Investigating the role of inhibition in healthy human motor system plasticity



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Abstract

Across our lifespan we encounter novel physical problems that must be overcome by learning to produce new movements, or to adapt the movements we already know. Learning or adaptation of these skills is the result of plastic changes occurring across a network of motor areas within the brain. The factors influencing these changes across the brain, and how they link to behaviour are not currently well understood. This multimodal thesis primarily focuses on elucidating the link between motor system inhibition, plasticity, and motor learning. It does so by using magnetic resonance spectroscopy (MRS) and transcranial magnetic stimulation (TMS) to measure inhibition, and using a variety of tasks to assess motor learning.

Firstly, the neurochemical changes induced by the 'plasticity enhancing' intervention, transcranial direct current stimulation (tDCS), were investigated. We found that tDCS caused changes in inhibition across the motor network, and that these changes were related to individuals' white matter microstructure. Next the effect of primary motor cortex (M1) tDCS on skill learning with the ipsilateral hand was probed. Here we found that anodal tDCS caused impairment of learning, but this may be offset, in part by decreases in inhibition occurring in the contralateral M1. Finally, we explored the effect of directly modulating inhibitory signalling on healthy human motor learning. We found that using a single, clinically-relevant, dose of baclofen to increase inhibitory signalling caused impairments in performance on an adaptation task. Behaviour changes in this task were related to the degree of inhibition change within the contralateral M1.

Taken together this work strengthens support for the theory that reducing inhibition within M1 is vitally important for allowing the normal plastic processes underpinning motor learning to occur. However, these results also highlight the importance of inhibition changes across the motor network. Investigating these changes, and how they affect network interactions is crucial if we are to understand how healthy motor learning occurs, and leverage this understanding to enhance learning in health and disease.

This thesis is approximately 48,000 words

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Related Publications

The below contain some material overlapping with Chapter 1 and Chapter 2

- **Johnstone, A.*,** Levenstein, J.*, Hinson, E., & Stagg, C. J., 2017. Neurochemical changes underpinning the development of adjunct therapies in recovery after stroke: A role for GABA?. In *Journal of Cerebral Blood Flow and Metabolism.*

- **Johnstone, A.,** Hinson, E., & Stagg, C. J., 2016. tDCS and Magnetic Resonance Imaging. In *Transcranial Direct Current Stimulation in Neuropsychiatric Disorders*

These publications contain some of the data and results also featured in Chapter 4

- Bachtiar, V.*, **Johnstone, A.*,** Berrington, A., Lemke, C., Johansen-Berg, H., Emir, U. and Stagg, C.J. 2018. Modulating regional motor cortical excitability with non-invasive brain stimulation results in neurochemical changes in bilateral motor cortices. *Journal of Neuroscience*

- Bachtiar, Velicia. 2014. Transcranial direct current stimulation of the human primary motor cortices. *DPhil Thesis, Clinical Neuroscience, University of Oxford*

The following contains aspects of the data included in Chapter 6

- Grigoras, Ioana. 2018. The effect of a single, clinically-relevant, dose of baclofen on human motor learning. *MSc Dissertation, Neuroscience, University of Oxford*

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine Triphosphate
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
$CMRO_2$	Cerebral Metabolic Rate of Oxygen Consumption
Cr	Creatine
CS	Conditioning Stimulus
CSF	Cerebrospinal Fluid
DA	Dopamine
DLPFC	Dorsolateral Prefrontal Cortex
dPMC	Dorsal Premotor Cortex
DWI	Diffusion Weighted Imaging
EEG	Electroencephalogram
EMG	Electromyography
FA	Fractional Anisotropy
FID	Free Induction Decay
GABA	γ-aminobutyric acid
GM	Grey Matter
ICF	Intra-Cortical Facilitation
ISI	Inter-Stimulus Interval
LICI	Long Interval Cortical Inhibition
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MD	Mean Diffusivity
MEP	Motor Evoked Potential
MPRAGE	Magnetisation Prepared Rapid Acquisition Gradient Echo
MRI	Magnetic Resonance Imaging
fMRI	Functional Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NA	Noradrenaline
NAA	N-acetyl Aspartate
NIBS	Non-Invasive Brain Stimulation
NMDA	*N*-methyl-D-aspartate

OEF	Oxygen Extraction Fraction
PAS	Paired Associative Stimulation
PET	Positron Emission Tomography
PMA	Premotor Areas
PMd	Dorsal Premotor Cortex
PMv	Ventral Premotor Cortex
PRESS	Point Resolved Spectroscopy
RF	Radio Frequency
RT	Reaction Time
SI	Stimulation Intensity
rSI	Resting Stimulation Intensity
aSI	Active Stimulation Intensity
S1	Primary Somatosensory Cortex
SICI	Short Interval Cortical Inhibition
SMA	Supplementary Motor Area
SMC	Supplementary Motor Cortex
SNR	Signal-To-Noise Ratio
SPL	Superior Parietal Lobule
SRTT	Serial Reaction Time Task
SSRI	Selective Serotonin Reuptake Inhibitor
tACS	Transcranial Alternating Current Stimulation
TBS	Theta-Burst Stimulation
iTBS	Intermittent Theta-Burst Stimulation
cTBS	Continuous Theta-Burst Stimulation
tDCS	Transcranial Direct Current Stimulation
TE	Echo Time
TMS	Transcranial Magnetic Stimulation
spTMS	Single-Pulse Transcranial Magnetic Stimulation
ppTMS	Paired-Pulse Transcranial Magnetic Stimulation
rTMS	Repetitive Transcranial Magnetic Stimulation
TR	Repetition Time
TS	Test Stimulus
VAPOR	Variable Power and Optimized Relaxation Delays
WM	White Matter

Chapter 1: Motor learning and the brain

"We have a brain for one reason and one reason only -- that's to produce adaptable and complex movements. Movement is the only way we have of affecting the world around us… I believe that to understand movement is to understand the whole brain. And therefore it's important to remember when you are studying memory, cognition, sensory processing, they're there for a reason, and that reason is action."

- Daniel Wolpert

Humans are capable of producing dexterous and flexible actions to a degree unmatched by any other species. From tying our shoelaces in the morning, to typing on keyboards at work, to flicking through the pages of a book as we fall asleep, we have created a world where fine motor skills, particularly of the hand, are essential to daily life. Loss of these abilities, caused by stroke or brain injury, can therefore have disastrous consequences on quality of life and independence, so improving methods for rehabilitation, or relearning of lost skills, is clearly of high importance. In the chronic phase of stroke, after any initial spontaneous biological tissue recovery has ceased, functional improvements, whether the result of true relearning or compensation, occur through a motor learning process (Krakauer, 2006). It is therefore vitally important that we understand how motor skill learning happens in the healthy brain, and the influence that internal and external factors can have on this learning, if we are to begin designing methods to improve rehabilitation. In this chapter the variety of approaches that are used to assess motor learning in humans will be discussed, as well as the physiological processes underlying this learning.

1.1 Assessing motor skill learning in the lab

Motor skill learning can be defined as improvements in the speed, accuracy or automaticity of producing a novel sequence of movements or muscle contractions. Different interpretations of this definition have given rise to the development of a number of paradigms for assessing motor skill learning in the lab. One set of approaches, referred to as motor sequence learning, focuses on assessing participant's ability to link together a sequence of well-practiced movements (Willingham et al., 1997). Another set of motor tasks focus on assessing improvements in the accuracy of visuomotor mapping, i.e. how a movement's force or direction translates into visual space. These visuomotor tasks fall into two main groups, force tracking tasks, and visuomotor adaptation tasks. This chapter will discuss each of these two main types of motor task, and what is known about the brain processes responsible for improvements in performance.

1.1.1 Motor sequence learning

The almost automatic pattern of movements required to type in your computer password was at some time acquired through a process of motor sequence learning. Arguably the simplest of the motor sequence learning tasks is known as the serial reaction time task (SRTT), where participants perform cued button presses using two or more fingers (Nissen and Bullemer, 1987). The cues presented follow a repeating sequence which the participant can either be informed of, termed explicit learning, or remain naïve to, termed implicit learning (Robertson et al., 2004). Sequences tend to be between 8-16 presses long, and can contain learnable temporal patterns as well as patterns of digit responses (Kornysheva and Diedrichsen, 2014). Learning can be quantified through improvements in reaction time, or improvements in speed-accuracy trade-offs, in response to the presented cues. These can be contrasted against changes in the same measures to cues presented in a random order, to enable removal of the effects of general performance changes unrelated to sequence learning.

As well as being an assessment of motor learning, the SRTT has also been argued also to rely on perceptual learning. Performance on the SRTT could be conceptualised as participants learning

the sequence of visual presented cues, and reaction times improving as participants are able to predict the visual cue and therefore respond to it more quickly (Robertson, 2007). While learning of SRTT-like tasks can occur in the perceptual domain (Dennis et al., 2006), learning has been demonstrated to have a motor component (Willingham, 1999). In addition to perceptual learning, if participants are aware of the presence of the sequence, either by instruction or by self-identification, then learning also involves declarative memory processes. It has been argued that the declarative and motor learning processes occur in parallel (Willingham and Goedert-Eschmann, 1999), but that the two forms of knowledge may interact (Brown and Robertson, 2007; Robertson et al., 2004; Tunovic et al., 2014). See Chapter 3 for a full discussion of this literature.

Other sequence learning task variants include the finger-tapping task where participants must produce a short, un-cued sequence of presses as quickly as possible (Wiestler and Diedrichsen, 2013). Finger-tapping tasks require the participant to have initial declarative knowledge of the sequence, normally around 5 presses long, but from then on performance improvements have less reliance on explicit learning processes.

The fact that most sequence learning tasks do not require learning of any novel movements, and instead rely on the linking together of well-learnt movements, can be considered both a strength and a weakness. Tasks can be easily repeated over multiple sessions by simply changing the order of the repeating sequence; however they lack ecological validity, as they do not require the learning of a novel movement.

1.1.2 Sensorimotor Mapping Tasks

Learning to adapt the strength or direction of your tennis serve in response to a strong wind can be thought of as an example of an adaptation of a sensorimotor mapping. These two aspects of sensorimotor mapping can be assessed in the lab by using either force tracking or visuomotor adaptation tasks. In force tracking tasks participants must learn to produce squeezes, pinches or

muscle contractions to control an onscreen cursor. They then train to guide the cursor to track a sequence of targets. These tasks require the learning of novel movement dynamics and novel visuomotor mapping, making them arguably closer to real-world motor learning. However this learning process can only occur once, meaning that repeating the task over multiple sessions can lead to large carry-over effects (Willerslev-Olsen et al., 2011). This issue can be circumvented by training participants on the visuomotor mapping prior to the first assessment of learning, and then altering the sequence of forces required across sessions. However this method removes the assessment of learning novel movement dynamics, and reduces the task to being equivalent to a more complex sequence learning task. Force tracking tasks have not been used in this thesis and so will not be discussed herein.

The other forms of sensorimotor mapping task involve visuomotor or force field adaptation. In these tasks participants make movements of a joystick, handle, or mouse to guide an onscreen cursor to target points. During these tasks a perturbation is introduced, either by creating force field to resist or alter the participants movement, or by changing the mapping of the produced movement relative to that of the displayed onscreen cursor. Like sequence learning tasks, visuomotor mapping tasks do not generally involve the acquisition of new muscle patterns, and rather depend on well-learnt movements.

Visuomotor adaptation relies mainly on implicit processes (Krakauer, 2009): participants can improve their performance without being aware of the task structure, or even aware that they are making systematic errors (Kagerer et al., 1997; Klassen et al., 2005). Despite this tasks can be manipulated to bring explicit learning processes in play (Taylor et al., 2014) which can alter both the learning (Mazzoni and Krakauer, 2006) and the retention of the visuomotor mapping (Haith et al., 2015). Under normal circumstances error reduction is thought to occur through minimising visual prediction error via a forward model. Such a model takes the efference copy of motor control signal and outputs the predicted position of the body, or cursor, based on previous knowledge about the body or cursor's position. This predicted position is then compared with

the actual position to produce the visual prediction error, which is then fed back to adjust the subsequent movement.

Adaptation to visuomotor perturbations tends to show poor generalisation to other settings, but can persist for multiple hours or even days (Criscimagna-Hemminger and Shadmehr, 2008), and produce aftereffects (Caithness et al., 2004), albeit with gradual unlearning. For comparison of adaptation at multiple timepoints it is therefore important that participants fully deadapt from the perturbation before retesting. This can occur in multiple ways, either by removing the perturbation and allowing the participant to readapt to the natural state, by eliminating movement errors through introducing movement clamp trials, or by removing visual feedback (Kitago et al., 2013). Another important point for consideration is the presence of savings, where previous exposure and adaptation to a perturbation results in faster adaptation when the perturbation is re-introduced. Savings during a second exposure tend to be reduced when participants undergo unlearning after first exposure via one of the mechanisms mentioned above, in addition to allowing decay of the learnt adaptation between exposures (Kitago et al., 2013).

1.2 Motor learning and the brain

Early studies of motor sequence learning and brain imaging found the involvement of a widely distributed motor network in performance of tasks (Grafton et al., 1992; Karni et al., 1995). A meta-analysis of functional magnetic resonance imaging (fMRI) activation in 70 motor learning studies has shown that left dorsal premotor cortex (dPMC), bilateral supplementary motor cortex (SMC: comprising both the supplementary motor area (SMA)-proper and pre-SMA), bilateral primary motor cortex (M1), left primary somatosensory cortex (S1), left superior parietal lobule (SPL), left thalamus, bilateral putamen, and multiple clusters in the cerebellum tend to show performance related changes in activation (Hardwick et al., 2013). These areas can be thought of as responsible for controlling movement, and contributing to learning, in a hierarchical nature, though this is likely an over-simplification. Strategy and planning of movements are controlled by areas of the association cortex, including the supplementary and premotor areas (PMA); the basal

ganglia for movement initiation; the cerebellum is responsible for error correction; and primary motor cortex for movement execution.

1.2.1 Supplementary motor and premotor areas

SMA and PMA are crucial areas for the preparation of movements. Neurons of the SMA will increase their firing rate around one second prior to movements of either hand, and lesions to the SMA will cause deficits in coordinating bimanual movements (Brinkman, 1981). It has been proposed that individual neurons within SMA may encode distinct movement transitions (Shima and Tanji, 1998) but recent meta-analysis has shown that SMA appears to play a domain general role, activating in response to many different tasks, all of which have a higher-order sequential representation (Cona and Semenza, 2017).

Similar to SMA, activity within premotor areas is higher during a state of motor readiness and then decreases once the movement has been executed (Weinrich and Wise, 1982). The premotor regions can be divided into ventral (PMv) and dorsal premotor cortex (PMd) which are thought to have distinct roles in preparing and executing movements (Chouinard and Paus, 2006; Davare et al., 2006; Rizzolatti et al., 1998; Rizzolatti and Luppino, 2001). The SMA and PMA form direct connection with the primary motor cortex and also with more frontal areas, but there is a second route that information can take between frontal areas and the SMA, through the basal ganglia.

In terms of sequence learning, previous data demonstrated that the premotor cortex modulates its activity once movement sequences become more complex in either their spatial or temporal structure (Bengtsson et al., 2004), or in the presence of both features (Sakai et al., 2002). Different premotor areas have been shown to selectively encode spatial and temporal sequence information (Kornysheva and Diedrichsen, 2014) and voxel pattern analysis can uncover sequence representations, in motor and premotor areas, which are specifically enhanced for trained sequences (Wiestler and Diedrichsen, 2013). Several studies have shown that there are reductions in premotor (Sale et al., 2017; Toni et al., 1998; Wu et al., 2004) and primary sensory

cortex (Sale et al., 2017; Seitz and Roland, 1992) activation as learning progresses, perhaps due to greater automaticity, though research has yet to directly tackle this question.

1.2.2 Basal Ganglia

The basal ganglia consist of a group of nuclei including the striatum, caudate, putamen, nucleus accumbens and globus pallidus. They are associated with a wide variety of functions including action selection, movement initiation, eye movements and motivation (Alexander et al., 1991, 1986; Kotz et al., 2009). The nuclei of the basal ganglia are interlinked in a complex network of GABAergic and glutamatergic connections, modulated by dopaminergic activity. Signals from the cortex arrive in the basal ganglia are channelled into at least two distinct pathways, which loop around the basal ganglia structures and back to the cortex in either an excitatory or inhibitory manner (Alexander and Crutcher, 1990; Middleton and Strick, 2000), perhaps allowing for calculations of probable reward, and selection and initiation of the optimal action (Hardwick 2013).

Recent lesion studies in rats have elucidated the role of basal ganglia nuclei in the learning of skilled movements. Neurons from rat M1 project directly to the dorsolateral striatum (Ebrahimi et al., 1992). Lesions within the dorsolateral striatum impair the performance of previously learnt movements, but lesions of the M1 to striatal connections impair the animals' ability to learn a new motor skill. It was concluded that the dorsolateral striatum was being tutored by the signalling from M1 and was then storing the stereotyped motor memory for execution directly to the spinal cord (Kawai et al., 2015).

1.2.3 The cerebellum

The cerebellum is an area of structural homogeneity but functional heterogeneity. Anatomical tracing studies in primates have demonstrated connections between the cerebellum and many diverse brain areas including prefrontal and parietal cortices, as well as motor areas (Kelly and

Strick, 2003). Similar diversity in connectivity has been demonstrated in humans both structurally (Jissendi et al., 2008) and functionally (O'Reilly et al., 2009; Buckner et al., 2011). This diverse connectivity pattern also appears to be mirrored in diverse functions: a recent fMRI study has demonstrated localised activation in the cerebellum in response to preforming tasks such as word comprehension and language processing, autobiographical and working memory, and memory tasks (King et al., 2018). Furthermore, individuals with lesions to the cerebellum exhibit a diverse range of behavioural impairments on tasks such as duration discrimination (Ivry et al., 2002; Penhune and Steele, 2012), attentional control (Courchesne et al., 1994; Townsend et al., 1999), emotion perception (Turner et al., 2007), and executive (Manes et al., 2009) and language function (Stoodley and Schmahmann, 2009). However perhaps the most well-studied role of the cerebellum is its contribution to error based learning (Albus, 1989, 1971; Marr, 1969).

The cerebellum has a highly organised structure where Purkinje cells, the main output cell of the cerebellum, receives weak inputs from the parallel fibres of granule cells, each of which contacts many thousands of Purkinje cells. Parallel fibres carry information from the ascending tracts of the spinal cord, subcortical nuclei and parts of the cortex (Jansen and Brodal, 1954). It has been proposed that these fibres generate a representation about the state of the body by incorporating sensory information with efference copies of motor commands. Purkinje cells also receive strong inputs from climbing fibres, with each climbing fibre contacting only a few Purkinje cells. These climbing fibres, which originate in the inferior olive, generate large responses in the Purkinje cell when an unexpected event or error is encountered. It is thought that the spiking of climbing fibres, indicating an error, causes a long-term depression between the parallel fibre and Purkinje cells synapses (Albus, 1989; Ito et al., 1982). This would mean a decrease in the strength of the weighting of the information about the state of the body coming from the parallel fibre. It is thought that this process can underlie both conditioning and adaptation learning.

Evidence from the motor deficits of cerebellar patients (Maschke et al., 2004; Miall, 1998; Morton and Bastian, 2006; Smith and Shadmehr, 2005; Tseng et al., 2007), experimentally induced lesions in non-human primates (Baizer and Glickstein, 1974; Martin et al., 1996; Weiner

et al., 1983) and from human brain stimulation studies (Galea et al., 2011) have demonstrated a role of the cerebellum in predictive control of movements and the correction of motor error. This is clearly of enormous importance in visuomotor adaptation tasks, which have been demonstrated to primarily recruit a cortico-cerebellar network (Doyon et al., 2009a, 2003; Doyon and Benali, 2005). Additionally, visuomotor adaptation style tasks have also been shown to induce plastic changes in the cerebellar cortex (Spampinato and Celnik, 2018).

As well as its clear role in adaptation learning, it seems that the cerebellum is also active in motor sequence task execution (Hardwick et al., 2013; King et al., 2018). Early in learning SRTT performance employs cortico-cerebellar circuits whereas after extended practice, cerebellar activation decreases (Wu, Kansaku and Hallett, 2004; Steele and Penhune, 2010; though see Seitz et al., 1990), and delayed recall involves cortico-striatal networks (Albouy et al., 2008; Debas et al., 2014; Doyon et al., 2009b, 2003; Doyon and Benali, 2005; Hikosaka et al., 2002; Peigneux et al., 2006). This links well with findings of increases in activation in sections of the basal ganglia (Seitz and Roland, 1992) seen with learning progression.

1.2.4 Primary motor cortex

M1 is the region with the most direct control of movement, with the large pyramidal Betz cells of layer 5 projecting directly onto motor neurons of the spinal cord. However there is some controversy about exactly how this control is exerted (Omrani et al., 2017). It was originally thought that a single pyramidal neuron from the monkey motor cortex contacted a single motor neuron pool, which innervated a single muscle, and that the rate of firing of pyramidal neurons was related to the degree of muscle contraction (Evarts, 1968). It has been shown that the activity of small numbers of monkey M1 neurons can be used to reconstruct the timecourse of electromyogram (EMG) measured activity within functional groups of muscles (Morrow and Miller, 2003), a finding supported by several other modelling studies (Mussa-Ivaldi, 1988; Todorov, 2000)

However, corticospinal motor neurons are only involved in a subset of movements of the target muscle (Griffin et al., 2015), going against the idea that M1 is coding the movement of individual muscles. Studies have instead argued that monkey M1 encodes muscle torque (Scott and Kalaska, 1997), 'synergies' (d'Avella and Bizzi, 2005), or pattern generation (Shenoy et al., 2013). A seminal paper by Georgopoulos showed that each monkey pyramidal neuron innervates a range of different muscles that are all involved in driving a limb towards a goal (Georgopoulos et al., 1982). It was proposed that the activity of each cell could be thought of as encoding a direction vector; the activity of a group of cells would then create a population vector encoding the direction of a desired movement (Georgopoulos et al., 1982). More recent research has shown that while there are a majority of neurons in monkey M1 that have a preferred direction in muscle space, there is also a minority who encode a preferred direction in Cartesian space (Morrow et al., 2007). This would mean that much of the motor cortex was actually active in every movement, and that the larger the active population of neurons the finer control of a movement could be achieved.

It has been postulated that perhaps there are different populations of cells within M1 that exert control over muscles in different ways (Omrani et al., 2017). Cells within M1 are not homogenous with respect to their outputs or inputs. In primates the majority of corticomotor neurons cells are found in caudal M1 (Rathelot and Strick, 2009), with only slower weaker corticomotor neurons and polysynaptic projections to motor neurons found within rostral M1 (Witham et al., 2016). Likewise, in terms of inputs caudal M1 tends to receive predominantly tactile sensory inputs from the periphery, whereas rostral M1 receives proprioceptive inputs (Wise and Tanji, 1981). These caudal, monosynaptic connections are present in humans, apes and only to a lesser extent in Old and New World monkeys such as the macaque, the most common model organism in this research. This may limit the ability to translate findings from monkey studies into the human motor system.

MRI studies performed in humans have demonstrated, somewhat unsurprisingly, that M1 shows increased activation during execution of a motor sequence task (Grafton et al., 1992). However

changes in M1 activation with repeated exposure to a sequence have also been demonstrated (Karni et al., 1998a, 1995; Ungerleider et al., 2002). Before training, two sequences activated roughly the same area and extent of M1. Then after initial training on one sequence the area activated became smaller (habituation), then larger (enhancement) with further experience, all within the first session (Karni et al., 1998a, 1995). This enhancement also persisted for several months, indicating long-term experience dependent plasticity in M1 after sequence learning.

Changes in task related neuronal activity are also seen within M1 of primates during motor adaptation tasks (Li et al., 2001; Wise et al., 1998), however these plastic changes in M1 have not been clearly seen in response to sensorimotor adaptation in humans (Spampinato and Celnik, 2018). It appears that M1 may be more important in the aftereffect or retention phase of visuomotor adaption in humans than in the learning *per se* (Galea et al., 2011; Hadipour-Niktarash et al., 2007; O'Shea et al., 2017).

Changes in M1 activation, and perhaps therefore the tutoring of basal ganglia nuclei, are not the only learning related change found in humans (Kawai et al., 2015); human M1 can also undergo a remapping of its corticospinal outputs. Transcranial magnetic stimulation (TMS) studies (see Section 2.2.2) have demonstrated increases in the size of an area representing a particular muscle after its training (Classen et al., 1998; Galea et al., 2009; Pascual-Leone et al., 1994; Perez et al., 2004), and the total active area of the primary motor cortex have been shown to increase as learning progresses (Karni et al., 1995). Evidence from animal studies has also shown that motor training causes reorganisation of M1 motor somatotopic mapping (Fu et al., 2012; Fu and Zuo, 2011; Kleim et al., 2004; Manto et al., 2006; Rogerson et al., 2014; Yu and Zuo, 2011). But how does this remapping occur?

1.3 Plasticity within M1

Plasticity in the human brain can be presumed to occur through the altering in strength of synaptic connections, through processes similar to long-term potentiation (LTP) and long-term

depression (LTD). These processes depend upon the timing of activation of glutamatergic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (*N*-methyl-D-aspartate) receptors on postsynaptic neurons. LTP results in the strengthening of synapses through the recruitment of a greater number of receptors on the postsynaptic membrane, through the creation of new synapses or the addition of dendritic spines.

Motor learning in animals has been demonstrated to induce at least two of these LTP-like outcomes in M1 pyramidal neurons: promoting synaptogenesis and leading to the formation of new dendritic spines (Hayashi-Takagi et al., 2015; Nemeth et al., 2010; Rogerson et al., 2014; Xu et al., 2009; Yang et al., 2009). These process likely occur within the horizontal connections spanning M1 (Jacobs and Donoghue, 1991): reorganisation of M1 in rats who had undergone facial nerve lesions was greater in M1 sites with strong horizontal connections to the deafferentated cortex (Huntley, 1997). Altering the synaptic efficacy of connections in response to certain patterns of activation may be a mechanism for allowing increased neural efficiency for performing trained movements, or even allowing remapping of M1 to increase the area recruited to perform a trained movement thereby allowing finer control.

1.4 GABA, plasticity and motor learning

γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain and, though not directly involved in the NMDA dependent mechanisms of LTP and LDP, has an established role in modulating cortical plasticity (Griffen and Maffei, 2014). GABA releasing cells constitute around 20% of cortical neurons, and their released neurotransmitter acts on receptors to increase membrane permeability to chloride. This causes hyperpolarisation, and therefore decreases the likelihood of action potential formation in the postsynaptic cell.

GABA released from the presynaptic terminals of one neuron can act on postsynaptic ionotropic $GABA_A$ receptors or metabotropic $GABA_B$ receptors. The binding to these receptors underlies 'phasic inhibition' of the postsynaptic neuron. However research has

demonstrated that GABA also exists in low concentration in extracellular spaces, activating presynaptic receptors, or at neighbouring synapses on other neurons. This results in persistent or 'tonic' activation of extrasynaptic GABA receptors resulting in overall lower probability of action potentials within the affected neurons. The relationship between tonic and phasic GABA release is complex and is not fully understood, but it is likely that the two pools have distinct roles in mediating plasticity.

Reduction in local GABAergic signalling has been proposed as a potential mechanism for unmasking silent synapses, allowing remapping, and underpinning motor learning within M1. Early (Hess et al., 1996; Trepel and Racine, 2000) and more recent animal studies (Chen et al., 2011, 2015; Donato et al., 2013) have demonstrated that administration of $GABA_A$ agonists blocked LTP-like plasticity. Pharmacological studies in humans (see Section 2.3.2) also further strengthen the link between GABA decreases and remapping: lorazepam, a $GABA_A$ agonist, inhibits practice-dependent enhancement of muscle representations within M1 (Ziemann et al., 2001), and both lorazepam (Ziemann et al., 1998a) and baclofen, a $GABA_B$ agonist, prevents M1 plasticity induction (McDonnell et al., 2007). Furthermore, interventions known to decrease M1 GABA such as ischemic nerve block (Levy et al., 2002), continuous theta burst stimulation (cTBS) (Stagg et al., 2009b) and anodal transcranial direct current stimulation (Kim et al., 2014a; Stagg et al., 2011a; Stagg et al., 2009a)(see Section 2.3.1 for more details), are all known to increase LTP-like plasticity within M1 (deafferentataion: Brasil-Neto et al., 1993; Ziemann, Hallett and Cohen, 1998; tDCS: Monte-Silva et al., 2013) or decrease LTD-like plasticity (cTBS: Huang et al., 2005).

As well as remapping M1, GABA concentration decreases also appear to be important for motor learning. In humans, decreases in magnetic resonance spectroscopy (MRS) measures GABA have been observed in the contralateral M1 during motor sequence learning, but not during performance of random button presses (Floyer-Lea et al., 2006; Kolasinski et al., 2018), though see (Chen et al., 2017). Lower levels of M1 GABA before learning also tended to associate with better motor learning ability (Kolasinski et al., 2018), faster reaction times (Stagg et al., 2011a)

and greater functional connectivity of the motor network (Bachtiar et al., 2015; Stagg et al., 2014). In addition the responsiveness of motor system GABA concentrations, as measured by GABA decreases during direct current stimulation, correlates with participants motor learning abilities in both a sequence learning task (Stagg et al., 2011a) and in visuomotor adaptation tasks (Kim et al., 2014a; O'Shea et al., 2017).

The physiological mechanisms underlying these decreases in GABA are not well understood. It may be that there is a decrease in the production (anabolism) of GABA from its precursor glutamate, or an increase in its breakdown (catabolism). Alternatively it may be that GABA molecules are transported to another brain region, or perhaps moved into other areas of the cell that are less visible to MRS and less accessible for use in neurotransmission.

1.5 Aims of this thesis

This thesis primarily aims to investigate the effects of inhibition on human motor system plasticity, vital for normal motor learning. It will begin by investigating task factors, specifically explicit sequence awareness, that influence how well motor sequence memories are retained (Chapter 3). The two subsequent chapters will focus on the effects of transcranial direct current stimulation (tDCS), a technique thought to enhance plasticity-like process, to alter brain chemistry and motor learning. In Chapter 4 the effects of tDCS on brain chemistry, both local and distant to the stimulation site, will be investigated. We will also probe the effects of brain connectivity on these chemical changes. The following chapter (Chapter 5) will explore the effect of applying tDCS to M1 during sequence learning with the ipsilateral hand. The study in the last experimental chapter the effect of a pharmacological intervention, the $GABA_B$ receptor agonist baclofen, on learning and retention of motor sequence and visuomotor adaptation tasks will be explored (Chapter 6). Finally, the results presented will be discussed along with future directions in Chapter 7.

Chapter 2: Methods for measuring inhibition, and interventions to influence inhibition and brain plasticity

In order to assess inhibition and to modulate inhibition or plasticity this thesis used a number of different modalities for recording from and stimulating the brain. Magnetic resonance spectroscopy (MRS) was used to measure neurochemical concentrations, including the inhibitory neurotransmitter GABA. Corticospinal excitability and activity at GABAergic and glutamatergic receptors within the primary motor cortices were measured using transcranial magnetic stimulation. Additionally, functional and structural connectivity were assessed using magnetic resonance imaging (MRI) methods. Transcranial direct current stimulation (tDCS) was used to stimulate the motor cortex in an attempt to modulate plasticity, neurochemistry and behaviour. A pharmacological intervention was also used to alter inhibition.

This chapter will discuss each of these methods in turn. For the measurement tools the mechanisms, and physiology underlying each will be covered, as will the limitations for data collection and interpretation. For the interventions, their mechanisms of action, and factors influencing their effects will also be discussed.

2.1 Methods to assess brain structure and function

2.1.1 Magnetic Resonance Imaging (MRI)

Since its first invention in the early 1970's MRI has proved to be an enormously valuable technique for imaging the human body, both for medical and research purposes. For neuroscientists MRI can provide a method to image the human brain *in vivo*, to trace the projections of white matter tracts, to record changes in regional blood oxygenation level under different conditions, and to measure the concentration of certain brain metabolites.

2.1.1.1 Physics of MRI

Most MRI techniques rely on measuring magnetisation produced by the positively charged nuclei of hydrogen atoms, protons, using applied magnetic fields. Many elementary particles, such as protons, exhibit a property known as spin, meaning that they rotate about a central axis. A spinning, positively charged proton has a magnetic moment, giving it a north and south pole. If a magnetic field (B_0) is introduced to a population of protons the protons will, on average, align their north and south poles with the magnetic field, creating a net magnetisation (M) among the protons. After the initial introduction of the field protons will oscillate, or precess, around alignment until they eventually come to rest in line with the field, a process known as relaxation. The frequency of these oscillations (ω_0) will depend on the strength of the applied magnetic field ($\omega_0 = \gamma B_0$, where B_0 is measured in Tesla (T)) scaled by the gyromagnetic ratio of protons (γ = 42.57 MHz/T).

The oscillation of these charged protons generates its own magnetic field that can then be detected by measuring the induced current within a coiled wire. Other nuclei also exhibit this phenomenon, for example phosphorus-31 and carbon-13, however their greater size relative to their charge gives them a smaller gyromagnetic ratio (γ), meaning they will oscillate at a lower frequency for a given applied magnetic field. This factor, in addition to their lower abundance

within the brain, means that there will be less signal produced for a given magnetic field strength compared with hydrogen MRI.

Within an MRI scanner, the B_0 field is induced along the direction of the bore of the magnet, and is used to describe the strength of the scanner, e.g. 1.5T, 3T or 7T. The direction of the B_0 field defines the longitudinal axis, and the transverse plane lies perpendicular to the B_0 field. If another magnetic field (B_1) is introduced perpendicular to the initial applied magnetic field at the protons' resonant oscillation frequency then the amplitude of the protons' oscillations will be enhanced, creating a greater measureable magnetic field signal. In MRI a rotating B_1 field is introduced within the transverse plane by transmit coils which cause the net magnetisation of the protons to be drawn away from alignment from the B_0 field into the transverse plane. This applied B_1 field can be referred to as the radio frequency (RF) pulse due to its frequency range. The degree to which the magnetism is shifted away from the longitudinal axis is known as the flip angle.

When the RF pulse is removed the protons will, on average, begin to realign with the longitudinal axis again, but will undergo a period of precession around the B_0 field at their resonant frequency. Protons will do this until they relax back to alignment with the magnetic field, a process that is described by two time constants (T1- describing the gain of magnetisation in the longitudinal axis, T2- describing the loss of magnetisation within the transverse plane). After an RF pulse is used to excite the magnetisation, there is then a waiting period known as the echo time (TE) before signal recording takes place using a receive RF coil. These signals can then be separated out using Fourier transformations. Protons within different environments will display different T1 and T2 relaxations, and so varying amounts of signal will be produced from each tissue type at a given echo time, allowing differentiation between tissues. See section 2.1.2 for more details. After a time delay known as the repetition time (TR) the process can be repeated and a new image obtained.

Gradient fields can be introduced that will add to, or subtract from, the B_0 field at different locations along their axis, changing the resonant frequencies of protons at different positions in space. This process allows for differentiation of signals across space.

2.1.2 Structural MRI

This group of techniques enables discrimination of different tissue types and structures within the brain. Structural MRI exploits the fact that protons within different tissue types exhibit different magnetic relaxation properties. By altering the parameters of a given pulse sequence, e.g. TR and TE, differing amounts of signal can be acquired from different tissue types, creating contrast between tissues.

2.1.2.1 T1-weighted images

T1 relaxation is the time constant defining the rate of recovery of magnetisation along the longitudinal axis (i.e. the re-alignment with the B_0 field) following an RF pulse. Hydrogen atoms bound tightly within their surroundings, e.g. hydrogen within fat tissue, will tend to release energy more quickly resulting in shorter T1 realignment time, than those loosely bound within water. In T1-weighted images cerebrospinal fluid (CSF) and dense bone appear dark, while areas with high fat content, e.g. white matter (WM), appear bright. This creates images with good contrast between neocortex and white matter. T1-weighted sequences tend to have short TR and short TE. Examples include Magnetisation Prepared Rapid Acquisition Gradient Echo (MPRAGE).

2.1.2.2 T2-weighted images

T2 relaxation is the time constant defining the loss of magnetisation in the transverse plane following the excitation caused by an RF pulse. T1 and T2 relaxation are independent processes, which happen simultaneously, but T2 is far shorter than T1. T2-weighted sequences have long TR and long TE in order to gain maximum contrast between CSF, which appears bright, and brain tissue, which appears dark.

In real biological systems the decay of magnetisation in the transverse plane is always far greater than would be predicted based on an idealised system. This rate is denoted T2* relaxation and can be thought of as the effective T2. The cause of the increased T2* is inhomogeneities in the main magnetic field either due to distortions created by biological tissues within the field, or defects in the magnet or the produced field. Inhomogeneities in the field experienced by different protons causes them to precess at different frequencies, and this results in a dephasing of the precession. This loss of coherence in the precession of protons results in a loss of overall signal, with the resulting signal loss described as the free induction decay (FID). T2*-weighted images can be used to detect haemorrhages or calcifications, and T2* sensitive sequences also form the basis for functional MRI (fMRI) using blood oxygenation level depended (BOLD) imaging.

2.1.3 Functional MRI (fMRI)

As well as using MRI to measure the brain structure, MRI can also been used to assess the changes in blood flow across the brain, thought to mirror changes in neuronal activity. This set of techniques relies on assessing differences in T2* decay caused by differing blood oxygenation.

An object within a magnetic field will exert an effect on the field known as the magnetic susceptibility. Differences in magnetic susceptibilities (χ) across tissue boundaries determine the amount of field inhomogeneity, with larger differences creating larger field disturbances. The susceptibility of grey matter, water and oxygenated blood is roughly equal ($\chi \sim -9 \times 10^{-6}$), whereas deoxygenated blood has a higher susceptibility ($\chi \sim -6 \times 10^{-6}$). This means that there is a relatively large difference in susceptibility between deoxygenated blood and surrounding tissues. In voxels containing a vessel carrying deoxygenated blood, there can be large inhomogeneities in magnetic field within the voxel, which in turn results in shorter T2* decay, and greater signal loss at a given TE. The difference in the signal obtained due to differences in oxygenation is exploited to create the BOLD contrast.

The cerebral metabolic rate of oxygen consumption ($CMRO_2$), i.e. the rate at which oxygen is utilised by the brain, is though to be a good measure of neuronal activity. Neuronal firing is a highly energetically costly process that uses adenosine triphosphate (ATP). This ATP must be created in via oxidative phosphorylation from adenosine diphosphate (ADP) in line with the cells energy demands. Oxidative phosphorylation requires oxygen, which is converted to water. It can therefore be assumed that increasing neuronal activity requires increased oxygen consumption. The vasculature supplying the brain is highly coupled with neuronal responses, whereby increasing neuronal activity causes increased cerebral blood flow (CBF) and blood volume (CBV) to the active areas. The amount of blood oxygenation in a given area is related to the cerebral blood flow (CBF) to that area, and also the oxygen extraction fraction (OEF), a measure of how much oxygen is removed from the blood in that region. There is a counterintuitive oxygenation increase when a region becomes more active, due to an over supplying of blood to the area. This means that by increasing activation in a given area, thereby increasing blood oxygenation, there is a lengthening of T2* decay and increase in BOLD signal.

The vascular response to changing neuronal activity is slightly delayed and can be described by the haemodynamic response function (Buxton et al., 2004). As there are baseline differences in BOLD signals across the brain, generally BOLD signal change in response to different conditions, stimuli or tasks is measured.

2.1.3.1 fMRI sequences

Echo planar imaging (EPI) is the most commonly used method for acquiring fMRI data, as it is sensitive to T2* and allows for fast acquisition across the whole brain. A number of different parameters (e.g. TE, slice thickness and orientation, field of view, matrix size) can be altered which can influence the degree of signal dropout, image distortion, BOLD sensitivity and SNR. Typical parameters for 3T fMRI were used in this thesis.

2.1.3.2 Task and resting state fMRI

Functional MRI can be used to examine changes in blood flow in response to task demands, task fMRI, and also to examine the functional connectivity in the brain at rest, known as resting state fMRI. For the task fMRI included in this thesis a blocked design was used where short periods of 'task' were interspersed between periods of 'rest'. This creates the highest contrast to noise ratio for the task related changes in BOLD. Resting state fMRI is based on measuring spontaneous low frequency fluctuations in the BOLD signal, and examining the synchronicity of activity across regions as a measure of their connectivity.

2.1.4 Diffusion Weighted Imaging (DWI)

DWI allows the mapping of white matter tracts within the brain by using the movement of water molecules to generate contrast within an image.

Within an unrestricted system molecules of water will move around at random, but their displacement away from a given point after a given time can, on average, be described by the diffusion coefficient (D). In these unrestricted conditions the diffusion of water is isotropic, meaning it will occur equally in any given direction. However, within a biological system, there are restrictions placed upon these water molecules by cellular structures, such as semi- or non-permeable membranes, which will change the pattern of diffusion of water molecules.

A typical neuron has a large bulbous cell body (GM) and a thin axon of white matter (WM) that tends to be aligned with other axons to form a tract. Water molecules can diffuse more freely along the direction of the axon than they can across the semi-permeable axonal membrane, meaning that movement of water within axons is anisotropic. The directionality of the diffusion of water can tell us, not only about tract orientation, but also about the fibre microstructure.

2.1.4.1 DWI sequences

DWI relies on the use of a technique known as spin echo. After the application of a first RF pulse there will be a FID in signal caused by the loss of magnetisation in the transverse plane. However, the contribution of T2* processes to this decay caused by field inhomogeneities, is largely reversible as the inhomogeneities are constant across time. If a second 180-degree RF pulse is applied at a given time after the first pulse, then this causes the precessing of protons to be reversed and to come back into phase after the same time delay again. This causes a second peak in signal intensity known as the spin echo.

In diffusion weighted sequences the field inhomogeneities within a given area are enhanced by applying a gradient field after the initial RF pulse. Applying a gradient field means that within a given voxel there will be a range of resonant frequencies, creating different frequencies of precession of protons, and therefore enhancing the dephasing of magnetisation within the voxel over time after the initial excitation pulse. By introducing a 180-degree pulse to a static system, while holding the gradient field constant, this dephasing could be reversed. However, in a biological system the water molecules are diffusing randomly through this gradient. This means that the magnetic field experienced by a given proton before and after the 180-degree spin echo pulse will be different, therefore dephasing will not be fully corrected and the signal will not be fully recovered. Using this technique more diffusion in the direction of the applied gradient results in more signal loss, which can be quantified by contrasting the signal with and without diffusion weighting produced by the applied field gradients.

This form of measurement can be repeated over and over again with a different diffusion weighting gradient direction to estimate diffusion in many different directions. By doing so the signal attenuation, and therefore the amount of diffusion in each direction in each voxel can be estimated. This tends to be done for around 64 directions. These images can then be combined along with an image where there is no diffusion weighting (the b=0 image) to create a diffusion tensor image.

Fitting a diffusion tensor is a method of quantifying the directionality of diffusion by using an ellipse to explain the average displacement of molecules from a given point, after a given time. This ellipse is described by eigenvectors, defining the axes of the ellipse, i.e. the direction of the underlying fibres, and eigenvalues, defining the size of the axes, i.e. the strength of diffusion. From the tensor we can extract several values, for example the mean diffusivity (MD), the average diffusion across all directions, or fractional anisotropy (FA), indicating how elongated the diffusion tensor is any particular direction, i.e. the degree directionality of diffusion.

By definition FA can vary between 0 and 1, with 0 meaning there are no constraints on diffusion, and 1 being diffusion in only one direction. FA tends to be lowest within regions such as the ventricles where water molecules can diffuse without constraint and is highest within highly ordered white matter tracts. FA is modulated by several different factors, for example, high axon packing and low axon diameter, meaning a greater membrane density perpendicular to the axon, results in higher FA values (Takahashi et al., 2002; Zatorre et al., 2012). Additionally damaged or absent myelin also tends to decrease FA values (Beaulieu and Allen, 1994; Gulani et al., 2001). It is generally interpreted as being as measure of 'white matter microstructure' (Sampaio-Baptista and Johansen-Berg, 2017).

2.1.4.2 DWI limitations

Diffusion gradients encode tiny displacements in water molecules, which means that they are very sensitive to any movement of the subject. This problem can be somewhat recovered by using single-shot echo-planar imaging, where the image is acquired in one shot. However, this can result in distortion and limits spatial resolution. Distortions can also be introduced by field inhomogeneities caused by eddy-currents, created in response to rapidly changing gradients within the scanner. These can largely be corrected using techniques such as FSL eddy correct (FMRIB software library). Finally, while DWI can detect changes or differences in white matter microstructure interpretation of measures can be difficult in areas where white matter fibres tend to cross.

2.2 Methods to measure neurochemistry

2.2.1 Magnetic Resonance Spectroscopy (MRS)

Unlike other forms of neuroimaging, MRS does not focus on the magnetic signal from protons within water molecules, but rather is interested in examining the signal produced from protons bound within other brain metabolites. This allows the examination of metabolic processes and brain chemistry rather than brain structure.

An isolated proton within a static magnetic field will not only be influenced by the applied B_0 field, it will also experience a local magnetic field produced by its spinning electron ($B_{local} = -\sigma B_0$), proportional to it chemical shielding constant (σ). The degree of this shielding depends on the chemical environment within which the proton is situated. For example, a proton bound within a water molecule will experience less chemical shielding than a free proton, as the oxygen molecule within water draws the electron towards it. This chemical shielding of the proton from the applied magnetic field creates a small offset to the resonance frequency ($\omega_0 = \gamma B_0(1-\sigma)$). This means that protons within different chemical environments will exhibit different resonant frequencies, which enables them to be distinguished from each other in the resulting recorded signal. As with other forms of neuroimaging this process can also be applied to carbon-13 and phosphorus-31, to enable identification of metabolites containing these molecules.

If the signal arising from a given region of tissue is plotted as a function of frequency, then a series of peaks in the signal can be seen, each corresponding to the nucleus of interest (a proton in H1 spectroscopy) bound in different molecules. The characteristic peaks and frequencies of many neurochemicals are known and can be identified from within the spectrum using Fourier transform. The concentration of a given neurochemical within the selected volume is proportional to the amplitude of its representative peak(s) within the spectrum, although accurate quantification often requires complex analysis approaches.

The metabolites of interest have concentrations around 1000x smaller than water. For this reason many hydrogen MRS sequences involve suppression of the water signal. Prior to the excitation pulse a frequency selective RF pulse will cause 90-degree flip of water molecules into the transverse plane. Another 'crusher' gradient is then applied to dephase the water spins. The following excitation pulse therefore causes the water molecules to be tipped back into the longitudinal plane preventing them from producing measurable signal. This technique is known as variable power and optimized relaxation delays sequence (VAPOR). Even with water suppression low concentrations of metabolites means low signal levels and so large voxels, in the order of several centimetres squared, are often required for accurate quantification. Sequences such as point resolved spectroscopy (PRESS) allow localisation of a voxel of interest.

The main metabolites of interest in this thesis, which can be quantified using MRS, are glutamate and GABA, the main excitatory and inhibitory neurotransmitters in the brain. Glutamate is present at relatively high concentrations (<12mM) within the brain (Choi et al., 2006) and is localised within cells, with only very low concentrations of glutamate found extracellularly (Danbolt, 2001). As well as it's role in neurotransmission, glutamate is involved in a number of metabolic processes. While the relative amount of glutamate that is used for neurotransmission varies largely across brain areas, the majority of glutamate molecules are used in basic cell metabolism. Therefore, it is likely that the majority of the glutamate signal measured using MRS does not represent glutamate used for neurotransmission.

As for GABA, the main inhibitory neurotransmitter is present in far lower concentrations (<1mM) (Petroff and Rothman, 1998) and is metabolised from glutamate by glutamate decarboxylase. GABA is also localised mainly within the cell bodies, with some presence in axon terminals and low concentrations extracellularly (Cavelier et al., 2005), and like glutamate, much of the brains' GABA is used in metabolism rather than neurotransmission (Rae et al., 2009). It is not clear whether the MRS signal is more or less sensitive to GABA within each of these pools, but it is likely that the majority of the MRS-measured GABA signal is produced by molecules present extrasynaptically but intracellularly (Dericioglu et al., 2008; Nasrallah et al., 2007; Rae et

al., 2009; Rae, 2014). This pool of metabolic GABA is thought to be accessible for use in neurotransmission however (Dericioglu et al., 2008), and so for the purposes of this thesis it will be referred to as a metric of inhibition.

Even before interpretation of the signals, quantifying both glutamate and GABA also presents challenges. At field strengths below 4T the resonances arising from the protons within glutamate, glutamine and GABA cannot be resolved using traditional sequences without editing methods. Increasing to a 7T field strength increases signal to noise ratio (SNR), and increased chemical shift dispersion, allowing for differentiation of signals (Otazo et al., 2006). Though the use of higher field strength is not without its drawbacks: greater B_0 field inhomogeneity and the potential for greater and B_1 inhomogeneity is present at 7T, both of which result in broadening of spectral linewidth, and reduce the accuracy and precision of metabolite quantification (Tkáč et al., 2009). Shimming, to optimise B_0 homogeneity over the voxel of interest can be employed, and in the study presented in Chapter 4 dynamic shim updating (DSU) was used to apply shim corrections in real time, between slice acquisitions, for the two voxels of interest being obtained (Blamire et al., 1996). Inhomogeneity in B_1 can also increase at higher field strengths; as for a given RF power, which needs to be restricted to avoid energy absorption resulting in heating, there is an increased level of spectral dispersion and poorer pulse bandwidth. Broad-band adiabatic RF pulses, a feature of sLASER sequences, can be used to create homogeneous excitation (Tannús and Garwood, 1997); and dielectric pads can be used to increase B_1 strength local to the pad without requiring increased power RF pulses (Teeuwisse et al., 2012).

Large voxels used in MRS tend to be composed of a variety of different tissue types: grey matter (GM), which includes the bulk of GABAergic neurons and therefore tends to have the highest GABA concentration; white matter (WM), which tends to contain less GABA; and cerebrospinal fluid (CSF) where there is virtually no GABA. Correcting for tissue composition can potentially improve the accuracy of GABA concentration quantification (Gasparovic et al., 2006; Mullins et al., 2014a), however there is controversy about the best way to perform these calculations as many methods counterintuitively increase the measure's dependence on GM fraction (Harris et

al., 2015). For this reason all of the within-subject, within-session GABA change results presented in this thesis are not GM corrected.

The concentrations of molecules of interest are often given as a ratio with the concentration of a molecule that tends to have low concentration variance within and between healthy individuals, for example myo-Inositol, *N*-acetylaspartate (NAA) or a combination of creatine (Cr) and phosphocreatine (pCr). In this thesis the total of creatine and phosphocreatine was used as a reference (Cr+PCr; tCr). Creatine in humans is derived from both diet and synthesis, and has an important role in regulating energy in the brain through interactions with adenosine triphosphate (ATP). Creatine levels tend to be highest within cortical grey matter, lower in white matter (McLean et al., 2000), and have highest concentrations the cerebellum (Maudsley et al., 2009; Pouwels and Frahm, 1998).

2.2.1.1 MRS Limitations

As discussed previously MRS measurements must be summed across a large spatial area in order to obtain sufficient signal from the molecules of interest, which only exist in low concentrations within the human brain. Voxel sizes of around 2x2x2cm are typically used, making measurements relatively spatially unspecific. MRS voxels centred over the motor cortex often also include regions of somatosensory cortex or premotor areas. Newly developed MRS imaging sequence have somewhat overcome this issue by enabling the acquisition of a 10mm thick slab of voxels with resolution of 5x5mm (Steel et al., 2018).

As discussed in more detail previously the interpretation of changes in MRS measurements of GABA and glutamate must be carefully considered. Only a fraction of the molecules of glutamate and GABA present within the brain, and visible to MRS, may be used in neurotransmission. Therefore, changes in these neurochemical's concentration as measured by MRS does not necessarily mean changes to excitatory or inhibitory signalling. For more details on

the relationships between MRS measures of neurochemical concentrations and TMS measures of inhibition and excitation see Section 2.2.3.

2.2.2 Transcranial Magnetic Stimulation (TMS)

The first use of transcranial magnetic stimulation (TMS) was by Barker, Jalinous and Freeston, (1985). They demonstrated that inducing a magnetic pulse, by passing a brief burst of high current through a flat coil positioned over the motor cortex, could cause a twitch within peripheral muscles. The magnetic pulse produced by the coil induces an electrical current in the nearby brain area via electromagnetic induction. If neurons are correctly orientated with respect to the direction of electric current induction, this will cause a depolarisation of the membrane. Depending on the state of the membrane potential initially, the depolarisation can cause the neuron to fire action potentials. It is believed that depolarisation primarily takes place in the axons of descending pyramidal cells (Fitzpatrick and Rothman, 2000), the main cell type within the corticospinal tract.

The TMS equipment used in this thesis was essentially unchanged from that used in 1985. Monophasic pulses were delivered via a 70mm flat coil from a DuoMAG MP-Paired Pulse TMS set-up (Rogue Resolutions/ Deymed). TMS can be used both as an intervention by applying a continuous train of pulses, and as a measurement tool. In these studies TMS was used as a measurement tool and so from herein only this aspect will be discussed.

2.2.2.1 Motor Evoked Potential (MEP) Production

If the TMS coil is held over the primary motor cortex, M1 the resulting neuronal discharge can cause motor evoked potentials (MEPs), triggered within muscles, which then be recorded via an electromyogram (EMG). The stimulation intensity required to elicit MEPs from within certain muscles varies highly between individuals, presumably due to differences in individual brain anatomy. Because of this, most studies will begin by determining the stimulation intensity, at a

given coil position, which produces MEPs of a certain size within the target muscle. This intensity can then be used in subsequent measures, and MEP amplitude can be recorded and examined relative to baseline. There are three basic physiological mechanisms thought to influence the size of MEPs within individuals: the number of recruited motor neurons in the spinal cord; the number of neurons discharging multiple times to the stimulus, which might be assumed to be related to the excitability of the cortex; and the synchronisation of the TMS induced motor discharges (Wassermann et al., 2008).

MEPs are produced as the result of of two or more signal components descending through the spinal cord, a short latency direct-wave (D-wave), produced by direct activation of corticospinal neurons; and one or more longer latency indirect-waves (I-waves), which are produced by indirect synaptic activation of these neurons (Patton & Amassian, 1960; Patton & Amassian, 1954) and occur at 1.5ms intervals after the D-wave. I-waves are induced preferentially if current is induced perpendicular to the central sulcus, i.e. in the posterior- anterior direction, whereas D-waves are elicited best by lateral-medial current (Kaneko et al., 1996; Sakai et al., 1997; Werhahn et al., 1994). The direction of current flow in the posterior-anterior direction is also important, with anterior to posterior (AP) and posterior to anterior (PA) current influencing different neuronal mechanisms (Ni et al., 2010), with AP tending only to recruit later I-waves (Day et al., 1989). Changes in motor cortical excitability have a greater effect on I-waves (Werhahn et al., 1994), for this reason figure-of-eight TMS coils are normally positioned with the long axis parallel with the central sulcus, therefore producing current in the brain from posterior to anterior direction and eliciting I-waves in the descending corticospinal tract neurons.

As well as measuring the MEP size for a given TMS intensity, some studies also examine participants' resting stimulation intensity rSI (often referred to in the literature as motor threshold- MT), the TMS intensity required to produce MEPs of a given size. The resting stimulation intensity (rSI) is defined as the minimum stimulus intensity that can produce a 0.5mV or 1mV MEP in a relaxed muscle in 5 out of 10 consecutive stimulation pulses (Rossini and Rossi, 2007). The active stimulation intensity (aSI) is the minimum stimulus intensity needed to

produce 0.2mV MEP during muscle contraction at around 30% of maximum strength (Rossini and Rossi, 2007).

MEP amplitude in response to single TMS pulses is altered by drugs acting on a variety of neurotransmitter systems, though the influence of many neurochemcials is not fully understood. For example drugs such as benzodiazepines and barbiturates (Inghilleri et al., 1996), both of which are $GABA_A$ receptor agonists, have been shown to depress high amplitude but not low amplitude MEPs. Similar results have also been shown for the dopamine (DA) receptor agonist cabergoline (Korchounov, Ilić, & Ziemann, 2007) and the noradrenaline (NA) antagonist, guanfacine (Korchounov et al., 2003). Furthermore drugs such as NA agonists (Boroojerdi et al., 2001; Ilic et al., 2002; Plewnia et al., 2006, 2004, 2001), selective serotonin reuptake inhibitors (SSRIs) (Gerdelat-Mas et al., 2005; Ilic et al., 2002), and muscarinic receptor M1 agonist, scopolamine (Di Lazzaro et al., 2000b), have all been seen to increase MEP amplitude.

2.2.2.2 Paired-pulse TMS measurements

Paired-pulse TMS is the more commonly used, and better understood TMS technique, for assessing neurochemical concentrations. Here, two TMS pulses are applied to the same location, the first a conditioning stimulus pulse (CS), and the second a supra-threshold test stimulus pulse (TS). As in single-pulse TMS, the supra-threshold test pulse excites (whether directly or indirectly) cortical pyramidal neurons which causes the production of an MEP; whereas the conditioning pulse activates cortical interneurons which have a modulatory effect on the descending pyramidal neurons.

Excitation of interneurons by conditioning stimulus pulses causes release of neurotransmitter from presynaptic terminals. These neurotransmitters then diffuse across the synapse to the dendrites of, among other neurons, the descending pyramidal neurons. Differences in activation and deactivation times of different receptors on the postsynaptic terminal mean that the influence of interneuron activity on the output of pyramidal neurons changes with time.

Therefore, by varying the inter-stimulus interval (ISI) between the conditioning and test pulses the influence of activity at different classes of receptors on the descending pyramidal neurons can be assessed. The difference in size between the resulting paired-pulse MEP, and an MEP elicited to a single test pulse at the same intensity, is thought to be proportional to activity within the targeted neurotransmitter receptor system. The most commonly used intervals include: 1-6ms, known as short-interval intra-cortical inhibition (SICI); 50-200ms, known as long-interval intra-cortical inhibition (LICI); and 10-12ms, known as intracortical facilitation (ICF).

SICI (ISI of 1-6ms) is thought to be mediated by ionotropic $GABA_A$ receptor activation. Several studies have shown that drugs acting as $GABA_A$ receptor agonists, for example the benzodiazepines, enhance SICI (Di Lazzaro et al., 2005, 2000a; Ilić et al., 2002; Ziemann et al., 1996), though small variations in the ISI appear to change the sensitivity to different receptor subtypes (Fisher et al., 2002; Roshan et al., 2003). In this thesis SICI protocols were used with 2.5ms ISI, thought to measure synaptic $GABA_A$ activation (Stagg et al., 2011a).

LICI (ISI of 50-200ms) (Valls-Solé et al., 1992) is thought to be dependent on metabotropic $GABA_B$ receptor mediated inhibition, with baclofen, a specific $GABA_B$ receptor agonist causing an increase in LICI magnitude (McDonnell et al., 2007). In further support of the distinction between SICI and LICI, use of the GABA reuptake inhibitor tiagabine has differential effect on the two intracortical inhibition types, causing an decrease in SICI magnitude and an increase in LICI magnitude (Werhahn et al., 1999). In this thesis LICI measures were recorded with ISI of 150ms.

As well as assessing GABA receptor activity, paired pulse protocols such as intracortical facilitation (ICF) can also be used to assess primarily glutamatergic receptor activity. ICF, where the ISI is normally around 10ms, is decreased by NMDA receptor antagonists (Schwenkreis et al., 1999; Ziemann et al., 1998b). However, this relationship appears to be more complex than that of SICI and LICI, with one study finding no effect of NMDA receptor agonists (Di Lazzaro et al., 2003), and others finding an increase in ICF with benzodiazepines (Inghilleri et al., 1996;

Mohammadi et al., 2006; Ziemann et al., 1996). These later studies indicate that there is a contribution of $GABA_A$ receptor mediated inhibition to the magnitude of ICF.

2.2.2.3 TMS limitations

TMS produced MEPs are a noisy measure, in part due to the fact that MEP sizes largely depend on the phase of on-going oscillatory activity with the stimulated area (Guerra et al., 2016; Nowak et al., 2017). For this reason multiple measures are taken at each timepoint and averaged to attempt to eliminate some of this noise. However, there are also many other nuisance factors which can influence MEP sizes, for example if the participant has their eyes open or closed (Chen et al., 2017). A recent study has also demonstrated that paired-pulse measures may be under conscious control (Koganemaru et al., 2018).

The individualised set up of TMS also introduces potential for error and operator dependence. To attempt to eliminate this within this the work presented in this thesis, the same operator took all measurements for the same participant. A final source of error and variability within TMS measurements is changes to the location of stimulation. For all experiments in this thesis the stimulation location was determined at the beginning of the session and guide marks were drawn on the participants scalp to enable re-location of the coil. Neuronavigation software systems exist to enable quantification of the degree of movement within and between measurements, and to guide coil placement. However, accurate neuronavigation requires the participant to wear a headband with position sensors for the duration of the session. In the experiments presented in this thesis, the study sessions were long and it was deemed likely that this headband would move during the session making the neuronavigation ineffective.

2.2.2.4 TMS safety considerations

TMS is widely considered to be a safe technique (Rossi et al., 2009), but has induced brief seizures in a small number (<20) of individuals worldwide. Because of these potential risks, international guidelines on the parameters for intensity, frequency and duration of TMS

stimulation were established (Rossi et al., 2009; Wassermann, 1998). To further ensure that risks are minimised, subjects are also required to fill out a TMS safety screening form before participating to rule out other contraindications to participation. These include generally agreed factors that might reduce seizure threshold, including: family history of epilepsy, a history of neurological or psychiatric conditions or transient lowering of seizure thresholds e.g. as a result of lack of sleep, high consumption of alcohol, caffeine, or through taking anti-malarial medication, SSRIs or roaccutane.

2.2.3 Relationship between TMS and MRS measures

The method by which TMS is normally set up, where stimulation intensities are tailored to individuals' motor thresholds does not lend itself to comparison of individual differences. In order to compare across individuals TMS input-output curves must be calculated. With this method the change in size of MEPs are measured in response to varying either the test pulse stimulation intensity, as a percentage of the resting motor stimulation intensity, for corticospinal excitability measures; or the conditioning stimulus intensity as a percentage of the active stimulation intensity, for paired-pulse measures. A curve can then be fit to the data and the maximum slope or other curve parameters can be extracted for further analysis.

Despite the clear results from drug studies demonstrating the dependence of various paired-pulse TMS measures on certain neurotransmitter systems, comparing paired-pulse TMS measures to MRS measures has yielded complex results. MRS measures glutamate levels have been reported to correlate with a variety of measures including a positive correlation with ICF, thought to be a measure of predominantly glutamatergic signalling, and IO curve slope, a measure of the degree of change in MEP size with increasing stimulation intensity (Stagg et al., 2011a),. However studies have also reported counterintuitive relationships with TMS derived measures that are thought to assess inhibition. One study found a negative correlation with single-pulse IO curve plateau, i.e. the maximum MEP size which could be thought of as a measure of cortical

excitability (Dyke et al., 2017b) and a positive correlation with the duration of cortical silent period, a measure of inhibition (Tremblay et al., 2012, with Glx).

In terms of GABAergic TMS measures, neither 2.5ms SICI, thought to be a measure of synaptic $GABA_A$ activation, LICI, a measure of $GABA_B$ activation, or a linear combination of the two showed a significant correlation with MRS-measured GABA levels (Stagg et al., 2011a). One study showed a significant correlation between MRS-measured GABA and 1ms SICI, suggested as a measure of extrasynaptic $GABA_A$ activation (Stagg et al., 2011a) however this has not been replicated (Dyke et al., 2017b).

The lack of correlation between these measures is perhaps unsurprising given the differences in the measures obtained from each modality. MRS obtains the overall concentration of the neurotransmitter within a large voxel of interest, and as has been previously discussed (see Section 2.2.1) it has long been known that there are multiple 'pools' of each neurotransmitter which may have varying visibility to MRS (Rae, 2014). It has been proposed that GABA currently in use for neurotransmission makes up only a small part of the MRS-measured GABA signal (Rae, 2014; Stagg et al., 2014).

2.3 Interventions to alter plasticity or inhibition

2.3.1 Transcranial direct current stimulation (tDCS)

Scientists have been stimulating the brain with direct current for over 200 years, since the discovery of 'animal electricity' and 'bimetallic electricity' by Luigi Galvani and Alessandro Volta. Giovanni Aldini, the nephew of Galvani was the first to consider applying currents to the human brain as a therapeutic tool. In 1801, for several weeks he applied progressively increasing intensities of stimulation on a 27-year-old patient with depression, which reportedly resulted in a complete cure (Parent, 2004). For the next 160 years the use of low current brain stimulation was largely forgotten until the publication of several animal studies showing that transcranial direct

current stimulation (tDCS) caused changes in brain activity and increased excitability (Bindman, Lippold and Redfearn, 1964; Purpura and McMurtry, 1965). In the early 2000s Nitsche and Paulus (Nitsche and Paulus, 2000) showed these same changes in excitability in humans using a stimulation set-up which is still commonly used in research now.

Modern tDCS involves the placement of two (or more) electrodes on the surface of the participants skin with at least one of the electrodes placed on the head. Current flows between the anodal, positively charged, electrode and the cathodal, negatively charged, electrode. This current, normally in the order of 1-2mA, is not sufficient to cause the depolarisation of neuronal membranes needed to produce action potentials, and so tDCS can be considered a neuromodulatory technique. Current from the electrodes is normally delivered through sponges soaked in NaCl solution, with a concentration of 15-140mM optimally suited to minimize discomfort (Hummel et al., 2005). Alternatively electrode cream can be used as a conductive medium between the scalp and the electrodes (Nitsche et al., 2010; Voss et al., 2014).

In most studies the current is ramped up over a period of 5-15 seconds in order to reduce participant discomfort. Despite this, many participants report tingling, itching or burning around the site of the electrodes, with fewer reporting pain at the stimulation site (Poreisz et al., 2007) . These experiences tend to be most prominent during the first 30 seconds of stimulation (Nitsche, Schauenburg, et al., 2003), and so sham stimulation tends to ramp up in the same way as real stimulation, but then ramp back down after 30 seconds (Gandiga et al., 2006). At 1mA this creates a sham stimulation experience that is statistically undistinguishable from real stimulation for naïve participants (Ambrus et al., 2012).

tDCS is technique with a very high number of degrees of freedom; the location of electrodes, direction of current flow, intensity of stimulation and duration can all be altered. Conclusions about the effect of tDCS on the human brain may not generalise across differences in these factors. tDCS has been used as an intervention in a wide variety of disorders, including depression (e.g. Fregni et al., 2006; see Nitsche et al., 2009 for review), schizophrenia (e.g.

Brunelin et al., 2012), bipolar disorder (Brunoni et al., 2011), stroke (e.g. Fregni et al., 2005), chronic pain (Fregni et al., 2006b); and has been shown to alter the behaviour of healthy individuals when performing motor tasks (e.g. Nitsche et al., 2003; Reis et al., 2009), working memory tasks (Fregni et al., 2005), and decision making tasks (e.g. Fecteau et al., 2007). Across these many studies the electrode montage can vary hugely depending on the brain areas the authors choose to target. As this thesis is most concerned with plasticity within the motor system, only those montages commonly used in these studies will be discussed, thought there is still a wide variation within this literature.

Studies using tDCS to stimulate motor regions tend to have at least one electrode placed above the primary motor cortex (M1), though there are also many studies examining the effect of cerebellar tDCS on motor adaptation type tasks (Ehsani et al., 2016; Ferrucci et al., 2013; Ferrucci and Priori, 2014; Galea et al., 2009; Galea et al., 2011; O'Shea et al., 2017; Ugawa et al., 1991). Unilateral motor tDCS tends to place one electrode over M1, with a second, so called reference electrode, placed over the contralateral supra-orbital ridge (SOR). In some cases the reference electrode is positioned extracephalically, for example the contralateral shoulder (Moliadze et al., 2010), collar bone (Elbert et al., 1981), upper arm (Clark et al., 2012; Coffman et al., 2012; Falcone et al., 2012; Priori et al., 2008), or leg (Meyer-Schwickerath and Magun, 1951). While it has been shown that extracephalic reference increases the focality of the tDCS effects over the cortical area (Im et al., 2012) and also ensure that there are no focal effects of the reference electrode location on brain activity directly, increasing the distance between the cortical and reference electrode causes a decrease in the effects of tDCS on cortical excitability (Moliadze et al., 2010). Bilateral motor cortex tDCS places one electrode on each of the primary motor cortices.

More recently a new montage, known as high-definition tDCS (HD-tDCS), has been developed on the basis of computational modelling of current distribution. In this montage multiple reference electrodes are arranged in a ring, centred around an active electrode (Borckardt et al.,

2012; Kuo et al., 2007a). HD-tDCS results in more localized current distributions, compared to standard tDCS protocols.

Within each of the two standard forms of motor cortex tDCS, unilateral and bilateral stimulation, the direction of stimulation can be altered. In anodal tDCS the anode is placed over M1 and the cathode is treated as the reference electrode, whereas in cathodal tDCS the direction of current application is reversed. In bilateral tDCS the anode and cathode can be placed on either of the M1s, and so the position must be specified. Early animal studies demonstrated that in the area stimulated by the anode neuronal membranes become more depolarised (Purpura and McMurtry, 1965) and therefore more action potential spikes will be produced in response to sensory stimulation (Bindman, Lippold and Redfearn, 1964). Additionally, findings from stimulation of isolated pyramidal neurons show that those axons running parallel to the current field were activated by anodal tDCS and inhibited by cathodal tDCS (Bindman, Lippold and Redfearn, 1964). A recent study has demonstrated that anodal tDCS applied to monkeys causes increases in neuronal firing rates of around 15%, and found evidence for decreases in inhibitory signalling (Bogaard et al., 2019). These results are line with work measuring cortical excitability in humans: anodal stimulation centred over M1 results in an increase corticospinal excitability, as measured by TMS induced MEP size in the contralateral hand (Nitsche and Paulus, 2000; O'Shea et al., 2014), whereas cathodal stimulation causes a decrease in corticospinal excitability (e.g. O'Shea et al., 2014), though there is high inter-individual variability (Wiethoff et al., 2014). The effect of bilateral stimulation on the cortical excitability of the stimulated areas is more complex (O'Shea et al., 2014) but will be discussed in more detail in subsequent chapters (see Chapter 5).

However, the simple assumption that anodal stimulation causes excitation and cathodal inhibition has been challenged. Neurons may display different polarity changes across different cell areas (Joucla and Yvert, 2009), for example there may be somatic depolarization alongside apical dendritic hyperpolarisation or vice versa. Furthermore, the pattern of these changes varies depending on axon morphology (Ranck, 1975; Tranchina and Nicholson, 1986; Arlotti et al., 2012; Kabakov et al., 2012; Marquez et al., 2013) . In human experiments the orientation of

current flow across a cortical area has been shown to influence the effects of tDCS on cortical excitability (Rawji et al., 2018). This effect of current flow direction may account in some part for the differences in effect on cortical excitability of unilateral anodal stimulation and the effects of the anode in bilateral stimulation (O'Shea et al., 2014).

The changes in membrane polarization in anodal tDCS have been shown, in animal studies, to be caused by an increase in intracellular calcium (Ca^{2+}) concentration (Bikson et al., 2004; Islam et al., 1995), which is necessary for the induction of short and long-term plasticity (Bennett et al., 2000). Drug studies in humans have shown that flunarizine, a calcium channel blocker, eliminated the excitability enhancement induced by anodal stimulation during and after tDCS (Liebetanz et al., 2002; Nitsche, Fricke, et al., 2003) and a similar result was found using the sodium channel blocker carbamazepine (Liebetanz et al., 2002; Nitsche et al., 2003a), though riluzole the preferential blocker of teterotoxin sensitive Na^{+} channels does not influence tDCS-induced changes in corticospinal excitability (Liebetanz et al., 2003).

tDCS can have effects which long outlast the stimulation time, and while changes to intracellular calcium and sodium appear to play a role (Nitsche, Fricke, et al., 2003), synaptic modulation of glutamatergic and GABAergic synapses also appears to be important (Stagg and Nitsche, 2011). Anodal stimulation increases intracortical facilitation, and application of dextromethorphane, an NMDA receptor antagonist blocks the excitability change after effects (Liebetanz et al., 2003, 2002; Lugon et al., 2015; Monte-Silva et al., 2013a). Furthermore d-cycloserine, a specific NMDA receptor agonist, increases the duration, though, not the magnitude of the aftereffects (Nitsche et al., 2004b).

Additionally, human spectroscopy studies have also shown neurochemical effects of tDCS, M1 anodal tDCS reduces local GABA concentration (Kim et al., 2014b; Stagg et al., 2009a). In line with this, anodal tDCS has been demonstrated to reduce SICI and increase intracortical facilitation (Nitsche et al., 2005), indicating that tDCS may be causing an increase in glutamatergic activity and a decrease in GABAergic activity. The effects of cathodal tDCS are less consistent, with one MRS study showing reduced glutamate and GABA concentrations in the

stimulated area (Stagg et al., 2009a), whereas Kim and colleagues (Kim et al., 2014b) observed no change in GABA with cathodal tDCS. These neurochemical changes will be discussed in more detail in Chapter 4. Results from combined tDCS and drug studies paint a more complicated picture of the role of GABA. The $GABA_A$ agonising benzodiazepine lorazepam causes a delay in the onset of the excitatory effects of anodal tDCS, however this late-onset effect is enhanced and prolonged (Nitsche et al., 2004c).

Work using other psychoactive drugs has demonstrated the involvement of other neurotransmitter and neuromodulator systems. The SSRI citalopram can enhance the beneficial effect of anodal tDCS on motor plasticity, and reverse the normally inhibitory effect of cathodal tDCS (Kuo et al., 2016), indicating that the serotonin system is also involved. Rivastigmine, an acetylcholinesterase inhibitor, abolishes anodal and stabilizes cathodal aftereffects on excitability (Kuo et al., 2007a). Finally the catecholaminergic system has also been implicated, with l-dopa, the DA precursor, causing anodal tDCS to become inhibitory and stabilising cathodal after-effects on excitability (Kuo et al., 2007b), and amphetamines, which increase DA and NA, causing significant enhancement and prolonging increases in anodal, tDCS-induced, long-lasting excitability enhancement (Nitsche et al., 2004a).

2.3.1.1 Factors influencing tDCS effects

As mentioned previously, tDCS can be applied with a huge number of degrees of freedom, and changes in its method or timing of application can have implications for its effects. Furthermore there are also several factors known to alter the effects across individuals.

One such factor affecting tDCS efficacy within individuals is changing current density, the ratio of injected current to electrode area (Miranda et al., 2009). Keeping the electrode size constant but increasing stimulus intensity from 0.2mA to 1mA caused increases in the MEP size after stimulation (Nitsche and Paulus, 2000). Other studies examining the influence of stimulation intensity on behavioural effects have found significant differences compared to sham only when using higher intensities than 1mA (Cuypers et al., 2013; Iyer et al., 2005). However increasing the

intensity of stimulation does not appear to have linear effects TMS-measured excitability: while 1mA cathodal tDCS has been found to reduce cortical excitability, 2mA cathodal tDCS for 20 minutes caused excitability enhancement (Batsikadze et al., 2013). Furthermore, it has been shown that 2mA tDCS performed over the motor cortex does not result in adequate participant or experimenter blinding (Kessler et al., 2012; O'Connell et al., 2012; though see Russo et al., 2013), and can result in participant discomfort and increased risk of seizures (Bikson et al., 2009; Rossi et al., 2009).

Reducing the size of electrodes also causes increases in current density, as well as focalising the tDCS generated cortical excitability shifts (Nitsche et al., 2007). The increase in M1 excitability with decreasing electrode size still holds even for constant current density, indicating that the effect of stimulation surrounding regions may have a negative impact on cortical excitability changes (Bastani et al., 2013). Additionally by increasing the size of the reference electrode stimulation of frontal areas becomes less inefficient, but tDCS generated excitability shifts do not change (Nitsche et al., 2007). Though the current density can be fixed across individuals, the actual current flow through a participants brain can vary by up to 100% (Laakso et al., 2015) due to individual differences in brain and skull anatomy (Opitz et al., 2015). The amount of induced electric field in the brains of individuals does appear to relate to the degree of tDCS-induced changes in corticospinal excitability, however the relationship is complex and varies with changing stimulation parameters (Laakso et al., 2018)

The duration of tDCS has also been shown to influence its effects: stimulation of at least 3 minutes at 1mA was required to induce after effects on cortical excitability (Nitsche and Paulus, 2000), with anodal stimulation of 9-13 minutes required to increase MEPs above baseline for longer than 10 minutes after stimulation (Nitsche and Paulus, 2001). Anodal stimulation of 11 or 13 minutes caused elevations of cortical excitability for at least 50 minutes (Nitsche and Paulus, 2001). Likewise, cathodal stimulation of 5 and 7 minutes resulted in cortical excitability reductions for several minutes post stimulation, whereas stimulation for 9 minutes induced aftereffects for up to an hour after stimulation (Nitsche, Schauenburg, et al., 2003). It has been

proposed that short duration stimulation, which does not produce aftereffects primarily depends on sub-threshold resting membrane potential changes which are not sufficient to alter TMS-measured corticospinal excitability, however stimulation induced aftereffects are caused by intracortical inhibition and facilitation which depend on synaptic activity (Nitsche et al., 2005).

Even keeping the stimulation parameters constant there is still a large degree of intra-individual variability in response to tDCS between sessions (Dyke et al., 2017a). It seems that the effect of tDCS depends on the participant's brain state before or during stimulation (Amadi et al., 2015a). Electroencephalography measured resting state connectivity between stimulated M1 and the parietal cortex has been shown to be a strong predictor of tDCS response (Hordacre et al., 2017). Whether the participant is engaged in a concurrent activity has also been shown to alter tDCS effects: it has been demonstrated that the facilitation of MEP size by anodal tDCS is not seen if stimulation is applied during motor task performance (Amadi et al., 2015a; Antal et al., 2007; Bortoletto et al., 2017), voluntary motor contraction (Thirugnanasambandam et al., 2011) or cognitive tasks (Antal et al., 2007). Furthermore, the task type appears to be important in modulating the effect of tDCS on cortical excitability (Shirota et al., 2017). This has been taken as evidence for the existence of a form of homeostatic plasticity whereby increased background activity leading to LTP-like synaptic changes prevents further facilitation. Similar results showing the influence of brain state have also been seen with cathodal stimulation (Antal et al., 2007; Shirota et al., 2017; Thirugnanasambandam et al., 2011).

At inter-individual level there are also factors influencing the effect of tDCS. It has been demonstrated that subjects with the common Val66Met polymorphism at the brain-derived neurotropic growth factor (BDNF) gene showed enhanced effects of anodal tDCS compared to Val66Val carriers (Antal et al., 2010). Gender may also play a role: greater faciliatory response to anodal tDCS (Chaieb et al., 2008), and greater and prolonged excitability depression in response to cathodal tDCS (Kuo et al., 2006) has been shown in females, though these studies did not replicate each other. While this is by no means an exhaustive list, these are some of the factors to be considered when designing tDCS experiments.

2.3.2 Pharmacological Interventions

As discussed, above, the effects of tDCS on brain plasticity and inhibition are not fully understood, and stimulation is likely influencing a number of different physiological processes simultaneously. To enable the specific manipulation of a certain neurotransmitter system pharmacological interventions must be used. Direct and specific modulation of GABA concentration in humans is not possible, however pharmacological interventions can be used to alter the activity at specific GABA receptors, with agonists or antagonists allowing up or down regulation of activity at specific GABAergic receptors.

A number of GABA agonists, with varying specificity for different receptor subtypes, are currently in use for a wide range of clinical problems, from anxiety to muscle spasticity. Agonist drugs tend to act at either ionotropic $GABA_A$ receptors or at metabotropic $GABA_B$ receptors, and are often specific to certain receptor subtypes. For example the drug zolpidem shows specificity for the $GABA_A$ alpha1 receptor, lorazepam is a non-specific $GABA_A$ agonist, gaboxadol acts at extrasynaptic GABA receptors, while baclofen is a $GABA_B$ agonist.

While pharmacological interventions have increased physiological specificity their effects are spatially non-specific. By influencing many brain areas, these interventions can have complicated, and often unexpected, effects on brain processes. In general GABA agonists tend to cause sedation and somnolence, and so these effects must be taken into consideration when designing studies. Any differences in motor learning or cognitive processes seen with these drugs may be the result of effects on attention or alertness, rather than changes to the process of interest *per se*.

GABA antagonists, which inhibit the action of GABA at receptors, also present difficulties for use in research as these drugs tend to cause stimulant and consultant effects. One newly developed extrasynaptic $GABA_A$ antagonist has been shown to be safe in humans, and to effectively increase cortical excitability (Darmani et al., 2016).

2.4 Summary

This thesis aims to investigate the influence of inhibition on plasticity within the human primary motor cortices. This section has described the multimodal methods used to take measurements of brain structure and physiology, and methods used to alter the brain physiology. MRS has been used to assess concentrations of GABA and glutamate within M1, and TMS has been used to assess activation at $GABA_A$, $GABA_B$ and glutamatergic receptors. Brain structure has been measured using structural MRI, and DWI, and brain functional connectivity was measured using resting state fMRI. tDCS and GABAergic drug interventions have been used to alter inhibition and plasticity.

Chapter 3: Offline improvements in motor skill depend on amount of practice, not explicit sequence knowledge or passage of time: an argument against wakeful consolidation

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Understanding the factors affecting how well we learn and retain motor skills is a major neuroscientific question with clear implications for clinical research. As well as skill levels improving during learning, skill on some motor tasks has been shown to spontaneously improve between practice sessions, a phenomenon known as consolidation. Consolidation on motor sequence learning tasks which contain an explicit declarative component have been shown to be dependent on sleep; whereas similar tasks which are learnt implicitly, i.e. without a declarative component, can additionally show consolidation across periods of wakefulness (Robertson et al., 2004).

However, in many of the studies comparing consolidation after explicit and implicit learning, equivalent levels of online skill across task types is achieved by giving differing amounts of practice; the implicit condition typically experiences around 40% more sequence repetitions than the explicit condition. While appropriately controlling for skill level, this approach has an inherent confound. We therefore investigated whether it was task type, or amount of practice that was the key factor in determining offline skill acquisition. We showed that controlling for amount of practice, specifically in the follow up session, eliminates any difference between implicit and explicit learning. Furthermore, improvements in task skill could be explained by continued relearning in the follow up session, therefore calling into question the existence of the phenomenon of wakeful consolidation on the SRTT.

3.1 Introduction

How we acquire new motor skills, for example when learning a new piano piece, or, potentially, in rehabilitation of motor skills after a brain injury such as a stroke, is a vital question in neuroscience. It is becoming understood that many external factors affect our ability to learn new skills, including the amount of practice and the instructions given. However, recent research has also highlighted the importance of the rest periods, as well as training sessions, in skill acquisition. It has been shown that in the hours following the learning of a new motor skill not only does skill memory become resistant to interference (Brashers-Krug et al., 1996), but skill performance may also improve without additional practice (Karni et al., 1998b; Karni and Sagi, 1993; Korman et al., 2003; McGaugh, 2000; Ungerleider et al., 2002; Walker et al., 2003). This process of offline stabilisation, or even improvement, in motor memory is termed consolidation.

For many motor skills, consolidation has been shown to be sleep-dependent (Maquet, 2001; Peigneux et al., 2001; Plihal and Born, 1997; Stickgold et al., 2001; Walker et al., 2002). However, it has been more recently been argued that consolidation can be observed in the absence of sleep under certain circumstances (Al-Sharman and Siengsukon, 2014; Robertson et al., 2004; Tunovic et al., 2014), a finding that has important implications for the design of optimal training interventions. One of the key factors that is thought to be important for wakeful consolidation is the absence of explicit knowledge; if participants are informed of the presence of a sequence in the serial reaction time task (SRTT) then consolidation is only seen across 12 hours which includes sleep; whereas participants who are unaware of the presence of a sequence, and therefore learn implicitly, show performance improvements both with and without sleep (Robertson et al., 2004).

Though the differences in consolidation between implicit and explicit versions of the SRTT have been replicated (Press et al., 2005; Tunovic et al., 2014), in order to ensure comparable skill levels between the explicit and implicit versions of the tasks, participants in the implicit condition in the above-mentioned studies were exposed to approximately 40% more repetitions of the sequence

than those in the explicit condition (Robertson et al., 2004). This difference in amount of practice is present in both the initial training session, and in the later re-test. While matching skill level is vital for meaningful comparisons to be made, this approach leaves open the possibility that it is the amount of practice, and not task type, that is primarily responsible for modulating wakeful consolidation.

This study therefore aimed to test the hypothesis that the degree of wakeful consolidation seen in a SRTT could be accounted for by the amount of practice performed, either in the morning training session or the evening retest session. As discussed in Chapter 1, the SRTT is widely used in the motor learning field, and is used in subsequent chapters of this thesis. It is therefore important that the normal learning and retention trajectory is well understood.

3.2 Methods

3.2.1 Participants

60 healthy, right-handed individuals between the ages of 18 and 30 years old were recruited (24 males, aged 19-30 years, mean age 23.18 years). We specifically recruited participants who did not play a keyboard instrument to a high level (defined as achieving UK ABRSM grade 6 or equivalent, and still being in regular practice). Handedness was assessed using the Edinburgh Handedness Questionnaire (Oldfield, 1971). Participants were assigned to one of four conditions: explicit-short-short (ESS), explicit-long-long (ELL), explicit-short-long (ESL) or implicit-long-long (ILL).

3.2.2 Experimental overview

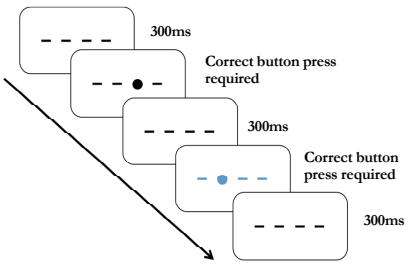
Participants came to the lab twice on the same day, once in the morning (between 8 and 10am) and returned 10 hours later. During both sessions they completed a button pressing reaction time task, the SRTT (Nissen and Bullemer, 1987).

3.2.3 SRTT set-up

Participants viewed the task on a laptop monitor (Dell, Round Rock, Latitude E5540) at a distance of approximately 40 cm, and made responses on an external 4-button box (Current Designs, Philadelphia, OTR-1X4-CR). Stimuli were presented using Psychtoolbox (PTB-3) (Brainard, 1997) for Matlab, and displayed using Matlab R2014b (The Mathworks Inc., Natick, USA). The task was designed to exactly replicate that used in Robertson, Pascual-Leone and Press, 2004. During the task 4 dashes were presented on the screen, arranged in a horizontal line (dash size=5x2mm, spacing=35mm). Participants were instructed that each of these dashes represented one of four buttons on the button box. They were asked to place the 4 fingers of their right hand on the 4 buttons of the box. During the experiment a filled circle (diameter=17mm) would appear at one of the 4 dash locations, signalling that the participant should respond by pressing the corresponding button. The circle cue remained onscreen until participants made the correct button press response, after which there was a 300ms interstimulus interval (ISI) before another circle cue appeared, see Figure 3.1A. The first response made to each cue was recorded.

During the morning session SRTT all participants performed three blocks of the task: an initial baseline test block, followed by a longer training block, and finally an 'AM test' block. In the evening session participants performed only a 'PM test' block. During all blocks of the task random sections were performed before and after a sequence section (see Figure 3.1C). Cues in the sequence section always followed the same 12-item pattern (2-3-1-4-3-2-4-1-3-4-2-1; where 1 is the index finger and 4 is the little finger) (Tunovic et al., 2014). Random sections consisted of 48 button presses presented pseudorandomly with the following constraints: there were no item repeats (e.g. no -1-1-), and each finger press occurred 3 times every 12 items. Eight unique random sections were used across the experiment; the order in which participants performed them was counterbalanced.

A



B

Condition Name	AM sequence repetitions (baseline, training, AM test)	PM sequence repetitions
Implicit- Long, Long (ILL) - Robertson et al., (2004) : Implicit	15, 25, 15	15
Explicit- Short, Short (ESS) - Robertson et al., (2004) : Explicit	9, 15, 9	9
Explicit- Long, Long (ELL)	15, 25, 15	15
Explicit- Short, Long (ESL)	9, 15, 9	15

C

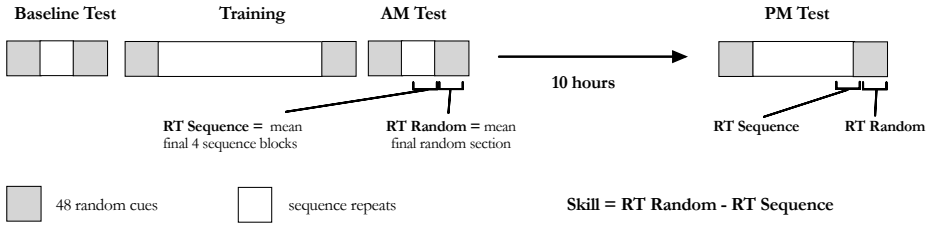


Figure 3.1 A, Task screen as seen by participant, identical to that used in Robertson et al., 2004. Blue cue signifies the beginning of the sequence; B, List of task conditions and number of sequence repeats. ILL and ESS condition identical to that used in Robertson et al., 2004 designed to be matched for skill level; C, Diagram of task protocol, including the method of calculating AM and PM skill.

In the explicit conditions the beginning of each sequence repetition was signalled to the participant by the first circle cue of the sequence appearing in blue; in the implicit condition all circles remained black. Participants in the explicit conditions were told the meaning of this blue signal before beginning the task but were not told about the sequence length or how sequences would be distributed among random blocks. Participants in the explicit conditions were also instructed to try to learn the sequence, and were told they would be asked to recall it in the evening session. Participants in the implicit condition were never informed about the presence of a sequence. In all other respects the implicit and explicit conditions were the same in both instruction and appearance. All participants were told to make responses as quickly and as accurately as possible.

The number of sequence repeats in each sequence block varied between the 'long' versions of the task and the 'short' versions. In the 'long' conditions there were 25 repeats of the sequence in the training block, and 15 repeats in each of the test blocks; in the 'short' condition there were 15 repeats in the training block, and 9 in each test block. Participants in the implicit-long-long (ILL)

and explicit-long-long (ELL) conditions had 'long' sessions in both the morning and evening; participants in the explicit-short-short (ESS) condition had 'short' sessions in both the morning and the evening; and participants in the explicit-short-long (ESL) had a 'short' morning session and a 'long' evening session (see Figure 3.1B).

Awareness of the sequence was assessed for every subject at the end of the evening session by asking participants to reproduce as much of the sequence as possible by free recall using the button box.

3.3 Analysis

In line previous literature only correct responses were included and response times (RTs) greater than 2.7 SDs above the mean for each block of the experiment were excluded. Accuracy was high (>95%) across all conditions and so could not be used to assess learning.

Significantly outlying participants in terms of morning session RT (Grubb's test $p < 0.05$) were also identified. One participant from the ESS condition was removed from further analysis on this basis. Two further participants from the ILL condition who were able to explicitly recall 7 and 10 items respectively were also removed. All other participants from the ILL group recalled 4 or fewer sequence items. Statistical testing was performed using SPSS Statistics Version 22 (IBM, Armonk), curve fitting was performed with Matlab 2015b (The Mathworks Inc., Natick, USA).

3.3.1 Skill measure calculation

As is common, skill was calculated as the difference between the mean RT in the final random section of the test blocks (i.e. final 48 random presses), and the mean response time of the preceding 4 sequence repetitions (i.e. final 48 sequence presses; Robertson et al., 2004, Tunovic

et al., 2014). A skill value was calculated for the final morning test block, (AM Skill) and for the evening test block (PM Skill).

3.3.2 Estimating ESS, ILS and ELS conditions

Using the above method, skill for the ILL, ELL and ESL conditions was calculated using the mean response time to the 12th, 13th, 14th and 15th repeats of the sequence, whereas in the ESS condition skill was calculated using the 6th, 7th, 8th and 9th repeat. To directly compare between conditions, we therefore calculated an 'Early PM Skill' measure for the ESL, ILL and ELL conditions where the participant's mean RT in the 6th, 7th, 8th and 9th sequence repeats was subtracted from the final random section RT. This approach allows us to estimate consolidation after the equivalent of a short evening practice session, i.e. creating estimates of ILS and ELS conditions, termed estimated ILS (eILS) and estimated ELS (eELS) respectively. As the ESS and estimated ESS (eESS) conditions had equivalent morning and evening sessions, these were analysed together, forming an ESS+eESS condition.

3.3.3 Learning Curve Estimation

In order to perform a model fitting analysis to generate learning curves, we combined the ESL and ESS conditions as they contained identical morning sessions. Model fitting was therefore performed on three conditions separately: ILL condition, ELL condition and a combined ESS+ESL condition.

Individual participant's RTs were log transformed (Ratcliff, 1993) to improve normality (logRT). Each participant's mean logRT for each sequence repetition was calculated, and then subtracted from their mean logRT in the first random section. Baselined mean logRT for each sequence repetition across the group was then calculated for each of the three conditions. The first repetition of the sequence in the training and AM test section were excluded from this analysis.

The baselined mean logRTs for each morning sequence repetition were then fitted with a two-term power function ($Y = a*x^b$) (Rickard et al., 2008) and extrapolated to account for the additional number of sequence repetitions in the evening session.

3.4 Results

3.4.1 Long implicit practice leads to greater offline skill retention than short explicit practice

We first wished to investigate whether offline skill improvements could be demonstrated in any experimental condition, and if so, whether it depended on the type (i.e. implicit or explicit) of learning performed. We first investigated differences in offline skill change across all four conditions using a Repeated Measures (RM) ANOVA with a between-subjects factor of Condition (ILL, ESS, ELL, ESL) and a within-subjects factor of Time (AM Skill, PM Skill). As predicted, we found that offline skill change differed between the four conditions [No significant main effect of Time ($F(1,53)=1.702$, $p=0.198$); no significant main effect of Condition ($F(3,53)=2.607$, $p=0.061$); significant Time by Condition interaction ($F(3,53)=5.847$, $p=0.002$); Figure 3.2].

We therefore went on to investigate whether this difference between conditions was dependent on the type of learning performed (i.e. varied between implicit and explicit conditions). We therefore contrasted offline skill change between the ILL and ESS conditions, chosen as they have previously been shown to be matched for skill at the end of the morning training session (Robertson et al., 2004; Tunovic et al., 2014), a finding which we replicated here by confirming that the skill levels at the end of the AM session were not significantly different ($t(25)=1.266$, $p=0.271$). To investigate differences in offline skill change a RM ANOVA was performed with a within-subjects factor of Time (AM Skill, PM Skill) and a between-subjects factor of Learning Type (Implicit [ILL], Explicit [ESS]). In line with previous reports, there was a difference in offline skill change between these conditions [significant main effect of Time ($F(1,25)=5.595$,

p=0.026); significant main effect of Learning Type (F(1,25)=0.011, p=0.917); significant Time by Learning Type interaction (F(1,25)=7.687, p=0.010)].

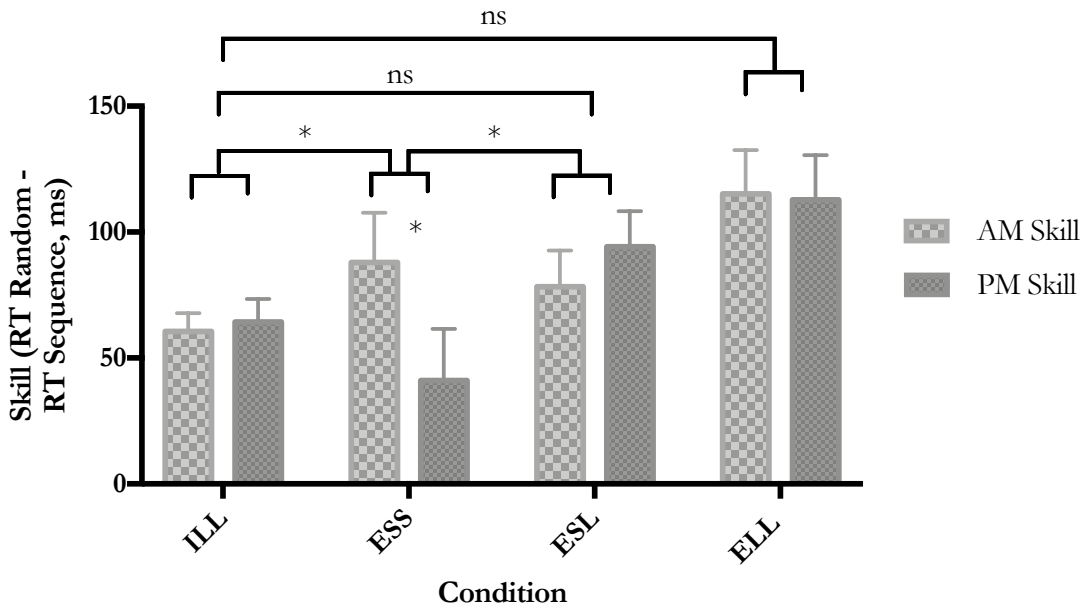


Figure 3.2 Skill level (RT final random section- RT final 4 sequence repeats) in milliseconds, in the AM and PM sessions, separated by condition. The ESS condition shows a significantly different pattern of change in skill than the other three conditions, none of which differ from each other.

Planned *post-hoc* tests revealed no significant change in skill in the ILL condition (t(12)=-0.375, p=0.715). However, we did observe a significant decrease in skill within the ESS condition (t(13)=3.135, p=0.008).

3.4.2 Practice, rather than task type, determines consolidation

In order to match skill levels at the end of the AM session, we used an implicit learning task with more practice blocks than its explicitly learnt equivalent. The difference between offline changes in skill in the ILL and ESS conditions could hence be due either to differences in the nature of the learning (explicit vs. implicit) or in the amount of practice (LL vs. SS), or both. To test whether differences persist when controlling for the amount of practice, we contrasted the ELL and ILL conditions, which were matched for the amount of practice. A RM ANOVA, with a within-subjects factor of Time *(*AM Skill, PM Skill), and a between-subjects factor of Learning Type (Implicit [ILL], Explicit [ELL]) demonstrated no significant difference in offline skill

change between implicit and explicit learning when amount of practice was matched [no significant main effect of Time ($F(1,26)=0.012$, $p=0.914$,); significant main effect of Learning Type ($F(1,26)=6.955$, $p=0.014$); no significant Time by Learning Type interaction ($F(1,26)=0.245$, $p=0.624$)]. This therefore suggests that the previously reported differences in wakeful consolidation between implicit and explicit learning could be explained by amount of practice, rather than by the type of learning.

3.4.3 More evening practice leads to better re-test performance.

Next, we investigated whether the difference in offline skill acquisition, as normally calculated, between the ILL and ESS conditions could be explained by differences in the length of the evening session learning session alone, i.e. the amount of re-exposure before the PM skill measure was sampled. We therefore contrasted the ESL and ILL conditions, which had differing amounts of morning practice but the same amount of evening practice, and showed no significant difference between consolidation [RM ANOVA with within-subjects factor Time (AM Skill, PM Skill) and between-subjects factor Learning Type (Implicit [ILL], Explicit [ESL]); no significant main effect of Time ($F(1,26)=1.596$, $p=0.218$); no significant main effect of Learning Type ($F(1,26)=2.417$, $p=0.132$); no significant interaction between Time and Learning Type ($F(1,26)=0.619$, $p=0.439$)].

To test the hypothesis that the amount of morning practice alone can influence this measure of offline skill acquisition, we compared the ESL and ELL conditions. There was no difference in skill consolidation between the two conditions, suggesting that the amount of morning practice does not significantly affect consolidation on this task [RM ANOVA with the within-subjects factor of Time: (AM Skill, PM Skill) and the between-subjects factor of Morning Practice: (Long [ELL], Short [ESL]): no significant main effect of Morning Practice: $F(1,28)=1.646$, $p=0.210$); no significant main effect of Time ($F(1,28)=0.957$, $p=0.336$); and no significant Time by Morning Practice interaction ($F(1,28)=1.745$, $p=0.197$)].

Can the significant difference in offline skill acquisition be explained solely by the amount of re-exposure in the PM session? To address this question, we contrasted the ESL and ESS conditions, which had different amounts of evening practice, but identical AM sessions. A RM ANOVA with a within-subjects factor of Time (AM Skill, PM Skill); and a between-subjects factor of Evening Re-exposure (Long [ESL], Short [ESS]) demonstrated that the commonly used measurement of degree of offline skill acquisition in the explicit learning condition may, at least in part, be driven by the amount of re-exposure during the evening session [no significant main effect of Time ($F(1,27)=2.696$, $p=0.112$); no significant main effect of Re-exposure ($F(1,27)=0.930$, $p=0.343$); significant interaction between Time and Re-exposure ($F(1,27)=11.153$, $p=0.002$)], driven by greater skill increases in the ESL than the ESS condition (t-test: $t(27)=-3.340$, $p=0.002$).

To further disentangle the effects of the amount of re-exposure in the evening from amount of practice in the morning on this measure of offline skill acquisition we performed a further analysis using a measure of 'Early PM Skill' (see Section 3.3.2). This gave us an estimated ESS (eESS), estimated ELS (eELS) and estimated ILS (eILS) condition using the data from the first part of the PM session in the ESL, ELL and ILL conditions respectively. We therefore had 3 conditions with differing amounts of morning practice or learning type, but the same amount of evening practice. We found that there was no difference in change in skill across these conditions, again suggesting that it is the amount of evening practice, rather than task type or morning practice, that primarily influences this measure of offline skill acquisition [Figure 3.3; RM ANOVA; within-subjects factor of Time (AM Skill, Early PM Skill) and between-subject factor of Condition ([ESS+eESS], eILS, eELS); significant main effect of Time ($F(1,54)=4.224$, $p=0.045$), significant main effect of Condition ($F(2,54)=3.818$, $p=0.028$,), no significant Time by Condition interaction ($F(2,54)=0.234$, $p=0.792$,)].

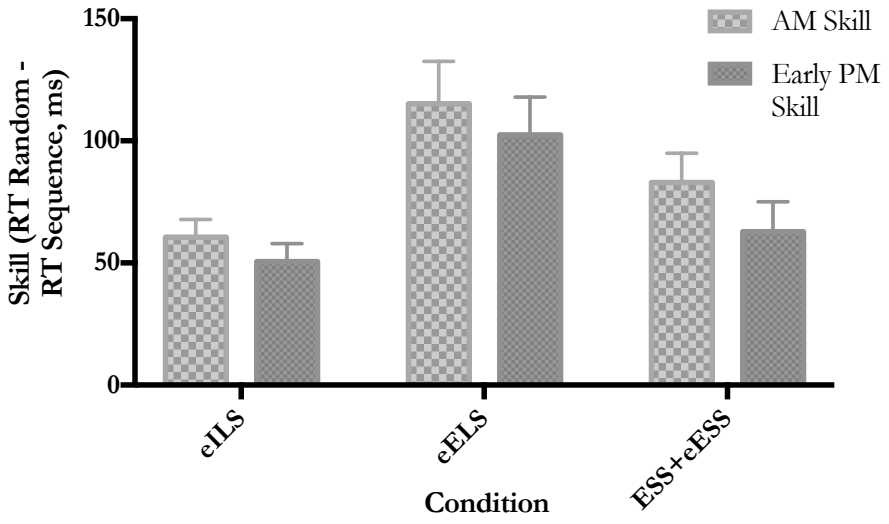


Figure 3.3 Skill level (RT final random section –RT for 6th, 7th, 8th and 9th sequence repeats) in milliseconds, in the AM and PM sessions. These results are for the estimated ILS, estimated ELS, and the combined ESS and estimated ESS condition. Demonstrating no significant differences in change in skill across the three conditions, where the amount of practice in the PM session before skill is sampled is equal.

3.4.4 Amount of practice determines learning, regardless of whether there is a break between AM and PM test sessions

Having shown that amount of practice on the SRTT sequence in the evening is sufficient to explain the differences in consolidation observed between conditions, we wanted to examine quantitatively whether the amount of additional practice alone, rather than the passage of time, might explain the improvements in performance seen between the AM test and PM test. We first fitted a learning curve (see Section 3.3.3) to the baseline mean logRTs from the sequence repetitions of the morning blocks, along with 95% prediction confidence intervals. The baselined mean logRTs for each sequence repeat from the PM test session were then plotted onto the same curve (Figure 3.4). The points that were used to calculate both the 'early skill' metric and those used to calculate the standardly used 'late skill' metric are highlighted, and the average of these 4 values is also plotted. If wakeful consolidation had occurred then it would be predicted that the logRT from the PM sessions would fall below the AM learning curve. As the ESS and ESL conditions contain the same amount of morning practice we combined them (ESS+ESL); the values for first 9 sequence repetitions of the PM session are the mean of the two conditions, the remaining 6 repetitions come from the ESL condition alone.

Examining the learning curves reveals that for both the ELL and the combined [ESS+ESL] conditions the mean baselined logRT of the 6th, 7th, 8th and 9th PM sequence repetitions (i.e. the early skill measure for ELL and ESL, and original skill for ESS) is higher than would be expected if these trials had occurred immediately after the morning session (i.e. performance is worse). However, in the ILL, ELL and ESS+ESL conditions the average RT of the 12th, 13th, 14th and 15th PM sequence repetition did not fall outside the range expected given a continuation of the morning learning. Therefore, our results appear to indicate that individuals' improvement in reaction time at the end of the evening tests across all conditions could be explained simply by the same learning process active in the morning session, rather than relying on an additional offline consolidation process.

3.4.5 Change in random RTs are not determined by amount of morning practice or condition type

Finally, we wished to investigate whether there might be a difference in general task performance, as opposed to specific sequence learning, between the conditions. We therefore performed a repeated measures (RM) ANOVA to examine whether the change in RT during the second random section of the AM Test and the PM test varied between conditions, with a within-subject factor of Time (AM Random RT, PM Random RT) and a between-subject factor of Condition (ILL, ESS, ELL, ESL). While RT to random presses tended to decrease between AM and PM test, and was lower in the groups with long morning session, there was no difference in change in Random RT between the groups [significant main effect of Time ($F(1,53)=16.535$, $p<0.001$); significant main effect of Condition ($F(3,53)=5.647$ $p=0.002$); no significant interaction between Time and Condition ($F(3,53)=2.192$, $p=0.100$, Figure 3.5)]. This demonstrates that while longer morning practice led to faster RTs in both the morning and evening sessions, any offline changes in skill seen were not merely a reflection of change in RT between the AM and PM sessions.

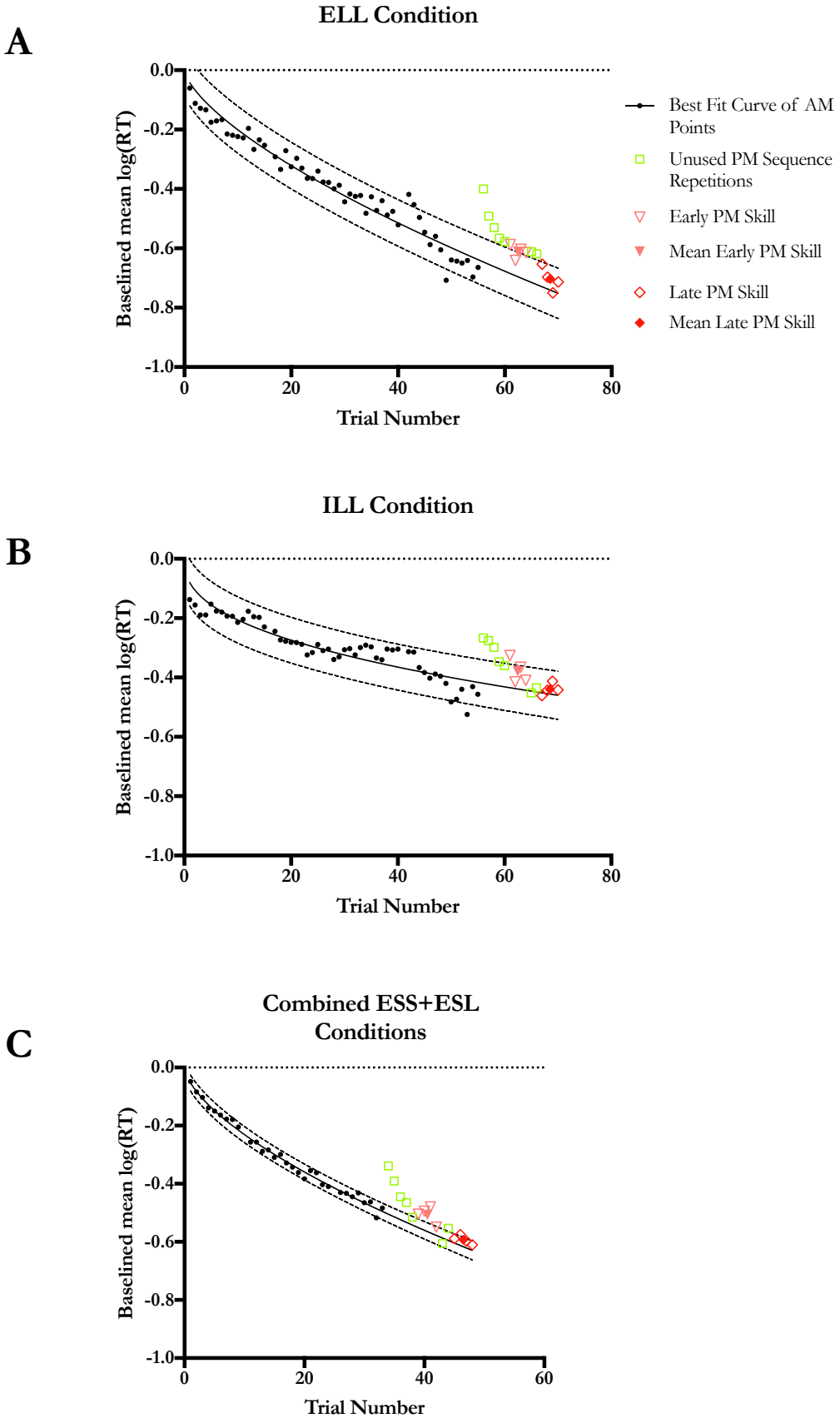


Figure 3.4 Learning curves fitted to the group mean log transformed morning RT data for the ELL (A), ILL (B), and combined ESS+ESL (C) conditions. On each of these curves the mean data points for the evening session are also plotted. The data points that were used to calculate Skill measures are highlighted.

We then went on to investigate whether task type affected the change general task performance over time. We therefore compared the ELL and ILL conditions, which vary by task type but are matched for amount of practice. A RM ANOVA with a within-subjects factor of Time (AM Random RT, PM Random RT), and a between subjects factor of Learning Type (Implicit-ILL, Explicit-ELL) demonstrated that learning type did not affect the change in task performance between the AM and PM sessions [significant effect of Time ($F(1,27)=4.737$, $p=0.038$; no significant main effect of Learning Type ($F(1,27)=0.005$ $p=0.945$), no significant Learning Type by Time interaction ($F(1,27)=0.751$ $p=0.394$)].

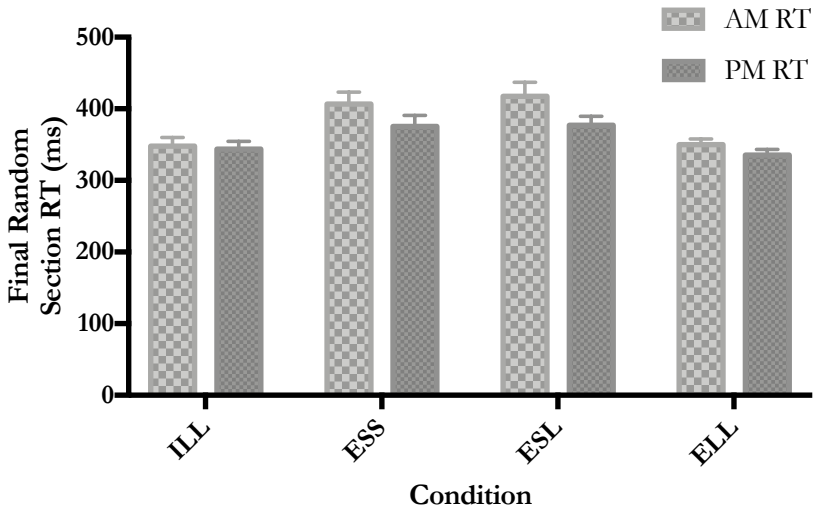


Figure 3.5 Mean reaction time (RT) for final random sections in both AM and PM sessions, separated by condition. There were no significant differences in change in RT across the conditions.

3.5 Discussion

This study aimed to understand the factors that affect wakeful offline skill acquisition. We demonstrated that, although there is a significant difference between offline skill acquisition in an implicit and explicit version of the commonly used motor sequence learning SRTT when tasks were matched for skill level at the end of the morning session, this difference could be explained parsimoniously by the amount of practice in the evening session.

Our results are in line with the only other published paper comparing consolidation between the implicit and explicit version of the SRTT where amount of practice, rather than skill, was matched (Sami et al., 2014). However, it is important to note that our results showed a generally lower level of consolidation across all conditions relative to previous studies (Robertson et al., 2004; Tunovic et al., 2014).

We propose that the amount of practice in the evening session is the most important factor in allowing apparent consolidation in skill to be demonstrated, at least for this implementation of the SRTT. We demonstrated no significant differences in the amount of offline skill acquisition between the explicit ELL and ESL conditions, which had a differing number of AM sequence repetitions but an equal number in the evening session. This explicit ESL condition showed a similar pattern of consolidation to the implicit ILL condition, where both groups showed maintenance of skill level; but a different pattern to the ESS condition, where skill significantly decreased between sessions, suggesting that evening practice is sufficient to explain the degree of apparent offline skill acquisition. Furthermore, when we calculated an early PM skill measure in the ELL and ILL conditions from the same sequence repetitions used to measure skill in the ESS condition, there were no significant differences between conditions.

In previous SRTT studies, the amount of practice in the implicit and explicit conditions was chosen so that task skill was matched at the end of the morning training session across learning types. In this study, by matching the amount of practice within the implicit and explicit conditions (ILL and ELL), we introduce a difference in skill at the end of the morning session between the two conditions. It could be argued that this greater level of initial skill may be responsible for the increased offline skill retention seen in the ELL condition. However, there was no statistical difference between offline skill retention in the ELL condition and that seen in ESL condition, which is matched for morning skill with the ILL and ESS groups, where the offline skill retention was reduced, making this explanation unlikely.

Examining in more detail how evening practice influences skill changes, we fitted learning curves to the response times from the AM session, and extrapolated these curves to give a predicted learning curve for the continuation of the learning. This approach allowed us to compare our observations in the PM session with predicted RTs if learning continued at the same rate as in the morning session. We found that, across all of the conditions, initial RT observed in the evening session was higher than would be predicted, but that RT decreased over the course of the evening session, ultimately reaching the level that would be predicted simply from a continuation of morning learning. This calls into question the use of the term "consolidation" within the context of the results presented here. The results presented here, and indeed across the literature, could be simply explained by just one learning process that is active in both the morning and evening sessions, albeit with some effect of forgetting in-between sessions. Using the commonly utilised methods presented here, our data does not support the idea of non-sleep dependent consolidation in either explicit or implicit sequence learning.

A similar argument has taken place surrounding sleep-dependent consolidation, with a recent meta-analysis (Pan and Rickard, 2015) finding that when moderating factors, such as averaging of trials, time of day and training duration, were controlled for there was no evidence that sleep could enhance learning. It has been proposed that sleep may be responsible for the stabilization of skills, rather than their enhancement (Nettersheim et al., 2015).

However, our results do not imply that there are no differences between explicit and implicit learning in terms of the processes involved in performing or learning these tasks. There has been much evidence from process-dissociation type experiments (Destrebecqz and Cleeremans, 2001; Perruchet et al., 1997; Song et al., 2007); brain imaging studies (Fletcher et al., 2005; Sami et al., 2014; Willingham et al., 2002); and TMS studies (Breton and Robertson, 2017; Hirano et al., 2017; Horn et al., 2005; Tunovic et al., 2014), showing that distinct learning process are at work during implicit and explicit motor sequence learning. We also cannot rule out that there may be differences in other aspects of consolidation, for example resistance to interference or savings in relearning, between the two task types (Ghilardi et al., 2009). Additionally, though we have

argued against the existence of wakeful consolidation on the SRTT under normal circumstances, there is still evidence to suggest that wakeful consolidation can be induced by applying magnetic (Tunovic et al., 2014) or electrical (Ehsani et al., 2016; Rumpf et al., 2017) brain stimulation at training.

3.6 Conclusions

In summary, we argue that previously observed differences between offline skill acquisition after implicit and explicit learning may be driven by the differing amount of practice on the task, rather than inherent differences between the two learning types. This finding may have important implications for the design of training regimens, as it suggests, perhaps unfortunately, that total amount of practice prior to skill measurement, rather than learning type, may be the major determinant of skill acquisition and retention.

Chapter 4: Application of non-invasive brain stimulation results in neurochemical changes in bilateral motor cortices

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Transcranial direct current stimulation (tDCS) is known to alter local neurochemistry in a polarity dependent manner (Stagg et al., 2011b), with these changes thought to underlie to concomitant behavioural changes (Stagg et al., 2011a). However, learning a novel motor skill is dependent both on regional changes within the primary motor cortex (M1) contralateral to the active hand, and also on modulation between and within anatomically distant but functionally connected brain regions. Inter-regional changes are particularly important in functional recovery after stroke, where critical plastic changes underpinning behavioural improvements are observed in both ipsilesional and contralesional M1s. However, the physiological mechanisms underpinning plasticity within other brain regions, most importantly the ipsilateral M1, are not well understood.

Here, we used concurrent two-voxel magnetic resonance spectroscopy (MRS) to simultaneously quantify changes in neurochemicals within left and right M1s, in healthy humans of both sexes, in response to tDCS applied to left M1. We demonstrated a decrease in GABA in both the stimulated (left) and non-stimulated (right) M1 after anodal tDCS, whereas a decrease in GABA was only observed in non-stimulated M1 after cathodal stimulation. This GABA decrease in the non-stimulated M1 during cathodal tDCS was negatively correlated with microstructure of M1:M1 callosal fibres, as quantified by diffusion MRI, suggesting that structural features of these fibres may mediate GABA decrease in the non-stimulated region. We found no significant changes in glutamate. Taken together, these findings shed light on interactions between the two major network nodes underpinning motor plasticity, offering a potential framework from which to optimise future interventions to improve motor function after stroke. The data for this chapter was collected by Velicia Bachtiar, and some of the results are published in Journal of Neuroscience (Bachtiar, Johnstone* et al., 2018).*

4.1 Introduction

It has consistently been shown that plasticity in the motor system, whether underlying the learning of novel motor skills, or their relearning after stroke, involves changes within and between a network of anatomically distributed motor regions. In particular, it is clear from studies examining the neural control of unilateral motor behaviours that the interaction between the two primary motor cortices (M1s) is vital for motor learning (Di Pino et al., 2014; Perez and Cohen, 2009; Reis et al., 2009). However, little is known about exactly how this interaction occurs, and what physiological changes in distant nodes underpin interregional plasticity. Here we tested this hypothesis by studying neurochemical changes underpinning plasticity in both M1s simultaneously, using transcranial direct current stimulation (tDCS).

As discussed in Chapter 2, anodal tDCS to M1 is thought to induce long-term potentiation (LTP)-like changes within the stimulated region (Fritsch et al., 2018; Hess et al., 1996; Monte-Silva et al., 2013b; Stagg and Nitsche, 2011) and has been demonstrated to affect regional neurochemistry in a manner similar to motor learning, most notably resulting in a regional decrease in GABA concentration (Bachtiar et al., 2015; Floyer-Lea et al., 2006; Stagg et al., 2011a; Stagg et al., 2009a; Stagg and Nitsche, 2011). The neurochemical changes induced by tDCS are reflected in behavioural changes, with anodal tDCS tending to improve learning if applied during practice of a motor task (Amadi et al., 2015a; Cuypers et al., 2013; Galea et al., 2011; Kim et al., 2014b; Nitsche et al., 2003c; Sriraman et al., 2014; Stagg et al., 2011a; Waters et al., 2017). This beneficial effect of anodal tDCS on motor learning has led to tDCS being suggested as a putative adjunct therapy to enhance motor rehabilitation after stroke, with some success in small proof-of-principle studies (Allman et al., 2016; Boggio et al., 2007; Fregni et al., 2005; Hummel et al., 2005; Hummel and Cohen, 2005; Kim et al., 2006; Lindenberg et al., 2010).

In addition to applying anodal tDCS to the ipsilesional hemisphere after stroke, some studies have shown that applying cathodal tDCS to the contralesional hemisphere, to reduce local activity here, also leads to behavioural improvements in the paretic hand. This functional

improvement is accompanied by increased activity in the ipsilesional M1, in line with the hypothesis of interhemispheric imbalance in this patient group (Fregni et al., 2005; Boggio et al., 2007; Kim et al., 2010; Stagg et al., 2012; O'Shea et al., 2014). However, the mechanism by which contralesional tDCS can increase activity in the contralateral, ipsilesional, M1 is not understood.

In this study we used a novel two-voxel magnetic resonance spectroscopy (MRS) approach to quantify GABA and glutamate changes concurrently within the left and right M1 during the three most commonly used tDCS montages: anodal to left M1; cathodal to left M1; and bilateral, where electrodes are placed over both M1s. We also examined how the neurochemical changes in the unstimulated hemisphere were related to white matter microstructure and the functional connectivity between the two M1s.

4.2 Methods

4.2.1 Participants

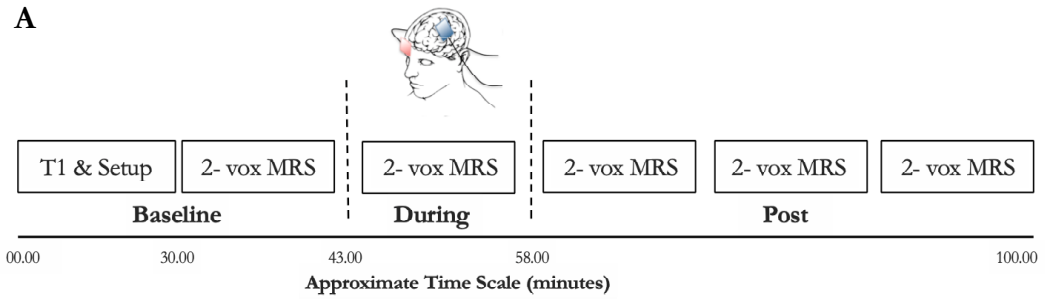
Twelve healthy participants (2 male; aged 20-32 years, mean age 25 years) gave their informed consent to participate in this study in accordance with ethical approval from the East London Research Ethics Committee (Ref: 10/H0703/50). All were right-handed as assessed by the Edinburgh Handedness Inventory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

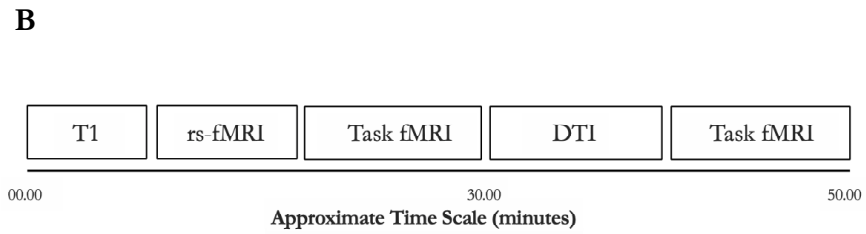
4.2.2 Experimental Overview

Participants undertook five experimental sessions: four of which were magnetic resonance spectroscopy (MRS) scans at 7T, to assess changes in GABA and glutamate concentrations in both M1s simultaneously due to either sham, anodal, or cathodal tDCS applied to the left M1, or to bilateral tDCS with the anode positioned over left M1.

In order to relate the magnitude of any changes in neurotransmitters across anatomically distant

regions to underlying structural and functional connectivity, we additionally acquired a resting state fMRI, task fMRI and DWI scan during a fifth imaging session performed at 3T.





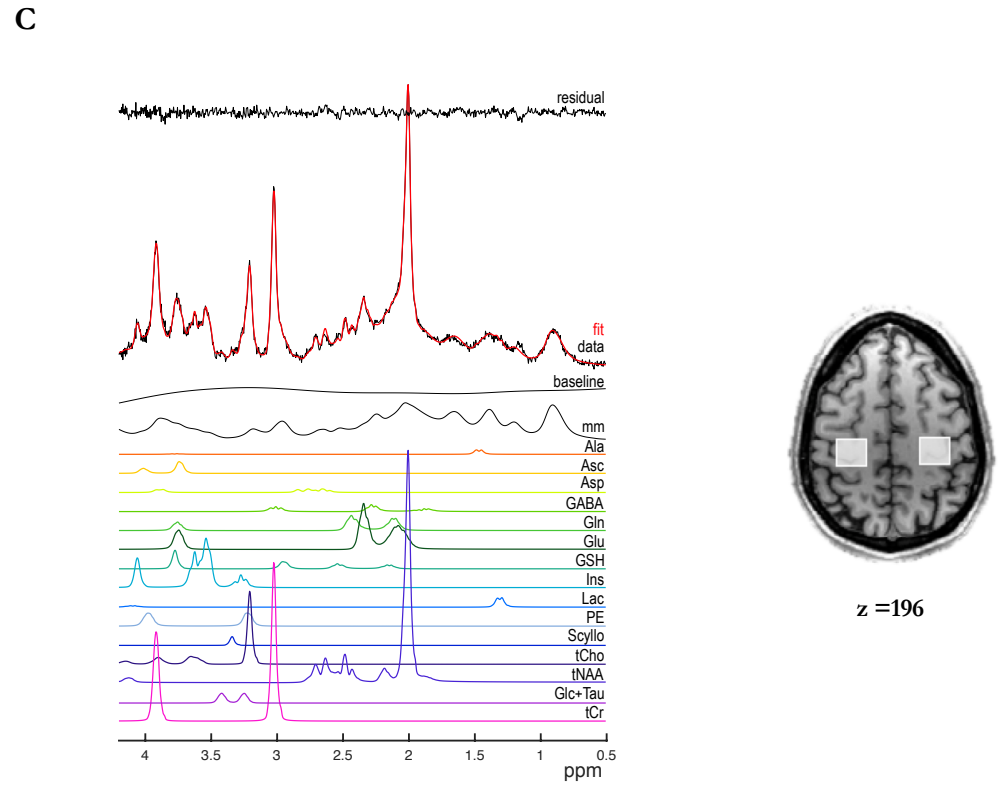


Figure 4.1 A, Schematic outlining the protocol of the 7T MRS and tDCS sessions. B, Schematic outlining the protocol of the 3T MRI session. C, A representative spectrum from one acquisition block from one voxel, as analysed by LCModel, showing the raw data, (red) as well as the individual metabolite fits (coloured lines) and the residual noise (black). Inset shows the location of both voxels from which MRS was acquired.

All 7T MRS-tDCS sessions were separated by at least one week and the order of the sessions was counterbalanced across the group. Two subjects did not complete the bilateral MRS-tDCS session due to personal time constraints. In each MRS-tDCS session, MRS spectra were acquired over five time points: at baseline, during tDCS, and at three timepoints post-tDCS (Post 1 (~1-13 mins post-tDCS), Post 2 (~14-26 mins post-tDCS), Post 3 (~27-39 mins post-tDCS)); see Figure 4.1 for experimental outline). Subjects watched a nature documentary for the duration of the experiment.

4.2.3 Transcranial direct current stimulation (tDCS)

A DC-Stimulator (Magstim, Ltd; UK) delivered a 1mA current to the brain via electrodes measuring 5 x 7 cm (Easycap, GmbH; Germany). For the anodal, cathodal, and sham sessions, one electrode was centred over the left M1, positioned 5 cm lateral to Cz and the other over the contralateral supraorbital ridge (SOR). For the bilateral session, the anode was placed over the left M1 and the cathode over the right M1, each electrode positioned 5 cm lateral to Cz. While this method of electrode placement does not take into account individuals' head sizes, we believe that differences in electrode placement between individuals is likely to be relatively minor.

High Chloride Electrolyte Gel (Easycap, GmbH; Germany) was used as the conducting medium between the scalp and electrodes. The electrodes contained 5 kΩ resistors and extension leads connected the stimulator, which was located outside of the magnetic field, to the subject positioned in the scanner. For the real stimulation conditions (anodal, cathodal, bilateral) the current was ramped up over 10 seconds and was then held at 1mA for 10 minutes, before being ramped down over 10 seconds. For sham stimulation the DC-stimulator was ramped up for 10 seconds then switched off, as previously described (Stagg et al., 2011b).

4.2.4 Magnetic resonance spectroscopy (MRS) acquisition

MRS data were acquired on a 7T Siemens MR system with a 32-channel receive array head-coil (Nova Medical, USA). T_1 weighted images (MPRAGE, 192x1mm axial slices,

TR/TE=2200/2.82ms, flip-angle=7◦, FOV 192x100) were used to place a 2x2x2cm voxel of interest over the left and right pre-central knobs, a known landmark for hand motor representation (Yousry et al., 1997).

A barium titanate-based dielectric pad with linear dimensions of 11 x 11cm and 5mm thick was positioned on the top of the head to increase B_1 efficiency within the volume of interest (VOI). B_1 maps were acquired using the actual flip angle imaging (AFI) sequence (FOV 240x240, TR1/2=6/30ms, TE=2.58ms, slice thickness=2.5mm, non-selective flip angle 60-degrees). When necessary, the dielectric pad was repositioned and the B_1 map repeated to achieve the best B_1 efficiency in the VOI (see Chapter 2 for more details).

Spectra were measured using the sLASER sequence (TR/TE=7000/30ms) (Öz and Tkáč, 2011) with VAPOR water suppression (pulse duration = 35ms) and 8 outer volume suppression (pulse duration = 4.6ms)(Tkáč et al., 1999). Spectra were acquired in an interleaved fashion from the two voxels (64 transients each) (Lemke et al., 2015). Each block of MRS data acquisition took approximately 12 minutes.

4.2.5 Magnetic resonance imaging (MRI) acquisition

MRI data were acquired using a 3T Siemens Verio MRI System and a 32 channel receive head coil. A whole-brain high-resolution structural image (1.0mm isotropic) was also collected using a multi gradient echo sequence to enable anatomic registration (TE1=1.79ms, TE2=3.65ms, TE3=5.51ms, TE4=7.37ms, TR= 2530ms, FOV=256 x256).

In order to relate the microstructure of the corpus callosum to the change in MRS metrics acquired at 7T, echo planar diffusion weighted imaging was acquired (diffusion directions=64, b-value=1500s/mm2, voxel dimensions=2x2x2mm, 64 slices, TR=8900ms, TE=91ms).

To assess functional connectivity resting state fMRI measures were collected. 200 echo planar volumes were acquired (54x3mm axial slices, TE=30ms, TR=3000ms, FOV=192×192mm). Subjects lay at rest with their eyes open for the duration of the resting state fMRI scan.

Task fMRI scans were also performed to enable functional localisation of M1 in each subject. 130 echo planar volumes were collected (3mm isotropic voxels, TE=30ms, TR=3000ms, FOV=192x192mm) while the participant performed visually cued hand tapping at 1Hz with either the left or right hand, interspersed with blocks of rest. Blocks of movement and rest were 30 seconds long.

4.3 Analysis

4.3.1 MRS analysis

As has been described previously (Lemke et al., 2015), standard pre-processing, including eddy current correction and zero-order phasing of array coil spectra, was performed using in-house scripts. Any residual water signal was removed from the metabolite spectra using Hankel-Lanczos singular value decomposition (HLSVD) (Cabanes et al., 2001). Neurochemicals were quantified using LCModel analysis (Provencher, 2001). The following criteria were applied for excluding spectra with poor quality: (i) Cramer-Rao lower bounds (CRLB)>50%, (ii) Water linewidths at full width at half maximum (FWHM)>15Hz, and (iii) Signal-to-noise ratio (SNR)<10. Of the 460 spectral blocks acquired (5 time-points from 2 voxels in 12 subjects with 3-4 sessions per subject), 15 blocks from 5 subjects were excluded on these criteria for the GABA measurements and two spectra from 1 subject were excluded for the glutamate measurement.

As is common, neurochemical concentrations are presented here as ratios relative to total creatine (tCr) (Mullins et al., 2014b; Soares and Law, 2009). To examine the relationship between individuals' baseline GABA levels and MRI measures, the mean baseline GABA:Cr value for right and left M1 in each subject across the sessions was calculated.

4.3.2 Task fMRI analysis

Analysis was performed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2012; Smith et al., 2004; Woolrich et al., 2009). Registration to high-resolution structural T1-weighted images was carried out using FLIRT. Registration from structural images to standard space was then further refined using FNIRT non-linear registration (Andersson et al., 2007a, 2007b) .

The following pre-statistics processing was then applied: motion correction using MCFLIRT (Jenkinson et al., 2002); non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; grand-mean intensity normalization for the entire 4D dataset by a single multiplicative factor; high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=57.0s). ICA-based exploratory analysis was carried out using MELODIC 3.0 (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) (Beckmann et al., 2005), in order to investigate the possible presence of unexpected artefacts or activation. FSL Motion Outliers was run on all functional scans to identify any timepoints corrupted by large motion. The resulting confound matrix was then included in the GLM to remove the effects of these timepoints on the resulting analyses.

For each participant, contrasts of Left Press>Rest and Right Press>Rest were performed with a Z threshold of 2.3 and cluster P threshold of 0.05. Group level analysis was then performed to find group average clusters of activation (Z threshold=2.3, cluster P threshold=0.05). These group-level clusters were then transformed into individuals' DWI space using FLIRT and FNIRT (Andersson et al., 2007a, 2007b), and were used as functionally defined M1 masks.

4.3.3 Resting state fMRI analysis

This data was also processed using FSL tools (Jenkinson et al., 2012; Smith et al., 2004). ICA was performed on participants' resting state data using MELODIC (Beckmann et al., 2005).

Components for each participant were then classified by eye into signal, noise or unknown, using MELVIEW. Components were classified as signal if spectral power lay primarily between 0 and 0.05Hz and signal clusters were within grey matter, but not solely distributed around the edges or in the location of large veins. Components were matched against figures in Salimi-Khorshidi et al., (2014) to assist in identification of noise components (Salimi-Khorshidi et al., 2014), which were then filtered out.

4.3.3.1 Seed-based analysis

Time series were extracted from individuals' functionally defined right and left M1s and correlation coefficients were calculated then transformed using Fischer's R to Z transformation. This created a measure of the degree of activation coherence between each individual's left and right M1s.

Additionally, the timeseries from individuals' functionally defined left M1 was used as a regressor in individual-level FEAT analyses to define areas showing correlated activity patterns. Timeseries were also taken from a region of 9 white matter voxels within the corpus callosum (centred around [44, 45, 43]), and 9 voxels within the CSF (centred around [47,66,42]). These two timeseries were included as regressors of no interest. The resulting individual-level maps of areas that showed correlated activity with the stimulated left M1 were then used in group-level FEAT analyses.

4.3.3.2 Resting state motor component analysis

Individual filtered resting state scans were transformed into standard space using FLIRT and FNIRT as described in Section 4.3.2. Group-level MELODIC was then run on individual participants filtered resting state scans. The number of components was limited to 20. Group-level noise components were removed leaving group average spatial signal components. The set of spatial maps from the group-average analysis was then used to generate subject specific versions of these spatial maps, and associated timeseries, using dual-regression (Beckmann et al., 2009; Filippini et al., 2009).

The motor component spatial map was identified by eye in the group analyses and was confirmed using cross-correlation with the 10 primary maps described in Smith et al., 2009 (Smith et al., 2009): our chosen motor resting state network showed the highest correlation with Smith network 6, which was defined as the "sensorimotor" network and included sensorimotor cortex, supplementary motor area and secondary somatosensory cortex. Figure 4.2 shows our group resting state motor network.

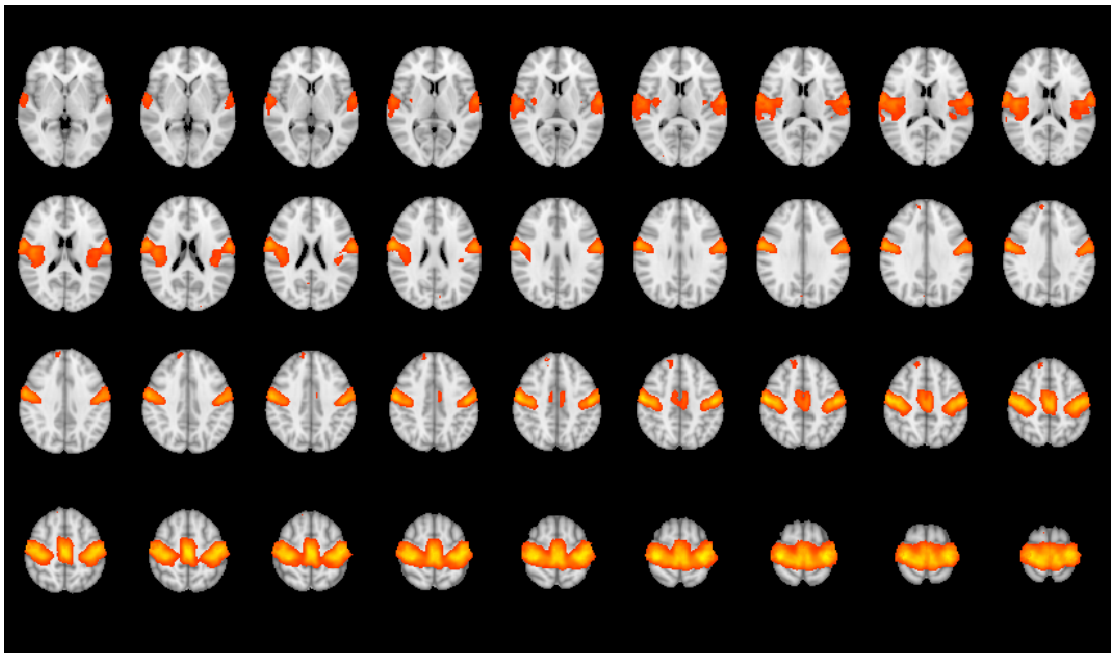


Figure 4.2 Group resting state motor network.

4.3.4 DWI analysis

Diffusion weighted scans were first corrected for eddy-current distortions using FSL eddycorrect, and then brain extracted using BET. Local diffusion tensors were then fit using the dtifit command in FSL. Markov Chain Monte Carlo sampling was then used to build the diffusion orientation distribution functions for each voxel (Behrens et al., 2007) using BEDPOSTX (Behrens et al., 2007, 2003), modelling a total of up to 2 fibres per voxel and using total of 1000 iterations.

4.3.4.1 Tractography

Tracts running from the corpus callosum to the motor cortices were estimated using PROBTRACKX2 (Behrens et al., 2007, 2003). A mask of the corpus callosum was created using the Juelich thr25 atlas and transformed into each participant's DWI space. The corpus callosum (CC) mask was used as the seed mask, with functionally defined bilateral M1s as a one-way waypoint, meaning that only pathways that passed through the corpus callosum and reached at least one M1 would be retained. A total of 5000 individual pathways were generated from each voxel within the seed mask, using a step-length of 0.5mm and a maximum of 2000 steps. A cosine curvature threshold of 0.2 (approximately 80 degrees) was used to limit how sharply pathways could deflect during tract generation. This procedure generated a tract map for each participant, in which voxel values represented the total number of pathways passing through each brain voxel.

For each participant, this tract map was then thresholded at >7500 and binarised, then the overlap between this mask and the CC mask was used to define a M1:M1 CC mask. The mean FA value across all voxels within this mask was then extracted and used in subsequent correlation analyses.

A first control region, the area of the CC carrying tracts connecting left M1 to right somatosensory cortex was defined using the method described above, but using HarvardOxford defined right somatosensory cortex and functional defined left M1 as way-points in PROBTRACKX2. This tract was thresholded to <25 and masked with the HarvardOxford CC mask. A second control region in right corticospinal tract (CST) was defined by using the right M1 as a seed point and right internal capsule as a waypoint, with the same parameters as for the M1:M1 tract. For each participant, this tract was thresholded at >2000, binarised, and then the region of interest was defined as the area within this tract mask falling within a 25x25x25 voxel volume around the centre of the internal capsule. This approach was taken to ensure the volume of the right CST mask and the M1:M1 CC mask were not significantly different (paired t-test, $t(11)=1.749$, $p=0.11$).

4.4 Results

We first wanted to test whether there were any baseline differences in neurochemical concentrations across sessions. We therefore performed a Repeated Measures ANOVA (RM ANOVA) with within-subject factors of Stimulation Condition (anodal, cathodal, bilateral, sham), Hemisphere (right M1, left M1), and Neurochemical (GABA, Glu). This demonstrated no significant main effect of Stimulation Condition ($F(3,21)=0.928$, $p=0.445$), no Stimulation by Hemisphere interaction ($F(3,21)=2.357$, $p=0.101$), and no significant Stimulation by Neurochemical interaction ($F(3,21)=2.515$, $p=0.086$). Therefore, to control for inter-subject differences in baseline neurochemical concentration, we calculated the change in each neurochemical from baseline at each timepoint for all subjects and sessions, and used these values in all subsequent analyses.

All neurochemical concentrations presented here are referenced to total creatine concentration (Cr+PCr; tCr). We therefore wished to test for any changes in creatine over time, which might potentially affect our results. We found no change in tCr over time in either M1 (RM ANOVA for each M1 separately with one factor of Stimulation Condition (anodal, cathodal, bilateral, sham) and one factor of Time (Baseline, During, Post 1, Post 2, Post 3); no main effect of Stimulation or Time, or Stimulation by Time interaction for either M1 (all $p>0.05$).

4.4.1 tDCS to the left M1 leads to significant changes in GABA in both hemispheres

We wished to investigate whether there were any significant changes in neurochemical concentrations in response to tDCS. RM ANOVA with within-subjects factors of Stimulation Condition (anodal, cathodal, bilateral, sham), Hemisphere (right M1, left M1), Neurochemical (glutamate, GABA), and Time (During, Post 1, Post 2, Post 3) demonstrated a significant main effect of Stimulation Condition ($F(3,15)=5.235$, $p=0.011$); no significant main effect of

Hemisphere (F1,5)=0.006, p=0.940; no main effect of Neurochemical (F(1,5)=0.890, p=0.890; and no significant main effect of Time (F(3,15)=0.904, p=0.463). Importantly, we demonstrated a significant interaction between Stimulation Condition and Neurochemical (F(3,15)=4.223, p=0.024) (see Supplementary Table 4.1 for full results).

To explore the effects of the different stimulation conditions further, we examined whether there were significant changes in the left and right M1s due to tDCS, when the neurochemicals and hemispheres were considered separately (RM ANOVAs, within-subjects factors of Stimulation Condition (anodal, cathodal, bilateral, sham), and Time (During, Post 1, Post 2, Post 3)). We found a significant main effect of Stimulation Condition on the change in GABA concentration within both left and right M1s [left M1 GABA (F(3,24)=4.739, p=0.016), and right M1 GABA (F(3,24)=7.513, p=0.001)]. There was no significant main effect of Time [(left M1 GABA: (F(3,15)=2.755, p=0.079; Right M1 GABA: (F(3,24)=0.030, p=0.993)], and no interaction between Stimulation Condition and Time [left M1 GABA: (F(9,45)=1.187, p=0.327); Right M1 GABA: (F(9,72)=1.311, p=0.246)] in either hemisphere.

However, tDCS did not significantly affect glutamate concentration within either hemisphere [left M1 glutamate: main effect of Stimulation Condition (F(3,27)= 2.110, p=0.122), main effect of Time (F(3,27)= 0.535, p=0.669), Stimulation Condition by Time interaction (F(9,81)= 0.597, p=0.796). Right M1 glutamate: main effect of Stimulation Condition (F(3,27)=1.329, p=0.118), main effect of Time (F(3,27)=1.236, p=0.316), Stimulation Condition by Time interaction (F(9,81)= 1.131, p=0.351)] .

4.4.2 Both anodal and cathodal tDCS to left M1 lead to a significant decrease in GABA in the unstimulated, right, M1

To investigate the changes in GABA in each hemisphere due to each stimulation protocol directly, we then directly tested each real stimulation condition against the sham session using a Repeated Measures ANOVAs with one factor of Stimulation Condition (real, sham), and one

factor of Time (During, Post 1, Post 2, Post 3). This approach demonstrated a decrease in GABA concentration in response to anodal tDCS compared to sham in both the stimulated left M1 [main effect of Stimulation Condition: $F(1,8)=8.964$, $p=0.017$, no significant main effect of Time: $F(3,24)=2.711$, $p=0.067$, no Stimulation by Time interaction: $F(3,24)=0.611$, $p=0.614$] and the non-stimulated right M1 [main effect of Stimulation Condition: $F(1,11)=7.898$, $p=0.017$, no significant effect of Time: $F(3,33)=1.278$, $p=0.298$, no Stimulation by Time interaction: $F(3,33)=0.816$, $p=0.494$; Figure 4.3A].

Cathodal tDCS did not modulate GABA within the stimulated left M1 [main effect of Stimulation Condition: $F(1,6)=1.410$, $p=0.280$, no significant main effect of Time: $F(3,18)=1.102$, $p=0.374$, no Stimulation Condition by Time interaction: $F(3,18)=0.517$, $p=0.676$], but there was a significant decrease in GABA within the non-stimulated right M1 [main effect of Stimulation Condition: $F(1,9)=13.106$, $p=0.006$; Figure 4.3B, no significant main effect of Time: $F(3,27)=1.530$, $p=0.229$; no Stimulation Condition by Time interaction: $F(3,27)=0.455$, $p=0.716$].

Bilateral tDCS did not modulate GABA within the anode-targeted left M1 [main effect of Stimulation Condition: $F(1,7)=0.894$, $p=0.376$, no significant main effect of Time: $F(3,21)=2.913$, $p=0.058$, no Stimulation by Time interaction: $F(3,21)=0.062$, $p=0.979$], but there was a significant decrease in GABA within the cathode-targeted right M1 [main effect of Stimulation Condition: $F(1,9)=8.058$, $p=0.019$; no significant effect of Time: $F(3,27)=0.382$, $p=0.767$; no Stimulation by Time interaction: $F(3,27)=2.149$, $p=0.117$; Figure 4.3C].

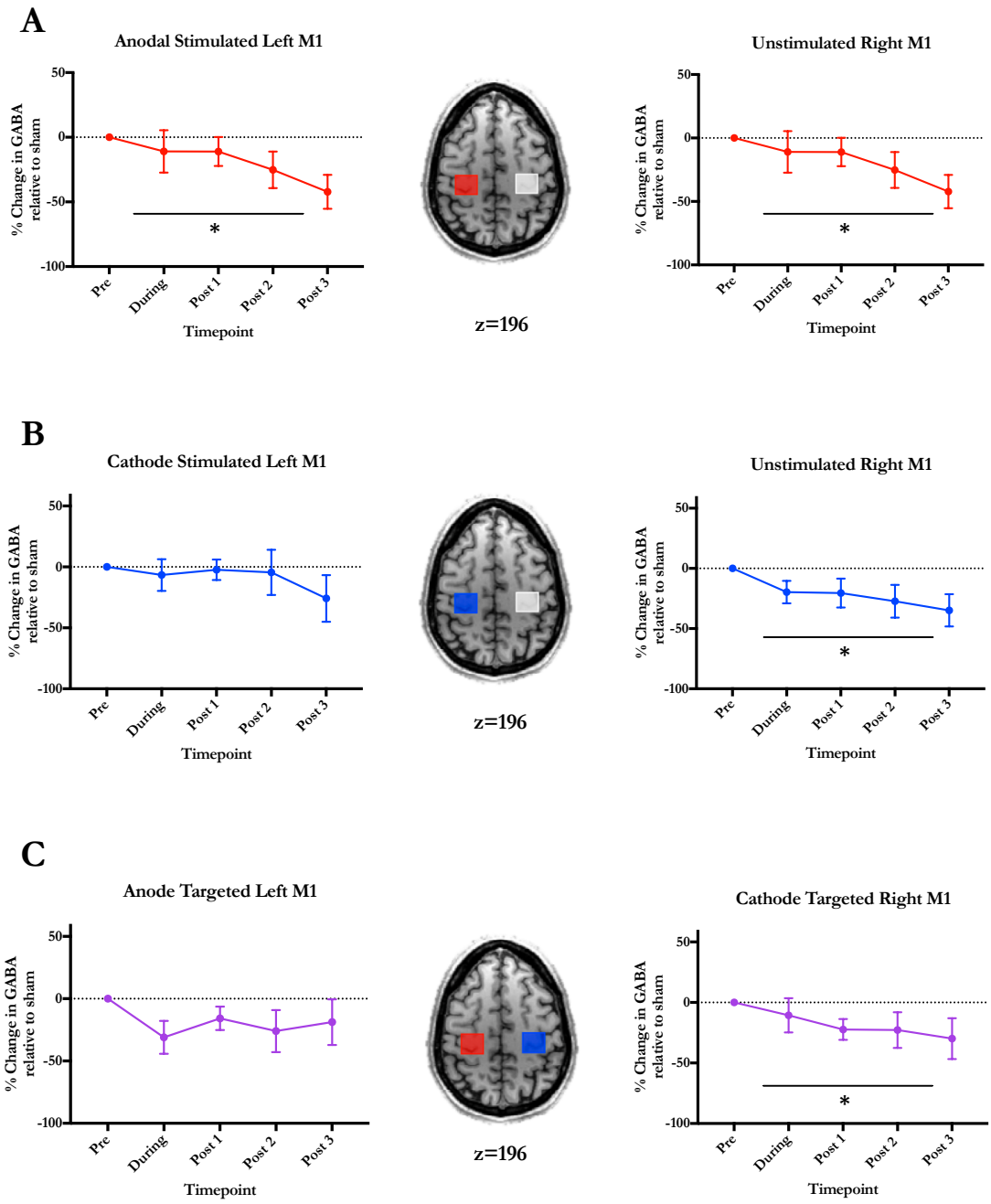


Figure 4.3 Percentage change in GABA during and after (A) anodal, (B) cathodal and (C) bilateral stimulation, relative to sham stimulation. Brain images showing location of MRS voxels, and location of stimulation. Red indicating anode placement, blue indicating cathode placement, and grey indicating no electrode placed. * Indicating a significant main effect of condition for each sham vs. real stimulation comparison of the baselined GABA concentrations. Error bars indicate standard error of the mean (SEM). A, decrease in GABA was observed in both stimulated and non-stimulated M1s in response to anodal tDCS (rM1 n=12, lM1 n=9). B, significant decrease in GABA was observed only within the non-stimulated, right, M1 in the cathodal tDCS condition (rM1 n=10, lM1 n=7). C, significant decrease in GABA was observed only within the cathode-targeted, right, M1 in response to bilateral tDCS condition (rM1 n=10, lM1 n=8).

4.4.3 Resting state fMRI measures do not relate to tDCS-related changed in GABA in the non-stimulated right M1

We first wished to investigate whether there was a correlation between unilateral tDCS-induced GABA changes within the two M1s. No significant relationship was demonstrated for either of the stimulation conditions [anodal: r=-0.014, n=12, p=0.965; cathodal tDCS: r=-0.268, n=10, p=0.453), see Figure 4.4]. However, given the strong functional and structural connectivity between the two M1s we decided to investigate whether individual differences in the degree of connectivity could explain the magnitude of non-stimulated hemisphere GABA changes in the unilateral stimulation conditions.

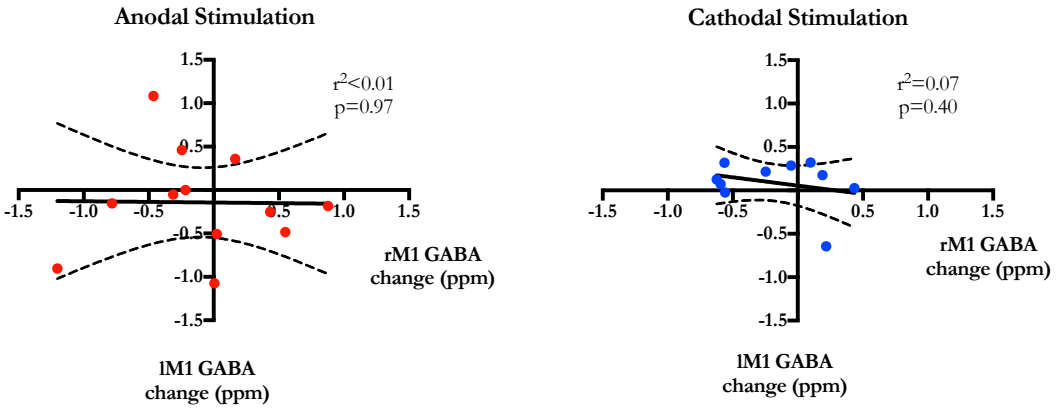


Figure 4.4 No significant correlations between change in lM1 and rM1 GABA during either of the unilateral stimulation conditions

A variety of measures were used to assess the functional connectivity between the two M1s. The r-to-z transformed correlation coefficient of activity timeseries between left and right M1s were calculated for each participant, and the correlation between these values and the GABA change in the non-stimulated right M1s was examined. There were no significant correlations with sham, anodal or cathodal induced change in GABA [anodal: r=-0.140, n=12 p=0.665; cathodal: r=-0.271, n=12, p=0.393; sham: r=-0.039, n=12, p=0.905].

Individual-level FEAT maps of areas showing correlated activity with left M1 timeseries were input into group-level FEAT analysis to look for relationships with right M1 GABA change. There were no areas that showed significant activation levels. Finally there was no significant

relationship between strength of activation in the motor resting state network and right M1 GABA change in any stimulation condition [anodal: r=-0.508, n=12, p=0.083; cathodal: r=-0.227, n=12, p=0.478; sham: r=0.202, n=12, p=0.529].

4.4.4 tDCS-induced GABA changes in non-stimulated, right, M1 are related to white matter microstructure.

We wished to investigate how change in GABA in the non-stimulated hemisphere during tDCS might relate to white matter microstructure between the two M1s. We demonstrated a significant relationship between the change in GABA during stimulation in the right (non-stimulated) M1 and white matter microstructure between the stimulated and non-stimulated M1s, as assessed by FA for cathodal tDCS [r=-0.610, n=12, p=0.035), but not for anodal tDCS (r=0.487, n=12, p=0.108), or in the sham control (r=-0.369, n=12, p=0.237; Figure 4.5].

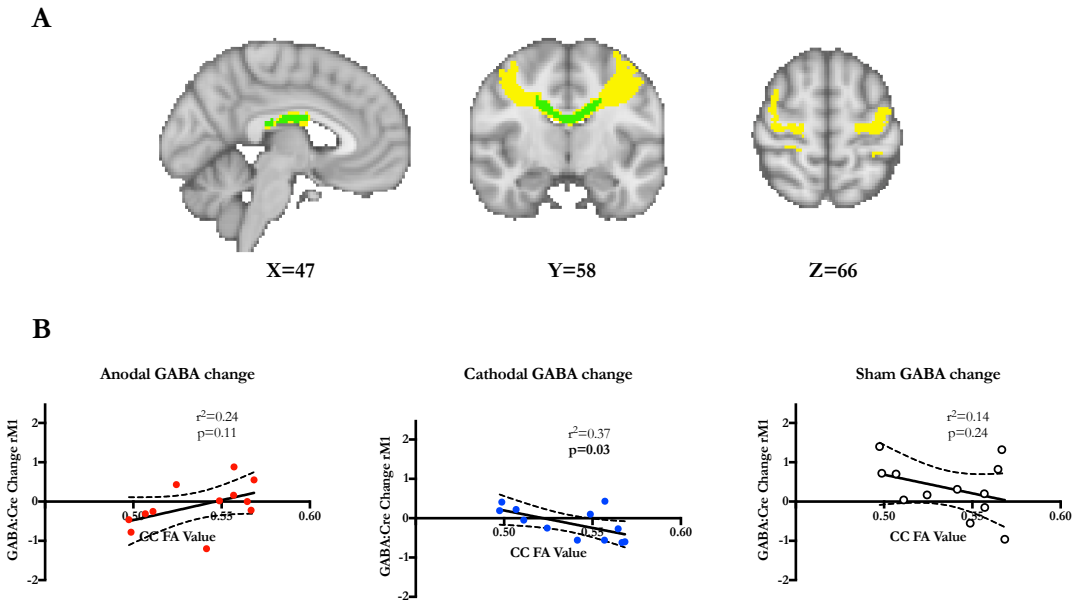


Figure 4.5 A, M1:M1 CC mask.–The M1:M1 tract is shown in yellow, the M1:M1 CC mask is shown in green. B, Cathodal tDCS-induced GABA change within right M1 correlates with FA in the M1:M1 CC mask. No significant relationship between M1:M1 CC FA, and change in GABA in rM1, was demonstrated during either anodal or cathodal tDCS.

To assess the anatomical specificity of this result, we investigated the relationship between FA within the two control regions, the section of CC linking left M1 to right somatosensory cortex

and the right CST, and change in GABA levels in right M1 in response to stimulation and demonstrated no significant correlations [CC: anodal: r=0.435, n=12 p=0.158; cathodal: r=-0.325, n=12, p=0.302; sham: r=-0.575, n=12, p=0.051. CST: anodal: r=0.160, n=12, p=0.620; cathodal: r=-0.069, n=12, p=0.831; sham: r=-0.138, n=12, p=0.668]. Furthermore, there were no significant correlations for any stimulation condition, between changes in glutamate levels in right M1 and FA in either the M1:M1 CC [anodal: r=0.382, n=12, p=0.221; cathodal: r=-0.472, n=12, p=0.121; sham: r=0.056, n=12, p=0.862] or CST mask [anodal: r=-0.096, n=12, p=0.767; cathodal: r=-0.453, n=12, p=0.139; sham: r=0.445, n=12, p=0.147].

4.5 Discussion

This study was performed to explore the physiological changes in both M1s induced by tDCS. We replicated previous findings (Bachtiar et al., 2015; Kim et al., 2014b; Stagg et al., 2011a; Stagg et al., 2009a) that anodal tDCS applied to M1 causes a decrease in MRS-measured GABA levels in the stimulated area of cortex, but we have also shown that this decrease is accompanied by a concurrent decrease in GABA in the non-stimulated M1. Cathodal tDCS led to a significant decrease in GABA in the non-stimulated right M1, though with no significant GABA changes in the stimulated left M1. Bilateral tDCS resulted in a GABA decrease only in the cathode-targeted right M1.

Our findings are in broad agreement with that of O'Shea and colleagues, who used TMS to examine changes to cortical excitability in both M1s after tDCS (O'Shea et al., 2014), demonstrating significant increases in corticospinal excitability in both hemispheres following anodal stimulation to left M1, consistent with our finding of a bilateral GABA decrease. The same study found a decrease in corticospinal excitability in the stimulated hemisphere in the cathodal condition, where we found no change in GABA, but an increase in excitability in non-stimulated hemisphere in line with our finding of a GABA decrease.

For all conditions in which a GABA decrease was observed the change persisted into the final MRS measurement. This indicates that tDCS-induced changes in neurochemicals endure for at

least 30 minutes post stimulation. This is perhaps unsurprising given that the effects of anodal tDCS on corticospinal excitability have been shown to outlast stimulation by up to 90 minutes (Nitsche and Paulus, 2001). Further studies, over longer timescales, are therefore required to determine the duration of tDCS-induced GABA changes.

The magnitude of decreases in GABA concentrations, if they were observed, also tended to be very large, with up to 50% reductions in concentration from baseline. While this may at first seem a dangerously large decrease in inhibition with the potential to induce seizures, as discussed in Chapter 2, much of the GABA signal obtained in MRS measures likely reflects molecules involved in metabolism rather than neurotransmission. Furthermore, cellular work has previously demonstrated that blocking GABA synthesis by around 40% does not impair synaptic inhibition under normal circumstances, though it did reduce inhibition under high stimulation conditions. This result was taken to indicate that there is a depletable pool of metabolic GABA that can be drawn upon for essential neurotransmission (Dericioglu et al., 2008).

4.5.1 No significant changes in glutamate concentration with any stimulation condition

We found no significant effect of stimulation on glutamate concentration in either hemisphere. A previous study has demonstrated changes in glutamate concentration in the stimulated region with cathodal tDCS to M1 (Stagg et al., 2009), but another did not find the same effect (Kim et al., 2014). A recent review examined the influence of factors such as field strength, experimental design and sequence on MRS-measured changes in glutamate (Mullins, 2018); it may be that differences in design and acquisition time of tDCS-MRS studies are responsible for the differing results.

4.5.2 Bilateral decrease in M1 GABA with anodal tDCS to left M1

Anodal tDCS has been shown to induce LTP-like plasticity in M1 in a manner at least similar to the mechanisms underpinning motor learning and recovery after stroke (Bindman et al., 1964a; Di Lazzaro et al., 2012; Stagg and Nitsche, 2011; Weiss et al., 1998). Here, we see a decrease in GABA in both the anodal stimulated and non-stimulated hemispheres, suggesting for the first time that a decrease in GABA outside the stimulated M1 may be important for motor plasticity. Decreases in GABA in M1 have been demonstrated when individuals practice a motor skill (Floyer-Lea et al., 2006), and tDCS-induced changes in MRS-measured GABA within M1 have been shown to correlate with individuals' ability to learn motor tasks (Kim et al., 2014b; Stagg et al., 2011a), whereas the same correlations were not seen with MRS-measured glutamate (Kim et al., 2014b). These results highlight the potential importance of change in GABA concentration in mediating motor plasticity, and examining the effect of GABA changes in both hemispheres during learning would be an important avenue to explore in future studies.

Here we found no significant relationship between the change in GABA in the non-stimulated hemisphere and the white matter microstructure between the two M1s in the anodal condition. Though the result is non-significant, and should not be over interpreted, the direction of the correlation was positive, indicating that those participants with higher FA showed less of a GABA decrease. If anodal stimulation were increasing excitability in the stimulated hemisphere then this positive correlation would be expected given an increase in interhemispheric inhibition between the two hemispheres; individuals with higher FA are experiencing a greater increase in inhibition in the non-stimulated hemisphere. However given there is an overall group level decrease in GABA in non-stimulated hemisphere (i.e. decrease in inhibition) it appears that, in large part, anodal tDCS is causing contralateral release of inhibition which is not mediated by the corpus callosum pathways projecting from the stimulated M1.

4.5.3 Magnitude of GABA decrease in the unstimulated hemisphere during cathodal tDCS relates to underlying white matter microstructure

Cathodal tDCS to the contralesional hemisphere has been suggested as a potential adjunct therapy in stroke recovery (Fregni et al., 2005; Boggio et al., 2007; Kim et al., 2010; Stagg et al., 2012). It has been hypothesised that cathodal tDCS improves function via a transcallosally mediated increase in excitatory activity in the contralateral M1 (Boggio et al., 2006; Fregni et al., 2005; Kim et al., 2010; O'Shea et al., 2014; Stagg et al., 2012), as cathodal tDCS has previously been demonstrated to decrease transcallosal inhibition between the motor cortices (Bradnam et al., 2010; Lang et al., 2004; Tazoe et al., 2014).

Here, we provide evidence to support this hypothesis by demonstrating that cathodal tDCS results in a decrease in inhibition in the contralateral M1, and, further, that this effect is positively correlated with the white matter microstructure between the two cortices; such that a higher FA value in the corpus callosum was linked to a greater decrease in GABA levels within the right, non-stimulated, M1. There is a strong positive correlation between an individual's transcallosal inhibition and the microstructure of the M1:M1 callosal tracts, as measured by FA (Fling et al., 2013; Wahl et al., 2007), and the link between greater FA within the corpus callosum and increased effect of tDCS in the non-stimulated hemisphere is broadly consistent with previous findings showing a link between FA values within transcallosal fibres and tDCS-induced fMRI laterality-index during a motor task (Lindenberg et al., 2013). Taken together these findings may provide an explanation for some of the variability in response to cathodal tDCS in the stroke recovery literature, and more generally (Chew et al., 2015; López-Alonso et al., 2015). They also raise a hypothesis to test: that stroke patients with relatively structurally intact callosal fibres (higher FA) may benefit from cathodal tDCS applied to the contralesional M1, whereas those with less structural integrity (lower FA) in their callosal fibres may respond better to other approaches.

4.5.4 The physiological effects of bilateral tDCS are not simply a sum of those underpinning anodal and cathodal tDCS

Bilateral tDCS led to a decrease in GABA only in the cathode-targeted M1. While this finding is somewhat difficult to interpret, it is important to highlight, in line with previous studies (O'Shea et al., 2014; Waters et al., 2017), that bilateral stimulation cannot simply be explained as the combined effect of the anode and cathode conditions. In the bilateral M1 montage, current flows transversely across the cortex, as opposed to the radial current flow direction in the unilateral montages. Recent work by Rawji and colleagues (Rawji et al., 2017) has shown that the orientation of current flow across the motor cortex influences the effect on corticospinal excitability, with current flowing orthogonal to the central sulcus eliciting the greatest changes. It has previously been proposed that this difference in current flow direction between the bilateral and unilateral montages may alter the influence of the tDCS polarity on behavioural (Waters et al., 2017) and neurophysiological (O'Shea et al., 2014) metrics, a hypothesis that would be supported by the data presented here. Taken together, these findings suggest that further research is needed before bilateral tDCS can be fully optimised as a neuroscientifically informed adjunct therapy in stroke recovery.

4.5.5 No relationship between GABA changes and resting state connectivity measures

Previous work has shown that baseline M1 GABA levels correlate with strength of activation in the resting state motor network (Bachtiar et al., 2015; Stagg et al., 2014), and that tDCS can modulate functional connectivity (Amadi et al., 2014; Bachtiar et al., 2015; Sehm et al., 2012). Here we set out to test whether a participant's baseline functional connectivity between the two M1s related to the degree of change of GABA within the non-stimulated right M1. We found no significant correlations between the right M1 GABA change in any of the stimulation conditions and any of our measures of resting state functional connectivity. This may be due to large time gaps between the MRS-tDCS and 3T imaging sessions (see Section 4.5.6).

4.5.6 Limitations

For all participants in this study stimulation was applied to the dominant hemisphere, and so it may not be possible to generalise these findings to individuals who are left-handed, or to stimulation of the non-dominant hemisphere.

Furthermore, as is common in many tDCS-MRS studies, we had a relatively small sample size. Replicating this paradigm with a larger sample size will be important to allow robust conclusions to be drawn.

Despite having 3 MRS measurements after the cessation of tDCS stimulation, the last being around 35 minutes post-tDCS, we did not see a return of GABA to baseline levels in the active stimulation conditions where changes were observed. To enable us to make statements about the timecourse of tDCS-related neurochemical changes, beyond saying that effects last for up to 35 minutes, longer scan times with more MRS measurements would be required.

Finally, there were large gaps in time between many of the participant's MRS-tDCS sessions and the 3T resting state and DTI session. It may be possible that during this time there could be substantial changes, particularly in functional connectivity, which might explain why we did not find relationships between functional connectivity metrics and changes in GABA in the unstimulated M1.

4.6 Conclusions

These results show for the first time that neurochemical changes are observed outside the targeted primary motor cortex during tDCS application in humans, supporting the role of the wider motor network in mediating tDCS-induced behavioural and physiological effects. The importance of the wider motor network in plasticity is further highlighted by the relationship

demonstrated between the anatomically distant effects of cathodal tDCS and the underlying white matter microstructure, findings that, taken together, begin to explain the putative effectiveness, and the well described variability, of tDCS in motor learning and stroke recovery.

Chapter 5: Perturbing ipsilateral M1 with non-invasive brain stimulation impairs healthy motor learning

Transcranial direct current stimulation (tDCS) has long been known to influence motor learning when applied contralateral to the performing hand (e.g. Nitsche, Liebetanz, et al., 2003), with these changes thought to be mediated by changes in corticospinal excitability and/or neurochemical concentration (Stagg and Nitsche, 2011). However it has been shown in the previous chapter and elsewhere (O'Shea et al., 2014) that these physiological changes induced by tDCS are not restricted to the stimulated hemisphere. Ipsilateral tDCS can improve motor recovery in stroke patients (e.g. Fregni et al., 2005), but how ipsilateral tDCS modulates learning in healthy volunteers and the physiological changes underpinning this are not well understood.

In this chapter anodal or cathodal tDCS was applied ipsilateral to the learning hand during performance of a serial reaction time task. Corticospinal excitability, $GABA_A$ and glutamatergic receptor activation were recorded from the non-stimulated, contralateral M1 using TMS. Anodal tDCS significantly impaired motor learning and resulted in a decrease in $GABA_A$ activation relative to sham stimulation. This decrease in $GABA_A$ activation correlated positively with behavioural improvements relative to sham. This indicates that perhaps the $GABA_A$ decreases were compensating, in part, for the behavioural impairment that may be mediated by another physiological change.

5.1 Introduction

Anodal transcranial direct current stimulation (tDCS) applied contralateral to the learning hand during performance of a motor task, for example a motor sequence learning task, has frequently been reported to result in improved learning of that task (Amadi et al., 2015b; Cuypers et al., 2013; Kantak et al., 2012; Nitsche et al., 2003c; Stagg et al., 2011b; Zimerman et al., 2013). Likewise studies have also demonstrated enhanced skill retention with tDCS (Cuypers et al., 2013; Kantak et al., 2012; Karok and Witney, 2013; Zimerman et al., 2013). It has been argued that these behavioural changes may be mediated by increases in corticospinal excitability and/or decreases in GABA concentration (Stagg et al., 2011a; Stagg and Nitsche, 2011). Those tDCS interventions which tend to result in improved learning when applied during task also induce GABA decreases (Stagg et al., 2011b; Stagg et al., 2009a) and increase corticospinal excitability (Di Lazzaro et al., 2013; Lang et al., 2004; Nitsche et al., 2003b) when applied at rest.

As demonstrated and discussed in the previous chapter (Chapter 4) the effects of tDCS on corticospinal excitability and neurochemistry are not isolated to the stimulated hemisphere. Despite the differing effects of anodal and cathodal tDCS on the stimulated cortex, we found that both anodal and cathodal tDCS decrease MRS-measured GABA in the non-stimulated M1. Likewise, a previous study has shown that both montages cause an increase in corticospinal excitability in the non-stimulated hemisphere (O'Shea et al., 2014).

If GABA decreases and/or corticospinal excitability increases are responsible for tDCS-induced behavioural improvements, then applying either anodal or cathodal tDCS to the hemisphere ipsilateral to the learning hand should improve learning on a concurrent task. This could be of potential use in individuals who have suffered a stroke, where large lesions have been demonstrated to distort the path of current flow, perhaps weakening the effects of tDCS applied to the lesioned hemisphere, and motivating application to the non-lesioned hemisphere.

Studies applying cathodal stimulation to the ipsilateral hemisphere with the aim of reducing the activation of this hemisphere, have tended to result in behavioural improvements both in healthy volunteers (Vines et al., 2008, 2006) and in stroke patients (Fregni et al., 2005; Hummel and Cohen, 2006; Boggio et al., 2007; Kim et al., 2010; Williams, Pascual-Leone and Fregni, 2010; Stagg et al., 2012; Takeuchi and Izumi, 2012; O'Shea et al., 2014). Similar motor learning improvements have been shown using 1Hz rTMS to inhibit the ipsilateral hemisphere (Kobayashi et al., 2004; Kobayashi, Théoret and Pascual-Leone, 2009). However despite causing increases in corticospinal excitability and decreases in magnetic resonance spectroscopy (MRS) measured GABA concentration, the same improvements in behavioural performance have not been reported using anodal ipsilateral tDCS (Vines et al., 2008, 2006), with some studies even finding a learning impairment (Keitel et al., 2018).

In this sham-controlled, within-subjects, single-blind study we attempted to reconcile the behavioural and physiological effects of tDCS applied to the ipsilateral hemisphere. We applied anodal or cathodal tDCS to right M1, during learning of a simple serial reaction time task (SRTT) with the right hand. We assessed the effect that this stimulation had on learning, and on skill retention after 6 hours of wakefulness. Corticospinal excitability, $GABA_A$ receptor activity, and glutamatergic activity within the contralateral left M1 were measured using transcranial magnetic stimulation (TMS). We predicted that, in line with the previous literature, we would see learning facilitation in the cathodal stimulation condition and impairment in the anodal condition. We predicted that application of tDCS during task would change the effects on physiology, and the behavioural changes would be accompanied by decreases (cathodal) or increases (anodal) in TMS-measured GABAergic inhibition in the left M1.

5.2 Methods

5.2.1 Participants

Twenty participants (9 male, aged 20-32 years, mean age 25.5 years) gave informed written consent to participate in this study in accordance with ethical approval from the Oxford University Central University Research Ethics Committee (CUREC: R30822/RE004). All were right-handed as determined by the Edinburgh Handedness Inventory (Oldfield, 1971) and none played a musical instrument to a high level (defined as having achieved UK ABRSM grade 6 or equivalent, and still in regular practice). All were within the University of Oxford inclusion criteria for TMS and tDCS stimulation.

5.2.2 Experimental overview

Participants came to the lab on three separate days, between 5-10 days apart (see Figure 5.1). On each day they attended the lab once in the morning (either 9am or 11am) and once in the afternoon (either 3pm or 5pm). During each morning session TMS motor thresholds were defined for the first dorsal interossei (FDI) muscle (see Section 5.2.5 for more details). TMS measurements of corticospinal excitability, $GABA_A$ receptor inhibition, and glutamatergic facilitation were then recorded at baseline in the left motor cortex (M1). Participants then performed a short familiarisation of the serial reaction time task (SRTT) with their right hand, and a second set of baseline TMS measurements were subsequently recorded.

Following this, participants performed the main learning portion of the SRTT while receiving either: (1) anodal ipsilateral tDCS, with the anode over the right M1 and cathode over the left supraorbital ridge; (2) cathodal ipsilateral tDCS, where the polarities were reversed; or (3) sham tDCS (Figure 5.1). TMS was recorded following the end of the training + tDCS portion (post-tDCS). A final task probe without tDCS was performed, and then TMS measurements were taken again immediately (5mins post), and at 10 minutes post cessation of the task (15mins post).

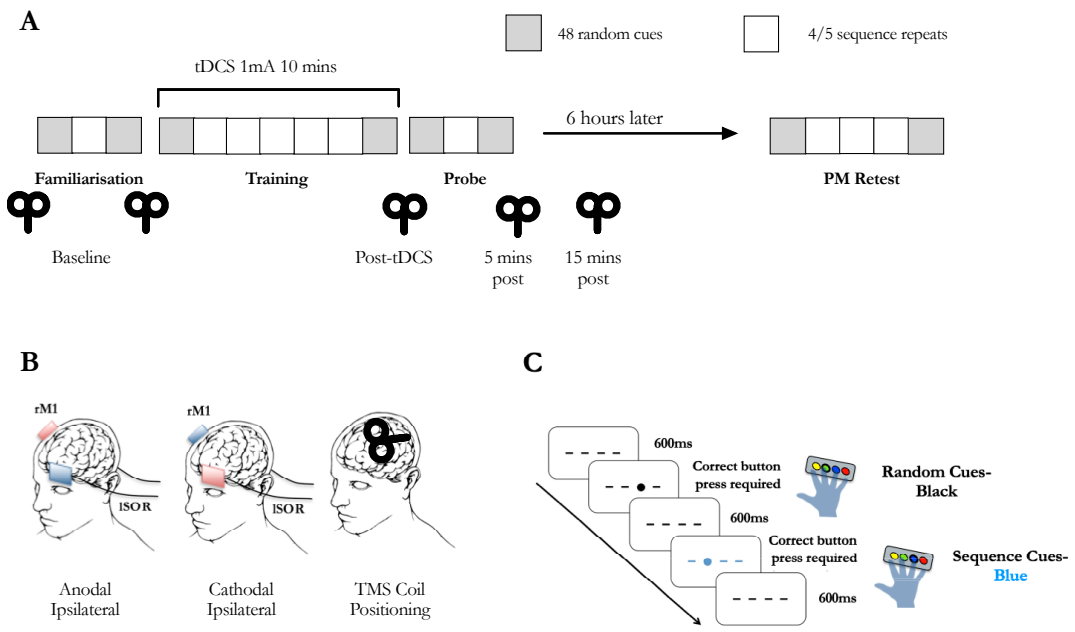


Figure 5.1 A, test session protocol. B, tDCS montages for the anodal and cathodal ipsilateral stimulation conditions and the positioning of the TMS coil over the M1 motor hotspot in the anterior-posterior direction. C, Task screen seen by participants, showing the black cues for random presses and the blue cues indicating sequence presses.

Finally, participants returned to the lab 6 hours after the beginning of their session for a PM retest session on the SRTT (PM retest) (see Figure 5.1A).

5.2.3 Serial reaction time task (SRTT)

Full details on the serial reaction time task can be found in Chapter 3. For this version of the task a 16-item sequence was used in order to increase the task difficulty as participants were performing it multiple times. In the initial familiarisation section and the probe section there was a block of 48 random presses followed by a block of 4 sequence repeats then another 48 random press block. The training section consisted of a random block, a sequence block of 25 sequence repeats, followed by another random block. The PM retest consisted of 15 sequence repeats flanked by random blocks. The response stimulus interval was 0.6 seconds (see Figure 5.1). During the main training block there were four 10 second breaks after sequence repeats 4, 10, 17 and 23.

The task was presented using PsychtoolBox (PTB-3) (Brainard, 1997) for MATLAB R2014b (The Mathworks Inc., Natick, USA) on a Dell Latitude E7470 laptop. Participants made responses using an external 4-button box (OTR-1X4-CR; Current Designs, Philadelphia, USA).

Five sequences were used (as several participants had to have sessions repeated) [sequence 1: 1,3,4,1,3,2,4,1,4,2,3,1,2,4,3,2; sequence 2: 3,2,3,4,1,2,4,3,1,4,2,1,3,2,4,1; sequence 3: 2,4,3,1,2,4,1,3,2,3,4,2,1,4,1,3; sequence 4: 1,3,2,1,4,3,1,2,4,1,3,4,2,3,2,4; sequence 5: 4,2,3,1,3,4,1,2,3,1,4,2,1,4,3,2].

5.2.4 Transcranial direct current stimulation (tDCS)

A DC-Stimulator (neuroConn GmbH; Germany) was used to apply current via 5 x 7 cm electrodes (neuroConn, GmbH; Germany), encased in saline-soaked sponges, and fixed to the head with rubber bands. In the real tDCS conditions stimulation was applied for 10 minutes at 1mA, with 10 seconds ramp up and 10 seconds ramp down. In the sham condition the current was held on for 30 seconds. One electrode was centred over the right M1 by positioning 5 cm lateral to mid-pre-central position (Cz). The reference electrode was placed over the left supraorbital ridge (SOR) (see Figure 5.1). The order of stimulation conditions, and the condition-sequence pairing were counterbalanced across the group.

5.2.5 Transcranial magnetic stimulation (TMS)

All TMS data were acquired using a monophasic DuoMAG machine connected to a 70mm figure-of-eight coil (Rogue Resolutions, Cardiff). The left M1 was targeted in all subjects, in all sessions. The motor hotspot, i.e. the position where MEPs could be elicited in the in the right FDI muscle at the lowest stimulator intensity, was determined before each session, and the TMS coil was held at 45° to the midsagittal line with the handle pointing posteriorly. The hotspot was marked by drawing guidelines onto the participants scalp to allow for the same coil placement across the measurements. For the duration of the TMS recordings the subjects were seated

comfortably with their hands relaxed on a pillow. Participants were instructed to keep their eyes open and look ahead at a fixation cross, presented on screen.

Surface EMG was recorded using disposable neonatal ECG electrodes (Henley's Medical, Welwyn Garden City, UK) from the FDI of the right hand using a belly-tendon montage with a ground electrode over the ulnar styloid process. Signals were sampled at 5 kHz, amplified, filtered (10Hz - 1kHz), and recorded using a Digitimer D440 4-Channel Isolated Amplifier (Digitimer Ltd, Welwyn Garden City, UK), a CED micro1401 A/D converter, and Signal software version 3.13 (Cambridge Electronic Design, Cambridge, UK). A Digitimer Humbug Noise Eliminator was used to reduce 50Hz noise in the EMG recordings (Digitimer, Welwyn Garden City, UK).

5.2.5.1 Stimulation intensities

The resting stimulation intensity and active stimulation intensity were determined at the beginning of each session. The resting stimulation intensity (rSI) was defined as the minimum stimulus intensity required for eliciting a mean MEP of ~1mV peak-to-peak amplitude in 10 consecutive trials in the relaxed FDI muscle. The active stimulation intensity (aSI) was defined as the minimum stimulus intensity necessary to evoke a 200μV peak-to-peak MEP in at least 5 of 10 consecutive trials while subjects maintained ~30% of the maximum contraction of the FDI.

5.2.5.2 TMS measurements

Corticospinal excitability (CE) was determined by measuring MEPs produced in response to the initial (rSI_{init}) determined at the beginning of the testing session. This intensity was not varied across the session.

Two paired-pulse protocols were performed in the study: SICI with an interstimulus interval (ISI) of 2.5ms to assess $GABA_A$ receptor activity (Di Lazzaro et al., 2006; Kujirai et al., 1993; Stagg et al., 2011b), and intracortical facilitation (ICF) with an ISI of 12ms as a measure of NMDA

receptor activity (Ziemann et al., 1996). The conditioning stimulus was set at 70% of the aSI and the test stimulus was set at the rSI for both protocols. The rSI used in these measurements was adjusted (rSI_{adj}) to account for potential changes in motor cortex excitability after tDCS/ motor performance.

5.2.5.3 TMS protocol

CE, $SICI_{2.5ms}$, and ICF were measured at two timepoints before tDCS, once pre-familiarisation, and once post-familiarisation (together referred to as baseline). The same measures were also taken at 3 timepoints after tDCS stimulation: immediately following stimulation (post-tDCS), after a final task probe (5 mins post tDCS), and 10 minutes after completion of the task (15 mins post tDCS). 10 single-pulse MEPs were recorded at the original rSI_{init} immediately before each TMS run. These pulses were used to calculate the single-pulse corticospinal excitability (CE) measurement. If the amplitude of the resulting MEP differed markedly from 1mV, then the stimulation intensity was altered accordingly (Nitsche et al., 2005; Amadi et al., 2015b; Nowak et al., 2017) before other measures were taken. Twelve of each rSI_{adj} , $SICI_{2.5ms}$ and ICF pulses were then delivered in a pseudorandomised order.

5.3 Analysis

5.3.1 Behavioural data analysis

As in previous chapters, in each section of the task response times (RT) greater than 2.7 times the standard deviation above the mean for each block were removed. The mean RT of correct button presses was then calculated for each random block. In the familiarisation and probe sections of the task the mean RT for all correct button presses of the 4 sequence repeats of the sequence block was calculated. Within the longer AM training and PM re-test sections the mean RT for correct responses were calculated for blocks of 5 sequence repeats.

Skill measures in the familiarisation and probe sections were calculated by subtracting the mean sequence block RT from the mean random block immediately following it. Skill measures were calculated at the beginning and end of the training and PM retest sections by calculating RT random $block_1$ – RT sequence $block_1$ and RT random $block_2$ – RT sequence $block_{final}$.

5.3.2 TMS analysis

Data cleaning was performed in line with previous TMS studies conducted in the lab (Nowak et al., 2017). Trials were excluded if there was pre-contraction in the target FDI muscle (EMG amplitude >0.1mV in the 50ms preceding the pulse). The peak-to-peak amplitude for each MEP occurring 10-40ms after the pulse was then calculated. Any MEPs outside of 2 SDs of the mean for each measurement type, for each timepoint, for each participant were rejected. These MEPs were then run through a single iteration Grubbs' test with a significance level of 0.05 in order to exclude any remaining significant outliers. $SICI_{2.5ms}$ and ICF are expressed as a fraction of the mean conditioned MEP amplitude over the mean unconditioned MEP amplitude (rSI_{adj}).

Baseline measurements were calculated by taking the mean corticospinal excitability, $SICI_{2.5ms}$, and ICF values recorded before and after task familiarisation. To investigate whether there was any difference in specific TMS measures before and after familiarisation, we performed RM ANOVAs on each TMS measure separately (within subjects factor of Stimulation Condition: anodal ipsilateral, cathodal ipsilateral, sham; and within subjects factor of Time: before familiarisation, after familiarisation). There was no significant difference in the corticospinal excitability or ICF measures between these two timepoints [main effect of Time- CE: $F(1,19)=3.920$, $p=0.062$; ICF: $F(2,38)=0.003$, $p=0.955$] and no Stimulation Condition by Time interaction- [CE: $F(2,38)=2.354$, $p=0.109$; ICF: $F(2,38)=0.598$, $p=0.555$]. However, there was an overall increase in SICI measurements between the two baseline measures (main effect of Time- $F(2,38)=4.425$, $p=0.049$), but no interaction between tDCS condition and time. ($F(2,38)=0.202$, $p=0.818$). We therefore used the mean of these two timepoints as our baseline measure.

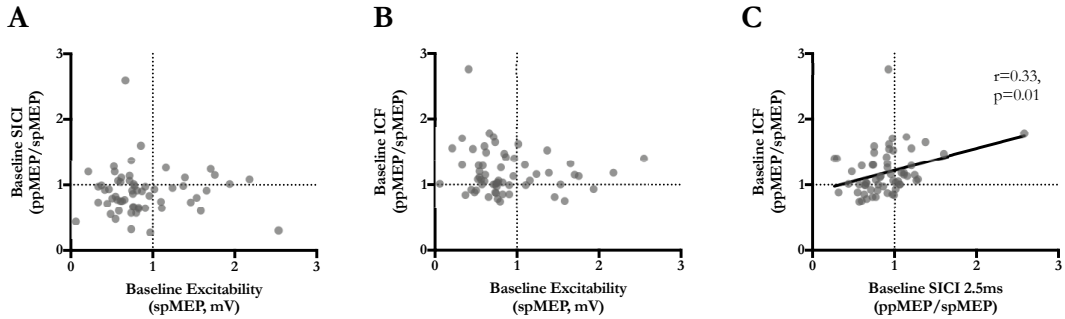


Figure 5.2 Correlations between baseline TMS measures. Partial correlations controlling for participant, revealed that there was a significant correlation between baseline SICI and ICF measures. This indicates that participants who show less GABAergic inhibition tend to show more glutamatergic facilitation.

These same RM ANOVAs also revealed that there was no significant baseline difference across the stimulation conditions in any measure [main effect of Stimulation Condition- CE: $F(2,38)=0.679$, $p=0.513$; SICI: $F(2,38)=0.020$, $p=0.981$; ICF: $F(2,38)=0.699$, $p=0.504$]. A single sample t-test of all corticospinal excitability baseline measurements revealed that they were not significantly different than 1mV ($t(59)=-1.861$, $p=0.068$). As would be expected we found that the baseline $SICI_{2.5ms}$ measurements were significantly below 1 [single sample t-test, $t(59)=-2.469$, $p=0.016$] indicating that there was significant $GABA_A$ inhibition at baseline with our protocol. The baseline ICF measurements were significantly above 1 [single sample t-test, $t(59)=4.157$, $p<0.001$] indicating that there was significant glutamatergic excitation at baseline. $SICI_{2.5ms}$ and ICF measures were initially correlated at baseline (using partial correlation controlling for participant ($r=0.333$, $n=20$ $p=0.010$; Figure 5.2A). Neither $SICI_{2.5ms}$ nor ICF showed a significant correlation with CE measures [SICI: $r=-0.024$, $n=20$, $p=0.857$; ICF: $r=-0.112$, $n=20$, $p=0.400$] (Figure 5.2B & C).

5.4 Results

5.4.1 Subjects were able to learn the task, but ipsilateral anodal tDCS significantly impaired online learning

We first wished to investigate whether tDCS affected online learning, i.e. learning during stimulation. We therefore performed an RM ANOVA on the skill measures, with within-

subjects factors of Training (2 levels: beginning of training, end of training) and Stimulation Condition (3 levels: sham, anodal ipsilateral, cathodal ipsilateral). This analysis revealed that skill tended to increase with training [main effect of Training- $F(1,19)=18.615$, $p<0.001$] but there was no overall difference in skill between the stimulation conditions [no main effect of Stimulation Condition-$F(2,38)=1.115$, $p=0.226$]. We found that there was a trend towards differences in the effect of training across stimulation conditions [Stimulation by Training interaction-$F(2,38)=3.203$, $p=0.052$].

Given we had a specific hypothesis that each of the real stimulation conditions may differ from sham we went on to perform follow up analyses. A RM ANOVA with one factor of Stimulation Condition (sham, anodal) and one factor of training (beginning, end) demonstrated a significant worsening of learning in the anodal group relative to sham [RM ANOVA: no main effect of Stimulation Condition ($F(1,19)=1.356$, $p=0.259$), main effect of Training ($F(1,19)=18.625$, $p<0.001$), and a significant interaction ($F(1,19)=7.658$, $p=0.012$)]. An equivalent analysis comparing cathodal tDCS and sham also showed a trend towards a worsening in the cathodal Stimulation Condition [RM ANOVA: no main effect of Stimulation Condition ($F(1,19)=3.152$, $p=0.092$), main effect of Training ($F(1,19)=17.432$, $p=0.001$), and interaction ($F(1,19)=4.142$, $p=0.056$)] (Figure 5.3).

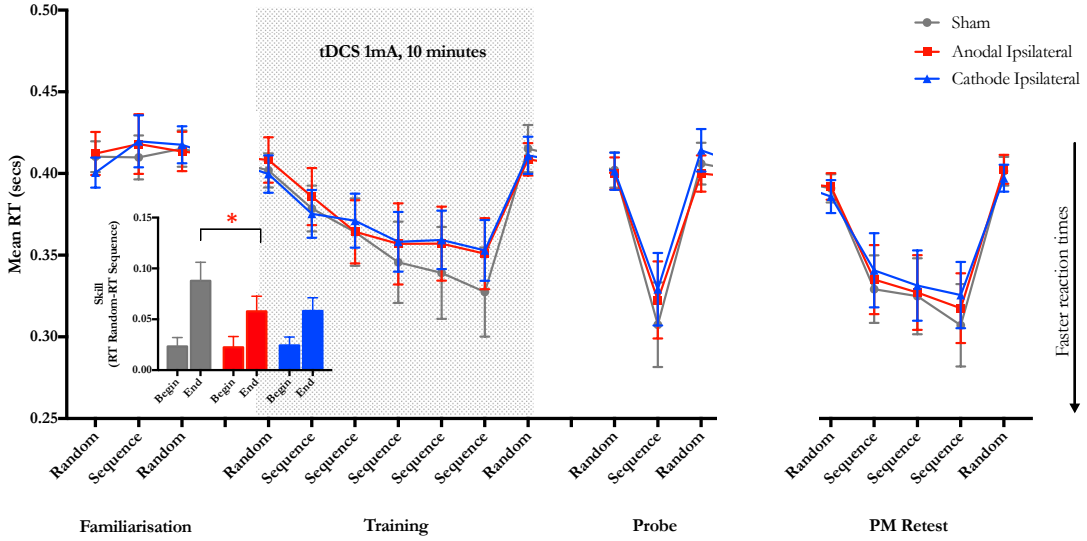


Figure 5.3 Main line graph shows mean RT for random and sequence blocks across the 3 stimulation conditions. Inset bar graph shows mean skill (RT random- RT sequence) in each condition at the beginning and end of the training section. Demonstrating a significant worsening of skill learning during the training

section of the task in the anodal ipsilateral condition relative to sham. Learning also showed a trend towards impairment in the cathodal ipsilateral condition.

We then went on to investigate the effects of tDCS on retention of the motor skill. A RM ANOVA was performed with one factor of Time (probe skill, PM retest initial skill) and one factor of Stimulation Condition (sham, anodal ipsilateral, cathodal ipsilateral). This allowed us to contrast the skill at probe, our best measure of learning at the end of the morning session, to skill at the beginning of the PM retest session. We found that there was an overall decrease in skill between the morning and afternoon sessions ($F(1,19)=31.455$, $p<0.001$), but that this was not modulated by Stimulation Condition (no main effect of Stimulation Condition – $F(2,38)=0.668$, $p=0.519$), Time by Stimulation Condition interaction: $F(2,38)=1.114$, $p=0.339$)

Finally, we wanted to explore whether tDCS had an effect on relearning of the task during the PM session (RM ANOVA: one factor of Relearning (PM retest initial skill, PM retest end skill; one factor of Stimulation Condition (sham, anodal ipsilateral, cathodal ipsilateral). Subjects showed significant relearning in the evening session ($F(1, 19)=31.584$, $p<0.001$), but there was no effect of Stimulation Condition ($F(2,38)=1.096$, $p=0.345$) and no significant interaction ($F(2,38)=0.120$, $p=0.887$).

5.4.2 Ipsilateral tDCS led to no significant change in cortical excitability

Next, we wished to investigate the effect of ipsilateral tDCS, applied during task performance, on our TMS derived neurophysiological metrics. We first performed a full RM ANOVA with the within subject's factors of Stimulation Condition (sham, anodal ipsilateral, cathodal ipsilateral) and Time (baseline, post-tDCS, 5mins post, 15mins post) and Measure (CE, SICI and ICF). For full results see Supplementary Table 5.1. There was a significant main effect of Measure ($F(2,38)=7.129$, $p=0.002$), and a significant Time by Measure interaction ($F(6,114)=3.206$, $p=0.006$).

We therefore performed RM ANOVAs for each of the TMS measurements (CE, SICI and ICF) separately, in each case with within subject's factors of Stimulation Condition (sham, anodal ipsilateral, cathodal ipsilateral) and Time (baseline, post-tDCS, 5mins post, 15mins post) to assess whether there were changes in these measures across the whole timecourse of the experiment. Analysis of corticospinal excitability measures revealed an overall tendency for CE to increase over time (main effect of Time- $F(3,57)=5.313$, $p=0.003$) but no overall differences in CE between the stimulation conditions (no main effect of Stimulation Condition $F(2,38)=1.041$, $p=0.363$). The change in CE measures across time did not differ significantly between the stimulation conditions (Stimulation Condition by Time interaction $F(6,114)=0.557$, $p=0.764$).

In order to increase our power to find transient effects in our TMS measures that were caused by tDCS we next performed a RM ANOVA with a within-subject factor of Stimulation Condition (sham, anodal ipsilateral, cathodal ipsilateral) but only examined the timepoints before and immediately after stimulation (baseline, post-tDCS). We found no significant effect of Stimulation Condition on cortical excitability (no significant effect of Stimulation Condition ($F(2,38)=0.139$, $p=0.871$), no significant effect of Time ($F(1,39)=1.164$, $p=0.294$), no significant Time by Stimulation Condition interaction ($F(2,38)=0.871$, $p=0.871$)) (Figure 5.4A).

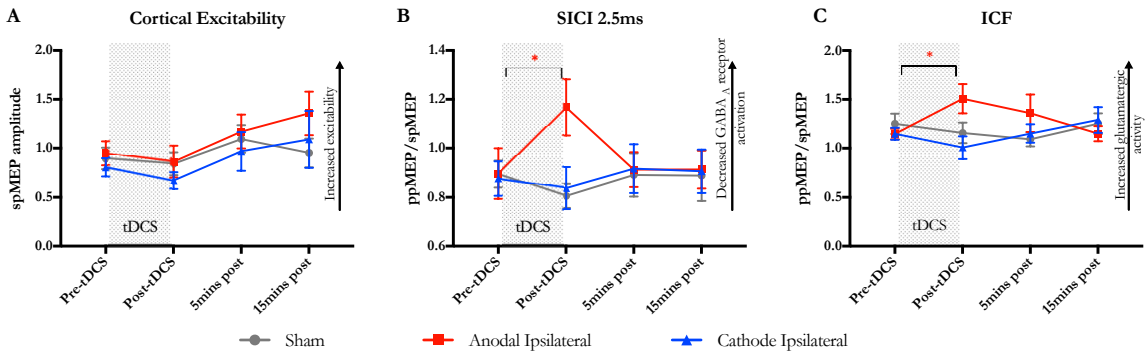


Figure 5.4 A, Cortical excitability measures (mean MEP amplitude in mV) across the stimulation conditions at all timepoints (baseline, post-tDCS, 5mins post, 15mins post). B, $SICI_{2.5ms}$ measure of $GABA_A$ mediated inhibition. Showing a decrease in GABAergic inhibition in the anodal ipsilateral condition after tDCS. C, ICF measure of glutamatergic facilitation. Showing an increase in glutamatergic activity in the anodal ipsilateral condition after tDCS.

5.4.3 Ipsilateral anodal tDCS combined with learning led to a significant decrease in $GABA_A$ activity compared with sham

Next, we performed the same series of analyses to investigate whether ipsilateral tDCS, when combined with a motor task, leads to a change in $GABA_A$ activity, as assessed by $SICI_{2.5ms}$ measurements. There was no significant effect of tDCS Condition when assessed across all four timepoints [no main effect of Stimulation Condition- $F(2,38)=1.895$, $p=0.164$; no main effect of Time- $F(3,57)=0.222$, $p=0.881$; no significant Stimulation Condition by Time interaction- $F(6,114)=1.476$, $p=0.193$].

However, $SICI_{2.5ms}$ was significantly modulated by tDCS when only the timepoints at baseline and immediately after stimulation were considered [main effect of Stimulation Condition ($F(2,38)=3.262$, $p=0.049$), main effect of Time ($F3,57)=0.560$, $p=0.464$), significant Stimulation Condition by Time interaction ($F(6,114)=3.398$, $p=0.044$)]. Follow up ANOVAs demonstrated that this was driven by a significant reduction in the $SICI_{2.5ms}$ measure of $GABA_A$ inhibition (i.e. an increase in the ratio of spMEP to ppMEP) in the anodal ipsilateral condition relative to sham {RM ANOVA: no main effect of Stimulation Condition $F(1,39)=3.892$, $p=0.063$; no main effect of Time $F(1,39)=1.236$, $p=0.280$; significant interaction $F(1,39)=6.589$, $p=0.019$] (Figure 5.4B). The $SICI_{2.5ms}$ measure did not reach a level significantly above 1 ($t(19)=1.454$, $p=0.162$).

5.4.4 Ipsilateral anodal tDCS combined with learning leads to a significant increase in ICF

Finally, we wished to perform the same set of analyses to investigate changes in ICF. Our first RM ANOVA revealed a significant effect of Stimulation Condition on the change in ICF measures over all four timepoints [no main effect of Stimulation Condition $F(2,38)=1.534$, $p=0.229$, or Time $F(3,57)=0.162$, $p=0.921$), Stimulation Condition by Time interaction ($F(6,114)=2.244$, $p=0.044$)]. This effect of Condition on ICF persisted when only examining the timepoint immediately following tDCS [main effect of Stimulation Condition $F(2,38)=3.503$,

p=0.040; no effect of Time F(1,39)=0.351, p=0.560; significant Stimulation Condition by Time interaction (F(1,39)=3.503, p=0.040)]. Follow up analysis demonstrated that, similarly to the change in $GABA_A$ activity, the observed increase in ICF was driven by an increase in facilitation the ipsilateral anodal condition relative to sham [RM ANOVA Stimulation Condition (sham, anodal ipsilateral) F(1,39)=1.433, p=0.246; Time (Baseline, post-tDCS) F(1,39)=1.670, p=0.212; interaction F(1,39)=5.228, p=0.034] (Figure 5.4C).

As ipsilateral anodal tDCS affected both $SICI_{2.5ms}$ and ICF, we then went on to investigate whether the changes in $SICI_{2.5ms}$ and ICF were correlated. Looking across all conditions there was a highly significant positive correlation between change in $SICI_{2.5ms}$ and ICF (partial correlation controlling for participant, r=0.586, n=20, p<0.001). When each stimulation condition was considered separately, these correlations between change in SICI 2.5ms and change in ICF remained significant for both the active stimulation conditions (Anodal: r=0.516, p=0.020; Cathodal: r=0.691, p=0.001) and was approaching significance in the sham condition (r=0.405, p=0.076) (Figure 5.5C).

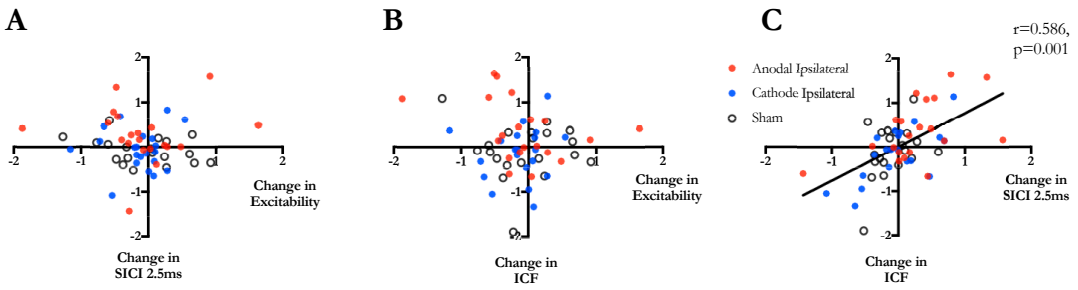


Figure 5.5 Correlations between change in TMS measures from baseline to post-tDCS. No significant correlations between change in single-pulse MEP measures of excitability and change in SICI or ICF measures. A significant correlation, controlling for participant, was however found between change and SICI and change in ICF measures indicating that a greater decrease in GABAergic activity was related to a greater increase in glutamatergic activity.

5.4.5 Ipsilateral anodal tDCS behavioural improvement is related to change in $GABA_A$ activity

Finally, we examined whether we could relate changes in our behavioural measures with changes in the TMS measures, particularly SICI. To this end, we used sham-corrected measures of

behavioural change during training (real learning change- sham learning change) and sham-corrected measures of neurophysiological changes. We demonstrated a significant correlation between sham-corrected change in $SICI_{2.5ms}$ and sham-corrected change in online skill in the anodal ipsilateral condition (r=0.542, n=20, p=0.014), such that subjects who showed a larger decrease in $GABA_A$ activity also showed a less impairment to learning with anodal tDCS (Figure 5.6). This correlation was not observed in the cathodal condition (r=0.036, n=20, p=0.880). Fisher's one-tailed r-to-z transformation revealed that the correlation coefficients of the $SICI_{2.5ms}$ and behaviour relationship were significantly greater in the anodal condition than in the cathodal condition (z=1.66, p=0.048, one-tailed).

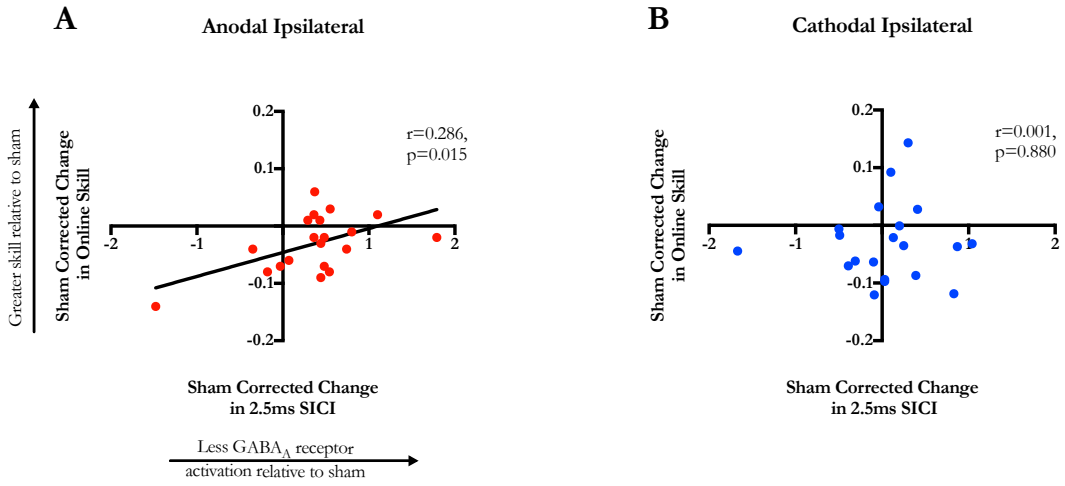


Figure 5.6 A, significant correlation between sham corrected change in online skill, and sham corrected change in $SICI_{2.5ms}$ in the anodal ipsilateral condition. Indicating that the tDCS-induced decrease in receptor activation relative to sham relates to greater skill. B, no such significant correlation in the cathodal ipsilateral condition.

There were no significant correlations between sham-corrected learning and sham-corrected ICF (anodal ipsilateral: r=0.139, n=20 p=0.558; cathodal ipsilateral: r=0.137, n=20, p=0.564) or corticospinal excitability changes (anodal ipsilateral: r=-0.052, n=20, p=0.827; cathodal ipsilateral: r=-0.307, n=20, p=0.188).

To further investigate whether the relationship between $SICI_{2.5ms}$ and behaviour was specific to $GABA_A$ activity, we performed a partial correlation controlling for sham-corrected ICF change, which did not change the pattern of results (anodal: r=0.531, n=20, p=0.019; cathodal: r=0.037, n=20, p=0.881). Additionally, the relationship between ICF and behaviour remained non-

significant when we performed a partial correlation controlling for $SICI_{2.5ms}$ (anodal: r=-0.065, n=20, p=0.793; cathodal: r=-0.11, n=20, p=0.964).

5.5 Discussion

This experiment was performed to investigate the effect of tDCS, applied during motor learning, ipsilateral to the learning hand, on behaviour and physiology of the contralateral cortex. In line with previous literature we found that anodal ipsilateral tDCS resulted in significant worsening of online learning compared to sham, but had no effect on the consolidation of skill. In line with the results in Chapter 4, but not with our predictions, we observed that anodal ipsilateral (right) M1 tDCS applied during learning also caused a significant, but transient, decrease in $GABA_A$ receptor activation in the contralateral (left) M1, and a correlated increase in glutamatergic receptor activity relative to sham. This ipsilateral anodal tDCS-induced decrease in GABAergic activity correlated with stimulation induced change in skill, such that larger decreases in contralateral M1 $GABA_A$ activity were associated with better learning relative to sham (Stagg et al., 2011a). A parsimonious explanation for these results would be that modulating ipsilateral excitability with anodal tDCS leads to behavioural worsening, but that this can be offset, at least in part, by modulation of contralateral GABA activity during learning. These results offer the beginning of a mechanistic explanation for the complex role of the ipsilateral M1 in motor learning.

Contrary to our prediction of behavioural improvements, we found the same pattern of behavioural results in the cathodal ipsilateral condition however the online worsening of learning had only a trend towards significance. These results indicate that perturbing activation in the ipsilateral M1 of healthy participants is detrimental to motor learning. We found no significant changes in corticospinal excitability or either of the TMS measures of receptor activation with cathodal ipsilateral tDCS.

5.5.1.1 Anodal and cathodal stimulation affect contralateral physiology differently during online task

Both anodal and cathodal stimulation, applied during rest, have been shown to increase corticospinal excitability (O'Shea et al., 2014) and decrease in MRS-measured GABA concentration (Chapter 4) in the contralateral hemisphere. Yet, here we found no stimulation-induced changes in corticospinal excitability in the contralateral hemisphere in either stimulation condition. We did however find an anodal stimulation-induced change in $SICI_{2.5ms}$, indicating a decrease in $GABA_A$ receptor mediated inhibition in this condition.

It is thought that the excitatory TMS effect normally seen in the non-stimulated hemisphere after cathodal tDCS is mediated by a reduction in excitability in the stimulated hemisphere, causing a decrease in interhemispheric inhibition. In line with this, as was shown in Chapter 4, the effect of cathodal tDCS on contralateral neurochemistry appears to be due to, in part, to interhemispheric connections passing through the corpus callosum. It has long been known that connections between M1s are mutually inhibitory (Chen, 2004; Curtis, 1940; Daskalakis et al., 2002; Ferbert et al., 1992; Ni et al., 2009), and cathodal tDCS has previously been demonstrated to decrease transcallosal inhibition between the motor cortices (Bradnam et al., 2010; Lang et al., 2004; Tazoe et al., 2014). However, in most of these studies tDCS has been applied during rest.

Performing unimanual muscle contractions, as was required during the SRTT, is known to alter not only the corticospinal excitability of both hemispheres (Liepert et al., 2001; Muellbacher et al., 2000), but also the levels of interhemispheric inhibition during muscle activation (Increase from active to resting: Vercauteren et al., 2008; Giovannelli et al., 2009. Decrease in both directions: Nelson et al., 2009) and after training (Decrease from resting to active: Shim et al., 2005). It has also been shown that the effects of tDCS on corticospinal excitability of the stimulated hemisphere vary depending on whether it is applied during contralateral task or rest (Amadi et al., 2015b), likely due to differing levels of baseline excitability. It is therefore perhaps unsurprising that the effect of tDCS on corticospinal excitability of the unstimulated hemisphere

during ipsilateral task may also vary, which may contribute to the differences between our findings, in both the anodal and cathodal condition, and previous literature where tDCS was applied during rest. Additionally, as previously discussed in Section 2.2.3, MRS and paired-pulse TMS assess fundamentally different brain aspects of physiology (neurochemical concentrations vs. receptor activation), and the two measures do not correlate well (Stagg et al., 2011a), which may also contribute to the differences in findings.

Despite this we did find a change in left M1 $SICI_{2.5ms}$ measurements in the anodal condition, indicating a decrease in $GABA_A$ receptor mediated inhibition, in line with the previous chapter findings of contralateral GABA concentration decreases with anodal stimulation (Chapter 4). In that chapter we also argued that the GABA decrease in the non-stimulated hemisphere during anodal tDCS appeared not to be related to corpus callosally mediated inhibition. It may be that this non-callosally mediated pathway may not be influenced by changes in activity or interhemispheric interference, and so this may be one possible reason why we found the same effect during task and at rest. It should be noted though that the decrease in TMS-measured GABA mediated inhibition found in this chapter was transient, whereas the decrease in MRS-measured GABA concentration shown in the previous chapter persisted for up to 35 minutes.

In the anodal ipsilateral condition we also found an increase in ICF, a measure of glutamatergic receptor activation. Changes in MRS-measured glutamate concentration are less frequently observed in response to M1 tDCS (Bachtiar et al., 2018; Kim et al., 2014b; Stagg et al., 2011a; Stagg et al., 2009a), and were not observed in the non-stimulated hemisphere during anodal tDCS in Chapter 4. In the data presented here both the baseline and tDCS-induced change in $SICI_{2.5ms}$ and ICF measures were highly positively correlated. It appears therefore that these measures, at least in our data, show some level of dependence.

5.5.2 Anodal Ipsilateral stimulation worsened learning, cathodal resulted in no significant change in motor learning

Here we replicated the detrimental effect of anodal ipsilateral stimulation on motor sequence learning, as recently shown by Keitel and colleagues (Keitel et al., 2018). There was not however any change to learning when measured immediately after the removal or tDCS (probe task) and nor was there any effect on retention of skill. Relatively few other papers have examined the effect of ipsilateral anodal tDCS on motor learning, with a tendency to find no significant change in skill (Vines et al., 2008, 2006). These papers used a repetitive key-pressing paradigm that may be less sensitive to small effects: the anodal ipsilateral condition caused slowing of reaction time specific to the sequence blocks of the task and only during stimulation. This small and transient effect may therefore have been missed in other studies. We saw a similar pattern of results in the cathodal ipsilateral condition, however the online effect did not reach significance.

Most previous work examining the effect of cathodal tDCS applied to the M1 ipsilateral to the performance-measured limb comes from stroke rehabilitation. Post-stroke recovery can be improved by cathodal tDCS ipsilateral to the stroke affected limb (Fregni et al., 2005; Hummel and Cohen, 2006; Boggio et al., 2007; Kim et al., 2010; Williams, Pascual-Leone and Fregni, 2010; Stagg et al., 2012; Takeuchi and Izumi, 2012; O'Shea et al., 2014). These findings are generally conceptualised as cathodal tDCS causing reduction in inhibition from the contra- to ipsilesional hemispheres, perhaps only possible in stroke patients due to abnormally high levels of interhemispheric inhibition between the ipsilateral, contralesional hemisphere and the ipsilesional hemisphere (Murase et al., 2004). In healthy participants while cathodal tDCS to the left hemisphere has been shown to improve learning with the left, non-dominant, hand (Vines et al., 2008, 2006), stimulation to the right hemisphere, as was used here, results in a non-significant decrease in learning with the dominant right hand (Vines et al., 2008). Once again these differing results between the equivalent protocols applied to different hands may reflect a resting asymmetry between the corticospinal excitability of the two hemispheres (De Gennaro et al.,

2004) and so this may influence the effects of tDCS on interhemispheric interference, and therefore the behavioural output.

Alternatively tDCS could be thought of as altering the activity of the stimulated ipsilateral cortex and affecting its more fine grained influence on behaviour (which may or may not be exerted through contact with the contralateral cortex (Ziemann et al., 1999)). It has been shown that the ipsilateral hemisphere can represent the planning and execution of finger movements (Berlot et al., 2018; Verstynen et al., 2005). Recordings from macaque demonstrate neurons M1 neurons that fire in response to both contra-and ipsilateral hand movements (Donchin et al., 2002; Tanji et al., 1988). In humans work from both ECoG (Fujiwara et al., 2017; Scherer et al., 2009) and fMRI (Diedrichsen et al., 2012) has shown that the identity of moved fingers can be decoded using only the activity patterns of the ipsilateral hemisphere, where the activation pattern is weaker but otherwise identical to that caused by the movements of the main hand (Diedrichsen et al., 2017, 2012). However from many of these studies it is not clear whether this activity in the ipsilateral hemisphere is truly functional or whether it is merely mirroring the representation in the contralateral cortex. Furthermore it is not known whether this activity is excitatory or inhibitory (Arthurs and Boniface, 2002) and so it is unclear how this would be influenced by altering excitability with tDCS.

5.5.3 $GABA_A$ receptor activity decreases are related to better learning in the anodal ipsilateral condition

Though we found a significant worsening of behaviour in the anodal ipsilateral condition and a significant increase in our $SICI_{2.5ms}$ measurements (indicating a decrease in GABAergic activity), we found a positive correlation between sham corrected change in $SICI_{2.5ms}$ and sham-corrected online learning in the anodal ipsilateral group. This meant that those participants who experienced greater decreases in $GABA_A$ receptor activation also tended to have better learning relative to the sham condition.

A parsimonious explanation for these results would be that some effect of the anodal stimulation, perhaps increasing ipsilateral excitability leads to behavioural worsening, but that this can be offset, at least in part, by changes in contralateral GABA activity during learning. It may be that the effect of anodal stimulation on the ipsilateral hemisphere is in some way detrimental to learning, while the effect on the contralateral hemisphere, resulting in a decrease in inhibition, is beneficial. Further experiments, collecting TMS or MRS measures from both hemispheres simultaneously, are needed to test this hypothesis.

5.5.4 Limitations

Due to time constraints, and a desire to keep the TMS measures as close in time as possible to the tDCS, we did not collect TMS measures from the contralateral hemisphere. This limits our conclusions that we are able to draw about the potential mechanisms of the behavioural changes, or the mechanisms behind the physiological changes, as it is not clear how the tDCS is influencing the stimulated hemisphere.

Once again, all of our participants in this experiment were right handed, performed the experiment with their right hand, and received tDCS to the right motor cortex. It is therefore not possible to know whether our results would generalise to stimulation to the other cortex, or hold for participants who were left-handed.

5.6 Conclusion

In this study we aimed to directly compare the effects applying tDCS to right M1 on the physiology of, and behaviour mediated by, left M1. Cathodal ipsilateral stimulation caused an approaching significant worsening of learning, while anodal ipsilateral stimulation caused a significant, but transient, worsening of learning on the SRTT relative to sham stimulation. There was also a significant increase in $SICI_{2.5ms}$ with anodal ipsilateral stimulation, indicating a decrease in $GABA_A$ mediated inhibition, which positively correlated with sham-corrected learning on the

SRTT. One explanation for these results in the anodal condition would be that modulating ipsilateral excitability leads to behavioural worsening, but that this can be offset, at least in part, by changes in contralateral GABA activity during learning. These results offer the beginning of a mechanistic explanation for the complex role of the ipsilateral M1 in motor learning, and strengthen the evidence for a link between GABA signalling changes in the contralateral M1 and motor learning.

Chapter 6: A single, clinically-relevant dose of the $GABA_B$ agonist baclofen impairs visuomotor adaptation and retention

The $GABA_B$ agonist baclofen is taken daily by thousands of stroke, brain injury and multiple sclerosis patients as a treatment for muscle spasticity. However a growing body of literature points to a decrease in GABAergic activity as a key feature of human motor skill learning, both in health and in recovery after brain injury (Floyer-Lea et al., 2006; Kolasinski et al., 2018; Stagg et al., 2011a). It has been shown that activating the GABA system with GABA agonists can impair motor system plasticity (McDonnell et al., 2007), but it is not well understood how this may impact behaviour.

In this experiment we investigated whether increasing activity at $GABA_B$ receptors using a single, clinically relevant, dose of baclofen impaired motor learning in young healthy participants. We found that taking 10mg baclofen resulted in reduced retention of the learnt visuomotor adaptation. State-space modelling of the behavioural data revealed that this was likely due to an increased reliance on fast system learning. This fast system adapts quickly when presented with error feedback, but also forgets quickly once this feedback is removed. Furthermore, baclofen-induced increases in the weighting of this fast system correlated with baclofen-induced increases in $GABA_B$ inhibition. This result confirms the importance of GABAergic inhibition in this form of adaptation learning, and may have implications for functional rehabilitation in patients who are taking baclofen. The data for this study was collected jointly by myself and Ioana Grigoras.

6.1 Introduction

As discussed in detail in Chapter 1, decreases in GABA concentration within M1 are observed during motor learning (Floyer-Lea et al., 2006; Kolasinski et al., 2018). Interventions that tend to decrease M1 GABA concentrations (Stagg et al., 2011; Stagg et al., 2009a) also tend to improve performance on sequence learning tasks (Buch et al., 2017; Nitsche et al., 2003) and increase retention of learnt visuomotor adaptation (O'Shea et al., 2017; Petitet et al., 2018). However from these studies it is unclear whether GABA decreases are *necessary* for the cortical plasticity underling motor learning to occur, or whether decreases in inhibitory GABA are merely a co-occurring, but not causally relevant, phenomenon. Direct manipulation of neurochemical concentrations is not possible in humans, but it is possible to pharmacologically modulate activity at specific receptors, an approach that can therefore be used to investigate the effect of modulating GABAergic signalling on human motor learning and plasticity.

Drugs that agonise GABA receptors have been shown to inhibit motor cortex LTP-like plasticity (Ziemann et al., 2006, 2001), however studies examining their effect on motor learning are lacking. This may, in part, be due to the sedative and somnolent effects of many GABA agonists, making well controlled comparisons between drug and placebo conditions difficult. From a clinical perspective $GABA_A$ agonists such as benzodiazepines are commonly prescribed to treat insomnia and anxiety, and so motor learning impairments are unlikely to be of critical importance to potential users. However this is not the case for the $GABA_B$ agonist baclofen, which is often prescribed to multiple sclerosis, stroke or brain injury patients as a treatment for spasticity. If baclofen does impair LTP-like plasticity and motor learning in humans this could negatively impact patient's motor rehabilitation.

Like other GABA agonists, baclofen has been demonstrated to impair LTP-like plasticity in the human motor cortex. In a small study of seven young healthy participants paired-associative stimulation (PAS), where electrical stimulation is applied to the median nerve followed after short time delay by a TMS pulse to the motor cortex, was used to induce LTP-like plasticity, measured

as a change in TMS-evoked MEP amplitude. In all seven participants 50mg of baclofen caused a reduction in PAS-induced LTP-like plasticity (McDonnell et al., 2007), indicating that increased activity at $GABA_B$ receptors does impair M1 plasticity.

In line with this, one previous study has examined the effect of 20mg baclofen on motor learning in healthy individuals (Willerslev-Olsen et al., 2011). In this within-subjects experiment participants performed a torque-tracking task with their ankle after receiving diazepam, baclofen or placebo. Participants showed impaired learning on the task in both drug conditions, however this finding is difficult to interpret as participants in the drug conditions, especially baclofen, also showed worse initial performance on the task. Subject drowsiness, or reductions in attention, which might plausibly explain this difference, were not measured and therefore could not be controlled for. Without ruling out a general worsening of attention it is not possible to make specific claims about the effect of baclofen on motor learning. Additionally, this study used a dose of 20mg that is far above the normally prescribed dose of 5-10mg, limiting the clinical translation of this finding.

In this study we performed a between-subjects, double-blind, placebo-controlled crossover study of the effect of a clinically relevant dose of 10mg baclofen on motor learning in young healthy participants. Participants were assessed on a variety of motor and sensory tasks. Motor learning ability was evaluated using a serial reaction time task, and a joystick based visuomotor adaptation task. An action selection task was used to assess participants' motor decision making and reaction time, and a temporal order judgement task was used to measure participants tactile acuity, which is also known to correlate with M1 GABA levels (Kolasinski et al., 2017) . Measures of declarative memory and mood were also recorded to examine non-motor effects of the drug. Finally, TMS measures of motor cortex excitability, activity at $GABA_A$ and $GABA_B$ receptors were recorded at multiple timepoints.

We had the following primary hypotheses for this study:

1. Baclofen will decrease motor system plasticity as measured by learning on the VMA and SRTT tasks

In the VMA task several measures of adaptation and retention can obtained. The degree to which participants reduce their error by adapting to an experienced perturbation is herein referred to as adaptation. The degree to which participants display this learnt adaption when visual feedback is removed is known as the adaptation aftereffect. The amount of retention of this learnt adaptation can be assessed at a later timepoint by exposing the participant to the same task but without a perturbation, and the change in error with this exposure is a measure of deadaptation. Finally the retention of the adaptation aftereffect can be measured at this later time from the participants' behaviour without visual feedback.

It has been proposed that the behaviour expressed during motor adaptation is controlled by two complementary adaptive processes occurring within the brain (Smith et al., 2006). These two processes occur over different timescales: a 'fast' system that learns quickly to adapt given error based feedback, but also forgets quickly once this feedback is removed; and a 'slow' system that learns slowly, but also forgets slowly. These two adaptive processes can be estimated from the data by applying a two-component state space model (Smith et al., 2006). Previous work has shown that MRS-measured M1 Glu:GABA ratios correlate with the relative weightings of these two systems, with higher relative weighing of the fast system being associated with greater inhibition. Higher relative weighing of the fast system was also associated with less adaptation aftereffect persistence (Petitet et al., 2018). On the basis of this evidence, and another paper showing a correlation between tDCS-induced GABA change and tDCS-induced change in adaptation aftereffect retention (O'Shea et al., 2017), we predicted that baclofen would decrease adaptation aftereffect and retention and shift the relative weighting of the two adaptation systems towards fast adaptation.

In the SRTT change in skill across time is the main measure of interest. Learning on the SRTT causes reductions in MRS-measured GABA concentration (Kolasinski et al., 2018), we therefore

predicted that by agonising $GABA_B$ receptors with baclofen we might see reductions in skill change.

2. Baclofen will modulate TMS-measured $GABA_B$ activity

We predicted that baclofen would reduce $LICI_{150ms}$ measures of $GABA_B$ receptor activation in line with previously published studies (McDonnell et al., 2007, 2006; Müller-Dahlhaus et al., 2008; Premoli et al., 2014). However, given the lower dose used in this study (10mg as opposed to 50mg) we expected our results to be much more modest in effect size.

3. The magnitude of these two changes will be related.

We predicted that changes in behaviour that we observed on either the VMA or SRTT task with baclofen would be correlated with baclofen-induced changes in $LICI_{150}$ measures of $GABA_B$ activity.

6.2 Methods

6.2.1 Participants

Twenty healthy volunteers (9 male, aged 21-32 years, mean age 24.9 years) participated in this study, after giving written informed consent to all experimental procedures, as approved by the Central University Research Ethics Committee (Ref: R55534/RE001). All participants were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and reported no history of neurological diseases, head injuries or other contraindication for transcranial magnetic stimulation (TMS). Participants were not taking any prescription medicines at the time of testing, with the exception of the oral contraceptive pill.

6.2.2 Experimental overview

Participants attended two testing days, one week apart. The protocol on both days was identical, other than the drug intervention given, either baclofen (10mg, Co-pharma, Watford, UK), or placebo (lactose/sucrose, Homeopathic Supply Company, Holt, UK). Prior to beginning the study, counterbalancing of drug condition, and day was performed by Dr Liliana Capitao (LC). Doses were over-encapsulated in gelatine capsules (Alchemists Apothecary, Derby, UK) and packaged into individual envelopes by LC. Envelopes were marked with the study information, participant number and day number. This ensured that researchers performing the data collection and analysis were blinded to drug condition. An equal number of participants received baclofen on the first day as those who received placebo, this was also counterbalanced within sexes.

All testing days began with safety screening at 9am followed by baseline mood questionnaire measures, baseline TMS measures, and task training. At around 10am participants took the drug, and there was then a 1 hour waiting period for the drug to reach peak plasma concentration. Motor tasks, memory tasks, mood questionnaires and TMS measures were then taken over the following 2 hours. This session, known from here on as the AM session, finished by 1pm. Participants remained in the testing centre for the next 3 hours, and then at 6 hours post-administration (~4pm) were subject to a final set of TMS measures, motor task assessments and mood questionnaires. This PM session was designed to assess retention of motor skills once the drug had washed-out, as the elimination half-life of baclofen in plasma is around 3-4 hours (see Figure 6.1).

All tasks were performed after drug administration in the AM session. This included the visuomotor adaptation task (VMA), the serial reaction time task (SRTT), the action selection (AS) task and the temporal order judgement (TOJ) task. Training prior to drug administration was performed on the TOJ and VMA task. The SRTT and VMA task were also performed in the PM session to assess retention.

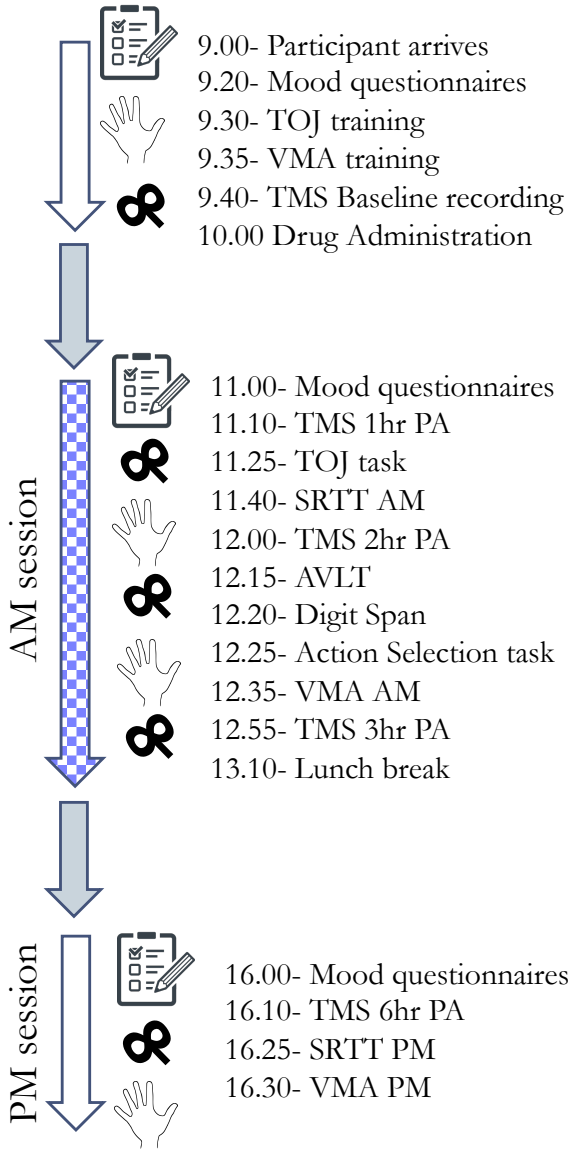


Figure 6.1 Timeline of testing day. White arrows represent task time in which the drug is not active, blue hatched arrow represents task time during baclofen peak plasma concentration, grey arrows represent breaks for the participant.

6.2.3 Transcranial magnetic stimulation (TMS)

All TMS data were acquired using a monophasic DuoMAG machine connected to a 70mm figure-of-eight coil (Rogue Resolutions, Cardiff). The left M1 was targeted in all subjects, in all sessions. The motor hotspot, i.e. the position where MEPs could be elicited in the in the right FDI muscle at the lowest stimulator intensity, was determined before each session, and the TMS coil was held at 45° to the midsagittal line with the handle pointing posteriorly. The hotspot was marked by drawing guidelines onto the participants scalp to allow for the same coil placement across the measurements. For the duration of the TMS recordings the subjects were seated

comfortably with their hands relaxed on a pillow. Participants were instructed to keep their eyes open and look ahead at a fixation cross, presented on screen.

Surface EMG was recorded, using disposable neonatal ECG electrodes (Henley's Medical, Welwyn Garden City, UK), from the FDI of the right hand using a belly-tendon montage with a ground electrode over the ulnar styloid process. Signals were sampled at 5 kHz, amplified, filtered (10Hz - 1kHz), and recorded using a Digitimer D440 4-Channel Isolated Amplifier (Digitimer, Welwyn Garden City, UK), a CED micro1401 A/D converter, and Signal software version 3.13 (Cambridge Electronic Design, Cambridge, UK). A Digitimer Humbug Noise Eliminator was used to reduce 50Hz noise in the EMG recordings (Digitimer, Welwyn Garden City, UK).

6.2.3.1 Stimulation intensities

The resting stimulation intensity and active stimulation intensity were determined at the beginning of each testing day. The resting stimulation intensity (rSI) was defined as the minimum stimulus intensity required for eliciting a mean MEP of ~1mV peak-to-peak amplitude in 10 consecutive trials in the relaxed FDI muscle. The active stimulation intensity (aSI) was defined as the minimum stimulus intensity necessary to evoke a 200μV peak-to-peak MEP in at least 5 of 10 consecutive trials while subjects maintained ~30% of the maximum contraction of the FDI.

6.2.3.2 TMS measurements

Corticospinal excitability (CE) was determined by measuring MEPs produced in response to the initial (rSI_{init}) determined at the beginning of the testing session. This intensity was not varied across the session.

Two paired-pulse protocols were performed in the study. SICI with an interstimulus interval (ISI) of 2.5ms to assess $GABA_A$ receptor activity (Di Lazzaro et al., 2006; Kujirai et al., 1993; Stagg et al., 2011a), where the conditioning stimulus was set at 70% of the aSI and the test stimulus was

set at the rSI. LICI with an ISI of 150ms was also recorded as a measure of $GABA_B$ inhibition (McDonnell et al., 2007, 2006; Müller-Dahlhaus et al., 2008), and both the test and conditioning stimuli were set at rSI. The rSI used in these measurements was adjusted (rSI_{adj}) to account for potential changes in motor cortex excitability due to drug administration or motor performance.

6.2.3.3 TMS protocol

CE, $SICI_{2.5ms,}$ and $LICI_{150ms}$ measures were collected at 5 timepoints per testing day: before drug administration (Baseline), 1h post administration (PA), 2h PA, 3h PA and 6h PA. At each time point, 10 single-pulse MEPs were recorded at the original rSI_{init} immediately before each TMS run. These pulses were used to calculate the single-pulse corticospinal excitability (CE) measurement. If the amplitude of the resulting MEP differed markedly from 1mV, then the stimulation intensity was altered accordingly (Nitsche et al., 2005; Amadi et al., 2015b; Nowak et al., 2017) before other measures were taken. Following this the SICI protocol was performed, where twelve of each rSI_{adj} and $SICI_{2.5ms}$ pulses were then delivered in a pseudorandomised order. Finally the LICI protocol was performed where twelve rSI_{adj} and $LICI_{150ms}$ pulses were delivered, again in a pseudorandomised order.

6.2.4 Sensory and motor tasks

All tasks were presented using a laptop monitor (Dell, Round Rock, Latitude E5540) at a distance of approximately 40 cm. For several of the tasks (TOJ, SRTT, VMA) the laptop was placed on a 15cm high wooden shield box, designed to occlude the participant's hand from their view (see Figure 6.2C). For the action selection task, the laptop was placed on the desk. Tasks were presented using either PsychoPy v2 (Peirce, 2007) (TOJ, VMA, Action Selection), or Psychtoolbox (PTB-3) (Brainard, 1997) for MATLAB, and displayed using MATLAB R2014b (The Mathworks Inc., Natick, USA) (SRTT).

6.2.4.1 Serial reaction time task (SRTT)

As in Chapter 3 participants performed a visually cued reaction time task, using their right hand, in order to assess motor sequence learning. The task was performed twice in each testing day, once at approximately 1.5h PA in the AM session and once at 6h PA in the PM session.

In this version of the task random blocks were made up of 48 button presses with cues presented in black, while the sequence blocks were made up of 5 repeats of a 12-item sequence with cues presented in blue on a white screen. In the AM session the familiarisation section contained one sequence block between two random blocks, the training section contained seven sequence blocks, then the post-training section again contained one sequence block flanked by random blocks. The PM session SRTT had 3 sequence blocks bookended by two random blocks. The sequences differed between the first and second testing day (Sequence day 1: 312423214134, sequence day 2: 342131241432). For one participant the computer crashed during the familiarisation section on day 1 and so this participant performed the task using a third sequence (sequence 3: 231434124213).

6.2.4.2 Visuomotor adaptation task (VMA)

This task was designed to measured participant's ability to adapt to a perturbation of visuomotor mapping. Full versions of the task were run twice during a testing day: once at approximately 2.5h PA in the AM session and once at 6.5h PA in the PM session. Participants also practiced on a short version of the VMA task before drug administration.

Participants used a joystick (Logitech, Lausanne, Switzerland; Attack 3) in their right hand to control a red cursor presented onscreen. At the beginning of each trial, with the joystick in the neutral position, the cursor would be positioned in the centre of the screen along with a yellow target dot. After a delay of 1 second the yellow target would move to one of 8 equally spaced locations on an imaginary circle with a radius of 4.5 cm around the centre of the screen (N, NE, E, SE, S, SW, W, NW). The targets appeared in each location randomly, within each set of 8

consecutive trials. The participant was instructed to make rapid "shooting" movements to guide their cursor through the target, similar to previously described tasks (Galea et al., 2011). After 2 seconds the target returned to the central position (see Figure 6.2A).

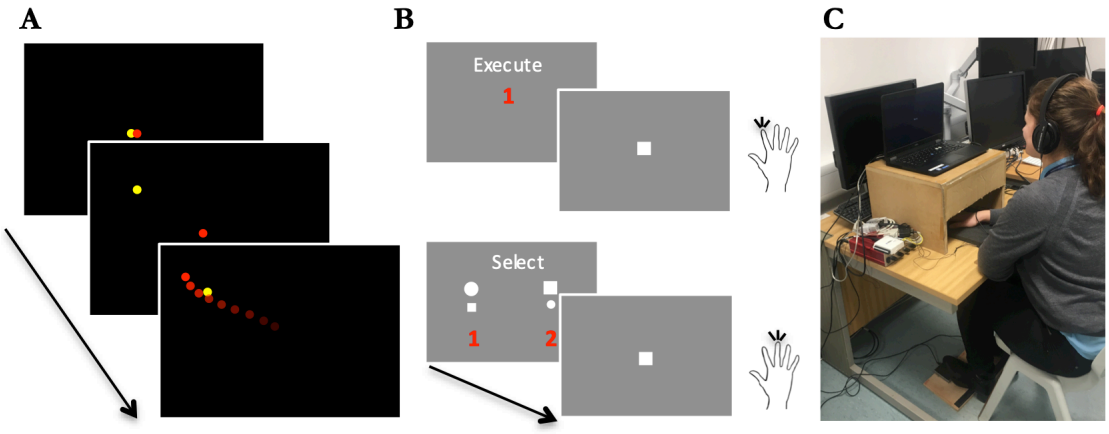


Figure 6.2 A, VMA task screen seen by participant during FB-on blocks. B, Action selection task screen, showing the instruction screen for the Execute and Select Blocks. C, Photo of TOJ set up, including the shield box, vibrotactors, headphones and foot pedals.

During the task there were blocks of feedback on (FB-on) and feedback off (FB-off) blocks. In trials during the FB-on blocks, the participant could see their cursor until it moved off screen, meaning that they could see trajectory of their movements and proximity to the target. In FB-off trials, the cursor would disappear as soon as the participant moved the joystick, meaning that they could not see the trajectory of their movement. Each block consisted of 40 trials (5 repeats, of the 8 different trial target locations), Both AM and PM task versions consisted of 5 FB-on blocks interspersed with 5 FB-off blocks. During the AM session, a 30° clockwise deviation was induced during the FB-on trials. Participants therefore began with 30° error in their movements, which they could reduce through adaptation. As discussed in Section 1.2.3 processing within cerebellum is thought to be crucial for this adaptation process. Recording the error in the FB-off trials in the AM session, where visual feedback and perturbation were removed, gave a measure of the adaptation aftereffect. Adaptation aftereffect is thought to be in influenced, at least in part by M1 GABA levels (O'Shea et al., 2017). Error in the PM session, where no perturbation was present, measured the degree to which participants retained the learnt adaptation and adaptation aftereffect. The pre-drug administration practice consisted of 2 FB-on repeats of the 8 trials

followed by 2 FB-off repeats. The purpose of the practice was to familiarise participants with the kinematics of the joystick and timing of the task, there was no perturbation introduced.

6.2.4.3 Action Selection (AS) Task

This task was performed to identify the participants' speed of movement selection. The task was performed approximately 2 hours after drug administration. Participants responded to onscreen cues by pressing keys on the laptop keyboard with the index and middle fingers of their right hand.

Participants were instructed to use their index finger to press 'o', which was renamed key 1 for the purpose of the experiment, and their middle finger to press 'p', named key 2. The task consisted of two types of blocks: Execute blocks where participants always executed the same movement, and Select blocks where participants had to make a choice about which movement to perform. Before an Execute block, participants would be instructed either "Execute 1" or "Execute 2" indicating that they should press key 1 or 2 in response to any cue that appeared on the screen. Cues could take the form of either circles or squares that were either large or small. During the Select blocks, participants were instructed to follow an abstract rule: press key "1" if the cue was a large circle or a small square or press key "2" if the cue was a small circle or a large square (see Figure 6.2B). The task consisted of two Execute blocks (one for each key) and two Select blocks, presented in a random order. Each block contained 50 trials. The inter-trial interval varied between 2 and 4 seconds to prevent anticipatory key presses.

6.2.4.4 Temporal order judgement (TOJ) task.

Participants performed the TOJ task, which was implemented similarly to Zampini et al., 2005, in order to assess tactile discrimination of the index and middle finger of the right hand. Participants were familiarised with a short version the task prior to drug administration and then performed the full version of the task approximately 1.25hrs PA.

Two vibrotactile stimuli were presented consecutively to the distal pad of their index and middle finger on the right hand using electromagnetic solenoid-type stimulators (Dancer Design, St Helens, UK). Stimuli were delivered to the two fingers at a variety of interstimulus intervals (ISIs): +300, +150, +100, +75, +50, +30, -30, -50, -75, -100, -150, -300 milliseconds. By convention, a positive interstimulus interval meant that the middle finger received the first stimulus. Participants were instructed to identify which finger received the first stimulus and press the corresponding foot pedal with right (middle finger), or left (index finger) foot. A 24-line digital input-output box (NI USB-6501; National Instruments, Austin, USA), a digital external soundcard (7.1; StarTech.com, London, Canada) and a digital-analogue converter and amplifier (Tactamp; Dancer Design, St Helens, UK) were used to drive the tactors from the laptop audio output.

Participants sat comfortably on a chair with their right arm resting on the table in the midline, with their index and middle finger on the two vibrotactor stimulators that delivered fixed amplitude supra-threshold pulses (20ms, 200Hz). The participant's hand was occluded by the shield box, and they wore headphones through which they heard pink noise (Figure 6.2C). This removed any visual or auditory information about the order of tactile stimulation. Participants saw a screen with the message "Ready" to announce the start of a trial, "Response" to cue the decision and "No response" in case of failure to register a response after 2 seconds. The 12 ISIs were each presented twice during the practice before drug administration and then 15 times for the full assessment (AM session), in a random order.

6.2.5 Other assessments

6.2.5.1 Bond-Lader visual analogue scale (VAS)

The participants were asked to fill in a Bond-Lader Visual Analogue Scale (VAS) three times on each day: before treatment administration (Baseline), one hour post-administration (1hr PA) and 6 hours post administration (6hr PA). Participants gave a subjective account of various aspects

their mood by marking their current state of mind on 16 different 100 mm analogue scales between two opposing emotions (e.g. Alert-Drowsy, Happy-Sad) (see Supplementary Table 6.1 for full list of categories).

6.2.5.2 Rey auditory-verbal learning test (AVLT)

Basic memory tests were included in the experimental design to assess for general effects of baclofen on cognition. Participants were asked to complete the Rey Auditory Verbal Learning Test (AVLT) approximately 2 hours after drug administration. The experimenter read the participant a list of 12 words (List A) at a rate of 1Hz (Rey, 1958; Spreen et al., 1998). The participant was then asked to recall as many words as they could. This procedure was carried out five times using the same list. Participants were then presented with a second list of words (List B) that they were given one chance to recall. Following this, participants were asked to recall List A for a sixth time (immediate list recall). They were then asked to execute a motor task (the Action Selection task) for approximately 10 minutes and, following this, were asked to recall List A again (delayed list recall).

6.2.5.3 Digit span test

Participants performed the digit span test, both forwards and backwards, approximately 2h after drug administration. During the forward section, the participants were instructed to repeat the digit sequence in the same order they heard it from the researcher, while during the backward section, they had to repeat the digit sequence in the reverse order.

Both forward and backward sections began with sequences of 2 digits in length. Participants were assessed on two sequences of each length, and if the participant recalled at least one of the sequences correctly then the task would proceed and they would be read sequences with an additional digit.

6.2.5.4 Blinding assessment

At the end of both testing days the participant and the experimenter who carried out the TMS measurements completed a blinding assessment form. On this form the participant/experimenter reported their guess of the treatment (baclofen/placebo), and marked their certainty about this guess on a 100mm analogue scale.

6.3 Analysis and results

Unless otherwise stated all pre-processing was performed using MATLAB R2018a (The Mathworks Inc., Natick, USA), and statistical tests performed in SPSS 25 (IBM corp, Armonk, USA). Analysis details are included with results.

6.3.1 TMS analysis and results

TMS data was collected from all but two participants, one due to equipment failure, and another due to high TMS thresholds. $LICI_{150ms}$ measurements were not recorded from a further two participants due to technical issues (see Supplementary Table 6.2 for included data sets for each participant).

Data cleaning was performed in line with previous TMS studies conducted in the group (Nowak et al., 2017). Trials were excluded if there was precontraction in the right FDI muscle (EMG amplitude >0.15mV in the 30ms preceding the pulse). Peak-to-peak amplitude for each MEP was quantified and MEPs greater than 3.5mV or outside of 2 SDs for mean for each measurement type, for each timepoint, for each participant were rejected. These MEPs were then run through a single iteration Grubbs' test with a significance level of 0.05 in order to exclude any remaining significant outliers. $SICI_{2.5ms}$ and $LICI_{150ms}$ are expressed as a fraction of the mean paired-pulse MEP amplitude over the mean single-pulse MEP amplitude (rSI_{adj}). TMS data was analysed using repeated measures ANOVAs and t-tests, as it was assumed that carry-over effects between testing days were unlikely.

6.3.1.1 TMS protocols were effective

We first needed to confirm that there was no significant difference in baseline single-pulse MEP amplitude between the baclofen and placebo condition (paired samples t-test: $t(17)=0.474$, $p=0.642$). There were also no significant baseline differences in $SICI_{2.5ms}$ measures of synaptic $GABA_A$ mediated inhibition ($t(17)=0.202$, $p=0.842$) or $LICI_{150ms}$ measures of $GABA_B$ mediated inhibition ($t(14)=0.700$, $p=0.495$).

We then went on to confirm that the MEP response elicited by rSI_{adj} pulses, i.e. the single-pulse measure against which the paired-pulse measures were contrasted, did not change across time. A single sample t-test revealed that the combined single-pulse rSI_{adj} MEP measures, used in both the SICI and LICI protocols, was significantly less than 1mV at baseline ($t(35)=3.883$, $p<0.001$), but that this was stable across the testing days as assessed by a repeated measures analysis of variance (RM ANOVA, 2 levels of Drug: placebo, baclofen; 5 levels of time: Baseline, 1hr PA, 2hr PA, 3hr PA, 6hr PA. No main effect of Time: $F(4,68)=2.206$, $p=0.078$). No main effect of Drug: $F(1,17)=1.004$, $p=0.330$, No significant Time by Drug interaction: $F(4,68)=2.501$, $p=0.050$). We then wanted to confirm that the paired pulse measures produced the expected effects. Single sample t-tests performed on the mean baseline measurements from both testing days demonstrated that both $SICI_{2.5ms}$ ($t(35)=5.19$, $p<0.001$) and $LICI_{150ms}$ ($t(29)=3.776$, $p<0.001$) measures produced significant inhibition.

6.3.1.2 Baclofen significantly reduced overall corticospinal excitability

Having established that the TMS measures were interpretable, we then went on to investigate the effects of baclofen on the TMS measures. Given there was no difference in MEP amplitude between the drug and placebo conditions, the corticospinal excitability (CE) measures for the subsequent timepoints (1hr PA, 2hr PA, 3hr PA, 6hr PA) were then baselined for further analysis.

The baseline measures from the three timepoints during the AM session were analysed using an RM ANOVA with a within-subjects factor of Drug (baclofen, placebo), and a within-subjects factor of Time (1hr PA, 2hr PA, 3hr PA). This revealed a significant reduction in corticospinal excitability with baclofen compared with placebo (Drug: $F(1,17)=4.929$ $p=0.040$). There was also a significant effect of Time ($F(2,34)=12.3$, $p<0.001$) driven by a dip in excitability at 2hr PA, but no significant Drug by Time interaction ($F(2,34)=0.6271$ $p=0.540$- Figure 6.3A).

A paired samples t-test performed on the baselined measures at 6hr PA showed that the baclofen-induced difference in corticospinal excitability was no longer present after drug washout ($t(17)=0.988$, $p=0.337$).

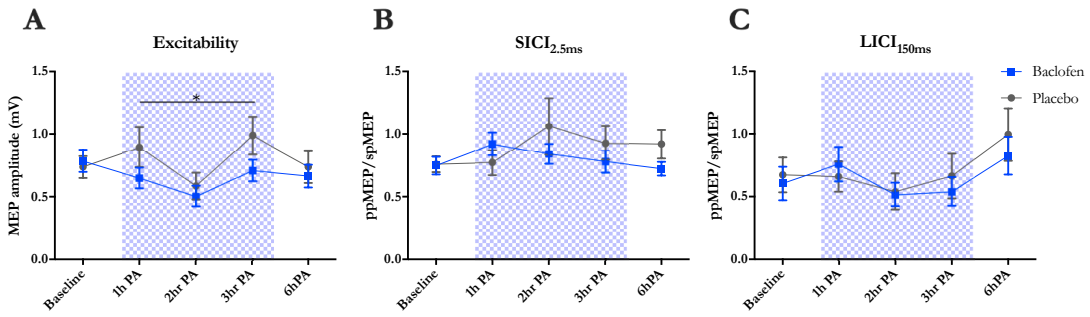


Figure 6.3 A, Decrease in corticospinal excitability during baclofen peak plasma concentration (blue hatch). Excitability returned to normal levels after drug washout at 6hrs PA. B, No change in $GABA_A$ receptor inhibition with baclofen. C, No change in TMS-measured $GABA_B$ receptor inhibition with baclofen.

6.3.1.3 No effect of baclofen on synaptic $GABA_A$ or $GABA_B$ mediated inhibition

We then went on to investigate the effects of baclofen on our TMS measures of $GABA_A$ and $GABA_B$ activity. The following analysis was performed on values normalised to baseline, as there was no difference in baselines between the two testing days.

Applying the same analysis (RM ANOVA, two levels of Drug (baclofen, placebo), three levels of Time (1hr PA, 2hr PA, 3hr PA) revealed that neither $SICI_{2.5ms}$ nor $LICI_{150ms}$ were significantly affected by baclofen during the AM session [$SICI_{2.5ms}$: $F(1,17)=0.263$, $p=0.615$. $LICI_{150ms}$: $F(1,14)=0.245$, $p=0.628$- Figure 6.3B & C]. There were also no significant changes in these measures with Time through the session [$SICI_{2.5ms}$: $F(2,34)=0.550$, $p=0.582$. $LICI_{150ms}$:

F(2,28)=2.29, p=0.120] or any interaction between Drug and Time [$SICI_{2.5ms}$: F(2, 34)=1.612, p=0.214. $LICI_{150ms}$: F(2,28)=0.968, p=0.392]

Finally, paired samples t-tests revealed that there were no differences between the drug conditions in the baselined paired-pulse measures at the 6hr PA timepoint [$SICI_{2.5ms}$: t(17)=0.2495, p=0.192. $LICI_{150ms}$: t(14)=0. 557, p=0.587].

6.3.2 Sensory and motor task analysis and results

We then went on to investigate the behavioural effects of baclofen. Motor and sensory tasks were analysed using linear mixed models (LMM) to enable drug effects to be examined while controlling for other potential confounding variables, such as day number. This approach also allows the inclusion of incomplete data sets.

6.3.2.1 SRTT analysis and results

Response time (RT) was calculated as the time from cue onset to button press. RTs of incorrect responses and RTs outside of 2.7 SDs of the mean value were excluded. Initial AM skill was calculated as the difference in mean RT between the random and sequence blocks during familiarisation. The same approach was used to calculate the final AM skill using the data from the post-training section. Skill at the beginning and end of the PM session (initial PM skill, final PM skill) was calculated by subtracting the first sequence block from the first random block, and the final sequence from the final random block in the PM session.

6.3.2.1.1 Baclofen did not significantly impact change in skill measures on the SRTT

To examine the effect of drug on change in SRTT learning initial AM skill and final AM skill were input into a LMM (fixed effects of Time (initial AM, final AM), Drug (baclofen, placebo), Day (1, 2), Time by Drug interaction, Day by Time interaction; random effect of Participant).

This revealed that skill on the task improved with training (Time: F(1,55)= 19.63, p<0.001). However, baclofen had no effect on the rate of learning in the AM session (Drug by Time interaction: F(1,55)=0.026, p=0.872- Figure 6.4). There was a significant effect of Day on the change in skill, driven by a greater improvement in skill on the second day (Day by Time interaction: F(1,55)=5.498, p=0.023). There were no overall differences in overall skill between drug conditions or testing days when accounting for other effects (Drug: F(1,55)=0.082, p=0.776. Day: F(1,55)=0.012, p=0.912).

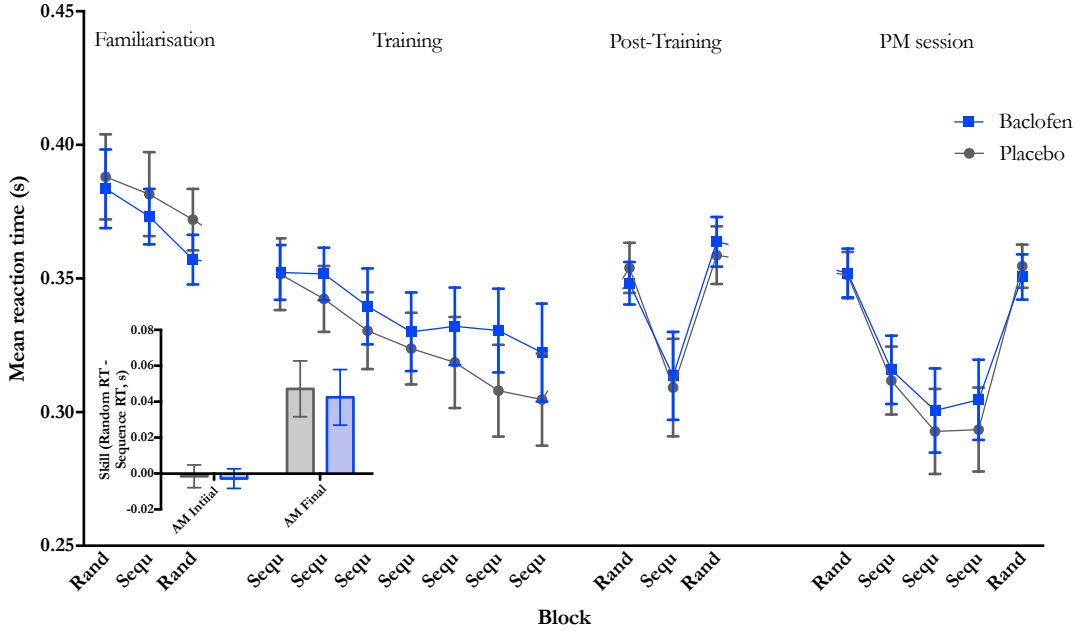


Figure 6.4 Mean reaction time for each block of the SRTT. There was learning on the task in both the drug conditions, but no significant difference in the change in skill across the AM session (inset), across the PM sessions, or in skill retention between the AM and PM sessions.

Similar analysis was performed to examine whether baclofen impacted skill retention between the AM and PM sessions using the final AM skill measure and the initial PM skill measure (LMM: Fixed effects of Time (final AM, initial PM), Drug (baclofen, placebo), Day (1, 2), Time by Drug interaction, Day by Time interaction; random effect of Participant). This also revealed that there was no effect on change in retention with baclofen (Drug by Time interaction: F(1,55)=0.006, p=0.936). No other effects in the model came to significance [Time: F(1,55)=0.526, p=0.471. Drug: F(1,55)=0.172, p=0.680. Day: F(1,55)=0.698, p=0.407. Day by Time interaction: F(1,55)=3.565, p=0.064].

Finally the change in skill between the two PM sessions was examined to determine whether baclofen impacted later relearning of the task in the PM session. (LMM: Fixed effects of Time (initial PM, final PM), Drug (baclofen, placebo), Day (1, 2), Time by Drug interaction, Day by Time interaction; random effect of Participant). There was no significant effect of baclofen on skill relearning (Drug by Time interaction: $F(1,55)=0.411$, $p=0.524$), or on any of the other effects in the model (Time: $F(1,55)=2.848$, $p=0.097$. Drug: $F(1,55)= 0.970$, $p=0.329$. Day: $F(1,55)=1.432$, $p=0.236$. Day by Time interaction: $F(1,55)= 0.013$, $p=0.908$).

6.3.2.2 VMA analysis and results

Performance on this task was measured by recording the error in degrees between the target and the point at which the cursor crossed through the imaginary circle the targets were placed on. Individual trials were removed from further analysis if the error was greater than ±67.5 degrees, indicating a movement was made towards the wrong target. A positive error indicates a displacement clockwise, therefore, due to the 30° clockwise deviation in the FB-on blocks of the AM version, the error in FB-on blocks is recorded as positive and the error in FB-off blocks, i.e. the adaptation after effect, is recorded as negative.

Poor quality data necessitated the removal of a number of participants from further analysis. Problems with the joystick to PC connection resulted in a failure to record movements in one dimension during some task runs (see Supplementary Figure 6.1). Additionally two sessions were removed because these participants rotated the joystick. In total, two participants had both AM sessions excluded from further analysis, and another three had one session removed. PM sessions were excluded for those participants whose AM sessions had been removed. One further participant had one PM session removed.

6.3.2.2.1 Baclofen significantly reduced AM adaptation aftereffect and reduced PM retention of visuomotor adaptation

We wished to investigate the effects of baclofen on both adaptation and retention metrics. Analysis was performed separately for the FB-on and FB-off blocks of the AM and PM sessions. Average angular error measurements for each of the 5 FB-on and FB-off blocks was calculated and input into LMMs (Fixed effects of Time (B1, B2, B3, B4, B5), Drug (baclofen, placebo), Day (1, 2), Time by Drug interaction, Day by Time interaction; random effect of Participant).

First we wished to investigate the effects of $GABA_B$ agonism on skill acquisition. In the AM session, FB-on blocks, participants were able to adapt and decrease their error across the blocks of the task (Time: $F(4,138.0)=6.125$, $p<0.001$), however there was no effect of baclofen on overall angular error (Drug: $F(1,138.0)=0.006$, $p=0.940$) or on the change in the error over the blocks (Drug by Time interaction: $F(4,138.0)=1.062$, $p=0.378$). There was also no effect of testing day ($F(1,15.3)=0.744$, $p=0.402$) nor a significant Day by Time interaction: ($F(4,138.0)=0.577$, $p=0.680$).

We then went on to look at the effects of baclofen on adaptation aftereffect, i.e. the degree to which participants continued to display the adaptation when feedback and perturbation was removed. As would be expected, in the FB-off blocks of the AM session participants showed a significant increase in adaptation after effect (i.e. increasing error) across the blocks of the task (Time: $F(4,134.0)=3.423$, $p=0.011$). In the baclofen condition the overall error across the FB-off blocks was significantly lower, indicating a reduction in adaptation aftereffect (Drug: $F(1,137.8)=6.133$, $p=0.014$; see Figure 6.5A). There was no difference in how this error changed across blocks between the drug conditions (Drug by Time interaction: $F(4,134.0)=0.366$, $p=0.833$) or in any other of the effects modelled (Day: $F(1,16.0)=0.059$, $p=0.812$. Day by Time interaction: $F(4,134.0)=0.302$, $p=0.876$).

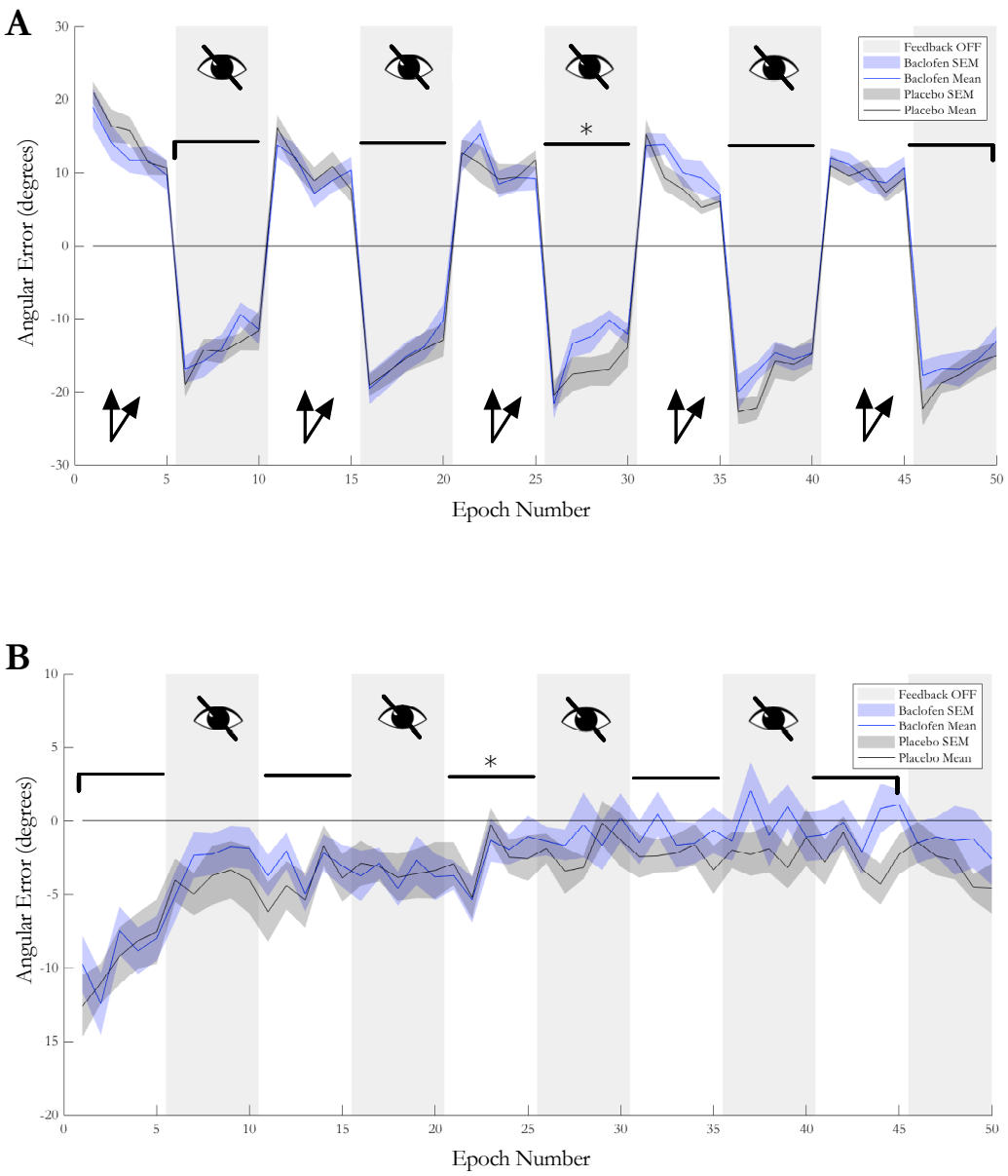


Figure 6.5 A, Group AM session data from visuomotor adaptation task. Showing a significant reduction in overall angular error in the baclofen condition across the FB-off blocks. B, Group PM session data, again demonstrating an overall reduction in angular error in the FB-on blocks with baclofen.

As expected, in the PM session we observed a significant deadaptation, measured as a decrease in angular error, across the FB-on blocks (Time: $F(4,123.8)= 37.383$, $p<0.001$), but there was a reduction in the overall retention of the adaptation (i.e. less angular error) in the baclofen condition (Drug: $F(1,130.7)= 4.138$, $p=0.044$; see Figure 6.5B). There were no other significant effects in the model (Day: $F(1,13.6)=4.138$, $p=0.356$. Drug by Time interaction: $F(4,123.8)=0.796$, $p=0.530$. Day by Time interaction: $F(4,123.8)=0.860$, $p=0.490$).

Finally, to examine the retention of the adaptation aftereffect the FB-off blocks in the PM session were analysed. Regardless of treatment condition there was a trend towards a reduction in aftereffect across blocks; (Time: $F(4,124.0)=2.418$, $p=0.052$). There was also a trend towards a reduction in after effect retention in the baclofen condition (Drug: $F(1,130.8)=12.891$, $p=0.091$). There were no other significant effects (Day: $F(1,13.9)= 0.740$, $p=0.564$. Drug by Time interaction: $F(4,124.0)=0.322$, $p=0.863$. Day by Time interaction: $F(4,124.0)=1.392$, $p=0.24$).

6.3.2.2.2 State space modelling of AM VMA data

The classical two-system model for adaptation data (Smith et al., 2006), where a fast and slow system are used to describe a participant's behaviour on an adaptation task, has recently been updated to add weightings describing the relative contributions of the two systems to the participant's behaviour (Petitet et al., 2018).This model was fit to the group mean data from each trial using MATLAB R2018a and optimised using fmincon (Figure 6.6A). The weighted two-state model fit to the group data had a lower Akaike's Information Criterion (AIC) than the non-weighted model ($\Delta AIC=-3.8$). Only subjects who had complete data sets from both AM sessions were included in this analysis.

$$e(n)=d-X(n)$$

$$X(n)= w_f x_f(n)+w_s x_s(n)$$

$$x_f(n)=A_f x_f(n-1)+ \Delta B_f e(n-1)$$

$$x_s(n)=A_s x_s(n-1)+ \Delta B_s e(n-1)$$

$$0<A_f<A_s<1;\ 0<B_s<B_f<1$$

$$0<w_f<2;\ 0<w_s<2;$$

Where $e(n)$ is pointing error on trial n; d is the perturbation; $X(n)$ is the degree of adaptation; x_f is the adaptation contribution from the fast system; x_s adaptation contribution from the slow

system; A is the retention rate; B is the learning rate; Δ reports whether feedback is on or off; w is the weighting parameters.

The learning and retention rates calculated from the group model fit (A_f=0.795, A_s=0.990, Bf=0.375, Bs=0.037) were input into weighted models that were fit to individual data from each AM session in order to obtain fast and slow system weightings for each subject for each day. A relative weighting parameter was also calculated $R_w=w_f/(w_f+w_s)$.

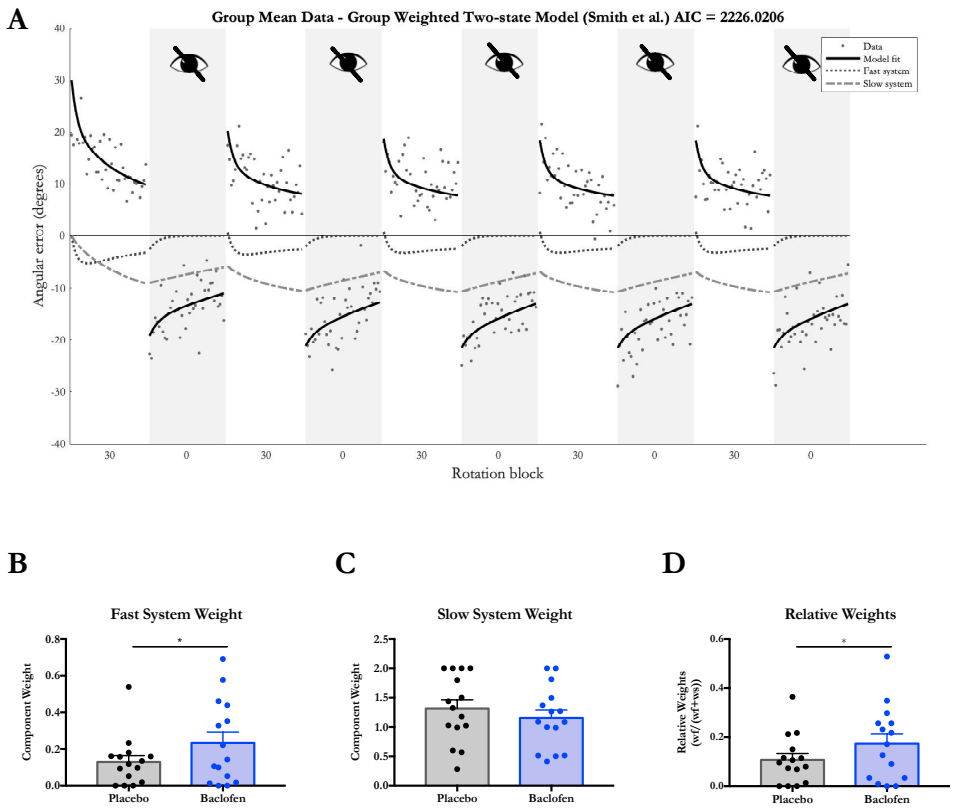


Figure 6.6 A, Weighted two state model fit to group data averaged across the baclofen and placebo conditions. B, C, D, Weighting of systems and relative weighting (wf/(wf+ws)), demonstrating an increase in fast system weighting in the baclofen condition.

6.3.2.2.3 Baclofen increased the weighting of the fast adaptation system

LMM analysis of the system weightings (Fixed effects of Drug (baclofen, placebo), Day (1, 2), random effect of Participant) revealed that there was a significant effect of Drug on the fast system weighting (w_f) (Drug: F(1,13)=5.470, p=0.036- Figure 6.6B) driven by an increase in the weighting in the baclofen condition. There was also trend towards significance of Day

(F(1,13)=4.633, p=0.051), as there was a tendency for the fast system to have a greater weight on the second day. The same analysis revealed no significant effect of either Drug (F(1,13)=2.939, p=0.110- Figure 6.6C) or Day (F(1,13)=0.246, p=0.628) on slow system weight. Finally, examining the relative weights revealed that there was a significant effect of both Drug (F(1,13)=5.914, p=0.030- Figure 6.6) and Day (F(1, 13)=4.737, p=0.049).

6.3.2.2.4 State space modelling weightings correlate with overall behavioural measures

In order to assess how the baclofen-induced change in weightings from the state space modelling related to the baclofen-induced overall error measures from the VMA task the measures were placebo corrected (baclofen measure- placebo measure) and Pearson's correlations were performed. The baclofen-induced weightings changes of both systems correlated with the baclofen-induced changes in overall error in the AM session of the VMA task (all $p<0.05$). The change in system weightings did not significantly correlate with the error in the PM sessions (see Table 6.1).

Table 6.1 Correlations between baclofen-induced change in total mean error across VMA conditions and the change weightings of state space model systems. Significant correlations between change in AM session error and the change in system weightings.

	Δ VMA w_f	Δ VMA w_s	Δ VMA Rw
Δ VMA AM FB-on error	r=-0.569, n=15 **p=0.027**	r=-0.707, n=15 **p=0.003**	r=-0.341, n=15 p=0.213
Δ VMA AM FB-off error	r=0.54, n=15 **p=0.038**	r=-0.748, n=15 **p=0.001**	r=0.646, n=15 p**=0.009**
Δ VMA PM FB-on error	r=0.023, n=14 p=0.938	r=0.496, n=15 *p=0.071*	r=-0.259, n=14 p=0.372
Δ VMA PM FB-off error	r=0.392, n=14 p=0.165	r=0.233, n=14 p=0.423	r=0.071, n=14 p=0.811

6.3.2.3 AS analysis and results

Response time (RT) was calculated as the time from the onset of the visual cue to a key press. In line with the pre-processing on the SRTT RTs of incorrect key presses and those outside 2.7 SDs of the mean for the block were excluded from the analysis. The mean RT for the Select and

Execute blocks was calculated, as was the difference between these (mean Select RT- mean Execute RT), a measure of 'action selection time'. Participants who showed an accuracy of below 70% in the Execute blocks, suggesting they applied the Select rule to the Execute blocks, were excluded. This resulted in data from both sessions being excluded in 1 participant, and a further 4 participants had data for one day excluded.

6.3.2.3.1 No effect of baclofen on reaction time or action selection time

Reaction time from the Execute blocks of the Action Selection task, and the 'action selection time' difference measure were input into separate LMMs with fixed effects of Drug (baclofen, placebo) and Day (1, 2) and random effects of Participant. There were no significant fixed effects on either 'action selection time' (Drug: $F(1,13)<0.001$, $p=0.994$. Day: $F(1,13)=0.934$, $p=0.349$- Figure 6.7) or Execute RT (Drug: $F(1,13)=1.145$, $p=0.303$. Day: $F(1,13)=0.003$, $p=0.958$- Figure 6.7B).

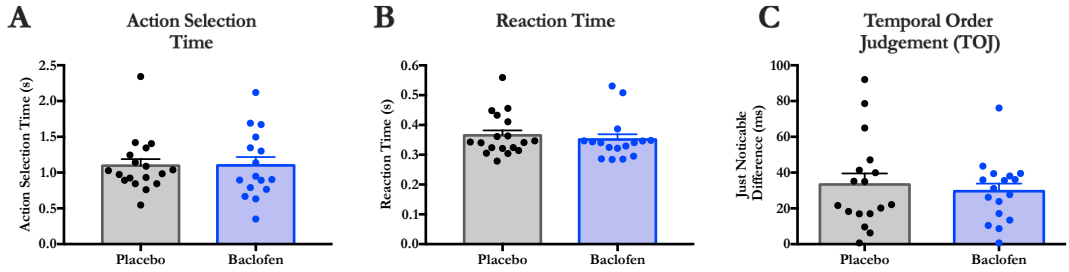


Figure 6.7 A, No change in action selection time (RT Select –RT Execute), measured in the AS task. B, No difference in reaction time, as measured in the Execute blocks of the AS task, with baclofen. C, No change in tactile acuity, as measured in the TOJ task, with baclofen.

6.3.2.4 TOJ analysis and results

The proportion of right-first (i.e. middle finger first) responses for each inter-stimulus interval in the TOJ task was recorded and a logistic function was fit using MATLAB. Poor curve fitting was defined as a goodness-of-fit (GOF) of $R^2<0.75$. For this task, the just noticeable difference (JND) is defined as half of the difference between two interstimulus intervals that result the 'middle finger first' decision in 75% and 25% of the trials, thus better performance at this task is indicated by smaller values for JND.

6.3.2.4.1 No effect of baclofen on tactile acuity

To determine whether there was an effect of baclofen on TOJ task performance a LMM approach was applied with fixed effects of Drug (baclofen, placebo) and Day (1, 2) and a random effect of Participant. This revealed no significant fixed effects of either Drug ($F(1,15)=0.112$, $p=0.743$- Figure 6.7C) or Day ($F(1,15)=0.016$, $p=0.902$).

6.3.3 Other assessments analysis and results

6.3.3.1 VAS mood questionnaire analysis and results

The mean of the two baseline measurements from each day, for each VAS question, of each participant was calculated. In order to reduce the dimensionality of the data, principal component analysis (PCA) was used on these 16 baseline measures to define two principle components that explained the most variance in a participant's mood. The principal component weights for both components for each of the mood measures are presented in Supplementary Table 6.1. The first component was predominantly influenced by the participant's score for feeling alert, strong, clear-headed, well-coordinated, energetic, quick-witted and attentive and the second component was predominantly influenced by the participant's score for feeling strong, tranquil, relaxed and happy (see Supplementary Table 6.1). The first component will further be referred to as the 'alertness component', and the second component as the 'hedonic component'.

The weights for each question, for each component, were applied to the data to calculate the scores for the two components for each participant at each timepoint on each testing day.

6.3.3.1.1 No significantly impact of baclofen on alertness or hedonic mood

There were no baseline differences in either the alertness mood component (paired samples between the sessions t-test: $t(19)=1.483$, $p=0.154$) or the hedonic ($t(19)=0.394$, $p=0.698$) mood

component. The mood measures at the following timepoints 1hr PA and 6hr PA were therefore baselined.

There were no significant differences in the changes in either alertness (paired samples t-test: t(19)=1.700, p=0.105) or hedonic mood (t(19)=0.507, p=0.618) at the 1hr PA timepoint between the drug conditions. There were also no significant differences between drug conditions at the 6hr PA timepoint in either component. (alertness: t(19)=1.706, p=0.104. hedonic: t(19)=0.029, p=0.977- Figure 6.8).

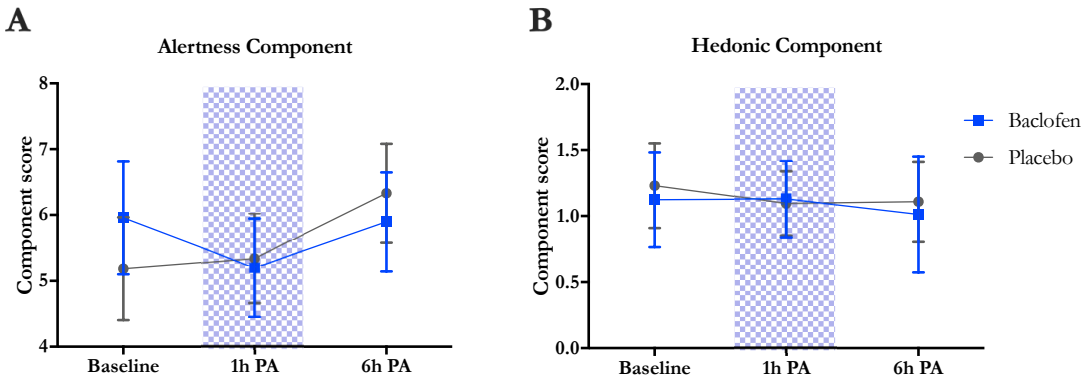


Figure 6.8 No significant change in either alertness of hedonic mood with baclofen, assessed using visual analogue scales of mood.

6.3.3.2 AVLT analysis and results

Performance on this task was quantified by how many words participants could recall on the first 5 repetitions of List A out a total number of 60, AVLT immediate recall. The ratio of the number of words correctly recalled in the delayed recall probe, to the number recalled at final immediate repeat was also calculated, AVLT delayed/immediate. One participant was excluded from analysis as they did not understand the task instructions on the first day. A different version of List A and List B were used on each testing day.

6.3.3.2.1 No significant effect of baclofen on auditory verbal learning

LMM analysis (fixed effects of Drug (baclofen, placebo), Day (1, 2), random effect of Participant) revealed no significant differences between the Drug conditions on overall AVLT

immediate recall (F(1,16.3)=1.894, p=0.187), or the AVLT delayed/immediate ratio (F(1,16.8)=0.016, p=0.899- Figure 6.9A & B). There were also no significant effects of Day (AVLT immediate: F(1,16.3)=0.035, p=0.854. AVLT delayed/immediate ratio: F(1,16.8)=1.912, p=0.185).

6.3.3.3 Digit span analysis and results

Performance at this task, for both the forward and backward sections, was measured by recording the highest number of digits at which the participant correctly recalled at least one sequence.

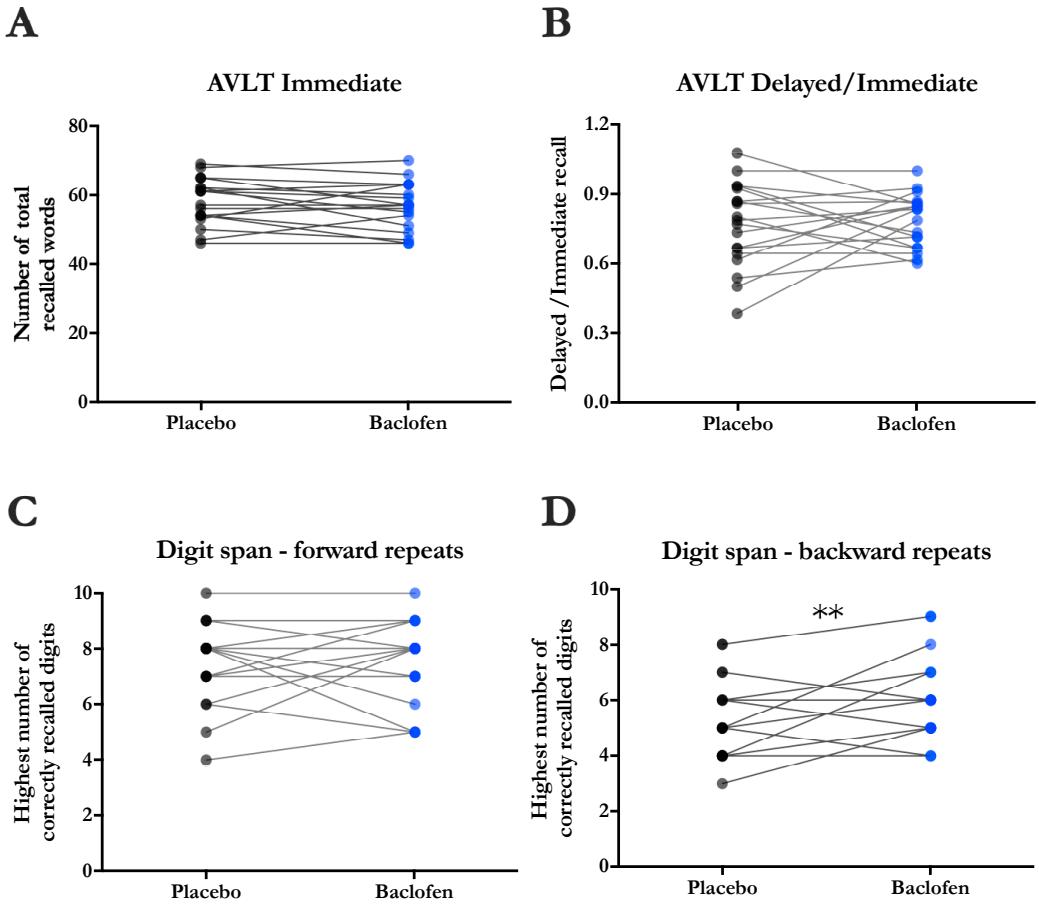


Figure 6.9 A & B, No change in either immediate or delayed recall on the AVLT. C, No change in forward digit span. D, Significant increase in backwards digit span with baclofen

6.3.3.3.2 Baclofen improved backwards digit span, but not forward span

There was no significant effect of Drug ($F(1,18)<0.001$, $p=1.000$- Figure 6.9C) or Day ($F(1,18)=0.095$, $p=0.761$) found on participants' forward digit span using a LMM (fixed effects of Drug (baclofen, placebo), Day (1, 2), random effect of Participant).

However, the same analysis revealed a highly significant effect of Drug on backwards digit span ($F(1,18)=8.691$, $p=0.009$- Figure 6.9D) driven by an increase in number of correctly recalled digits in the baclofen condition. There was no significant effect of Day ($F(1,18)=1.893$, $p=0.186$).

6.3.3.4 Blinding

Blinding indices (BI) were calculated using the method outlined in Bang et al., 2004 (Bang et al., 2004). This calculated an index on a scale of -1 to 1 for each drug condition, where -1 indicates completely opposite guessing, 1 indicated completely perfect guessing and 0 indicates perfect blinding.

Only the data from day 2 was included in this analysis as this was deemed to be the time when participants would have the most information to make a guess about their drug condition. If the participant or the experimenter indicated that they were less than 50 % certain about a response this was coded as a 'don't know' response for the blinding index calculation.

6.3.3.4.1 Participants and experimenters were blind to drug condition

For the baclofen condition on day 2 participants' BI was calculated as -0.3 (CI -0.78 – 0.18), and for the placebo condition the BI was 0.3 (CI -0.18 – 0.78). 95% confidence intervals (CI) for both treatment conditions did cross 0. This indicated that the study was well blinding in both drug treatment arms but that participants had a tendency to guess that they had placebo regardless of treatment condition (see Figure 6.10).

For the experimenters the BI for the baclofen condition was -0.1 (CI -0.53 – 0.33), and for Placebo was -0.2 (CI -0.57 – 0.17). Again for both treatment arms the 95% confidence intervals on the BI measures crossed 0, indicating good blinding.

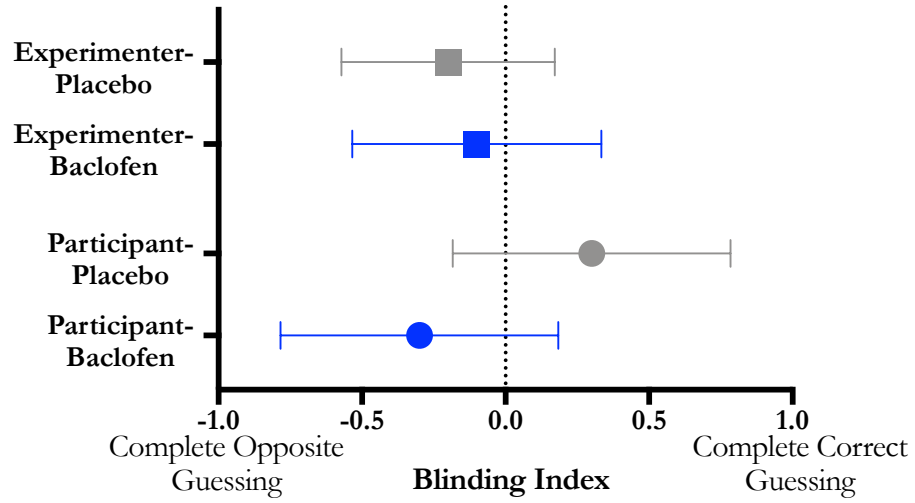


Figure 6.10 Experimenters and participants average blinding index score with 95% confidence intervals. Both experimenters and participants were adequately blinded to both drug conditions

6.3.4 Brain-behaviour correlations

Given the significant effects of baclofen on various aspects of the VMA, we went on to investigate whether we could establish a direct relationship between the degree of change in $GABA_B$ activity due to baclofen, and the effect of baclofen on behaviour on a subject-by-subject basis. We therefore correlated behavioural metrics with change in the ppTMS $LICI_{150ms}$, our measure of $GABA_B$ activity. In order to determine the specificity of our results, we additionally performed correlations between the other TMS measures and behaviour.

Firstly, to determine the degree of baclofen-related change in the TMS measures the average for each of the measures across the peak-plasma concentration timepoints (1hr PA, 2hr PA, 3hr PA) was calculated for both the baclofen and placebo day. This was subtracted from the baseline measure to obtain the average change in measure across the AM testing session. This average change measure was then placebo corrected (baclofen change – placebo change).

Placebo correction was also applied to measures from the two motor learning tasks. From the VMA task the placebo-corrected mean angular error from the FB-on and FB-off blocks of the AM session, and the mean change in angular error for the two block types (block 5 error- block 1 error) was also calculated. Finally the placebo-corrected fast and slow system weights, and the relative system weights were calculated. Full results of these Pearson's correlations can be seen in Table 6.2.

Table 6.2 Brain and behaviour correlations. Significant correlation between the baclofen-induced change in $LICI_{150ms}$ measure and the baclofen-induced change in relative weights of the two systems in the state-space model.

	Δ Excitability	Δ $SICI_{2.5ms}$	Δ $LICI_{150ms}$
Δ VMA AM FB-on error	r=-0.139, n=14 p=0.635	r=-0.194, n=14 p=0.507	r=0.081, n=11 p=0.813
Δ VMA AM FB-off error	r=-0.230, n=14 p=0.428	r=0.025, n=14 p=0.507	r=-0.409, n=11 p=0.212
Δ VMA w_f	r=-0.075, n=14 p=0.798	r=0.162, n=14 p=0.580	r=-0.588, n=11 *p=0.057*
Δ VMA w_s	r=0.234, n=14 p=0.418	r=0.193, n=14 p=0.509	r=0.349, n=11 p=0.293
Δ VMA Rw	r=-0.039, n=14 p=0.893	r=0.056, n=14 p=0.849	r=-0.660, n=11 **p=0.027**
Δ VMA AM FB-on (change B1-B5)	r=-0.155, n=14 p=0.596	r=-0.146, n=14 p=0.618	R=0.546, n=11 *p=0.082*
Δ VMA AM FB-off (change B1-B5)	r=-0.233, n=14 p=0.444	r=-0.170, n=14 p=0.562	r=0.260, n=11 p=0.441

6.3.4.2 Relationship between baclofen-induced $GABA_B$ change and fast system weighting in VMA

The only correlation to reach uncorrected statistical significance was the negative relationship between placebo-corrected $LICI_{150ms}$ and placebo-corrected VMA Rw (r=-0.660, n=11 p=0.027, see Figure 6.11). There were also trends towards a significant relationship between $LICI_{150ms}$ and the fast system weighting parameter (r=-0.588, n=11 p=0.057) and VMA FB-on improvement (r=0.546, n=11 p=0.082).

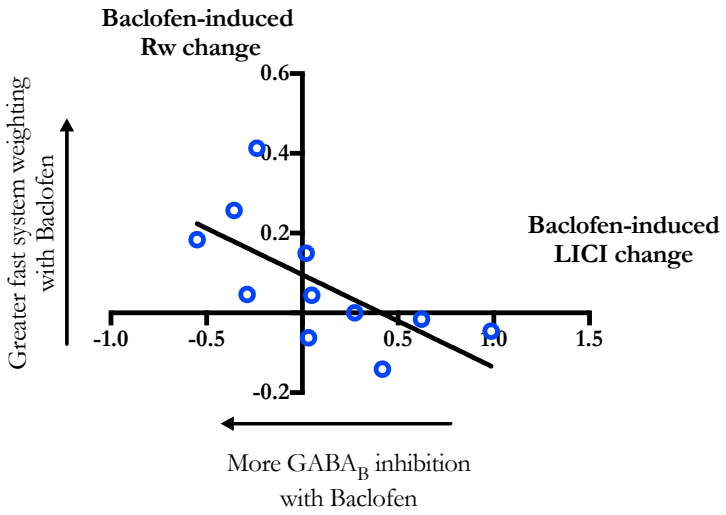


Figure 6.11 Significant correlation (r=-0.660, n=11, p=0.027) between baclofen-induced increases in $GABA_B$ inhibition and baclofen-induced increase in relative weighting towards the fast system.

6.3.5 Motor and 'control measure' correlations

The relationships between motor task and measures designed to assess changes in participants mood or memory were also analysed (see Table 6.3). This was done to assess whether the changes in participants motor skill measures could be related to any baclofen-induced effect on mood.

Table 6.3 No significant correlations between baclofen-induced change in learning measures and the changes in mood or memory measures.

	Δ Alertness component change	Δ Hedonic component change
Δ VMA AM FB-on error	r=0.061, n=15, p=0.828	r=0.160, n=15, p=0.570
Δ VMA AM FB-off error	r=-0.117, n=15, p=0.677	r=0.483, n=15, *p=0.069*
Δ VMA w1	r=-0.105, n=15, p=0.709	r=0.310, n=15, p=0.261
Δ VMA w2	r=-0.207, n=15, p=0.460	r=-0.202, n=15, p=0.471
Δ VMA Rw	r=0.043, n=15, p=0.879	r=0.292, n=15, p=0.292

6.3.5.2 No significant correlations between baclofen-induced mood or memory changes and changes in VMA performance

None of these correlations reached significance ($p<0.05$). There was however a trend towards a correlation between the placebo-corrected hedonic component change from baseline and VMA AM FB-off error ($R=0.483$, $n=15$, $p=0.069$).

6.4 Discussion

This experiment was designed to identify whether a single clinically relevant dose of the $GABA_B$ agonist baclofen impacted motor learning in healthy humans. We found that baclofen caused significant impairments to visuomotor adaptation aftereffect, and also reduced retention of the learnt adaptation. State-space modelling of the data revealed that baclofen increased the weighting of the fast adaptation system, and this change in relative weighting was correlated with the degree that baclofen increased TMS-measured $GABA_B$ mediated inhibition. Baclofen did not result in significant changes to sequence learning ability as measured using the SRTT.

6.4.1 Baclofen resulted in group-level decreases in corticospinal excitability

Here we found that baclofen resulted in a significant lowering of corticospinal excitability change relative to sham during peak-plasma concentration, but this effect was not present after drug washout. We found no overall group-level effects on TMS-measured $GABA_A$ or $GABA_B$ receptor activation.

Previous literature examining the baclofen-induced changes in TMS measures has reliably reported increases in $GABA_B$ mediated inhibition (McDonnell et al., 2007, 2006; Müller-Dahlhaus et al., 2008), with some also showing a decrease in $GABA_A$ mediated inhibition (McDonnell et al., 2006; Ziemann et al., 1996) . These studies used a much larger dose of 50mg

baclofen, and so the lower dose used in this study may be the reason for the lack of overall group effect on $LICI_{150ms}$ measured $GABA_B$ inhibition.

Changes to corticospinal excitability, as found here, have not generally been demonstrated elsewhere in the literature (McDonnell et al., 2007, 2006), however baclofen has been shown to alter paired-associative stimulation induced corticospinal excitability changes (McDonnell et al., 2007). TMS measures at the 2hr PA and 3hr PA timepoints were performed immediately following performance of motor tasks, and so it could be that baclofen impacts the way in which task performance affects corticospinal excitability, rather than influencing resting corticospinal excitability itself. Again, differences in dosage could also play a role; a higher dose may counterintuitively have a lesser effect on corticospinal excitability if it were to cause compensatory or homeostatic processes to become active.

6.4.2 Baclofen caused reductions in adaptation aftereffects and adaptation retention

Participants performed the visuomotor adaptation task to assess learning and retention of a visuomotor transformation. Across conditions participants showed significant learning of the perturbation, as evidenced by reducing angular error across the AM FB-on blocks, and increasing the aftereffect expression (increasing angular error) across the AM FB-off blocks. Likewise the PM session resulted in significant washout of the learnt adaptation across the groups (error reduction in the FB-on blocks). There was a significant reduction in the degree of expression of adaptation aftereffect with baclofen, as measured by a smaller in error in the AM FB-off blocks. Baclofen also resulted in less retention of the adaptation, indicated by lower error in the PM FB-on blocks.

This result was further probed by applying a weighted state-space modelling approach to determine the contribution of different adaptation systems to the observed behaviour (Petitet et al., 2018). A weighted two-system model was first applied to the group level data, then group

learning and retention rate parameters were used to fit weighted models to individual data. This identified that a 'fast adaptation system', which learns quickly and forgets quickly, has an increased weighting with baclofen, while there is no significant change to the 'slow system' weighting. This increased weight of a system that tends to forget quickly when feedback information is removed may explain the pattern of reduced retention in the baclofen condition.

The increase in relative weighting of the fast system with baclofen was also correlated with the degree of baclofen-induced change in $GABA_B$ receptor activation, whereby greater increases in $GABA_B$ activity were related to greater increases in fast system weighting. It has previously been reported that a greater MRS-measured excitation: inhibition ratio within M1 (i.e. more excitation) is related to a greater contribution of the slow-system to behaviour in a prism adaptation paradigm (Petitet et al., 2018). Our finding of greater contribution of fast-system weighting with increased inhibition, while not completely in line with this, complements this finding well. It may be that the tonic levels of excitation and inhibition measured in using MRS, across individuals, influences the weighting of the slow system. Whereas altering phasic $GABA_B$ mediated inhibition within individuals, as was done here, influences fast-system weighting.

As discussed more fully in Chapter 1, adaptation learning processes are thought to involve a network of brain regions including M1 and the cerebellum. While we recoded TMS measures of $GABA_B$ activity within M1 we did not measure any neurophysiological changes within the cerebellum, where there are many baclofen sensitive $GABA_B$ receptors (Wilkin et al., 1981). It is likely that change in activation at these receptors also influences the behavioural changes, but further research is needed on this.

6.4.3 No significant effect of Baclofen on SRTT learning or retention

Participants performed the serial reaction time task as an assessment of motor sequence learning, which has been shown to result in decreases in MRS-measured M1 GABA concentrations (Floyer-Lea et al., 2006; Kolasinski et al., 2018). Across the conditions, participants demonstrated

learning on the task, but baclofen did not appear to modulate this learning, or the retention of the skill. The results showed that participants tended to learn the task better during the second experimental day, though it is not clear whether this is a true effect of day or an effect of differences in learnability of sequences, as sequence 1 was always used on day 1. Regardless of the cause, by using a randomised, crossover design this effect of day should be balanced across the baclofen and placebo conditions. Furthermore, use of a linear mixed model approach to the analysis should result in the variance associated with day differences to be removed from the analysis of drug related differences.

6.4.4 Baclofen did not significantly influence tactile acuity

Previous literature has demonstrated that MRS-measured sensorimotor GABA concentration correlates with perceptual acuity, such that individuals with higher tonic GABA levels have better performance on the TOJ task. This relationship is mediated by the tuning of finger representational activity within the somatosensory cortex: higher GABA levels, relate to narrower tuning of finger representations, which in turn relates to better tactile acuity (Kolasinski et al., 2017). This led us to hypothesise that baclofen would improve TOJ performance by increasing GABA mediated inhibition. However it may be the case that a phasic increase in GABAergic inhibition caused by baclofen administration does influence the tuning that is mediated by tonic GABA levels over longer time scales. Perhaps with sustained drug administration, or if the TOJ task was performed later in the testing day, meaning that GABAergic inhibition would have been increased for a longer time before measurement, these effects may have been demonstrated.

6.4.5 Baclofen improved backwards digit span

Motor tasks such as the SRTT rely, to some extent, on explicit memory processes. For this reason, simple memory tasks, without any motor requirements, were included in this experiment with the objective of ruling out a general effect on memory as an explanation for any motor sequence learning differences. While we did not find any effect of baclofen on verbal memory

performance or on forward digit span, we did find a baclofen-induced improvement in backwards digit span. The backwards digit span task is widely considered a working memory task, because it differs from the other short-term memory tasks in that it not only requires recall but also mental manipulation of the material.

There has been little research into the effect of baclofen on working memory in humans, with only a few case studies reporting baclofen-induced global amnesia (e.g. Grande, Loeser and Samii, 2008; Zeman et al., 2016). Research in rodents also tends to point to memory impairments (e.g. DeSousa et al., 1994; Zarrindast et al., 2001, 2004). Further research, combining baclofen with final grained memory tasks in humans is required if we are to fully understand this potentially interesting finding.

GABAergic signalling is thought play a crucial role in the formation and maintenance of cortical associations underpinning memory in humans (Barron et al., 2016), however it is not well understood how changes in inhibition influence behaviour. One recent study has investigated the relationship between dorsolateral prefrontal cortex (DLPFC) GABA concentrations and working memory across individuals. This study found that higher GABA levels were related to higher memory load capacity on a novel working memory study (Yoon et al., 2016). Increasing GABAergic signalling in the DLPFC may be a mechanism through which baclofen could improve working memory, but this explanation is speculative and requires further testing.

6.4.6 The study was well blinded with no significant changes to mood

To be able to make claims about the impact of baclofen on motor learning specifically it is important to rule out changes to alertness or motivation that could result in learning differences. Tiredness, sleepiness and nausea are frequently experienced side effects of baclofen and were reported in the only other published study investigating the effect of 20mg baclofen on motor learning (Willerslev-Olsen et al., 2011). In this study, with a lower dose of 10mg, participant's alertness and hedonic mood was not significantly influenced by drug condition. No participants

in our study reported any adverse side effects, and assessment of treatment guesses revealed that participants were very poor at identifying when they had taken baclofen. Taken together with the absence of significant correlations between control measures of mood and baclofen-induced motor changes, it seems unlikely that our results could be explained by domain general changes in alertness.

6.4.7 Limitations

A primary limitation in this study is a small sample size: though 20 participants completed both experimental days there were relatively few for whom data from all tasks and TMS measures were included in the final analysis, given the technical difficulty of acquiring these data. As such many of the correlations in particular are performed on only a small number of participants and therefore should be replicated before strong conclusions can be drawn.

In terms of assessing participant's mood across the days, all our mood measures were taken following a period of free time for the participant. So while we observed no changes in the mood measures that we obtained while participants were 'at rest', it cannot be ruled out that there may be some interaction effect of task performance and drug on mood, resulting in mood changes only during 'task'.

Finally, in follow up experiments some aspects of the task design should be refined. In the PM VMA task it would have been beneficial to have a FB-off block come before the first FB-on block. This might have created a more sensitive measure of retention of the adaptation aftereffect.

6.5 Conclusion

A clinically relevant dose of baclofen resulted in reduced retention of learnt visuomotor adaptation. State-space modelling of the behavioural data revealed that this was likely due to an increased reliance on fast system learning. This fast system adapts quickly when presented with

error feedback, but also forgets quickly once this feedback is removed. Furthermore, baclofen-induced increases in the weighting of this fast system correlated with baclofen-induced increases in $GABA_B$ inhibition. This result confirms the importance of GABAergic inhibition in this form of adaptation learning, and may have implications for functional rehabilitation in patients who are taking baclofen.

Chapter 7: Reduction of inhibition in contralateral M1 is important for motor learning, but as are changes across motor network – a discussion

This thesis makes steps towards understanding the how inhibition in the motor network influences human motor plasticity. It did so by primarily by investigating the factors affecting how healthy humans learn and remember new motor skills, a process thought to be reliant on brain plasticity. Several methods were used to manipulate how motor skills were learnt and retained. Initially the effect of explicit task knowledge on retention of motor skill was assessed, and then the remainder of the thesis explored the effect of interventions known to alter brain chemistry. Applying tDCS to the primary motor cortex resulted in changes in inhibitory GABA concentrations both within the stimulated cortex and in the contralateral non-stimulated M1. Similarly, tDCS applied to one M1 was shown to influence learning with the ipsilateral hand, and these changes were related to GABA activity levels in the contralateral M1. Finally, we demonstrated that a single dose of the GABA agonist baclofen caused impairments in performance on a visuomotor adaptation task, and these performance changes were related to the degree of change in $GABA_B$ receptor activation in contralateral M1.

Taken together this work highlights the importance of inhibition reductions in the contralateral M1 in facilitating normal motor learning and adaptation. However this thesis also highlights the importance changes in inhibition and excitation across the whole motor network. This chapter discusses the main findings of each chapter, the overall conclusions of the thesis, and potential avenues for future research.

7.1 Chapter Overview

Chapter 1 of this thesis began with a review of the tasks commonly used to assess motor learning in the lab. Particular focus was given to sequence learning tasks, and visuomotor adaptation tasks, each of which assesses quite different aspects of motor learning and relies on a different network of brain areas. However, despite their differences stimulation induced changes in both sequence learning and visuomotor adaptation have been shown to correlate with stimulation-induced changes in MRS-measured GABA within the contralateral primary motor cortex. The majority of the experimental work in this thesis aimed to explore further the interventions that can be used to change plasticity processes or GABA levels within M1, and the effect these interventions have on motor learning.

Chapter 2 discussed the methods used to record from and stimulate the brain. Brain structure and function were evaluated using a multimodal MRI techniques including structural MRI, DWI and resting state and task fMRI. Neurochemical concentrations were assessed using MRS, and corticospinal excitability and activity at neurotransmitter receptors was measured using TMS. tDCS was used as a brain stimulation technique with the aim of altering brain chemistry. For each method the physical and biological mechanisms were discussed. Particular focus was given to the physiological basis of MRS and TMS measures of neurochemistry, how the measures differ and whether they relate in any way.

Chapter 3 investigated the effect of manipulating participants' explicit awareness of the structure of a commonly used motor sequence learning task, on the retention of their learnt motor skill across a period of wakefulness. Previous work had shown that skill on an implicitly learnt SRTT could show spontaneous improvements across periods of wakefulness, whereas skill on an explicitly learnt SRTT did not. Here we found that if amount of practice on the tasks was controlled for then this difference was eliminated. We also found no evidence for any spontaneous improvement in skill across either learning type, and rather found that participants tended to only partially retain the learnt skill, and then relearn and improve upon re-exposure.

The SRTT was used in all subsequent experiments to assess sequence learning and retention. An explicit version was always used to allow for repeated measures designs.

Chapter 4 examined the effect of applying unilateral or bilateral M1 tDCS on neurochemistry in bilateral M1s. It also explored whether changes in neurochemistry in the non-stimulated M1 could be explained by structural or functional connectivity between the two motor cortices. We found that anodal tDCS resulted in GABA decreases bilaterally; cathodal tDCS caused a decrease in GABA in only the non-stimulated hemisphere; and bilateral stimulation resulted in a decrease in GABA in only the cathode-targeted right M1. Corpus callosum microstructure, as measured using DWI, related to the change in unilateral cathodal tDCS-induced GABA change in the non-stimulated M1. This study demonstrated for the first time that M1 tDCS induces changes in neurochemical concentration outside of the stimulated area and that, in the cathodal condition, this may be related to the structural connectivity between the two M1s.

Chapter 5 built on the findings of the previous chapter, and the theory that altering GABA levels with tDCS may be the causal mechanism behind tDCS-related learning improvements. Here anodal or cathodal tDCS was applied to the M1 ipsilateral to the hand performing a concurrent SRTT. We found that ipsilateral cathodal tDCS did not result in any behavioural or TMS-measured changes. However ipsilateral anodal tDCS resulted in learning impairments on the SRTT, as well as reductions in TMS-measured $GABA_A$ receptor activation and increases in glutamatergic receptor activation. The stimulation-induced decrease in skill with anodal stimulation was correlated with less reduction in $GABA_A$ receptor activation. It may be that modulating ipsilateral processing with anodal stimulation leads to behavioural worsening, but that this can be offset, at least in part, by changes in contralateral GABA activity during learning

Chapter 6 used a pharmacological intervention to directly assess the role of $GABA_B$ receptor activation on a variety motor learning and sensory perception measures using a double-blind, crossover pharmacological intervention experiment. A single clinically-relevant, 10mg dose of the $GABA_B$ agonist baclofen caused a reduction in the retention of a learnt visuomotor adaptation.

State-space modelling of the behavioural data showed that participants on baclofen tended to rely more on a fast adaptation system which learns quickly and forgets quickly, and the degree to which baclofen changed this reliance correlated with the Baclofen-induced changes in TMS-measured $GABA_B$ receptor activation. We did not find a significant effect of baclofen on learning or retention of the SRTT, but we did find the surprising result of a highly significant improvement on the backwards digit span task.

7.2 Main conclusions from chapters

1. There is no significant difference between daytime consolidation on implicit and explicit versions of the SRTT if the amount of practice is controlled for
    - The term consolidation may be inappropriate, as performance changes periods of wakefulness performance did not spontaneously improve

2. tDCS results in neurochemical changes outside of the directly stimulated area
    - Anodal stimulation results in long lasting decreases in GABA concentration in non-stimulated M1 when applied during rest, and transient decreases in $GABA_A$ mediated inhibition when during task
    - It may be that anodal stimulation is influencing the non-stimulated cortex through two competing mechanisms, one an increase in transcallosal inhibition caused by increasing excitability in the stimulated hemisphere, and another resulting in inhibition decreases that is not callosally mediated. Though this suggestion is speculative and requires further research.
    - Cathodal stimulation results in GABA concentration decreases in the non-stimulated hemisphere when applied during rest, but the effects seem to differ when it is applied during task

3. Increasing $GABA_B$ mediated inhibition through administration of 10mg baclofen results in impaired retention of visuomotor adaptation learning.

- This could have implications for patients taking baclofen who are currently undergoing rehabilitation.
- Baclofen may also have positive effects on some aspects of memory.

7.3 Overall comments

The results of these studies, taken in the context of the existing literature, point towards inhibition reductions within the contralateral M1 being crucial for normal motor learning to occur. It has already been shown that contralateral M1 GABA concentrations tend to decrease when motor learning occurs (Floyer-Lea et al., 2006; Kolasinski et al., 2018), and that stimulation induced decreases in GABA concentration correlate with the changes in behaviour (Kim et al., 2014b; O'Shea et al., 2017; Stagg et al., 2011a). Here we again found this same correlation, more GABA decrease relating to better learning, but with stimulation applied to the ipsilateral hemisphere (Chapter 5). However a valid criticism of this work from brain stimulation studies is that a correlation between physiology and behaviour changes does not mean the two are causally linked. This does not in itself provide evidence that change in inhibition is needed for motor learning, it may be that tDCS is influencing a process which in turn is responsible for changing both GABA levels and behaviour.

The work presented in Chapter 6 of this thesis does directly examine how influencing GABA levels changes motor learning. We found that by pharmacologically increasing the activity at $GABA_B$ receptors, i.e. mimicking the effect of a higher GABA concentration, we impaired performance on a visuomotor adaptation task, and these changes were related to changing $GABA_B$ receptor activation in contralateral M1. This provides strong evidence in support of the theory that reducing contralateral M1 inhibition levels is crucial for allowing normal motor learning to occur, perhaps by facilitating plasticity within this region. However as we only took inhibition measurements from contralateral M1, while baclofen was affecting the whole brain, we

cannot rule out that it is another brain area that is ultimately in controls motor learning ability and contralateral M1 inhibition levels.

The work from Chapter 3 and Chapter 4 highlights the fact that the contralateral M1 cannot be considered alone. The two motor cortices are highly connected and both display some functional relevance in unimanual behaviour (Di Pino et al., 2014; Perez and Cohen, 2009; Reis et al., 2009). Further more, interventions designed to target one M1 will affect the other. We therefore must consider changes across the whole motor network if we are to understand how healthy motor learning occurs, and leverage this understanding to enhance learning in health and disease.

7.4 Next steps for future research

7.4.1 Assessing the effects of tDCS across the motor network

It is clear from this work that tDCS does not only affect the region directly underneath the electrode, but rather influences a wide network of brain areas. Likewise, motor learning is not only controlled by M1. It has not previously been possible to use MRS to accurately measure changes in neurochemical concentrations across multiple brain areas simultaneously, however developments in MRS imaging have recently made this possible (Steel et al., 2018).

To enable full understanding of the relationship between, brain physiology and motor learning, and how this is perturbed by tDCS, a number of questions must be tackled. How does the neurochemical milieu across the brain, under normal circumstances, influence motor learning? How does tDCS affect this milieu, and do these effects differ if tDCS is applied during task vs. during rest? How does tDCS-induced neurochemical change in specific areas, and relative change across the network of motor areas, relate to tDCS-induced changes in motor learning? Are the effects the same in patients suffering from motor impairments? By answering these questions we can push forward the development of tDCS or other techniques to enhance motor learning and rehabilitation.

7.4.2 Assessing the effects of pharmacological interventions across the motor network

Further research is also needed to fully understand the results from the baclofen project. Again it would be interesting to assess how neurochemical concentrations across the motor network change with baclofen, using MRS imaging, and how this relates to motor learning changes. The effect of baclofen on MRS-measured GABA is not easy to predict. The filling of $GABA_B$ receptors binding sites with baclofen could result in more unbound GABA molecules, which might be more visible to MRS, resulting in a measurement of higher GABA concentration. Conversely the increase in activation at $GABA_B$ receptors could result in a negative feedback loop causing reductions in GABA production

7.4.3 Does baclofen impair rehabilitation in patients ?

The results presented in Chapter 6 demonstrate that a single dose of baclofen, a drug taken multiple times a day by many thousands of patients, can impair performance on a motor adaptation task. A clear follow-up would be to examine whether there are difference in motor rehabilitation outcomes in patients taking baclofen compared with a matched control group.

7.4.4 Understanding the effect of 10mg baclofen on memory

A final avenue for future research is to further investigate the effect of baclofen on memory. The finding of an improvement in performance of healthy, young participants on the backwards digit span task is potentially exciting. Further work using more fine-grained assessments of working memory, executive functioning and attention processes is needed before we can fully understand the effect that baclofen is having.

Supplementary Table 4.1: Results of RM-ANOVA of baselined neurochemical change with within-subjects factors of Stimulation Condition (anodal, cathodal, bilateral, sham), Hemisphere (right M1, left M1), Neurochemical (glutamate, GABA), and Time (During, Post 1, Post 2, Post 3)

Effect	df	F	*p*	η^2
Stimulation Condition	3	5.235	*.011*	.511
Error (Stimulation Condition)	15			
Hemisphere	1	.006	*.940*	.001
Error (Hemisphere)	5			
Neurotransmitter	1	.890	*.389*	.151
Error (Neurotransmitter)	5			
Time	3	.904	*.462*	.153
Error (Time)	15			
Stimulation Condition*Hemisphere	3	.538	*.663*	.097
Error (Stimulation Condition * Hemisphere)	15			
Stimulation Condition * Neurotransmitter	3	2.101	*.024*	.458
Error (Stimulation Condition * Neurotransmitter)	15			
Hemisphere* Neurotransmitter	1	.002	*.969*	<.001
Error (Hemisphere * Neurotransmitter)	5			
Stimulation Condition * Time	9	1.648	*.130*	.248
Error (Stimulation Condition * Time)	45			
Hemisphere* Time	3	1.120	*.372*	.183
Error (Hemisphere * Time)	15			
Time* Neurotransmitter	3	.047	*.618*	.109
Error (Time * Neurotransmitter)	15			
Stimulation Condition *Hemisphere* Neurotransmitter	3	.550	*.407*	.171
Error (Stimulation Condition * Hemisphere *Neurotransmitter)	15			
Stimulation Condition *Hemisphere* Time	9	.306	*.969*	.058
Error (Stimulation Condition * Hemisphere * Time)	45			
Hemisphere * Neurotransmitter * Time	3	.787	*.520*	.136
Error (Stimulation Condition * Neurotransmitter *time)	15			
Stimulation Condition * Neurotransmitter * Time	9	.885	*.545*	.150
Error (Stimulation Condition * Neurotransmitter * Time)	45			
Stimulation Condition * Neurotransmitter * Hemisphere*Time	9	1.671	*.125*	.250
Error (Stimulation Condition Neurotransmitter * Hemisphere * Time)	45			

Supplementary Table 5.1: Results of full RM ANOVA of TMS results with the within subject's factors of Condition (sham, anodal ipsilateral, cathodal ipsilateral) and Time (baseline, post-tDCS, 5mins post, 15mins post) and Measure (CE, SICI and ICF).

Effect	df	F	*p*	η^2
Stimulation Condition	3	5.235	*.011*	.511
Error (Stimulation Condition)	15			
Hemisphere	1	.006	*.940*	.001
Error (Hemisphere)	5			
Neurotransmitter	1	.890	*.389*	.151
Error (Neurotransmitter)	5			
Time	3	.904	*.462*	.153
Error (Time)	15			
Stimulation Condition*Hemisphere	3	.538	*.663*	.097
Error (Stimulation Condition * Hemisphere)	15			
Stimulation Condition * Neurotransmitter	3	2.101	*.024*	.458
Error (Stimulation Condition * Neurotransmitter)	15			
Hemisphere* Neurotransmitter	1	.002	*.969*	<.001
Error (Hemisphere * Neurotransmitter)	5			
Stimulation Condition * Time	9	1.648	*.130*	.248
Error (Stimulation Condition * Time)	45			
Hemisphere* Time	3	1.120	*.372*	.183
Error (Hemisphere * Time)	15			
Time* Neurotransmitter	3	.047	*.618*	.109
Error (Time * Neurotransmitter)	15			
Stimulation Condition *Hemisphere* Neurotransmitter	3	.550	*.407*	.171
Error (Stimulation Condition * Hemisphere *neurochemical)	15			
Stimulation Condition *Hemisphere* Time	9	.306	*.969*	.058
Error (Stimulation Condition * Hemisphere * Time)	45			
Hemisphere * Neurotransmitter * Time	3	.787	*.520*	.136
Error (Stimulation Condition * Neurotransmitter *time)	15			
Stimulation Condition * Neurotransmitter * Time	9	.885	*.545*	.150
Error (Stimulation Condition * Neurotransmitter * Time)	45			
Stimulation Condition * Neurotransmitter * Hemisphere*Time	9	1.671	*.125*	.250
Error (Stimulation Condition Neurotransmitter * Hemisphere * Time)	45			

Supplementary Table 6.2: Weightings of the individual mood measures for each of the two principal components.

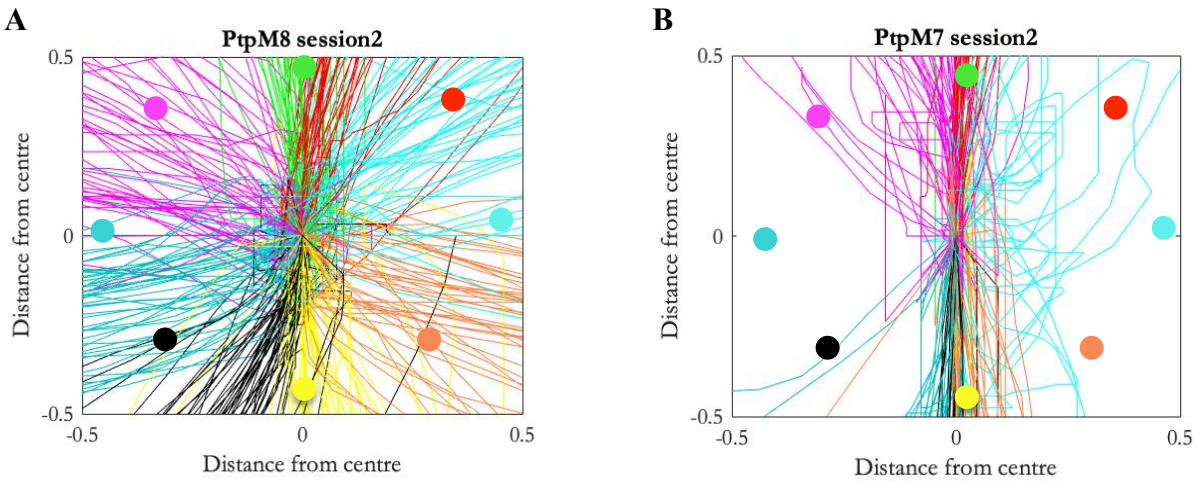
	Alertness Component	Hedonic Component
Alert	0.318	-0.113
Calm	0.045	0.064
Strong	0.265	0.253
Clear Headed	0.341	-0.150
Well-coordinated	0.324	-0.101
Energetic	0.303	-0.078
Contented	0.124	0.250
Tranquil	0.158	0.459
Quick-witted	0.350	-0.206
Relaxed	0.159	0.646
Attentive	0.333	-0.176
Proficient	0.262	-0.104
Happy	0.195	0.304
Friendly	0.189	-0.095
Interested	0.175	-0.021
Sociable	0.206	-0.059

Supplementary Table 6.1: Showing the measures for each participant that have been included in analysis. White indicates complete and included data sets. Grey indicates a completed data set on one testing day. Black indicates no data for this participant

	TMS			**TOJ**	**AS**	**SRTT**		**VMA**	
	CE	SICI	LICI			AM	PM	AM	PM
F1									
F2									
F3									
F4									
F5									
F6									
F7									
F8									
F9									
F10									
F11									
M1									
M2									
M3									
M4									
M5									
M6									
M7									
M8									
M9									

Supplementary Table 6.3: Results of RM ANOVA on TMS results with 2 levels of Drug (baclofen, placebo), 3 levels of TMS measure (Corticospinal Excitability, $SICI_{2.5ms}$, $LICI_{150ms}$) and 5 levels of Time (Baseline, 1hr PA, 2hr PA, 3hr PA, 6hr PA).

Effect	df	F	*p*	η^2
Drug	2	2.980	*.106*	.176
Error (Drug)	14			
TMS Measure	2	.830	*.446*	.056
Error (TMS Measure)	38			
Time	4	1.927	*.119*	.121
Error (Time)	56			
Drug * TMS Measure	2	.142	*.868*	.010
Error (Drug * TMS Measure)	28			
Drug * Time	4	.735	*.572*	.050
Error (Drug * Time)	56			
Time* TMS Measure	8	3.079	*.004*	.180
Error (Time * TMS Measure)	112			
Drug * TMS Measure * Time	8	0.775	*.626*	.052
Error (Drug * TMS Measure * Time)	112			



Supplementary Figure 6.1: A, example of a good data set showing the trajectories to each of the target locations in the AM FB-off section. B, example of a bad data set where data has failed to record in one dimension.

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