehabilitation has the aim of either relearning a skill the individual was able to perform effortlessly prior to the stroke (recovery of function), or finding alternative ways to achieve the same final end goal using novel movements and/or different muscle groups (compensatory function). These two processes are evidently very different, and the extent to

which each individual will focus on one or the other is personal to them, the characteristics of their stroke symptoms, and their rehabilitation programme. For the purposes of this thesis, when discussing rehabilitation, I am referring to the recovery of previously achievable action (either through recovery of damaged brain regions, or recruitment of other regions), rather than development of a novel alternative strategy using different muscles to achieve the same final goal.

tDCS as a potential adjunct therapy post-stroke

tDCS has obvious attractiveness for use as an adjunct therapy in stroke rehabilitation. The possibility of augmenting gold-standard rehabilitative methods by boosting motor cortex plasticity could lead to improved outcomes for stroke survivors, and lessen the burden on Health Service rehabilitation programs. Understanding the physiological basis of any observed changes when tDCS is performed in a stroke survivor cohort is therefore of significant interest for the development of these novel adjunct therapies.

Figure 1.3: tDCS in stroke rehabilitation: Andrew Marr receiving tDCS while undergoing motor rehabilitation as part of his investigation of adjunct therapies for stroke rehabilitation. Credit: Love Productions / Andrew Marr: My Brain and Me

In line with characteristic patterns of post-stroke cortical activation during affected and unaffected hand movement, many investigations of tDCS in stroke survivors have focussed on increasing activity (via anodal tDCS) in the affected, ipsilesional, hemisphere or reducing activity (via cathodal tDCS) in the less-affected, contralesional, hemisphere. These methods work towards the re-balancing of interhemispheric inhibition, which may be disrupted post-stroke (Ward, 2005; Hummel and Cohen, 2006). While there is a large body of research demonstrating promising effects of both ipsilesional anodal (Butler *et al.*, 2013; Khedr *et al.*, 2013; Allman *et al.*, 2016) or contralesional cathodal tDCS (Kim *et al.*, 2010; Khedr *et al.*, 2013), it is likely that the story is far more complex, due at least in part to the debated role of the contralesional motor cortex in stroke recovery. Furthermore, a number of studies have failed to show any beneficial effect of either anodal or cathodal tDCS on functional recovery post stroke (Geroin *et al.*, 2011; Hesse *et al.*, 2011). It has additionally been suggested that bilateral tDCS (ipsilesional anode, contralesional cathode) may have effects above those of either unilateral stimulation montage. A small number of studies have investigated this theory in stroke survivors, demonstrating mixed success (Lindenberg *et al.*, 2010; Bolognini *et al.*, 2011; Di Lazzaro *et al.*, 2014; Lefebvre *et al.*, 2014; O'Shea *et al.*, 2014). Multiple meta-analyses have been performed, with the majority finding small-to-moderate effect sizes (between ~0.4 and ~0.6) in favour of tDCS improving motor rehabilitation outcomes (Butler *et al.*, 2013; Kang *et al.*, 2015; Chhatbar *et al.*, 2016; Elsner *et al.*, 2017). This, however, is not true of all meta-analyses, with Tedesco Triccas and colleagues reporting no significant effect size in favour of tDCS improvement of either motor impairment or activity performance (Tedesco Triccas *et al.*, 2016). These meta-analyses together suggest a modest improvement to upper limb rehabilitation with adjunct tDCS, however this is not conclusive.

The role of the contralesional hemisphere in motor rehabilitation

Numerous functional magnetic resonance imaging (fMRI) and positron-emission tomography (PET) imaging studies in humans have identified a transient increase in contralesional brain activity during movement of the paretic upper limb following a stroke (first observed by Chollet *et al.*, 1991; for a full review, see Calautti and Baron, 2003), however the cause, and purpose of this increase is highly debated. It is difficult to assess whether this contralesional activity is adaptive (and a manifestation of beneficial cortical reorganisation), or maladaptive (and therefore preventative of recovery). Two main opposing models of the role of contralesional activity have been postulated (The Interhemispheric Competition Model and The Adaptive Plasticity Model), and are discussed in more detail below. From recent literature, it is likely that there is a place for both of these models, but in different cohorts of stroke survivors. Currently, however, it is still difficult to identify characteristics of these cohorts, and therefore which model suits which stroke survivors.

Interhemispheric Competition Model

The Interhemispheric Competition Model suggests that the increased activity observed across the contralesional hemisphere during movement of the paretic limb is preventative of full recovery. Cortical damage caused by the stroke lesion causes reduced activity within the ipsilesional primary motor cortex, resulting in disinhibition of the contralesional hemisphere, and therefore increased transcallosal inhibition on the ipsilesional hemisphere. This is supported by evidence that those stroke survivors with the highest contralesional activity during movement of the paretic upper limb are those with the least residual function (Ward *et al.*, 2003). Furthermore, the same researchers have shown that an increasing degree of return shift to a more normal unilateral (ipsilesional) activity has been linked to the increased improvement of functional recovery within the same period of time (Ward *et al.*, 2003).

Based on the logic of the Interhemispheric Competition Model, rebalancing of the hemispheres should be a key aspect of rehabilitation methods. An example of rebalancing is the use of NIBS methods to either up-regulate activity in the ipsilesional or down-regulate in the contralesional hemisphere should result in improved behavioural performance (Nowak *et al.*, 2009) (Figure 1.5). Application of this model in practice has been demonstrated to be successful (Fregni *et al.*, 2005; Hummel and Cohen, 2005; Stagg *et al.*, 2012). It should be noted that success has most widely been reported in mild or moderately impaired stroke survivors.

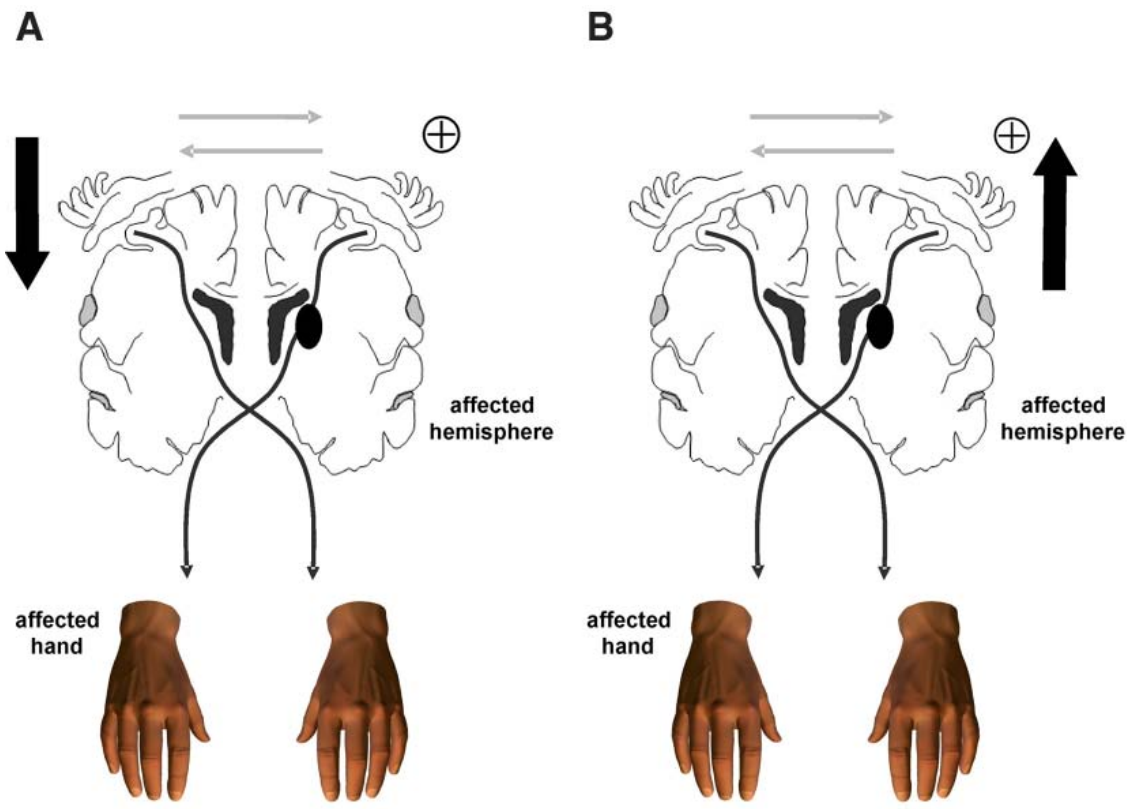

Figure 1.5: Schematic of the Interhemispheric Competition Model (Taken from Nowak et al. (2009), Figure 2)**:** Identification of the possible targets for modulatory NIBS to augment recovery alongside rehabilitation. **A:** Inhibition of the contralesional primary motor cortex (indicated by down arrow) or **B:** Facilitation of the ipsilesional primary motor cortex (indicated by up arrow) should prevent imbalance of transcallosal communication between ipsilesional and contralesional hemispheres, therefore encouraging recovery.

Adaptive Plasticity Model

The Adaptive Plasticity Model of post-stroke recovery postulates that residual cortical networks substitute for lost function following a stroke through a mechanism of beneficial cortical reorganisation. For many stroke survivors, it is suggested that remapping is of functional benefit, as evidenced by inhibitory TMS to the contralesional hemisphere resulting in a negative effect on behavioural performance in stroke cohorts (Johansen-Berg *et al.*, 2002; Lotze *et al.*, 2006). Higher fMRI activation in the contralesional primary motor cortex has been observed in subacute stroke survivors with good functional recovery when compared to healthy controls, which supports the adaptive plasticity model and suggests that contralesional activity does not prevent a good level of functional recovery (Bütefisch *et al.*, 2005; Schaechter and Perdue, 2008). Furthermore, there is some limited evidence of a supportive function of contralesional hemispheric activity in a subset of chronic stroke survivors (Lotze *et al.*, 2006). Particularly in stroke survivors with severe impairment (such as very low corticospinal tract integrity), there is a greater observed contralesional activity (Ward *et al.*, 2003). It has therefore been argued that there is an adaptive role of the contralesional hemisphere, particularly in stroke survivors with more severe functional impairments (Bradnam *et al.*, 2013).

Selecting the best rehabilitation programme

Understanding the role of the contralesional hemisphere after stroke is a crucial step for successful rehabilitation, particularly when considering combining conventional therapies with adjunct techniques such as tDCS. While both the Interhemispheric Competition Model, and the Adaptive Plasticity Model have been used with some success to rebalance the hemispheres, it is becoming increasingly clear that this is not a one-size-fits-all problem. Multiple articles have now postulated that multiple factors should be taken into account when deciding which model to use for designing rehabilitation programmes (Stinear *et al.*, 2007;

Bradnam *et al*, 2013; Plow *et al.*, 2016; McCambridge *et al.*, 2018). These include time since stroke (Volz *et al.*, 2017), cortical damage severity (Kim and Jones, 2010; Touvykine *et al.*, 2016), corticospinal tract integrity (often assessed by diffusion scanning or by the presence/absence of an MEP) (Stinear *et al.*, 2007; Lotze *et al.*, 2012), and residual function/degree of impairment (Ward *et al.*, 2003).

Unfortunately, the majority of clinicians will not have access to many (let alone all) of the methods used within research laboratories to assess this multitude of factors. A priority within the neurorehabilitation field is to develop simple algorithms (see Figure 1.6 for an example from Stinear *et al.*, 2007), which can quickly and easily identify (within a range of probability) a best rehabilitation programme for each individual stroke survivor. It has been shown that the most commonly accessible factors available (current clinical scores and time since onset of stroke) are not sufficient to predict functional potential, unless combined with neurophysiological and imaging data (Stinear *et al.*, 2007).

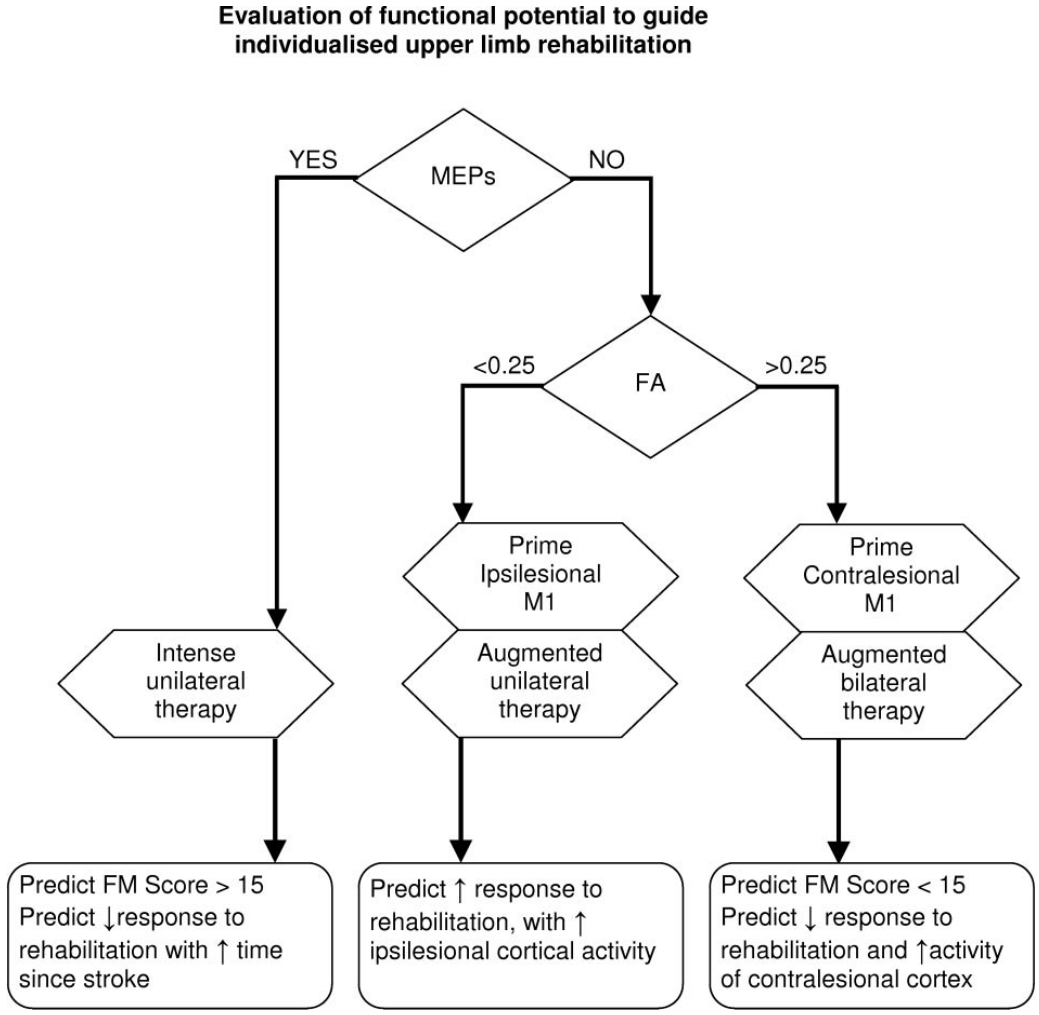

Figure 1.6: An algorithm to predict functional recovery of the upper limb and provide targeted rehabilitation post stroke (taken from Stinear et al., (2007), Figure 4): An example of a simple algorithm which enables a prediction of functional ability (Fugl Meyer (FM) score), recovery potential, and a suggested rehabilitation method for stroke survivors based on presence of MEP, and fractional anisotropy (FA) of the corticospinal tract.

Aims of this thesis

This thesis aims to elucidate the physiology of motor learning in healthy controls, and compare it between groups of healthy controls and chronic stroke survivors. Motor skill learning is a key component of the motor rehabilitation process, and much of our theories for improving rehabilitation programmes are based around methods which augment and improve motor skill learning in young healthy participants. However, if there is a fundamental difference in the physiology underpinning motor skill learning between healthy controls and stroke survivors, then we are already limiting the potential of these rehabilitation programmes before we begin administering them. Within this thesis, I mainly focus on a role of inhibition within the motor cortex using a multimodal MRI and NIBS approach. By combining techniques, I have been able to measure brain structure, function and metabolites as well as behavioural performance across healthy controls (both young and older adults) and in chronic stroke survivors. It is my hope that by investigating the motor skill learning process at a physiological level, we might be able to improve the rehabilitation of the many thousands of stroke survivors with motor impairments even many months to years after their stroke.

In Chapter Two, I will describe the methods I have used within this thesis, particularly non-invasive brain stimulation methods of tDCS and TMS used within Chapter Four and Five. I will also describe the different methods of magnetic resonance imaging and spectroscopy used within Chapter Three, Four and Six. Finally, I will discuss the various methods for assessing motor learning which are used within all Chapters of this thesis.

In Chapter Three, I have performed an MRS study to assess dynamic changes in GABA within the sensorimotor cortex during performance of a learning task. I replicate a previous finding

(Floyer-Lea, 2006) that a reduction in GABA is observed only during performance of a learning task, and not during a non-learning version of the same task, nor a resting condition.

Chapter Four contains the results of a study performed to assess the timing effect of cathodal tDCS delivered to the contralateral (left) hemisphere either prior to, or during performance of a motor learning task. Using concurrent fMRI, I demonstrate an increase in learning related activity in the hemisphere ipsilateral to the task-performing hand when stimulation is delivered prior to task performance. A follow-up to this study is presented in Chapter Five, where cathodal tDCS is again used to modulate performance of a motor learning task. In this chapter, TMS is used to assess bilateral cortical excitability and neurotransmitter systems, finding relationships between baseline response time (RT) and change in left hemisphere cortical excitability, and rate of learning and change in right hemisphere glutamatergic signalling.

In the final experimental chapter of this thesis, Chapter Six, a novel motor task is used to assess performance in both chronic stroke survivors and an age-matched cohort of healthy older adults. Participants also completed a multimodal MRI scan to assess brain structure and function, as well as neurotransmitter systems. A subsection of the data collected within the MRI scan is included within this thesis – the results of two functional MRI tasks and the magnetic resonance spectroscopy results.

As a point of note, whenever I reference "contralateral" or "ipsilateral" hemispheres (without specifically references to a hand/tDCS stimulation) these refer to the hemisphere which is contra/ipsilateral to the hand performing a task.

Chapter 2: Methods

This thesis includes studies using multimodal methods of understanding and modulating human motor learning. Magnetic Resonance methods allow for unparalleled ability to study the structure and function of the brain *in vivo*. This thesis presents data using various modalities in both high (3T) (Chapters Four and Six) and ultra-high (7T) (Chapter Three) field research MRI scanners. In addition, cortical excitability and neurotransmitter systems have been assessed using different protocols of transcranial magnetic stimulation (TMS) (Chapter Five). Within Chapters Four and Five of this thesis, transcranial direct current stimulation (tDCS) is used to modulate cortical excitability in order to alter the normal process of motor learning.

Magnetic Resonance Acquisition Methods

Magnetic Resonance (MR) techniques have been used within medicine and research to image the human body since their first development in the 1970s. MR Imaging (MRI) of the brain allows for non-invasive study of its structure and function *in vivo*. Various methods utilising MR allow neuroscientists to study structure (of grey and white matter), function (via blood oxygenation changes) and metabolite concentrations.

MR, at its core, harnesses the magnetic properties of the positively charged nucleus of the hydrogen atom. Other nuclei can be used in a similar method (including Carbon-13 and Phosphorus-31), however their lower concentrations mean a significantly lower signal can be detected. While these other molecules are used within aspects of brain MR (particularly within spectroscopy), the vast majority of MR uses the proton as its source of signal.

All nuclei with an odd number of protons will exhibit spin, a property whereby the particle rotates around a central axis. In the absence of a magnetic field, these spins are oriented randomly, producing no net magnetisation in any particular direction. However, when placed in a magnetic field (such as the main B_0 field of the MR scanner) protons align themselves with the magnetic field, and will rotate (or "precess") around this field direction. In MR, the B_0 field is induced in the direction of the bore of the MR scanner, and is referred to as the z-axis for imaging.

Creating MR Image Contrasts

In order to measure a usable signal for imaging from the protons, a second, temporary, weaker, magnetic field (B_1) is applied in a perpendicular direction to the B_0 field. This is known as a Radio Frequency (RF) pulse. On application of the RF pulse, the protons will flip away

from the B_0 direction into the *xy* plane, and will then precess around a direction determined by the combined fields. Following the RF pulse, the protons will slowly return to precess only around the main B_0 field in a process called relaxation. Relaxation can be split into gain of magnetisation in the longitudinal (z or B_0 plane) axis (known as T1) and the loss of magnetisation in the lateral (*xy*) plane (known at T2) (Bloch, 1946). An additional lateral relaxation time constant, T2*, reflects the combination of the loss of magnetisation in the lateral *xy* plane, along with effects of inhomogeneities in the B_0 field (Chavhan *et al.*, 2009). Together, this loss of magnetisation and field homogeneity lead to dephasing of precession in neighbouring nuclei. The T1 and T2 relaxation processes are independent of one another, and occur over different time frames. Protons within different tissues (e.g. water (or Cerebrospinal Fluid, CSF), grey matter (GM), muscle, fat or bone), and within different regions of the same tissue, will have different T1 and T2 relaxations. These differences are due to the large array of microscopic and macroscopic structures, which differentially affect the behaviour of the protons in water molecules, the main contributor to the MRI signal. This therefore allows for differentiation between them. By harnessing the differences in T1 and T2 relaxation times within, and between tissue types, it is therefore possible to design MR sequences to visualise regions of interest.

Signal recording within the MR scanner takes place using an RF receive coil. The RF coil quantifies the electromagnetic radiation emitted from the protons at time points after the application of the RF pulse. The time between application of a RF pulse and signal recording is called the Echo Time (TE), and the time interval between RF pulses is known as the repetition time (TR).

Slice Selection

In order to create an image using MRI, it is necessary to select the region of space to be targeted with the RF pulse, and then to localise the origin of the recorded signal change from the relaxing protons. Slice selection and spatial encoding are performed using the Gradient Coils of the MR scanner. Induction of rapidly changing magnetic fields by the Gradient Coils limits the region of excitation of the RF pulse. By altering the outputs of the Gradient Coils, it is possible to target single 'slices' at a time, or to target multiple equally-spaced 'slices' simultaneously.

Structural MRI

Structural MRI sequences are designed to enable discrimination between tissue types. T1-weighted sequences and T2-weighted sequences exploit the differing characteristics of the T1 and T2 relaxations of protons within different tissue types. In T1-weighted images of the brain, white matter appears bright, due to the high fat content of the myelin (and therefore tightly bound hydrogen atoms which exhibit a short T1 relaxation). By contrast, CSF and bone appear dark, due to the loosely bound hydrogen atoms. In T2 weighted images, the contrasts are reversed, such that CSF appears light and brain tissue appears much darker. T1-weighted images are useful in the assessment and visualisation of normal brain anatomy, while T2-weighted images hold advantages for observing pathologies such as haemorrhages. The T1-weighted sequence used in this thesis is the Magnetisation Prepared RApid Gradient Echo (MPRAGE) sequence (Mugler and Brookeman, 1990). This sequence uses a short TR to maximise the T1 effect, and short TE to minimise the T2 effect. Collectively this produces a good contrast between grey and white matter within the cortex.

Functional MRI

In addition to visualising brain structure, MRI can also be used to observe patterns of neuronal activity across the brain, and temporal changes in this activity, via assessment of changes in blood flow. This is performed using Blood Oxygen Level Dependent (BOLD) MRI, which takes advantage of the differing magnetic properties of oxygenated and deoxygenated haemoglobin in blood. The basis of the BOLD signal is highly complex, and is thought to reflect many different aspects of neural activity and metabolism. However, in essence, The BOLD signal is often assumed to be related to neural firing as follows: Neuronal activity requires energy in the form of adenosine triphosphate (ATP). ATP synthesis involves the oxidative phosphorylation of adenosine diphosphate, requiring oxygen. Increased neuronal activity therefore necessarily requires increased oxygen delivery, which is reflected by increased cerebral blood flow to the active brain area. Increased neuronal activity is therefore associated with increased oxyhaemoglobin and an increased BOLD signal. There is a delay between the neuronal activity and the response of the vascular system to deliver oxygenated blood. This can be modelled by the haemodynamic response function (HRF). When using BOLD fMRI to assess regionally specific brain functions, changes are recorded in response to stimuli or tasks in order to take the HRF into account.

Magnetic Resonance Spectroscopy (MRS)

Quantification of neurotransmitter concentrations in the human brain *in vivo* can be performed using MRS. Neurochemicals within a region of interest can be identified based on differences in their molecular structure, allowing for quantification of concentration of neurochemicals of interest. In the clinical setting, MRS can provide relevant information for diagnosis of tumours, and various metabolic or systemic diseases. Spectra are plotted based on the frequency of the structural components, with peaks representing different

neurochemicals. The magnitude of these peaks is representative of the relative concentration of each neurochemical. Hydrogen (^{1}H-MRS) is the most commonly targeted nucleus for MRS, however it is also possible to perform MRS on an extensive range of other nuclei, including phosphorus (^{31}P-MRS), carbon (^{13}C-MRS), sodium (^{23}Na-MRS), potassium (^{39}K-MRS) and fluorine MRS (^{19}F-MRS) *in vivo* in the human brain. ^{1}H-MRS does not require additional MR hardware, as protons are high in natural abundance and their gyromagnetic ratio is large, giving a relatively high signal to noise (Blüml, 2013). All MRS performed within this thesis is ^{1}H-MRS.

Principles of Magnetic Resonance Spectroscopy

The peaks in the resultant spectra produced during MRS represent the concentrations of a number of different metabolites within the region of interest. The variation in shift of the peaks (known as chemical shift) is produced as a result of shielding of the central nucleus by its surrounding protons. The proximity of the protons to their central nuclei results in shielding from the main B_0 magnetic field of the MR scanner. The subtle variations in this shielding resulting from differing molecular structures in each metabolite can be identified through peak assignment, and form the basis for the spectral separation used to plot an MRS spectrum. Each metabolite may have multiple nuclei with surrounding protons, therefore resulting in complexes of multiple peaks relating to each metabolite within the final spectrum. The MRS spectrum is additionally modulated by J-coupling. J-coupling is the interaction between groups of protons on neighbouring nuclei within the same molecule. The combination of J-coupling, and chemical shift will produce a biochemical fingerprint for each metabolite, which is specific to the field strength of the MR scanner, and the sequence type and acquisition parameters of the MRS performed.

Single Voxel MRS

Single voxel MRS allows for neurochemical quantification within a single large voxel of interest (VOI) placed within the cortex while suppressing signal from outside this region. The size of this VOI is normally reasonably large (in the region of 8 - 32 cm^3) and therefore the quantification values recorded are representative of the relatively large portion of cortex covered by the VOI. The VOI is typically manually placed based on a T1-weighted structural scan, to cover an anatomical region of interest. For sensorimotor regions, this is often the hand-knob, which was first identified histologically by Yousry *et al.* (1997) and is characteristically the centre point of activation on the central sulcus observed using BOLD functional MRI activity during movement of the hand. Irrespective of the region of voxel placement, it is important to place the VOI within the cortex of the brain, to prevent macromolecule contamination from the dura.

Benefits and limitations of Ultra-High Field MRS

It is difficult to quantify low abundance metabolites, such as GABA, whose signal overlaps with that of more abundant metabolites (including Creatine, NAA and macromolecules) at lower field strengths. In order to quantify GABA, edited sequences such as MEGA-PRESS were developed (Mescher *et al.*, 1998), which are commonly used at lower field strengths such as 3T. However, these edited approaches require two acquisitions for each measurement and therefore have a low Signal-to-Noise Ratio (SNR) (for further details, see Blüml, 2013). Increasingly, therefore, studies have employed ultra-high field (7T) scanners to accurately quantify GABA at higher field strengths, as the increased frequency resolution afforded allows for adequate spectral separation.

There are additional benefits of performing MRS at Ultra-High (7-Tesla) MRI: the higher field strength gives a much greater SNR, allowing for an increased temporal and spatial resolution. 7-Tesla MRS therefore allows for more accurate quantification of the less abundant neurochemicals over a much finer time scale without the necessary use of edited MRS sequences. Of particular relevance to this thesis, ultra-high field MRS also allows for separate quantification of Glutamate and Glutamine, due to its increased spectral resolution. We therefore have used the non-editing semi-LASER sequence at 7T in Chapter Three to quantify GABA and glutamate (van de Bank *et al.*, 2015). Edited MRS sequences are available for 7T, such as the MEGA-sLASER sequence (Scheenen *et al.*, 2008), but these are difficult to implement.

As with all ultra-high field MRI, there are limitations to 7T MRS, most commonly caused by side effects of the high B_0 field strength such as spatial distortion and drop out caused by susceptibility artefacts near the frontal sinuses and skull base. These distortions can therefore introduce difficulties with shimming due to inhomogeneities across the brain. At higher field, inhomogeneity in the B_1 field results in additional signal inhomogeneity, in particular a reduced signal in the periphery of the brain (Webb and Collins, 2010). This reduced signal makes quantification of already low concentration metabolites (such as GABA) much more difficult. In order to combat this, dielectric pads can be used to locally increase signal (for example in the motor cortex) in favour of other regions of less interest (Teeuwisse, Brink and Webb, 2012; Lemke *et al.*, 2015). In line with recent studies from our centre (Lemke *et al.*, 2015; Barron *et al.*, 2016; Kolasinski *et al.*, 2017; Frangou *et al.*, 2019), a dielectric pad is used for the MRS reported in Chapter Three.

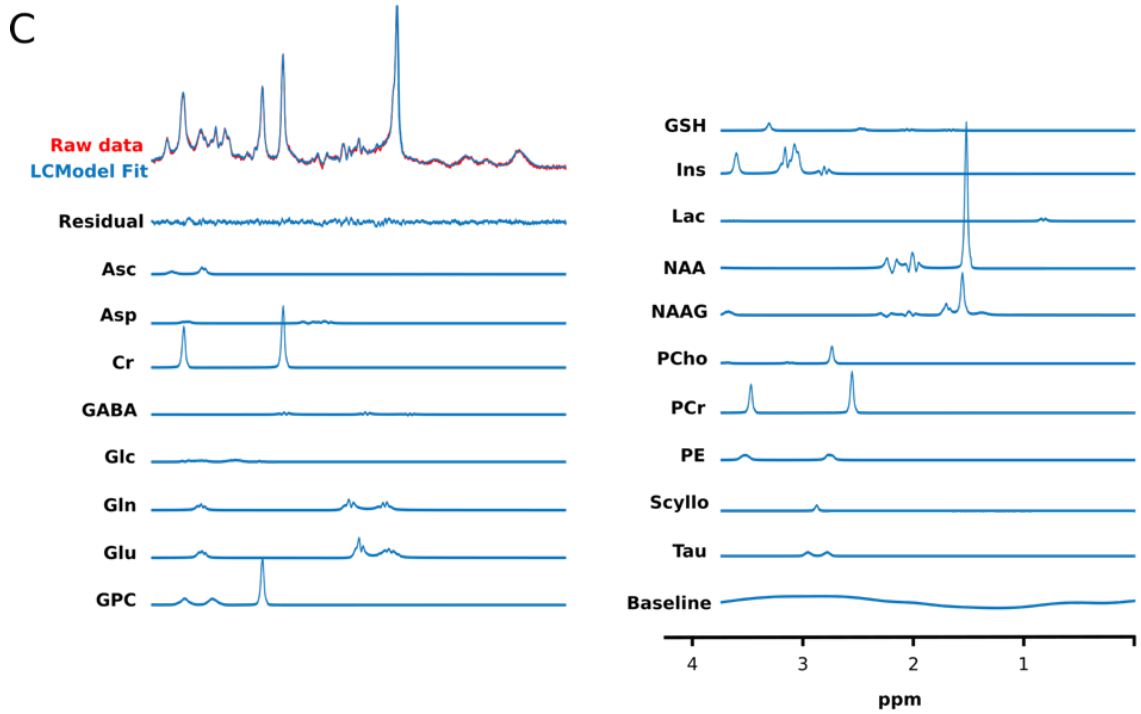

Figure 2.1: Representative MRS Spectrum (taken from Kolasinski et al. (2018), Figure 1: C (see also Chapter Three)): Representative MRS acquisition from sensorimotor cortex voxel at 7-Tesla, including the LCModel fit for neurochemicals.

Magnetic Resonance Spectroscopic Imaging (MRSI)

Magnetic Resonance Spectroscopic Imaging (MRSI), also known as Chemical Shift Imaging (CSI), simultaneously collects an array of multiple MRS voxels within a larger region of interest than single voxel MRS (Öz *et al.*, 2014). This allows for the creation of metabolic maps, demonstrating distribution of metabolites across a larger region of tissue. MRSI can be performed in 2D or 3D. The higher spatial resolution and larger total coverage area are obvious benefits of MRSI, however the technical limitations of the sequence have, until recently, resulted in prohibitively long set up and acquisition times, especially for clinical populations (Zhu and Barker, 2011). Additionally, MRSI is more sensitive to participant motion that SVS, and spectral contamination between neighbouring voxels is common in multi-voxel MRS, requiring additional and more complex analysis approaches (Zhu and Barker, 2011).

Recent work has demonstrated use of density-weighted concentric ring trajectory (DW-CRT) MRSI at both ultra-high (≥ 7-Tesla) (Chiew *et al.*, 2018; Hingerl *et al.*, 2018) and more recently at 3T (Steel *et al.*, 2018). This method allows for both improved artefact reduction (by sampling k-space using a density weighed pattern), and decreased acquisition time (using concentric ring trajectory method for phase encoding) (Steel *et al.*, 2018). It is now feasible to perform MRSI at a higher resolution than single voxel MRS (e.g. multi voxel array, with each voxel measuring 0.5 x 0.5 x 15 mm), and in both a healthy and disease cohort, at 3T (Steel *et al.*, 2018). Chapter Six of this thesis uses the relatively novel 2D MRSI approach published by Steel and colleagues (2018) with a region of interest focused on the bilateral primary motor cortices and surrounding cortical area.

Static vs. Dynamic MRS (/or 'snap-shot' vs. 'functional' MRS)

Most traditionally, MRS is performed to provide a static 'snap-shot' of the neurochemistry within the region of interest at a certain time point. Typically, MRS averages the metabolite concentrations across a number of acquisitions (usually totalling in the region of ~5 minutes). This method allows for cross-sectional assessment of metabolites with behavioural or other MR assessed metrics, as well as pre/during/post intervention investigation. Across the brain, this method has proved useful in studying how the resting metabolite concentrations within a brain region can predict performance in a behavioural task dependent on that region (higher GABA in primary sensorimotor cortex predicts better tactile discrimination: Kolasinski *et al.*, 2017), and can be related to structural (higher hippocampal NAA correlates with greater left ventrolateral prefrontal cortical grey matter: Steinke *et al.*, 2017) or functional measures at rest (lower sensorimotor cortex GABA is predictive of stronger resting motor network strength: Stagg *et al.*, 2014).

Recording the dynamic changes of the metabolite systems is limited by the temporal resolution of MRS. Previous work has recorded changes in metabolite concentrations across two or more discrete time points (either pre/post task or an intervention), and related these changes to behaviour (Stagg, Bachtiar and Johansen-Berg, 2011a; Sampaio-Baptista *et al.*, 2015), however this is not a true measure of the dynamics of metabolite change over the course of task performance. More recently, a small number of studies have acquired multiple time points of MRS during performance of a task (Floyer-Lea, 2006; Chen *et al.*, 2017), however these studies rely on a long task duration (of around 30 minutes) in order to record multiple MRS acquisitions. In Chapter Three of this thesis, dynamics of the motor cortex metabolite system are assessed by acquisition of multiple MRS blocks in succession, with each block lasting approximately 5-6 minutes. While our approach still has a relatively poor temporal resolution, it is a step forward compared to previous literature (Floyer-Lea, 2006; Chen et al., 2017). Further developments of true 'functional' magnetic resonance spectroscopy (fMRS) have demonstrated an ability to assess neurotransmitter fluctuations and BOLD fMRI simultaneously, and over a much finer temporal resolution (in the range of a few seconds rather than minutes). This is undoubtedly an exciting step for neuroimaging methods, however this method is still undergoing significant development, and is limited as to which metabolites which can be reliably quantified (Ip et al., 2017, 2019; Stanley and Raz, 2018).

Magnetic Resonance Analysis Methods

Magnetic Resonance Spectroscopy Analysis

Water Suppression

Water is many times more abundant than any other metabolite in the human brain. In the healthy brain, water accounts for approximately 83% of grey matter (GM), and 70% of white

matter (WM). By contrast, concentrations of the metabolites of interest for MRS research (such as Glutamate and GABA) are between 10^3 and 10^4 times lower. When unaccounted for the water peak will therefore make the quantification of other metabolites impossible. However, as the resonant frequency of the protons in water is known (4.65 ppm), it is possible to measure and then suppress the signal from water within the selected MRS ROI, without affecting the signal from metabolites of interest. The process of water suppression during MRS is performed by a sequence called VAriable Power and Optimised Relaxation Delay (VAPOR) (Tkáč *et al.*, 1999), which delivers a selective RF pulse to prevent water molecules from creating a measurable signal.

Metabolites Detected by MRS

Approximately 15-20 metabolites can be detected in the human brain by MRS . The main metabolites of interest within this thesis are GABA, and to a lesser extent, Glutamate (Table 2.2). Their roles as the main inhibitory and excitatory neurotransmitters mean they are of key interest during research of synaptic plasticity, and by extension motor learning. Glutamate is present at much higher concentrations than GABA (~12 mM (Choi *et al.*, 2006) vs. <1 mM (Petroff and Rothman, 1998)). When performing MRS at 3T, it is difficult to separate the spectra for Glutamate and Glutamine, and therefore is often reported as a combined measure of "Glx".

Table 2.2: Average metabolite ranges in the healthy human for metabolites of interest within this thesis

Neurochemical	Abbreviation	Range (mmol L^{-1})
Gamma-aminobutyric acid	GABA	1.0 – 2.0
Creatine	Cr	4.5 – 10.5
Glutamate	Glu	3.0 – 6.0
Glutamine	Gln	6.0 – 12.5
N-acetylaspartate	NAA	7.5 – 17.0
Phosphocreatine	PCr	3.0 – 5.5

Table adapted from Graff (2007)

Reporting MRS-measured metabolite concentrations

Even though the amplitude of the peaks within an MR spectrum reflects the absolute concentration of any given metabolite, it can be influenced by a wide variety of factors, including voxel position, subject movement, and B_0 inhomogeneity. It is therefore important to reference the metabolite of interest to another, more stable metabolite within the same voxel. Within the literature, this reference metabolite varies. The most commonly used is total creatine (tCr), calculated as the sum of creatine (Cr) and phosphocreatine (PCr) within the VOI. The concentration of PCr is relatively stable in the healthy adult human brain, therefore making it a good reference. Other signals used include *N*-acetylaspartate (NAA) or water. NAA is relatively stable within the healthy human brain, but reflects neuronal mitochondrial activity and therefore might be hypothesised to change during task performance. Referencing neurochemicals to a simultaneously-acquired reference metabolite also reduces the influence of movement or potential changes in water concentration that might occur during activity (Gasparovic *et al.*, 2006). Therefore, for all MRS reported in this thesis, tCr is used as the reference metabolite.

Due to its relatively large size, the selected VOI will contain a mixture of grey and white matter, and potentially some CSF. Correction for tissue composition is common with the MRS literature and can improve accuracy of metabolite reporting (Gasparovic *et al.*, 2006; Mullins *et al.*, 2014), however the methodology for this process is not standardised, and different methods are preferred by different groups (Harris, Puts and Edden, 2015). The method most commonly performed and reported in Oxford (Stagg, Bachtiar and Johansen-Berg, 2011a; Stagg *et al.*, 2014; Bachtiar *et al.*, 2015; Kolasinski *et al.*, 2017) calculates the contributions of GM, WM and CSF to the VOI using FMRIB's Automated Segmentation Tool (FAST) (Zhang, Brady and Smith, 2001).

Within this thesis, metabolites of interest are corrected for GM as follows:

$$GM\ Corrected\ GABA = \frac{\frac{[Absolute\ GABA]}{[GM]}}{[GM] + [WM] + [CSF]}$$

In addition, total Creatine (to which metabolites of interest are reported as a ratio) are corrected for total brain volume within the VOI as follows:

$$Brain\ volume\ corrected\ tCR = \frac{\frac{[Absolute\ Cr] + [Absolute\ PCr]}{[GM] + [WM]}}{[GM] + [WM] + [CSF]}$$

Functional Magnetic Resonance Imaging Analysis

fMRI analysis methods are varied, and are often dictated by task design. A block design is the simplest, and most efficient way to deliver, and perform a task during fMRI. Periods of task performance are interspersed with periods of rest, in order to create a contrast in BOLD

activity. Block designs provide robust activation and allow for straightforward analysis pipelines. Event-related designs are advantageous for some tasks as they are significantly more flexible in terms of task design, and can provide a higher temporal resolution. However, these advantages are less relevant for the tasks in this thesis, and therefore a block design is used for all tasks (in Chapters 3, 4 and 6).

fMRI Preprocessing

All fMRI processing within this thesis is performed using tools from the FMRIB Software Library (FSL), in particular using FMRIB's Expert Analysis Tool (FEAT) (Woolrich *et al.*, 2001, 2004). The initial steps of fMRI analysis involve preprocessing the data, including motion correction using MCFLIRT (Jenkinson *et al.*, 2002), B0 Unwarping using Boundary Based Registration (BBR) (Greve and Fischl, 2009), brain extraction using Brain Extraction Tool (BET) (Jenkinson, Pechaud and Smith, 2005) and registration to standard space using FMRIB's Linear Registration Tool (FLIRT) (Jenkinson and Smith, 2001; Jenkinson *et al.*, 2002) or FMRIB's Non-Linear Registration Tool (FNIRT) (Andersson, Jenkinson and Smith, 2007).

Independent Component Analysis (ICA)

Independent Component Analysis (ICA) can be used to decompose data into discrete spatial and temporal components which can then be classified (either automatically or manually) as either signal or noise, before further processing.

ICA can either be performed manually following component identification using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components)) (Beckmann and Smith, 2003) or automatically using FIX (FMRIB's ICA-based Xnoiseifier)

(Griffanti *et al.*, 2014; Salimi-Khorshidi *et al.*, 2014). Recently, a fully automated ICA component identifier, and classification program ICA-AROMA (Automatic Removal of Motion Artefacts) which completes both steps, and robustly identifies motion induced signal variation in both function and resting state MRI data (Pruim, Mennes, Buitelaar, *et al.*, 2015; Pruim, Mennes, van Rooij, *et al.*, 2015).

First and Higher Level FEAT Analyses

A first level analysis is used to analyse an individual session's data. It combines the preprocessing steps mentioned above and statistical steps to model the task design. Higher Level FEAT analyses combine outputs from multiple First Level FEAT analyses to assess group level results, either across several sessions, or across multiple subjects. Full methodological details for First and Higher Level stages of fMRI analysis are given within the relevant Methods section of each experimental chapter.

Non-Invasive Brain Stimulation (NIBS) Methods

Non-invasive brain stimulation (NIBS) methods are valuable tools for measuring physiological and neurochemical environments within the cortex and for modulating cortical activity. Within this thesis, Transcranial Magnetic Stimulation (TMS) is used for the former while Transcranial Direct Current Stimulation (tDCS) is used for the latter. The combined use of these two techniques (either with each other, or with other methods such as MRI or electroencephalography (EEG)) allows for investigation of how exogenous modulation of cortical excitability can affect physiology and behaviour. Within the motor learning literature, investigation and understanding of the effects of tDCS on healthy motor learning is a crucial early step towards the hoped-for use of NIBS methods for adjunct treatment in motor

rehabilitation after stroke. Detailed investigation of the physiological changes induced by NIBS methods such as tDCS is necessary before these methods can be translated to a wider clinical population. In recent years, the initial promise of NIBS techniques (particularly tDCS) has been marred by claims of non-reproducibility and interindividual variation within the field. For this reason, the careful selection of stimulation parameters and attempts to perform replications of findings is paramount.

Transcranial Magnetic Stimulation (TMS)

Transcranial Magnetic Stimulation (TMS) is a non-invasive brain stimulation technique which can be used as either a method of a physiological recording, for interference of normal neuronal firing, or for modulation of cortical excitability. TMS has the potential to be used alongside motor rehabilitation after stroke, and is FDA approved for the treatment of depression. However, within this thesis, TMS is used a as method of physiological recording using single and paired pulse protocols to assess aspects of cortical excitability and neurotransmitter systems.

Underlying Physiology

During TMS, neuronal stimulation is produced by the generation of an electrical current within a coiled wire (within the TMS coil) which produces a magnetic field. Pulses of electrical current induce a brief magnetic field directed through the scalp onto the surface of the brain. The magnetic field induces neuronal depolarisation in the region of cortex under the TMS coil. If the size of the stimulating current is high enough, the induced depolarisation will result in an action potential within the underlying neurons. TMS of the motor cortex is relatively easy to perform, as the primary motor cortex is represented on the cortical surface, and has relatively easy to record outcomes (the MEP, discussed below).

TMS can be performed with a variety of different coil types. The TMS reported within this thesis is all performed with a figure-of-eight coil, allowing for relatively focal stimulation. The targeted region of cortex with a figure-of-eight coil is smaller than with a circular coil, therefore allowing for more targeted and reliable stimulation (Fleming *et al.*, 2012).

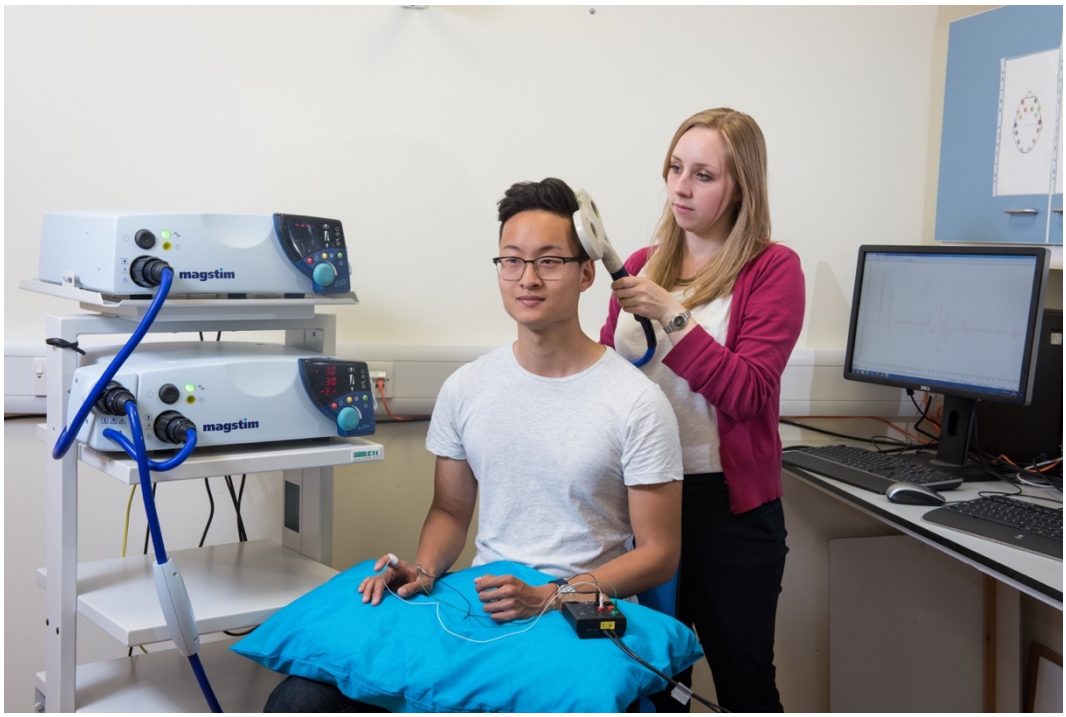

Figure 2.3: Transcranial Magnetic Stimulation Photo Credit to John Cairns

Motor Evoked Potential (MEP)

When a single pulse of TMS of sufficient intensity is delivered to the motor cortex, an action potential is induced in corticospinal neurons under the coil. These neurons make direct glutamatergic monosynaptic connections with spinal motor neurons, which propagate along peripheral motor axons. The resulting motor response can be recorded as the MEP, measured using surface electromyography (EMG). The size of an MEP (measured as the peak-to-peak amplitude of the EMG recording) is one of the main methods of assessment for TMS studies. While maintaining a consistent coil position, changes to the size of an MEP can be induced

by changes to stimulator output, frequency of repeated TMS stimulation and by different paired pulse TMS protocols. The MEP size can also be inadvertently altered by changes to coil position, angle and rotation. For this reason, changes to coil position should be avoided once the ideal motor hotspot location has been chosen.

Single-pulse TMS

Single-pulse TMS (spTMS) involves delivery of a single discharge of the stimulator over a target region of cortex. The target location and discharge intensity can be modulated in order to assess different components of cortical excitability. A single pulse of TMS of sufficient intensity discharged over the appropriate region (e.g. the hand area) of M1 will elicit an MEP in the contralateral side (e.g. the hand).

Resting Motor Threshold (rMT)

The lowest stimulator output intensity required to produce an MEP of at least 50 μV in at least five out of ten consecutive trials while the participant sits at rest is known as the resting motor threshold (rMT). It reflects a general measure of corticospinal tract excitability and is typically increased in the affected hemisphere following stroke (McDonnell and Stinear, 2017).

Active Motor Threshold (aMT)

The Active Motor Threshold (aMT) is defined as the lowest stimulator output intensity required to produce an MEP of at least 200 μV with a cortical silent period following the MEP, while the participant maintains an isometric contraction of the target muscle in at least five of ten consecutive trials. For paired-pulse protocols within Chapter Five of this thesis, 70% of the aMT was used as the conditioning stimulus (CS) intensity while targeting the first dorsal interosseous (FDI).

1 mV Motor Threshold (MT_{1mV})

The 1 mV Motor Threshold is defined as the lowest stimulator output required to produce an MEP measuring 1 mV (1000 μV) in five of ten consecutive trials while the participant sits at rest. For paired-pulse protocols within Chapter Five of this thesis, MT_{1mV} was used as the test stimulus (TS) intensity.

Paired-pulse TMS (ppTMS)

Paired-pulse TMS (ppTMS) is used to assess cortical excitation and inhibition within the cortex. ppTMS can be used to assess either inter-regional interactions through the use of two coils, or intra-regional interactions through a single coil. This thesis uses only intra-regional ppTMS.

Intra-regional ppTMS involves the application of two TMS pulses via the same coil, to the same cortical location (Kujirai *et al.*, 1993). The first pulse is a Conditioning Stimulus (CS) followed by a Test Stimulus (TS). The interval between the two pulses (the interstimulus interval (ISI)), as well as the size of the CS dictates whether the ppTMS protocol results in inhibition or facilitation of the resulting MEP (Figure 2.4). It is thought that intercortical connections via either inhibitory or excitatory interneurons onto pyramidal tract neurons mediate the ppTMS effect in an ISI-dependent manner (Kujirai *et al.*, 1993; Ziemann, Rothwell and Ridding, 1996). Due to this ISI-dependency, comparison of the peak-to-peak amplitude of a ppTMS evoked MEP in relation to that of an MEP resulting from a single (non-conditioned) TMS pulse allows for assessment of neurotransmitter systems within the target cortex. This thesis uses ppTMS protocols to assess inhibition using Short-Interval Cortical Inhibition (SICI), and excitation using Intracortical Facilitation (ICF).

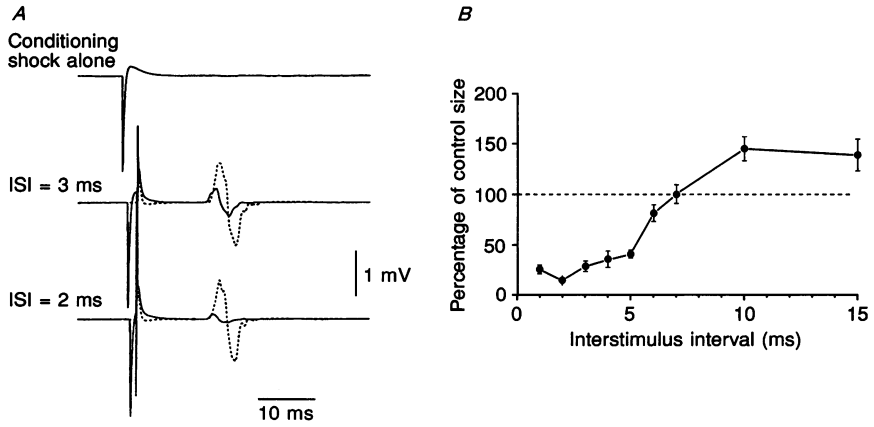

Figure 2.4: The EMG response to TMS (taken from Kujirau et al. (1993), Figure 1): **A:** EMG response to TMS in relaxed first dorsal interosseous (FDI) muscle are inhibited by a prior, subthreshold, stimulus. B: The mean (± 1 SEM) suppression or facilitation of MEP in FDI muscle with increasing ISI as a percentage of an unconditioned MEP from a single TMS pulse.

Short-Interval Intracortical Inhibition (SICI)

ppTMS with a short ISI, where the CS is set at approximately 70% of the aMT, results in reduction of the resulting MEP compared to a non-conditioned TS. This is known as Short-Interval Intracortical Inhibition (SICI). SICI can be used to target different sub-pools of the inhibitory neurotransmitter system by using an ISI ranging from 1 to 4 ms. Two distinct phases of SICI have been identified, with ISIs of 1 ms and between 2-4 ms (Fisher *et al.*, 2002; Roshan, Paradiso and Chen, 2003). SICI at 1 ms occurs at significantly lower conditioning stimulus intensity than SICI at 2.5 ms (Fisher *et al.*, 2002), and are differentially effected by voluntary contraction of the target muscle (greater effect at 2.5 ms) (Fisher *et al.*, 2002). Finally, the magnitude of inhibition at the two phases of SICI do not correlate (Roshan, Paradiso and Chen, 2003), indicating that distinct physiological processes underpin each ppTMS protocol.

SICI performed with an ISI of between 2-4 ms is dependent on $GABA_A$ receptor activity. Lorazepam and Diazepam (both $GABA_A$ receptor agonists), augmented the inhibitory effect of SICI supporting this as the pathway of action (Ziemann *et al.*, 1996; Di Lazzaro *et al.*, 2000; Ilić *et al.*, 2002). There is some suggestion that glutamatergic signalling may play a role in

reducing SICI effects, as application of an NMDA antagonist has been shown to enhance SICI (Ziemann *et al.*, 1998; Schwenkreis *et al.*, 1999). Furthermore, neuromodulators of dopamine, noradrenaline, serotonin have all been shown to influence SICI (for a full review of TMS and drug interactions, see Ziemann, 2004).

The neurophysiological mechanisms of 1 ms SICI are less well understood, with inhibition likely caused by refractive inhibition of the cortex following the conditioning pulse rather than synaptic GABAergic transmission (Ziemann *et al.*, 1996; Fisher *et al.*, 2002). SICI 1 ms has been largely unaffected by both neurotransmitter and neuromodulator pharmacological interventions in those studies which have differentiated between the SICI processes (Ziemann, 2004). SICI 1 ms is known to be GABAergic as it is suppressed to a similar extent as SICI 2.5 ms during the silent period following MEP induction, a period known to be caused by synaptic inhibition (Ni, Gunraj and Chen, 2007). Furthermore, SICI 1 ms is physiologically distinct from SICI 2-4 ms (Fisher *et al.*, 2002; Roshan, Paradiso and Chen, 2003) and not affected by $GABA_A$ agonists (Ziemann *et al.*, 1996). A relationship between SICI 1 ms and MRS-measured GABA has been observed by Stagg and colleagues (2011) , whereby higher GABA concentrations were associated with more inhibition (Figure 2.5), however this has not been consistently observed (Dyke *et al.*, 2017). It is therefore possible that SICI 1 ms and MRS-measured GABA are at least partial reflections of a the same pool of the inhibitory neurotransmitter, which is likely to be tonic extra-synaptic GABA (Stagg, Bachtiar and Johansen-Berg, 2011b; Stagg, Bestmann, *et al.*, 2011; Rae, 2014).

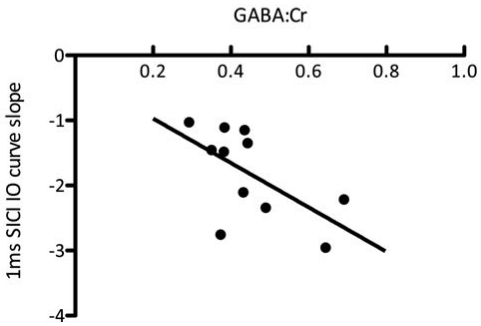

Figure 2.5: Relationship between MRS GABA and SICI 1 ms (taken from Stagg et al. (2011), Figure 3D): Significant relationship between MRS measured GABA in Left M1 and slope of the SICI 1ms recruitment curve ($r = -0.79$, $p = 0.006$). Lower numbers reflect greater cortical inhibition. Data points are individual subjects.

It should be noted that a clear inhibition of the TS induced MEP is only observed when the CS strength is around 60-80% of the TS. Higher intensity CS pulses will induce MEP facilitation, even at short ISIs (Ziemann *et al.*, 1996; Ilić *et al.*, 2002). ppTMS protocols with a suprathreshold CS and longer ISIs of between 50 and 200 ms will also induce MEP inhibition (so called Long-interval Intracortical Inhibition, LICI), however these protocols are thought to be reflective of a distinct inhibitory system from that which underpins SICI, which is mediated by the presynaptic metabotropic $GABA_B$ receptor (Sanger, Garg and Chen, 2001).

Intracortical Facilitation (ICF)

ppTMS with an ISI of 7-20 ms, where the CS is set to approximately 70% of the aMT results in an increase of the resulting MEP compared to a non-conditioned TS. The net facilitatory effects of the ICF ppTMS protocol is suggested to reflect a combination of both increase facilitation and weakening inhibition (Ziemann *et al.*, 1996). Pharmacological investigations have shown reduction in the ICF effect by both NMDA antagonists (Ziemann *et al.*, 1998) and $GABA_A$ agonists (Ziemann *et al.*, 1996).

Safety of TMS

TMS is considered to be a generally safe technique when performed within published guidelines (Rossi *et al.*, 2009). The most serious potential adverse effect of TMS is induction of an epileptic seizure. The few cases of TMS induced seizures have predominantly occurred following repetitive TMS (rTMS, not used for the work in this thesis) and have occurred in high-risk individuals, or when TMS has been performed outside safety limits. Based on published guidelines, participants for TMS experiments are screened for contraindications which may increase likelihood of a seizure. This includes personal or close family history of seizures or medications which lower the seizure threshold. Participants are also screened for factors which may increase likelihood of other adverse effects of TMS (including fainting/syncope, headaches, migraine or tinnitus). Participants are asked to avoid alcohol for the 24 hours preceding a TMS session, not to consume caffeine before the session and to get a good night's sleep the night before the session – in order to minimise chances of adverse effects of the stimulation. In general, spTMS and ppTMS are very well tolerated in healthy volunteer participants, particularly when performed on the primary motor cortex as the stimulator output required is relatively low. In stroke patients, TMS of the lesioned hemisphere often requires a significantly higher stimulation intensity due to corticospinal tract damage. Higher intensities of TMS can produce increased cutaneous sensations or trigeminal nerve stimulation and hence anecdotally can be less well tolerated. Furthermore, other brain regions (such as the cerebellum) have anecdotally been reported to be less well tolerated.

Transcranial Direct Current Stimulation

The use of electrical currents to influence human behaviour dates back to the Greco-Roman period when cures for gout, headache and pain were sought from electricity generated from electric fish. Since those early medical investigations, electrical stimulation has taken many

forms and been developed far beyond its *fishy* origins (sorry, not sorry…). During the Edwardian and Victorian period, usage of electrical stimulation machines grew in popularity as a treatment for multiple ailments, but also for the induction of euphoria or improvement of mental performance. It was during this period that the first physician-led suggestions of dosage and possible side effects were reported, including the burning and shock effects of higher (> 10 mA) currents, and potential adverse effects including headache, dizziness and nausea.

The development of 'modern' electrical stimulation came following invention of the battery in 1799, and this led to more systematic study of the effect of the electrical current on the human. Electroconvulsive Therapy (ECT) is probably the best example of the first widely used therapeutically administered electricity. The first real application of ECT was performed in 1938 on a patient with schizophrenia. Usage of ECT decreased following the development of more advanced neuroleptic medications, and following strong anti-ECT campaigns. Nevertheless, ECT is still regarded as a highly effective therapy in treatment resistant depression.

tDCS differs greatly from ECT, as it induces a subthreshold modulation effect on the cortex via delivery of a continuous current (rather than deliberate induction of seizures using an alternating current). The current used for tDCS is much lower (typically in the range of ~0.25-2 mA, compared to 800-900 mA used in modern ECT). tDCS also does not requiring patient sedation or provision of muscle relaxants, and the stimulation is very well tolerated in the majority of participants. Unlike other stimulation methods, tDCS induces weak, static fields which do not have the strength to induce action potentials.

tDCS exerts its effect on the cortex by increasing or decreasing the polarisation of neuronal membranes. Thus, it was initially suggested that the neuronal firing rate was modulated depending on current direction. The now classical studies performed by Michael Nitsche and Walter Paulus in the late 1990s and early 2000s found that prolonged electrical stimulation by tDCS lasting a few tens of minutes would induce long lasting, and polarity specific changes to cortical excitability as assessed by TMS (Nitsche and Paulus, 2000). The specific effects of tDCS can be affected by a complex interplay of electrode placement, electrode size, current intensity and duration of stimulation. Since these initial investigations, the usage of tDCS for neurophysiological modulation has exponentially increased, with potential applications stretching to practically every corner of medical and psychiatric research. The relative low cost and practical ease of tDCS application in a research setting, and the appeal of potential adjunct therapeutic use in a home setting, has made the combination of tDCS with existing behavioural interventions one of the most widely investigated rehabilitative procedures of our age. However it should be noted that as more research is performed with larger participant groups, and meta-analyses of existing trials are reported, findings are less conclusive than initially anticipated.

Practical Application of tDCS

The basic components of the tDCS setup are remarkably simple. A current is delivered from a battery operated direct current stimulator, with attached electrodes. Classically, two electrodes are used (an anode and a cathode), although recent developments have used multiple electrodes of one type surrounding a single electrode of the other type to control the application of current (Dmochowski *et al.*, 2011; Edwards *et al.*, 2013; Bortoletto *et al.*, 2016). Typical standard tDCS electrodes measure between 25 cm^2 and 35 cm^2, resulting in a relatively large region of cortex being targeted by the stimulation. Electrodes are attached to the scalp

of the subject, and conduction of current is provided by use of saline soaked sponges, or conductive gel/paste. The tDCS stimulator can be controlled to provide a consistent pre-determined current intensity (usually between 1 – 2 mA), for a set period of time (in the order of 10 – 20 minutes).

Most studies use either anatomical landmarks, or functional localisation with TMS or fMRI to determine electrode location. For motor cortex stimulation (as is used in this thesis), one electrode is conventionally placed over the primary motor cortex, via use of the 10-20 EEG system. The primary motor cortex is approximately located at C3 (left) or C4 (right), which is 5 cm lateral of Cz (the vertex).

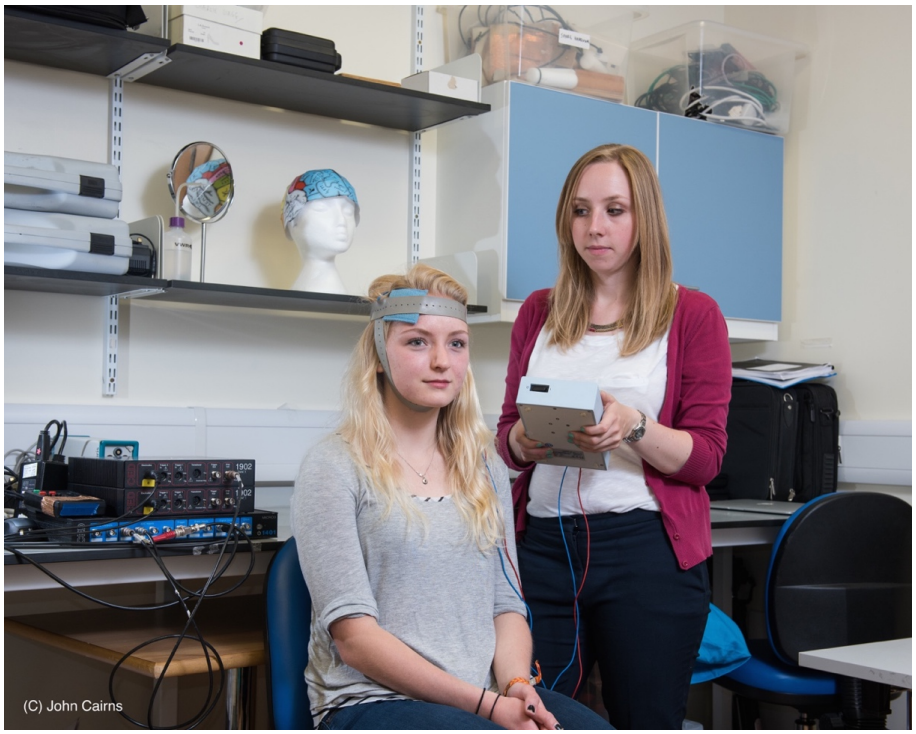

Figure 2.6: Transcranial Direct Current Stimulation to the motor cortex Photo credit to John Cairns

Combining tDCS and MRI

tDCS can be combined safely with MRI either in a sequential or concurrent approach. In a sequential approach, the stimulation is delivered outside of the scanner, with the participant

placed in the scanner before and/or after application of stimulation. Alternatively, stimulation can be safely delivered within the bore of the scanner during concurrent scanning (or during rest). Performing scanning and stimulation concurrently has obvious advantages, as it reduces the logistical and timing issues associated with moving a subject in and out of the MRI scanner. It also increases reproducibility of scanning, as setup of regions or voxels of interest can be maintained for the pre/during/post stimulation scanning. Concurrent tDCS with MRI scanning is used with Chapter Four of this thesis.

Integration of the tDCS kit to the MRI environment requires extra considerations, both in terms of safety considerations and potential adverse effects to scanning quality. Full details of these considerations can be found in a technical guide to tDCS by many world leading tDCS researchers (Woods *et al.*, 2016). Concurrent tDCS/MRI required specialised kit, which is tested within the MRI environment at the appropriate field strength. Electrodes should be fitted with high-ohmic resistors to prevent eddy-current induction. Care should also be taken with electrode leads, and associated extension leads to prevent RF burns, and eddy-current inducing loops. Electrode conductance within the MRI environment should also be considered, as saline soaked sponges will dry out given the longer duration of an MRI scan compared to standard tDCS protocols. Conductive EEG paste is a viable alternative to saline sponges when performing tDCS in the MR environment.

Safety and Ethical Considerations of tDCS

As with TMS, tDCS is considered to be a safe method of non-invasive neuronal modulation. Extensive safety guidelines have been published regarding the use of tDCS in healthy subject volunteers, as well as in patient groups (Bikson, Datta and Elwassif, 2009; Bikson *et al.*, 2016). The most commonly reported subject discomfort is a tingling sensation under the electrodes.

This occurs most commonly during the period in which stimulation is ramped up to its stable current intensity.

The attractiveness of tDCS for cortical modulation with possible therapeutic benefit has led to increased ethical considerations of its application, and accessibility. There have been concerns raised regarding the commercial availability of at-home electrical stimulation kits, particularly targeted at those hoping to take advantage of the gains associated with improved concentration, reaction time and alertness (Wurzman *et al.*, 2016).

Methods for Assessing Motor Learning

Serial Reaction Time Task (SRTT)

Sequence learning tasks are probably the commonest motor learning tasks in research, and involve a participant being instructed to press buttons in response to cues presented on a screen. Cues are presented in a repeated sequence which can vary in length (usually between 8 and 16 cues long) (Nissen and Bullemer, 1987). This task, the SRTT, can be performed explicitly or implicitly, depending on whether or not the participant is informed of the presence of a repeating sequence. The effect of explicit vs. implicit SRTT on performance is debated, with some suggestion that this can lead to modification of the skill-learning benefits and consolidation (Robertson, Pascual-Leone and Press, 2004). For all versions of the SRTT, learning is measured as improvement in performance, either through reduction in response time to the presented cue, or through improved accuracy, or both. Comparison of response time to cues presented in a random order vs. those cues presented in the learned sequence order can inform of sequence specificity of the behavioural improvement.

Button press sequence learning tasks such as the SRTT involve performance of non-novel discrete movements, and the skill improvement is demonstrated by ability to link movements together with increased speed and reduced errors. Such tasks can be performed easily in a repeated sessions study as the generalised skill is usually well known to the participants. The learnable aspect of the task (e.g. a sequence of button presses) can be changed between sessions to create a different task.

A SRTT task is used within Chapters Three, Four and Five of this thesis, when studying motor skill learning in young healthy adults. The SRTT is ideally designed for performance within the MRI scanner environment (as seen for Chapters Three and Four) as it has simple visual stimuli and results in minimal head movement which could affect image quality. For Chapters Four and Five it is also important to have a task which is repeatable across multiple testing sessions as participants would each take part in three sessions.

Force Tracker Task (FTT)

A Force Tracker Task (FTT) requires a participant to master a continuous modulation of force to track a target, rather than responding to discrete cues as they appear on the screen (Floyer-Lea and Matthews, 2005; Reis *et al.*, 2009). In general, performance of these tasks requires a participant to control movement of a cursor on a computer screen via the use of a control interface, such as a dynamometer (force-transducer). Alternative versions of this task have used a joystick, or similar gaming-controller to control cursor position (which therefore removes the "force" aspect of the FTT, but maintains the "tracking"). In a similar pattern to the SRTT task, both general and sequence-specific skill learning can be probed using the FTT with the use of predictable and unpredictable patterns of movement. It is also possible to probe task retention as a measure of learning.

Tracker tasks often require learning of both a novel movement dynamic and novel visuomotor mapping skill, differentiating them from button-press sequence tasks such as the SRTT. This makes these tasks more difficult to perform in repeated sessions, as the generalised skill learning of the novel movement dynamic will show greater session effects. Nevertheless, it would be possible to mitigate these effects to some extent by the use of baseline training blocks or a baseline training session to allow participants to reach a performance plateau.

Surface Electromyography (EMG) Controlled FTT

Within Chapter Six, a modified FTT task is designed and implemented for use in healthy controls (both young and old), and in chronic stroke survivors. This modified version uses direct recording of surface EMG from a muscle of choice to act as the driver of the task. Smoothed amplitude of the EMG activity is translated into the height of the cursor on the screen, such that increasing activation will move the cursor up the screen, whereas decreasing activation will drop it down the screen. It is has previously been shown that participants performing a similar task using either EMG (a pair of electrodes on the biceps and triceps muscles of the upper arm), a joystick, or force-transducer were equally able to perform the task across each interface (J Lobo-Prat *et al.*, 2014). A tracker style of task is chosen for this study as the novel movement dynamic required would be more representative of a "real-world" learning environment.

Chapter 3: Measuring Learning-Specific Changes in Motor Cortical GABA Using Ultra-High Field MRS

Abstract

Decreases in local inhibitory signalling are thought to be crucial for induction of motor cortical plasticity in humans, and this disinhibition has been linked to the inhibitory neurotransmitter GABA. One previous study has used Magnetic Resonance Spectroscopy (MRS) to measure GABA concentration in the sensorimotor cortex during motor learning, and demonstrated a significant decrease during learning. Here, ultra-high field MRS at 7 Tesla was used to observe a decrease in GABA concentration in the sensorimotor cortex during motor skill learning. No such change was observed for the excitatory neurotransmitter Glutamate. Furthermore, there was no change in GABA in participants who performed a non-learning version of the same task, or lay at rest, suggesting the observed change is learning-specific. Finally, sensorimotor GABA early in task performance was found to be correlated with the magnitude of behavioural improvement. Together, these results support a hypothesis for a reduction in cortical inhibition being a necessary step for the induction of motor cortical plasticity.

Chapter Acknowledgements

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Kolasinski J.*, **Hinson E. L.*,** Zand A. P. D., Rizov A., Emir U. E. and Stagg C. J. The dynamics of cortical GABA in human motor learning. *J. Physiol.* (2018). doi:10.1113/JP276626

*Co-first authors

The experiment described in this Chapter was conceptualised by James Kolasinski, Charlotte J. Stagg and myself. The MRS sequence was developed by Uzay Emir. Data acquisition was additionally supported by Amir Puyan Divanbeighi Zand and Assen Risov (both undergraduate FHS Students).

Introduction

There is a strong body of evidence suggesting a critical role for inhibition, specifically a reduction in inhibitory tone, in the induction of plasticity within M1. Animal studies have established that decreases in this extra-synaptic, tonic inhibition, also referred to as GABAergic tone, is necessary for LTP-like plasticity to take place in M1 (Castro-Alamancos, Donoghue and Connors, 1995; Trepel and Racine, 2000). It has also been suggested that a decrease in cortical inhibitory tone is central to the process of human motor learning (Bachtiar and Stagg, 2014).

In human studies, disinhibition of M1 has been found during and following motor learning using TMS (Rosenkranz *et al.*, 2007; Coxon, Peat and Byblow, 2014). It is known that the GABAergic system is responsive to exogenous modulation via tDCS (Stagg *et al.*, 2009; Stagg, Bachtiar and Johansen-Berg, 2011a), and that the magnitude of GABAergic response to tDCS is correlated with the degree of motor learning in the same participants (Stagg, Bachtiar and Johansen-Berg, 2011a). Over a longer learning period of a number of weeks, the size of GABAergic change has been correlated with the connectivity changes in the resting motor network such that GABA decreases were associated with greater increases in network strength (Sampaio-Baptista *et al.*, 2015).

One study has previously shown a reduction in Magnetic Resonance Spectroscopy (MRS) measured GABA during the period of motor learning (Floyer-Lea, 2006). Despite papers further investigating the relationship between GABA in the motor system, and human motor learning ability (Stagg, Bachtiar and Johansen-Berg, 2011a), there have been no subsequent MRS studies which have investigated GABA changes during motor skill learning. Here, a

successful replication and extension of Floyer-Lea and colleagues' previous findings is presented. By taking advantage of the improved spatial and temporal resolution of ultra-high field MRS at 7-Tesla, it was possible to investigate the dynamics of change in the GABAergic system during motor learning with a higher temporal resolution than has previously been reported. Based on previous findings, it was hypothesised that a reduction in GABA would be observed within the primary motor cortex that would specific to learning (Floyer-Lea, 2006), and that there would be a relationship between the change in GABA concentration which would be predictive of the degree of learning (Stagg, Bachtiar and Johansen-Berg, 2011a).

Methods

Participants

51 healthy participants gave written informed consent in accordance with local ethical approval (University of Oxford: MSD-IDREC-C1-2014-100). All participants were right handed (Oldfield, 1971) with no contraindication to MRI scanning at 7T. Participants were allocated to one of three scan types, with no participants recruited to more than one group (Figure 3.1A). A between-subjects design was used to prevent any generalised task-specific skill improvement from affecting behavioural and neurochemical measurements. No participants had taken part in a study using a SRTT with the same learning sequence before. Full participant demographics can be found in Table 3.2.

- **Learning**: Performance of repeating sequence SRTT during MRS acquisition.
- **Movement**: Performance of random-order SRTT during MRS acquisition.
- **Rest**: No task performed. Participants watched nature documentary.

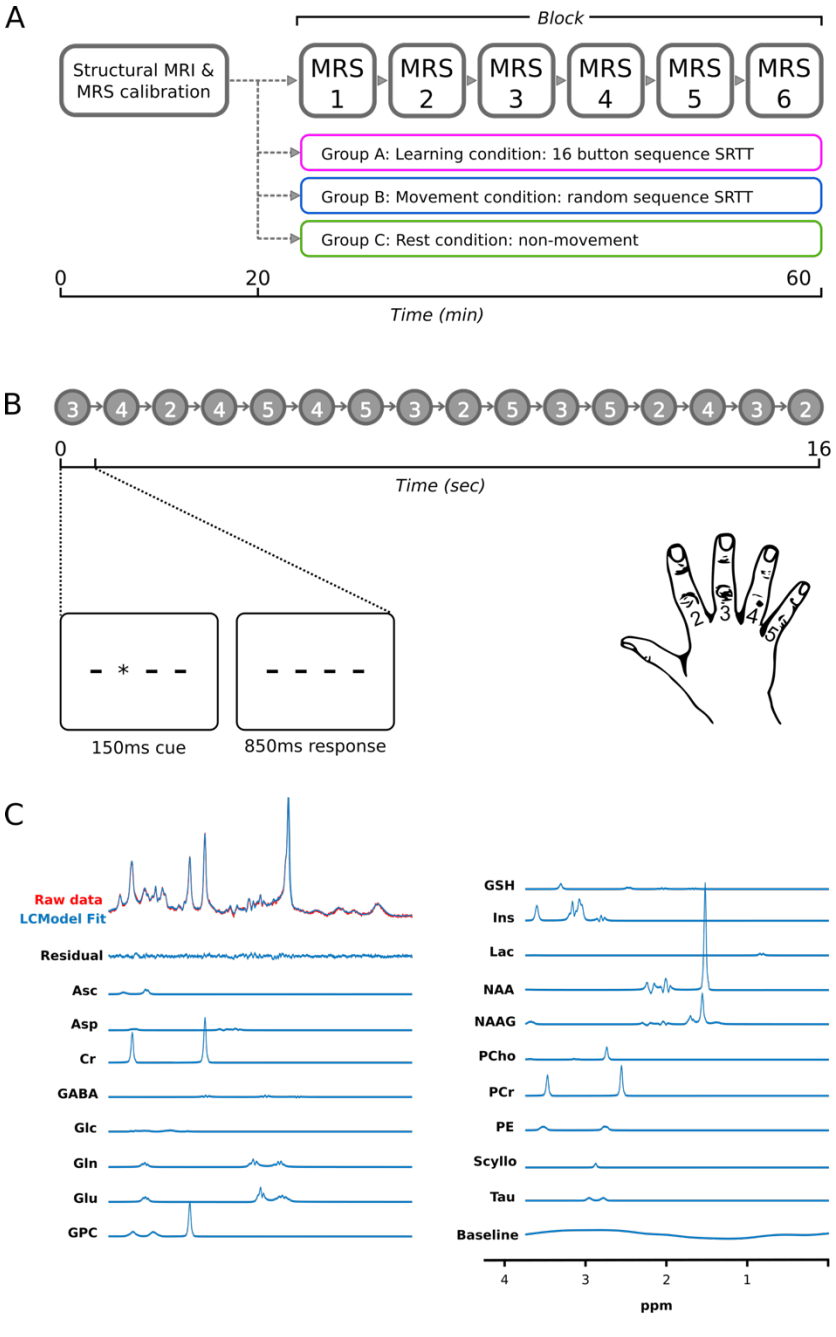

Figure 3.1: Study outline and MRS method summary:

A: MRS data were acquired in six independent blocks during a concurrent task which differed across the three experimental groups: the Learning group performed a 16-button press, repeating serial reaction time task; the Movement group performed a serial reaction time task without a repeating sequence; the Rest group passively observed a video.

B: The Learning group performed the same 16-button press sequence with three repeats per epoch (48 s), with each epoch separated by a 12 s rest period. MRS data were acquired in each block (64 averages) and analysed using LCModel (Provencher, 2001).

C: Representative acquisition from one participant in one M1 block, including model fit.

D: M1 MRS voxels were centred over the left (contralateral) hand knob illustrated in as heatmaps in the three experimental groups: colour bars represent the number of participants.

Table 3.2: Participant information:

	Learning	Movement	Rest
Completed MRI	18	19	14
Excluded (based on MRS CRLB outlier)	6	7	2
Excluded (based on Grubbs test of MRS measured GABA)	0	0	1
Excluded (based on Grubbs test of behavioural data)	1	0	0
Final Analysis	**11**	**12**	**11**
Age (years, mean ±SD)	24.2 ±3.7	24.6 ±4.1	24.2 ±4.8
Gender	7 female	6 female	5 female

Note: Participants were excluded if they had fewer than 6 complete MRS data points with satisfactory Cramér-Rao lower bound (CRLB) of <50%, if their GABA values were statistical outliers.

MR Data

MR Data Acquisition

MRI and MRS data were acquired using a 7-Tesla Siemens Magnetom System (Siemens, Erlangen, Germany) with a 32-channel head coil (Nova Medical Inc., Wilmington, MA, USA). A dielectric pad (barium titanate: 0.5 x 11 x 11 cm) was positioned dorsally on the scalp over the left central sulcus to increase B1 efficiency in the M1 VOI (Lemke *et al.*, 2015). B1 efficiency was imaged using actual flip angle imaging (AFI): field of view = 240 x 240, TRI = 6 ms, TR2 = 30 ms, TE = 2.58 ms, non-selective flip angle = 60°, slice thickness = 2.5 mm. B1 efficiency was assessed during scanning using the AFI scan, and the dielectric pad was moved if required.

Structural MRI scanning was performed to enable MRS VOI placement, and analysis. Structural MRI data were acquired using a Magnetization Prepared Rapid Acquisition Gradient Echo (MP-RAGE) sequence: TR = 2200 ms, TE = 2.82 ms, slice thickness = 1.0 mm, in-plane resolution = 1.0 x 1.0 mm, GRAPPA factor = 2.

MRS data were acquired using a semi-LASER sequence (Localisation by Adiabatic SElective Refocusing) (van de Bank *et al.*, 2015): TR = 5000 ms, TE = 36 ms, Voxel size = 20 x 20 x 20 mm, 64 averages per block, Total Acquisition time = 5 minutes 20 seconds. VAPOR (VAriable Power RF pulses with Optimized Relaxation delays) water suppression was performed (Tkáč *et al.*, 1999). The VOI was manually placed in the left hemisphere, using the anatomical hand knob as a guide (Yousry *et al.*, 1997) (Figure 3.1D), avoiding the dura and ventricles. MRS data were acquired in six serial blocks, of approximately 5 minutes each, from the same VOI location. Both the Learning and Movement groups performed a SRTT during MRS acquisition. The Rest group watched a video, and indicated continued alertness between MRS blocks using a single verbally-cued button press.

MRS Data Analysis

MRS data analysis was performed in order to calculate an independent measure of neurochemical concentration for each of the six MRS blocks. For all blocks, neurochemical concentration is expressed as a ratio of total creatine and corrected for brain tissue proportions within the VOI.

An unsuppressed water signal from the same VOI was collected following the acquisition of the six MRS blocks and used to correct the neurochemical data for the six MRS block separately. Data then underwent eddy current correction and zero-order phasing of array coil

spectra using in-house scripts. Residual water signal was removed using Hankel-Lanczos singular value decomposition (Cabanes *et al.*, 2001). LCModel was used to quantify concentration of neurotransmitters within the chemical shift range of 0.5 – 4.2 ppm ((Provencher, 2001). Exclusion criteria for data quality metrics were as follows: Cramér-Rao Lower Bounds (CRLB) > 50 %, Signal-to-Noise (SNR) < 30, water linewidths at full width and half maximum (FWHM) > 15 Hz. Data quality metrics were recorded for each block to assess change over the total time course of MRS acquisition.

The proportion of GM, WM and CSF in the VOI of the participant's MPRAGE scan were calculated using FAST (Zhang, Brady and Smith, 2001). Neurotransmitter concentrations were corrected for the proportion of GM, WM and/or CSF in the VOI. GABA and Glutamate were corrected for the proportion of GM, as the majority of these neurotransmitters are found within GM. Total Creatine (tCr), including creatine (Cr) and phosphocreatine (PCr) was corrected for the total volume of brain tissue (GM and WM) in the VOI. Thus neurotransmitters are reported as GABA:tCr and Glutamate:tCr.

Behavioural Task

SRTT Stimuli

The Learning and Movement groups both performed the SRTT. Visual cues were presented for 150 ms with an 850 ms ISI (Figure 3.1B). Each task block consisted of 48 cues with a 12 second rest period between blocks. Participants in the Learning group were explicitly informed to expect a repeating sequence, which they should try to learn, but not told the length of the sequence (16-item sequence repeated three times per task block). In the Movement condition, participants were explicitly told not to expect a sequence, and that they

should just try to respond as quickly and accurately when cues appeared. In the Movement condition, the three repeats of the 16-item sequence were randomised to produce a different 48 order of cues for each task block. For the Rest group, participants lay at rest and indicated alertness using a verballed cued single button press using the button box between MRS blocks. A nature documentary (with no subtitles or sound) was displayed to maximise chance of continued alertness.

SRTT Analysis

In the Learning and Movement groups, SRTT behavioural data was analysed to quantify Response Time (RT). RT was calculated as the time in milliseconds from visual cue onset to a correct button press. Trials with anticipatory responses (where a correct response was given prior to the visual cue) and incorrect first button presses, even if the correct button was subsequently pressed, were excluded. RT data were divided into six blocks corresponding to the length of the six MRS blocks. Median RT was calculated for each block. A motor learning metric was calculated as the percentage change from median RT in block 1 to an average RT across blocks 4 to 6. In addition, a measure of "peak performance" was calculated as the percentage change from block 1 to the lowest median RT. Task accuracy was measured by the number of correct button presses per block.

Motor Learning Metric

$$= \frac{RT(4-6) - RT(1)}{RT(1)} \times 100\%$$

Peak Performance Metric

$$= \frac{RT(best) - RT(1)}{RT(1)} \times 100\%$$

Results

Participants in the learning group were able to improve performance on the SRTT

In order to assess change in performance on the Serial Reaction Time Task, a two-way mixed ANOVA was performed, with factors of Group (Learning, Movement) and Block (1-6) (Figure 3.3). A significant interaction of Group*Block was observed: $F_{(1.49,\ 31.24)} = 10.52$, $p = 0.001$, partial $\eta^2 = 0.334$ (Greenhouse Geisser corrected). Furthermore, for the Learning group, there was a significant main effect of Block on RT: $F_{(1.41,\ 14.0)} = 9.33$, $p = 0.005$, partial $\eta^2 = 0.483$. *Post hoc* paired-samples *t* tests revealed significant reduction in RT for Blocks 4 and 6, when compared to Block 1 ($p < 0.05$, Bonferroni adjusted). For the Movement group, there was no significant main effect of Block on RT: $F_{(5,\ 55)} = 0.89$, $p = 0.496$, partial $\eta^2 = 0.075$.

There was no difference in task accuracy between the Learning and Movement groups at the start of task performance (independent samples *t*-test $t_{(21)} = -0.670$, $p = 0.510$) , nor any significant change in task accuracy during the course of task performance (two-way mixed ANOVA with factors of Group (Learning, Movement) and Block (1 – 6), no interaction of Group*Block ($F_{(1.12,\ 23.73)} = 3.22$, $p = 0.081$, partial $\eta^2 = 0.133$), nor main effect of Block ($F_{(1.13,\ 23.73)} = 3.85$, $p = 0.060$, partial $\eta^2 = 0.155$). This suggests that improvement in RT (demonstrated as a decrease in time) in the learning was not associated with a decrease in accuracy of performance.

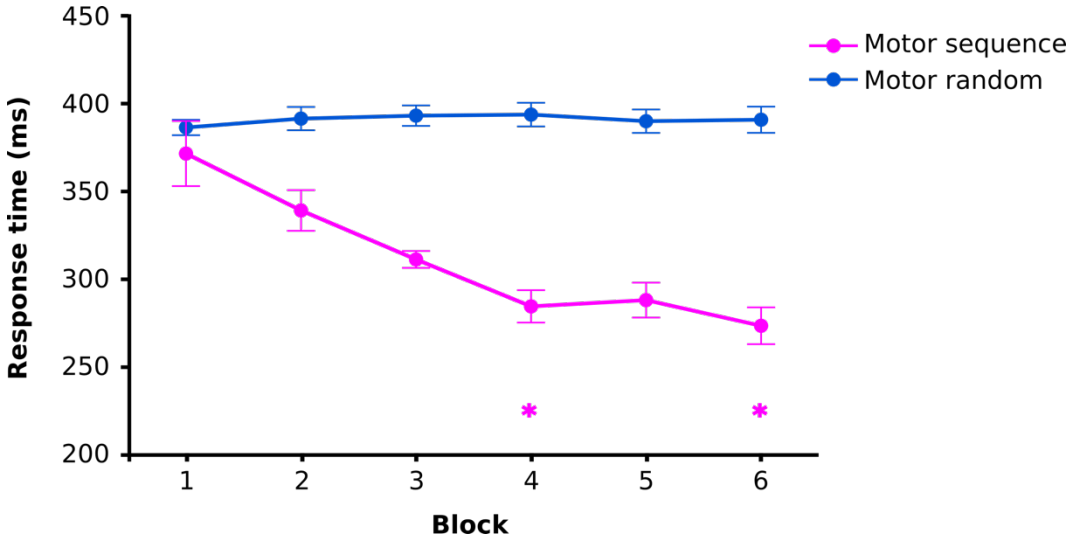

Figure 3.3: Learning of motor sequence serial reaction time task: Group mean response time data showing a decrease in response times in the Learning SRTT as the participants learned the four-button 16-press sequence (magenta). No equivalent learning was observed in the Movement group SRTT of equivalent duration, which contained no repeating sequence (blue). Two-way mixed ANOVA: experimental group (Learning or Movement) as between-subjects factor and block (blocks 1–6) as within-subjects factor: $F_{(1.49, 31.24)} = 10.52$, $p = 0.001$, partial $\eta^2 = 0.334$ (Greenhouse–Geisser corrected). This effect was driven by a reduction in response time in the Learning group (main effect of block: $F_{(1.4, 14.0)} = 9.33$, $p = 0.005$, partial $\eta^2 = 0.483$, Greenhouse–Geisser corrected).

* $p < 0.05$ Bonferroni-adjusted post hoc pairwise comparison compared with block 1. Within-subject standard error bars calculated across each group (Cousineau, 2005).

Motor sequence learning is associated with reduction in M1 GABA concentration

The results of the MRS analysis is presented in Figure 3.4. There was a significant difference in the changes in GABA between the groups: a two-way mixed ANOVA on GABA:tCr data revealed a significant interaction between Group (Learning, Movement, Rest) and Block (1-6): $F_{(10, 155)} = 2.03$, $p = 0.034$, partial $\eta^2 = 0.116$. Investigation of simple main effects revealed a main effect of Block for the Learning group: $F_{(5, 50)} = 4.16$, $p = 0.003$, partial $\eta^2 = 0.294$. *Post hoc* paired samples *t* tests revealed a significant reduction in GABA:tCr in block 6 compared to block 1 ($p = 0.011$, multiple comparisons adjusted). There was no significant main effect

of Block for the Movement group ($F_{(5, 55)}$ = 1.16, p = 0.339, partial η^2 = 0.0.096, nor for the Rest group ($F_{(5, 50)}$ = 0.226, p = 0.0.949, partial η^2 = 0.022.

An identical analysis was performed on the Glu:tCr MRS data and no significant interaction of Group (Learning, Movement, Rest) and Block (1-6): $F_{(10, 155)}$ = 0.780, p = 0.648, partial η^2 = 0.048 was demonstrated.

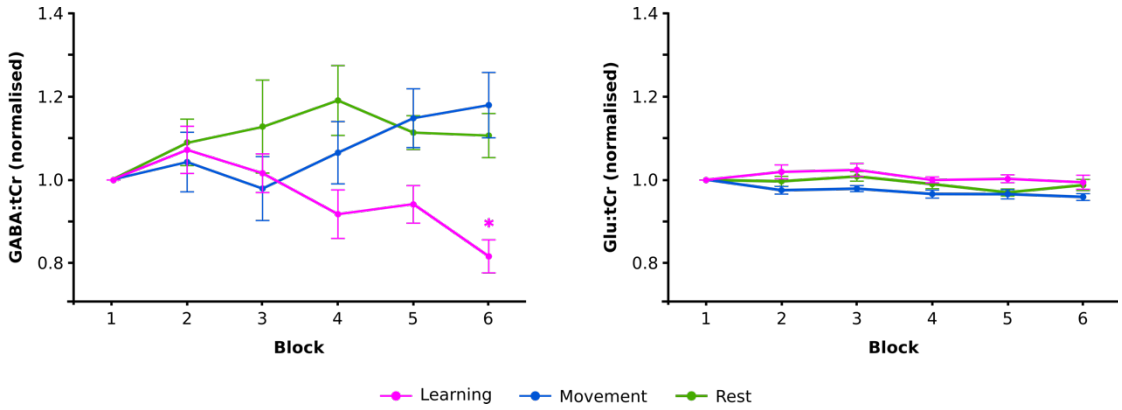

Figure 3.4: Motor learning is associated with a reduction in motor cortex GABA concentrations: Motor learning is associated with a reduction in motor cortex GABA concentrations: Group mean GABA:tCr (left) and Glu:tCr (right) concentrations presented normalised to block 1 for six serial MRS acquisitions measured during task performance. During motor sequence learning, a reduction in the concentration of motor cortex GABA:tCr is observed (pink) that is not seen in a motor task of equivalent duration lacking a learnable sequence (blue), nor during a passive resting task of the same duration (green). Two-way mixed ANOVA with one factor of experimental group (Learning, Movement, or Rest) and one factor of block (1–6): $F_{(10, 155)}$ = 2.03, p = 0.034, partial η^2 = 0.116. This effect was driven by a drop in GABA:tCr concentration in the Learning group (simple main effect of block: $F_{(5, 50)}$ = 4.16, p = 0.003, partial η^2 = 0.294). Equivalent measures of glutamate showed no evidence of a change specific to the Learning group: a two-way mixed ANOVA revealed no significant interaction between experimental group (Learning, Movement or Rest) and time on Glu:tCr: $F_{(10, 155)}$ = 0.780, p = 0.648, partial η^2 = 0.048.

* p < 0.05 Bonferroni-adjusted post hoc pairwise comparison compared with block 1. Mixed ANOVAs were conducted using raw non-normalised data; for visualisation purposes only, data were normalised to block 1. Within-subject standard error bars were calculated across each group (Cousineau, 2005).

To assess if observed GABA:tCr changes were driven by longitudinal changes in data quality which could be associated with serially collected MRS from the same VOI, two-way mixed ANOVAs were run on the SNR, CRLB and FWHM from the LCModel output (Figure 3.5). No significant interactions were found between Group (Learning, Movement, Rest) and

Block (1-6) for SNR ($F_{(10, 155)}$ = 0.725, p = 0.658, partial η^2 = 0.045), CRLB ($F_{(10, 155)}$ = 1.252, p = 0.273, partial η^2 = 0.075) or FWHM ($F_{(10, 155)}$ = 0.800, p = 0.602, partial η^2 = 0.049).

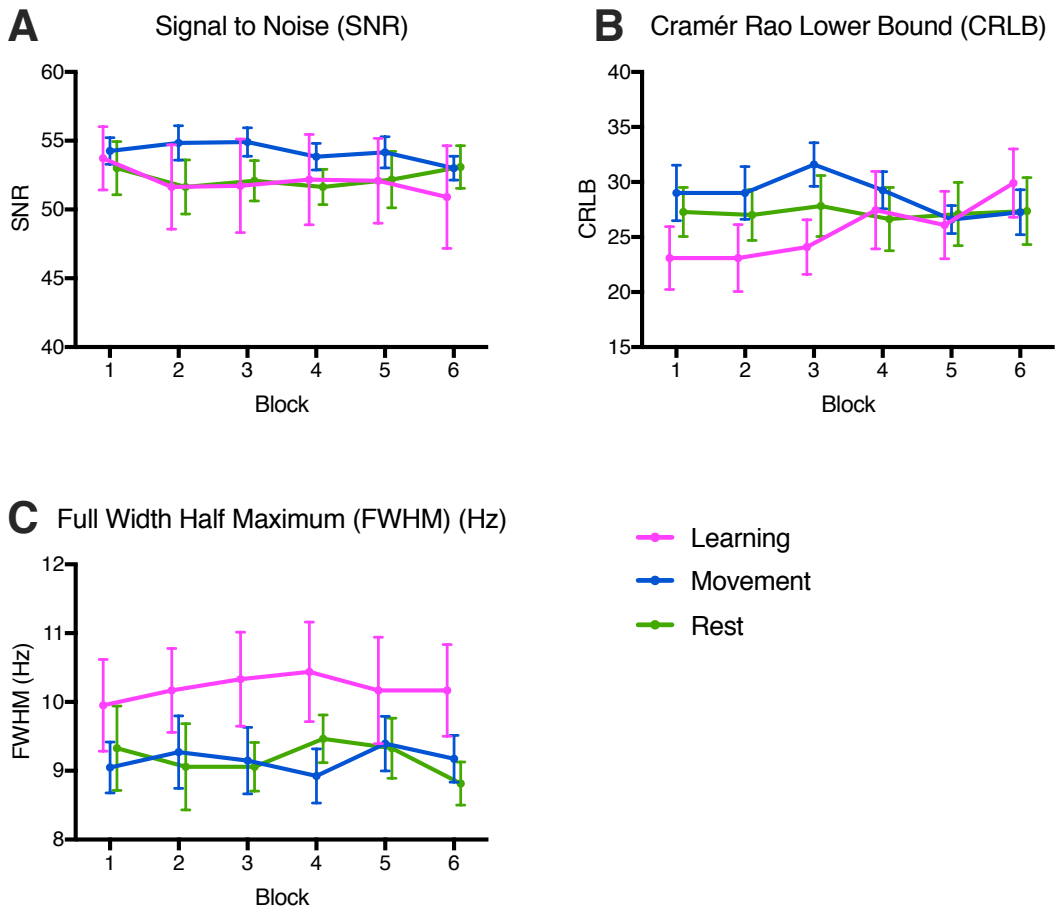

Figure 3.5: Data quality metrics for MRS data: Assessment of data quality metrics for MRS data across period of task performance (Learning/Movement) or Rest. Two-way mixed ANOVAs with one factor of experimental group (Learning, Movement, or Rest) and one factor of block (1–6) were performed, with no significant interactions observed for Signal to Noise (SNR) ($F_{(10, 155)}$ = 0.725, p = 0.658 , partial η^2 = 0.045), Cramér Rao Lower Bound (CRLB) ($F_{(10, 155)}$ = 1.252, p = 0.273, partial η^2 = 0.075) or Full Width Half Maximum (FWHM) ($F_{(10, 155)}$ = 0.800, p = 0.602 , partial η^2 = 0.049).

Early M1 GABA:tCr concentration is predictive of subsequent learning performance

Despite previous null findings based on resting MRS measured GABA prior to task performance, it was investigated if GABA:tCr concentration during the early stage of task performance was predictive of subsequent learning (Stagg, Bachtiar and Johansen-Berg,

2011a). This analysis was performed on the 11 subjects previously analysed in the Learning group, plus an additional four who had GABA:tCr CRLB values < 50% for Block 1 (Correlation analysis n = 15). These participants had been excluded from previous analysis as one or more of their six collected MRS Blocks had a CRLB value greater than the cut-off of 50%.

There was a significant correlation between Block 1 GABA:tCr and the percentage change in RT associated with learning: $r(15) = 0.515$, $p = 0.049$ (Figure 3.6A). This reflected that subjects with lower levels of GABA:tCr in Block 1 of the task were those who showed greater subsequent learning. The same relationship was tested for Glutamate, with no significant relationship observed: $r(15) = -0.180$, $p = 0.520$). Furthermore, the correlation for GABA:tCr with learning was significantly stronger than the Glu:tCr relationship (Hittner's $Z = 2.08$, $p = 0.038$). These correlations persisted with GABA:tCr values uncorrected for grey matter percentage, with the percentage included as a covariate in a partial correlation ($r(15) = 0.571$, $p = 0.033$). RT in block 1 was not itself a predictor of subsequent performance ($r(11) = 0.482$), $p = 0.07$), however the trend suggested that those with slower RT during block 1 may go on to learn less. There was no correlation between magnitude of GABA:tCr change and magnitude of learning ($r(11) = 0.056$, $p = 0.88$).

For the secondary measure of learning, a peak performance measure observing the best block performance of each subject between blocks 2 and 6 was calculated as a percentage change from RT in block 1. There was a correlation between GABA:tCr in Block 1, and peak performance, such that lower GABA:tCr reflected greater reduction in RT at peak performance: $r(15) = 0.613$, $p = 0.015$) (Figure 3.6B). The same relationship was not observed between Glu:tCr and peak performance: $r(15) = -0.172$, $p = 0.540$.

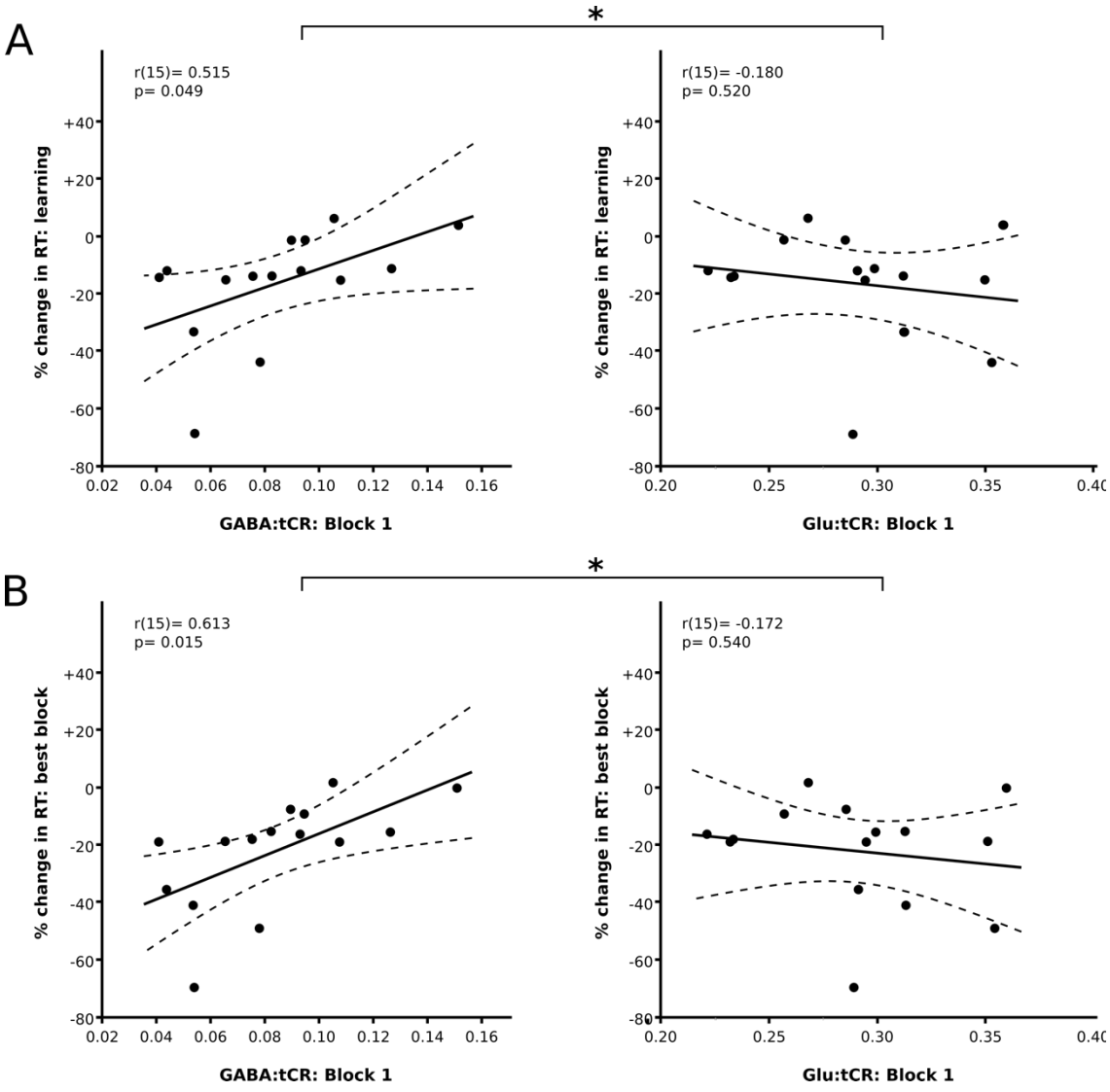

Figure 3.6: Early concentrations of GABA:tCr are correlated with the magnitude of subsequent motor learning:

A: A positive correlation was observed between the concentration of GABA:tCr in and RT reduction due to learning, defined as the percentage change from RT in block 1 to the median RT in sequence blocks 4–6.

B: Early concentrations of GABA:tCr are strongly correlated with the percentage change in reaction time in the best block, defined as the block (from blocks 2–6) in which the median reaction time was lowest. The same pattern was not observed with equivalent concentrations of excitatory glutamate (Glu:tCr).

* The correlation of GABA:tCr with learning and task performance differed significantly from equivalent correlations between Glu:tCr and the same behavioural measures: Hittner's Z ($p < 0.05$).

Discussion

This study provides evidence for a reduction in GABA within the sensorimotor cortex that is specific to a group of participants performing an explicit motor sequence learning task. A decrease in GABA was not seen in two control conditions, one where participants performed similar movements but without the sequence learning component, and a second where participants lay at rest. Furthermore, this finding was specific to the inhibitory neurotransmitter, GABA, and was not observed for the excitatory neurotransmitter, Glutamate. In addition, the concentration of GABA in the sensorimotor cortex measured very early in the learning process was predictive of learning performance. Together these results strongly implicate GABA, and sensorimotor inhibition in the learning process, and specifically sensorimotor disinhibition (or the reduction in inhibition).

The findings of this study are in line with a previous study, demonstrating a learning specific reduction in GABA during a force tracking learning task, but not during a non-learning equivalent task (Floyer-Lea, 2006). A recent study has reported a decrease in motor cortical GABA during a non-learning motor task (Chen *et al.*, 2017), in which participants performed a visually-cued bi-manual fist-clenching task. It is difficult to interpret the results of this recent study, as it is logical to suggest that bi-manual tasks will exhibit a different balance of cortical activity, due to the combination of bilateral activity and consequential interhemispheric signalling (Koeneke *et al.*, 2004). Furthermore, a fist-clenching task, even when performed unimanually, will very likely exhibit a different pattern of activation across the motor and cognitive cortical networks to a highly dexterous, motor skill learning task such as the SRTT. SRTT tasks have been used to study a broad range of cognitive and behavioural processes

and therefore is far more complex than a less complex task (such as fist-clenching) (Robertson, 2007).

It has been argued that the GABA MRS signal most closely reflects extracellular GABA concentration which is linked to maintenance of tonic inhibition within the cortex (Stagg *et al.*, 2011). A number of studies have failed to find a relationship between MRS GABA and well-established TMS measures of phasic GABAergic synaptic transmission (namely SICI with an interval of 2.5 or 3 ms) (Stagg *et al.*, 2011; Tremblay *et al.*, 2013; Dyke *et al.*, 2017). The GABA in this phasic signalling pool is bound to macromolecules and contained within vesicles, making it less visible to MRS (Stagg *et al.*, 2011; Stagg, Bachtiar and Johansen-Berg, 2011b). By contrast, some evidence exists suggesting a relationship between MRS GABA and tonic GABAergic tone (as measured with SICI 1 ms) (Stagg *et al.*, 2011). However, subsequent attempts to replicate this finding have been unsuccessful, making it difficult to conclusively state the origin of the MRS GABA signal (Dyke *et al.*, 2017).

The two main pools of GABA within the neuron are cytoplastic GABA (primarily used within metabolism) and vesicular GABA (used for neurotransmission). In addition, GABA within the extracellular space is thought to exert a neuromodulatory role, acting on presynaptic GABA-A receptors, lowering the probability of action potential generation (Farrant and Nusser, 2005). Although it is not possible to directly measure GABA pools using MRS, reduction in tonic GABA concentration observed here during learning is likely to result from a reduced phasic signalling, and therefore a reduced overspill. This will have a resultant effect of disinhibition on neighbouring neuronal membrane refractory periods, and thus alter the activity of those neurons. This theory is in line with mouse studies which have observed a reduction in axonal boutons on inhibitory interneurons following induction of motor training

(Chen *et al.*, 2015) and rat studies observing increased unmasking of latent connections which could facilitate plastic change (Huntley, 1997).

This study assessed two different metrics of performance on the SRTT. In a cross-sectional correlative analysis, early MRS GABA was found to be predictive of both overall learning performance (percentage change in RT from start to plateau performance) and 'peak' performance (quickest block median RT observed). The latter measure allowed for observation of inter-participant differences, as it was observed that participants reached their peak performance at different times within the task performance period.

No relationship was observed between the magnitude of GABA change across task performance, and learning. Based on previous findings from our group (Stagg, Bachtiar and Johansen-Berg, 2011a), it might have predicted that this would have been observed, however it is sensible to be cautious of drawing firm conclusions from this null finding. It is most likely that disinhibition of the motor system via reduction in GABA is not the sole physiological driver of learning, even though no significant relationships were observed here for other neurotransmitters. In addition, we cannot rule out that the lack of this observation is caused by a limitation of our MRS design: the first MRS acquisition reported here is an average neurotransmitter signal from the first part of task performance (just under 5 minutes of task performance), rather than being a 'true' baseline MRS measure recorded at rest. Based on findings reported in previous literature, those participants with the most responsive GABAergic systems are likely to be those who demonstrate the most learning on the SRTT (Stagg, Bachtiar and Johansen-Berg, 2011a). By this logic, these same participants could experience a physiologically significant reduction in GABA early during the learning process, which would occur during the first recorded MRS block. By not able to directly test the change

in neurotransmitter concentration from a resting baseline, we are unable to test this hypothesis directly here.

No change was observed in the Glutamate concentration for any of the three conditions, and nor were any significant relationships observed between individual subject Glutamate concentrations, and behavioural performance. It is possible that due to the broad range of processes in which Glutamate plays a key role within the cortex, a subtle change specifically associated with motor skill learning was masked within the relatively large volume of the VOI. Furthermore, MRS is not sensitive to structural changes at the synapse such as neurotransmitter receptor density which will have an influence on learning related signalling. The MRS sequence used here used a short echo time, however recent evidence has suggested that short TE MRS can have a reduced sensitivity to changes in Glutamate signalling (Mullins, 2018).

Conclusions

The work presented here provides an important replication and extension of a previous finding published by Floyer Lea and colleagues, demonstrating a learning specific disinhibition during performance of a dexterous, explicit motor skill learning task (Floyer-Lea, 2006). A reduction in the inhibitory neurotransmitter, GABA, was observed only in the group of participants performing a learning task, and not in a group performing an identical task lacking the learning component, nor in a group who lay at rest for the duration of MRS acquisition. Furthermore, it was possible to demonstrate that a cross-sectional relationship exists between sensorimotor GABA concentration early in learning and the magnitude of behavioural improvement later in task performance. Previous work has predominantly used MRS to

provide a 'static' snapshot of the neurochemical environment within a region of interest, and relate this to behavioural measures. Here, we have demonstrated the ability to record dynamic changes (although still with a limited temporal resolution) within the same region of interest over a period of task performance, while harnessing the benefits of performing MRS at ultra-high field. These results, when taken together, support suggestions that a dynamic disinhibition of the motor system is an important step within the neuroplastic process underpinning skill acquisition.

Chapter 4: Using NIBS and fMRI to study the role of ipsilateral M1 in human motor learning

Abstract

The role of the contralesional motor cortex in stroke recovery is highly debated, but difficult to study due to heterogeneity of the clinical population. "Virtual lesions" created in healthy subjects using transcranial magnetic stimulation (TMS) are sub-optimal in the context of motor learning tasks involving use of the hand due to production of supra-threshold motor evoked potentials (MEPs). Here, the potential of cathodal transcranial Direct Current Stimulation (ctDCS) is studied in the context of inducing a down regulation of primary motor cortex as a "virtual lesion" method to test the hypothesis that compensatory activity may occur in the contralateral M1. Cathodal tDCS was performed before and during motor task performance concurrent with functional MRI (fMRI). Each subject participated in three experimental sessions. Data were analysed using Linear Mixed Modelling. Cathodal tDCS delivered prior to learning of the motor task resulted in an increase in learning-related fMRI activity in the contralateral M1.

Chapter Acknowledgements

The work presented in this chapter was collected prior to commencement of my DPhil. The original data collection was performed by Onn Shaun Thein (with supervision from Charlotte Stagg) in 2010/2011. His contribution to this chapter in the form of data collection is therefore acknowledged.

Introduction

A number of neuroimaging studies have demonstrated an increase in activity within the ipsilateral M1 during movements of the affected upper limb after stroke (Chollet *et al.*, 1991; Cramer *et al.*, 2001; Johansen-Berg *et al.*, 2002). It has also been demonstrated that this activation is related to the initial residual motor function after stroke, where those stroke survivors with the poorest motor function have the greatest contralesional M1 activity (Ward *et al.*, 2003) and also to the degree of recovery, such that those stroke survivors who have the best recovery over time are those who show correlated decreases in contralesional M1 activity (Ward *et al.*, 2003). Furthermore, disruption of the contralesional hemisphere using transcranial magnetic stimulation (TMS) impairs function of the affected hand in stroke patients (Johansen-Berg *et al.*, 2002; Lotze *et al.*, 2006).

There is, however, conflicting evidence as to whether this increased activity is beneficial or detrimental to the recovery of function. The commonly cited model of interhemispheric imbalance suggests that following brain injury from a stroke, the normal balance of the two hemispheres is disrupted and the level of interhemispheric inhibition from the contralesional cortex is greater than the excitation from the ipsilesional cortex, resulting in impairment of the stroke-affected hand (Murase *et al.*, 2004; Nowak *et al.*, 2009). Conversely, there is evidence that the contralesional M1 acts to compensate for reduced ipsilesional activity by contributing to motor function, rather than impairing it, particularly in poorly recovered patients (Johansen-Berg *et al.*, 2002; Jaillard *et al.*, 2005; Gerloff *et al.*, 2006).

Studying the role of the contralesional M1 after stroke is inherently problematic due to the heterogeneity of the clinical population. Lesion volume and location can differ greatly between individuals. It is also evident that the effects of the lesion are not limited to brain

regions spatially within the lesion as evidenced by cortical reorganisation following the stroke (Grefkes and Ward, 2014). By developing an experimental model in a healthy population, it would become possible to study the role of the contralesional cortex in the presence of a more homogenous disruption of activity. To this end, NIBS techniques have been suggested as a "virtual lesion" method to temporarily and specifically disrupt activity within the stimulated M1 (Pascual-Leone, Bartres-Faz and Keenan, 1999; Pascual-Leone, Walsh and Rothwell, 2000). Within the literature, "virtual lesions" are predominantly performed using either low-frequency inhibitory rTMS, or single carefully timed single TMS pulses to disrupt a specific process (for reviews see: Pascual-Leone, Bartres-Faz and Keenan, 1999; Pascual-Leone, Walsh and Rothwell, 2000). Single pulse TMS virtual lesions have a clear use for identifying precisely when a particular neural activity contributes to behaviour while rTMS act to down-regulate a region of cortex for a period of time. rTMS virtual lesions hold a number of advantages over true lesion studies in patient cohorts, for example they can be performed in homogenous (healthy) populations, across repeated sessions and allow for precise cortical targeting (Pascual-Leone, Bartres-Faz and Keenan, 1999). Use of cathodal tDCS for a similar method is much less used, very probably due to the inconsistent findings of its effects (Ammann, Spampinato and Márquez-Ruiz, 2016) and relative non-specificity of its stimulation area. However, for studies of the motor cortex, rTMS virtual lesions are difficult to administer, resulting in supra-threshold MEPs which could disrupt movement, as well as audible 'clicks' which might act to otherwise distract the participant (Bolognini and Ro, 2010).

As discussed in Chapter One, tDCS involves passing a low current (in the range of 1-2 mA) via two large electrodes placed on the scalp. tDCS has been demonstrated to have polarity-specific effects on cortical excitability that outlast the stimulation period for tens of minutes (Nitsche and Paulus, 2000, 2001), and specifically, cathodal tDCS (ctDCS) has previously been shown to slow learning of an explicit SRTT (Stagg, Jayaram, *et al.*, 2011) although there have

been a number of studies which have failed to find this effect with other motor learning tasks (Michael A Nitsche *et al.*, 2003; Reis *et al.*, 2009). Taken together, this suggests that tDCS could be a potentially useful technique to investigate the role of ipsilateral M1 during (or following) down-regulation of contralateral M1.

Here, ctDCS and fMRI were combined in healthy adults (as has previously been successfully performed (Stagg *et al.*, 2009, 2014; Bachtiar *et al.*, 2015)) to study online effects of the stimulating current. Combining tDCS with fMRI concurrently provides an unrivalled opportunity to visualise the effects of tDCS on brain activity, and on task performance without temporal methodological limitations. Due to possible timing- and state-dependent effects of tDCS (Stagg, Jayaram, *et al.*, 2011), tDCS was delivered either prior to, or during, task performance.

Specifically, this study aimed to test the hypothesis that inhibiting left M1 with ctDCS when learning an explicit sequence task with the right hand would lead to a compensatory increase in activity of the unstimulated, right M1. Identification of any areas where this compensatory activity is seen would not only help to shed light on the direct effects of transcranial stimulation on the wider motor network, but also potentially help narrow down options for future targets of adjunctive tDCS as a therapy for stroke rehabilitation.

Methods

Participants

17 healthy adults (nine male, mean age 21, range 19-25 years) gave written informed consent (East London REC: 10/H0704/30). Participants were right-handed (Oldfield, 1971) and had

no history of neurological or psychiatric disorder, nor contraindications to MRI scanning or brain stimulation. Participants completed 3 scanning conditions, each separated by at least one week:

- **Prior**: ctDCS administered at rest, immediately before task performance
- **During**: ctDCS administered during task performance
- **Sham**: sham tDCS administered during task performance

Due to scheduling constraints (preventing participation in all sessions) and exclusion of sessions based on behavioural data, final analysis numbers are as follows:

Table 4.1: Final participant numbers

	Prior	During	Sham
Completed MRI	13	16	17
Excluded (based on behavioural data)	0	2	1
Final Analysis	**13**	**14**	**16**

Numbers of participants recruited and subsequently included in analysis. Not all participants were scanned in all sessions. Participants were excluded based on behavioural data if there were insufficient correct responses.

This study was originally performed as a 12 participant study, including the "During" and "Sham" conditions. The third tDCS condition ("Prior") was subsequently added for eight of the original 12 participants, along with all three conditions for a further four participants, and two conditions for one participant. This additional condition was added as an exploratory condition as, at the time of data collection, there was emerging evidence that the timing of tDCS delivery could influence its effects (Stagg, Jayaram, *et al.*, 2011). This therefore resulted in a non-counterbalanced order of scan completion across the 17 participants. All behavioural analysis is therefore performed as a Linear Mixed Model, to take into account the unbalanced Session Order and Sequence as well as factors of interest: tDCS Condition and SRTT Block. A full breakdown of tDCS condition and Sequence order can be found in Table 4.2.

Table 4.2: Participation details: Breakdown of tDCS condition and Sequence for all participants.

	Session 1		Session 2		Session 3	
	tDCS	Sequence	tDCS	Sequence	tDCS	Sequence
P01	During	3	Sham	1	N/A	
P02	Sham	3	During	1	Prior	2
P03	During	3	Sham	1	N/A	
P04	Sham	3	During	1	Prior	2
P05	During	1	Sham	3	Prior	2
P06	Sham	1	During	3	Prior	2
P07	Sham	3	During*	1	Prior	2
P08	Sham	1	During	3	Prior	2
P09	Sham	1	During	3	Prior	2
P10	Sham	1	During	3	Prior	2
P11	During*	1	Sham	3	N/A	
P12	During	1	Sham	3	N/A	
P13	Sham	2	Prior	3	During	1
P14	Prior	3	During	1	Sham	2
P15	Sham*	1	During	2	Prior	3
P16	Sham	1	Prior	3	N/A	
P17	Sham	1	During	3	Prior	2

NB: Participant P01 – P12 completed the original study, participants P12 – P17 were added later, along with Session 3 for P01 – P12 who were able to participate. An asterisk (*) indicates those participants who's data was excluded based on behavioural data.

Table 4.3: Sequence information: Summary of tDCS and Sequence across all participants.

	Prior	During	Sham	TOTAL
Sequence 1	0	6	8	**14**
Sequence 2	9	1	2	**12**
Sequence 3	4	7	6	**17**
TOTAL	**13**	**14**	**16**	

Behavioural Task

SRTT Stimuli

All participants performed the SRTT in each session. Visual cues were presented for 850 ms with an 150 ms ISI (Figure 4.4). Each task block consisted of 30 cues, with a 30 second rest period between blocks. 17 task blocks were performed, comprising 15 sequence blocks and two random blocks (Block 1 and Block 15). The random blocks were used to test for non-sequence specific skill learning. All participants were explicitly informed to expect a repeating sequence, which they should try to learn, but not told the length of the sequence (10-item sequence repeating three times per block). Participants performed the same task in each testing session, with a different learnable sequence in each session. The sequences were matched for difficulty (see supplementary data in Chapter Eight).

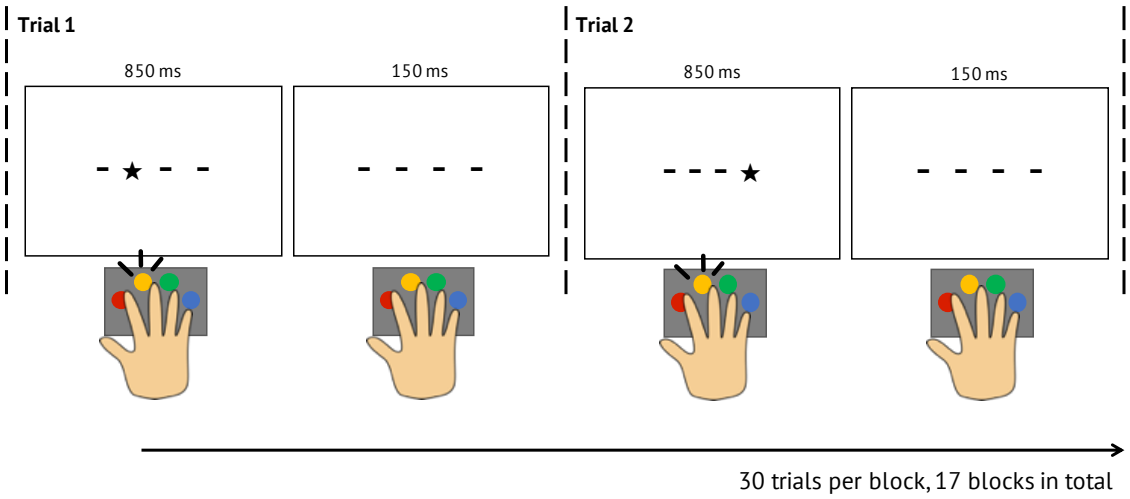

Figure 4.4: Serial Reaction Time Task Design: All participants performed the SRTT during each session. The task consisted of a 10-item sequence which repeated 3 times per block, with 30 second rest between each block. Stimuli were presented for 850 ms with a 150 ms ISI. Participants were explicitly informed to expect a sequence, and told that the sequence would be different in each session.

SRTT Analysis

SRTT behavioural data was analysed to quantify Response Time (RT). RT was calculated as the time in milliseconds from visual cue onset to a correct button press. Trials with anticipatory responses (where a correct response was given prior to the visual cue) and incorrect first button presses, even if the correct button was subsequently pressed, were excluded. Mean RT and standard deviation were calculated for each block. RTs ±2 standard deviations from the mean were excluded. Blocks with fewer than 50% correct responses were removed. Participants with >5 blocks removed were excluded from further analysis (Table 4.1).

Transcranial Direct Current Stimulation (tDCS)

tDCS was performed during the MRI scan without altering the position of the subject, as previously reported (Stagg *et al.*, 2009, 2014; Bachtiar *et al.*, 2015). Cathodal tDCS (1 mA, 20 minutes) or sham tDCS was delivered *in situ* during MRI using a DC-Stimulator (NeuroConn, GmbH, Germany) via two 5x7 cm MR-compatible electrodes, each fitted with 5 kΩ resistors. Low chloride EEG paste (EasyCap, Germany, GmbH) was used as a conducting medium. The cathode was placed over left M1 (centred approximately 5cm lateral to Cz, according to the international 10-20 system of electrode placement), and the anode over contralateral (right) supraorbital ridge. Electrodes were held in place using rubber straps and a fabric swimming cap for the duration of the scan. Extension cables (built in-house) were used to connect the electrode wires to the stimulator (which was kept in the MR Scanner control room at all times) via the penetration panel. Cables for each electrode were kept separate from each other and ran parallel to, and without touching, the participant, in order to prevent eddy currents. The extension cables remained unplugged from the stimulator during the scan, except for the 20 minute stimulation period.

For the "During" and "Sham" conditions, tDCS was delivered concurrently to performance of the SRTT. For the "Prior" condition, the stimulation was delivered directly preceding task performance, while participants lay at rest. In all cases, stimulation was ramped up over 10 seconds. For sham stimulation, the current was switched off for the remainder of the stimulation time (Stagg *et al.*, 2009).

MR Data Acquisition

Subjects were scanned in a 3T Vario MRI scanner (Siemens Medical Systems, NJ, USA) with a 32-channel head coil. Head movement was minimised by use of foam padding. Scans included a T1 weighted image (1x1x1 mm isotropic resolution, T_E/T_R = 4.68 ms/2040 ms, FOV = 174x192, Flip angle = 8°) and functional MRI collected during task performance: 371 volumes, 3x3x3 mm resolution, T_E/T_R = 30 ms/3000 ms, FOV = 64x64.

Functional MRI Analysis

fMRI data was analysed using FMRIB Software Library (FSL) tools (Smith *et al.*, 2004; Woolrich *et al.*, 2009; Jenkinson *et al.*, 2012) as described in Chapter Two.

A first-level model for each subject consisted of three regressors. The first was a boxcar regressor with 30 second alternating on and off blocks to model motor-related activity and was identical for all three stimulation conditions. The second regressor modelled the learning blocks, calculated using the average mean RT for each sequence block across participants for each of the three stimulation conditions separately, in order to model the expected signal drop over time as a consequence of repeated performance of a simple repetitive motor task (Warach *et al.*, 1992; Karni *et al.*, 1995, 1998). The final regressor modelled the random blocks (blocks 1 and 15), and was calculated using the same method as the learning regressor but for

the non-sequence blocks. A higher-level mixed-effects analysis was performed to investigate voxel-wise differences between the three stimulation conditions.

Results

SRTT Results

Linear Mixed Model Setup

Due to removal of some blocks of the SRTT during pre-processing, and the lack of counterbalancing across sessions, a Linear Mixed Model (LMM) analysis was performed with a Random effect of "Participant", Fixed Effects of "Session Order", "Sequence", "tDCS Condition", "SRTT Block" and a Fixed effect interaction of "tDCS Condition * SRTT Block".

Linear Mixed Model of full SRTT Results

The results of the LMM are displayed in Table 4.5, with significant main effects of Session Order ($F_{(2, 633.551)} = 4.020$, $p = 0.018$), Sequence ($F_{(2, 632.616)} = 9.349$, $p < 0.001$) and SRTT Block ($F_{(2, 630.971)} = 32.052$, $p < 0.001$). There was no significant main effect of tDCS ($F_{(2, 635.304)} = 0.962$, $p = 0.383$), nor interaction of tDCS*Block ($F_{(2, 630.970)} = 0.535$, $p = 0.984$).

Table 4.5: Linear Mixed Model Results for SRTT RT data

Source	Numerator d.f.	Denominator d.f.	F value	Significance
Intercept	1	15.944	318.903	<0.001
Session Order	2	633.551	4.020	0.018
Sequence	2	632.616	9.349	<0.001
tDCS Condition	2	635.304	0.962	0.383
SRTT Block	16	630.971	32.052	<0.001
tDCS Condition * SRTT Block	32	630.970	0.535	0.984

No effect of tDCS, session or sequence on rate of learning or peak performance

Additional analysis of SRTT learning metrics revealed no significant main effect of tDCS on peak performance (defined as the minimum RT achieved, $F_{(2, 21.362)} = 0.448$, $p = 0.645$) (Figure 4.6B), nor the rate of learning (defined as the linear slope of best fit of sequence blocks, $F_{(2, 21.874)} = 2.131$, $p = 0.143$) (Figure 4.6C).

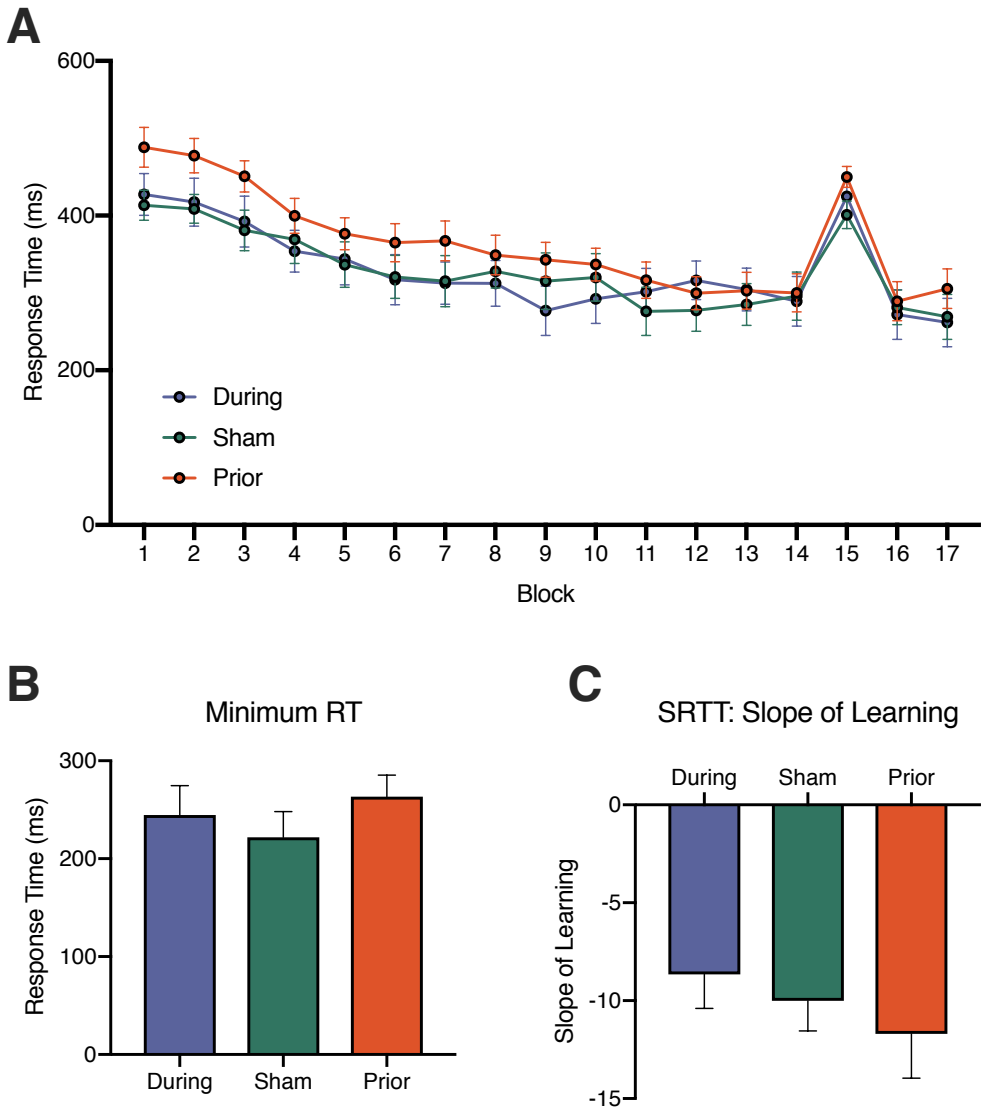

Figure 4.6: No significant effect of tDCS on Response Time in the SRTT: A: Participants showed a significant improvement in RT over the duration of task performance (main effect of Block), with no significant main effect of tDCS. Blocks 1 and 15 are Random blocks, where visual cues were presented in a random order. All other blocks are Sequence blocks where the cues were presented in a repeating sequence which participants were explicitly told to expect, and learn. Error bars indicate SEM for each task block. **B:** There was no effect of tDCS on peak performance (defined as minimum RT achieved), **C:** nor the rate of learning (slope of linear best fit).

fMRI Results

Group Mean: Motor Activity vs. Learning Activity

In order to assess the cortical activity due to performance of a simple motor task with the right hand, I assessed the motor-related activation across all three stimulation conditions in

response to the motor boxcar regressor. This revealed expected activation of left motor, bilateral frontoparietal and bilateral visual areas, as well as subcortical motor networks (basal ganglia including striatum) and bilateral cerebellum (Figure 4.7A). A similar analysis for the learning boxcar regressor demonstrated a similar but more limited activation than for the motor regressor, including similar bilateral frontoparietal regions and bilateral visual areas (Figure 4.7B). A more limited activation of left primary motor cortex was observed for learning related activity (Figure 4.7B) than motor related activity (Figure 4.7A), which is consistent with previous literature suggesting a greater primary motor cortex role for performance of simple motor execution, rather than for sequence performance (Krakauer *et al.*, 2019; Wong and Krakauer, 2019; Yokoi and Diedrichsen, 2019).

Cathodal tDCS delivered prior to learning increases ipsilateral primary motor cortex activity

To assess changes in learning related activation between tDCS stimulation conditions, a tripled two group difference ("Tripled T-Test") higher level Feat analysis was performed. This allowed for pair-wise comparisons of all three conditions with each other. When comparing the "Prior" and "Sham" conditions, a significant increase in right hemisphere (ipsilateral to task performing hand) primary motor cortex learning-related activity was observed (Figure 4.8). This was no observed for any other pairs of conditions. The cluster was not present when pre-threshold masking was performed with the group mean learning activation, meaning that the cluster lay outside the group mean areas for learning-related activation.

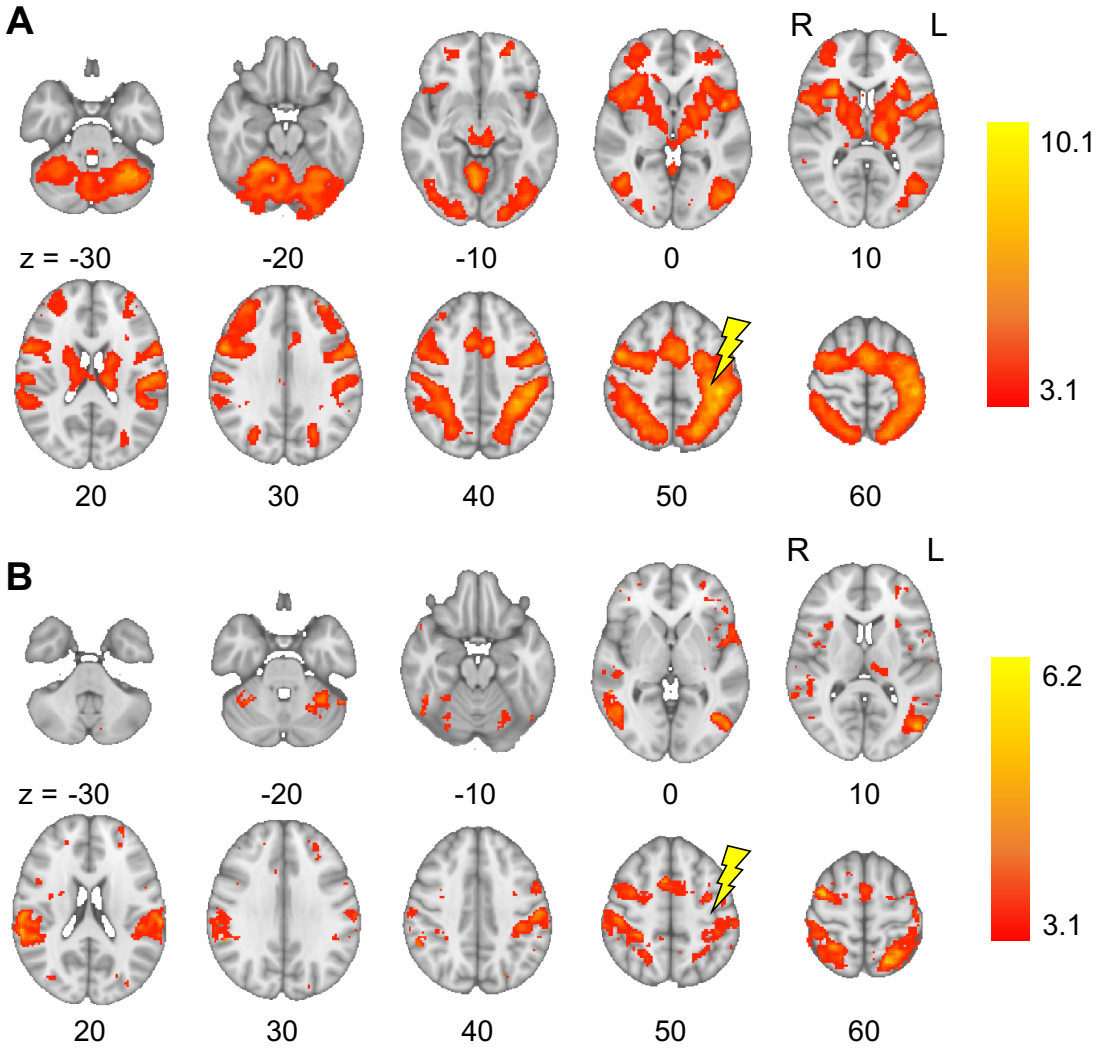

Figure 4.7: Group Mean fMRI activations for movement (A) and learning (B): A distributed network of activation was observed for movement, with a similar, more limited network observed for the group mean of learning specific activity. Lightning Bolt indicates location of ctDCS simulation, colour bar indicates range of z-statistic values.

The degree of activity change does not relate to behavioural performance

In order to explore if the degree of activity change was related to performance on the SRTT, the average zstat within a mask of the above result was extracted for each stimulation condition. This was compared to behavioural metrics (minimum RT and slope of learning). There were no significant correlations for any stimulation conditions and either metric (Spearman's correlations all $p>0.05$).

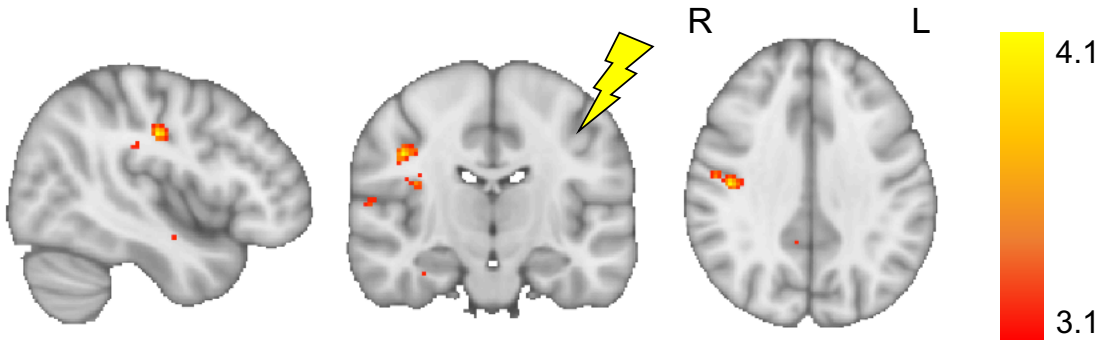

Figure 4.8: Significant increase in learning related activity within the right primary motor cortex when comparing the Prior condition to Sham. Peak of activity is at x = 44, y = -16, z = 34. Lightning Bolt indicates location of ctDCS simulation, colour bar indicates range of z-statistic values.

Discussion

Overview

This study was performed to investigate the role of the distributed cortical motor network during learning of a simple motor task during or following a period of down-regulation of the M1 primarily responsible for the task. Additionally, this study allowed investigation of the feasibility of cathodal tDCS as a "virtual lesion" method to influence behaviour. Concurrent tDCS and fMRI was performed in order to observe concurrent cortical activation and behaviour, something which is much more difficult to achieve using other forms of virtual lesions, such as TMS. Cathodal stimulation applied directly before performance of a simple, visually cued motor task was found to result in increased activation within the ipsilateral motor network, when compared to sham stimulation. However, there was no significant effect on learning.

The finding that cathodal tDCS delivered before a motor task has a significant slowing on reaction time is consistent with a previous finding (Stagg et al. 2011), however this previous

work had also demonstrated a main effect of cathodal tDCS (both online and offline), causing slowing of learning, which was not observed here (no main effect of tDCS condition on RTs across task duration). It is possible that these differences could be due to the type of task used, as although both use a similar SRTT, the overall task (and stimulation) period differed (20 minutes here versus 10 minutes previously), as did the lengths of the resting block (30 seconds here versus 12 seconds previously).

Of the small number of studies which have used online cathodal tDCS to modulate motor learning performance, the majority have reported null results (Antal *et al.*, 2004; Galea and Celnik, 2009; Ambrus *et al.*, 2016), while others have reported a negative effect (or a trend to the same result) of online cathodal tDCS on performance (Michael A Nitsche *et al.*, 2003; Stagg, Jayaram, *et al.*, 2011), or a significant difference between anodal and cathodal stimulation (Reis *et al.*, 2009). For a full and detailed review of effects of different tDCS conditions on different motor learning task see Ammann et al. (2016).

In the results presented here, I have only observed a significant effect of stimulation on brain activity following offline stimulation, without a similar effect during online stimulation. No significant differences in brain activity were found between online left hemisphere ctDCS and sham tDCS. The observed increase in contralateral (right hemisphere) activity when comparing the offline ctDCS condition and sham could be suggested to be compensatory, meaning that learning performance is not impaired.

Virtual Lesion Methods

Within this chapter, the inhibitory effects of cathodal tDCS are harnessed to assess if compensatory activations can be observed within the non-stimulated hemisphere during task

performance. Such "virtual lesion" methods have predominantly used either low-frequency inhibitory rTMS, or single carefully timed single TMS pulses to disrupt a specific process (Pascual-Leone, Bartres-Faz and Keenan, 1999; Pascual-Leone, Walsh and Rothwell, 2000). Cathodal tDCS has been used to a lesser extent, however is more applicable for down-regulation of motor task performance as TMS methods often result in supra-threshold MEPs which could disrupt movement, as well as audible 'clicks' which might act to otherwise distract the participant (Bolognini and Ro, 2010). The neurophysiology of cathodal tDCS is less well understood than inhibitory TMS protocols, and there are mixed results of its effects on motor learning (Ammann, Spampinato and Márquez-Ruiz, 2016). Here, I did not observe any significant reductions in brain activity in the stimulated hemisphere for either stimulation condition when compared to sham. This could, however, be explained by the simultaneous performance of a motor task, performed with the right hand, which would drive activity in the left hemisphere. It is possible that down-regulations of brain activity might have been observed during rest, or even during a non-learning version of the task, however this was not assessed here.

Ipsilateral activation during motor tasks

Activity within the ipsilateral motor cortex has been demonstrated by some fMRI studies during unilateral hand movement of healthy individuals (Kim *et al.*, 1993; Rao *et al.*, 1993; Singh *et al.*, 1998). This seems to be more common for the non-dominant hand (Ziemann and Hallett, 2001), or during higher complexity tasks (Hummel, Kirsammer and Gerloff, 2003; Verstynen *et al.*, 2005; Buetefisch *et al.*, 2014). In the results presented here, significantly greater ipsilateral activity was observed when cathodal tDCS had been delivered prior to task performance. It is possible that the a decrease in activity caused by offline ctDCS required a

more global increase in activity during learning (i.e. across both the ipsilateral and contralateral hemispheres.

Limitations

The findings of the study reported here should be treated with some caution, as there are a number of methodological limitations, which were outside of my control (due to the data being collected prior to the commencement of my DPhil). Consequently, I have attempted to address these limitations within the analysis, however it is likely that these compensations will themselves be limited. Firstly, and most importantly, the "Prior" stimulation condition was added as a third condition after collection of 12 datasets for the "During" and "Sham" conditions in order to assess the timing effects of stimulation. At the time of data collection (Summer 2010), the evidence for timing specific effects of tDCS was emerging (Stagg, Jayaram, *et al.*, 2011), and hence the "Prior" condition was added as an additional exploratory condition. In order to address the possible impact of the effect of Session, a Linear Mixed Model was performed on the behavioural data.

A main effect of Sequence was found during the LMM analysis of RT data. The sequences used within this study (and within a follow up study discussed in Chapter Five) were tested prior to the original data collection (and retested more recently: see supplementary data in Chapter Eight), with no significant difference observed between performance of each sequence. It is possible that the change in environment between these tests (from outside to inside the MRI scanner) may have changed performance of the task, however this is difficult to assess.

Future Directions

The results of the study presented here suggest that compensatory increases in brain activity in the right hemisphere during task performance may prevent detriments to behavioural performance. It is not, however, possible to assess if the increased activity is indeed compensatory, and specifically related to task performance. Further investigation is performed in Chapter Five, where ctDCS is used to attempt to suppress this ipsilateral behaviour.

Another possible future direction following this study, would be a fully counter-balanced repetition, with a non-learning condition. From Chapter Three, it is known that the neurochemical changes within left M1 are specific to a learning related task condition and therefore by adding a non-learning condition to this study, it would be possible to see if there is a compensatory ipsilateral activity when learning is not required.

Conclusions

This is the first study, to my knowledge, to combine tDCS and fMRI to assess the effects of NIBS on motor learning performance, and attempt to elucidate the possible locations of compensatory activity during task performance. There are clear limitations of this study, which make the results difficult to interpret with a great degree of certainty. The combination of a lack of tDCS effect on behavioural performance, and a significant increase in ipsilateral (right) hemisphere brain activity suggest that compensatory activity in the non-task dominant hemisphere may prevent detriments to behavioural performance. This result is in line with observations from post-stroke literature, where increases in contralesional activity are observed during movement of the stroke affected limb (Chollet *et al.*, 1991; Cramer *et al.*, 2001; Johansen-Berg *et al.*, 2002).

Chapter 5: Investigating the role of ipsilateral primary motor cortex during human motor learning

Abstract

In Chapter Four, combined cathodal tDCS and functional MRI during performance of a motor learning task revealed an increase in ipsilateral hemisphere activity when stimulation was delivered offline, prior to task performance. In this Chapter, a follow up study is presented using ctDCS and bilateral TMS to attempt to elucidate the neurophysiological underpinning and behavioural role of this ipsilateral activity increase. While the results of the previous Chapter are not directly replicated here (namely no changes to cortical excitability are observed following tDCS), relationships were observed between TMS measures and behaviour following offline tDCS. Initial behavioural performance (measured as response time in the first task block) was found to correlate with change in cortical excitability in the stimulated left hemisphere. Additionally, the rate of learning was found to correlate with change in glutamatergic signalling in the non-stimulated right hemisphere, which could be hypothesised to link to the observed fMRI activity increase seen in Chapter Four.

Chapter Acknowledgements

The experiment presented here was collected with assistance from Francesca Back, an undergraduate FHS student. A subset of the data presented in this chapter was submitted by Francesca for her FHS project report.

Introduction

In Chapter Four, a significant increase in ipsilateral (right) hemisphere activity was observed while participants performed a sequence learning task following offline left M1 ctDCS. This activity was significantly greater than in a sham condition, and no similar pattern of activity was observed during online left M1 stimulation. It was therefore speculated that this increase in activity might be compensatory, however neuroimaging alone cannot inform as to whether or not this reflects compensatory activity. Here, therefore NIBS approaches are used to investigate the behavioural effects of suppressing this increase in ipsilateral activity.

In Chapter Four, tDCS was used to decrease excitability in the left (contralateral) motor cortex during motor learning with the right hand, with the aim of producing a "virtual lesion". In line with this goal, the increase in activity in ipsilateral M1 during a movement task in healthy individuals after cathodal tDCS to the contralateral hemisphere is reminiscent of the pattern of activity observed in the contralesional hemisphere of stroke survivors during movement of their affected upper limb. However, the specific role of this contralesional activity post stroke is the subject of debate, with both adaptive and maladaptive theories posed within existing literature (Feydy *et al.*, 2002; Johansen-Berg *et al.*, 2002; Hummel and Cohen, 2005; Nowak *et al.*, 2009; Bradnam, Stinear and Byblow, 2013).

In the healthy brain, physiological processes that underpin motor learning are not limited to the contralateral primary motor cortex. The degree of ipsilateral M1 activation in the healthy brain may increase for more complex tasks, or those which require a higher degree of accuracy (Hummel, Kirsammer and Gerloff, 2003; Buetefisch *et al.*, 2014). Meta-analyses of functional MRI studies using a range of sequence and sensorimotor tasks have demonstrated that a wide network of cortical, subcortical and cerebellar areas are involved in motor tasks, with some

differences in recruitment of specific brain areas for different task types (Hardwick *et al.*, 2013). Changes in this distributed network have been observed following both short and long term learning. Floyer-Lea and colleagues (2005) used fMRI to dissociate the brain activation networks involved in both processes. Additionally, long-term changes have been observed in both white matter connectivity (Sampaio-Baptista *et al.*, 2013) and resting motor network strength (Sampaio-Baptista *et al.*, 2015) following motor skill learning paradigms.

However, several studies have postulated that increased contralesional M1 activity is associated with poorer recovery post stroke (Chollet *et al.*, 1991; Ward, Brown, A. J. Thompson, *et al.*, 2003; Ward, Brown, A.J. Thompson, *et al.*, 2003) and that the magnitude of interhemispheric imbalance correlates with functional impairment (Ward, Brown, A. J. Thompson, *et al.*, 2003; Murase *et al.*, 2004). There is, however an evidence from some stroke survivors that the contralesional hemisphere may be supportive of post-stroke motor function (Johansen-Berg *et al.*, 2002; Lotze *et al.*, 2006). Further, it may be that in patients with severe corticospinal tract disruption, the contralesional activity may be required to allow even minimal recovery (Bradnam, Stinear and Byblow, 2013). For a more extensive discussion of the role of the contralesional motor cortex in stroke recovery, see Butefische, (2015). It may be, therefore that the increased activity in ipsilateral M1 during learning after a virtual lesion to contralateral M1 observed in Chapter Four may reflect compensatory activity.

Here, using a sham-controlled, within-subject design, the behavioural relevance of an increase in ipsilateral M1 activity is explored, along with the concurrent neurophysiological changes, during performance of the SRTT. Based on the findings of Chapter Four, and existing literature, it was hypothesised that left M1 cathodal tDCS applied before motor learning (off-line; i.e. Cathodal-Sham) would result in increased right hemisphere corticospinal excitability compared with sham stimulation. As an extension to the previous findings, it was

hypothesised that right M1 cathodal tDCS applied online during motor learning, following Left M1 cathodal tDCS (i.e. Cathodal-Cathodal) would lead to a decrease in learning rate.

Methods

Participants

17 participants gave informed consent to take part in the study in accordance with Central University Research Ethics Committee approval (Ethics Ref: University of Oxford R30822/RE001 and R58733/RE001). All participants were right handed (as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971)), with no history of neurological or psychiatric disorders, or contraindications to TMS or tDCS. Four of the consented participants were excluded during their first testing session (due to adverse response to TMS, or no TMS hotspot) and their data was not included in any analysis. Of the 17 participants consented, 13 completed at least one session, 10 completed all three sessions. Data from all available sessions were used for SRTT analysis (n = 13). Data from participants who completed all sessions were used for TMS analysis (n = 10) to ensure all participants had a sham control.

Experiment Outline

Participants completed three testing sessions with sessions separated by at least one week. Two blocks of tDCS (Block 1 and Block 2) were delivered in each session. In each tDCS block, sham or cathodal tDCS (stDCS / ctDCS) was delivered. The SRTT was performed during tDCS Block 2. TMS protocols were performed bilaterally at three time points (at baseline (T1), between the two blocks of tDCS and before SRTT performance (T2), and after the second tDCS block and SRTT performance (T3), see Figure 5.1).

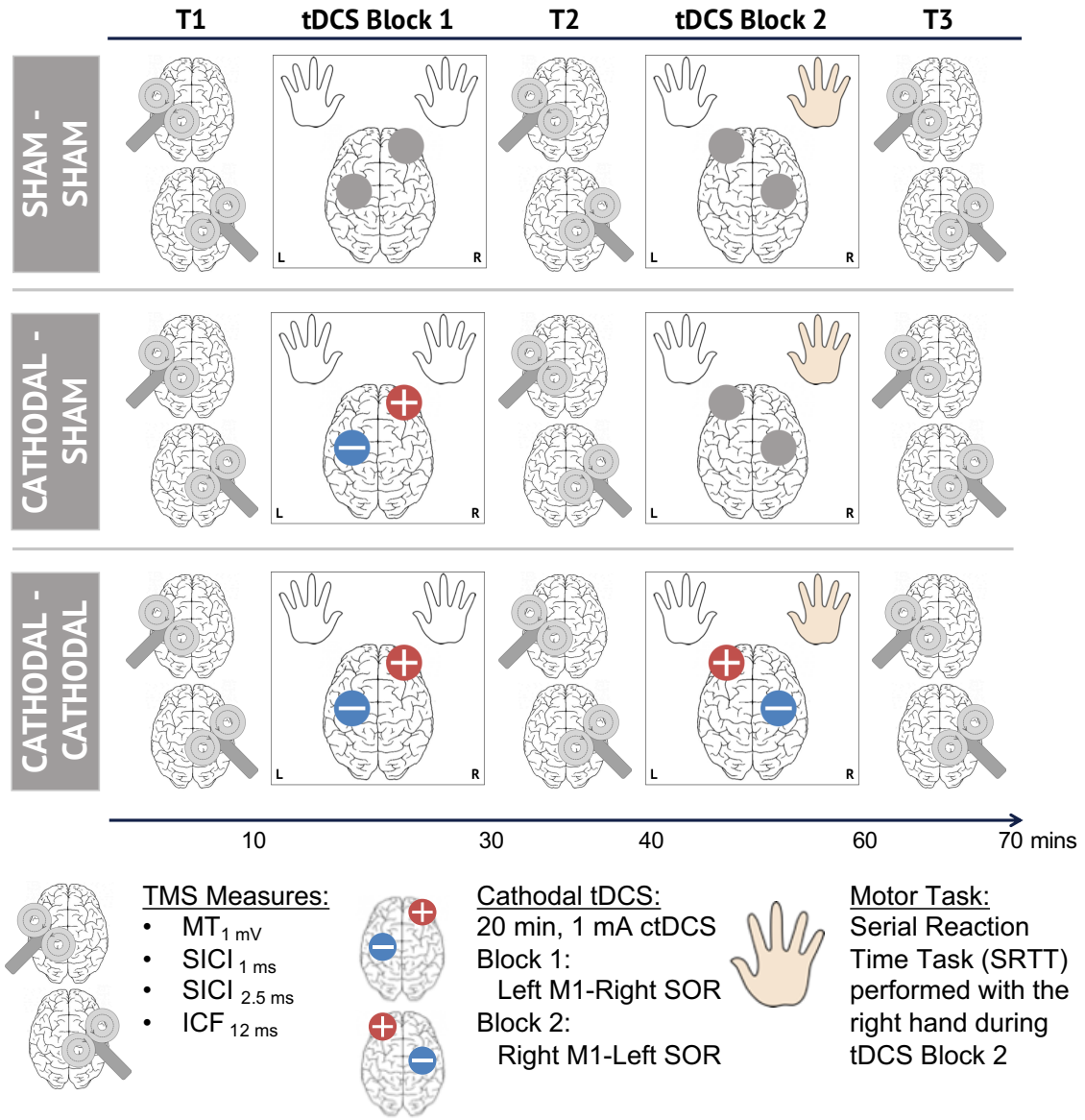

Figure 5.1: Experiment Outline: Participants completed three testing sessions in a counterbalanced order, each separated by at least one week. In each session, participants received TMS at three timepoints (T1, T2 and T3) and two block of tDCS (tDCS Block 1 and tDCS Block 2). TMS Measures consisted of spTMS at the 1 mV threshold (MT_{1mV}), ppTMS measures of inhibition ($SICI_{1ms}$ and $SICI_{2.5ms}$) and a ppTMS measure of facilitation (ICF). Cathodal tDCS was performed for 20 minutes at 1mA. The Serial Reaction Time Task (SRTT) was performed with the right hand during tDCS Block 2.

Serial Reaction Time Task (SRTT)

All participants completed a visually cued SRTT in each session. In each session, participants were seated comfortably in front of a laptop, with the four fingers of the right hand resting on an external button box (Current Designs). Four horizontal lines were presented on the screen, with the cue to press a button indicated by one of the lines changing to a circle.

Participants were instructed to press the corresponding button of the button box with the corresponding finger as quickly and accurately as possible, after the cue appeared on the screen.

The SRTT task design was identical to that described in Chapter Four. In short, participants performed a visually cued SRTT comprising of 17 blocks. Each block lasted 30 seconds and consisted of 30 button presses. Cues were presented for 850 ms with an 150 ms interstimulus interval. Task blocks were separated by a 30 second enforced break. Blocks 1 and 15 of the task were random blocks, with no learnable sequence. All other blocks consisted of three repeats of a 10-item sequence, which participants were explicitly informed to expect, and to learn. After task performance, participants were asked to recall the sequence to demonstrate learning.

SRTT Analysis

Response Time (RT) was calculated as the time of cue onset to the time of correct button press. Anticipatory responses (i.e. those where a correct response was recorded before the cue appeared), and those where the first recorded button press was incorrect were excluded. Any blocks with fewer than 15 correct responses were removed.

During pre-processing of the RT data from the SRTT, two participants had a very low number of recorded 'correct responses' (< 10 correct of 30) in a number of blocks. This was predominantly caused by anticipatory responses where the correct button press was recorded before the cue appeared. This was recorded as an incorrect response, even though the participant was capable of learning the required sequence (as demonstrated by correct explicit recall of the sequence at the end of the SRTT performance). Therefore, only the blocks with

> 10 correct responses were included from the behavioural data analysis. All TMS data was used for these participants.

Transcranial Magnetic Stimulation (TMS)

TMS Data Acquisition

TMS was delivered using a 70 mm figure-of-eight coil attached to a DuoMag Monophasic TMS machine (BrainBox Ltd, Cardiff). Surface electromyography (EMG) was recorded from left and right first dorsal interosseous (FDI) muscle using disposable neonatal ECG electrodes (Henley's Medical) in a belly-tendon montage. The ground electrode was placed on the ulnar styloid process of the same hand. Signals were sampled at 5 kHz, amplified, filtered (10 Hz – 1 kHz), and recorded using a Digitmer D440 4-channel Isolater Amplifier (Digitimer), a CED micro 1401 A/D converter, and Signal software version 3.13 (Cambridge Electronic Design). A Digitimer Hum Bug Noise Eliminator was used to reduce 50 Hz noise in the EMG recording (Digitimer).

The TMS coil was held at 45° to the midsagittal line, with the handle pointing posteriorly. The location of the motor hotspot (i.e. the optimal scalp position for TMS as assessed during baseline thresholding) was marked on the participant's scalp for ease of coil relocation in each block.

Motor Thresholds

At the start of each session, the 1mV Motor Threshold (MT_{1mV}) and Active Motor Threshold (aMT) were recorded from both left and right M1 as defined in Chapter Two. The values for MT_{1mV} and aMT were used to set the Test Stimulus (100% MT_{1mV}) and Conditioning Stimulus (70% aMT) for the paired-pulse TMS (ppTMS) protocols during the main experiment.

Paired Pulse TMS Acquisition

ppTMS was performed at three time points (Figure 5.1). At each time point, TMS was performed on each hemisphere consecutively. The order of hemispheres was kept consistent within a subject across sessions and counterbalanced across participants.

At the start of T1 a few ppTMS pulses were performed, and if necessary the CS was altered in order to ensure facilitation with ICF and prevent complete inhibition in SICI. This CS intensity was then used for the remainder of the study.

At the start of each ppTMS block, 10 spTMS pulses were recorded at the initial MT_{1mV} in order to assess cortical excitability changes across the experiment. If the observed peak-to-peak MEP size differed from 1 mV then the stimulator output was altered to still produce a 1 mV MEP before beginning the ppTMS protocol (Nitsche *et al.*, 2005; Amadi *et al.*, 2015; Nowak *et al.*, 2017).

The ppTMS protocol consisted of 12 spTMS pulses at the TS stimulator output, 12 $SICI_{1ms}$, 12 $SICI_{2.5ms}$ and 12 ICF (with a 12 ms ISI) ppTMS pulses delivered in a pseudorandomised order via the Signal software so that four consecutive TMS pulses consisted of one of each type of pulse.

MEP Analysis

MEPs from the FDI contralateral to the stimulated hemisphere were recorded using EMG. MEPs were pre-processed to remove trials with precontraction of the FDI (EMG amplitude > 0.15 mV immediately preceding the TMS pulse). Peak-to-peak amplitude of the MEP was

calculated and all MEPs higher than 2 standard deviations from the mean for each TMS protocol condition were excluded.

ppTMS measures ($SICI_{1ms}$, $SICI_{2.5ms}$ and ICF) were calculated as a ratio of the mean spTMS induced MEP for the same hemisphere at the same time point:

$$Ratio\ of\ ppTMS\ measure = \frac{mean\ ppTMS\ MEP}{mean\ spTMS\ MEP}$$

Transcranial Direct Current Stimulation (tDCS)

tDCS was performed twice during each session. Participants received either cathodal or sham tDCS in each block. Participants were blinded to the type of stimulation received. All tDCS was performed with the cathode over M1 and the anode electrode over the contralateral SOR. tDCS was delivered via 5x7 cm conductive rubber electrodes in saline-soaked sponges. The electrodes were attached to a DC Stimulator (NeuroConn). In tDCS Block 1, the electrodes were placed over Left M1 and Right SOR. In tDCS Block 2, the electrodes were placed over Right M1 and Left SOR (See Figure 5.1).

For both tDCS blocks, stimulation was ramped up over 10 seconds to a current of 1 mA. The real stimulation lasted 20 minutes before being ramped down over 10 seconds. For sham stimulation, the current was ramped up to 1 mA and run for ~30 seconds before being ramped down and remaining off for the remainder of the 20 minutes. In Block 2, the subject was given instructions for the SRTT before commencing tDCS. Once participants were comfortable they began the task. In most sessions, the task finished slightly before the tDCS. The subject was then asked to sit at rest until the end of the stimulation period.

Statistical Analysis

Statistical analyses were performed using MATLAB 8 software (version R2018b; The MathWorks), Prism (version 8; GraphPad software), and SPSS (version 24; IBM). Greenhouse-Geisser corrections were applied as required. Effect size estimates were computed using partial η squared (partial η^2) for RM-ANOVAs and Cohen's d_z for *t* tests. All correlations were performed as Spearman's correlations.

Linear Mixed Model Setup

As preprocessing of the SRTT data resulted in some blocks being removed, and I wished to account for session order effects an LMM analysis was performed, with a Random effect of 'Participant', Fixed effects of 'Session Order', 'Sequence', 'tDCS Condition', 'SRTT Block' and a Fixed effects interaction of 'tDCS Condition*Block'.

Results

Baseline TMS & SRTT Analysis

No systematic differences in TMS thresholds between sessions

We first wished to investigate whether there were any systematic differences between sessions at baseline, in terms of TMS metrics. For all participants with three complete sessions (n = 10), two RM-ANOVAs were performed on the MT_{1mV} and aMT values with factors of Hemisphere (Left, Right) and tDCS Condition (S-S, C-S, C-C). There was no main effect of Hemisphere (MT_{1mV}: $F_{(1, 9)} = 1.270$, p=0.289, partial $\eta^2 = 0.124$; aMT: $F_{(1, 9)} = 0.418$, p=0.534, partial $\eta^2 = 0.044$) or tDCS Condition (MT_{1mV}: $F_{(1.691, 15.220)} = 0.634$, p=0.519, partial $\eta^2 = 0.066$; aMT: $F_{(1.301, 11.705)} = 0.039$, p=0.902, partial $\eta^2 = 0.004$), nor interaction of Hemisphere*tDCS

Condition (MT_{1mV}: $F_{(1.247, 11.227)} = 0.537$, $p=0.517$, partial $\eta^2 = 0.056$; aMT: $F_{(1.754, 15.782)} = 1.062$, $p=0.361$, partial $\eta^2 = 0.106$).

No change to spTMS induced MEP at T1 between sessions

Using the 10 spTMS pulses taken prior to commencing the ppTMS protocol at T1, an RM-ANOVA was run with factors of Hemisphere (Left, Right) and tDCS Condition (S-S, C-S, C-C). There was a main effect of Hemisphere ($F_{(1, 9)} = 8.033$, $p=0.020$), but no main effect of tDCS Condition ($F_{(1.945, 17.509)} = 1.092$, $p=0.356$) or interaction of Hemisphere*tDCS Condition ($F_{(1.563, 14.068)} = 0.917$, $p=0.399$).

All ppTMS protocols other than Left Hemisphere ICF were effective at T1

We then wished to confirm if the ppTMS protocols used were resulting in the anticipated effects on the MEP sizes.

As there was no significant difference in the spTMS induced MEP across the tDCS conditions for each hemisphere, the mean MEP sizes were calculated at Timepoint 1 (T1) across all three tDCS Conditions for each TMS protocol (spTMS, $SICI_{1ms}$, $SICI_{2.5ms}$, ICF) to achieve four values per participant. These values were then compared using 2-tailed paired samples t-tests to test if the ppTMS protocols were having the desired effect on MEP size.

For Right M1, all three ppTMS protocols produced the expected effects on MEP amplitude (Table 5.2). For Left M1, the two inhibitory ppTMS protocols produced the expected effect on MEP amplitude, however the facilitatory ICF ppTMS protocol did not produce significantly larger MEP sizes when compared to the spTMS induced MEP (Table 5.2). We therefore excluded left hemisphere ICF from the rest of our analyses.

Table 5.2: Baseline ppTMS protocol check

Right M1	Paired T-Test Result	Working?
spTMS vs. $SICI_{2.5ms}$		
spTMS: 0.724 ± 0.221	t=3.412, p=0.008	Yes
$SICI_{2.5ms}$: 0.514 ± 0.168		
spTMS vs. $SICI_{1ms}$		
spTMS: 0.724 ± 0.221	t=6.409, p=0.000	Yes
$SICI_{1ms}$: 0.176 ± 0.124		
spTMS vs. ICF		
spTMS: 0.724 ± 0.221	t=-2.679, p=0.025	Yes
ICF: 0.817 ± 0.260		
Left M1		
spTMS vs. $SICI_{2.5ms}$		
spTMS: 0.967 ± 0.409	t=3.599, p=0.006	Yes
$SICI_{2.5ms}$: 0.695 ± 0.315		
spTMS vs. $SICI_{1ms}$		
spTMS: 0.967 ± 0.409	t=5.504, p=0.000	Yes
$SICI_{1ms}$: 0.323 ± 0.274		
spTMS vs. ICF		
spTMS: 0.967 ± 0.409	t=-0.413, p=0.689	No
ICF: 0.999 ± 0.401		

Right M1:, All three ppTMS protocols produced the desired effects when compared to the unconditioned Right M1 spTMS. **Left M1**: The two SICI protocols produced the desired inhibition of MEP size, however the facilitatory ICF protocol failed to increase MEP size as anticipated.

SRTT Results

Linear Mixed Model of full SRTT Results

An LMM of all RT data for all task blocks revealed main effects of Session Order ($F_{(2, 452.528)} = 12.042$, $p < 0.001$), tDCS Condition ($F_{(2, 451.994)} = 4.143$, $p = 0.016$) and SRTT Block ($F_{(16, 442.832)} = 34.382$, $p < 0.001$). There was no main effect of Sequence, nor an interaction of tDCS Condition*Block (Figure 5.3).

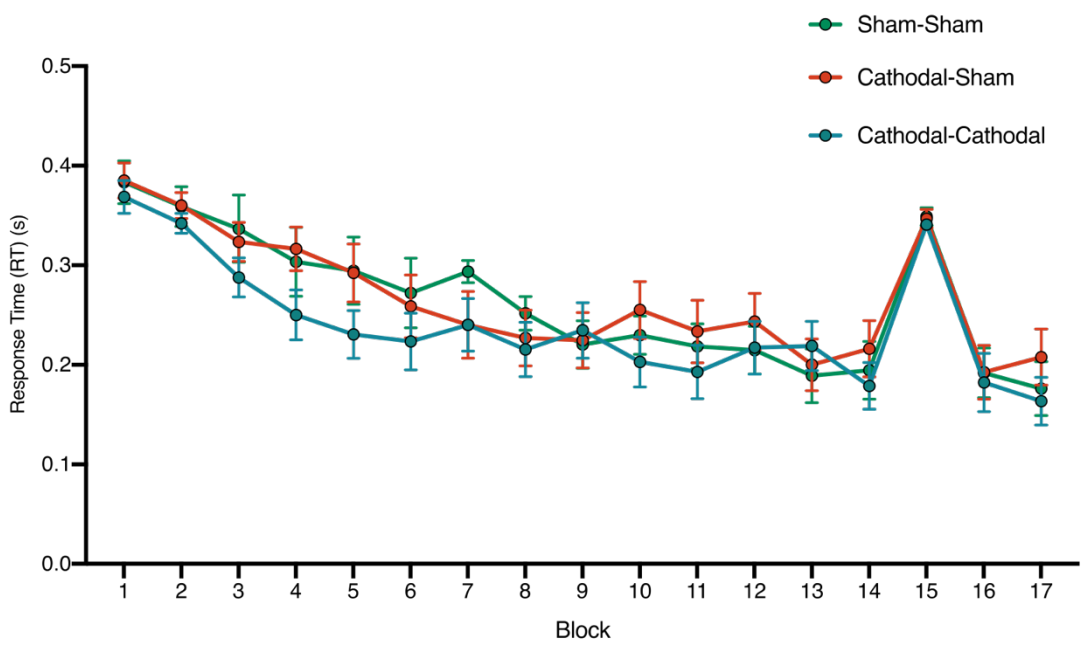

Figure 5.3: Response Time (RT) data for the SRTT: Participants showed a significant improvement in RT over the duration of task performance (main effect of Block), with a significant main effect of tDCS. Blocks 1 and 15 are Random blocks with all other blocks as Sequence blocks. Error bars indicate SEM for each task block.

No effect of tDCS, session or sequence on rate of learning or peak performance

Additional analysis of SRTT learning metrics revealed no significant main effects of tDCS, session or sequence. For rate of learning (measured as the slope of linear best fit across all sequence blocks), there was no main effect of tDCS ($F_{(2, 16.205)} = 0.106$, $p = 0.154$), nor session ($F_{(2, 16.274)} = 0.696$, $p = 0.513$), or sequence ($F_{(2, 15.013)} = 0.622$, $p = 0.550$). For peak performance (assessed as minimum RT achieved) there was no main effects of tDCS ($F_{(2, 14)} = 1.960$, $p = 0.178$), nor session ($F_{(2, 14)} = 0.255$, $p = 0.779$) or sequence ($F_{(2, 14)} = 0.458$, $p = 0.642$).

No effect of tDCS on generalised skill learning

In order to test for generalised, non-sequence specific, improvement in performance across task duration, a LMM was run on the Random Blocks alone. This revealed no significant main effect of tDCS ($F_{(1, 47.275)} = 1.024$, $p = 0.367$), nor interaction of tDCS*Block ($F_{(2, 43.283)} = 0.868$,

p = 0.427). There was a significant main effect of Block ($F_{(1, 43.283)}$ = 21.689, p<0.0001), however follow up LMM analysis on each of Blocks 1 and 15 separately showed no main effects of tDCS (p > 0.2). There was also no main effect of tDCS when performing an LMM analysis on the change in RT from B1 to B15 ($F_{(2, 16.223)}$= 0.235, p = 0.794).

TMS Results

Cathodal tDCS had no effect on cortical excitability

Cathodal tDCS has previously been reported to lead to a decrease in cortical excitability when performed at rest. In order to test the effect of left M1 cathodal tDCS on corticospinal excitability, the change in MEP amplitude elicited by spTMS at the MT_{1mV} intensity from timepoint 1 to 2 (e.g. MEP_{T2} – MEP_{T1}) and timepoint 2 to 3 (e.g. MEP_{T3} – MEP_{T2}) was assessed. An RM-ANOVA with factors of Hemisphere and tDCS Condition (S-S, C-S, C-C) was performed for each change in timepoint separately. For MEP_{T2} – MEP_{T1}, no significant main effect of Hemisphere ($F_{(1, 9)}$ = 1.322, p = 0.280, partial η^2 = 0.128) or tDCS ($F_{(1.734, 15.606)}$ = 0.808, p = 0.900, partial η^2 = 0.009) were observed, nor an interaction of Hemisphere*tDCS Condition ($F_{(1.674, 15.064)}$ = 1.084, p = 0.352, partial η^2 = 0.107) (Figure 5.4A). For MEP_{T3} – MEP_{T2}, no significant main effect of Hemisphere ($F_{(1, 9)}$ = 0.277, p = 0.611, partial η^2 = 0.030), or tDCS ($F_{(1.929, 17.362)}$ = 0.113, p = 0.888, partial η^2 = 0.012) were observed, nor an interaction of Hemisphere*tDCS Condition ($F_{(1.445, 13.004)}$ = 0.721, p = 0.461, partial η^2 = 0.074) (Figure 5.4B). This therefore demonstrates that for this study, there was no significant changes in cortical excitability after cathodal tDCS.

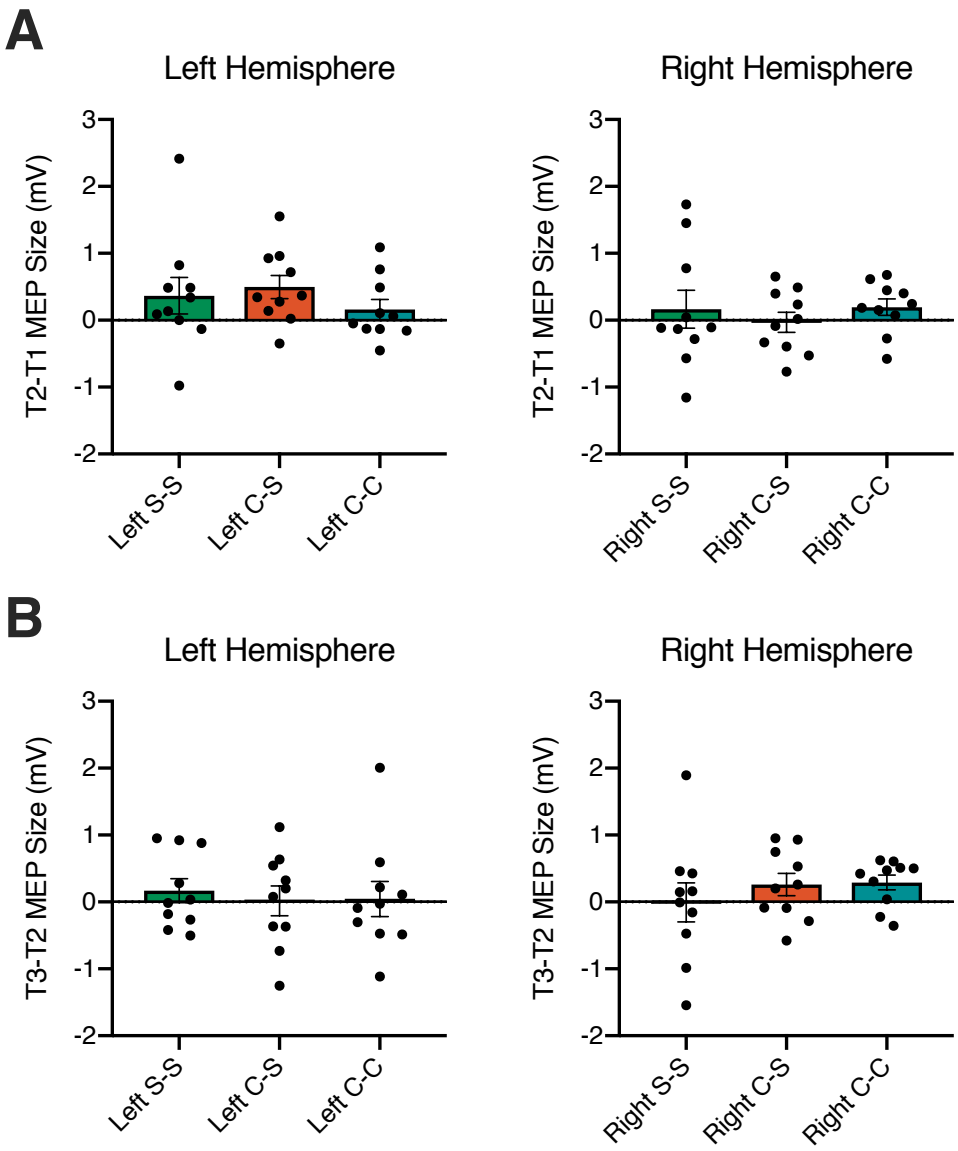

Figure 5.4: Change in cortical excitability: Change in cortical excitability (measured as change in MEP size elicited by spTMS at the MT_{1mV} threshold before each ppTMS block from T1 to T2 (**A**) and T2 to T3 (**B**). RM-ANOVAs for each hemisphere revealed no significant main effects or interactions. Data points indicate individual subjects, error bars indicate group SEM.

Relationships between SRTT and TMS

Block 1 RT predicts change in left hemisphere cortical excitability during task performance following cathodal tDCS

Despite the lack of an overall significant effect of cathodal tDCS on cortical excitability, I wished to explore whether there was a relationship between aspects of the behavioural task and changes in cortical excitability on a subject-by-subject basis. I therefore assessed relationships between timepoints (e.g. MEP_{T2} – MEP_{T1} and MEP_{T3} – MEP_{T2}) and the previously used behavioural metrics (Block 1 RT, minimum RT and slope of learning).

In the Cathodal-Sham condition, initial response time (in Block 1 of the task) was predictive of left hemisphere cortical excitability change from T2 to T3 (MEP_{T3} – MEP_{T2}): Spearman's correlation: $r = -0.9151$, $p = 0.0005$ (Bonferroni corrected p value = 0.0083, based on six comparisons: three tDCS stimulation conditions for two hemispheres) (Figure 5.5, top centre panel). Those participants with the quickest RT during Block 1 were those who went on to show the greatest increase in cortical excitability from T2 to T3 within the left hemisphere. There were no similar correlations found for other stimulation conditions, and the Cathodal-Sham condition was significantly different to both other stimulation conditions in the left hemisphere and to the correlation for the Cathodal-Sham condition in the right hemisphere. $C\text{-}S_{left}$ vs. $S\text{-}S_{left}$: $z = 3.44$, $p = 0.0006$; $C\text{-}S_{left}$ vs. $C\text{-}C_{left}$: $z = -3.07$, $p = 0.0021$; $C\text{-}S_{left}$ vs. $C\text{-}S_{right}$: $z = -2.97$, $p = 0.0032$. Furthermore, there was no relationships between MEP change and the other metrics of learning (minimum RT and slope of learning).

The observed significant relationship between initial RT and cortical excitability change could suggest that those participants who experienced the most minimal effects of the ctDCS prior

to task performance (hence lower initial RT) were also therefore able to increase their left hemisphere cortical excitability during task performance.

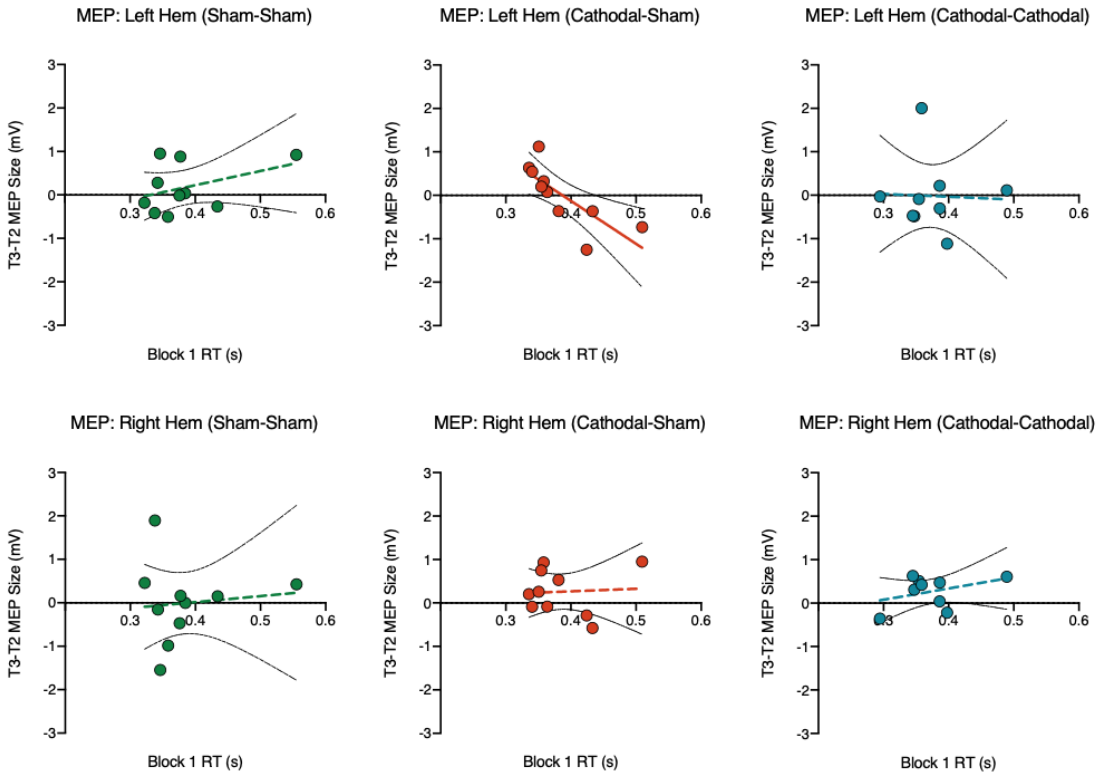

Figure 5.5: Relationships between initial RT and change in cortical excitability: Block 1 RT was found to be predictive of cortical excitability change over the duration of task performance for left hemisphere Cathodal-Sham condition (centre top). This relationship was significantly different to other stimulation conditions in the left hemisphere (top row), and the Cathodal-Sham condition in the right hemisphere (centre bottom).

Change in right hemisphere ICF correlates with rate of learning following cathodal tDCS to left hemisphere

As a relationship had been observed between behavioural performance and cortical excitability change, I wanted to explore if there were any further relationships with learning and neurotransmitter system changes using the ppTMS measures. I tested change in each ppTMS measure from T2 to T3 with the previously used behavioural metrics (Block 1 RT, minimum RT and slope of learning). No correlations were found between either $SICI_{1ms}$ and $SICI_{2.5ms}$ and any of the behavioural metrics..

A relationship was observed between rate of learning (slope of learning) and change in ICF induced MEP size in the right hemisphere (Spearman's correlation: r = -0.9515, p = 0.0001) such that those participants with the best learning were also those who showed greatest increase in the right hemisphere glutamate measure (ICF) (Figure 5.6, centre panel). A trend to the opposite relationship was observed in the right hemisphere Cathodal-Cathodal condition (r = 0.7818, p = 0.0105), however this did not meet the required significance level when corrected for multiple comparisons (Bonferroni Corrected p value = 0.0033, based on 15 comparisons: three stimulation conditions for five ppTMS metrics (Left $SICI_{1ms}$, Left $SICI_{2.5ms}$, Right ICF, Right $SICI_{1ms}$ and Right $SICI_{2.5ms}$)). Left hemisphere ICF was not included due to the failure of this protocol to induce significant MEP facilitation at T1.

The correlation for C-S_{right} was significantly different to the other stimulation conditions in the right hemisphere (C-S_{left} vs. S-S_{left}: r = 3.15, p = 0.0016; C-S_{left} vs. C-C_{left}: r = -5.42, p <0.0001). This result suggests that following left hemisphere ctDCS, those participants who learn best are those who are also able increase excitatory signalling within the task-non-dominant hemisphere. This is supportive of the results presented in Chapter Four, where after left hemisphere ctDCS, there was an observed increase in learning related activity in the right hemisphere.

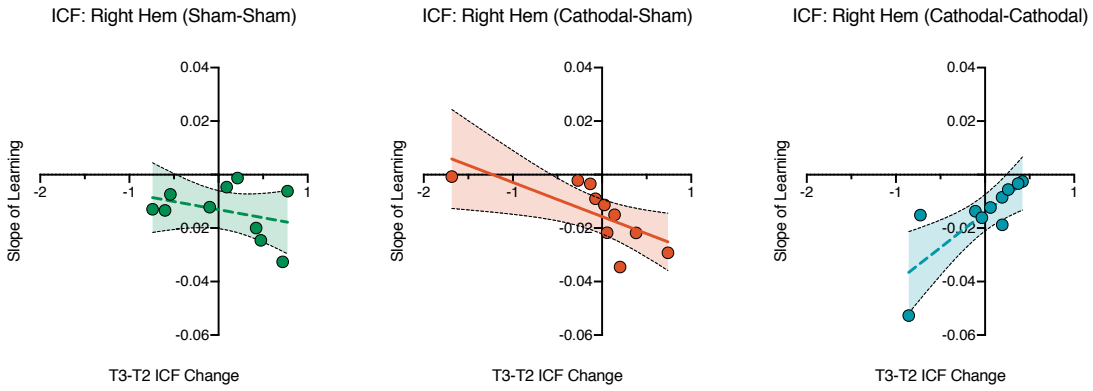

Figure 5.6: Significant correlation between ICF change and rate of learning in Cathodal-Sham: Within the Cathodal-Sham condition, the rate of learning (slope of learning) correlated with change in right hemisphere ICF induced MEP size over duration of SRTT performance. This correlation was significantly different to other stimulation conditions.

Discussion

This study was performed as a follow up to the cathodal tDCS and fMRI study detailed in Chapter Four in order to further investigate the behavioural and physiological effects of cathodal tDCS delivered prior to motor task performance. In Chapter Four, ctDCS delivered to left M1 directly preceding task performance resulted in an increase in learning related activity in the non-stimulated hemisphere during task performance with the ipsilateral hand. I therefore wished to support this finding using TMS measures of cortical excitability, and assess the bilateral neurochemical changes which accompanied any behavioural effects of the tDCS protocols. A replication condition (Cathodal-Sham) was used alongside a sham control (Sham-Sham), we also added a second stimulation condition (Cathodal-Cathodal) to attempt to counteract the hypothesised increase in right hemisphere activity.

In this experiment, I was unable to observe the hypothesised increase in right M1 excitability following offline ctDCS, but I was able to demonstrate tDCS condition-specific relationships between behavioural performance and measures of cortical excitability and neurotransmitters systems using TMS. In the Cathodal-Sham condition, initial RT in the SRTT demonstrated a

relationship with subsequent changes in left hemisphere cortical excitability such that participants with the quickest RT in Block 1 of the SRTT showed the biggest increase in cortical excitability from pre to post task TMS measures. In addition, the rate of learning in the same stimulation condition was correlated with the change in predominantly glutamatergic signalling in the non-stimulated (right) hemisphere such that those participants who showed the greatest increase in right M1 ICF were also those who showed the quickest rate of learning over the course of the SRTT.

The results of this chapter, while not in line with previous findings, or the hypotheses, do allow us to make interesting inferences regarding the effect of ctDCS on the cortical environment and on motor learning behaviour. It is feasible that the lack of replication is due to methodological differences between the two studies (namely that there was a timing delay before commencing the SRTT, potentially disrupting the ctDCS effect on initial RT, and the lack of recording of cortical excitability measures during SRTT performance – neither of which were avoidable).

The effect of tDCS on sequence motor learning

The LMM on RT across all participants and all blocks of the SRTT revealed a significant main effect of tDCS. This effect persisted when the LMM was performed on the sequence blocks alone (but not on random blocks alone), suggesting that the effect was specific to sequence learning rather than generalised learning. However, further assessment of elements of SRTT performance displayed no main effects of tDCS condition on initial response time or peak performance, nor did it affect the rate of learning. It is possible that the elements of SRTT performance used here were not specific to the tDCS induced changes in behaviour, and that other metrics may have accounted for the observed tDCS effects in the main LMM analysis.

Cathodal stimulation before task performance did not alter cortical excitability, neurotransmitter systems

No significant changes in cortical excitability were observed across the experiment. This includes both hemispheres, from T1 to T2 and from T2 to T3. Previously, changes in cortical excitability have been observed in both the stimulated and non-stimulated hemisphere following cathodal tDCS (Nitsche and Paulus, 2000; O'Shea *et al.*, 2014), however this has not been consistently observed (N. Lang *et al.*, 2004), and was not observed here. Furthermore, previous studies have demonstrated changes to bilateral GABAergic systems following unilateral cathodal tDCS using MRS (Bachtiar *et al.*, 2018). However, this has not been consistently observed, with some showing no effect of ctDCS on cortical excitability (Varoli *et al.*, 2018). These results highlight the complexity of the literature surrounding cathodal tDCS, as although the initial investigation of its on cortical excitability were shown to be polarity-dependent (Nitsche and Paulus, 2000, 2001), the directionality of the effects have since been demonstrated to be more complex (Batsikadze *et al.*, 2013; Samani *et al.*, 2019). Specifically, it has been found in one study that increasing the intensity of ctDCS from 1 to 2 mA reverses the effect on cortical excitability from inhibitory to excitatory (Batsikadze *et al.*, 2013).

The non-linearities of cathodal tDCS have been investigated in a recent extensive study, in which motor cortical excitability was assessed for up to ~36 hours after three different stimulation intensities and durations (Samani *et al.*, 2019). The authors found that 1 and 3 mA stimulation intensities resulted in a significant reduction in cortical excitability for up to 1 hour post-tDCS across durations of 15, 20 and 30 minutes of stimulation. The results for 2 mA

were more complex, with no discernible change to MEP size for 15 and 30 minute durations, and a significant increase in cortical excitability for 20 minutes of 2 mA ctDCS.

Further investigation of the cortical excitability changes of the same protocol as used here (1 mA, 20 minutes) within the same paper revealed significant decreases in MEP size when compared to sham (but not baseline) at 5 and 15 minutes post tDCS (but not at 1, 10 or 20 minutes post tDCS). This highlights the possible difficulties in assessing offline effects of cathodal stimulation, which could be present at certain intervals post-stimulation, but not at others.

Rate of learning linked to changes in right hemispheric glutamatergic signalling following cathodal tDCS

A strong relationship was found between an individual participants rate of learning, and the change in glutamatergic signalling in the right hemisphere in the Cathodal-Sham condition such that those participants with the quickest rate of learning were those who showed the greatest increase in right hemisphere glutamate. Within the right hemisphere, no relationship was observed in the Sham-Sham condition, and a non-significant trend in the opposite direction in the Cathodal-Cathodal condition. The significant Cathodal-Sham correlation was significantly different to relationships in both other stimulation conditions.

Although this is not a direct replication, this result links with the main result of Chapter Four, where an increase in right hemisphere activity was observed during learning following offline left hemisphere ctDCS. There is a possibility that the relationship observed here between rate of learning and change in glutamatergic signalling is a similar mechanism to that observed within the main finding of Chapter Four. The measure of ICF change is a much more subtle

measure than cortical excitability change (spTMS induced MEP size) and reflects a change in the balance of neurotransmitter systems.

Limitations of this study

There are a number of limitations of this study, most notably a significant effect of session order on behavioural performance. Due to non-experimental issues with obtaining ethical approval for the Cathodal-Cathodal session, a number of recruited participants completed the Sham-Sham and Cathodal-Sham conditions before the Cathodal-Cathodal condition, and timing issues prevented additional participants being recruited to fully counterbalance this study. A session effect would be expected based on previous assessment of this version of the SRTT (see supplementary data in Chapter Eight), as participants gain explicit awareness of the task demands, and therefore generalised task skill. In order to compensate for this, all SRTT analysis performed using a LMM analysis which accounts for variance in multiple variables of non-interest when analysing a variable of interest (e.g., accounting for variability in "Session" when assessing effect of "tDCS Condition").

In this study, I have not demonstrated clear changes in cortical excitability following tDCS, or motor learning (or a combination). While it is feasible that the findings of Chapter Four are false positive findings, methodological limitations of this study (in particular timing delays between ceasing offline tDCS and commencing the SRTT, and inability to record TMS during SRTT performance) could go a long way towards explaining this lack of replication. We could have performed TMS assessments of cortical excitability during task performance, which may have increased the likelihood of observing an increase in right hemisphere activation. However, performing TMS concurrently with the SRTT is suspected to interfere with behavioural performance (through induction of suprathreshold muscle twitches which can be

distracting, and impair task performance – even if they were induced in the non-task performing hand).

Future Directions

A simple follow-up to the experiments described here would be a non-TMS replication, to test if the timing break alone was sufficiently long to allow for any tDCS washout. This would potentially allow for investigation of whether the failed replication could be due to the delivery of bilateral TMS protocols between offline tDCS and the SRTT. Furthermore, a Sham-Cathodal session (which was not included here in order to limit the number of sessions participant were required to complete) would potentially help unpick the role of the right hemisphere in SRTT performance.

Conclusions

Despite its limitations, this study is, to my knowledge, the first attempt to assess the effects of cathodal tDCS on motor learning performance on the SRTT, while assessing changes in cortical excitability and neurotransmitters systems across both hemispheres. The results presented here allow the possibility to make inferences regarding the effect of ctDCS on the cortical environment and on motor learning behaviour which have not been possible before. I have shown relationships between behavioural performance and cortical excitability change, and between rate of learning and neurotransmitter system changes – both following offline ctDCS stimulation. The latter of these results demonstrates that learning related changes can be observed in the ipsilateral hemisphere during motor learning in healthy adults. These results could guide future work to investigate the role of the ipsilateral hemisphere in SRTT

performance, which in turn could guide investigation of the role of the contralesional hemisphere in motor rehabilitation after cortical damage following stroke.

Chapter 6: Development of a novel EMG Force Tracker Task to study motor learning in chronic stroke

Abstract

Understanding the process of motor learning following stroke is a crucial step towards improving motor rehabilitation, however many existing studies exhibit methodological limitations preventing the most severely impaired due to the requirement for volitional movement of the hand/arm or to interact with a control interface for task performance. Here, results are presented of a short proof-of-principle study run in a small group of chronic stroke survivors and age-matched healthy controls, to assess the feasibility of performance of an EMG-controlled force tracker task (EMG-FTT). Both stroke survivors and age-matched controls were able to improve performance at the group level, however controls performed significantly better at both baseline and in improvement. Importantly, within the stroke survivor cohort, neither metric of task performance could be predicted by residual function of the stroke affected upper limb. All participants additionally underwent a multimodal 3T MRI scan to assess differences in functional activity and neurochemical systems between groups. A significant correlation was found between GABA concentration in the left and right primary motor cortices of healthy controls, which was not found in stroke survivors. Furthermore, significant differences in the laterality index of brain activity during hand movement was found for healthy controls, but not stroke survivors. Neither neurotransmitter or functional brain activity was found to correlate with behavioural performance.

Chapter Acknowledgements

The work presented in this chapter is, more so than the others, a culmination of a number of people's expertise.

The EMG Force Tracker Task, and the methods for recording both behavioural and EMG data would not have been possible without the help of Alek Pogosyan, who developed the software used, and wrote the task code.

The MRSI sequence used here was developed by Uzay Emir, and the analysis pipeline was adapted and explained by Will Clarke.

Data collection was greatly assisted by Fylis van Horssen, an MSc Biomedical Sciences student from Radboud University, Nijmegen.

Introduction

Relearning of motor skills is a key component of motor rehabilitation during recovery after a stroke, and consequently research into if (and if so, how) motor control and motor learning processes are altered following a stroke are key factors which impact development of rehabilitation strategies (Krakauer, 2006; Hubbard *et al.*, 2009). Motor rehabilitation is often described as a process of relearning a motor skill, and relearning of a skill can be suggested to be similar to a process of learning a skill for the first time, or the process of learning to use a skill in a new way. It is therefore logical, to some extent, to study healthy motor learning and make suggestions on how these findings could benefit motor rehabilitation after stroke. While this has merits, it ignores the possibility that the learning process is fundamentally changed following a stroke. Assessment of motor learning ability post stroke is not a simple task, and there is a relatively limited literature assessing motor learning ability post stroke, particularly involving direct comparisons of ability between stroke survivors and age matched healthy controls. Methodologically, the assessment of learning in stroke survivors is difficult to perform, with an incredibly heterogenous population and residual functional abilities which can range from zero movement to normal movement. Despite these difficulties, there is an existing literature of motor learning post-stroke which has informed the study outlined in this Chapter.

From the post-stroke motor learning literature, it has been widely suggested that learning is not itself abolished, although performance decrements have been reported. It has been reported that, compared to age-matched controls, chronic stroke survivors have slower response times (Boyd *et al.*, 2007; Fleming, Newham and Rothwell, 2016), slower movement speeds (Pohl and Winstein, 1999; Pohl *et al.*, 2001; Fleming, Newham and Rothwell, 2016) and slower rates of learning (Wadden *et al.*, 2017).

It is often difficult to interpret results of post-stroke motor learning studies, with limitations possibly preventing full translation of any findings into useful advances for rehabilitation. A number of studies have assessed motor learning using the less-affected upper limb for task performance (Pohl *et al.*, 2001; Boyd *et al.*, 2007; Wadden *et al.*, 2017) which could potentially confound differences in results due to hand dominance, therefore limiting translation to true recovery of the affected limb. In terms of participants, it is common for post-stroke motor learning studies to recruit only mild and moderately impaired groups of stroke survivors due to the use of tasks requiring dexterous movements (Pohl *et al.*, 2006; Boyd *et al.*, 2007; Fleming, Newham and Rothwell, 2016; Wadden *et al.*, 2017), and in some cases no age-matched healthy controls (Lefebvre *et al.*, 2012; Zimerman *et al.*, 2012).

Motor learning studies most commonly require repeated performance of a skilled (and often dexterous) movement, usually via a device to detect movement and relay information to a computer. These control interfaces can take the form of button boxes, joysticks or pinch or grip force transducers and therefore require a certain level of voluntary movement to perform the task. A number of studies have used adapted versions of more "classic" motor learning tasks in order to combat possible floor/ceiling effects. Examples include use of whole arm or hand movements rather than more dexterous finger movement (Pohl *et al.*, 2001, 2006; Boyd *et al.*, 2007; Fleming, Newham and Rothwell, 2016), however even with these adaptations, the most severely impaired stroke survivors are prevented from participating due to the requirement for volitional movement of the hand/arm or to interact with a device.

For a number of years, an increasingly common control interface for movement-assistive devices and prosthetics has been surface electromyography (EMG) (Schultz and Kuiken, 2011; Lobo-Prat *et al.*, 2014). Through recording the residual EMG from one of more muscles,

the user is able to control multiple aspects of the item's use. By using similar engineering methods, EMG-driven rehabilitation devices have been successfully developed and used within stroke cohorts as a rehabilitation tool and adjunct therapy for movement of various joints, including the wrist (Lambelet *et al.*, 2017), ankle (Ferris *et al.*, 2006; Koller *et al.*, 2015) and knee (Peng *et al.*, 2016). Such devices are attractive within rehabilitation as they can improve efficiency and deliver longer endurance, and higher intensity rehabilitation at a lower running cost than traditional manual repetitive therapies (Wilkins *et al.*, 2017).

Despite the relatively wide use of EMG-controlled devices within rehabilitation of both the upper (Lo and Xie, 2012; Wu *et al.*, 2018) and lower limbs (Shi *et al.*, 2019), there is very little investigation of their use as a control interface for motor learning. In healthy controls, EMG has been shown to perform as well as force-transduction and joystick control in a force tracker task (Lobo-Prat *et al.*, 2014). In their study, Lobo-Prat and colleagues used a visuomotor tracking task in a cohort of healthy participants. Task performance was assessed for tracking error, effort required and the maximum frequency at which participants could accurately track the cursor (maximum performance frequency). In this case, EMG was found to be superior to force-transducer and joystick based interfaces for both tracking error and maximum performance frequency, however it required a higher effort (measured as percentage of maximum voluntary contraction) than the force-transducer interface. Overall, the authors reported that an EMG control interface was a viable alternative control interface for their task.

Within this chapter, I will present results of a proof-of-principle study run in a small group of chronic stroke survivors and age-matched healthy controls, to assess the feasibility of performance of an EMG-controlled force tracker task (EMG-FTT). In addition to the motor task, all participants underwent a multimodal 3T MR scan on the same day as task

performance in order to assess variability in structure, function and neurochemistry between participants and explore whether these relate to learning performance.

From the literature, there is evidence that post-stroke motor function is closely associated with functional cortical activation during affected limb movement (Ward *et al.*, 2003) and neurochemistry (Blicher *et al.*, 2015). Furthermore, the degree of contralesional activation has been related to learning performance (Boyd, Vidoni and Wessel, 2010) and recovery of motor function over time (Ward *et al.*, 2003).

I hypothesised that all participants would be able to perform, and improve performance on the EMG-FTT, however I predicted seeing a difference in performance between the stroke survivors and age-matched controls. For stroke survivors, it was expected that residual functional ability in the affected upper limb (as assessed with the Action Research Arm Test, ARAT) would predict neither initial performance, nor change in performance over time as the EMG-FTT is not reliant on volitional movement. Given the expectation that task performance would not be predicted by residual function, I wanted to explore if an aspect of functional activation (such as the degree of contralesional activation during movement of the affected hand (Boyd, Vidoni and Wessel, 2010)) would help predict EMG-FTT performance. Finally, based on previous literature it was expected that there would be differences in the laterality indices of functional activation and neurochemicals (Blicher *et al.*, 2015; Mooney *et al.*, 2019) between the stroke and control cohorts.

Methods

Participants

11 chronic stroke and six age-matched healthy controls (see Table 6.2 for participant details) gave written informed consent in accordance with Central University Research Ethics Committee approval (Ethics Ref: University of Oxford R59721/RE001). All participants were right handed (prior to stroke for patient cohort) (Oldfield, 1971). No healthy controls had a history of any neurological or psychiatric disorder. Stroke survivors were at least six months post first ischemic or haemorrhagic stroke, with no other neurological or psychiatric disorder. All participants had no contraindications to MRI scanning at 3 Tesla. Participants completed two testing sessions (MRI Scan and Behavioural Testing) on the same day.

Questionnaires

All participants completed a number of questionnaires and functional tests. The Action Research Arm Test (ARAT) (Lyle, 1981) and 9-Hole Peg Test (9-HPT) were used to test upper limb function and finger dexterity. The Montreal Cognitive Assessment (MoCA) was used as a tool to assess for mild cognitive impairment (Nasreddine *et al.*, 2005). The Epworth Sleepiness Scale (ESS) was used to assess self-reported daytime sleepiness within participants (Johns, 1991).

At two points during performance of the EMG-FTT, participants rated their current level of sleepiness and fatigue on a Visual Analogue Scale (VAS). After training on the EMG-FTT, all participants completed a questionnaire to self-report their understanding and performance of the task (See Table 6.1 for questions). Participants responded to statements about the task using a five point Likert scale ranging from "Strongly Disagree" to "Strongly Agree".

Table 6.1: EMG-FTT Participant Feedback Questions

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
I understood the goal of the task	1	2	3	4	5
I found it was easy to concentrate on the task	1	2	3	4	5
I found the task difficult to perform	1	2	3	4	5
I think I got better at the task over time	1	2	3	4	5
I found the task tiring physically	1	2	3	4	5
I found the task tiring mentally	1	2	3	4	5

5-point Likert scale used to assess participants understanding and self-reported performance of the task

Table 6.2: Participant demographics

Participant	Age at testing (years)	Gender	Time since stroke (months)	Affected Hand	ARAT		9HPT		MoCA	ESS
STROKE SURVIVORS					**Affected**	**Unaffected**	**Affected**	**Unaffected**		
S01	41	M	103	R	21	57	0	24	25*	7
S02	74	F	45	L	45	57	18	24	26	22
S03	63	M	133	L	23	57	0	24	19	6
S04	61	M	14	R	43	57	16	17	22*	11
S05+	42	M	63	R	10	57	0	21	14*	0
S06	78	M	158	L	11	57	0	22	24	8
S07	62	M	102	L	1	57	0	24	23	7
S08	60	M	98	L	17	57	0	24	28	7
S09	57	F	183	L	28	57	0	28	23	14
S10	65	M	34	L	21	57	0	18	18	10
S11	54	F	156	L	10	57	0	27	27	14
Med:	**59.7**	**-**			**21**	**57**	**0**	**24**	**24**	**8**
Range:	**41 - 78**	**3 F, 8 M**			**1 - 45**	**57 - 57**	**0 - 18**	**17 - 28**	**14 - 27**	**0 - 22**
AGE-MATCHED CONTROLS					**Left**	**Right**	**Left**	**Right**		
C01	76	M	-	-	57	57	25	23	29	5
C02	80	M	-	-	56	54	22	27	30	3
C03	66	F	-	-	57	56	24	22	28	5
C04	69	M	-	-	57	56	19	21	30	8
C05	70	F	-	-	57	57	25	25	30	2
C06	54	M	-	-	57	57	27	27	30	17
Med:	**69.2**	**-**			**57**	**56.5**	**24.5**	**24**	**30**	**5**
Range:	**54 - 80**	**2 F, 4 M**			**56 - 57**	**56 – 57**	**19 - 27**	**21 - 27**	**28 - 30**	**2 - 17**

ARAT: Action Research Arm Test (Lyle, 1981) (scored by researcher, max score 57, higher scores indicate greater function); **9HPT:** 9-Hole Peg Test (number of pegs inserted and removed in 30 s); **MoCA:** Montreal Cognitive Assessment (Nasreddine *et al.*, 2005) (scored by researcher, max score = 30, score < 26 indicates some mild cognitive impairment), stroke survivors with aphasia symptoms indicated with asterisk (*); **ESS:** Epworth Sleep Scale (Johns, 1991) (self-reported, max score = 24, score > 10 indicates higher than normal daytime sleep). **R** = Right, **L** = Left, **M** = Male, **F** = Female, **SD** = Standard Deviation. Participant S05 did not undergo MRI scan due to scan fault (indicated with +).

MR Data

MR Data Acquisition

Due to scanner faults, one stroke survivor did not complete the MRI scan. All other participants (n = 10 stroke, n = 6 controls) were scanned in a 3T Prisma MRI scanner (Siemens Medical Systems, NJ, USA) with a 32-channel head coil. Head movement was minimised by use of foam padding. Stroke survivors were offered additional padding and/or strapping to aid comfort and support of their paretic upper limb. All participants completed the full MRI scanner protocol.

To enable placement of the MRSI slab during the scan, and perform *post hoc* lesion mapping, structural MRI data were acquired with a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence (Mugler and Brookeman, 1990): TR = 1900 ms, TE = 3.96 ms, voxel size = 1.0 × 1.0 × 1.0 mm, flip angle = 8°, total slices = 192. Functional MRI scanning during task performance used Multiband 6: TR = 933 ms, TE = 33.40 ms, voxel size = 2.0 × 2.0 × 2.0 mm, flip angle = 60°, GRAPPA Factor = 6, number of slices = 72.

In addition to the scans described above, resting fMRI and diffusion weighted imaging were acquired for all participants. These scans are outside the scope of this thesis. These scans were matched to the UK BioBank protocol (for full details, see www.fmrib.ox.ac.uk/ukbiobank/protocol).

MR Data Processing

For stroke survivors, lesion masks were manually drawn from the T1 scan using MRIcron (Rorden and Brett, 2000) and smoothed with a 2 mm kernel in three dimensions (Figure 6.3). Masks were drawn to include lesioned and perilesional tissue, in order to prevent possible

registration issues. Two of the 10 stroke survivors with MRI data had lesions in the left hemisphere, and these scans were flipped to align all lesions in the same hemisphere.

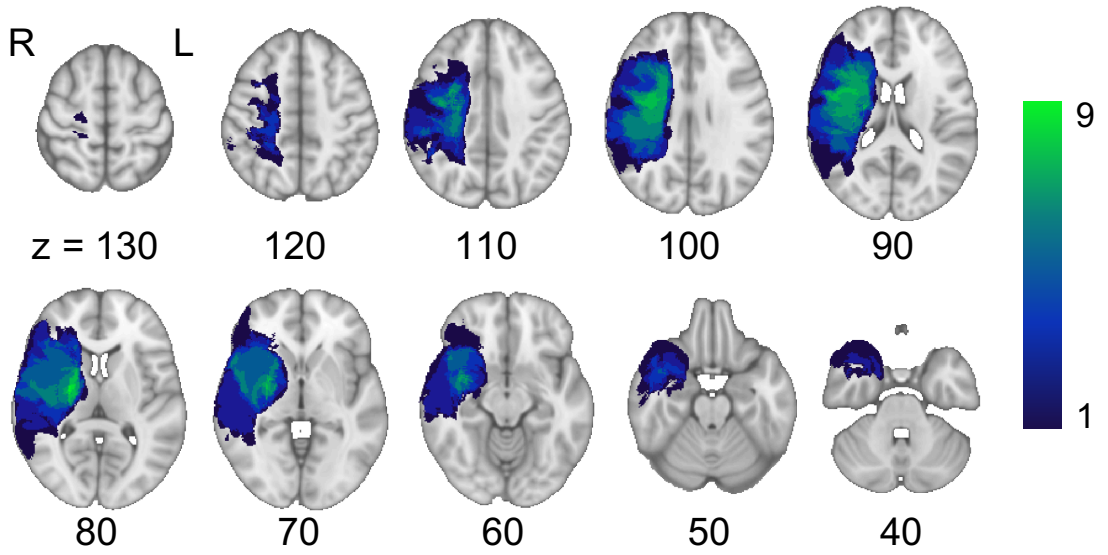

Figure 6.3: Lesion overlap in standard space: Two of the ten stroke survivors with MR data had left hemisphere lesions, the scans for these subjects were flipped to place lesions in the right hemisphere for all participants. Colour bar indicates the number of subjects.

The Anatomical Processing Script (fsl_anat) within FSL was run on T1 images to reorient images to standard space and run bias field correction. The options to automatically crop (robustfov, --nocrop), registration to standard space (FLIRT, --noreg and FNIRT --nononligreg), segmentation of cortical and subcortical tissue (FAST, --noseg and FIRST --nosubcortseg) were switched off. Optimized brain extraction script for patients' brains (optiBET) (Lutkenhoff *et al.*, 2014) was run for all subjects (both stroke survivors and controls) followed by registration to standard space (using FLIRT/FNIRT) taking into account the lesion mask for stroke survivors.

Magnetic Resonance Spectroscopic Imaging (MRSI)

MRSI Acquisition

Three MRSI scans (time per scan 4.5 minutes = 13.5 minutes total) were collected sequentially using a non-water suppressed semi-LASER DW-CRT sequence as previously described (Steel *et al.*, 2018). During MRSI acquisition, participants watched a nature documentary (Blue Planet 2, BBC).

2D MRSI scans were collected from a single slice manually positioned using the T1-weighted MP-RAGE image. The MRSI grid (85 x 35 x 15 mm) was positioned to cover both hand knobs in the posterior corners, with the slab extending to cover the pre- and post-central gyrus and supplementary motor areas.

Non-water-suppressed metabolite-cycling MRSI was acquired using parameters described in Emir *et al.* (2017) and Steel *et al.* (2018). The three independent scans were acquired sequentially and averaged together to form the final image set.

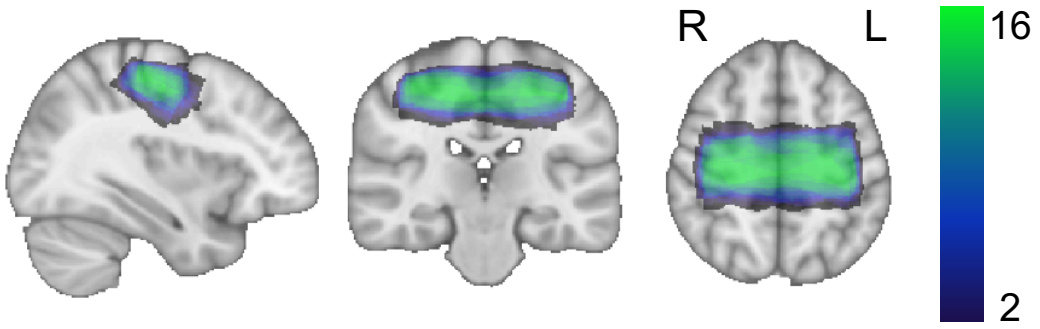

Figure 6.4: MRSI overlap in standard space: Slices taken at x = 35, y = -25, z = 50. Colour bar indicates the number of subjects.

MRSI Preprocessing and Analysis

The LCModel package was used to quantify metabolites for each MRSI voxel. Full details of the MRSI preprocessing and analysis are outside the scope of this chapter and are detailed in a recent publication by Steel and colleagues (2018).

Following LCModel fitting, metabolite maps were registered to each participant's native T1 space using FLIRT (Jenkinson and Smith, 2001; Jenkinson *et al.*, 2002), and resampled to 1.0 × 1.0 × 1.0 mm resolution using nearest-neighbour interpolation (Figure 6.5).

Voxels within the metabolite map which did not satisfy quality assurance (QA) values were removed from further analysis (exclusion values were as follows: CRLB > 50%, FWHM > 15 Hz, SNR < 30). In addition, for stroke survivors, voxels which overlapped with the delineated lesion were also removed from further analysis (Figure 6.5).

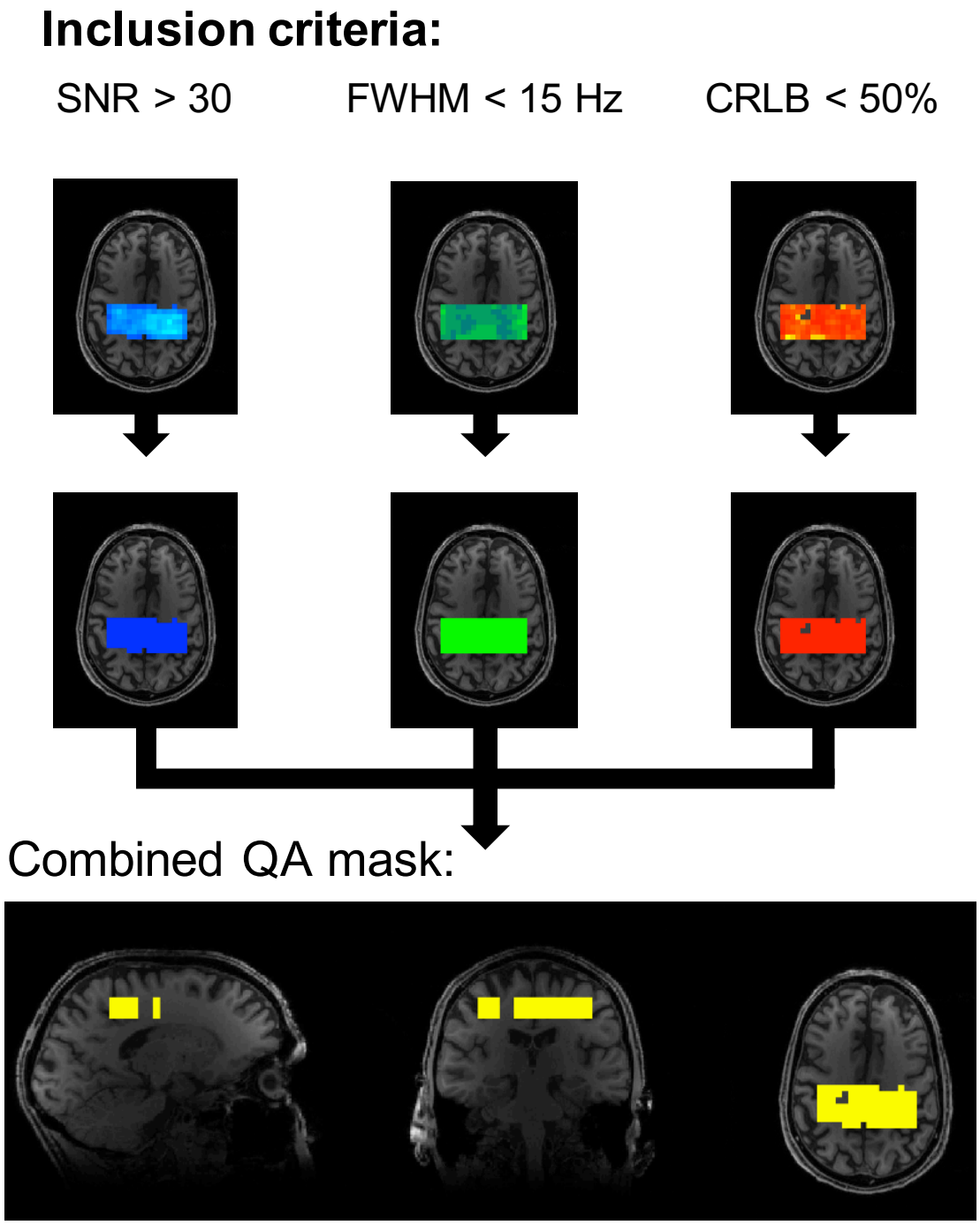

Figure 6.5: Method of creating quality assurance (QA) masks for MRSI analysis: Separate thresholded masks were made for each of the QA metrics: Signal to Noise (SNR) (Blue), Linewidth (Full Width, Half Maximum: FWHM) (Green) and Cramér Rao Lower Bound (CRLB) (Red). These masks were binarised and combined to create a subject and metabolite specific QA mask (Yellow). For stroke survivors, an additional mask of the participant's lesion was also included. Voxels were required to satisfy all QA metrics to be included in metabolite analysis.

Functional MRI Tasks

All participants performed two motor tasks during functional MRI scanning: a passive and an active movement task. Movement and rest blocks alternated in 30 second blocks, with three blocks per side. For both tasks, stroke survivors performed three blocks with the less-affected hand first, followed by the affected hand while the order of hand performance for controls was counterbalanced within the control cohort.

Active Movement Task

For the active task, participants performed self-paced squeezes of a stress ball with the number of squeezes per block recorded by researcher. Participants were cued which hand to use via on-screen cues reading "RIGHT", "LEFT" or "REST". Participants were instructed to squeeze at a pace and pressure which felt comfortable, and to match these factors between hands as far as possible. Participants were given the opportunity to briefly practice the active task outside the scanner to find a comfortable rate of squeezing.

Passive Movement Task

For the passive task, passive extension/flexion of the wrist was performed by the researcher, cued by a 0.33 Hz auditory cue delivered via MR safe headphones. Participants were instructed to keep both hands relaxed for the full task duration, and did not view any visual stimuli during the task.

fMRI Task Analysis

Preprocessing and statistical analysis of functional MRI task data was performed using tools from the FMRIB Software Library (FSL) (www.fsl.fmrib.ox.ac.uk/fsl), including motion correction using MCFLIRT (Jenkinson *et al.*, 2002) and denoising using AROMA (Pruim,

Mennes, Buitelaar, *et al.*, 2015; Pruim, Mennes, van Rooij, *et al.*, 2015) and MELODIC (Beckmann and Smith, 2003) (See Methods Chapter for details of preprocessing steps). For the First-Level statistical analysis, a boxcar regressor modelling the 30-s task and rest blocks was used to create first-level statistical maps for each participant. Higher-Level fixed-effects analysis was performed to assess difference in activation maps between performing hands, tasks and groups. Due to the low subject numbers in this pilot study, a fixed-effects design was used, which is more sensitive to activation, but restricts the inferences that can be made from any results – i.e. any results are representative of the participants tested, but not necessarily of the wider population.

Creation of Group Mean ROIs

In order to perform Laterality Index (LI) analysis of functional MRI data and for more accurate extraction of metabolite information from task-specific regions of cortex, data-driven regions of interest (ROI) masks were derived from the Active task data. Group mean clusters for left (affected) and right (less-affected) hand movement were created from the group mean Active task data of all participants (stroke and controls combined). The resulting clusters for left and right hand movement were thresholded at 50% of their maximum zstat value (Left hand maximum zstat = 20.3, threshold at 10.15; Right hand maximum zstat = 36.9, threshold at 18.45) and then multiplied by an M1/S1 anatomical mask. The resulting masks were similar in size (left hemisphere = 974 voxels, 7792 mm^3); right hemisphere = 939 voxels, 7512 mm^3). Standard space masks of ROIs can be seen in Figure 6.6.

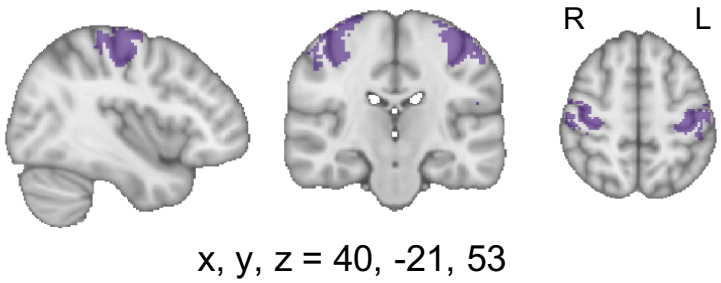

Figure 6.6: Data-driven ROIs in standard space: M1/S1 ROIs created from group mean data of left and right hand movement from the group mean (stroke survivors and controls combined) of the Active Task.

Left and right hemisphere masks were then transformed into each subject's native space using an inverse of the native-to-standard warp from Feat registration. Native space masks were dilated with a 0.2 mm kernel and re-binarised to create subject-specific data-driven ROIs (see Figure 6.13 for all subject's masks).

Laterality Index (LI)

The percentage change in activation was extracted for both hemispheres during performance of the active and passive tasks using the group mean derived ROIs in Featquery (Feat results interrogation tool within FSL). A Laterality Index (LI) was calculated for both right and left hand movement as the task-dominant hemisphere (contralateral to moving hand) minus the task-non-dominant hemisphere (ipsilateral to moving hand). Lateralised activation in favour of the hemisphere contralateral to the moving hand (as is expected with unimanual movement in the healthy brain) would therefore be shown as a positive value, with a value close to zero indicating a more bilateral activation. A negative value indicates a lateralised activation in favour of the hemisphere ipsilateral to the moving hand.

Electromyography-driven Force Tracker Task (EMG-FTT)

EMG-FTT Task Design

All participants completed the EMG-FTT. Stroke survivors used their affected upper limb, and controls used the non-dominant (left) arm. Surface EMG of both arms was recorded including the extensor carpi radialis (ECR) of both arms and the flexor carpi radialis (FCR) of the task performing arm, however only the task performing arm ECR was used as a task driver.

Participants sat comfortably in front of a computer screen with arms resting on a pillow on their lap to encourage complete muscle relaxation (Figure 6.7B). The task screen (Figure 6.7A) displayed three coloured circles in the centre of the screen. A large blue circle (approx. 20 mm diameter) indicated the resting, "home" space of the screen. A white circle (approx. 10 mm diameter) moved in a fixed amplitude sinusoid in a 1-dimensional line in the midline of the screen. Target speed and range of movement (~ 75% of screen height) was fixed for all participants. A single trial of the task consisted of three full phase cycles of the target cursor, and lasted 20 seconds with 15 seconds rest between trials. Participants were instructed to track the movement of the white target circle by moving the red cursor (approx. 7.5 mm diameter) through controlled modulation of muscle contraction. Movement of the hand, wrist and arm were not restricted or recorded during task performance, although participants were instructed to keep the non-task performing arm as relaxed as possible. Movement of the task-performing limb was not restricted in order to more closely replicate natural movement. It was expected that different participants would produce different movements and fatigue at different rates. For this reason, EMG signals were passively recorded from the FCR of the ipsilateral and ECR of the contralateral arm to that performing the task. These traces were

monitored by the experimenter during task performance for excessive activity. If necessary, the task was paused to allow for relaxation of the ipsilateral FCR and contralateral ECR.

Participants completed 8 trials per block of the task, with age-matched controls completing 12 blocks of the task, and stroke survivors completing a minimum of three task blocks (Figure 6.7D). Prior to task commencement, all participants completed three sustained maximal voluntary contractions (MVC) (each lasting ~2 seconds) of the task driver muscle (ECR of affected/left arm). The average EMG of these MVCs was calculated, and the maximum contraction required to reach the top of the screen was set at 40% MVC.

EMG-FTT Piloting

Prior to the data collection reported in this chapter, pilot sessions were run on the EMG-FTT with two stroke survivor participants in order to develop the task parameters. These participants had previously taken part in multiple other studies for our group and were invited to give extensive (and honest) feedback. Within these sessions the following parameters were altered and finalised:

- **Target trace design**: both a fixed amplitude and varied amplitude trace were piloted. Both participants reported that the fixed amplitude was sufficiently difficult and so this was used for the main study.
- **Maximum voluntary contraction**: various percentages of the MVC were piloted as the force required to reach the top of the screen. In previous heathy control pilots, 15-20% of MVC had been used however the two stroke survivors reported better cursor control with a higher MVC percentage as the top of the screen. This piloting resulted in the value of 40% MVC being used to reach the top of the screen.

- **Speed of target**: the cursor speed was slowed down in order to increase the likelihood of controlled tracking, rather than production of short bursts of muscle contraction. Both patients reported that this improved their perceived control of the cursor.
- **Range of target movement:** based on feedback from piloting, we reduced the range of target movement to ~75% of screen height. This removed the necessity to completely return to a relaxed EMG signal during tracking (as the tracker icon never overlapped with the "home" space). It was found that this encouraged continuous modulation of muscle contraction, rather than repeated short contraction bursts.

EMG-FTT Acquisition

EMG signals were sampled using a TMSi Porti32 amplifier (2084 Hz sampling rate, Twente Medical Systems International, Oldenzaal, The Netherlands) and recorded using in-house software (RecEEG©, developed by A. Pogosyan, Oxford). Spike 2 (Cambridge Electronic Design Limited, Cambridge, UK) was used to extract and preprocess behavioural data (target position, non-rectified error and EMG). All age-matched control participants completed 12 blocks of the task. Stroke survivors all completed a minimum of three blocks (median: 5 blocks, range: 3 to 10 blocks), and were informed they could stop when they felt fatigued.

EMG-FTT Analysis

Target position and non-rectified error were preprocessed within Spike2 using DC remove (300 ms) and smoothed (100 ms) and written to separate files. EMG data were preprocessed using DC remove (300 ms), rectified and then smoothed (100 ms). Cursor position was subsequently calculated in Matlab (R2018b, MathWorks, Massachusetts, USA) as target position + non-rectified error. The first second of the target and error traces were removed to account for initial errors caused by delayed response to the start of trials.

All traces were visually inspected for recording errors. Within each task block, the median rectified error for each trial was calculated, followed by the median rectified error within each block was calculated and used for further analysis.

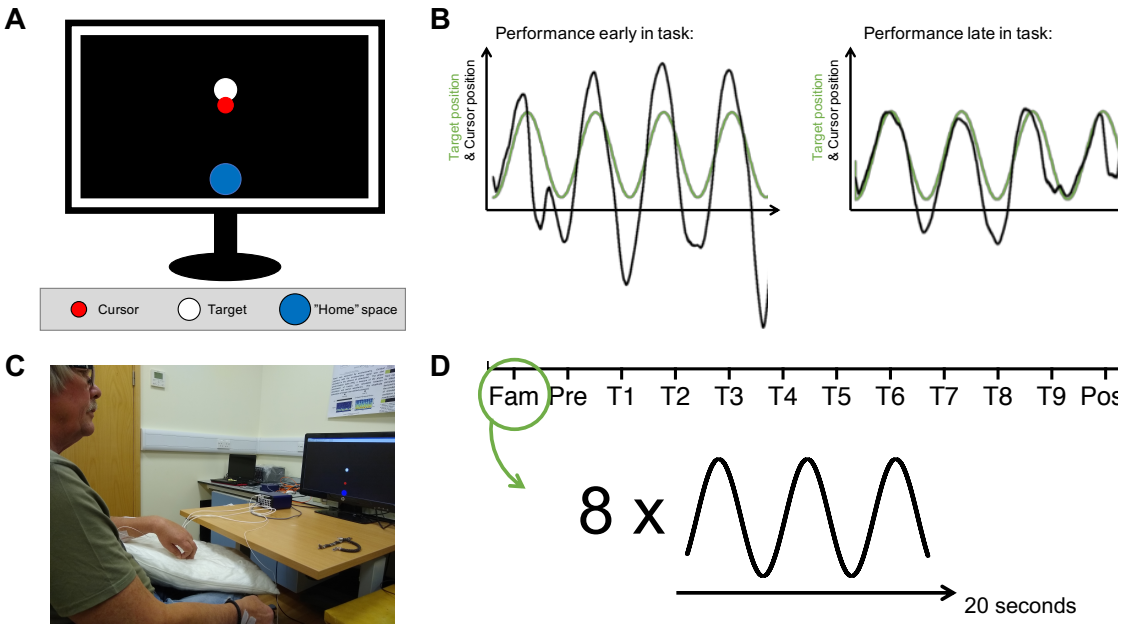

Figure 6.7: EMG-FTT Task Design:

A: Visual display seen by participants during the task. Participants were instructed to track the movement of the white target circle as accurately as possible by moving the red cursor. The blue circle indicated the "home" space where participants returned between trials.

B: Example traces of performance early and late in task (representative data from pilot session in a young healthy participant). In early learning, it was expected that participants would make large over- and under-shoot errors, with incorrectly timed direction changes. Later on in training, the amplitude of movements should be more closely matched to the target cue.

C: A stroke survivor participant performing the EMG-FTT. Photo used with permission.

D: Overall task outline: Participants completed 8 trials per block, with each trial consisting of a 20 second period of movement (three phases of movement) with a 15 second rest between trials. Blocks were performed as two single blocks with ample rest periods (Familiarisation and Pre), followed by three triplets with shorter breaks (T1-2-3, T4-5-6 and T7-8-9), and one final single block (Post). All age-matched older adults completed all 12 blocks, stroke survivors were allowed to stop earlier if fatigued.

Results

Functional Tests and Questionnaires

Action Research Arm Test (ARAT)

As expected, as a group, stroke survivors showed significantly lower in scores on the ARAT for their affected upper limb (median = 21, range 1-45), compared to the less-affected limb (median = 57, range 57-57) (Wilcoxon sum of signed ranks (W) = 66, p = 0.001). No such difference was seen for age-matched controls (Left: median = 57, range 56-57; Right: median = 56.5, range = 56-57) (W = -6, p = 0.250), however it should be noted that some participants did not score full marks (most commonly on the "pinch" tasks). The difference in ARAT score between limbs (less affected minus affected, or right minus left) was calculated and a significant difference was found between groups, with stroke survivors showing a greater difference between limbs (Mann Whitney U = 0, $p < 0.0001$).

9-Hole Peg Test (9-HPT)

Only two of the stroke survivors were able to perform the 9-HPT with their affected hand. These were those with the highest residual function as assessed by the ARAT (scoring 43 and 45 out of 57). There was no significant difference between the number of pegs inserted and removed by the less-affected hand of stroke survivors (median = 24, range 17-28) and either hand of age-matched controls (Left: median =24.5, range 19-27; Right: median = 24, range 21-27) (vs. right hand: U = 28, p = 0.6387; vs. left hand: U = 26.5, p = 0.5364), nor between the hands of the age-matched controls (W = 2, $p > 0.999$).

Montreal Cognitive Assessment (MoCA)

Stroke survivors scored significantly lower on the MoCA compared to age-matched controls (Stroke: median = 24, range 14-27; Control: median = 30, range 28-30) ($U = 0.5$, $p = 0.0002$). This was also the case when those participants with aphasia symptoms ($n = 3$), were excluded ($U = 0.5$, $p = 0.001$).

Epworth Sleepiness Scale (ESS)

There was no significant difference in the ESS scores between stroke survivors (median = 8, range 0-22) and age-matched controls (median = 5, range = 2-17) ($U = 19.5$, $p = 0.1890$).

EMG-FTT Results

There was a significant difference in the number of blocks completed between the two groups ($U = 0$, $p < 0.0001$). All age-matched controls completed 12 blocks, whereas the number of blocks completed by stroke survivors ranged from 3 to 10 (median number of blocks completed = 5).

EMG-FTT Task Feedback

There was no significant difference in responses to the self-report task feedback questionnaire between the stroke survivors and age-matched control groups (Mann-Whitney test: $p > 0.1$) indicating that both groups found the task similar, and neither group found it more tiring (either physically or mentally). It should be noted that stroke survivors were allowed to stop the task when they felt fatigued, and therefore had, as a group, completed fewer blocks than the age-matched controls which may have affected their self-reported physical or mental fatigue.

Baseline Performance

A significant difference between stroke and controls was observed between the median error within the first block of the task (U = 4, p = 0.0019), with a higher error for stroke survivors as expected (Figure 6.8A). Within the stroke survivor group, there was no relationship between the error within the baseline block, and ARAT score for their stroke affected limb (Spearman's r = -0.3379, p = 0.3074 (Figure 6.8B)).

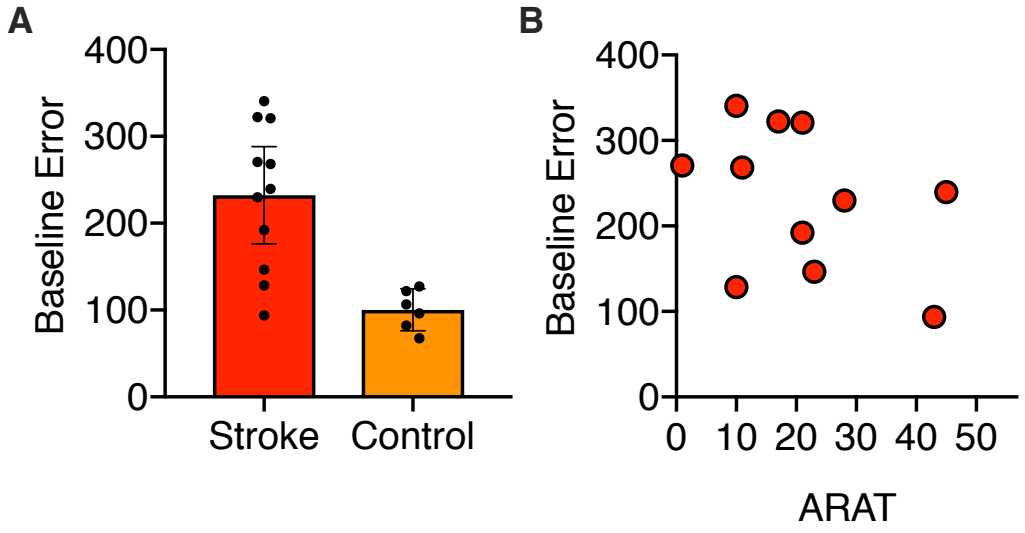

Figure 6.8: Baseline Results of EMG-FTT: A: There was a significant difference in median error between groups during the first block of the task (p = 0.0019); **B:** There was no relationship between the median error within the stroke survivor cohort and the residual function of the affected upper limb (ARAT score).

Task Improvement

As a group, age-matched controls were able to improve their performance over time (RM ANOVA, Main Effect of Block: $F_{(1.966, 4634.596)} = 5.321$, p = 0.028). A LMM was performed for all subjects, with factors of Group (stroke, control) and Block (1-12), finding a significant main effect of Group ($F_{(1, 15.011)} = 17.763$, p = 0.001) and no overall main effect of Block ($F_{(11, 97.979)} = 0.350$, p = 0.971), nor interaction of Group*Block ($F_{(9, 98.011)} = 1.291$, p = 0.251).

A participant's best block was calculated as the block in which the median error was lowest, no matter where this occurred within task performance. The percentage change from baseline to the best block was calculated. There was a significant difference in this percentage change between stroke survivors and age-matched controls (U =5, p = 0.0028), such that stroke survivors showed a smaller percentage change (Figure 6.9A). However both groups showed significant improvement (Stroke: W = -28, p = 0.0156, Controls: W = -21, p = 0.0312). As with baseline error, within the stroke cohort, the percentage change in error was not found to correlate with ARAT score (Spearman's r = -0.2571, p = 0.4391) (Figure 6.9B).

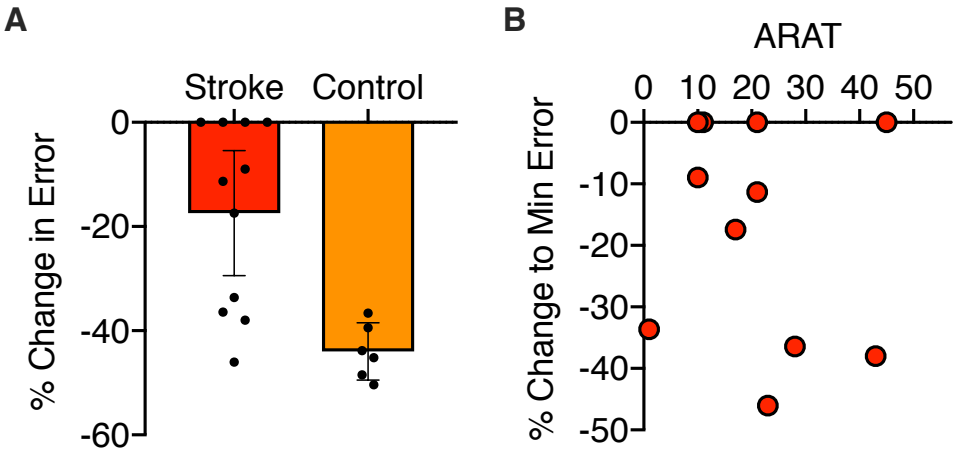

Figure 6.9: Improvement results of EMG-FTT: A: There was a significant difference in percentage change from baseline to best block between groups (p = 0.0028); **B:** There was no relationship between the percentage change within the stroke survivor cohort and the residual function of the affected upper limb (ARAT score).

Further visual inspection of the percentage change results revealed a subset of four stroke participants who showed a similar percentage improvement to the age-matched control group, and a second subset of four participants who made no improvement in their performance after the first block of the task. Within both of these groups, there was a range of functional impairment, suggesting that learning was independent of upper limb function.

Functional MRI Results

Active Task

There was no significant difference in the average number of squeezes between the affected (median = 7, range = 0-18.7) and unaffected (median = 10.5, range = 3-18.3) hand in the stroke survivor cohort, nor between the left (median = 13.85, range = 10.7-23.7) and right (median = 13.85, range = 10.3-21.7) hands of age-matched controls (Stroke: W = 15, p = 0.4297, Control: W = -10, p = 0.25). Furthermore, when comparing across groups, both performed a similar number of squeezes (U = 23, p = 0.4518).

Higher level Feat analyses were run in FSL to examine the activation patterns during movement of each hand, and activation differences between groups. A comparison was performed between movement of the affected hand of stroke survivors, and the matched (left) hand in age-matched controls. As controls were moving their non-dominant hand, a bilateral activation pattern was observed (Figure 6.10A), however there was greater activation in their contralateral (right) hemisphere than the ipsilateral hemisphere. By contrast, stroke survivors showed a more balanced bilateral pattern of activation when actively moving their affected hand, with similar levels of activity in each hemisphere (Figure 6.10B). Subtractions of the activation patterns in stroke survivors from age-matched controls revealed significantly higher contralateral (right) hemisphere activation in controls on movement of the left hand (Figure 6.10C), and the opposite subtraction revealed a small area of ipsilateral (left) hemisphere activation which was greater for stroke survivors than controls (Figure 6.10D).

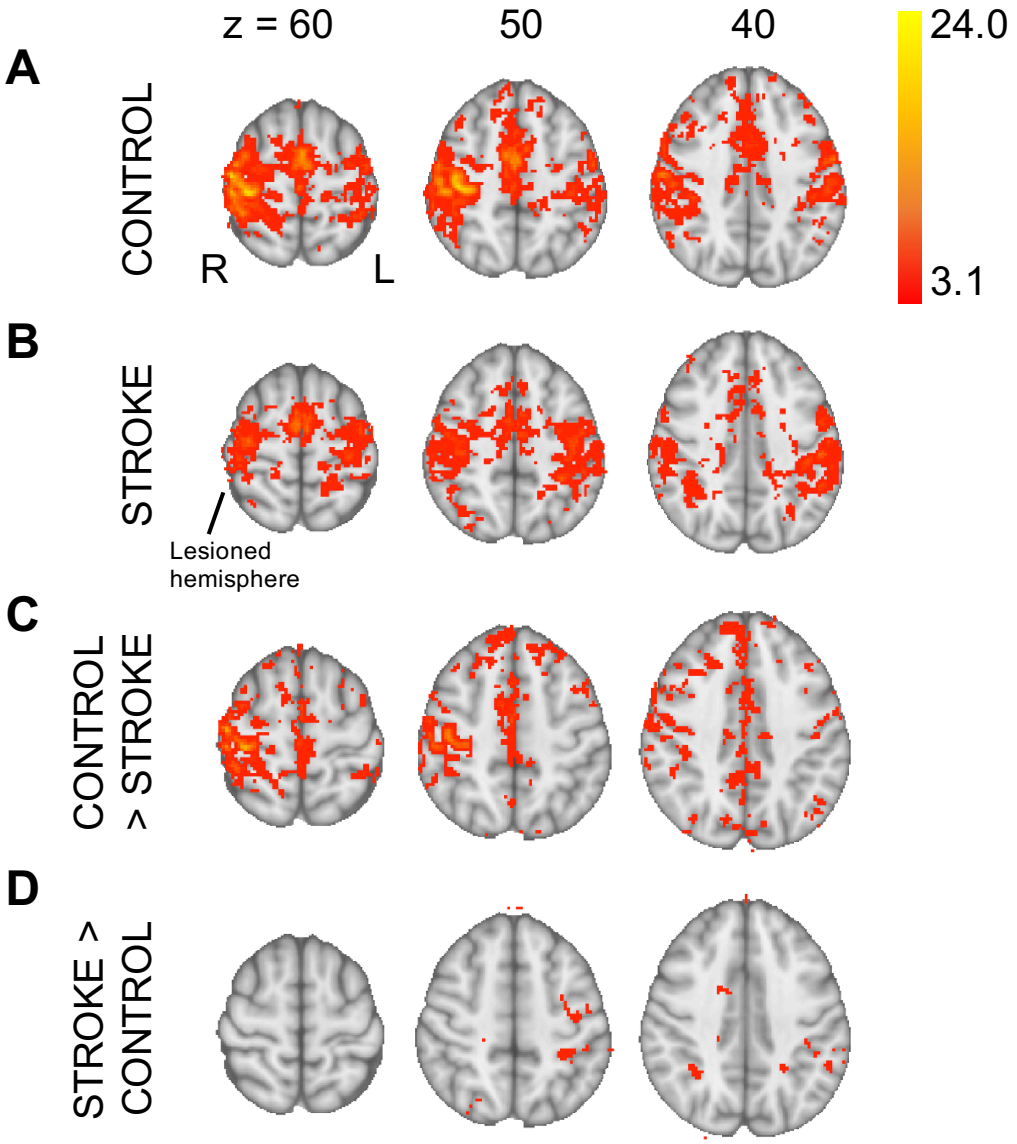

Figure 6.10: Group Mean activations for movement of the left hand in controls and the affected hand in stroke survivors: A: Group mean activation for age-matched controls showing higher contralateral (right) hemisphere activation. Some ipsilateral (left) hemisphere activation likely due to movement of the non-dominant hand. **B:** Stroke survivors show a much more balanced bilateral activation during movement of their affected hand. **C:** Activation map for areas more active in controls than stroke survivors, showing higher activity in the contralateral (right) hemisphere). **D:** Activation map for areas more active in stroke survivors than controls showing a small amount of ipsilateral (left) hemisphere activation.

In order to perform further investigation of which areas may be additionally active in stroke survivors during affected hand movement I performed a second higher level analysis, to contrast the Stroke>Control for Left>Right Hand movement (i.e. a contrast of contrasts). This revealed a region within the left hemisphere which was more active in stroke survivors when moving the affected compared to unaffected hand (Figure 6.11A).

A mask of this region was the created, and the average zstat value extracted for movement of each of the left and right hands for both groups (Figure 6.11B). Age matched controls showed greater activation within this ROI during right hand movement than left (Figure 6.11B, orange bars), whereas stroke survivors showed a more similarly matched activation during affected and less-affected hand movement (Figure 6.11B, red bars).

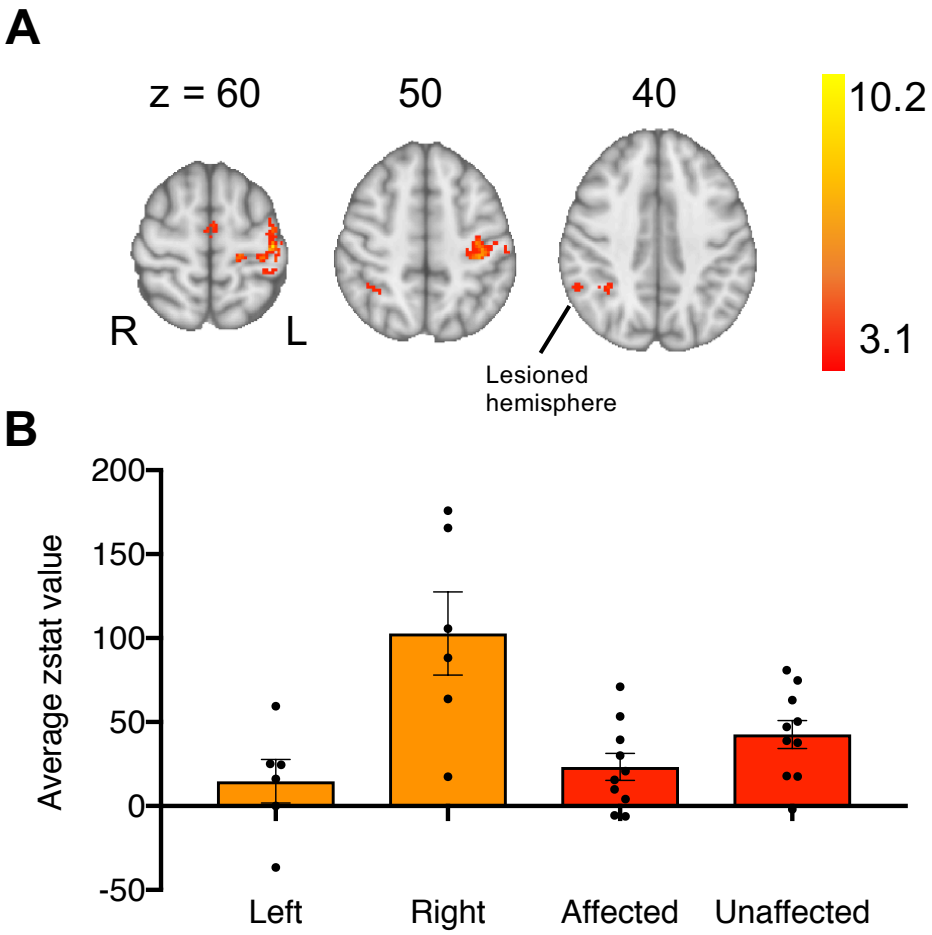

Figure 6.11: Increased left hemisphere activation in stroke survivors vs. controls during left (affected) hand movement: A: Stroke survivors show greater activity than age-matched controls in ipsilateral (left hemisphere) primary motor cortex when comparing areas which are more active during left (affected) hand movement than during right (unaffected) hand movement (i.e. (Stroke left>right) > (Control left>right); **B:** Bar chart showing the mean zstat values for movement of each hand in controls (orange) and stroke survivors (red). Error bars show standard deviation. As expected, controls show much higher activation of this left hemisphere region during movement of the right hand, whereas stroke survivors show a much more similar level of activation within this region during movement of both the affected and unaffected hand.

Laterality Index (LI) of Functional MRI Tasks

Using the data-driven ROI masks from the group mean activation, the percentage change in activity was extracted for left and right hand movement in both the stroke and age-matched control groups. The LI value is positive if the activity is lateralised in favour of hemisphere contralateral to the moving hand, close to zero if activity is bilateral, and negative if lateralised to the ipsilateral hemisphere.

For the Active Task (Figure 6.12), there was no difference in the LI for movement of the left and right hand within the age-matched controls ($W = 5$, $p = 0.6875$), however stroke survivors showed significantly more lateralised activations for movement of the less-affected hand than the affected hand ($W = 43$, $p = 0.0273$). For the Passive task (Figure 6.12), there was no significant difference for either controls ($W = 7$, $p = 0.5625$) nor stroke survivors ($W = 31$, $p = 0.1309$).

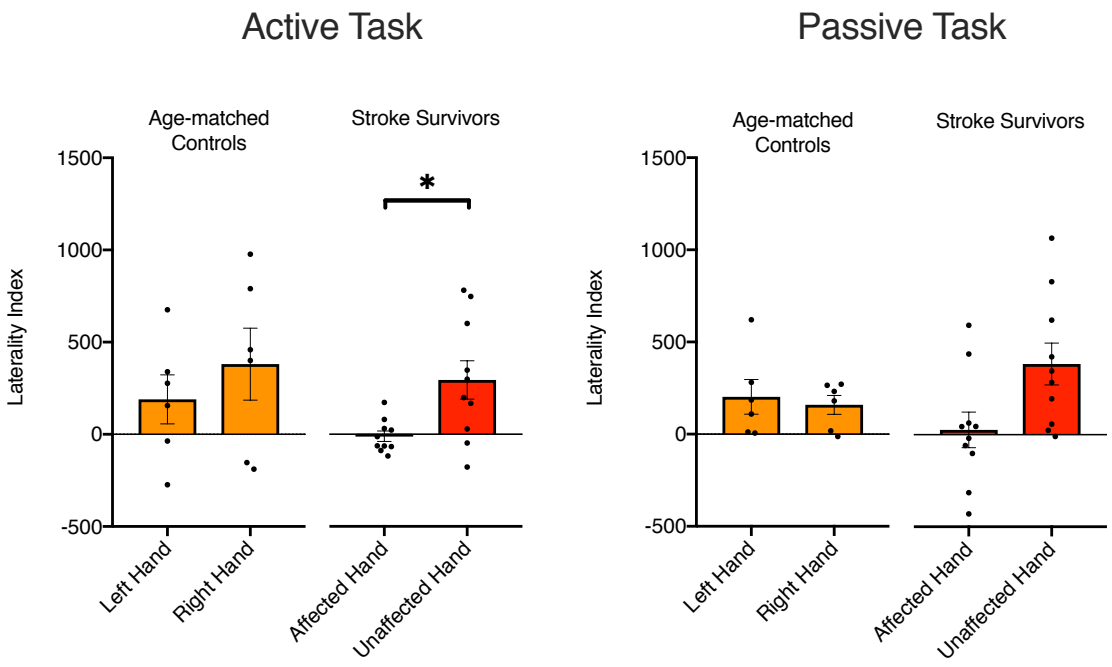

Figure 6.12: Laterality Index: Laterality Index for Active and Passive Tasks, calculated as the percentage change in the hemisphere contralateral to movement minus ipsilateral hemisphere. Asterisk (*) indicates significant difference between LIs ($p < 0.05$). Bars show mean ± SD.

No relationship between laterality index and task performance

In order to assess if there was any relationship between the degree of functional activation and performance on the EMG-FTT, the laterality index for movement of the task performing hand (affected hand in stroke survivors and left hand in controls) was compared to performance metrics of the EMG-FTT (baseline error and percentage change in error). There was no correlation between LI and either metric for either stroke (Baseline Spearman's $r = -0.3455$, $p = 0.3304$; Percentage change Spearman's $r = 0.2939$, $p = 0.4058$) or controls (Baseline Spearman's $r = 0.08571$, $p = 0.9194$; Percentage change Spearman's $r = -0.1429$, $p = 0.8028$).

MRSI Results

Extraction of metabolite concentrations

To assess differences in metabolite concentrations between groups and hemispheres, the average metabolite concentration within grey matter voxels of the MRSI/Feat ROI overlap region was extracted for GABA and Glx. In a small number of cases, it was not possible to identify any voxels which satisfied these criteria (S07, Right Hemisphere, all metabolites; S08, Left Hemisphere, all metabolites; S03, Right Hemisphere GABA only). Overlaps of the MRSI slabs, and Feat ROIs for each subject can be seen in Figure 6.13.

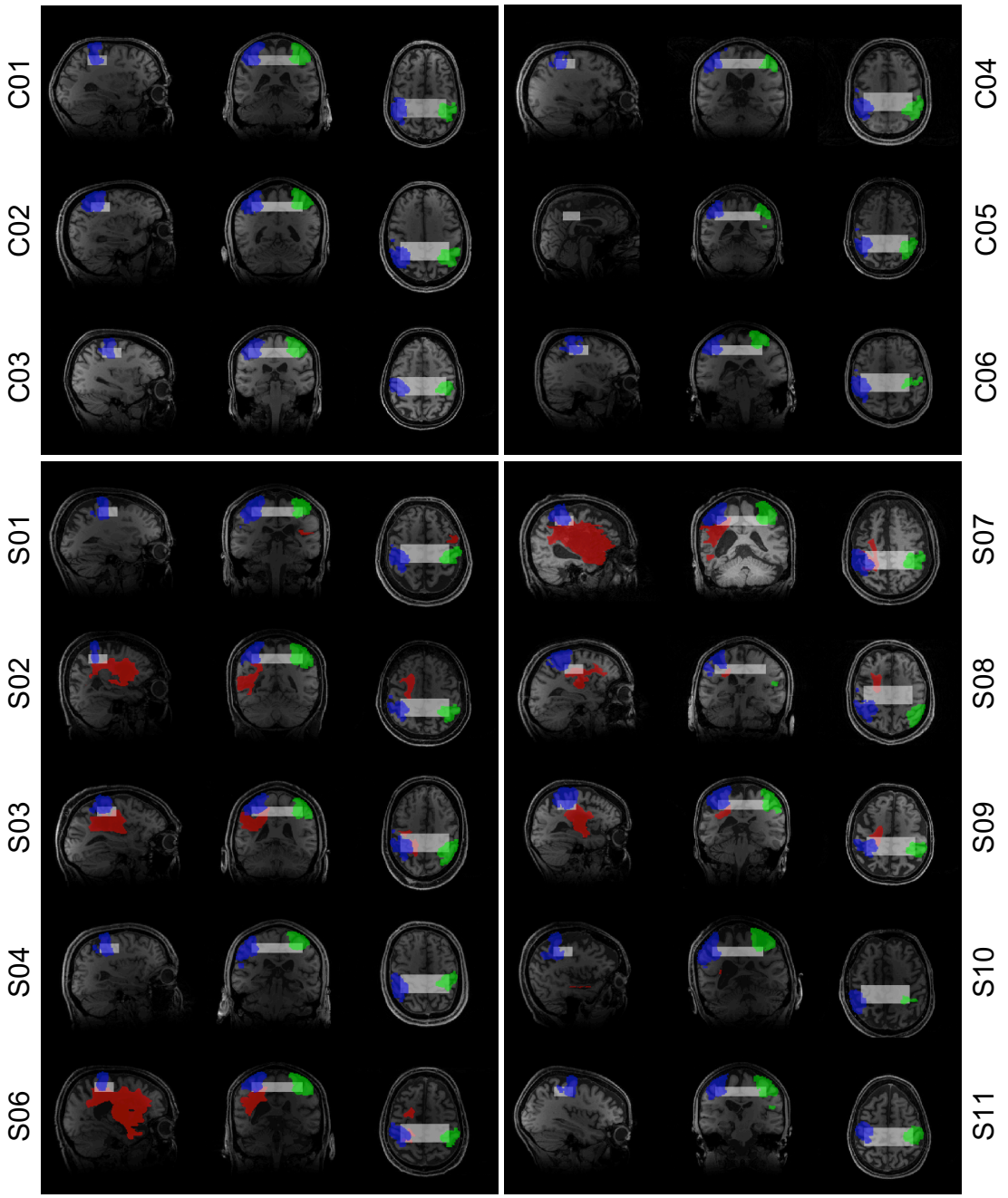

Figure 6.13: Overlaps of Feat ROIs and MRSI Slab: Overlaps for all subjects showing the data driven ROIs from Feat analysis in native space (Left hem = green, Right hem = blue), along with the MRSI slab location (white) and lesion masks for stroke survivors (red). S01 and S04 have left hemisphere lesions, all other stroke survivors have right hemisphere lesions.

Number of extracted voxels differs between Hemisphere, but not Group or Metabolite

A Linear Mixed Model (LMM) was run to check for any effects of Group (stroke vs. control), Metabolite (GABA, Glx) and Hemisphere (left, right) on the number of voxels included in the analysis. A main effect of Hemisphere was observed ($F_{(1, 36.388)} = 5.086$, $p = 0.030$). Within the Left Hemisphere, a main effect of Metabolite was observed ($F_{(1, 13)} = 9.000$, $p = 0.010$), however there was no main effect of Group ($F_{(1, 13)} = 1.510$, $p = 0.241$). There was no main effect of Group for similar analyses of Left Hemisphere GABA ($p = 0.256$) or Glx ($p = 0.246$). There were no main effects nor interactions of Group and Metabolite within the Right Hemisphere.

Neither GABA or Glx differ in concentration between Group nor Hemisphere

LMMs were run on the extracted metabolite concentrations for GABA and Glx separately, with factors of Group and Hemisphere). There were no significant main effects or interactions of Group and Hemisphere for either GABA or Glx (Figure 6.14): $GABA_{Group}$: $F_{(1, 12.579)} = 0.069$, $p = 0.796$, $GABA_{Hem}$: $F_{(1, 11.512)} = 1.615$, $p = 0.229$, $GABA_{Group*Hem}$: $F_{(1, 11.512)} = 0.101$, $p = 0.756$. Glx_{Group}: $F_{(1, 13.889)} = 0.688$, $p = 0.421$, Glx_{Hem}: $F_{(1, 13.709)} = 0.593$, $p = 0.346$, $Glx_{Group*Hem}$: $F_{(1, 13.709)} = 0.474$, $p = 0.503$.

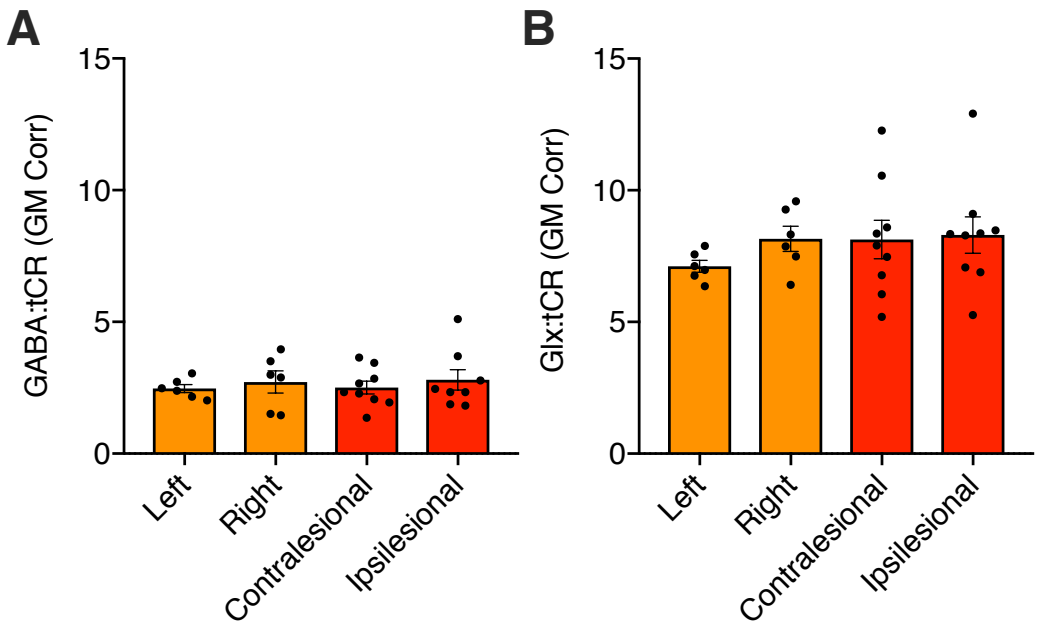

Figure 6.14: No difference in metabolite concentration between Groups or Hemispheres: LMM analysis of metabolite concentration revealed a significant main effect of Metabolite ($p < 0.0001$), but no main effects or interactions of Group or Hemisphere ($p > 0.2$) Bars show mean ± SD.

GABA concentration is correlated between hemispheres in controls, but not in stroke survivors

In order to assess if there was any relationship between concentrations of GABA in the left and right hemispheres, Spearman's correlations were run on both age-matched controls ($n = 6$) and stroke survivor's ($n = 7$) data. Due to missing data, three stroke survivors were excluded from this analysis. A significant correlation was found for age-matched control data (Spearman's $r = 0.943$, $p = 0.017$), but not for stroke survivor data (Spearman's $r = 0.536$, $p = 0.236$) (Figure 6.15). The two correlations were not significantly different when performing a two-tail Fisher's r-to-z transformation ($z = 1.53$, $p = 0.126$).

There were no correlations between hemispheres for either age-matched controls or stroke survivors for Glx (Controls: Spearman's $r = -0.771$, $p = 0.103$; Stroke: Spearman's $r = 0.476$, $p = 0.243$).

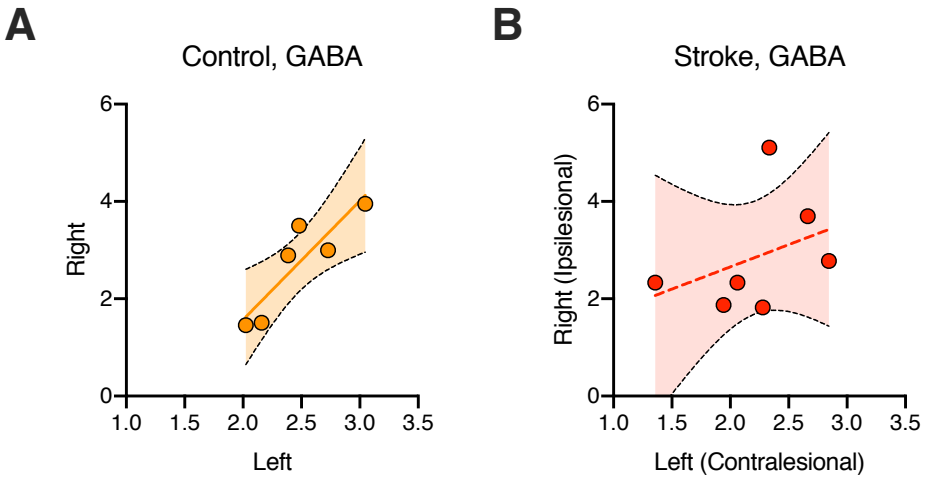

Figure 6.15: GABA concentration is correlated between hemispheres for age-matched controls, but not stroke survivors: GABA concentration was correlated between left and right hemispheres for age-matched controls (Spearman's r = 0.943, p = 0.017, n = 6) but not for stroke survivors (Spearman's r = 0.536, p = 0.236, n = 7). The two correlations are not significantly different from one another (Fisher's r-to-z: z = 1.53, p = 0.126).

No relationship between metabolite concentration and task performance

In order to assess if there was any relationship between the metabolite concentration and performance on the EMG-FTT, the concentrations of GABA and Glx in the hemisphere contralateral to the task performing hand was compared to performance metrics of the EMG-FTT (baseline error and percentage change in error). For GABA, there was no correlation between concentration and either metric for either stroke (Baseline Spearman's r = 0.3571, p = 0.3894; Percentage change Spearman's r = 0.1015, p = 0.8155) or controls (Baseline Spearman's r = -0.6000, p = 0.2417; Percentage change Spearman's r = -0.2571, p = 0.6583). For Glx, there were also no correlations between concentration and either metric for stroke (Baseline Spearman's r = -0.3500, p = 0.3586; Percentage change Spearman's r = -0.1741, p = 0.6521) or controls (Baseline Spearman's r = -0.2571, p = 0.6583; Percentage change Spearman's r = -0.02857, p > 0.9999).

Discussion

Overall summary

Here, results are presented for a preliminary, proof-of-principle study in a small cohort of chronic stroke survivors and age-matched controls. The main aim of this study was the development and testing of a novel motor skill task: the EMG-FTT. This task has been designed to overcome limitations within the stroke motor learning literature which could impact the interpretation of findings. By using surface EMG as a control interface for driving a motor learning task, participation is not restricted by functional ability in the stroke-affected limb.

As hoped, all participants understood, and were able to perform the EMG-FTT. At the group level, both age-matched controls and stroke survivors reduced their error from the first block of their task. On closer visual inspection of data, four of the stroke cohort did not improve beyond their baseline performance, and a further four were able to improve their performance by a similar percentage to the age-matched controls. For these subsets of participants, neither baseline performance nor improvement could be predicted by residual function in the stroke-affected upper limb. This is a crucial component of designing a motor learning task for use with stroke cohorts, as understanding changes in motor learning ability may help inform motor rehabilitation paradigms.

Functional MRI Tasks

As previously shown, during active movement of the affected limb, stroke survivors showed a more bilateral functional activation. This was not the case for age-matched controls, who showed lateralised activations for movement of both the left and right hands. While the degree of contralesional activation during affected hand movement was not related to EMG-FTT

performance, it has previously been demonstrated that the degree of activation is closely linked to residual function (Ward, Brown, A. J. Thompson, *et al.*, 2003) and longitudinal recovery (Ward, Brown, A.J. Thompson, *et al.*, 2003).

Magnetic Resonance Spectroscopic Imaging (MRSI)

Concentrations of neurochemicals such as GABA within the healthy human brain have been shown to be closely linked to behavioural performance (Edden *et al.*, 2009; Boy *et al.*, 2010; Sumner *et al.*, 2010; Stagg, Bachtiar and Johansen-Berg, 2011a; Kolasinski *et al.*, 2017), and a small number of studies have demonstrated differences between healthy and stroke cohorts in metabolite concentrations (Blicher *et al.*, 2015; Mooney *et al.*, 2019). These studies have previously only been able to measure an averaged neurotransmitter concentration in an anatomical region of interest specified by the researcher. In this study, we have implemented MRSI, which allows for higher resolution mapping of neurotransmitter concentrations across a larger section of cortex. Previously, MRSI has been hampered by long set up and acquisition times, as well as increased sensitivity to motion artefacts (see Chapter Two for further details). Recent developments have demonstrated that MRSI methods can be feasible in both healthy and patient cohorts (Steel *et al.*, 2018).

Here, MRSI is performed in a stroke cohort for the first time, demonstrating feasibility of the technique. Furthermore, due to the higher resolution of MRSI, it has been possible to extract metabolite concentration from the grey matter voxels that overlap with regions activated during a movement task. This allows for a much more specific quantification of metabolites from a region of cortex which is specifically involved in task performance. This method does have limitations, as highlighted by the small number of participants for whom metabolite concentration could not be extracted. This was due to a mixture of reasons, either lack of

overlap between the data-driven ROI and the MRSI slab, or removal of voxels within quality assurance checks.

Unlike a previous studies (Blicher *et al.*, 2015; Mooney *et al.*, 2019), this study did not show differences in the concentration of GABA between healthy controls and stroke survivors. It is feasible that this is due to the MRSI method used. In both previous studies, single-voxel spectroscopy was used with a relatively large region of cortex covered (in the region of 2 cm^3), whereas a more specific region of cortex is examined here. Furthermore, Blicher et al. studied a population of stroke survivors between three and 12 months post stroke, whereas the median time since stroke of the population here is over eight years post stroke. To date, no work has assessed changes in neurotransmitter concentrations across the course of stroke recovery, and therefore it is possible that neurotransmitter systems are different between sub-acute and chronic stages.

In this pilot study, no relationships were observed between neurotransmitter concentration at rest, and task performance (either baseline error or percentage change in error). Work in healthy controls from my thesis (Chapter Three / Kolasinski *et al.*, 2018) and elsewhere within the literature (Edden *et al.*, 2009; Stagg, Bachtiar and Johansen-Berg, 2011a; Kolasinski *et al.*, 2017; Frangou *et al.*, 2019) have related GABAergic concentration in the brain to aspects of behavioural task performance, however no such relationships were seen here.

The EMG-FTT: Thoughts, limitations and future directions

The data presented here for the EMG-FTT is limited, and there are a large number of potential future directions for this task to take. This study and it's analysis was designed to be a relatively simple proof-of-principle study in order to assess the task's feasibility and potential for use within a stroke cohort. Consequently, there are a number of aspects of the task's

design, data collection and analysis which can be developed, improved and investigated in the future.

Data collection

Within this cohort, the ECR was used as the task driver muscle for the EMG-FTT. This was chosen based on pilot testing sessions with young healthy and chronic stroke volunteers. It was found that in the pilot sessions and in the majority of the participants tested, the ECR was both much easier to record from, and participants found it easier to modulate than alternatives (such as the FCR). For one participant, they acknowledged that they personally would have preferred to use the FCR, as they have very low voluntary activation of the ECR in their affected arm.

Within the stroke cohort especially, compensatory activations are often seen during movement post-stroke, for example excessive use of the wrist, elbow and shoulder during movement of the hand. This was observed here, as well as wide ranging voluntary movements within the control cohort. I did not control for, nor restrict voluntary movement during task performance, however this is something that potentially should be controlled in future experiments. I did not want to use any equipment to restrict voluntary movement, as I wanted participants to perform as natural movements as possible. It is highly possible that by not restricting movement, co-activation of the ECR and FCR of the task-performing arm will have occurred. It is therefore possible that stretch-response of the FCR will have inhibited activation of the ECR, resulting in accelerated fatigue of the ECR. This will have altered the EMG signature of the ECR which in turn could affect task performance (as accuracy of tracking is reliant on controlled contraction/relaxation of the task-driver muscle). Very recent work by Mooney and colleagues (Mooney *et al.*, 2020) has reported successful use of a sequential isometric force task in chronic stroke survivors which could inform alterations of

task design. However, it should be noted that the reported ARAT scores of those stroke survivors in the Mooney study is 38-57 is much higher than those in the present study (range for stroke survivors in this study is 1-45). This is likely to be driven by the recording of neurophysiological measurements using TMS which would prevent a majority of low ARAT scoring stroke survivors from participating.

Data analysis

The analysis performed on the EMG-FTT data here is limited, with only the median error within each block examined. As this is an incredibly highly sampled dataset, with both error and EMG recordings taken, there is a large scope for examination of behavioural differences between groups, which is not fully explored here. The results presented here demonstrate a proof-of-principle for the feasibility of this novel task in both an older adult and chronic stroke cohort. Furthermore, as hoped, there was no relationship between residual functional ability (as measured by the ARAT) and individual performance. However, it is possible that the ARAT is not sensitive enough to distinguish differences between people with moderate severity who may use different compensation strategies, and therefore assessments of impairment (e.g. Fugl-Meyer assessment) may be an alternative measure to consider in the future.

The EMG recordings taken during task performance have not been analysed as part of this thesis due to time and space restrictions. This will be an incredibly rich dataset to examine, and will hopefully play an important role in deciding the future directions of this task. Additionally, the EMG dataset will be useful for investigating changes in co-activation of the ECR and FCR of the task-performing arm. This will subsequently inform any further data collection using this task, and any potential future studies. By examining the details of muscle activation patterns within the driver muscle, and co-activation patterns or muscle synergies

between the recorded muscles, it may be possible to identify other factors which affect behaviour, and show change associated with improvement in performance. In particular, those stroke survivors who show mirrored EMG signals in the contralateral ECR during task performance may also be those with reduced corticospinal tract integrity, and lower residual function in the affected limb. Another possible future development could be combined electroencephalography (EEG) recording during task performance in order to study cortico-muscular coherence, which has previously been shown to be disrupted in stroke cohorts (Rossiter *et al.*, 2013; Liu, Sheng and Liu, 2019).

Future directions

In the future, it would be prudent to perform detailed comparison of the EMG-FTT across age groups and to examine a large population of stroke survivors with a range of time since stroke, and residual functional levels. A main limitation of previous motor learning literature within the stroke population are the relatively small recruitment ranges. Most studies will recruit only acute, sub-acute or chronic stroke survivors, and a selected range of upper limb impairment based on functional assessments. The development of a flexible and intuitive task, such as the EMG-FTT may allow for more detailed investigation of the motor learning process after stroke. Furthermore, the comparison of different control interfaces, such as the more commonly used joysticks and force-transducers, with surface EMG will allow for better characterisation of the task, and it's feasibility of use within healthy and patient populations.

Chapter 7: Discussion

Overview of this thesis

This thesis comprises a series of experiments performed to expand our current understanding of the neurophysiological underpinning of motor learning in health and disease. These experiments are particularly concerned with the role of inhibition in healthy motor learning (Chapter Three), and the role of the ipsilateral (or contralesional) hemisphere in learning. Chapters Four and Five use cathodal tDCS to investigate the ipsilateral motor cortex in healthy subjects, while Chapter Six introduces a novel motor learning task for use in both stroke survivors and controls.

Chapter One of this thesis began by outlining the current literature surrounding neuroplasticity in the motor system and the role of inhibition (and specifically the role of GABA) in human motor learning. I then discussed how researchers have identified methods to modulate learning in both the healthy and post-stroke brain in attempts to further elucidate how we might improve practice for motor rehabilitation after stroke. NIBS methods such as tDCS have been used relatively extensively to alter motor skill learning and have attracted attention for their potential as an adjunct therapy for motor rehabilitation. The final section of my introduction to this thesis highlighted how our understanding of motor learning in the post-stroke brain is a relatively understudied area of research, and how gaining a more detailed understanding of the role of the contralesional hemisphere might enable development of better algorithms to design the best rehabilitation strategies.

In Chapter Two, I have detailed the methods I have used within my experimental chapters. I have been lucky to use a wide variety of methods throughout my DPhil, including multimodal

MR scanning at both 3 and 7 Tesla and NIBS techniques including tDCS and TMS. As motor learning is a core element of the work of this thesis, I have given a brief overview of the two task types used within my research. The SRTT is used for the experiments in Chapters 3, 4 and 5, while Chapter Six details the early pilot work I have performed using a novel motor task: the EMG-FTT.

The experimental work of my thesis begins in Chapter Three, where ultra-high field MRS is used to study dynamic changes in GABA within the sensorimotor cortex during motor skill learning. This is an extension of previous work (Floyer-Lea, 2006) and has been published during the course of my DPhil (Kolasinski *et al.*, 2018). Here, I found a significant reduction in the concentration of GABA was specific to a motor skill learning task, and not observed during a non-learning version of the same task, nor when participants lay at rest in the scanner.

Chapters Four and Five report a pair of experiments to assess the role of the ipsilateral motor cortex during motor learning in the presence of a virtual lesion caused by cathodal tDCS targeted to the task-dominant left sensorimotor cortex. The data for the first study (Chapter Four) was collected prior to the commencement of my DPhil, however my analysis of this data has found a significant increase in ipsilateral hemisphere activity during task performance, which is only seen when cathodal tDCS is delivered offline, prior to the task. The same result is not seen with online left hemisphere tDCS. Chapter Five then detailed a follow up experiment I performed to elucidate the neurophysiological underpinnings and possible behavioural role of this ipsilateral activity. In this study, TMS was used to measure bilateral cortical excitability and neurotransmitters systems before and after both cathodal tDCS and motor learning. While no changes in cortical excitability were observed in this study, relationships were found between behaviour and TMS measures, which could provide insight

into the neurophysiological changes caused by cathodal tDCS and how these may relate to behaviour.

In the final experimental chapter of my thesis (Chapter Six), I have presented the results of a proof-of-principle study in a small cohort of chronic stroke and age-matched healthy controls. Motor learning literature in stroke survivor populations is a relatively understudied area of research despite the clear links from motor learning to motor rehabilitation. The majority of studies performed to asses motor learning post-stroke have not studied those with the more severe motor impairments, due to their inability to perform the volitional movements necessary for task performance. I have demonstrated that the EMG-FTT can be performed by stroke survivors across a range of functional abilities, and that their residual ability is not correlated with performance. In this Chapter, I have also reported results from a multimodal 3T MR scan performed on the same participants, demonstrating the feasibility for use of recently developed MRSI sequences in a stroke cohort and, for the first time, extracted metabolite concentrations from grey matter voxels which directly overlap with regions activated during a movement task. This allows for a much more specific quantification of metabolites from a region of cortex which is specifically involved in task performance. By developing tasks which can assess motor learning ability, and MR methods which can measures changes in brain structure, function and neurochemistry, we will be able to explore if and how motor learning neurophysiology changes following a stroke, thereby enabling better development of adjunct therapies to improve rehabilitation.

Future directions

Assessment of the role of ipsilateral motor cortex in healthy motor learning

The results of Chapters Four and Five highlight that left-hemisphere cathodal tDCS delivered prior to task performance seems to result in changes within the non-stimulated, right, hemisphere. These results could be strengthened by further work, as both studies were performed in small cohorts and display relatively heterogenous results across participants. The brain stimulation literature has, over the course of my DPhil, reported ever more inter- and intrasubject variability (López-Alonso *et al.*, 2014; Wiethoff, Hamada and Rothwell, 2014; Chew, Ho and Loo, 2015; Dyke *et al.*, 2016), which is an important factor when considering possible translation of these techniques to a clinical setting.

Further investigation of the usefulness of the EMG-FTT

The results presented in Chapter Six using the EMG-FTT in a small chronic stroke cohort are clearly a small introduction to the possible uses for this task. Important future work should be done to assess performance on the EMG-FTT in a much larger stroke cohort, with a wide range of residual functional ability and at different times since stroke (in particular at the acute or subacute stages where the majority of rehabilitation takes place). More careful classification of functional abilities (for example by use of the Upper-Limb portion of the Fugl-Meyer Assessment rather than the ARAT) would enable better interpretation of any results. Furthermore, direct comparison of EMG with other control interfaces (such as joysticks and force transducers) should be performed in both healthy controls and stroke survivor cohorts, to allow for better characterisation of the task itself, and its feasibility for all participant populations.

The role of the contralesional motor cortex in stroke recovery

The contralesional hemisphere's role in motor recovery after stroke is already a widely investigated area, with various theories for its adaptive or maladaptive role discussed extensively within the literature. More recent research has suggested that, as would probably be expected, there is no clear role for this part of the brain following a stroke. It seems for some stroke survivors the contralesional hemisphere may prevent true recovery within the ipsilesional hemisphere whereas for others it is the only hope for regaining any movement in the paretic upper limb. A key step for future research is the development of our understanding of what neurophysiological systems might predict into which group stroke survivors will fall. Development of methods such as the MRSI used in Chapter Six, and tasks which can assess learning potential (such as the EMG-FTT) could be beneficial for this goal.

Key findings from this thesis

- In Chapter Three, I reported evidence of a reduction in left sensorimotor GABA which was specific to performance of a motor learning task. The methodology used (multiple sequential blocks of 7T-MRS) is a significant advancement on previous similar findings. Furthermore, a significant relationship was observed between magnitude of GABA and behavioural performance, which provide insight into the neurochemical correlates of cortical plasticity associated with motor learning. This work has been published during the course of my DPhil: Kolasinski J.*, **Hinson E. L.*,** Zand A. P. D., Rizov A., Emir U. E. and Stagg C. J. The dynamics of cortical GABA in human motor learning. *J. Physiol.* (2018).
- In Chapter Four, offline cathodal tDCS to the left hemisphere delivered prior to task performance with the right hand resulted in increases in remote right hemisphere

learning related activity, without local left hemisphere effects. The same result was not observed with online left hemisphere cathodal tDCS.

- In Chapter Five, I showed relationships between behavioural performance and changes in neurophysiological measures of cortical excitability and neurotransmitter systems in response to the same tDCS paradigm used in Chapter Four. This provides interesting insight to how the remote effects observed in Chapter Four might be produced.
- In Chapter Six, I have discussed a new motor task I developed to assess learning ability in both stroke survivor and healthy participant cohorts. This is a novel task, and as such, the findings are limited. However, I have shown the ability of all participants to perform the task.
- Also in Chapter Six, I have used very recently developed MRSI techniques in a stroke cohort for the first time. The results of this study demonstrate the feasibility to perform this method in a patient cohort, and the potential for this technique to be used further to investigate neurotransmitter concentrations across the brain, rather than in more limited areas as is possible with conventional single voxel MRS methods.

Taken together, the findings of this thesis provide additional insight into the process of motor learning in the healthy brain, both during normal motor learning and when cortical excitability is modulated using NIBS techniques such as tDCS. These findings are important when considering how motor rehabilitation strategies for stroke survivors are developed. It is critically important that we understand how healthy learning takes place, and how that process is changed following a stroke.

Chapter 8: Supplementary Data

Assessing sequence difficulty for Serial Reaction Time Task

In Chapters 4 and 5, a Serial Reaction Time Task (SRTT) is performed as a method to assess the effects of cathodal tDCS on motor skill learning. Here, the sequences used within for the SRTT are tested to confirm equivalent difficulty of learning.

Methods

Participants

10 participants (mean age 20.6 ± 0.5 years, 5 female) gave informed consent to take part in the study in accordance with Central University Research Ethics Committee approval (Ethics Ref: University of Oxford MSD-IDREC-C1-2015-235). All participants were right handed (as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971)), with no history of neurological or psychiatric disorders, or contraindications to TMS or tDCS.

Experiment Outline

All participants completed a behavioural motor learning task (the Serial Reaction Time Task (SRTT) as previously described (Robertson, 2007; Stagg, Bachtiar and Johansen-Berg, 2011a)) in three testing sessions, each separated by at least seven days.

Task details were identical to those described in Chapters 4 and 5. In short, participants performed a visually cued SRTT comprising of 17 blocks. Each block lasted 30 seconds and consisted of 30 button presses. Cues were presented for 850 ms with an 150 ms interstimulus interval. Task blocks were separated by a 30 second enforced break. Blocks 1 and 15 of the

task were random blocks, with no learnable sequence. All other blocks consisted of three repeats of a 10-item sequence, which participants were explicitly informed to expect, and to learn. After task performance, participants were asked to recall the sequence to demonstrate learning.

SRTT Analysis

Response Time (RT) was calculated as the time of cue onset to the time of correct button press. Anticipatory responses (i.e. those where a correct response was recorded before the cue appeared), and those where the first recorded button press was incorrect were excluded. Any blocks with fewer than 10 correct responses were removed.

During pre-processing of the RT data one participants had a very low number of recorded 'correct responses' (< 10 correct from 30) in a number of blocks. This was caused by anticipatory responses where the correct button press was recorded before the cue appeared. This was recorded as an incorrect response, even though the participant was capable of learning the required sequence (as demonstrated by correct explicit recall of the sequence at the end of the SRTT performance). Therefore, data from this subject was excluded from further analysis.

Statistical Analysis

Data preprocessing was performed in MATLAB 8 software (version R2018b; The MathWorks). Statistical analyses were performed Prism (version 8; GraphPad software), and SPSS (version 24; IBM). Greenhouse-Geisser corrections were applied as required. Effect size estimates were computed using partial η squared (partial η^2) for RM-ANOVAs and Cohen's d_z for *t* tests.

Results

SRTT Results

No difference between sequences

A Repeated Measures-ANOVA with factors of Block and Sequence revealed the expected main effect of block, suggesting that subjects learnt the sequence. ($F_{(3.623, 28.983)} = 41.612$, $p < 0.001$, partial $\eta^2 = 0.839$) (Figure 8.1A), However, there was no main effect of sequence ($F_{(1.941, 15.527)} = 0.118$, $p = 0.884$, partial $\eta^2 = 0.015$), nor interaction of Block*Sequence ($F_{(5.437, 43.496)} = 0.718$, $p = 0.624$, partial $\eta^2 = 0.082$), suggesting that there was no significant difference between the sequences.

Rate of learning was calculated as the gradient of a linear best fit line fitted to all sequence blocks. "Peak Performance" was defined as the minimum RT achieved, no matter which block this occurred in. No difference in rate of learning was observed between the three sequences (RM-ANOVA testing for effect of sequence on rate of learning: $F_{(1.458, 11.660)} = 0.275$, $p = 0.696$, partial $\eta^2 = 0.033$) (Figure 8.1B), and no significant difference in Peak Performance RT between the sequences (RM-ANOVA testing for effect of sequence on Minimum RT: $F_{(1.391, 11.125)} = 0.149$, $p = 0.787$, partial $\eta^2 = 0.018$) (Figure 8.1C).

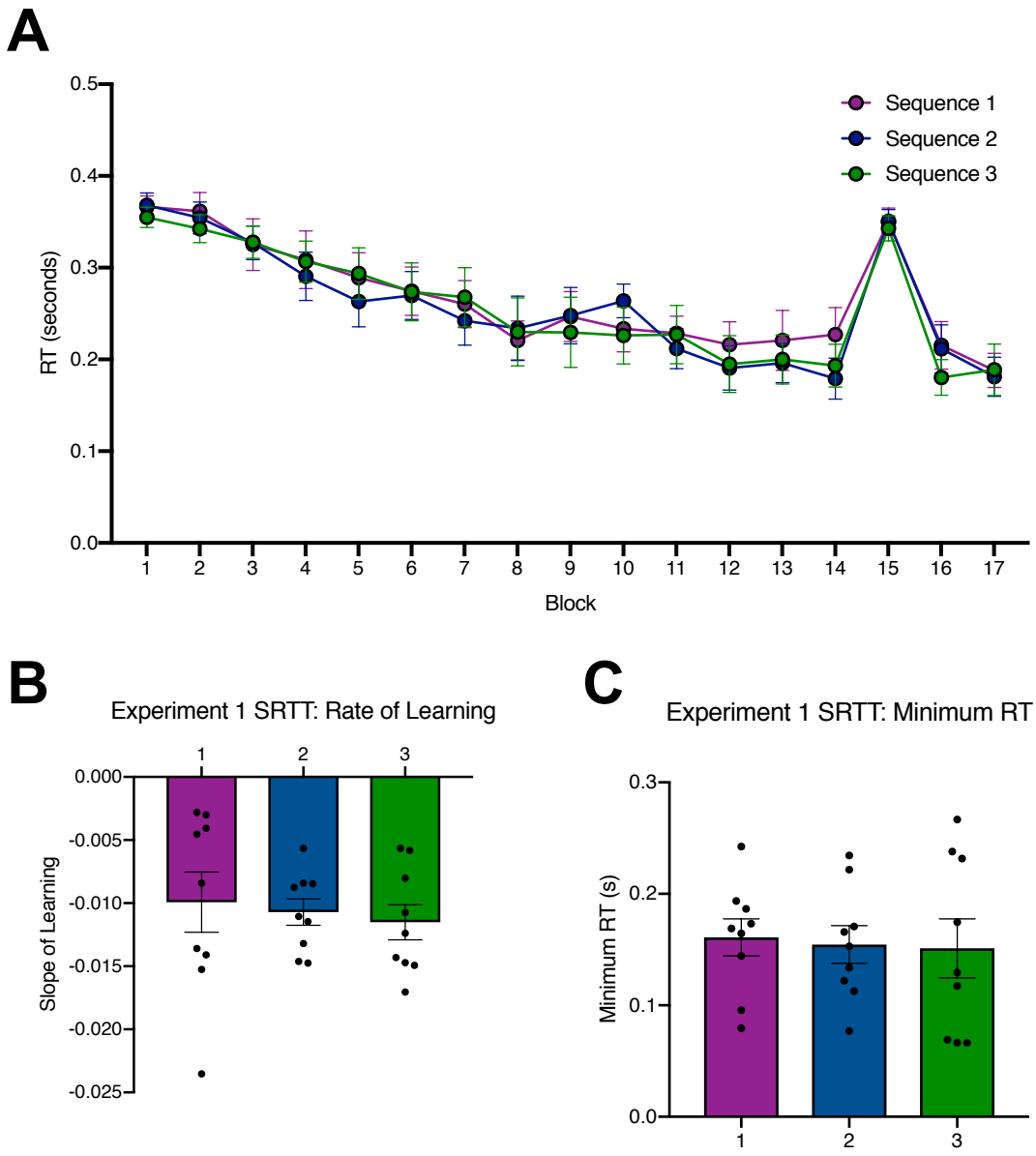

Figure 8.1: SRTT Response Time (RT) data split by tDCS condition: A: RT data showed a significant main effect of Block, with no significant main effect of Sequence. Blocks 1 and 15 are Random blocks (visual cues in a random order). All other blocks are Sequence blocks (cues presented in a repeating sequence with explicit instructions to expect, and learn). **B:** No effect of sequence on rate of learning (slope of linear best fit for sequence blocks). **C:** No effect of sequence on peak performance (minimum RT achieved). Error bars = SEM. Data points in B/C indicate individual subjects.

Significant effect of session order on SRTT performance

A second RM-ANOVA with factors of Block and Session was performed to check for session-on-session improvement. A significant main effect of block was found ($F_{(3.623, 28.983)}$ = 41.612, $p < 0.001$, partial $\eta^2 = 0.839$) as above, reflecting learning. There was also a significant

main effect of session ($F_{(1.571, 12.556)} = 53.109$, $p < 0.001$, partial $\eta^2 = 0.869$) and a significant interaction of Block*Session ($F_{(5.778, 46.225)} = 3.102$, $p = 0.013$, partial $\eta^2 = 0.279$) (Figure 8.2A).

Follow up paired T-Tests revealed RT in Block 1 of Session 1 was significantly slower than the same block in both Session 2 ($t = 2.320$, $p = 0.049$, $d_z = 0.773$), and Session 3 ($t = 4.126$, $p = 0.003$, $d_z = 1.376$). There was no significant difference between Block 1 RTs for Session 2 and 3 ($t = 0.640$, $p = 0.540$, $d_z = 0.213$) (Figure 8.2B). Given these differences between sessions in terms of behaviour, session number was included in all analyses of behavioural data in Chapters 4 and 5.

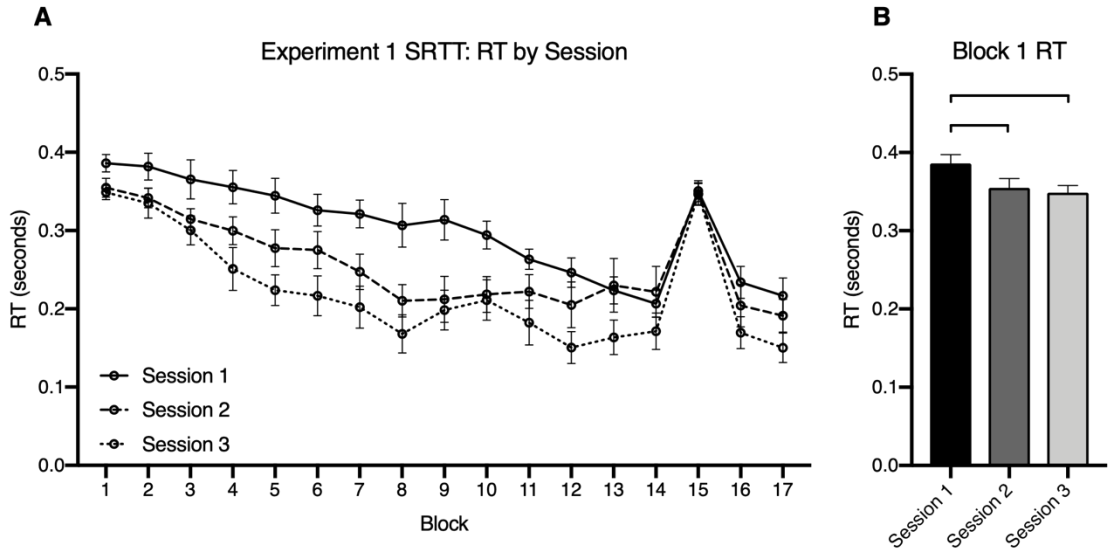

Figure 8.2: SRTT Response Time (RT) data split by session: A: Significant main effects of Block and Session. Error bars indicate SEM for each block. **B:** Paired T-Tests revealed Block 1 RT for testing Session 1 was significantly slower than both Session 2 and 3. Bars indicate mean RT across participants, error bars are SEM.

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