Investigations of Learning Induced Changes in Corticospinal Excitability in Healthy Human.

by

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Abstract

In the healthy human the primary driver for neuroplastic change is experience, in the form of learning and memory. Visuomotor learning has been shown as an effective experimental paradigm for inducing neuroplasticity, which is expressed as changes in corticospinal excitability (CSE). The present thesis uses the transcranial magnetic stimulation (TMS) stimulus response (SR) curve to assess learning induced changes in CSE. The first study presents a means of rapidly acquiring the TMS SR curve. Study two compares learning induced modulation of CSE between proximal and distal muscles. Study three assesses the influence of hand preference on learning induced changes in CSE. The results of study one indicate that it is possible to acquire the TMS SR curve in under two minutes. Studies two and three suggest distal muscles have a greater capacity for CSE modulation and this modulation of CSE is invariant to hand preference. Importantly, there is considerable variability in learning induced modulation of CSE. This thesis presents a novel paradigm for rapidly acquiring the TMS SR curve. It also highlights an important point for future studies of learning induced neuroplasticity – there is considerable variability in the neuroplastic response to a single session of visuomotor learning.

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List of Abbreviations

Transcranial Magnetic Stimulation	TMS
Repetitive Transcranial Magnetic Stimulation	rTMS
Motor Evoked Potential	MEP
Corticospinal Excitability	CSE
First Dorsal Interosseus	FDI
Abductor Digiti Minimi	ADM
Biceps Brachii	BB
Abductor Policis Brevis	APB
Stimulus Response	SR
Interstimulus Interval	ISI
Root Mean Square	RMS
Electromyography	EMG
Non-Invasive Brain Stimulation	NIBS
Paired Associative Stimulation	PAS
Theta Burst Transcranial Magnetic Stimulation	TBS
Maximal Voluntary Contraction	MVC
Maximal Compound Muscle Action Potential	M_{max}
One Way Repeated Measures ANOVA	1w-RMANOVA
Two Way Repeated Measures ANOVA	2w-RMANOVA
Central Nervous System	CNS
Maximal Motor Evoked Potential	MaxMEP
Long Term Potentiation	LTP
Long Term Depression	LTD
N-Methyl-D-aspartic acid	NMDA
Bienenstock Cooper Munrow	BCM
Hoffmann Reflex	H-Reflex
Serial Reaction Time Task	SSRT
Functional Magnetic Resonance Imaging	fMRI
Conditioned Stimulus	CS
Unconditioned Stimulus	UCS
Conditioned Response	CR
Transcranial Direct Current Stimulation	TDCS
Blood Oxygen Level Dependent	BOLD
Transcranial Electrical Stimulation	TES
Resting Motor Threshold	rMT
Active Motor Threshold	aMT
Electroencephalogram	EEG
Magentoencephalogram	MEG
Maximal Stimulator Output	MSO
Intraclass Correlation Coefficient	ICC
Area Under Curve	AuC
Gamma-Aminobutyric Acid	GABA
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor	AMPA
Standard Deviation	S.D.
Brain Derived Neurotrophic Factor	BDNF

Chapter I – Preamble

"Every man can, if he so desires, become the sculptor of his own brain."

- Santiago Ramón y Cajal 1923

Chapter I – Preamble

The purpose of this chapter is to provide the reader with information about the organisation of this PhD thesis, the rationale for the research and the goals for the PhD projects.

Organisation of This PhD Thesis

This PhD thesis contains seven chapters and one appendix. This thesis starts by presenting the rationale for the research alongside the aims, objectives and research questions addressed here. Following there is an introductory chapter which introduces the reader to key concepts and terms used throughout the thesis. Chapter III presents the reader with summary information for the general methods used to address the research questions. Chapters IV, V and VI provide detailed reports for each of the experiments that compose this thesis. Chapter VII provides a general discussion of research findings, including limitations of the work, considerations and future directions.

Rationale for Research

Arguably one of the most interesting features of the central nervous system (CNS) is the capacity to change it structure and function in response to intrinsic and extrinsic cues. Changes in the CNS following prolonged training on activities such as volley ball (Tyè *et al.*, 2005), ballet dancing (Nielsen *et al.*, 1993) and playing a musical instrument (Meister *et al.*, 2005) are well documented. Neuroplasticity induced by learning involves functional changes such as modulation of synaptic efficacy in the corticospinal tract and the unmasking of functionally silent neural pathways or structural changes including the generation of new synapses and neurones (Taub *et al.*, 2002). This ability

of the CNS to adapt is termed 'neuroplasticity,' and plasticity underlies our ability to adapt existing and/ or acquire novel motor skills as well as the functional recovery seen after injury or illnesses like stroke (Thickbroom *et al.*, 2004; Traversa *et al.*, 1997). However, there is a dark side to neuroplasticity, it can be maladaptive; excessive neuroplasticity is seen in many neurological conditions such as chronic pain (Sessle, 2000) and dystonias (Classen, 2003). Given that neuroplasticity underlies our ability to learn and recover after illness there is great interest in the factors which modulate the brains neuroplastic responses and whether these can be harnessed to maximise therapeutic effects.

Recent advances in neurophysiology have given us non-invasive means of investigating neuroplastic changes (Nitsche & Paulus, 2000; Stefan *et al.*, 2000; Barker *et al.*, 1985; Huang *et al.*, 2005). Studies which use these techniques to investigate learning induce neuroplastic changes typically report effects at the group level; comparing changes in two or more distinct conditions. There has been little systematic study of the individual differences in the neuroplastic changes induced by learning and the factors which may affect these individual differences. Furthermore, some methods for assessing neuroplastic changes, specifically the transcranial magnetic stimulation (TMS) stimulus response (SR) curve, need to be improved if they are to be clinically useful. This thesis presents a method for rapidly acquiring SR curves in less than two minutes and two more experiments, the first compares the neuroplastic response to visuomotor learning in proximal and distal muscles and the second examines the influence of hand preference on learning induced changes in corticospinal excitability (CSE). Combined these studies present valuable information which can be used to inform future studies

through aiding power calculations and informing investigators of the efficacy and reliability of visuomotor tracking task.

PhD Project Goals

There is a large focus in the TMS literature toward the study of the differences in response of the CNS to neuroplasticity inducing protocols between patient and healthy populations. However, one of the most striking aspects of the human CNS response to NIBS is the degree to which individuals differ. This has gone largely understudied, especially for neuroplasticity induced via motor learning. The overall goal of this thesis was to provide a novel method for rapidly acquiring the TMS SR curve and to apply this to examine factors which affect learning induced neuroplasticity.

A limitation of the traditional method of SR curve acquisition is the time required to collect the necessary data. SR curves are used to measure the state of CSE which is known to fluctuate within the time required to acquire a curve, typically in excess of ten minutes (Barsi *et al.*, 2008; Malcolm *et al.*, 2006; Pitcher *et al.*, 2003). While the source of these fluctuations in CSE is unknown ,it is believed not to arise from autonomic (Filippi *et al.*, 2000), cardiac (Ellaway *et al.*, 1998), or respiratory (Ellaway *et al.*, 1998) signals. However, the amplitude of MEPs used to construct SR curves is known to be mediated by attention (Rosenkranz & Rothwell, 2006; Rosenkranz & Rothwell, 2004) and drowsiness (Andersen *et al.*, 2008). Therefore, in order for SR curves to provide an accurate reflection of CSE, SR curves should be acquired in the shortest possible time. To that end I set out to investigate the feasibility of reducing SR curve acquisition time

by determining the optimal inter-stimulus interval (ISI) and minimum number of stimuli required to construct a representative SR curve.

Since their introduction in the mid 1990's, SR curves have become increasingly used in studies of motor learning. Typically these studies examine changes in CSE for the first dorsal interosseous (FDI) (McAllister *et al.*, 2011; Cirillo *et al.*, 2011). There has been little study of whether proximal muscles undergo similar neuroplastic changes following visuomotor learning (Lundbye-Jensen *et al.*, 2005) and no within subject comparison between the two muscles. Comparing learning induced neuroplasticity in the proximal and distal representations of the upper limb might allow us to speculate about the contributions of the different regions of the motor cortex involved during whole limb movements such as reach to grasp. To that end I set out to compare learning induced neuroplasticity in first dorsal interosseous (FDI) and biceps brachii (BB).

Similarly to the influence of muscle choice, the influence of hand preference has been understudied specifically in the context of skill learning. The majority of studies report no significant differences in learning induced neuroplastic changes between hands (Garry *et al.*, 2004; Gallasch *et al.*, 2009). That said, Cirillo *et al.*, (2010) found a 21% greater facilitation of motor evoked potentials (MEP) following ballistic motor learning in the non-dominant hand despite a 40% greater increase in performance for the dominant hand. All three of these studies used a ballistic motor learning task. I set out to investigate differences in neuroplastic changes between hands using a visuomotor learning task previously shown to modulate CSE (Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005; Cirillo *et al.*, 2011). The aim of this study was to investigate the influence of

hand preference on learning induced modulation of CSE. Additionally, based on observations from the previous study, I sought to determine the percentage of people who exhibit increased CSE after a single session of visuomotor learning.

Chapter II – Introduction

Chapter II – Introduction

The following chapter provides an overview of neuroplasticity and its mechanisms, followed by a discussion of paradigms used to induce neuroplasticity naturally via motor learning, and artificially by non-invasive brain stimulation (NIBS) techniques.

What is Neuroplasticity?

The CNS has a wide variety of functions including receiving sensory input, storing memories, managing motor plans, generating higher conscious thoughts and controlling posture and balance (Bennett *et al.,* 2007). One key property of the CNS which enables it to achieve such a diverse range of functions is its ability to adapt or change i.e. it is plastic (McGaugh *et al.,* 1995). The structure and function of the CNS are able to adapt to the task at hand, allowing the acquisition of a new skills. This ability is termed 'neuroplasticity,' and can be defined as "the ability of the CNS to respond to intrinsic and extrinsic stimuli by reorganising its structure, function and connections," (Cramer *et al.,* 2011). Neuroplasticity encompasses molecular changes at the level of the synapse modifying their functional strength to changes at the systemic level altering structure of the nervous system (Bennett *et al.,* 2007).

With regards to the nervous system the term 'plasticity' was introduced by noted neuroscientist William James (1890) in The Principles of Psychology, here James used the term in reference to the ease of modifying human behaviour;

> *"Plasticity, then, in the widest sense of the word means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all*

> *at once... Organic matter, especially nervous tissue, seems endowed with a very ordinary degree of plasticity of this sort: so that without hesitation lay down our first proposition the following, that the phenomena of habit living beings are due to the plasticity of the organic materials of which their bodies are composed."*
>
> - (James, 1890).

Four years later the plastic nature of the CNS was acknowledged by arguably one of the seminal neuroscientists of the past century, Nobel Prize winning Santiago Ramón y Cajal (1852 – 1934). Here Cajal suggested an explanation in his Croonian Lecture; La Fine Structure des Centres Nervuex of 1894:

> *Brain gymnastics are not likely to improve the organization of the brain by increasing the number of cells because, as we know, nervous elements have, since their embryological stages, lost their ability to proliferate; but we can admit as very plausible that mental exercise induces in the brain regions that are most active a greater development of the protoplasmic mechanisms and of the system of (nervous) collaterals. In this way, connections already created between certain groups of cells would be significantly strengthened through the multiplication of terminal twigs of the protoplasmic appendices and of the nervous collaterals; but also, completely new intercellular connections could be established by the neoformation of collaterals and protoplasmic expansions.*
>
> - (Ramòn Y Cajal, 1894 - English translation).

Cajal later extended the notion of plasticity providing a neural substrate (Ramòn y Cajal, S. 1904 in Pascual - Leone *et al.*, 2005). When examining the acquisition of new skills Cajal wrote;

> *The labor of a pianist [. . .] is inaccessible for the uneducated man as the acquisition of new skill requires many years of mental and physical practice. In order to fully understand this complex phenomenon it becomes necessary to admit, in addition to the reinforcement of pre-established organic pathways, the formation of new pathways through ramification and progressive growth of the dendritic arborization and the nervous terminals.*
>
> - (Ramòn Y Cajal, 1904 - English translation).

Cajal's overriding message can be surmised as neuroplasticity is expressed as new connections between synapses and/ or changes in the efficacy of transmission across existing synapses. This view was overshadowed by Cajal's later opinion, 'everything may die, nothing may be regenerated' (Ramòn Y Cajal, 1928). This view of an inflexible, unchangeable nervous system prevailed for most of the 20th century only being re-examined as evidence of the capacity for CNS plasticity began to grow in the mid 20th century. This thesis will first discuss neuroplasticity.

In 1949, Donald Hebb introduced an important model for how memories are encoded at the level of the synapse (Hebb, 1949). Hebb suggested that repetitive activation of a presynaptic neurone simultaneously with a post synaptic neurone leads to increase in the strength of the synaptic connections between both neurones. This led to notion of

Hebbian plasticity, surmised as 'nerves that fire together, wire together,' and has become the cornerstone of neuroplasticity.

Lømo provided the biological substrate for Hebbs theory (Lømo, 1966); Lømo reported that tetanic stimulation results in a frequency dependent potentiation of hippocampal neurones of anaesthetised rabbits, the results of which led to the theory of long term potentiation (LTP) (Lømo, 1966). LTP came to prominence when further studies reported that synaptic potentiation following tetanic stimulation outlasts the period of stimulation (Bliss & Lømo, 1973; Bliss & Gardner-Medwin, 1973). LTP can be defined as a long-lasting, in excess of one hour, increase in synaptic strength following a short period of high frequency repetitive stimulation (50 – 200 Hz) (Bailey *et al.*, 1996). These changes in synaptic strength arise from increased neurotransmitter release and increased receptor expression.

The inverse phenomenon, a reduction in synaptic strength also exists and is termed 'long-term depression' (LTD). LTD is induced following short periods of low frequency repetitive stimulation and similarly to LTP depends on NMDA receptors (Lovinger, 2010; Kullmann & Lamsa, 2011). Activity dependent LTD in hippocampal CA1 slices *in vivo* was first demonstrated by Lynch *et al.*, (1977) and in the dentate gyrus *in vivo* by Levy and Steward (1979).

Early studies noted two distinct forms of LTD each with a distinct induction mechanism (Escobar & Derrick, 2007). Homosynaptic LTD is used in reference to LTD that follows synaptic activity and is induced by repetitive low frequency stimulation (Bear,

1999). Homosyanptic LTD has distinct parallels to LTP; it is input specific, depends upon NMDA receptors and it is associative (Mulkey *et al.*, 1993; Christie & Abraham, 1992; Debanne & Thompson, 1996). Additionally, LTD can be observed when synaptic activity or LTP occurs at adjacent synapses (Hulme *et al.*, 2013). This form of LTD is known as heterosynaptic LTD and occurs at synapses which are not active. Heterosynaptic LTD is most evident in the perforant projections to the dentate, here LTP induction in one set of afferents, such as the medial perforant pathway, induces heterosynaptic LTD in a separate set of afferents, the lateral perforant pathway (Doyere *et al.*, 1997; Abraham *et al.*, 1994).

Bliss and Cooke (2011) put forward five key features of LTP/LTD which make them appealing biological substrates for Hebbian plasticity;

I. **Rapid induction**: LTP/LTD can be induced rapidly by one or more brief tetanic stimuli.

II. **Input specificity**: LTP/LTD once induced occurs only in synapses which have been stimulated.

III. **Associativity**: Weak inputs can induce LTP/LTD in the presence of strong inputs depending on precise timing.

IV. **Cooperativity**: Multiple weak inputs can summate in space and/ or time to induce LTP/LTD.

V. **Long-lasting**: The effects are immediate and long-lasting.

These characteristics of LTP/LTD dictate the function of neural networks and computational models based on Hebbian principles and define the benchmark against which alternative models of neuroplasticity are assessed.

There are variants of synaptic plasticity which do not conform to the Hebbian form of LTP/ LTD. An example of non-classical LTP/ LTD is the Marr-Albus model which describes synaptic plasticity between the parallel fibres and purkinje cells in the cerebellum (Marr, 1969; Albus, 1971). Here, the synapse undergoes LTD, as opposed to LTP, during simultaneous firings of both neurones (Ito, 2006; Ito, 1982). In addition, a non-NMDA receptor dependent variant of LTP has been demonstrated in the visual cortex (Petrozzino & Connor, 1994; Grover & Yan, 1999).

Metaplasticity

The Hebbian nature of synaptic plasticity has a notable limitation - it is unstable. This instability was first recognised by Bienenstock *et al.* (1982) who modelled the orientation and selectivity of neurones in the visual cortex and noted that neurones in a purely Hebbian model would differentiate into synapses saturated by LTP or LTD. Bienenstock *et al.* proposed a theory, now known as BCM theory, where a history of high activity in the post-synaptic neurone makes LTP induction more difficult and LTD induction easier with the inverse for a history of minimal activity in the post-synaptic neurone also true (Bienenstock *et al.*, 1982; Bear, 1995). BCM theory is illustrated below in figure 1.

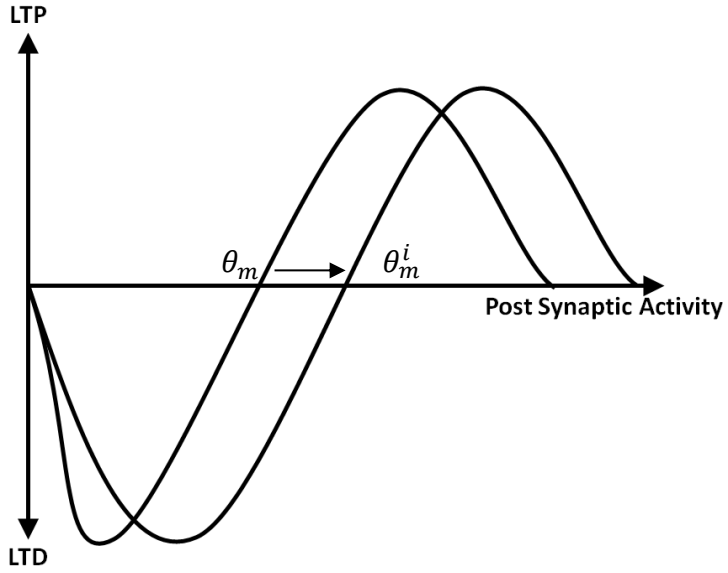

Figure 1. Bienenstock-Cooper-Munrow theory. The y-axis represents changes in synaptic strength and the x-axis represents either post-synaptic activity, frequency of stimulation or post-synaptic intracellular calcium concentration. ϑ_m represents the crossover point between inducing LTP or LTD, and shifts as a function of the history of previous activity in the post-synaptic neurone (adapted from Bienenstock *et al.*, 1982).

The threshold for synaptic plasticity 'θ_m' shifts leftward along the x-axis after a period of low synaptic activity and to the right after periods of high synaptic activity. BCM theory places a negative feedback loop to changes in synaptic gain preventing excessive LTP leading to hyperexcitability (Stanton, 1996). BCM theory, with its sliding threshold for LTP/ LTD induction is commonly known as 'metaplasticity' - the plasticity of synaptic plasticity (Abraham & Bear, 1996). There is experimental evidence for BCM theory in the visual cortex; deafferentation changes NMDA receptor composition in favour of LTD induction (Philpot *et al.*, 2003; Philpot *et al.*, 2001) while experience dependent plasticity alters NMDA receptors in favour LTP induction (Kirkwood *et al.*, 1996). There is indirect experimental evidence for BCM theory in the hippocampus and in the primary motor cortex (Harms *et al.*, 2008; Whitlock *et al.*, 2006; Rioult-Pedotti *et al.*, 2000; Rioult-Pedotti *et al.*, 1998). It is notable that prior

experience dependent plasticity in the primary motor cortex occludes subsequent LTP induction.

According to BCM theory, LTP induction shifts ϑ^m to right making it harder to further induce LTP and, as a result reduces the bi-directionality of synaptic plasticity. A homeostatic feedback loop is required to return ϑ^m to baseline. This feedback loop is termed 'homeostatic plasticity'. In homeostatic plasticity the efficacy of synaptic transmission scales up after periods of low synaptic activity and down after periods of high activity (Turrigiano, 1999; Leslie *et al.*, 2001). This scaling in the efficacy of synaptic transmission is related to modifications of the glutamate receptors on the post synaptic neurone (Watt *et al.*, 2000; Wierenga *et al.*, 2005) and post synaptic ion channels (Misonou *et al.*, 2004). It is important to note that homeostatic plasticity has a timeframe in the order of hours and as such is unlikely to play a role in the early phases of motor learning and plasticity (Ziemann *et al.*, 2004).

The majority of studies examining synaptic plasticity have been performed using *in vitro* hippocampal preparation. However, recent work using TMS in the conscious human has shown that it is possible to modulate synaptic plasticity for periods which outlast the stimulation (Chen *et al.*, 1997; Nitsche & Paulus, 2000; Stefan *et al.*, 2000; Huang *et al.*, 2005). In a recent study, Huang *et al.*, (2010) used theta burst stimulation (TBS), a patterned form of repetitive TMS to study reversal of plastic like effects of the stimulation in the conscious human. Huang *et al.*, report it is possible to reverse LTP using an inhibitory form of TBS as well as to reverse LTD using an excitatory form of TBS. The efficacy of the second, depotentiating/ repotentiating, intervention was time

dependent, the intervention was only effective when given one minute after the initial intervention, and there was no effect when the second intervention was given ten minutes after the first. Additionally, the second depotentiating/ repotentiating intervention was ineffective in modulating synaptic plasticity of its own accord.

Structural Plasticity

Distinct regions of the brain such as the primary motor cortex are capable of considerable structural plasticity (Nudo & Milliken, 1996; Pascual - Leone *et al.*, 1995; Nudo *et al.*, 1996). Structural plasticity occurs through three mechanisms, the unmasking of functionally silent synapses (Isaac *et al.*, 1995; Ward *et al.*, 2006; Atwood & Wojtowicz, 1999) synaptogenesis (Geinisman *et al.*, 1991) and neurogenesis (Altman & Das, 1967; Eriksson *et al.*, 1998; Bernier *et al.*, 2002).

Much of the early focus around functionally silent synapses arose from a concerted effort to understand the early changes that occur during LTP induction. Functionally silent synapses are synapses where glutamate release fails to induce an observable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated depolarisation of the post synaptic neurone, although an NMDA receptor mediated depolarisation still possible (Kullmann, 1994). Following LTP induction, AMPA receptors detect greater quantities of neurotransmitter during synaptic transmission (Kullmann, 1994). The changes in receptors which occur on the post synaptic neurone during LTP induction could be explained by the insertion of AMPA receptors that under basal conditions would remain silent (Kullmann, 1994; Isaac *et al.*, 1995; Laio *et al.*, 1995).

Synaptogenesis is an increase in the number of synapses and/or dendritic spines per neurone. Xu *et al.* (2009) were able to show synaptogenesis after a single session of training on a food retrieval task in the rat model. The authors report a strong correlation between the number of dendritic spines formed and the number of successful trials in the first training session. Synaptogenesis was specific to the motor learning task, activity alone did not result in any changes. Furthermore, synaptogenesis was specific to the first exposure to the learning task, the rats showed no increase upon re-exposure after a period of non-training. However, synaptogenesis could be observed if a second novel task was introduced. Changes in synaptogenesis and synaptic morphology have further been linked with LTP induction (Bourne & Harris, 2011; Bruel-Jungerman *et al.*, 2007; Muller *et al.*, 2002) providing a link back to the principles of Hebbian plasticity.

Santiago Ramón y Cajal's 1928 proposition that 'everything may die, nothing may be regenerated,' remained a founding principle of clinical neuroscience until the mid 1990's. It has been suggested there were four key reasons that neuroscientists were resistant to the idea of neurogenesis prior to the 1990's; the progressive worsening of symptoms in neurodegenerative conditions, an adaptive system could disrupt established behaviours and the ability to recall memories, additionally the scientific methods of the time were inadequate (Colucci-D'Amato et al., 2006). Neurogenesis is defined as the process of generating functional neurones from precursor cells (Ming & Song, 2011). Magavi et al., (2000) provided proof of concept for neurogenesis in the adult mammalian hippocampus. There is limited evidence for neurogenesis in the

neocortex (Bonfanti & Peretto, 2011), striatum (Pytte et al., 2012) and amygdala (Canales, 2013). Neurogenesis has also been demonstrated in corticospinal neurones in the rat model (Chen et al., 2004). Importantly, the evidence for the role of neurogenesis in learning and memory in man remains ambiguous at best (Castilla-Ortega et al., 2011; Koehl & Abrous, 2011).

The concept that our higher cognitive functions are attributed to specific regions of the brain can be traced back into antiquity however, the theory was not widely accepted until the 19th century. Emanuel Swedenborg is widely credited as the first individual to write about cortical localisation (Ramstrom, 1910; Norrsell, 2007; Akert & Hammond, 1962; Gibson, 1967; Toksvig, 1948). In 1740 Swedenborg localised the motor centres in a region incorporating the precentral and some of the post central gyrus (Taylor, 2003). Although Paul Broca is often credited with localising the speech area to the anterior frontal lobes he was not the first, Jean-Baptiste Bouillaud had previously used clinical observations and post-mortem findings to argue that speech functions were located in the anterior frontal lobes. In 1825 Bouillaud wrote:

> *"In the brain there are several special organs… In particular, the movements of speech are regulated by a special cerebral centre, distinct and independent. Loss of speech depends sometimes on loss of memory for words, sometimes of want of the muscular movements of which speech is composed…The nerves animating the muscles, which combine in the production of speech, arise from the anterior lobes or at any rate possess the necessary communications with them."*
>
> - (Bouillaud S, 1825 in Head, 1963)

Importantly, Bouillaud did not identify that the left hemisphere is dominant for speech in most people; right-hemispheric lesions rarely lead to speech disorders . Paul Broca, openly credited as champion of cortical localisation, emphasised the fact that the speech areas are located in the anterior lobes but he also suggested that anterior lobes may be response for other functions including; judgement, reflection and abstraction. Broca presented his seminal paper on cortical localisation of speech in 1861 (Broca, 1861).

Broca's localisation of the speech area to the anterior lobes is considered one of the most important papers in the history of cortical localisation; its laboratory counterpart, the discovery of the motor cortex, has to be of equal value. Here Fristch and Hitzig (1870) electrical stimulated the dog cortex and found distinctive cortical regions which when stimulated triggered movements in muscles in contralateral limbs. In further experiments, Fritsch and Hitzig used the scalpel hand to ablate the area triggering forepaw movement, the lesions did not abolish voluntary movements in the forepaw however motor control of the forepaw was impaired (Finger, 2010).

In 1937 Penfield and Boldrey presented the concept of the sensory and motor homunculi (Penfield & Boldrey, 1937). The homunculi are illustrations of the cortical areas given over to processing sensory input and controlling motor functions of the human body, i.e. part of the cortex is responsible for abducting the index finger or receiving sensory input from the palm of the hand. These homunculi are not fixed, they are capable of considerable neuroplastic change via the unmasking of functionally silent synapses, synaptogenesis and neurogenesis in response to intrinsic and extrinsic cues.

Each digiti has a distinct representation on the sensory and motor homunculi however, the boundaries are not fixed. Dystonia can be defined as "involuntary sustained muscle contractions producing twisting and squeezing movements and abnormal postures," (Evatt *et al.*, 2011) and is suggested to be a disorder of neuroplasticity. Quartarone *et al.*, (2003) investigated the plastic response to paired associative stimulation (PAS) in participants with writers cramp, a form of task specific focal hand dystonia, compared with healthy controls. The volunteers with writers cramp exhibit a larger response to PAS. What is more, the response was not focal to abductor pollicis brevis (APB) – volunteers with writer's cramp exhibited a response in FDI as well as APB.

Merzenich *et al.*, (1984) demonstrated that the area representing digit three in the squirrel monkey somatosensory cortex shrinks rapidly following amputation. However, following a period of sensory discrimination training, the representation of digits two and three increased (Jenkins *et al.*, 1990). In the primary motor cortex, Nudo *et al.* (1996) examined structural plasticity following a unilateral ischemic lesion of the cortical area responsible for the intrinsic muscles of a hand of adult squirrel monkeys. Nudo *et al.*, report a use-dependent change in the cortical representation of the paretic hand; the cortical area of paretic hand expanded into the surrounding cortical tissue in those forced to use the paretic limb. This expansion of the cortical area responsible for the paretic limb was accompanied with increasing skilled functioning of the paretic hand.

Studies examining motor learning induced neuroplasticity in man have shown that as little as a single session of training on a novel motor task can induce neuroplastic changes (Muellbacher *et al.*, 2001; Pascual - Leone *et al.*, 1995; Classen *et al.*, 1998). In these studies neuroplasticity was expressed as a change in the excitability of the corticospinal connection for the muscles involved in the task. Changes in CSE observed in these studies are suggested to occur due to LTP (Muellbacher *et al.*, 2001; Pascual - Leone *et al.*, 1995; Classen *et al.*, 1998).

It should be noted that neuroplastic changes in the primary motor cortex are driven by the acquisition of a novel motor skill – repetitive use alone is not sufficient to evoke neuroplastic changes. Experiments in the animal model have shown neuroplastic changes are correlated with performance improvements (Xu *et al.*, 2009; Nudo & Milliken, 1996; Jenkins *et al.*, 1990). However, when animals are exposed to tasks which require minimal learning there are no observable neuroplastic changes (Kleim *et al.*, 2002; Plautz *et al.*, 2000). These results suggest that learning is the primary driver in the development of functionally relevant neuroplasticity.

It should be noted that neuroplastic changes associated with novel skill acquisition are not limited to supraspinal structures – the spinal cord is capable of neuroplastic change. During both maturation as well as later life the spinal cord changes its structure and function in response to ascending stimuli from the periphery or descending stimuli from supraspinal structures. Similar to plasticity in the brain, spinal cord plasticity involves neuronal and synaptic changes mediated by LTP and changes in neuronal morphology and excitability (reviewed in Wolpaw & Tennissen, 2001).

The acquisition of a novel motor skill is linked with structural and functional changes in neuronal circuits of the spinal cord. These changes in spinal circuitry can be probed using the spinal stretch reflex or its electrical analogue, the Hoffman or H-reflex (Hoffmann, 1910). The spinal stretch reflex is a monosynaptic reflex evoked by a mechanical stretch of the muscle spindles. The pathway consists of the Ia afferents from the muscle spindle, the synapse onto the motorneurone and the motorneurone itself. The H-reflex is evoked using a low-intensity electrical stimulus to an afferent nerve and the H-reflex utilises a similar pathway except it bypasses muscle spindle and fusimotor activity (Knikou, 2008; Zehr, 2002; Misiaszek, 2003). The pathway used in both these reflexes is engaged in both simple and complex behaviours, therefore changes in reflex activity will influence behaviour and changes in behaviour will influence reflexes (Wolpaw, 2006).

There are many examples in the literature where changes in behaviour have been linked to changes in reflex amplitudes (Casabona *et al.*, 1990; Goode & Van Hoven, 1982; Nielsen *et al.*, 1993; Rochongar *et al.*, 1979). Nielsen *et al.*, studied the H-reflex in the soleus muscle of sedentary, moderately active, extremely active participants as well as professional ballet dancers. The authors report reflex amplitudes were largest in the most active participants. However, the lowest reflex amplitude were detected in the ballet dancers, which were even lower than the sedentary volunteers. The authors conclude that this reduction in reflex amplitude occurred due to the regular cocontractive postures adopted during ballet dancing. Further lab based studies have demonstrated it does not take many years of training to modulate H-reflex amplitudes.

Thompson *et al.,* (2009) demonstrated it is possible to up or down-regulate H-reflex amplitude volitionally with as little as three, twenty minute training sessions.

Neuroplastic changes which occur following the acquisition of a novel motor skill are expressed as changes in CSE. In context of the nervous system the term excitability can be traced back to noted neuroscientist Jerzy Konorski who states;

> *"The application of a stimulus leads to changes of a twofold kind in the nervous system... The first property, by virtue of which the nerve cells react to the incoming impulse… we call excitability, and… changes arising… because of this property we shall call changes due to excitability. The second property, by virtue of which certain permanent functional transformations arise in particular systems of neurons as a result of appropriate stimuli or their combination, we shall call plasticity and the corresponding changes plastic changes."*
>
> - *(Konorski, 1948)*

Here Konorski is describing how the propensity of neurones to fire an action potential, there excitability, can change. Since Konorski's initial proposition studies have gone on to suggest LTP or LTP like processes as the casual mechanism behind observed changes in excitability (for review see Ziemann *et al.*, 2008). The details of LTP have been discussed previously however they can be surmised as an increase in neurotransmitter release, insertion of receptors onto the post synaptic membrane and increased sensitivity to neurotransmitters (Bennett *et al.*, 2007; Kandel *et al.*, 2000). For ease of understanding, CSE is best thought of as a gain function on the efficacy of synaptic transmission down the corticospinal tract. An increase in CSE, after an intervention such a motor learning, scales up the efficacy of synaptic transmission and leads to an increase in MEP amplitude by many studies (Rossini *et al.*, 2008). Likewise, following an inhibitory NIBS protocol such as PAS10, there is a lowering of CSE as evidenced by a reduction in MEP amplitude (for review see Carson & Kennedy, 2013).

Inducing Neuroplasticity

Neuroplasticity underlies the functional increases seen in the acquisition of a novel motor skill. However, NIBS techniques can also be used to induce neuroplasticity. This thesis will now go on to introduce some of the common paradigms used to induce neuroplasticity.

Motor Learning

Traditional classifications of learning differentiate between learning explicit and implicit knowledge. Motor learning is categorised as implicit learning as complex information is learnt without the ability to provide conscious verbal recollection of what was learnt. There are few universally accepted definitions of motor learning although many groups have attempted to define motor learning with a pragmatic definition;

> *"Motor learning does not need to be rigidly defined in order to be effectively studied. Instead it is better thought of as a fuzzy category that includes skill acquisition, motor adaptation, such as prism adaptation, and decision making, that is, the ability to select the correct movement in the proper context. A motor skill is the ability to plan and execute a movement goal."*
>
> - (Krakauer, 2006).

Other groups have opted for a more mechanistic description;

> *"Motor learning takes many forms, including: (1) learning over generations that becomes encoded in the genome, is epigenetically expressed as instincts and reflexes and contributes to learned (conditioned) reflexes; (2) learning new skills to augment your inherited motor repertoire, and adapting those skills to maintain performance at a given level; and (3) learning what movements to make and when to make them."*
>
> - (Shadmehr & Wise, 2005).

For the purposes of this thesis, I have chosen to use the broadest definition of motor learning; a lasting change in performance shaped by previous experience. This is encompassed within the following definition:

> *"Learning involves changes in behaviour that arise from interaction with the environment and is distinct from maturation, which involves changes that occur independent of such interaction."*
>
> - (Wolpert *et al.*, 2003).

A key component of these definitions is that they involve changes in motor performance as a function of practice. As performance can be measured in numerous ways depending on the goal, intrinsic within each definition is the recognition that optimising performance is specific to the task and the goal. Consequently studies of motor learning require an understanding of the different paradigms used.

Common Motor Learning Paradigms

There is an increasing number of motor learning paradigms being used and this thesis will now go on to review some of the common ones:

Sequence Learning

Sequence learning was first used in the study of motor learning by Nissen and Bullermer (1987) as the serial reaction time task (SRTT). In this task, participants perform a repeating series of button presses and their reaction times become progressively faster, participants are then presented with a different series of button presses and their reaction time is slower. The variance in reaction times between the learnt and novel sequences provides an index of motor learning. This task spawned numerous variants which attempt to overcome its deficiencies by using mixed or probabilistic sequences, non-spatial colour cues and measurements using the non-dominant hand or with more complex movements.

Sequence learning is the most established and widely used learning paradigm in the study of motor learning. It is not without its limitations which include inability to generalise findings and a lack of ecological validity especially when used clinically (Muslimovic *et al.*, 2007). Studies involving functional neuroimaging have shown the dorsolateral prefrontal cortex, supplementary motor areas and cerebellum are involved in this type of learning (Toni *et al.*, 1998; Grafton *et al.*, 1995).

Ballistic Motor Learning

Ballistic motor learning as a motor learning paradigm was first presented by Muellbacher *et al.,* (2001) who demonstrated that repeated voluntary thumb abductions increase peak thumb acceleration. This task has been shown to induce neuroplastic changes focal to the primary motor cortex (Muellbacher *et al.*, 2002). Specifically, neuroplastic changes were seen in the cortical representation and CSE of the APB (Classen *et al.*, 1998; Kaelin - Lang *et al.*, 2005; Lotze *et al.*, 2003; Muellbacher *et al.*, 2001). Repeated thumb abductions has been linked with changes in activation patterns observed on functional magnetic imaging (fMRI) (Karni *et al.*, 1998).

Visuomotor Transformation

Visuomotor transformation is a broad category of motor learning tasks that involve the participant performing a motor task while the visual and/ or sensory feedback is transformed using displacements, rotations, inversions, mirroring and depth distortions. Learning in this manner combines visual or proprioceptive feedback with motor learning (Amiez *et al.*, 2012). Examples of classical visuomotor transformation tasks include mirror drawing (Corkin, 1968) and rotor pursuit (Ammons, 1951; Ammons *et al.*, 1958). As technology has advanced rotor pursuit tasks have evolved to use styluses and computer screens allowing researchers to manipulate the visual feedback independent of proprioceptive feedback. This category of learning tasks has been shown to involve primary motor cortex, basal ganglia, cerebellum, premotor cortex, supplementary motor area, inferior frontal cortex, dorsolateral prefrontal cortex and inferior parietal cortex (Chouinard & Goodale, 2009; Yamada *et al.*, 2010).

Force Field Adaptation

Force field adaptations involve the participant wearing a robotic exoskeleton while making reaching movements, the resistance provided by the exoskeleton during these movements is subsequently altered (Shadmehr & Mussa - Ivaldi, 1994). The dynamics of these reaching movements are affected by the force field; early movement trajectories are heavily distorted however, with practice, participants learn to make movements resembling near normal movements in free space. The mechanism of learning in force field adaptations has been explained as a system whereby the CNS builds an internal model of the force field and adapts motor behaviours accordingly 'using a intrinsic system of coordinates with sensors and actuators,' (Shadmehr & Mussa - Ivaldi, 1994; Conditt *et al.*, 1997). Studies using neuroimaging have shown that even hours after the task has ceased the performance improvement is retained (Bhushan & Shadmehr, 1999). There are changes to the cortical activation patterns with notable increases in premotor, parietal and cerebellar cortices recruited (Nezafat *et al.*, 2001; Shadmehr & Holcomb, 1997). Interventional studies have suggested that learning in this manner does not depend on the primary motor cortices (Baraduc *et al.*, 2004).

Force field adaptations have been used to study control of the ankle during locomotion. Several studies have demonstrated that the lower limb is capable of a motor recalibration while walking in a force field during normal gait (Blanchette & Bouyer, 2009; Noel *et al.*, 2009; Noble & Prentice, 2006; Lam *et al.*, 2006; Fortin *et al.*, 2009; Emken & Reinkensmeyer, 2005). Similarly to studies examining the upper limb, the movement trajectory of the leg is distorted by the force field, however, with practice, participants adapt to the perturbation and restore near normal walking kinematics.

Locomotor Adaptation

Within the field of motor learning there is a large focus towards the upper limb and hands; there are limited studies examining motor learning in the core and lower limbs. Work with split-belt treadmills and in our lab using split-crank cycling is beginning to correct this bias. In split-belt treadmill tasks, participants learn to walk on a treadmill with each lower limb on a different belt thereby allowing each leg to walk at different rates (Morton & Bastian, 2006). The split-belt treadmill task involves neuroplastic adaptations in spinal, supraspinal and cortical structures as well as their descending commands. Despite its limited experimental use, data from split-belt treadmill tasks has been able to differentiate between the functional networks which control different walking cadences (Choi & Bastian, 2007).

Classical Conditioning

Classical conditioning is a form of motor learning that was discovered accidentally by noted physician Ivan Pavlov (Pavlov, 1927). Pavlov discovered that a conditioned stimulus (CS) paired with an unconditioned stimulus (UCS) resulted in a motor response known as the unconditioned response (UR). If the UCS and CS are repeatedly presented in pairs eventually they will evoke a motor reflex response, known as the conditioned reflex (CR).

Since Pavlov's demonstration of classical conditioning the paradigm has evolved into the eye-blink classical conditioning reflex (Gormezano, 1966; Telford & Anderson, 1932; Cason, 1922). Here the CS takes the form of an auditory tone played shortly

before the UCS which is either a puff of air delivered to the cornea or an electrical stimulus delivered to the superorbital nerve. Repeated exposure to the paired CS and UCS results in the participant blinking, the CR.

The physiology and brain structures involved in classical conditioning are well understood with central roles attributed to the pontine structures, inferior olives and the cerebellar nuclei (Gerwig *et al.*, 2005; Wada *et al.*, 2007; Gerwig *et al.*, 2007). Decerebrate animals have been shown capable of acquiring this conditioning (Jirenhead *et al.*, 2007) and this form of learning is spared in anterograde amnesia (Clark & Squire, 1998). Classical conditioning is considered a primitive form of learning relatively confined to the brainstem and cerebellum. Classical conditioning paradigms are used to study different forms of learning and memory.

Aimed Rapid Movements

All rapid aiming movements are subject to the psychomotor principle of Fitts Law (1954) which describes the inverse relationship between speed and accuracy in reaching movements, and it is expressed mathematically as:

$$\mathrm{MT} = a + b\ \mathrm{ID}$$

where MT is movement time, ID is index of difficulty, *a* and *b* are coefficients

$$\mathrm{ID} = \log_2\left(\frac{2\mathrm{A}}{\mathrm{W}}\right)$$

where A is distance between the starting point to the centre of the target, and W is the width of the target

Fitts Law demonstrates the trade-off between speed and accuracy when making rapid pointing actions. In studies of motor learning coefficient *b* is of interest as it approximates to the reduction in movement time for a task with a given degree of difficulty (Schmidt & Lee, 2005; Kelso, 1984). Repeated practice is correlated with a reduction in this coefficient. It has been suggested that a change in coefficient *b* reflects motor learning (Cohen, 2008). It is important to note that the elderly have a raised *b* (Welford *et al.*, 1969; Goggin & Meeuwsen, 1992), although it is unclear whether this is due to changes within the CNS or peripheral mechanical factors. An example of one of the inherent limitations of this paradigm is that peripheral mechanical factors affect coefficient *b*, thereby limiting its use in pathophysiological conditions such as spasticity.

Explicit Learning in Motor Learning

Despite anterograde amnesia following a bilateral temporal lobectomy amnesic H.M could still acquire novel motor skills (Scoville & Milner, 1957; Corkin, 1968). This observation gave rise to the opinion that there is a segregation of the neuronal circuits involved in explicit declarative knowledge and implicit motor skills. This segregation has been demonstrated for numerous motor learning paradigms including visuomotor transformation (Corkin, 1968), sequence learning (Vandenberghe *et al.*, 2006; Reber & Squire, 1994) and eye-blink classical conditioning (Woodruff-Pak *et al.*, 1996; Clark & Squire, 1998). Importantly, this segregation does not exclude a role for explicit

knowledge or simultaneous explicit learning in modulating implicit learning, in fact there is evidence of an interaction during sequence learning (Brown & Robertson, 2007; Reber & Squire, 1998; Boyd & Winstein, 2004; Vandenberghe *et al.*, 2006). This demonstrates the importance of understanding the role of explicit knowledge and motivation when designing paradigms to study motor learning (Vandenberghe *et al.*, 2006; Wilkinson & Jahanshahi, 2007).

Adaptation versus Learning

Shadmehr and Wise (2005) attempted to sub-classify motor learning paradigms and provided a key distinction between the acquisition of a new motor skill and motor adaptations;

I. Acquiring a new motor skill involves the expansion of motor repertoire or learning of a new motor program which can be generalised onto other tasks.

II. Adaptation involves the repurposing of an existing motor skill to maintain performance.

It is clear that sequence and visuomotor learning examine motor learning while force field and locomotor adaptations examine motor adaptation. Difficulty lies in classifying paradigms like classical conditioning and ballistic learning. Classical conditioning is so hard to classify as it is abstract with no obvious ecological validity. Ballistic motor learning resembles motor adaptations though it could be argued that a new force vector is learnt.

The respective motor learning paradigms rely on different aspects of the sensorimotor systems. Questions remain as to whether they work using similar mechanisms of neuroplasticity and how these paradigms relate to more ecologically valid forms of learning such as learning how to ride a bike, play a musical instrument or neurorehabilitation.

Stages of Motor Learning

The stages of motor learning were first described in 1967 with differing systems involved in the respective stages (Fitts & Posner, 1967);

I. **Verbal cognitive stage**. This is the first stage of motor learning and it has a large cognitive component. This stage is characterised by large error and variable performance, often termed 'familiarisation' and is dependent on attention and higher cognition.

II. **Association stage**. This is intermediate phase and is often referred to as the 'refining phase'. This stage is characterised by decreasing variability in performance and errors. This decrease in performance variability is believed to be due to the development of associations between sensory cues with movements which more closely achieve goals. Most experimental paradigms test performance in this phase of learning.

III. **Autonomous stage.** This is the final stage of motor learning and is not achieved by everyone. This stage is characterised by increasing cognitive and motor efficiency with limited decreases in performance variability.

The different stages of motor learning rely on different cognitive or motor 'modules' and so the various stages are likely to rely on different brain regions and different types of neuroplastic change. The staged nature of this time course may also be relevant in the process of consolidation where memories are becoming more resistant to disruption over time; for example ballistic motor learning is initially dependent on the primary motor cortex but becomes consolidated after six hours (Muellbacher *et al.*, 2002).

The majority of motor learning paradigms examine the intermediate phase of learning but it is important to consider that some participants may be performing at different stages depending on their prior familiarity with the task. For some paradigms, for example classical conditioning, it is difficult to perceive such a staged process. The staged nature of motor learning reflects the dynamic nature of the process whereby different processes and cortical structures play a variety of roles at different points during the learning process.

The Link between Plasticity and Motor Learning

It is a commonly held belief that there is a link between motor learning and neuroplasticity. Martin *et al.* (2000) put forward the suggestion that the following five criteria must be met to link synaptic plasticity and learning:

I. **Correlation:** The behavioural parameters of learning should be correlated with some but not all of the properties of synaptic plasticity.

II. **Induction:** Learning should be associated with the induction of measurable changes in synaptic efficacy at the appropriate synapses and the induction of changes in relevant synapses should result in apparent memories.

III. **Occlusion:** Saturation of synaptic plasticity in a network should destroy the pattern of trace strengths corresponding to established memories and occlude new memories.

IV. **Intervention**: Blockade or enhancement of synaptic plasticity, achieved by pharmacological, genetic or other manipulations, should have commensurate effects on learning or memory.

V. **Erasure:** Erasure of synaptic plasticity should, at least, shortly after learning induce forgetting.

In the animal model, the evidence for correlation comes from the demonstration of learning induced functional reorganisation in the primary motor cortex of the monkey following learning (Nudo & Milliken, 1996; Nudo *et al.*, 2001; Nudo *et al.*, 1996). The evidence for induction comes from the demonstration that LTP induced in a spike timing dependent manner in the freely behaving primate primary motor cortex resulted in changes in the motor representation of movements (Jackson *et al.*, 2006). The evidence for occlusion comes from the demonstration that prior motor learning impairs any subsequent LTP induction in the horizontal connections of the primary motor cortex (Sanes & Donoghue, 2000; Rioult-Pedotti *et al.*, 2000; Rioult-Pedotti *et al.*, 1998). Cortical stimulation paired with motor learning has been shown to promote the re-emergence of cortical maps (Plautz *et al.*, 2003), additionally pairing cortical stimulation and rehabilitative training has been shown to increase functional recovery

and promote structural plasticity (Adkins-Muir & Jones, 2003) satisfying the intervention criterion. There is a lack of evidence satisfying the erasure criterion. As a result of the evidence outlined here I would suggest there is a strong link between plasticity and motor learning in animals.

Proof of the relationship between human neuroplasticity and motor learning in man is also incomplete according to the criteria set forward by Martin *et al.,* (2000). The evidence satisfying the correlation criterion comes from studies showing pharmacologically inhibiting and disinhibiting agents are associated with lower rates of motor learning (Ziemann *et al.*, 2006; Nitsche *et al.*, 2012). The evidence satisfying the induction criterion comes from studies which have shown that basic tasks such as sequence learning can be enhanced using TDCS (Nitsche *et al.*, 2003), even some complex tasks such as mathematical learning can be enhanced (Snowball *et al.*, 2013). Induction is challenging to examine experimentally; induction of artificial plasticity is less likely to code information as usefully as effective motor learning which is likely to require encoding information across multiple neural networks. The need for multiple networks is demonstrated by a study showing how motor practice alters MEPs and sensorimotor organisation while paired-associative stimulation only affects MEPs (Rosenkranz & Rothwell, 2006). Inducing plasticity in the primary motor cortex produces very subtle behavioural changes while by definition most motor learning paradigms show much more obvious behavioural changes (Ridding & Ziemann, 2010; Muellbacher *et al.*, 2001). There is an abundance of evidence satisfying the occlusion criterion of motor learning and neuroplasticity. Ziemann and colleagues examined how various paradigms interact with one another and report that prior ballistic motor

learning prevents subsequent induction of plasticity via repetitive TMS (rTMS) (Ziemann *et al.*, 2004). Stefan and colleagues also showed PAS occlusion after force adaptation motor training (Stefan *et al.*, 2006). Visuomotor, ballistic and sequence learning have been shown to induce changes in neuronal excitability and structural plasticity, this is termed practice-dependent plasticity (Lundbye-Jensen *et al.*, 2005; Snowball *et al.*, 2013; Pascual - Leone *et al.*, 1995). These changes in representation are thought to be due to changes in synaptic efficacy probably involving LTP analogous to animal models of practice-dependent plasticity (Ziemann *et al.*, 2001; Boroojerdi *et al.*, 2001b). Changes in excitability and plasticity are accompanied with increased blood oxygen level dependent (BOLD) signal suggestive of increased neural activity (Lotze *et al.*, 2003). fMRI BOLD changes correlate to changes in synaptic plasticity probably involving LTP (Ziemann *et al.*, 2001; Boroojerdi *et al.*, 2001b). Taub and colleagues demonstrated that an association between increased plasticity and behavioural outcomes following constraint induced movement therapy (Mark *et al.*, 2006). Thus I suggest there is ample evidence to satisfy the intervention criterion. The erasure criterion is satisfied as Muellbacher *et al.* (2002) demonstrated that performance improvements following ballistic motor learning are abolished by 1 Hz rTMS delivered to the motor cortex shortly after training.

The relationship between neuroplasticity and motor learning is further complicated by the numerous motor learning tasks used within the field and that these different paradigms are reliant on different systems making contribution depending on the paradigm being used.

Artificially Induced Neuroplasticity

Motor learning is not the only way to induce neuroplasticity, NIBS techniques can also be used to induce neuroplasticity. Over the past decade there has been considerable interest in using NIBS techniques as adjuncts to augment neuroplasticity induction during the learning and/ or rehabilitation processes (for review see Ridding & Rothwell, 2007). This thesis will now go on to discuss the use of rTMS, TBS, PAS and transcranial direct current stimulation (TDCS) to induce neuroplasticity.

TMS is a non invasive means of activating the cortex using a transient magnetic field (Barker *et al.*, 1985). When magnetic stimuli are presented at 1 Hz or greater they modulate the excitability of the cortex for a period of time after the stimulation ceases; with 1 Hz depressing activity and 5 Hz or greater increasing cortical excitability. rTMS has been extensively used in the study of motor learning and memory formation (for review see Censor & Cohen, 2010). Briefly, rTMS has revealed the sleep dependency for offline consolidation of performance gains (Walker *et al.*, 2002; Korman *et al.*, 2007), the brain areas involved in performance consolidation (Zangen *et al.*, 2005), the time course of consolidation (Karni & Sagi, 1993; Korman *et al.*, 2007) and the mechanism underlying reconsolidation (Nader & Hardt, 2009).

There has been great interest in the therapeutic application of TMS for neuro- and psychiatric rehabilitation (for review see Ridding & Rothwell, 2007). The paradigms used in these studies typically involve many stimuli; in excess of 1,000 delivered over multiple days for a long period of time (Cortes *et al.*, 2012; Corti *et al.*, 2012; Hao *et al.*, 2013). These studies have a severe weakness – there is large inter- and

intra-individual variability in how the cortex responds to rTMS (Maeda *et al.*, 2000). However, theta burst stimulation or TBS is a recently developed form of patterned rTMS (Huang *et al.*, 2005). TBS involves delivering high frequency pulses delivered at 50 Hz, repeated at 5 Hz. Huang *et al.* (2005) described two forms of TBS, continuous TBS (cTBS) and intermittent (iTBS), both paradigms use subthreshold stimulation intensities to modulate CSE. cTBS uses 200 triplet bursts with each burst in the triplet delivered at 50 Hz and the triplets delivered at 5 Hz.. iTBS is comparable to cTBS however there are breaks of eight seconds after twenty triplets. Both iTBS and cTBS are illustrated below in figure 2. The patterned nature of TBS is advantageous over conventional rTMS as it uses lower stimulation intensities which raise the spatial specificity while the higher frequencies reduce the duration of the stimulation required to modulate CSE (Zafar *et al.*, 2008).

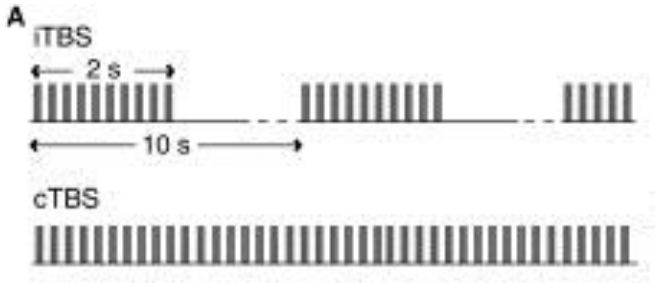

Figure 2. Theta burst stimulation protocols (Adapted from Huang et al., 2005).

In his 1949 book, The Organisation of Behaviour, Donald Hebb proposed a hypothesis to explain the adaptation of neurones during the learning process. Hebbs model of synaptic plasticity is summarised in the quote below:

> *"When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased."*
>
> - (Hebb, 1949)

The Hebbian model of synaptic plasticity is undoubtedly one of the seminal theories of neuroscience - forming the cornerstone of our understanding of the CNS. PAS is a NIBS paradigm based upon the Hebbian model of synaptic plasticity (for review see Carson & Kennedy, 2013). PAS involves pairing peripheral electrical stimuli with central magnetic stimuli to induce neuroplasticity (Stefan *et al.*, 2000). When the stimuli are given at 10 ms ISI PAS has an inhibitory effect whereas a 25 ms ISI has an excitatory effect (Stefan *et al.*, 2000). LTP and LTD are suggested as the principle mechanism behind the neuroplastic effects seen following PAS. Following the initial report of PAS by Stefan *et al.,* (2000) there has been numerous studies examining the efficacy of different ISI (Wolters *et al.*, 2005; Kumpulainen *et al.*, 2012), the efficacy of PAS in different muscles (Stefan *et al.*, 2000; Carson *et al.*, 2013) and the efficacy of PAS in different clinical populations (Monte-Silva *et al.*, 2009; Bologna *et al.*, 2012).

TDCS involves applying weak direct current to the cortex and has been shown to modulate CSE for a period of time which outlasts the stimulation (Nitsche & Paulus, 2000). Cathodal stimulation has inhibitory effects on cortical activity while anodal stimulation has excitatory effects. Reis *et al.,* (2009) examined the effect of TDCS on the time course of novel skill acquisition over a three month period. Reis *et al.,* report a

significantly greater acquisition of the skill in those who had TDCS and this greater performance increase was retained for up to three months. A recent meta-analysis of randomised control trials examining the use of TDCS during stroke rehabilitation found TDCS to be effective for stroke patients in the chronic period with mild to moderate motor impairments (Marquez *et al.*, 2013). TDCS has also been shown to be an effective adjunct to rehabilitation in a variety of neurological diseases and disorders including traumatic brain injury (Dermirtas-Tatlidede *et al.*, 2012) and Alzheimer's disease (Freitas *et al.*, 2011).

Motor learning is a natural means of inducing neuroplasticity, however plasticity can also be induce artificially via NIBS paradigms. These paradigms can be rapid cortical magnetic stimulation, simple or patterned as well as central magnetic stimulation paired with peripheral electrical stimulation. Even the application of simple 1 mA current can induce neuroplastic changes. This thesis will move on to discuss methods of assessing neuroplasticity.

Assessing Neuroplasticity

I have previously described how NIBS techniques are used to induce neuroplasticity however they can also be used to assess neuroplasticity. This thesis will now go on to briefly discuss the use of TMS to assess neuroplastic changes. Additionally although they're not directly used in this thesis it presents a brief explanation of TES, TDCS,EEG and fMRI as they are alternate means of assessing neuroplastic changes commonly employed in the literature.

Transcranial Electrical Stimulation

Until 1980, stimulating the exposed cortex during neurosurgery was the only means of stimulating the corticospinal pathway in man. NIBS was made possible in 1980 when Merton and Morton presented transcranial electrical stimulation (TES) (Merton & Morton, 1980). TES involves passing a large direct current between two electrodes placed over the motor cortex. TES activates corticospinal neurones at either, the cell body or the axon hillock (Day *et al.,* 1989a; Day *et al.,* 1987). However, TES has a notable floor. Due to the high resistance of the scalp, skin and skull one needs to give a high intensity stimulus to activate the corticospinal neurones. These high intensity stimuli also activate nocioceptive afferents and facial muscles to cause significant pain and discomfort to the participant.

Transcranial Magnetic Stimulation

Barker *et al.* (1985) demonstrated it possible to activate the primary motor cortex and corticospinal tract non-invasively with a transient magnetic field. This idea was by no means new, the first experiments regarding the efficacy of magnetic fields in man date back to the work of d'Arsonval in the 1890's (d'Arsonval A. 1896 in Pascual - Leone *et al.,* 2002).

TMS uses a coil carrying a large transient voltage around the coil of wire which induces a magnetic flux perpendicular to the direction of travel of the electrical current. If the coil is placed on the scalp the magnetic field will induce an electrical current in the tissue beneath (Pascual - Leone *et al.,* 2002; Barker *et al.,* 1985). The magnetic field

passes painlessly into the cortex and the technique is non-invasive (Pascual - Leone *et al.*, 2002).

When applied over the primary motor cortex TMS evokes a MEP in surface electromyography (EMG) of the peripheral muscle represented in the area the primary motor cortex being stimulated (Day *et al.*, 1989a). Epidural recordings from corticospinal neurones demonstrate MEPs consist of two components, a D wave and several I waves with more I waves seen at higher stimulation intensities (see figure 3 for illustration). D waves represent direct activation of corticospinal neurones while I waves represent indirect or trans-synaptic activation of the same neurones (Di Lazzaro *et al.*, 2004). An MEP is the summation of the discharge of multiple motor units (Cortes *et al.*, 2012).

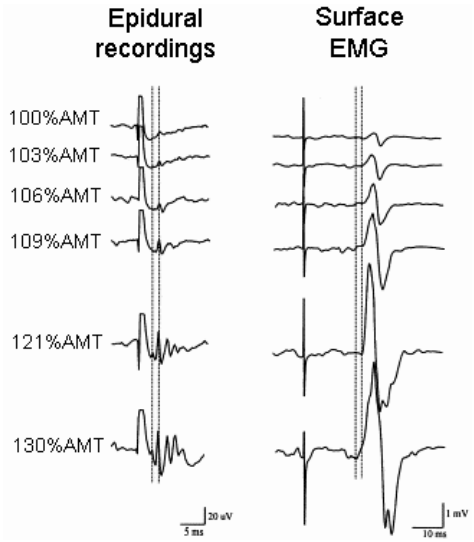

Figure 3. Epidural volleys and motor evoked potentials recorded after a single TMS pulse over the primary motor cortex (Adapted from Di Lazzaro et al., 2004).

Single pulse TMS can be used to evoke an MEP in the contralateral muscle of interest. The threshold for MEP induction, the motor threshold, and MEP amplitude are commonly used probes for CSE.

Motor threshold is defined as the minimum stimulation intensity required to evoke a MEP in the muscle of interest. Motor threshold can be assessed either in a resting muscle (rMT) or an active muscle (aMT). rMT is defined the minimum stimulation intensity to evoke MEPs of greater than or equal to 50 μV on 5 out of 10 occasions (Rossini *et al.*, 1994). aMT is defined similarly except MEPs must be greater than 200 μV and clearly distinguishable from the background EMG (Rossini *et al.*, 1994). In TMS experiments motor thresholds are commonly used a reference point to standardise stimulation intensities between participants. Motor thresholds should be considered as a good marker of intrinsic membrane characteristics/ axonal excitability for corticospinal neurones (Ziemann *et al.*, 1996; Chen *et al.*, 1997). Motor thresholds are influenced by neural inputs to corticospinal neurones, tonic inhibitory or excitatory drives as well as the excitability of motor neurones in the spinal cord and neuromuscular junction (Chen *et al.*, 1997).

In order to assess changes in CSE, MEP amplitudes are commonly taken before and after an intervention such as motor learning. Changes in MEP amplitude may reflect changes in synaptic efficacy in the neural circuits recruited by TMS or by more neural circuits being recruited by the TMS pulse. Changes in MEP amplitude may also be explained by changes in synaptic excitability and/ or intrinsic excitability of the spinal motor neurones activated by the TMS pulse. Descending volleys generated using TMS

pulses travel along the corticospinal pathway, therefore measures of sub-cortical or spinal excitability are commonly combined with TMS to better aid the interpretation of changes in MEP amplitudes. Common measures include brain stem or cervicomedullary stimulation, H-reflexes and F-waves, however, these techniques are not without their limitations. H-reflexes involve electrical stimulation of group 1a afferents (Knikou, 2008). H-reflexes can be influenced by a host of different mechanisms, rather than motor neurone excitability including pre-synaptic inhibition, post activation depression and disynaptic reciprocal inhibition (Zehr, 2002; Misiaszek, 2003; Knikou, 2008). F-waves reflect activation of a small number of spinal motor neurones and may not truly represent all neurones activated by TMS. Cervicomedullary stimulation involves passing large electrical currents between the mastoid processes, which is painful. That said, cervicomedullary stimulation is suggested to activate similar pathways to TMS without the cortical component of TMS (Ugawa *et al.*, 1991; Taylor *et al.*, 2006). Cervicomedullary stimulation is considered the gold-standard for assessing subcortical changes subcortical excitability (Ugawa *et al.*, 1991).

Since its introduction in the mid 1980's by Barker *et al.*, (1985) TMS has evolved into numerous techniques for assessing and altering the structure and function of the CNS. Some of the common TMS techniques have been summarised below in table 1.

Table 1. Summary of TMS methods

Measurement	Description	Reference
Cortical Mapping	Suprathreshold single pulse stimulation which relates the size of the motor evoked potentials to the position of stimulation.	(Amassian *et al.*, 1989b; Wilson *et al.*, 1993; Levy *et al.*, 1991)
Stimulus Response Curves	Stimulating over motor cortex at various intensities and plotting the size of motor evoked response against the stimulus intensity	(Valls-Solé *et al.*, 1994; Boroojerdi *et al.*, 2001a; Devanne *et al.*, 1997)
Short Interval Intracortical inhibition (SICI)	Subthreshold conditioning pulse 2-3 ms before test TMS pulse.	(Kujirai *et al.*, 1993)
Long Interval Intracortical inhibition (LICI)	Suprathreshold conditioning pulse 100 – 200 ms before test TMS pulse.	(Valls - Solé *et al.*, 1992; Wassermann *et al.*, 1996a)
Intracortical facilitation (ICF)	Subthreshold conditioning TMS pulse 8-15 ms before test TMS pulse.	(Kujirai *et al.*, 1993)
Paired Associative Stimulation (PAS)	Low frequency repetitive peripheral nerve stimulation paired timed TMS over contralateral motor cortex	(Stefan *et al.*, 2000)
Short latency afferent inhibition (SAI)	Peripheral conditioning electrical stimulation 20-24 ms before test TMS pulse.	(Tokimura *et al.*, 2000)
Long latency afferent inhibition (LAI)	Peripheral conditioning electrical stimulation 50 – 100 ms before test TMS pulse.	(Sailer *et al.*, 2002)
Interhemispheric inhibition (IHI)	Suprathreshold conditioning TMS pulse to contralateral motor cortex 8-40 ms before test TMS pulse.	(Ferbert *et al.*, 1992)
Interhemispheric facilitation (IHF)	Near threshold conditioning TMS pulse to contralateral motor cortex 10 ms before test TMS pulse.	(Mochizuki *et al.*, 2004; Bäumer *et al.*, 2006)
Ipsilateral premotor inhibition	Near threshold conditioning TMS pulse to ipsilateral premotor area 6-8 ms before test TMS pulse.	(Civardi *et al.*, 2001)
Interhemispheric premotor inhibition	Near threshold conditioning TMS pulse to contralateral premotor area 8-10 ms before test TMS pulse.	(Mochizuki *et al.*, 2004; Bäumer *et al.*, 2006
Posterior parietal motor inhibition	Near threshold conditioning TMS pulse to ipsilateral motor cortex 3-10 ms before test TMS pulse	(Koch *et al.*, 2007)
Repetitive (rTMS)	TMS pulse applied at greater than or equal to 1 Hz.	(Wassermann *et al.*, 1996a)
Theta burst (TBS)	High frequency (50 Hz) pulses repeated at	(Huang *et al.*, 2005)

	5 Hz	
Quadripulse stimulation	Trains of 2-4 monophasic TMS pulses delivered at 1.5 ms intervals	(Hamada *et al.*, 2007)
Subthreshold Inhibition	Subthreshold pulses delivered at 1Hz	(Davey *et al.*, 1994)

TMS has been used extensively to study the CNS. It is beyond the scope of this thesis to critically detail all these papers, instead I have chosen to present three seminal papers, George *et al.*, (1995), Pascual-Leone *et al.*, (1995), and Amassian et al., (1989a).

Much of the TMS literature is devoted to the therapeutic application of TMS to treat depression. Much of this literature can trace its route back to George *et al.*, (1995). In this study George *et al.*, targeted rTMS towards the left prefrontal cortex and observed a significant increase in mood following treatment with TMS. In this study the authors related improvements in mood and the concomitant increase in cerebral blood flow following rTMS. Pascual-Leone *et al.*, (1995) presented a seminal paper on the neuroplastic response to the acquisition of novel skill. Over the course of a five day training programme the authors studied the plastic response to mental and physical practice on a sequence learning task. The investigators mapped the cortical representation of the finger flexor and extensor muscles using TMS. The authors report several interesting phenomenon. First, they note that mental practice alone was sufficient to improve performance on the sequence learning task although these improvements were significantly less than the physical practice cohort. Secondly, the authors report that mental practice of the sequence learning task resulted in similar levels of neuroplastic change compared with the physical practice cohort. TMS has

applied to understand how structures within the brain alter function when they are knocked out using virtual lesions. The virtual lesion technique was first presented by Amassian *et al.,* (1989a) in the visual cortex and Day *et al.,* (1989b) in the motor cortex. The virtual lesion technique is best thought of as introducing noise to the system. If a population are involved in a given task, applying a TMS pulse is very unlikely to selectively stimulate the same population of neurones involved in a given task (Walsh & Cowey, 2000). Rather, the TMS pulse introduces neural activity that is random with respect to the goal-state of this population of neurones (Walsh & Cowey, 2000). Simply put, the virtual lesion technique induces disorder rather order in neuronal processing of information. The virtual lesion technique has been used to gleam information regarding the temporal relationship between different cortical regions to a specific behaviour (Pascaul-Leone *et al.*, 2000).

Electroencephalography and Magnetoencephalography

The electroencephalogram, or EEG, is a non-invasive means of recording the electrical activity of the cortex. The EEG is a recording of the differences in the electrical potential between different points on the scalp (Pond, 1967). The rhythmic pattern of the EEG is generated by cyclical changes in the membrane potentials of the neurones underlying each electrode. The potentials recorded during an EEG arise from the cortex, specifically the large pyramidal cells in layers IV and V of the cortex (Pond, 1967). Magnetoencephalography is a complimentary technique which detects magnetic correlate of the electrical activity of the cortex (Vecchiato *et al.*, 2011). Recently, in an attempt to increase the efficacy of TBS, Brownjohn *et al.,* (2014) used EEG to record the theta frequency and then, delivered TBS at this individualise frequency. Brownjohn

et al., compared the individualised TBS with conventional TBS and report that individualising TBS does not induce a significantly greater modulation of MEP amplitude compared with conventional TBS.

Functional Magnetic Imaging

fMRI is a common paradigm used to assess neuroplastic changes. BOLD is by far the most common fMRI paradigm and has dominated the field since its inception (Arthurs & Boniface, 2002). BOLD fMRI uses haemoglobin as a contrast agent, relying on the differences in magnetisation between oxy- and deoxyhaemoglobin. BOLD fMRI provides an indirect measure of neural activity via the assumed haedynamic correlate. The BOLD fMRI signal has several key features; the response of the CNS to a stimulus; the relationship between the neural activity and the haemodynamic response, the haemodynamic response itself and the way the signal is detected by the scanner. An example of the application of fMRI to assess plasticity is the recent study by Saiote et al., (2013). Here the authors examined the neuroplastic effects of two different transcranial electrical stimulation (TES) paradigms on the early and late phase of motor learning. The authors assess the plastic effect of these two interventions use fMRI, the authors report changes in brain activation similar to those reported in other studies (for review see Kelly *et al.*, 2006) however, authors report that adding high frequency noise to the system using TES facilitates motor learning whereas adding low frequency noise hinders motor learning.

Summary

It is possible to change the structure and functioning of the cerebral cortex naturally through learning novel skills and artificially via NIBS techniques. The neuroplastic changes are suggested to occur via LTP or LTP like processes. This thesis employs motor learning and TMS techniques to assess changes in CSE in the healthy human. This thesis now moves on to introduce the methods used to assess motor learning and learning induced neuroplasticity.

Chapter III – General Methods

Chapter III – General Methods

This chapter details the TMS SR curve acquisition protocol use in all three studies of this thesis as well as the visuomotor learning paradigm used in studies two and three of this thesis. The purpose of this chapter is to provide a summary of the methods common to one or more experiment in this thesis, to consider the advantages and disadvantages of each method and permit the reader to evaluate the results of the studies presented. The precise details of each experiment, the rationale, the protocol, findings and discussion will be reported in the respective chapters.

All study protocols were approved by the University of Birmingham's science, technology, engineering and mathematics ethics committee (ERN_11-0444) and all experimental procedures were conducted in accordance with the declaration of Helsinki (World Medical Organisation, 1996). In study one investigated the feasibility of reducing the acquisition time of the SR curve. In study two, I used the rapid SR curve acquisition protocol to assess learning induced modulation of CSE in proximal and distal muscles. Study three investigated the influence of hand preference on learning induced modulation of CSE.

All studies in this thesis focused on healthy participants with no history of neuromuscular disease. Healthy participants with a mean age of 22 ± 4 years were recruited for the studies in this thesis (n=76, 60% female, 58 right hand dominant, sample of convenience). Bespoke samples were recruited for each experiment, no participants completed more than one experiment. Detailed characteristic for each of the study populations is reported in the relevant chapters. Before being allowed to

participate in a study, participants were given verbal and written information so as to inform consent. Participants were screened for contradictions to TMS using a modified version of the TMS adult safety questionnaire, a copy has been included as appendix four for clarity (Keel *et al.*, 2001). For studies two and three participants handedness was determined using the Edinburgh handedness inventory (Oldfield, 1971). Participants were reminded of the right to withdraw from the study at any point without reason or fear of repercussion. No adverse events were reported following the use of TMS in the studies of this thesis.

Visuomotor Learning

Studies two and three assessed changes in CSE following a short period of visuomotor learning. This particular visuomotor learning task was chosen as it has been previously shown to modulate CSE in a wide variety of muscles including FDI, BB and TA (Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005; Cirillo *et al.*, 2011). However, the task was adapted to use isometric force rather than joint position was the input signal. This adaptation was chosen as increasing levels of force have shown to increase the level of activity within the primary motor cortex (Wexler *et al.*, 1997). Additionally there was a pragmatic reason behind the adapation, the necessary goniomoters to study FDI were unavailable whereas the force transducers were available.

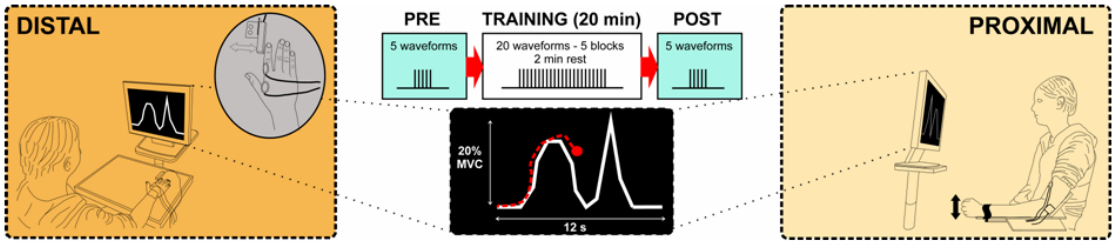

Figure 4. Experimental setup for visuomotor learning in distal and proximal muscles. Participants were asked to track waveforms through isometric elbow flexion and finger abductions with varying degrees of force. The target waveform is shown in white and the participants trace in red on the centre panel. Performance was quantified using root mean square error between the target and actual traces. Forces were recorded as displayed in the left and right panels. Five waveforms were used to assess performance prior to and post training. During the training participants were provided with performance feedback in the form of a bar chart. The bar chart was updated after each waveform, showing how their performance improved across the five training blocks. Thanks go to Mark van de Ruit for the figure.

The visuomotor learning task used in this thesis involved tracking waveformsthrough isometric contractions of either FDI or BB. For studies involving BB force was recorded using a custom made torsion bar (setup shown in figure 4). For studies involving FDI force was recorded using the NL 62 - 5 kg force transducer (Digitimer Ltd, Welwyn Garden City, UK).

Participants were asked to track a target waveform with a cursor shown on the computer screen through isometric index finger abductions or elbow flexions of varying degrees of force, the experimental set-up is illustrated below in figure 4. Increasing force made the cursor rise, shown as the red dot in figure 4, on the screen and decreasing made it fall. Participants were able to control the vertical movement of the cursor, movement in the x-axis was pre-defined in the matlab code. At the beginning of the task participants were verbally instructed how to complete the task, participants were not allowed to practice the task prior to commencing the training. The training protocol used in this thesis is illustrated by in centre portion of figure 4.

In each session participants performed five blocks of training, each 4 minutes with 2 minutes rest between blocks to minimise fatigue. Each training block consisted of

tracking 20 waveforms, each lasting 12 seconds. To further minimise fatigue the waveform amplitudes were normalised between 0 and 20 % maximal voluntary contraction (MVC) for each participant. Furthermore, the first and last second of each waveform returned to 0 %MVC. The participant's performance on the task was assessed prior to and post training using separate blocks of assessment waveforms shown in figure 4.

Online performance feedback, in the form of absolute error, was given to control for fluctuations in attention during learning. Error feedback was given in the form of a bar chart displayed on the computer screen after each waveform. The bar chart was updated after each waveform so the participant saw their performance improvement for each waveform across all training blocks. The force signal was high pass filtered at 30 Hz, amplified x1000, digitised at 4 kHz and stored on a computer for offline analysis. Performance was quantified post hoc using the RMS error between the target trace and the actual cursor position.

Electromyography

Surface electrodes (Blue Sensor N, Ambu®, Denmark) were placed in a bipolar montage over the muscle of interest. Care was taken to ensure accordance with the SENIAM guidelines. The EMG signals were band-pass filtered (0.5 - 2 kHz), sampled at 5 kHz, and amplified using custom amplifiers. All data were stored on a computer for offline analysis.

Transcranial Magnetic Stimulation

For all studies of this thesis motor evoked potentials (MEP) were elicited with a biphasic TMS pulse (Magstim Rapid^2, The Magstim Company, Dyfed, UK) from a custom-made 90mm *'figure of eight'* coil (batwing design; type no. 15411, Magstim company, Dyfed, UK). Magnetic stimuli were delivered over the cortical area which evoked maximal MEPs in the particular muscle of interest, commonly referred to as the '*hotspot'*. The coil was positioned over the respective hotspot with the handle pointing backwards at an angle of 45° from the midline (Brasil - Neto *et al.*, 1992) inducing a posterior-anterior current (Kammer *et al.*, 2001). Coil position and orientation were monitored in real time using frameless stereotaxy (Brainsight, Rogue Research Inc). In an attempt to control for attention mediated variation in MEP amplitude participants were asked to ensure the coil was as close to the hotspot as possible with the aid of feedback from the Brainsight system.

Neuronavigation

In neuroscience many studies apply TMS to stimulate specific regions of neurones and observe the functional significance of doing so. However, this poses significant challenges for studies which require stimulation over several sessions – how does one ensure the same regions of the brain are being stimulated in each session? To overcome this confound, the BrainSight neuronavigation system (Rogue Research Inc) was used for all studies of this thesis. Here, the participant and the TMS coil wear infrared reflective markers to monitor their position in space. The system is calibrated by marking sights that are common to the scan and to the participants head, such as the tip of the nose. The calibration process defines the position of the head with respect to

infrared marker and sights marked during the calibration. BrainSight uses infrared markers worn by the participant and the calibration sights together with the infrared marker on the coil to monitor, in real-time, the position and orientation of the coil. The use of neuronavigation in TMS experiments has been shown to enhance the efficacy of stimulation as well as decreasing variability and latency and increase MEP amplitudes (Julkunen *et al.*, 2009; Sparing *et al.*, 2008; Rankin & Stokes, 1998; Gugino *et al.*, 2001).

Safety of Transcranial Magnetic Stimulation

Single pulse and rTMS are widely considered to be safe and well tolerated in man. Tissue heating during single pulse TMS is less than 0.1°C (Ruohonen & Ilmoniemi, 2002), and the total exposure to the magnetic field during TMS and rTMS is considered too small to pose a risk to participants (Rossi *et al.*, 2009). Mild transient headaches are the most commonly reported side-effect of TMS (Rossi *et al.*, 2009). A recent study examining mild adverse events to TMS reported an incidence of approximately 5% for mild headaches in a sample of 1270 TMS sessions (Maizey *et al.*, 2013). The most serious adverse event associated with TMS, particularly with regards to high frequency rTMS due to its excitatory after effects, is TMS induced seizure. It should be noted that these events are rare – a recent review of TMS safety reported 16 incidences of TMS induced seizures (Rossi *et al.*, 2009). It is important to highlight that these events occurred where participants had a family history of epilepsy or were using pro-convulsant medications (Bernabeu *et al.*, 2004; Tharayil *et al.*, 2005). Since the introduction of internationally recognised safety guidelines (Wassermann, 1998; Wassermann *et al.*, 1996a) and a safety screen for contraindications to TMS (Keel *et al.*,

2001) the incidence of TMS induced seizures has dropped markedly. There has been a single reported incidence of TMS induced seizure in an otherwise healthy participant (Kratz *et al.*, 2011). There has been great interest in using rTMS to treat psychiatric disorders, these treatments typically involve many stimuli, delivered on a daily or weekly basis. Importantly, there are no document serious adverse events to chronic exposure to rTMS (Janiack *et al.*, 2008; Rossi *et al.*, 2009). Given that TMS is in its infancy further work is warranted to evaluate the safety of TMS, particularly through longitudinal studies.

Stimulus Response Curves

MEP amplitudes increase in a sigmoidal manner with increasing stimulation intensities; this led to the conception of the TMS SR curve (Valls-Solé *et al.*, 1994; Devanne *et al.*, 1997; Boroojerdi *et al.*, 2001a). Since its introduction in the mid 1990's the SR curve has been increasingly used in studies of motor learning and neurorehabilitation.

SR curves were initially proposed by Devanne *et al.,* (1997). The authors used a binned acquisition protocol whereby the SR relationship was established by delivering successive magnetic stimuli at increasing stimulator intensity starting just below motor threshold and ending when the stimulator's maximum output was reached (for an example of an SR curve acquired using binned acquisition please refer to figure 2 of Devanne *et al.,* (1997). MEPs were acquired by recording a set of 5-10 stimuli at different stimulation intensity levels grouped in steps of 2-10 % of the maximum stimulator output (MSO). The responses for each stimuli intensity level were then averaged and a Boltzmann-like model fit to the mean data using a nonlinear least mean-

square algorithm (Devanne *et al.*, 1997). This protocol for acquiring SR curves has become commonplace in motor control and neurorehabilitation research.

Devanne *et al.*, (1997) suggest "the order of the presentation of stimuli has no effect on the form of the [stimulus response] relationship or its parameters," however, recent research has begun to question this statement. Möller *et al.*, (2009) examined the effect of three different acquisition protocols on the SR curve. Möller *et al.*, used binned increasing (similar to Devanne *et al.*, 1997), binned decreasing and a random protocol (for an example of an SR curve acquired using binned acquisition please refer to figure 2 of Devanne *et al.*, (1997). SR curves acquired with the binned decreasing protocol were significantly different compared to those acquired with the binned increasing protocol while curves acquired with the random protocol ran between the two binned protocols. This paper demonstrates it is possible to influence SR curves with the acquisition protocol. The random acquisition protocol recommend by Möller *et al.*, (2009) was adopted for all studies of this thesis.

Reliability of Transcranial Magnetic Stimulation Stimulus Response Curve

Carroll *et al.* (2001) assessed the reliability of SR curves. The authors report ICCs for the SR curve parameters ranging between 0.72 and 0.96 based upon this finding the authors suggest SR curves are a reliable means of assessing CSE in the passive and active muscle. Malcolm *et al.* (2006) report SR curves in healthy volunteers acquired twice in a two week period have an intraclass correlation coefficient of between 0.60 and 0.83. Carson *et al.* (2013) recommend using 'area under the curve' (AuC) analysis

when assessing SR curves in interventional studies. I would suggest SR curves are a reliable method for assessing CSE in healthy populations.

Physiology of Transcranial Magnetic Stimulation Stimulus Response Curve

The parameters SR curve (illustrated in figure 6) reflects aspects of the underlying physiology of the CNS. rMT denotes the excitability and membrane channel characteristics of the cortico-cortical axons and their excitatory synapses within the motor cortex (Ziemann, 2004). The gradient of the sigmoid is suggested to mirror the magnitude of the cortical representation and the distribution of excitability within the corticospinal projection (Siebner & Rothwell, 2003). AuC analysis has also been used to provide a robust measure of the corticospinal output and projection strength (Pitcher *et al.*, 2009; Carson *et al.*, 2013; Talelli *et al.*, 2008).

Limitations of Stimulus Response Curves

The use of single pulse TMS to acquire SR curves is subject to the same limitations as cortical mapping i.e. changes in SR curve parameters reflect changes in both excitability and cortical topography. Moreover, suprathreshold stimuli evoke compound MEP and changes in compound MEP amplitudes reflect changes along the entire corticospinal pathway. Changes in MEP amplitude may be mediated by subcortical structures or spinal motor neurones. Additionally TMS mapping may detect an uneven expansion of the muscles representation on the motor cortex or a change of centre of gravity within the map.

Stimulus Response Curve Acquisition Protocol

SR curves were collected in all studies of this thesis. The protocol for acquiring SR curves is illustrated in figure 5, a representative example has been included as figure 6. TMS was delivered using intensities between 80 %rMT and 100 %MSO. The first stimulation intensity was chosen at random between the specified limits for each participant, with the subsequent stimuli delivered within -5 %MSO to +30 %MSO of the previous stimulus. This range was used to ensure the stimulator would not misfire when decreasing stimulation intensity at the shorter ISI. rMT was defined as the minimum TMS intensity required to evoke a reproducible MEP of greater than 50 µV in 5 of 10 consecutive trials (Rossini *et al.*, 1994).

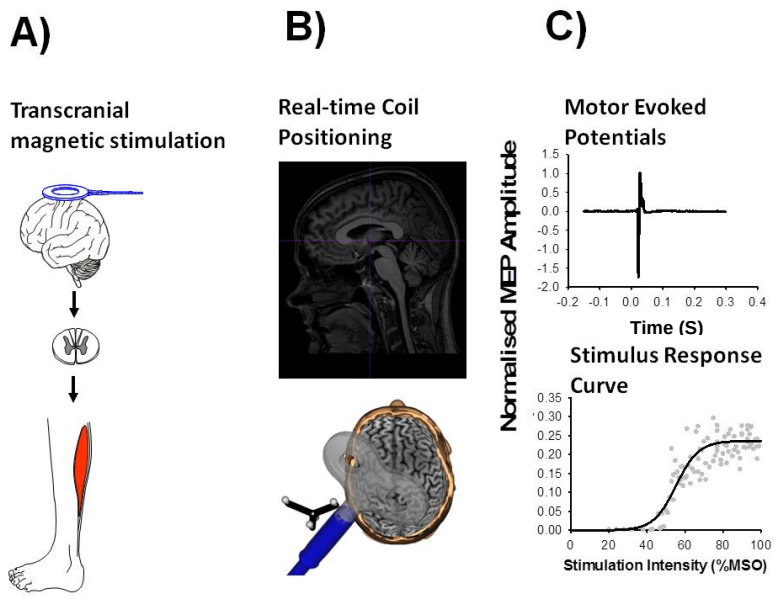

Figure 5. The procedure for generating a stimulus response curve. TMS is applied over the hotspot induces contraction a 'Motor Evoked Potential', in the contralateral muscle due to excitation of corticospinal and spinal motorneurones (A). The coil position and orientation with respect to the hotspot were monitored in real-time using frameless stereotaxy (BrainSight, Rogue Research Inc) (B). An exemplar motor evoked potential is shown in the upper section of panel (C) The amplitude of the motor evoked potential varies as a function of the TMS intensity. This relationship between amplitude and intensities is shown in the bottom figure of panel C as the grey dots which represent peak to peak amplitude of motor evoked potentials. The black line is the stimulus response curve. Each stimulus response curve is characterised by these parameters MEP_{min}, MEP_{max}, I_{50} and Slope.

Stimulus Response Curve Fitting

The MEP was defined as the peak to peak amplitude in the recorded EMG response between 20 and 60 ms after the presentation of the stimuli. The MEP amplitudes were plotted against stimulation intensity and the relationship modelled using a four-parameter Boltzmann sigmoid function:

$$MEP(I) = MEP_{\min} + \frac{MEP_{\max} - MEP_{\min}}{1 + e^{\frac{I_{50} - I}{S}}}$$

where MEP_{min} and MEP_{max} are the minimum and maximum asymptotes of the function; I_{50} is the percentage of maximal stimulator output at which the MEP is mid-way between MEP_{min} and MEP_{max} and S is the steepness of the relationship at I_{50}. The parameters of the SR curves are illustrated below in figure 6. MEP_{max} and MEP_{min} are illustrated by the green and red arrows while the I_{50} is illustrated by the blue arrow and the slope by the yellow line.

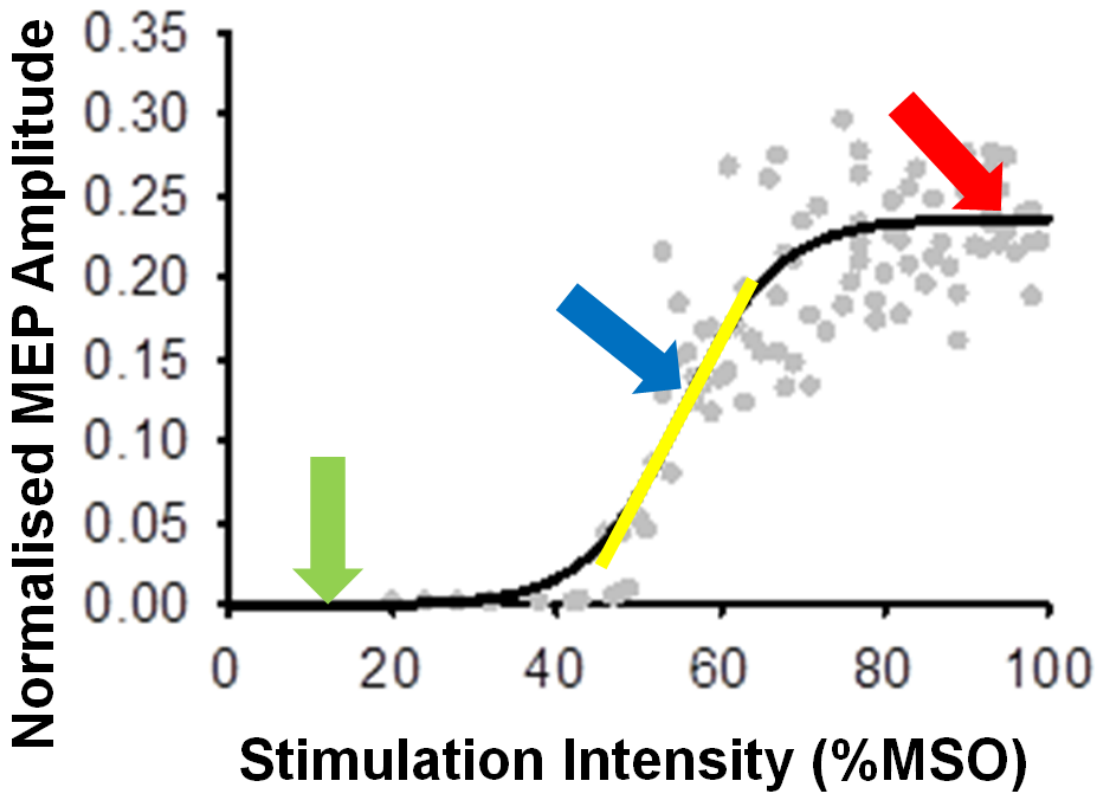

Figure 6. The parameters of the transcranial magnetic stimulation stimulus response curve. The red arrow denotes MEPmax, I50 and slope are donated by blue arrow and yellow line respectively. MEPmin is represented by the green arrow.

This model was fitted to our data with a Levenberg-Marquardt nonlinear least mean-square algorithm. Initial parameters were set to the following: MEP_{min} = min (MEP(I)), MEP_{max} = max(MEP(I)), I_{50} = 60 %MSO, and Slope = 5. Parameter constrains were applied as follows: MEP_{min} > 0; 25 < I_{50} 95 %MSO.

Repetitive Transcranial Magnetic Stimulation

rTMS was used in study one of this thesis to control for the neuromodulatory effects of rapid rate stimulation. rTMS is defined as trains of multiple stimuli delivered at greater than or equal to 1 Hz (Wassermann, 1998). Experimentally rTMS is used with a wide variety of scopes in equally varied situations; examples include to induce plasticity in

attempts to unpick the mechanism behind neuroplasticity (Wang *et al.*, 1996; Ziemann *et al.*, 2002) and to induce virtual lesions within the cortex to study the relationship between structure and function (Hilgetag *et al.*, 2001; Pascual - Leone *et al.*, 1999). The ability to induce physiological effects which outlast the period of stimulation differentiates rTMS from single pulse TMS.

The aim of study one was to reduce the acquisition time of the TMS SR curve. However, one cannot simply deliver stimuli as fast as possible due to the well-documented phenomenon whereby low frequency (1 Hz) repetitive TMS (rTMS) depresses cortical excitability (Chen *et al.*, 1997; Muellbacher *et al.*, 2000; Tergau *et al.*, 1997; Fitzgerald *et al.*, 2002). Consequently, it would be reasonable to postulate that SR curves constructed with MEPs that were acquired with short inter-stimulus intervals (ISIs) to be depressed. Such a depression would result in a curve with a lower plateau, a larger inflection point, and a milder slope. So, to control for the frequency dependent modulation of MEP amplitudes, focal rTMS was delivered to the left primary motor cortex with a Magstim Rapid^2 (Magstim Company, Dyfed, UK) using a custom made 90mm 'figure of eight' coil (batwing design; type no. 15411, Magstim company, Dyfed, UK). The stimulus was biphasic with a pulse width of 400 μs. The coil position and orientation were the same for all single pulse TMS studies. Coil position and orientation were monitored in real-time using frameless stereotaxy (Brainsight, Rogue Research Inc). I delivered three trains of 180 stimuli at 120% rMT, at a frequency of 1 Hz. This protocol is in accordance to the internationally recognised safety guidelines (Wassermann, 1998; Wassermann *et al.*, 1996a).

Data Normalisation

To ensure any conclusions drawn from statistical analysis are valid the data should be normalised. The process of normalisation allows the investigators to minimise the influence of unwanted variability, allowing the investigators to focus on the interesting variability. For example, normalising MEPs obtained in the active muscle to the maximal compound muscle action potential removes the influence of increase in amplitude and allows the team focus on any changes that have occurred as a result of activating the muscle.

In study one, MEP amplitudes were normalised to the maximal evoked response (M_{max}) to a peripheral nerve stimulus in the muscle of interest. This response was obtained using an electrical stimulus (Digitimer DS7AH, Digitimer Ltd, Welwyn Garden City, UK) delivered to the median or ulnar nerve, for FDI and abductor digiti minimi (ADM) respectively, or to Erb's point (for BB). In all cases, M_{max} was determined using supramaximal stimuli. The response was quantified as the peak to peak amplitude between 2-10 ms after the presentation of the stimulus. For studies two and three, based upon feedback from participants that electrical stimulation at Erb's point was painful, the normalisation process was changed. MEP amplitudes were normalised to the maximal MEP amplitude. To acquire maximal MEP I delivered 10 stimuli at 100 %MSO to the hotspot and then averaged the responses. All SR curves within the thesis are presented as plots of normalised MEP amplitude against %MSO. Normalising the x-axis to other factors such as %rMT did not significantly alter the result.

Data Analysis

In all studies involving motor learning performance was quantified as the RMS error between the target trace and the actual cursor position. Performance feedback was given at the end of each learning block, and analysed post-hoc as a marker of learning. The formula for calculating RMS error is shown below.

$$RMS\ Error = \sqrt{\frac{\sum |(x - y))|^2}{N_x}}$$

where x is the force transducer output and y is the target waveform

Individual MEPs were excluded from the construction of SR curves if there RMS EMG in the 100 ms prior to stimulation was greater than twice the mean RMS for that data set. MEPs were also excluded from SR curve fitting if they lay outside of the 95% prediction interval from the calculated curve fit. For study 1 all MEPs were normalised against the electrically elicited M_{max}. For studies two and three all MEPs were normalised to the maximal MEP. The normalisation measure was changed as participants reported electrical stimulation at Erb's point very painful.

To account for variability in background muscle activation, individual MEPs were excluded from the construction of SR curves or statistical analysis if their respective RMS EMG in the 100 ms prior to stimulation was greater than twice the mean RMS for that dataset. SR curves were excluded from the statistical analysis if the r^2 was less than 0.7 or the upper plateau was not achieved.

All statistical testing was conducted with NCSS 2007 v07.1.4 (Hintze, 2007), and all tests were considered significant at an alpha of 0.05. For the studies of this thesis area under the SR curve and the parameters of the SR curve were used as the dependent variables for statistical analysis. Results are reported as mean ± 1 standard deviation (S.D) unless otherwise stated.

Chapter IV – Rapid Acquisition of Transcranial Magnetic Stimulation Stimulus Response Curve

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Abstract

Transcranial magnetic stimulation is frequently used to construct stimulus response (SR) curves in studies of motor learning and rehabilitation. A drawback of the established method is the time required for data acquisition, which is frequently greater than a participant's ability to maintain attention. The technique is therefore difficult to use in the clinical setting. The aim of this study was to reduce the time of curve acquisition by determining the minimum acquisition time and number of stimuli required to acquire an SR curve.

SR curves were acquired from first dorsal interosseus (FDI) and abductor digiti minimi (ADM) at 6 interstimulus intervals (ISI) between 1.4 and 4 s in 12 participants. To determine if low-frequency rTMS might affect the SR curve, MEP amplitudes were monitored before and after 3 min of 1 Hz rTMS delivered at 120% of resting motor threshold in 12 participants. Finally, SR curves were acquired from FDI, ADM and Biceps Brachii (BB) in 12 participants, and the minimum number of stimuli was calculated using a sequential MEP elimination process.

There were no significant differences between curves acquired with 1.4 s ISI and any other ISI. Low frequency rTMS did not significantly depress MEP amplitude ($p=0.87$). On average, 61 ± 18 (FDI), 60 ± 16 (ADM) and 59 ± 16 (BB) MEPs were needed to construct a representative SR curve.

This study demonstrates that reliable SR curves may be acquired in less than 2 min. At this rate, SR curves become a clinically feasible method for assessing corticospinal excitability in research and rehabilitation settings.

Introduction

Transcranial magnetic simulation (TMS) is frequently used to assess the state of corticospinal excitability (CSE). Since its introduction in the mid 1990's, the stimulus response (SR) curve has become increasingly used in studies of motor learning and neurorehabilitation (Devanne *et al.*, 1997; Valls - Solé *et al.*, 1992; Boroojerdi *et al.*, 2001a). SR curves are typically acquired by delivering multiple stimuli in pseudorandomised bins of stimulation intensity. A full range of stimulation intensities are delivered from just below motor threshold until either the motor evoked potential (MEP) amplitude plateaus or the maximum stimulator output (MSO) is reached. The MEP amplitudes for each stimulus intensity level are then averaged and a 3, 4 or 5 (Barsi *et al.*, 2008; Malcolm *et al.*, 2006; Pitcher *et al.*, 2003) parameter Boltzmann-like model is fit to the mean data using a nonlinear least squares algorithm to produce the SR curve (Devanne *et al.*, 1997; Valls - Solé *et al.*, 1992; Boroojerdi *et al.*, 2001a).

A limitation of the traditional method of curve acquisition is the time required to collect the data. SR curves are used to measure the state of CSE which is known to fluctuate within the time required to acquire a curve, typically in excess of 10 minutes. The source of this fluctuation in CSE is not known however it is believed not to arise from autonomic (Filippi *et al.*, 2000), cardiac (Ellaway *et al.*, 1998), or respiratory (Ellaway *et al.*, 1998) signals. Furthermore, the amplitude of MEPs used to construct SR curves is known to be mediated by attention (Rosenkranz & Rothwell, 2006; Rosenkranz & Rothwell, 2004) and drowsiness (Andersen *et al.*, 2008). Therefore, in order for SR curves to provide an accurate reflection of CSE, SR curves should be acquired in the shortest possible time. Additionally, due to their lengthy acquisition time SR curves are

not practical in studies of motor learning, where one expects short-term changes in CSE, or in the clinical setting.

Commonly, investigators attempt to reduce acquisition time by reducing either the number of stimuli per bin or the number of bins (Liepert *et al.*, 2003; Ward *et al.*, 2006; Pearce *et al.*, 2012; Ray *et al.*, 2002). However, there has been no systematic study of the minimum number of stimuli required for an SR curve. Additionally, the use of blocked acquisition protocols has been shown to influence the acquisition of SR curve. SR curves acquired using blocked decreasing stimulation intensity protocols were shifted significantly to the left suggestive of increased CSE when compared to a blocked increasing protocol, showing that it is possible to significantly alter the SR curve via the method used to acquire them (Möller *et al.*, 2009). Möller *et al.* (2009) recommend acquiring SR curves using stimulation intensities determined randomly on a pulse-by-pulse basis. When seeking to reduce the acquisition time, one cannot simply deliver stimuli as fast as possible due to the well-documented phenomenon whereby low frequency (1 Hz) repetitive TMS (rTMS) depresses cortical excitability (Chen *et al.*, 1997; Muellbacher *et al.*, 2000; Tergau *et al.*, 1997; Fitzgerald *et al.*, 2002). Consequently, it would be reasonable to postulate that SR curves constructed with MEPs that were acquired with short interstimulus intervals (ISIs) to be depressed. Such a depression would result in a curve with a lower plateau, a larger inflection point, and a milder slope. MEPs from proximal muscles are also known to have greater variability than those of distal muscles (Kiers *et al.*, 1993), and this variability is likely to be reflected in the SR curve. Consequently, proximal muscles may require more stimuli and thus more time, to produce a curve that accurately reflects CSE.

The aim of the present study was to reduce the acquisition time of the SR curve by determining the shortest ISI at which stimuli may be presented without depressing CSE and by determining the minimum number of stimuli required to acquire an SR curve. To determine the influence of ISI on the SR curves I tested the hypothesis that the inflection point would increase while angle of the slope and plateau decreases with ISI. Second, to control for the effect of frequency dependent depression in CSE I tested the hypothesis that 3 minutes of 1 Hz rTMS would depress the amplitude of the MEP. Finally, I aimed to determine the minimum number of stimuli required for a representative SR curve and I tested the hypothesis that SR curves acquired from proximal muscles would require more stimuli than those of distal muscles.

Methods

Participants

Healthy participants with a mean age of 21 ± 3 years were recruited for the study (n = 36, 18 male, 75% right handed, sample of convenience)**.** All participants were screened for TMS safety with a modified version of the TMS adult safety survey (Keel *et al.*, 2001) and all gave informed written consent. The study protocol was approved by the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN_11-0444) and all experiments were conducted in accordance with the Declaration of Helsinki.

Electromyography

Surface electrodes (Blue Sensor N, Ambu®, Denmark) were placed in a bipolar montage over the First Dorsal Interosseus (FDI), Abductor Digiti Minimi (ADM), and Biceps Brachii (BB). The EMG signals were band-pass filtered (0.5 - 2 kHz), sampled at 5 kHz, and amplified using custom amplifiers. All data were stored on a computer for offline analysis.

Transcranial Magnetic Stimulation

Motor evoked potentials (MEP) were elicited with a biphasic TMS pulse (Magstim Rapid^2, The Magstim Company, Dyfed, UK) from a custom-made 90mm *'figure of eight'* coil (batwing design; type no. 15411, Magstim company, Dyfed, UK). Magnetic stimuli were delivered over the cortical area which evoked maximal MEPs in the particular muscle of interest, commonly referred to as the '*hotspot'*. The coil was positioned over the respective hotspot with the handle pointing backwards at an angle of

45° from the midline (Brasil - Neto *et al.*, 1992) inducing a posterior-anterior current (Kammer *et al.*, 2001). Coil position and orientation were monitored in real time using frameless stereotaxy (Brainsight, Rogue Research Inc). In an attempt to control for attention mediated variation in MEP amplitude participants were asked to ensure the coil was as close to the hotspot as possible with the aid of feedback from the Brainsight system.

Stimulus Response Curve Modelling

SR curves were collected in experiments 1 and 3. MEP amplitudes were plotted against stimulation intensity and the relationship modelled using a four-parameter Boltzmann sigmoid function:

$$MEP(I) = MEP_{\min} + \frac{MEP_{\max} - MEP_{\min}}{1 + e^{\frac{I_{50} - I}{S}}}$$

where MEP_{min} and MEP_{max} are the minimum and maximum asymptotes of the function; I_{50} is the percentage of maximal stimulator output (% MSO) at which the MEP is mid-way between MEP_{min} and MEP_{max} and S is the steepness of the relationship at I_{50}.

This model was fitted to our data with a Levenberg-Marquardt nonlinear least mean-square algorithm. Initial parameters were set to the following: MEP_{min} = min (MEP(I)), MEP_{max} = max(MEP(I)), I_{50} = 60 %MSO, and Slope = 5. Parameter constrains were applied as follows: MEP_{min} > 0; 25 < I_{50} 95 %MSO.

Experimental Protocols

The Effect of Interstimulus Interval on the Stimulus Response Curve

In 12 participants, data were acquired to assess the effect of ISI on SR curve acquisition. Data were acquired from FDI and ADM at; 1.4, 1.6, 1.8, 2, 3 and 4 s ISI. SR curves were acquired using varying stimulation intensities determined pseudorandomly on a pulse-by-pulse basis between 80 % resting motor threshold (rMT) until 100 %MSO. The first stimulation intensity was chosen at random between the specified limits for each participant, with the subsequent stimuli delivered within -5 %MSO to +30 %MSO of the previous stimulus. This range was used to ensure the stimulator would not misfire when decreasing stimulation intensity at the shorter interstimulus intervals. Each SR curve was constructed from 100 MEPs.

Each participant underwent 2 sessions separated by at least 7 days. All participants completed both sessions. Three SR curves were acquired from each muscle during each visit with a randomised presentation of ISIs to prevent an ordering effect.

Corticospinal Excitability following 1 Hz rTMS

In 12 participants MEP amplitudes were monitored pre and post a 3 min bout of 1 Hz rTMS delivered at 120 %rMT to determine if a short bout of rTMS would depress CSE. MEPs were elicited pre and post rTMS from the right FDI, using TMS delivered at 120 %rMT with an ISI of a 10 s. This protocol was repeated three times in a single session with no fewer than 5 min between each train of stimuli to avoid potential cumulative effects due to the rTMS.

Minimum Number Required for a Stimulus Response Curve

The minimum number of stimuli required to construct a representative SR curve was determined in 12 participants. Data were acquired from FDI, ADM and BB with a 5 s ISI. SR curve were acquired using varying stimulation intensities determined pseudorandomly on a pulse-by-pulse basis between 80% rMT and 100 %MSO. The stimuli were pseudorandomised in the same manner as the previous experiment. Each SR curve was constructed from 120 MEPs and 3 curves were acquired from each muscle.

Data Analysis

The amplitude of the MEP was defined as the peak to peak amplitude in the recorded EMG response between 20 and 60 ms after the presentation of the magnetic stimulus. All MEPs obtained with the coil more than 2.5 mm and/or 5° away from the hotspot were removed from analysis. In total, I removed 17 MEPs across all experiments as a result of this procedure. To account for variability in background muscle activation, individual MEPs were excluded from the construction of SR curves if their respective root mean square (RMS) EMG in the 100 ms prior to stimulation was greater than twice the mean RMS for that dataset. SR curves were excluded from the statistical analysis if there r^2 was less than or equal to 0.7 or the upper plateau was not achieved in the curve. To ensure valid statistical comparisons across participants, MEP amplitudes were normalised to the maximal evoked response (M_{max}) to a peripheral nerve stimulus in the muscle of interest. This response was obtained using an electrical stimulus (Digitimer DS7AH, Digitimer Ltd, Welwyn Garden City, UK) delivered to the median or ulnar nerve (for FDI and ADM, respectively) or to Erb's point (for BB). In all cases, M_{max}

was determined using supramaximal stimuli. The response was quantified as the peak to peak amplitude between 2—10 ms after the presentation of the stimulus.

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4 (Hintze, 2007), and all tests were considered significant at an alpha of 0.05. Results are reported as mean ± standard deviation (S.D).

A two-way repeated measures ANOVA (2w-rmANOVA) using within subject factors muscle (FDI and ADM) and interstimulus interval (*1.4, 1.6, 1.8, 2, 3 and 4 s*) was used to assess the effect of stimulation frequency on each of the four parameters of SR curves. A 2w-rmANOVA using within subject factors *time (Baseline, 1 Min Post, 2 Min Post and 3 Min Post)* x *trial number* (*1, 2 and 3*) was used to assess the effect 3 minutes of rTMS on the amplitude of MEPs. An iterative data elimination process, illustrated in figure 7 was used to determine the minimum number of stimuli required when acquiring SR curves. The minimum of stimuli required for an SR curve was defined as the number of stimuli at the iteration in the data elimination process where the new curve fit left the 95% confidence intervals calculated for the original curve fit and remained outside on three consecutive iterations. A 1w-rmANOVA with the factor muscle (*FDI, ADM and BB*) was used to test the hypothesis that SR curves from proximal muscles would require more stimuli than curves from distal muscles. Furthermore, I also examined the test-retest reliability of the parameters of the SR curve generated with the minimum number of MEPs using the intraclass correlation coefficient (ICC), following McGraw and Wong (1996).

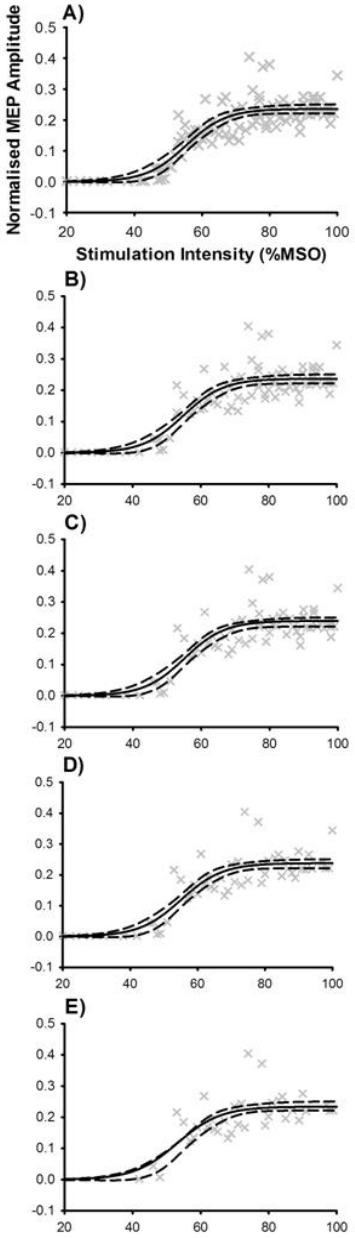

Figure 7. A representative example of the data elimination process used to calculate the minimum number of stimuli. A) All 100 MEPs are used to calculate the SR curve (solid line) with 95% confidence interval of the fit (dashed lines). B-E) 84, 68, 52 and 36 data points used for the SR curve fit (solid line), superimposed with the original fit's confidence interval (dashed lines).

Results

The Stimulus Response Curve is Invariant to Interstimulus Interval

In order to determine the effect of ISI on the SR curve, data were acquired from FDI and ADM at six different ISIs. Representative data from a single participant is illustrated in figure 8. SR curves were observed to be highly reproducible within and between sessions. Statistical analysis revealed the parameters of the SR curve to be invariant of ISI; MEP_{min} ($F_{5, 12} = 0.60$, $p = 0.70$), MEP_{max} ($F_{5, 12} = 1.31$, $p = 0.26$), I_{50} ($F_{5, 12} = 1.34$, $p = 0.25$), Slope ($F_{5, 12} = 0.96$, $p = 0.44$) and r^2 ($F_{5, 12}=1.56$, $p=0.17$).

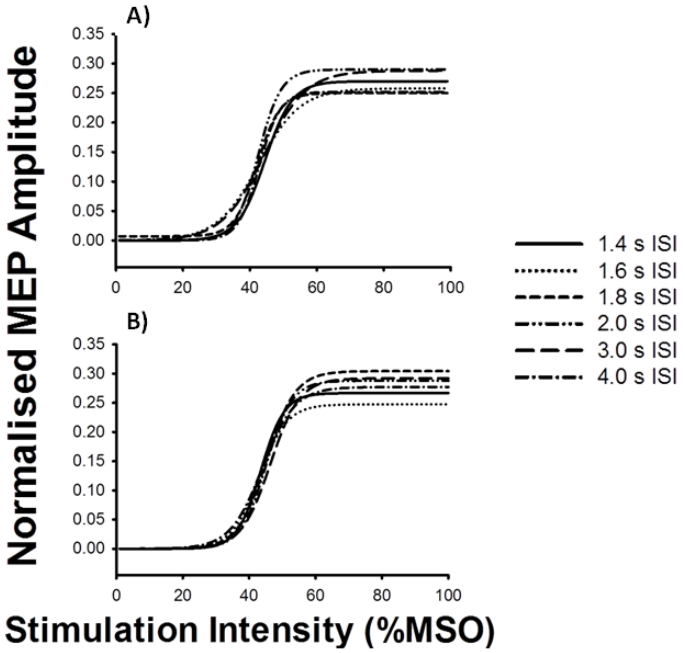

Figure 8. Representative data from a single participant showing the effect of interstimulus interval on the stimulus response curve. All MEP amplitudes were normalised to M_{max}. Figures (A) and (B) illustrate the data for FDI and ADM, respectively. A two way repeated measures ANOVA indicates the SR curve parameters are invariant to interstimulus interval for both muscles ($p > 0.05$).

A Short Bout of 1 Hz rTMS does not Depress Corticospinal Excitability

In order to control for the influence of frequency dependent depression in CSE on MEP amplitude, MEP amplitudes were compared pre and post 3 min of rTMS. Figure 9A is a

representative dataset from a single participant while figure 9B depicts the grouped data. Statistical analysis revealed that 3 min of 1 Hz rTMS did not significantly depress CSE as indicated by MEP amplitude ($F_{3,84} = 0.94$, $p = 0.39$), nor was there a cumulative effect of the repetitions of the protocol on MEP amplitude ($F_{2, 84} = 0.31$, $p = 0.82$) or any interaction *time point* x *trial number* ($F_{3,84} = 0.97$, $p = 0.45$).

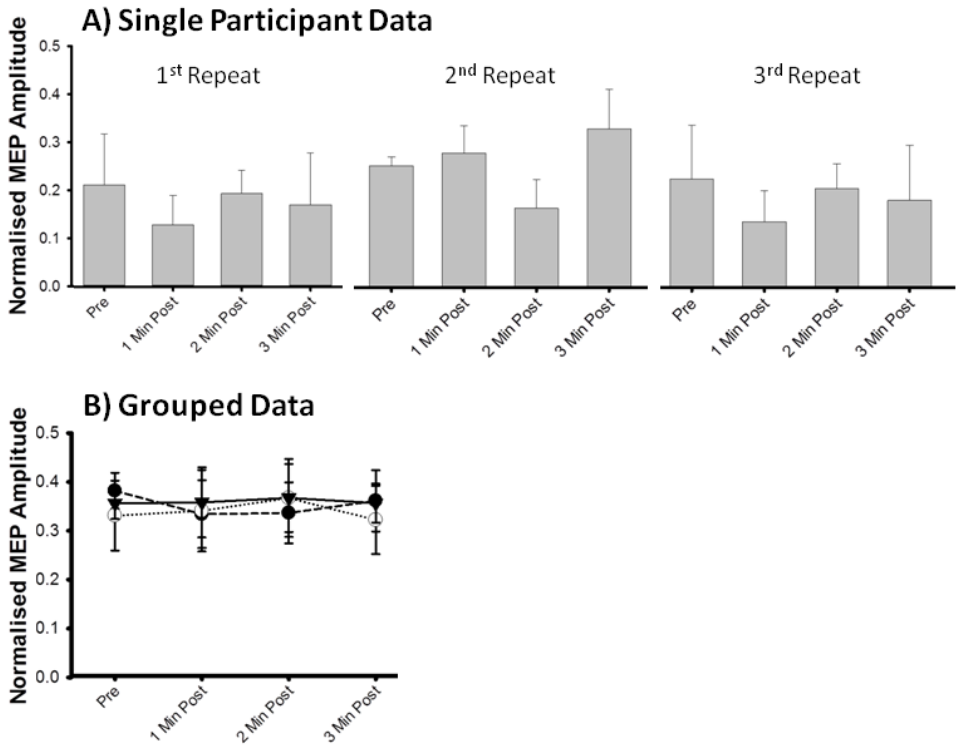

Figure 9. Mean MEP amplitude before and after a 3 min period of 1 Hz rTMS at 120% rMT. All MEP amplitudes were normalised to the electrically elicited Mmax. (A) Representative data from 1 participant showing it is possible to reduce MEP amplitude although this effect is neither robust nor consistent. (B) Group data showing the lack of effect of 3 minutes of rTMS on MEP amplitude (filled circles, open circles and triangles represent the first, second and third repeats, respectively)

Minimum Number Required for Stimulus Response Curves

The minimum number of stimuli needed to acquire an SR curve for FDI, ADM and BB is shown in figure 10. The box-plot shows the group 25 and 75 percentile (box) with mean (thick black line) and 1 S.D. (error bars). Across all participants, the number of

stimuli needed to produce an SR curve was 61 ± 18 (FDI), 60 ± 16 (ADM), and 59 ± 16 (BB). A 1w-ANOVA comparing the minimum number of stimuli between muscles revealed that there was no significant difference between muscles ($F_{2,108} = 0.01$, $p = 0.99$). In all cases the correlation was either strong or very strong, indicating very good reliability. These results are summarised in Table 1.

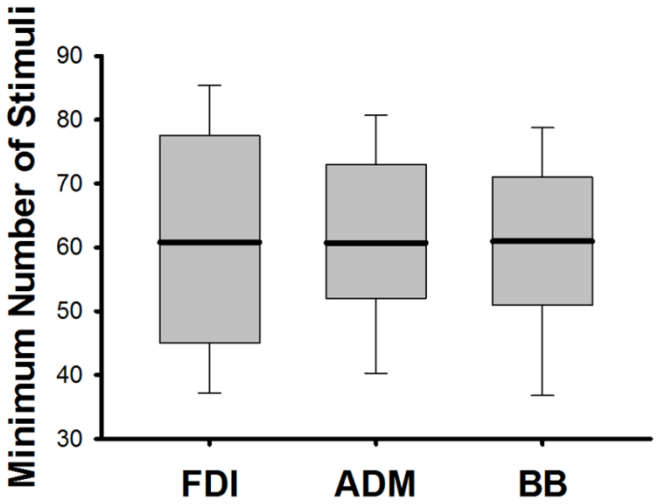

Figure 10. Group results for the minimum number of stimuli needed when acquiring SR curves for first dorsal interosseus (FDI), abductor digiti minimi (ADM) and biceps brachii (BB). The box-plot shows the group 25 and 75 percentiles (box) with means (thick black line) and 1 S.D from the mean (error bars). On average, the number of stimuli needed to produce a representative SR curve was 61 ± 18 (FDI), 60 ± 16 (ADM), and 59 ± 16 (BB).

Table 2. Intraclass correlation coefficients describing the test-retest reliability of the parameters of three stimulus response curves generated with the minimum number of stimuli in a single session in first dorsal interosseus (FDI), abductor digiti minimi (ADM) and bicep brachii (BB). In all cases the correlation ranges from strong to very strong, indicating very good reliability.

Muscle	Parameter	ICC
FDI	MEP_{max}	0.87
	Slope	0.76
	I_{50}	0.90
	MEP_{min}	0.74
ADM	MEP_{max}	0.91
	Slope	0.90
	I_{50}	0.87
	MEP_{min}	0.85
BB	MEP_{max}	0.79
	Slope	0.88
	I_{50}	0.72
	MEP_{min}	0.83

Discussion

The present study demonstrates it is feasible to acquire a reliable SR curves in less than 2 minutes. This was made possible by reducing the ISI and determining the minimum number of stimuli required to acquire an SR curve. To determine the influence of ISI on SR curve acquisition I tested the hypothesis that CSE would be depressed with shorter ISIs, as evidenced by an increased I_{50} and decreased slope, and a lower plateau (i.e. decreased MEP_{max}). This hypothesis was not supported by the data, indicating that SR curves are invariant to stimuli delivered at or slower than 1.4 s ISI. Due to the well-known depressive effect of 1 Hz rTMS on CSE, I also tested the hypothesis that a short bout (3 min) of 1 Hz rTMS would decrease MEP amplitude. This hypothesis was also not supported by the data, suggesting that low-frequency rTMS requires more than 3 min to depress CSE. Finally, I aimed to determine the minimum number of stimuli required for an SR curve. Due to the greater variability of proximal muscles compared with distal muscles (Kiers *et al.*, 1993), I hypothesised that SR curves acquired from proximal muscles would require more stimuli. All muscles tested in the present study required approximately 60 stimuli to produce a curve and there were no significant differences found between muscles. The present study demonstrates that it is possible to acquire reliable SR curves with on average 76 stimuli (gross mean + 1 S.D) and that these stimuli can be delivered using an ISI equal to or greater than 1.4 s.

How Fast can one Stimulate?

All participants tolerated the rTMS protocol well and there were no complications at any of the stimulation frequencies. However, during pilot testing participants frequently reported difficulty maintaining attention when SR curves were acquired using 5 s ISI.

Accordingly, to avoid increased MEP variability as a result of CSE arising from changes in attention (Rosenkranz & Rothwell, 2006; Rosenkranz & Rothwell, 2004) and/or drowsiness (Andersen *et al.*, 2008), I opted to use a maximum ISI of 4 s. In addition, participants in our pilot testing reported stimuli delivered at 1 and 1.2 s ISI to be uncomfortable, with some participants reporting an increase in anxiety, which has previously been shown to increase CSE (Greenberg *et al.*, 2000). For these reasons, the minimum ISI in the present study was capped at 1.4 s.

The relationship between ISI and MEP amplitude is well documented, with 1 Hz rTMS demonstrated to reduce CSE (Chen *et al.*, 1997; Tergau *et al.*, 1997; Muellbacher *et al.*, 2000; Fitzgerald *et al.*, 2002). In contrast, when stimuli are delivered at an ISI of less than 0.2 s, TMS has been shown to increase CSE (Pascual-Leone *et al.*, 1994; Tergau *et al.*, 1997). These studies typically involve long trains of stimuli requiring several minutes of constant stimulation at intensities above rMT. For example, Chen *et al.*, (1997) demonstrated that 15 minutes of 1 Hz rTMS depressed the amplitude of MEPs by approximately 20% for 15 minutes after stimulation was ceased. As our interest was reducing SR curve acquisition time, I investigated how the motor cortex would respond to 3 minutes of 1 Hz rTMS at 120 %rMT. Our results are consistent with those of Maeda *et al.*, (2000), who report being unable to evoke a robust and significant depression of CSE following 240 stimuli delivered at 120 %rMT. They attributed their result to the relatively small number of stimuli and suggested that, in order to combat the inter-individual variability and evoke a consistent depression of CSE, it may be necessary to administer in excess of 1,600 stimuli at suprathreshold intensities to significantly overcome the variability in the modulatory effects of rTMS on CSE.

There is a remote possibility that the test pulses delivered after each short bout of rTMS may have reduced or abolished the depressive effect of the rTMS. An example of such metaplasticity has been observed by Huang et al. (2010), who used theta-burst stimulation (TBS) to abolish corticospinal excitability by presenting an inhibitory TBS paradigm one minute after an excitatory TBS paradigm. It is possible that the test pulses used to assess corticospinal excitability following each 3 min bout of rTMS similarly induced metaplasticity. Whilst I cannot exclude this possibility, I believe a similar effect is unlikely because several other studies have used similar test TMS pulses following rTMS and have shown changes in corticospinal excitability (Chen *et al.*, 1997; Fitzgerald *et al.*, 2002; Maeda *et al.*, 2000). However, in each of these cases, the number of stimuli and length of exposure to rTMS was much greater than that used in the present study.

The lack of effect of stimulation frequency on the parameters of the SR curve could be attributed to the low number of stimuli involved in SR curve acquisition and/ or the nature of stimulation during SR curve acquisition. When stimulation intensity is randomised, both sub- and suprathreshold stimuli are delivered to the motor cortex. This contrasts with stimuli designed to inhibit cortical drive, which are delivered as long trains of suprathreshold stimuli, typically in excess of 800 stimuli (Chen *et al.*, 1997; Tergau *et al.*, 1997; Muellbacher *et al.*, 2000; Pascual-Leone *et al.*, 1994; Fitzgerald *et al.*, 2002; Maeda *et al.*, 2000), which far exceed the number of stimuli required to construct the SR curve. As a result, it is very unlikely that stimuli delivered in the manner proposed in the present study to obtain an SR curve would depress CSE.

Minimum Number of Stimuli Required for the Stimulus Response Curve

Our data suggest that as few as 32 but as many as 96 MEPs are required to produce an SR curve. It is interesting to note that there is considerable overlap in the minimum number of stimuli between the proximal and distal muscles assessed here. This surprising result suggests that the expected proximal-distal variance in MEP amplitude variability does not have an effect on the number of stimuli required to acquire an SR curve. Importantly, SR curves generated with the reduced number of stimuli were found to be highly reproducible within a single session, with the ICC across all parameters and muscles ranging from a strong to very strong correlation. This result suggests that rapidly acquired SR curves are a reliable means of assessing CSE.

Benefits of Reduced Acquisition Time

The present study demonstrates that it is feasible to significantly reduce the time required to acquire the SR curve. By reducing acquisition time, the temporal resolution of this method is increased such that changes in CSE following motor learning and/or rehabilitation may be more accurately determined. A second benefit to reduced acquisition time is that attention moderated variations in MEP amplitude (Kamke *et al.*, 2012) are diminished. Finally, our demonstration that SR curves can be acquired in less than two minutes means that the technique becomes much more suitable for use in elderly and patients populations where, at present, attention deficits limit the use of TMS.

Is it possible to further reduce the acquisition time of SR curves? Yes, I would suggest it is possible to further reduce the acquisition time of SR curves. Any further reductions in ISI are not the way to proceed as our participants reported SR curves acquired using ISIs less than 1.4 s ISI anxiety inducing, and anxiety has been shown to raise (Greenberg *et al.*, 2000). Rather, to further reduce the acquisition time of SR curves without skewing the curve one needs to reduce the variability within MEPs. The sources intra-individual variability within MEPs are well documented, with factors such as coil position and orientation (Ellaway *et al.*, 1998), varying desynchronisation of the efferent volley (Magistris *et al.*, 1998), stimulation frequency (Maeda *et al.*, 2000), stimulation intensity (Fitzgerald *et al.*, 2002) and subthreshold activation of corticospinal neurons (Wassermann, 2002), all known to contribute. In order to minimise the influence of this variability participants are commonly asked to maintain a low level of muscle activation (e.g. 5-10% maximum voluntary contraction) (Rösler, 2001; Wassermann *et al.*, 1996b). Holding a low level precontraction in the muscle of interest has been demonstrated reduce the relative variability and facilitate in MEP amplitudes without saturating the response (Carroll *et al.*, 2001; Rösler, 2001; Hess *et al.*, 1987). Therefore, one could postulate that the minimum number of stimuli required for an SR curve could be further reduced if SR curves are acquired during a stable precontraction in the muscle of interest, however further work is warranted to experimentally valid this hypothesis.

Conclusions and Recommendations

In summary, I have demonstrated the feasibility of acquiring stimulus response curves in less than 2 min. The present study demonstrated that it is possible to construct an SR

curve with, on average, 76 stimuli (gross mean + 1 S.D), that these stimuli can be delivered using an ISI less than or equal to 1.4 s without affecting the acquisition of SR curves and that the resulting curves are a reliable means of assessing corticospinal excitability.

Chapter V – Learning Induced Plasticity in Proximal and Distal Muscles

Abstract

Distal muscles have larger cortical representation in the primary motor cortex and more corticospinal projections from the primary motor cortex compared with proximal muscles. This increased representation and larger projection could suggest a greater potential for learning induced changes in CSE for distal muscles compared to proximal muscles. The aim of this study was to investigate changes in CSE following visuomotor learning in proximal and distal muscles.

In 16 healthy participants, I acquired three stimulus response (SR) curves for first dorsal interosseus (FDI) and biceps brachii (BB). All participants were assessed before and after an isometric visuomotor tracking task involving either index finger abduction or elbow flexion. Motor learning performance was quantified by the root-mean-square tracking error across five different waveforms.

Across all participants, tracking error decreased by 23 ± 17% for FDI and 28 ± 17% for BB indicating the task was learnt ($p<0.05$) with no significant difference between muscles ($p>0.05$). CSE changes were variable between muscles; 4 participants exhibited increased CSE in FDI and only 2 for BB respectively.

There were a greater number of participants in whom CSE increased for the distal muscle whilst motor learning performance was not significantly different between muscles. It is important to note that so few participants exhibited increased CSE following motor learning suggesting individual differences should be better reported in

studies of motor learning involving TMS. In addition, the inter-individual differences in the changes in CSE should be further examined.

Introduction

The neural pathways involved in voluntary motor control are constantly changing according to the demand from our lives. This capacity for change termed neuroplasticity can be defined as "the ability of the CNS to respond to intrinsic and extrinsic stimuli by reorganising its structure, function and connections" (Cramer *et al.*, 2011) and it underlies our ability to adapt existing behaviours and/ or acquire novel skills through practice. Examples of skills shown to induce neuroplastic change include reading Braille (Pascual-Leone & Torres, 1993), playing a musical instrument (Schlaug *et al.*, 1995) and ballet dancing (Nielsen *et al.*, 1993). Experimental studies of motor learning have shown that it is possible to induce neuroplastic changes after a single session of training on a ballistic learning task (Muellbacher *et al.*, 2001), a sequence learning task (Pascual - Leone *et al.*, 1995) and a visuomotor learning task (Perez *et al.*, 2004) respectively. Neuroplasticity induced following learning is expressed as a change in the efficacy of synaptic transmission in the corticospinal tract for the muscles involved in the task. This change in synaptic efficacy is often referred to as a change in corticospinal excitability (CSE).

It is unclear whether learning induced changes in CSE are influenced by the anatomical and physiological differences between the regions of the primary motor cortex controlling proximal and distal muscles of the upper limb. Proximal muscle representations are known to have fewer corticospinal projections (Palmer & Ashby, 1992) and these projections arise from a smaller region of the motor cortex (Penfield & Boldrey, 1937; Wassermann *et al.*, 1992). Comparing learning induced changes in CSE in the proximal and distal representations of the upper limb might permit us to speculate

about the contributions of the different regions of the motor cortex during rehabilitation of whole limb movements such as reach to grasp. To date this subject has received little examination, only Krutky & Perreault (2007) have attempted to address this issue. They trained participants elbow, wrist and finger on a ballistic motor learning task and report that induced changes were greatest in distal muscles compared with proximal muscles.

The aim of the present study was to compare learning induced changes in CSE for the neural pathways controlling proximal and distal muscles of the upper limb in context of visuomotor learning. I tested the hypothesis that there would be a greater change in CSE for the neural pathways of finger compared with the BB as indicated by increases in area under the curve (AuC), MEP_{max}, slope and a decrease in I_{50}. To test this hypothesis I had participants complete two sessions on a visuomotor tracking task, one on their BB the other on the FDI. CSE was assessed using the TMS SR curve.

Methods

Participants

Healthy participants with a mean age of 22 ± 5 years were recruited for the study (n=17, 59% female, 14 right hand dominant, sample of convenience). All participants were screened for contraindications to TMS using a modified version of TMS adult safety screen (Keel *et al.*, 2001). All participants gave informed written consent to participate in the study. Participants were excluded from the study if their rMT was greater than 70% maximal stimulator output (MSO). The study protocol was approved by the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN_11-0444) and all experiments were conducted in accordance with the Declaration of Helsinki. Participants hand preference was determined using the Edinburgh Handedness Inventory (Oldfield, 1971).

Electromyography

Surface electrodes (Blue Sensor N, Ambu®, Denmark) were placed in a bipolar montage over FDI and BB muscle in the dominant limb. The electromyography signals were band-pass filtered (10 - 1 kHz), sampled at 4 kHz and amplified using custom amplifiers. All data were stored on a computer for offline analysis.

Transcranial Magnetic Stimulation

MEPs were elicited with a biphasic TMS pulse (Magstim Rapid^2, The Magstim Company, Dyfed, UK) from a custom-made 90mm *'figure of eight'* coil (batwing design; type no. 15411, Magstim company, Dyfed, UK). Magnetic stimuli were delivered over the cortical area that evoked maximal MEPs in the FDI or BB, referred to

as the '*hot spot'*. The coil was positioned over the respective hot spot with the handle pointing backwards at an angle of 45° from the midline (Brasil - Neto *et al.*, 1992) inducing a posterior-anterior current (Kammer *et al.*, 2001). Coil position and orientation were monitored in real time using frameless stereotaxy (Brainsight, Rogue Research Inc).

Stimulus Response Curves

SR curves were acquired using a computer-controlled semi-automated procedure (for detailed explanation see Mathias *et al.*, 2014). Briefly, SR curves were acquired using varying stimulation intensities determined pseudorandomly on a pulse-by-pulse basis between 80 %rMT until 100 %MSO. All curves were acquired in less than 2 min. rMT was defined as the minimum stimulation intensity required to elicit MEPs of greater than or equal to 50 µV on 5 out of 10 occasions in the relaxed muscle (Rossini *et al.*, 1991). MEP amplitudes were plotted against stimulation intensity and the relationship modelled using a four-parameter Boltzmann sigmoid function:

$$MEP(I) = MEP_{\min} + \frac{MEP_{\max} - MEP_{\min}}{1 + e^{\frac{I_{50} - I}{S}}}$$

where MEP_{min} and MEP_{max} are the minimum and maximum asymptotes of the function, I_{50} is the %MSO at which the MEP is mid-way between MEP_{min} and MEP_{max} and S is the steepness of the relationship at I_{50}.

Visuomotor Training

The visuomotor training task used in the present study was based upon a task previously shown to increase CSE (McAllister *et al.*, 2011; Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005). The task involved tracking a target waveform with a cursor shown on the computer screen through isometric of varying degrees of force. At the beginning of the task participants were verbally instructed how to complete the task, participants were not allowed to practice the task prior to commencing the training.

In each session participants performed five blocks of training, each block lasting 4 min with 2 min rest between blocks to minimise fatigue. Each training block consisted of tracking twenty waveforms, each lasting twelve seconds. To minimise the influence of fatigue the waveform amplitudes were normalised to 20 %MVC for each participant, furthermore, the first and last second of each waveform returned 0 %MVC. The participants performance on the task was assessed prior to and post training using separate blocks of five assessment waveforms.

For studies of involving FDI, participants were seated in a comfortable chair with the hand outstretched approximately 20 cm in front of them and the distal inter-phalangeal joint placed against the force transducer (NL 62 - 5 kg, Digitimer Ltd, Welwyn Garden City, UK) and secured in place with Velcro. A drawing of the experimental set-up has been included as figure 11 for clarity. For studies involving BB, participants were seated in a comfortable chair with their forearm supported in a custom rig. Participants were instructed to keep their wrist in the neutral position during the learning. A strap was attached over the wrist to secure the arm in place. Force was recorded using a

custom made torsion bar. The force signal was high pass filtered at 30 Hz, amplified x1000, digitised at 4 kHz and stored on a computer of offline analysis.

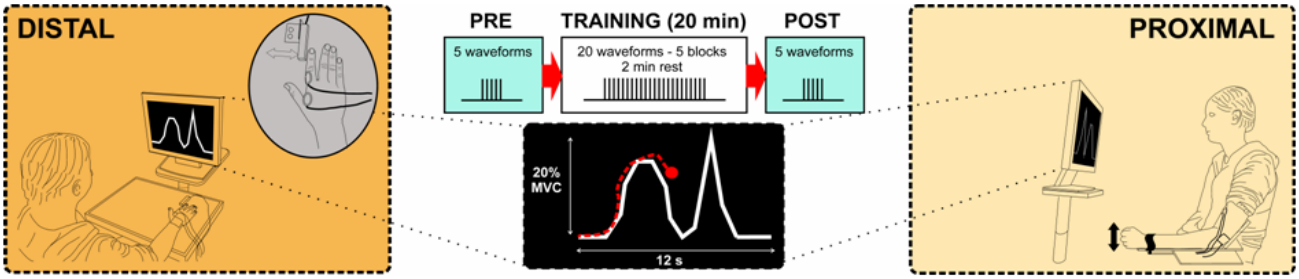

Figure 11. Experimental setup for visuomotor learning in Biceps Brachii and First Dorsal Interosseous. Participants were instructed to track the waveforms (white line in centre section) using isometric of the relevant muscles (track shown as red line). The training paradigm consisted of 5 assessment waveforms delivered either side of 5 blocks of training. Each training block consisted of 20 waveforms delivered in a random order. Performance was quantified as the absolute error between the target and actual waveform. Thanks to Mark van de Ruit for the figure.

Online performance feedback in the form of absolute error was given to control for fluctuations in attention during the learning. Error feedback was given in the form of a bar chart displayed on the computer screen after each waveform. The bar chart was updated so after each waveform so the participant saw their improvement in performance across each training block for each waveform.

Protocol

Participants completed two sessions examining learning induced changes in CSE in proximal and distal muscles. Participants were randomly allocated to either proximal muscle (Biceps Brachii) or FDI in the first session. At the beginning of each session, I determined participant's MVC and rMT for muscle studied. To examine learning-induced changes in CSE I acquired three TMS SR curves prior to and post

training in all participants. To control for the influence of fatigue I acquired MVC after training as well. Participants completed the protocol on the other muscle no less than seven days after their first session.

Data Analysis

In order to assess learning during the visuomotor tracking task I used RMS error between the actual and target force. RMS error is reported as the sum of RMS error between target and actual waveforms across all points of each waveform. A lower RMS error in the post training assessment was used as a marker of learning (Perez *et al.*, 2004; Cirillo *et al.*, 2011; Lundbye-Jensen *et al.*, 2005).

The MEP amplitude was defined as the peak-to-peak amplitude in the recorded EMG response between 20-60 ms after the presentation of the magnetic stimulus. To account for variability in background muscle activation, individual MEPs were excluded from the construction of SR curves if their respective RMS EMG in the 100 ms prior to stimulation was greater than twice the mean RMS for that dataset. SR curves were excluded from the statistical analysis if r^2 was less than 0.7 or the upper plateau of the curve was not visible. The dependent variables used in the statistical analysis of learning induced changes in CSE were AuC, MEP_{max}, I_{50} and slope. MEP amplitudes were normalised to MaxMEP. To acquire MaxMEP I delivered 10 stimuli at 100 %MSO to the hotspot and then averaged the responses.

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4 (Hintze, 2007) and all tests were considered significant at an alpha of 0.05. Results are reported as mean ± S.D.

To assess examine visuomotor learning I used a 2w-rmANOVA with factors muscle (*proximal, distal*) x time *(pre training, post training)* on the RMS error in the baseline and post assessment sections of the visuomotor learning. To assess learning induced changes in CSE I used a 2w-rmANOVA with factors muscle (*proximal, distal*) x time *(pre training, post training)* on AuC, MEP_{max}, I_{50} and slope following learning. To control for the effect of fatigue I used a 2w-rmANOVA with factors muscle (*proximal, distal*) x time *(pre training, post training)* on MVC. Intra-individual variability in learning induced changes in CSE was assessed using intraclass correlation coefficients (ICC) on the change in AuC, MEP_{max}, I_{50} and slope between muscles.

Results

Motor Learning

In order to determine whether motor learning had occurred performance data were collected from the FDI and BB of 16 participants. A representative example of the improvement in visuomotor tracking has been included below as figure 12A. Statistical analysis revealed that performance significantly improved (i.e. mean RMS error decreased) as a result of training ($F_{1,15}$ = 61.21, p =<0.001) illustrated in figure 12B. However there was no effect for *muscle* ($F_{1,15}$ = 3.32, p =0.09) or interaction *time x muscle* ($F_{1,15}$ = 0.68, p =0.42).

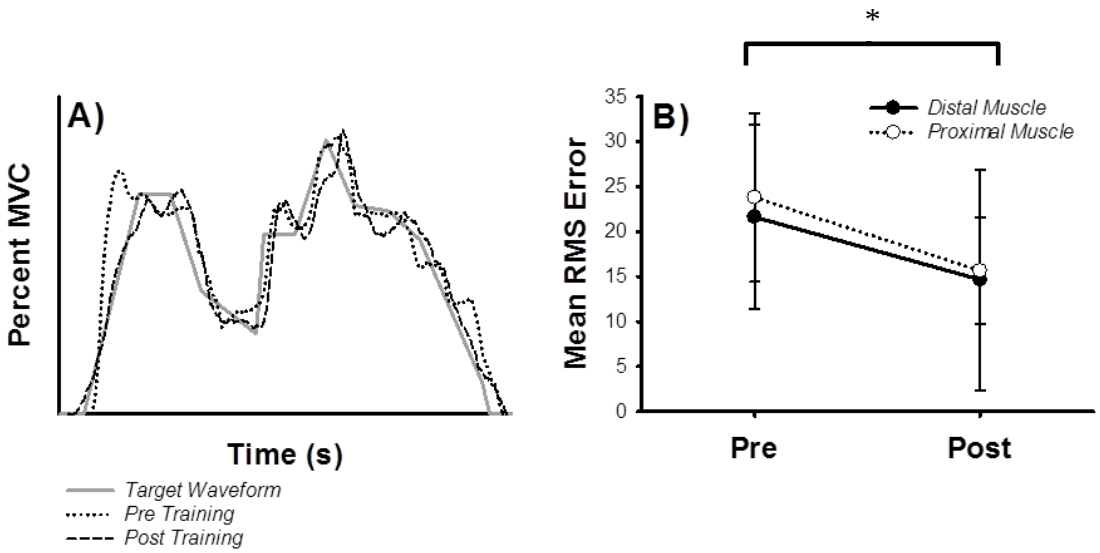

Figure 12. The improvement in visuomotor tracking in proximal and distal muscles. A) A representative example of the improvement in visuomotor tracking from the FDI of a single participant. The solid grey line shows the target waveform the participant was asked to track, the dotted line shows the participants performance prior to training and the solid black line shows the performance after training. B) Group data showing the improvement in performance in BB and FDI of 16 participants. Filled circles represent the dominant hand and open circles the non-dominant hand. Performance significantly improved after training (p = <0.001) however there was no significant differences in performance improvements between muscles (p =0.09).

Learning Induced Changes in Corticospinal Excitability

In order to determine the plastic effect of motor learning on CSE SR curves were acquired prior to and post training from the BB and FDI of 16 participants. Auc was used as the primary marker of neuroplastic change, while changes in MEP_{max}, I50, slope and AuC served as secondary markers of changes in CSE. Group AuC is represented in figure 13. The expected increase in CSE was observed to be variable. Statistical analysis of the grouped data showed a significant effect for muscle on AuC ($F_{1,15} = 8.59$, $p = 0.01$). There was no significant effect for time nor an interaction effect for any of the dependent variables assessed here. The results of the 2w-rmANOVA are summarised in table 3.

Table 3. Summary of statistically analysis for learning induced changes in corticospinal excitability for FDI and BB. * denotes $p = <0.05$ ** denotes $p = <0.01$.

	Time		*Muscle*		*Time x Muscle*	
AuC	$F_{1,15} = 0.74$	p = 0.40	$F_{1,15} = 8.59$	p = 0.01*	$F_{1,15} = 0.70$	p = 0.41
MEP_{max}	$F_{1,15} = 0.75$	p = 0.40	$F_{1,15} = 2.47$	p = 0.14	$F_{1,15} = 0.82$	p = 0.38
I_{50}	$F_{1,15} = 0.18$	p = 0.68	$F_{1,15} = 22.3$	p = <0.001**	$F_{1,15} = 0.01$	p = 0.93
Slope	$F_{1,15} = 0.31$	p = 0.59	$F_{1,15} = 3.9$	p = 0.067	$F_{1,15} = 0.69$	p = 0.42

Learning induced changes in CSE were observed to variable, variability was greatest in BB. Our data suggest there is an internal inconsistency in the corticospinal response to visuomotor training of the proximal and distal muscles of the same limb - an increase in CSE following training on BB was not indicative of an increase for FDI following FDI training and vice versa. SR curves for BB were observed to be more variable compared with FDI. This variability is illustrated by the larger S.D. for BB in figure 19. There

were no significant correlations between the changes in any of the parameters assessed in either muscle.

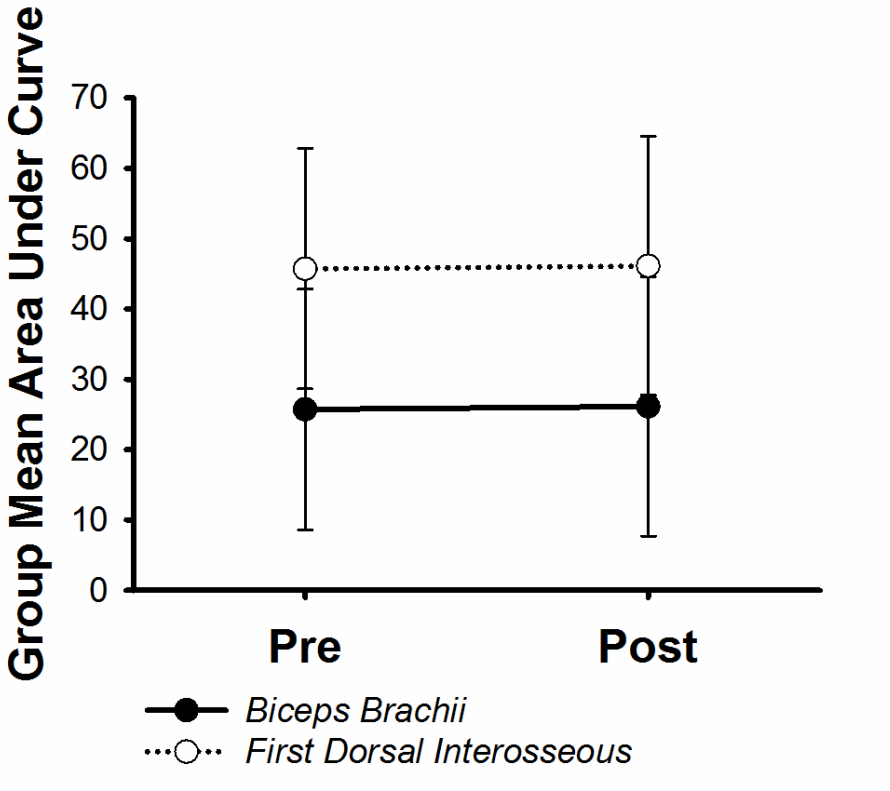

Figure 13. The effect of visuomotor learning on area under the Stimulus Response Curve. Statistical analysis showed a significant effect for muscle on AuC ($F_{1,15}$ = 8.59, p = 0.01). MEP amplitudes are normalised to the corresponding averaged max MEP.

It is logical to ask whether this internal inconsistency in the corticospinal response to visuomotor learning stems from increased variability in our measure of CSE for proximal muscles. Furthermore, ICC analysis on the dependent variables prior to learning in BB points towards a high degree of reliability in our measure of CSE [MEP_{max} (ICC = 0.95, p = 0.08), I_{50} (ICC = 0.84, p = 0.056), slope (ICC = 0.641, p = 0.06) and AuC (ICC = 0.972, p = 0.13).

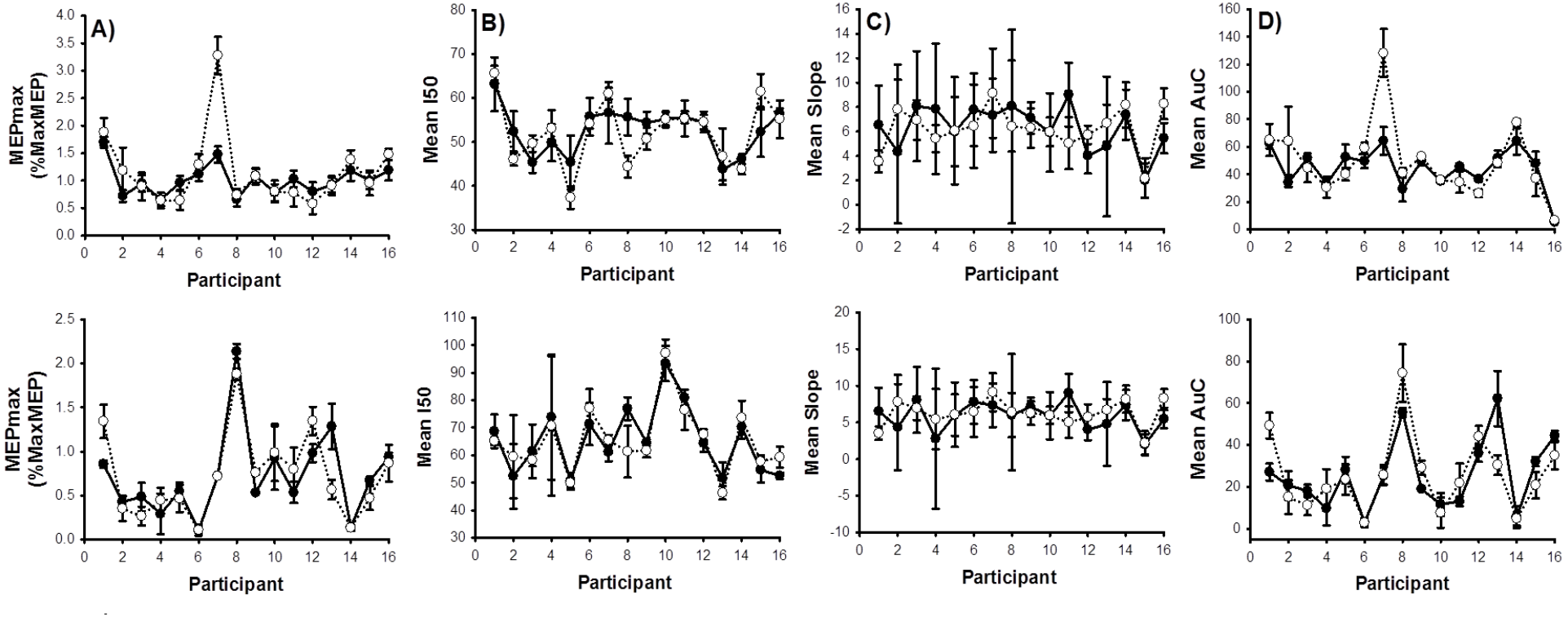

Figure 14. The effect of visuomotor learning on corticospinal excitability. Closed circles represent baseline measures and open post training. Results are reported as mean ± 1S.D. Top row represents data from FDI, the bottom BB respectively. A) represents MEP_{max} B) represents I_{50} C) represents slope and D) represents area under the curve.

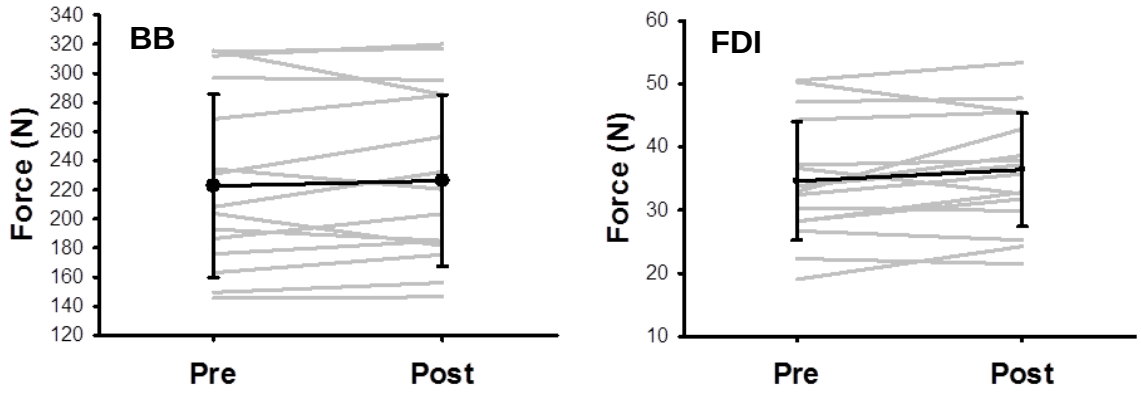

Figure 15. Changes in maximal voluntary contraction following visuomotor learning. Grey lines represent data from each participant and black lines represent group data. A) Represent data from BB and B) represents. Statistical analysis revealed there was no change in maximal voluntary contraction after visuomotor training (p = 0.72).

In order to control for the effect of fatigue on CSE I recorded MVC from FDI and BB prior to and post training in all 16 participants. Group mean plus individual MVC prior to and after training is illustrated in figure 15. MVC did not decrease as a result of learning [FDI (pre training 34.6 ± 9.4, post training 36.4 ± 9.0), BB (pre training 222.6 ± 62.9, post training 226.3 ± 58.9)]. Statistical analysis revealed that no significant difference in MVC as a result of training ($F_{2, 31}$ = 0.74, p = 0.72).It is logical to ask whether any of the factors known to modulate plastic change influenced the results presented here. Using the data within the TMS adult safety screen, logistical regression analysis was used to examine the effect of caffeine consumption, gender and sleep deprivation on the plastic changes associated with visuomotor learning. The results are summarised below in table 4.

Table 4. Logistic regression analysis of factors which may affect plastic changes in proximal and distal muscles.

Factor	B	SE	Wald	Sig.	Exp (B)
Gender	0.219	.415	.486	.459	.89
Caffeine	.109	0.241	.205	.65	1.12
Sleep deprived	.241	.315	.588	.443	.786

Discussion

This study set out to investigate whether proximal muscles have the same capacity for learning induced increases in CSE as distal muscles of the upper limb. To that end, SR curves were acquired from BB and FDI prior to and post training in 16 healthy participants. Changes in AuC, MEPmax, slope, and I_{50} were used as markers of use dependent plasticity. I tested the hypothesis that there would be a greater change in CSE for the neural pathways of finger compared with the BB as indicated by increases in AuC, MEP_{max}, slope, and a decrease in I_{50}.Our data do not support this hypothesis, I observed no statistically significant changes in any parameters assessed here for either muscle as a result of training. The present study highlights an important point – a single session of visuomotor tracking, as described in the present study, is not a reliable means of modulating CSE in all participants, this variability is illustrated in figure 14.

The present study specifically compares motor skill learning induced changes in CSE in both proximal and distal muscles. Previous studies have shown that learning induced changes in CSE are graded with the largest change in distal muscles of the upper limb following training on a ballistic motor learning task (Krutky & Perreault, 2007). However, the present study I observed no such gradient. Most notably I observed a degree of variability the corticospinal response to motor learning, illustrated in figure 14. This variability is underreported in the field and certainly warrants further examination.

In the present study I observed greater variability for learning induced changes in CSE for BB, it is likely the differences between the regions of the motor cortex controlling

proximal and distal muscles of the upper limb contributed to this variability. There are three key differences between the regions of the cortex given over to the control of distal and proximal muscles. First, large amounts of the motor cortex are given over to control over distal muscles, whereas relatively small amounts are devoted to control of proximal muscles (Penfield & Boldrey, 1937; Wassermann *et al.*, 1992). Second, the corticospinal projection to distal muscles of the upper limb is much greater compared with more proximal muscles (Palmer & Ashby, 1992) and third, these corticospinal projections are known to play a greater role in control of the hand compared with the more proximal muscles (Turton & Lemon, 1999). Different regions of the motor cortex may have varying capacity for learning induced neuroplasticity, studies involving TMS induced changes in CSE (Martin *et al.*, 2006) and use-dependent changes in interhemispheric inhibition (Sohn *et al.*, 2003) were shown to be more effective in distal muscles of the upper limb compared with proximal muscles.

It is important to state I am not suggesting that learning induced modulation of CSE do not occur in the corticospinal structures which control of proximal muscles. In the present study I have shown an improvement in performance following a single measure of training with BB, previous research has shown this to be a good marker of motor learning (Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005; McAllister *et al.*, 2011). Previous studies have shown that prolonged skilled use of the proximal musculature in a sporting context leads to an expansion of their representation on the motor cortex (Tyè *et al.*, 2005). Experimental studies of motor learning have shown that the proximal musculature of the upper limb is capable of neuroplastic change during reach to grasp movements in a force field (Shadmehr & Mussa - Ivaldi, 1994) following a similar dose

of training to the present study. The variable findings in the present study could be explained by the possibility that changes in CSE following visuomotor learning with different muscles occur with different time, alternatively that learning induced changes in CSE for proximal muscles are not readily observable using TMS.

Learning induced neuroplasticity may occur in other cortical regions and neural pathways when proximal muscles are trained. The prefrontal cortex has been suggested to play an important role in the acquisition of a novel motor skill (Shadmehr & Holcomb, 1997). Additionally, there are numerous indirect and ipsilateral neural pathways involved in the control of proximal muscles. Neural pathways which have the potential to play a role in learning induced changes in proximal muscles include small diameter corticospinal pathway (Colebatch *et al.*, 1990), corticobulbospinal pathway (Colebatch *et al.*, 1990) and corticoreticulospinal pathway (Ziemann *et al.*, 1999) respectively. Had any learning induced plasticity occurred in these pathways it would not have been assessed in the present study. These pathways are involved in generating the response to ipsilateral TMS, consequently there may be some value in assessing the response to ipsilateral TMS after motor learning.

I acknowledge the possibility that brief exposure to the visuomotor learning paradigm, 28 min in total, used in the present study may have been insufficient to modulate CSE for the neural structures and pathways controlling BB compared with those control FDI respectively. In short, there may be different dose response characteristic between FDI and BB for learning induced modulation of CSE. Previous research has shown that BB is capable of neuroplastic change following continued exposure to a paradigm similar to

that used in the present study (Lundbye-Jensen *et al.*, 2005), further research is warranted to compare the dose response characteristics for distal and proximal muscles of the upper limb.

What is interesting, yet poorly understood, is why CSE decreased for some participants following a protocol designed to raise it. Several factors including age, gender, attention and genetics are known to influence synaptic plasticity (for review see Ridding & Ziemann, 2010). In order to minimise the influence of these factors I used a randomly selected sample of convenience, selected from a narrow age range and used only healthy participants. In recent study of TMS induced changes in CSE Hamada *et al.*, (2013) suggest an alternative explanation for the variability in CSE changes. Hamada *et al.*, suggest the variability is due, at least in part, to different interneurone networks stimulated at different times of the day in different people (Hamada *et al.*, 2013). The authors report a significant correlation between induced changes in CSE and MEP latency following a TMS pulse which induces an anterior-posterior current across the central sulcus. This correlation accounted for approximately 50% of the variability in the response. Whether the same postulation, MEP latencies following TMS pulses, which induce an anterior-posterior current across the central sulcus, could predict learning induced changes in CSE is certainly an interesting proposition and warrants further examination. However, I also acknowledge the possibility that any changes in CSE were within the measurement error of the SR curve acquisition method.

Fluctuations in participants' attention within and between sessions has to be considered as a potential confound for this experiment. Attention has previously been shown to

modulate MEP amplitudes (Ellaway *et al.*, 1998; Funase *et al.*, 1999) and the magnitude of PAS induced changes in CSE (Rosenkranz & Rothwell, 2006; Stefan *et al.*, 2004). In the present study SR curves were acquired in less than two minutes, minimising the opportunity for fluctuations in attention to influence the SR curve. To control for attention mediated fluctuations in CSE the participants were provided real-time feedback of coil position and orientation and instructed to keep the coil in the correct position and orientation during SR curve acquisition. To control for fluctuations in attention during the motor learning task participants were provided with online feedback of tracking error in the respective waveforms.

Conclusions

In summary the findings presented here suggest that studies assuming that CSE is modulated following a single session of visuomotor learning may be misleading and further work to examine the dose response relationship between motor learning changes in CSE is warranted. Additionally, I observed no relationship in CSE modulation following learning in the BB and FDI in the same limb – an increase in CSE for FDI was not indicative of an increase for BB and vice versa. The mechanism behind this asymmetrical response remains to be determined.

Chapter VI – Hand Preference and Learning induced Plasticity

Abstract

Learning-induced changes in corticospinal excitability (CSE) are variable both between and within participants. However, apart from Muellbacher *et al.,* (*Exp Brain Res. 136, 4* 2001) who demonstrated that changes in CSE following ballistic motor learning are variable, the topic has received little rigorous examination. The aim of this study was to evaluate the inter- and intra-individual variability in learning-induced changes in CSE.

I assessed CSE using the transcranial magnetic stimulation (TMS) stimulus-response (SR) curves. SR curves were acquired from first dorsal interosseus in the dominant and non-dominant hands of 21 healthy participants before and after training. In this study, training involved a visuomotor tracking task that has previously been shown to induce changes in CSE (Perez *et al*., *Exp Brain Res.* 159, *2* 2004). The participants' hands were tested in a random order and the sessions were separated by 7 days. A reduction in tracking error served as a marker of learning.

Tracking error significantly decreased in all participants and in both hands as a result of learning ($p<0.05$). However, learning-induced changes in CSE were observed to be variable. CSE was seen to increase, remain the same or decrease in 50%, 30% and 20%, of the participants respectively. Additionally, there were no significant differences in the SR curve parameters between the dominant and non-dominant hands after training ($p>0.05$ for all parameters).

This study highlights an important consideration for future studies of motor learning; CSE does not increase for everyone following visuomotor tracking. There is

considerable variability in the magnitude and direction of learning induced changes in CSE and these changes are independent of hand dominance.

Introduction

During the acquisition of a novel motor skill, playing the piano for example, there is an increase in corticospinal excitability (CSE) for muscles involved in the task (Pascual - Leone *et al.*, 1995; Nudo *et al.*, 1996; Classen *et al.*, 1998; Muellbacher *et al.*, 2001; Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005). This effect is typically reported at a group level, there is a paucity of evidence regarding the variability in CSE modulation following motor learning at the level of the individual. This is an important factor to consider as it has implications for therapeutic interventions in neurorehabilitation.

To the best of my knowledge there has been little systematic study into the variability in the neuroplastic response to motor learning. Muellbacher *et al.,* (2001) observed no increase in MEP amplitude 30 min after training on a ballistic motor learning task in 4 out of 10 participants.

Importantly, non-invasive brain stimulation (NIBS) techniques such as theta burst transcranial magnetic stimulation (TBS) (Huang *et al.*, 2005) and paired associative stimulation (PAS) (Stefan *et al.*, 2000) are known to modulate CSE, this modulations is said to utilise similar mechanisms to motor learning induce modulation of CSE, namely long-term potentiation (Cooke & Bliss, 2006). Changes in CSE following NIBS is used as a marker of neuroplasticity, akin to learning induced neuroplasticity, however, not everyone expresses increases in CSE following TBS or PAS respectively. Reports estimate that 50 - 62.5% of people express increases in CSE following TBS (McAllister *et al.*, 2013; Martin *et al.*, 2006) and 52 - 75% following PAS (Vallence *et al.*, 2013; Müller-Dahlhaus *et al.*, 2008) respectively. If learning induced and NIBS changes in

CSE do share a common mechanism it would be reasonable to expect similar levels of variability in learning induced changes in CSE.

The evidence examining the significance of hand preference on neuroplasticity is equivocal. At an anatomical level, studies involving MEG and magnetic resonance imaging have shown the cortical representation of distal hand muscle in the dominant hand are greater compared to the non-dominant hand (Guye *et al.*, 2003; Volkmann *et al.*, 1998). Studies involving transcranial magnetic stimulation (TMS) have shown asymmetries in resting motor threshold (rMT) and intracortical inhibition between hemispheres (Triggs *et al.*, 1999; Civardi *et al.*, 2000). However, these asymmetries do not translate to a functional level. The majority of studies found no significant differences in learning-induced and NIBS-induced changes in CSE between hemispheres (Ridding & Flavel, 2006; Garry *et al.*, 2004; Gallasch *et al.*, 2009). That said, Cirillo *et al.*, (2010) found a 21% greater facilitation of motor evoked potentials (MEP) following ballistic motor learning in the non-dominant hand despite a 40% greater increase in performance for the dominant hand. As a result, further study is warranted to examine the significance of hand preference on learning-induced changes in CSE.

The aim of the present study was to investigate any inter- and intra-individual variability in learning-induced changes in CSE. I sought to determine the percentage of people who exhibit increased CSE after visuomotor learning. Secondly, I hypothesised that learning induced changes in CSE would be invariant to hand preferences.

Methods

Participants

Healthy participants with a mean age of 22 ± 3 years were recruited for the study (n=21, 60% female, 20 right hand dominant, sample of convenience). All participants were screened for contraindications to TMS using a modified version of TMS adult safety screen (Keel *et al.*, 2001). All participants gave informed written consent to participate in the study. Participants were excluded from the study if resting motor threshold (rMT) was greater than 70% maximal stimulator output (MSO). The study protocol was approved by the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN_11-0444) and all experiments were conducted in accordance with the Declaration of Helsinki. Participants hand preference was determined using the Edinburgh Handedness Inventory (Oldfield, 1971).

Experimental Protocol

Participants completed two sessions examining the variability in changes in CSE following motor learning. Participants were randomly allocated to either dominant or non-dominant hand in the first session. At the beginning of each session, I determined the participant's maximal voluntary contraction (MVC) and rMT for first dorsal interosseous (FDI). To examine learning-induced changes in CSE I acquired three TMS stimulus response (SR) curves prior to, and post training in all participants. To control for the effect of fatigue on the muscle maximal compound muscle action potentials (M_{max}) were acquired prior to, and post training in all participants. Participants completed the protocol on their other hand no less than seven days after their first session. The experimental protocol is illustrated in figure 16.

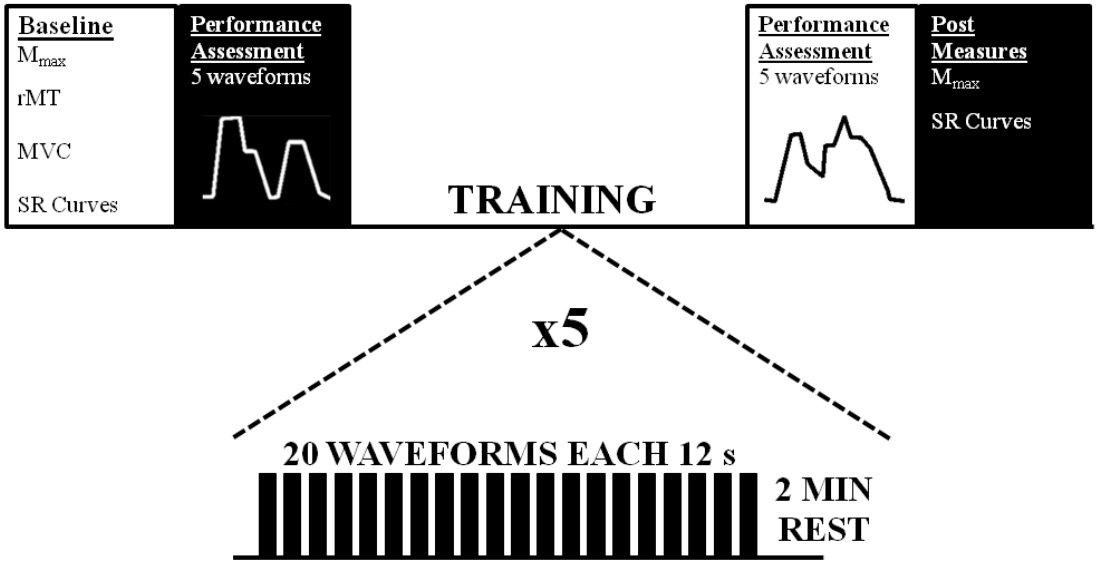

Figure 16. Schematic representation of the experimental protocol with measures obtained prior to and post training. Baseline measures include assessment of maximal compound muscle action potentials (M_{max}), maximal voluntary contraction (MVC) and resting motor threshold (rMT). TMS stimulus response curves were acquired as I have described previously (for detailed explanation see Mathias *et al.*, 2014). Performance assessment consisted of tracking five unseen waveforms, example waveforms are shown in the box. Training was divided into five blocks which consisted of tracking 20 waveforms, each 12 seconds long and the waveforms were presented in a random order in each block. Each training block was punctuated by 2 minutes of rest. Each block on the training line represents a waveform. Performance was assessed after training using the same waveforms in the performance assessment prior to training.

Electromyography

Surface electrodes (Blue Sensor N, Ambu®, Denmark) were placed in a bipolar montage over FDI muscle. The electromyography signals were band-pass filtered (10 - 1 kHz), sampled at 4 kHz and amplified using custom amplifiers. All data were stored on a computer for offline analysis.

Transcranial Magnetic Stimulation

MEPs were elicited with a biphasic TMS pulse (Magstim Rapid^2, The Magstim Company, Dyfed, UK) from a custom-made 90mm *'figure of eight'* coil (batwing design; type no. 15411, Magstim company, Dyfed, UK). Magnetic stimuli were delivered over the cortical area that evoked maximal MEPs in the FDI, commonly

referred to as the '*hot spot'*. The coil was positioned over the hot spot with the handle pointing backwards at an angle of 45° from the midline (Brasil - Neto *et al.*, 1992) inducing a posterior-anterior current (Kammer *et al.*, 2001). Coil position and orientation were monitored in real time using frameless stereotaxy (Brainsight, Rogue Research Inc). For all TMS assessments the hand was fully supinated and fixed with Velcro to a firm board.

Stimulus Response Curves

SR curves were acquired using the rapid method I have described previously (for detailed explanation see Mathias *et al.*, 2014). Briefly, SR curves were acquired using varying stimulation intensities determined pseudorandomly on a pulse-by-pulse basis between 80 %rMT until 100 %MSO. rMT was defined as the minimum stimulation intensity required to elicit MEPs of greater than or equal to 50 µV on five out of ten occasions in the relaxed muscle (Rossini *et al.*, 1994). MEP amplitudes were plotted against stimulation intensity and the relationship modelled using a four-parameter Boltzmann sigmoid function:

$$MEP(I) = MEP_{\min} + \frac{MEP_{\max} - MEP_{\min}}{1 + e^{\frac{I_{50} - I}{S}}}$$

where MEP_{min} and MEP_{max} are the minimum and maximum asymptotes of the function; I_{50} is the %MSO at which the MEP is mid-way between MEP_{min} and MEP_{max} and S is the steepness of the relationship at I_{50}.

Maximal Compound Muscle Action Potentials

In order to control for the effect of fatigue on the trained muscle I recorded M_{max} following a peripheral nerve stimulus. This response was obtained using an electrical stimulus (Digitimer DS7AH, Digitimer Ltd, Welwyn Garden City, UK) delivered to the median nerve. M_{max} were determined using supramaximal stimuli. The response was quantified as the peak to peak amplitude between 2-10 ms after the presentation of the stimulus. In order to ensure valid statistical comparisons all motor evoked potentials were normalised to the M_{max} acquired pre training.

Visuomotor Training

The visuomotor training task used in the present study was based upon a task previously shown to increase CSE (McAllister *et al.*, 2011; Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005). The participants hand was fully supinated on the board and the distal inter-phalangeal joint placed against the force transducer (NL 62 - 5 kg, Digitimer Ltd, Welwyn Garden City, UK) and secured in place with Velcro. Participants were asked to track a target waveform with a cursor shown on the computer screen through isometric index finger abductions of varying degrees of force, the experimental set-up is illustrated below in figure 16. At the beginning of the task participants were verbally instructed how to complete the task, participants were not allowed to practice the task prior to commencing the training.

In each session participants performed five blocks of training, each block lasting 4 min with 2 min rest between blocks to minimise fatigue. Each training block consisted of tracking twenty waveforms, each lasting 12 s. To further minimise the influence of

fatigue the waveform amplitudes were normalised to 20 %MVC for each participant, furthermore, the first and last second of each waveform returned 0 %MVC. The participant's performance on the task was assessed prior to and post training using separate blocks of five assessment waveforms shown in figure 16.

Online performance feedback in the form of absolute error was given to control for fluctuations in attention during learning. Error feedback was given in the form of a bar chart displayed on the computer screen after each waveform. The bar chart was updated after each waveform so the participant saw their performance improvement across for each waveform across all training blocks. Figure 22C illustrates performance feedback. The force signal was high pass filtered at 30 Hz, amplified x1000, digitised at 4 kHz and stored on a computer of offline analysis.

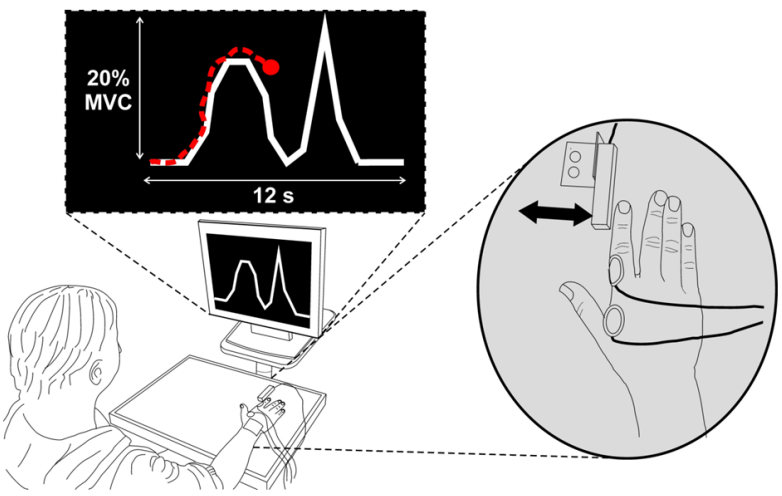

Figure 17. The experimental set up for visuomotor learning in first dorsal interosseous. The participant tracked the white with isometric contractions of varying degrees of force (tracking shown as red line). Performance was quantified as the RMS error between the target and participants trace. Thanks go to Mark van de Ruit for the figure.

Data Analysis

In order to assess learning during the visuomotor tracking task I used root mean square (RMS) error between the actual and target force. RMS error is reported as the sum of RMS error between target and actual waveforms across all points of each waveform. A lower RMS error in the post training assessment was used as a marker of learning (Perez *et al.*, 2004; Cirillo *et al.*, 2011; Lundbye-Jensen *et al.*, 2005).

The MEP amplitude was defined as the peak to peak amplitude in the recorded EMG response between 20 and 60 ms after the presentation of the magnetic stimulus. To account for variability in background muscle activation, individual MEPs were excluded from the construction of SR curves if their respective RMS EMG in the 100 ms prior to stimulation was greater than twice the mean RMS for that dataset. SR curves were excluded from the statistical analysis if r^2 was less than 0.7 or the upper plateau of the curve was not visible. AuC was used as primary outcome measure in the present study as it reliably characterises the whole SR curve (Carson *et al.*, 2013). MEP_{max}, I_{50} and slope parameters were used as secondary outcome measures.

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4 (Hintze, 2007) and all tests were considered significant at an alpha of 0.05. Results are reported as mean ± S.D.

To assess examine visuomotor learning I used a two-way repeated measure analysis of variance (2w-rmANOVA) with factors hand preference (*left*, *right*) × time *(pre training, post training)* on the RMS error in the baseline and post assessment

sections of the visuomotor learning. To assess learning induced changes in CSE a 2w-rmANOVA with factors hand preference (*left, right*) × time *(pre training, post training)* was used to assess changes in AuC, MEP_{max}, I_{50} and slope following learning. To control for the effect of fatigue on the trained muscle I used a 2w-rmANOVA with factors hand preference (*left, right*) × time *(pre training, post training)* on M_{max} amplitudes. Intra-individual variability in learning induced changes in CSE was assessed using intraclass correlation coefficients (ICC) on the change in AuC and change in the MEP_{max}, I_{50} and slope parameters of the SR curve between hands.

Results

All procedures were well tolerated and no adverse events were recorded in the present study. Data from the non-dominant hands of 2 participants is missing as they were lost to follow-up after the first session, their data has been included for analysis were appropriated.

Motor Learning

In order to determine whether learning had occurred, data were collected from dominant and non-dominant FDIs of 21 participants. A representative example of the improvement in visuomotor tracking has been included as figure 18A. Statistical analysis of all data revealed a significant effect for *time* on RMS error ($F_{1,19}$ = 31.93, p = <0.001) illustrated in figure 18B. However, there was no main effect for hand preference ($F_{1,19}$ = 1.94, p =0.18) or an interaction *time x hand preference* ($F_{1,19}$ = 3.42, p = 0.08).

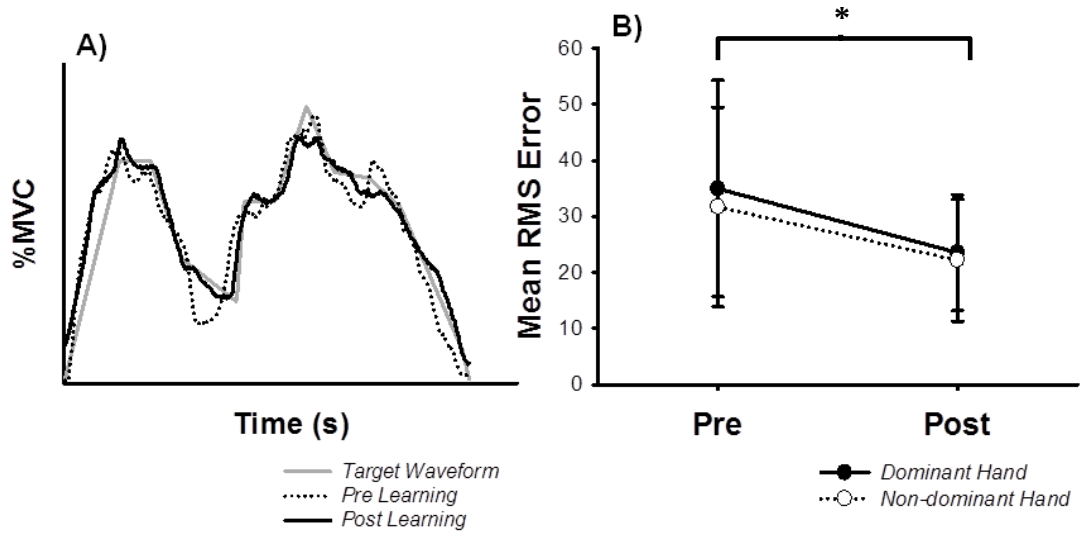

Figure 18. The improvement in visuomotor tracking. A) A representative example of the improvement in visuomotor tracking from the dominant hand of a participant. The solid grey line shows the target waveform the participant was asked to track, the dotted line shows the participants performance prior to training and the solid black line shows the performance after training. B) Group data showing the improvement in performance in the dominant and non-dominant hands of 21 participants. Filled circles represent the dominant hand and open circles the non-dominant hand. Performance significantly improved after training (p = <0.001) however there was no significant differences in performance improvements between hands (p =0.18).

Learning Induced Changes in Corticospinal Excitability

In order to determine effect of motor learning on CSE, SR curves were acquired prior to and post training from FDI in the dominant and non-dominant hands of 21 participants. AuC was used as the primary marker of neuroplastic change, changes in MEP_{max}, I_{50}, slope were used as secondary markers of changes in CSE. Group changes in AuC are illustrated in figure 19. The expected increases in CSE as a result of learning were observed to be variable. Statistical analysis revealed a main effect for *time* on AuC ($F_{1,20}$ = 4.77, p = 0.04) and main effects for hand preference on MEP_{max} ($F_{1,20}$ = 7.26, p = 0.01) and AuC ($F_{1,20}$ = 8.56, p = <0.01). There was no interaction between factors, the results of the 2w-rmANOVA are summarised in table 4.

Table 5. Summary of statistical analysis for learning induced changes in CSE and hand preferences. * denotes $p = <0.05$, ** denotes $p = \leq 0.01$.

	Time		*Hand Preference*		*Time x Hand Preference*	
AuC	$F_{1,20}$ = 4.77	p = 0.04*	$F_{1,20}$ = 8.56	p = <0.01**	$F_{1,20}$ = 2.43	p = 0.13
MEP_{max}	$F_{1,20}$ = 3.23	p = 0.09	$F_{1,20}$ = 7.26	p = 0.01**	$F_{1,20}$ = 2.98	p = 0.09
I_{50}	$F_{1,20}$ = 0.58	p = 0.46	$F_{1,20}$ = 0.01	p = 0.92	$F_{1,20}$ = 0.19	p = 0.67
Slope	$F_{1,20}$ = 0.44	p = 0.51	$F_{1,20}$ = 0.11	p = 0.75	$F_{1,20}$ = 0.17	p = 0.68

Learning induced changes in CSE were observed to be variable within individuals and are illustrated in figure 20. Learning-induced changes in CSE were observed to be variable, with MEP_{max} increasing, remaining the same or decreasing in 50%, 30% and 20%, of the participants respectively. There were no significant correlations between the change in RMS error and change in AuC for either hand [dominant hand (r = 0.35, p = 0.12), non-dominant hand (r = 0.95, p = 0.68)]. ICC analysis on the change in the dependent variables showed a high degree of variability between hands [MEP_{max} (*ICC* = 0.274, p = 0.240), I_{50} (*ICC* = 0.228, p = 0.284), slope (*ICC* = 0.189, p = 0.321) and AuC (*ICC* = 0.195, p = 0.877)]. There was no correlation between rMT and change in AuC (r = 0.01, p = 0.95).

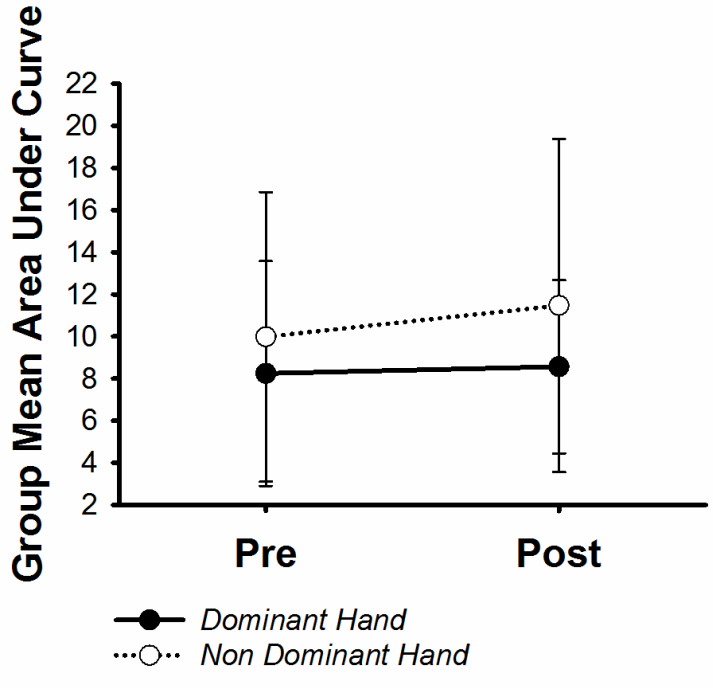

Figure 19. The effect of visuomotor learning on corticospinal excitability of the dominant and non-dominant hand. Data presented here is group mean area under the curve. There were no significant effects for time point and hand preference or any time point by hand preference interactions. Individual data is presented in figure 20

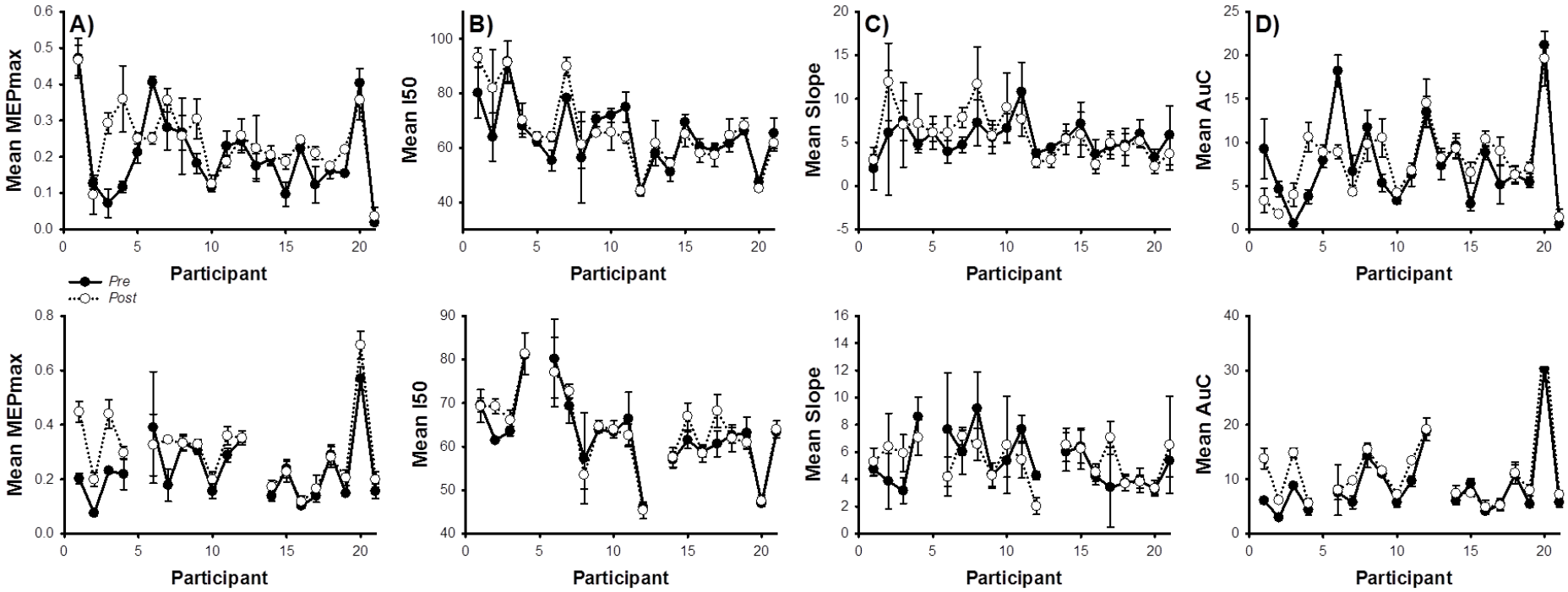

Figure 20. The effect of visuomotor learning on corticospinal excitability. Close circles represent baseline measures and open post training. Results are reported as mean ± 1S.D. Top row represents data from the dominant hand and the bottom represents data from the non-dominant hand. A) represents MEPmax B) represents I50 C) represents slope and D) represents area under the curve. Data for the non-dominant hands of two participants is missing as they were lost to follow up.

It is logical to ask whether this intra-individual variability in the corticospinal response to motor learning, illustrated in figure 20, stems from unreliability in our TMS measure of CSE. ICC analysis of rMT between hands indicates a high reliability (*ICC* = 0.637, $p = 0.04$). Furthermore, ICC on the dependent variables at prior to learning between hands points towards a high degree of reliability in our measure of CSE [MEP_{max} (*ICC* = 0.45, $p = 0.027$), I_{50} (*ICC* = 0.613, $p = <0.01$), slope (*ICC* = 0.345, $p = 0.058$) and AuC (*ICC* = 0.782, $p = <0.001$)]. Further, logistical regression analysis was used to determine the caffeine consumption, gender and sleep deprivation on CSE changes.

Table 6. Logistic regression analysis of factors which may have influence plastic change in visuomotor learning

Factor	B	SE	Wald	Sig.	Exp (B)
Caffeine	.72	.71	1.019	.313	1.074
Gender	.81	.69	0.899	.542	.995
Sleep deprived	.77	.74	1.087	.297	1.08

To control for the effect of fatigue on the trained muscle I acquired M_{max} from FDI of the dominant and non-dominant hands prior to and post training in 21 participants. Statistical analysis revealed M_{max} amplitudes to invariant to visuomotor learning ($F_{2,31} = 1.24$, $p = 0.73$) illustrated below as figure 21.

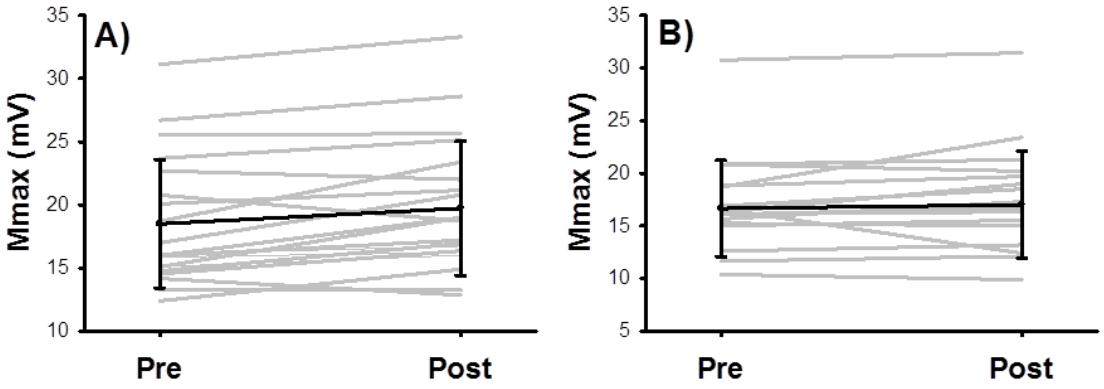

Figure 21. Changes in the maximal compound muscle action potentials following visuomotor learning. Grey lines represent data from each participant and black lines represent group data. A) represent data from the dominant hand and B) represents the non-dominant hand. Statistical analysis revealed there were no significant changes in the neuromuscular junction after visuomotor training ($p = 0.73$).

Discussion

The novel finding of this study is that there is both intra- and inter-individual variability in magnitude of learning induced changes in CSE in the context of skill learning. I sought to determine the percentage of people who exhibit increased CSE after visuomotor learning as indicated by increased AUC, MEP_{max} and slope with a concomitant decrease in I_{50}. MEP_{max} and AuC increased, remained the same or decreased in 50%, 30% and 20% of the participants respectively. Whereas I_{50} increased, remained the same or decreased in 20%, 45% and 35% of participants respectively and slope increased, remained the same or decreased in 30%, 40% and 30% of participants respectively. Learning induced modulation of CSE was invariant of hand preference. Interestingly, in those who exhibit an increase in CSE following visuomotor learning, an increase in CSE for the dominant hand did not predict an increase in CSE for the non-dominant hand following visuomotor learning and vice versa. To examine the intra-individual variability in the corticospinal response to motor learning I tested the hypothesis that changes in CSE following motor learning would be invariant of hand preference. Our data support this hypothesis, ICC analysis on the change in the parameters between hands revealed no significant correlations.

Hand Preference and Learning induced changes in Corticospinal Excitability

In the present study I observed no significant differences in the capacity for CSE modulation between hands following visuomotor learning. The neurophysiological asymmetries between hands are well documented (for review see Hammond, 2002), yet these differences do not clearly translate to the functional level. Several studies have reported no significant differences in changes in CSE modulation between hands

following PAS and motor learning respectively (Ridding & Flavel, 2006; Garry *et al.*, 2004; Gallasch *et al.*, 2009). Whereas, Cirillo *et al.*, (2010) observed a significantly greater increase in CSE for the non-dominant hand following training on a ballistic motor learning task. In the present study I observed no significant differences in CSE modulation between hands. These differences in results between the present study and Cirillo *et al.*, (2010) may be due to the paradigms used in the respective studies. Visuomotor motor tracking, used in the present study, is a much more complex task involving multiple cortical regions whereas ballistic learning involves numerous repetitions of a single planar movement arising from one muscle. The nature of the movements is very different between tasks, as the name implies, ballistic learning involves very brisk, rapid movements which a repeated throughout the session. Visuomotor learning involves lots of gradual increases in force with varying rates of change, perhaps standardising the rate of force increase across all waveforms in the current experiment would have induced comparable results?

Inter-individual Variability

Previous studies have shown that visuomotor tracking is an effective means of modulating CSE however these changes are typically reported at a group level (Cirillo *et al.*, 2011; Perez *et al.*, 2004; McAllister *et al.*, 2011; Lundbye-Jensen *et al.*, 2005). The present study points toward a large degree of inter-individual in the magnitude of learning induced changes in CSE (see figure 20). This high degree of variability is reflected in the non-significant effects and lack of interaction between factors summarised in table 4. The response rates observed in the present study, approximately 50% of participants, is comparable to changes in CSE induced following NIBS (Martin

et al., 2006; McAllister *et al.*, 2013; Müller-Dahlhaus *et al.*, 2008; Sale *et al.*, 2007). Further, similar to Sale *et al.*, (2007) who studied plasticity induced by PAS, I observed no correlation between changes in AuC and rMT suggesting this variability is not a consequence of greater variability in the MEP.

What is interesting, yet poorly understood, is why CSE decreased for some participants following a protocol designed to raise it. Several factors including age, gender, attention and genetics are known to influence synaptic plasticity (for review see Ridding & Ziemann, 2010). In order to minimise the influence of these factors I used a randomly selected sample of convenience, selected from a narrow age range and used only healthy participants. It is without doubt these factors, despite our best efforts to control them, have influenced the results in the present study although I would suggest any impact from these parameters is minimal. In recent study of TMS induced changes in CSE Hamada *et al.*, (2013) suggest an alternative explanation for the variability in CSE changes. Hamada *et al.*, suggest the variability is due, at least in part, to different interneurone networks stimulated at different times of the day in different people (Hamada *et al.*, 2013). The authors report a significant correlation between induced changes in CSE and MEP latency following a TMS pulse which induces an anterior-posterior current across the central sulcus. This correlation accounted for approximately 50% of the variability in the response. Whether the same postulation, MEP latencies following TMS pulses, which induce an anterior-posterior current across the central sulcus, could predict learning induced changes in CSE is certainly an interesting proposition and warrants further examination.

Intra-individual Variability

My data suggest there is an internal inconsistency in the corticospinal response to visuomotor learning – an increase in CSE following motor learning for the dominant hand does not mean the non-dominant hand will respond in the same way and vice versa.

The internal inconsistency in the corticospinal response to visuomotor learning cannot be explained by differences in the basal level of CSE between hemispheres. Although Daligadu *et al.*, (2013) reported differences between in the TMS SR curve of the dominant and non-dominant, I failed to observe similar findings. In the present study ICC analysis of the SR curves between hands at baseline was high ($ICC \geq 0.35$). Further, the factor *time* in the 2w-rmANOVA analysing changes in CSE was only significant for AuC. Combined, this suggests no differences in CSE between hands prior to learning. That said it is important to highlight that the SR curves were collected using different methodologies. In the present study I used a rapid acquisition method over a range of intensities (Mathias *et al.*, 2014) whereas Daligadu *et al.*, (2013) used a binned acquisition protocol which has previously been shown to influence the SR curve (Möller *et al.*, 2009).

Additionally, differences in performance changes between the hands cannot explain the internal inconsistency in the corticospinal response to visuomotor learning. In the present study I observed no significant differences in the change in tracking error between hands and there was no significant correlation between changes in performance and changes in CSE.

The internal inconsistency in the corticospinal response to learning induced changes in CSE could possibly be explained by different characteristics for learning induced modulation of CSE between the dominant and non-dominant hands. Previous studies have shown that learning induced changes in CSE are subject to dose response relationship, with more training sessions inducing larger changes in CSE (Lundbye-Jensen *et al.*, 2005; Pascual - Leone *et al.*, 1995). However, both studies report significant modulation of CSE after a single session of motor learning. I have to acknowledge the possibility that a single session of training might be insufficient for modulating CSE between hands.

Fluctuations in participants' attention within and between sessions has to be considered as a potential confound for this experiment. Attention has previously been shown to modulate MEP amplitudes (Ellaway *et al.*, 1998; Funase *et al.*, 1999) and the magnitude of PAS induced changes in CSE (Rosenkranz & Rothwell, 2006; Stefan *et al.*, 2004). In the present study SR curves were acquired in less than two minutes, minimising the opportunity for fluctuations in attention to influence the SR curve. To control for attention mediated fluctuations in CSE I asked the participants were provided real-time feedback of coil position and orientation and instructed to keep the coil in the correct position and orientation during SR curve acquisition. To control for fluctuations in attention during the motor learning task participants were provided with online feedback of tracking error in the respective waveforms.

It would be remiss not to highlight that, in the hand preference study the population tested was predominantly right hand dominant according to the Edinburgh Handedness Inventory. This is without doubt a limitation of this work. In an attempt to minimise the influence of this, I regressed the change in AuC onto the laterality quotient (the degree of handedness)

Conclusions

In summary, this study confirm the suggestions that visuomotor learning is an effective means of modulating CSE at the group level but is variable at the level of individual participants. Further, there is an inconsistency in the corticospinal response to visuomotor learning between hemispheres - an increase in CSE following visuomotor learning for the dominant hand is not indicative of an increase in CSE following visuomotor learning in the non-dominant hand and vice versa. The findings presented here suggest that studies assuming that CSE is modulated following a single session of visuomotor learning may be misleading and further work to examine the dose response relationship between motor learning changes in CSE is warranted. The mechanism behind this asymmetrical response remains to be determined.

Chapter VII – General Discussion

Chapter VII - General Discussion

Structure of the chapter

The purpose of this chapter is to provide an integrated discussion of research contained within this thesis, discussions specific to each experiment can be found in the relevant chapters. This chapter starts by presenting a summary statement of the principles results for this thesis. It moves on to present an integrated discussion of the findings contained within the work. This chapter suggests how the work adds value to the literature, and impacts on future research practices. In the interest of completeness further experimental questions are suggested and the chapter finishes with some concluding remarks.

Summary statement of results

This thesis contains three experimental chapters. The aim of the first study in this thesis was to reduce the acquisition time of the TMS SR curve. The principle results of this chapter are that the SR curve is invariant to ISI, on average, 76 stimuli are required to construct a representative SR curve and 3 min of rTMS does not have a reliable neuromodulatory effect. This means that SR curves can be acquired in less than 2 min: 76 stimuli delivered with a 1.4 s ISI leads to a 106 s acquisition time. The final two chapters sought to examine the influence of hand preference and muscle choice on learning induced neuroplasticity. Both experiments report a non-significant effect for hand preference and proximal-distal muscles on plasticity induced following visuomotor learning.

Rapid acquisition of TMS SR curve

This work provides proof of concept for reducing the TMS SR curve acquisition time in healthy control participants. It provides a novel means of rapidly acquiring the necessary data to construct a TMS SR curve in less than two minutes. ICC analysis of SR curves constructed using the minimum number of stimuli indicates this is a reliable means of acquiring TMS SR curves.

Despite the well documented increased variability in MEPs for proximal muscles (Rossi *et al.,* 1999) ICC analysis indicates that SR curves can be reliably constructed with 76 stimuli in BB, similarly to FDI and ADM. Given the inclusion/ exclusion criteria and SR curve acquisition protocol used in this study (rMT had to be ≤ 70 %MSO) this result is not surprising. Assuming a participant had a rMT of 50% MSO and acquiring SR curves as described would mean there would be 1.09 MEPs per %MSO. This measurement density may explain why the average minimum number is very similar for all muscles examined.

It would be remiss not to document some of the participant feedback obtained during the ISI experiment and technical considerations which arose during the development work. During pilot testing SR curves were acquired using a 1.0 and 1.2 s ISI and participants reported this to be uncomfortable and anxiety inducing. As anxiety has been shown to influence CSE (Greenberg *et al.,* 2000) the decision was made to drop these protocols from our investigations. On a technical note, it may be possible to further reduce the acquisition time of SR curves. Typical parameters of the TMS pulse include a rise time of the order 0.1 ms, a peak field of approximately 1 Tesla (depending on a

number of factors including local anatomy and coil geometry) and a magnetic field of several hundred joules. The circuitry used to generate the magnetic field pulses is usually based on a capacitor discharge system with typical peak coil currents in the range of several kiloamps and discharge voltages of up to a few kilovolts. There is an important trade-off with shorter ISIs, the stimulator will misfire more (receive a trigger but not deliver a stimulus) when trying to drop to a lower intensity. Misfires occur due to the capacitors in the stimulator not fully discharging before any subsequent triggers arrive. Using the biphasic Magstim Rapid^2 it is possible to reduce the incidence of stimulator misfires by imposing limits in the code controlling the stimulator which mean any subsequent stimuli cannot be more than -10 %MSO of the previous intensity. However, this restricts investigators to biphasic pulses which have been shown to influence rMT and MEP amplitude (Sommer *et al.,* 2006). This is an important technical point should any teams attempt to further reduce the acquisition time of the TMS SR curve.

This experiment demonstrates the proof of principle for reducing the acquisition time of the TMS SR curve in healthy participants and refers to the method as a 'clinically feasible means of acquiring TMS SR curves'. It is important to highlight that in this context clinically feasibility refers to the acquisition time of the TMS SR curve not to using the rapid acquisition protocol in clinical populations. The method presented here has not been validated in elderly or clinical populations and we know that ageing and disease states alter TMS SR curves (Cacchio *et al.,* 2009, 2011; Smith *et al.,* 2011, Pitcher *et al.,* 2003). Prior to being used in any clinical or elderly populations the acquisition protocol needs to be validated, there must be a comparison of the rapid

acquisition and the conventional binned acquisition protocol in the elderly and disease states.

The TMS SR curve is a plasticity assessment technique with very few safety risks, SR curves are being applied in a growing number of diverse situations. For example, SR curves have been used to assess plasticity after learning (Perez *et al.,* 2004; Lundbye-Jensen *et al.,* 2005), to identify mechanism of NIBS induced plasticity (Maeda *et al.,* 2000, 2002), and to prognosticate recovery after stroke (Cacchio *et al.,* 2011; Huyn *et al.,* 2013; Harris-Love *et al.,* 2013) each of these studies used a different acquisition protocol. These acquisition protocols remain commonplace within the literature (examples include Smith *et al.,* 2010; Boudreau *et al.,* 2013; Crupi *et al.,* 2013) despite the demonstration that they influence the SR curve (Möller *et al.,* 2009). To ensure rigorous findings and facilitate comparison of data from separate studies methodological standardisation is required. Standardising the method for acquiring the TMS SR curve could improve data validity and make it easier to compare data between studies. The standardisation protocol described utilises the protocol shown to be optimal by Möller *et al.,* builds upon that to reduce the acquisition time and overcomes the variability in the minimum number of stimuli required.

Non-significant effect of hand preference and muscle choice on neuroplasticity

A multitude factors combine to result in plastic change, factors include age, lifestyle, gender and history of synaptic activity. In light of the non-significant effect of training on neuroplasticity presented in both the learning experiments it seems opportune to discuss some of these factors and their potential influence on the learning experiments.

Age and neuroplasticity

The decline in memory and capacity for learning associated with ageing is well documented. Furthermore, studies have provided a biological substrate for this effect, suggesting the ageing process influences an individual's capacity for synaptic plasticity – in particular LTP (for review see Barnes, 2003). Several studies have used inhibitory PAS to study the capacity of the aged motor cortex for plasticity and it is widely accepted the capacity for plasticity decreases with age. For example, Müller-Dahlhaus *et al.*, (2008) report the magnitude of inhibitory PAS effect was greatest the young participants compared to the elderly. Fathi *et al.*, (2010) expanded on this, conducting a three way comparison between young, middle aged, and elderly cohorts and report significant LTP like responses following inhibitory PAS in the young and middle aged cohort. Fathi *et al.*, were unable to elicit the expected inhibition of CSE in the elderly participants. Tecchio *et al.*, (2008) report similar findings, however the authors report there was evidence for change in the elderly, post menopausal, women. It should be noted that any influence of age on plastic changes in the present work is minimal as all populations were drawn from samples of convenience (undergraduate students) with very narrow age ranges.

Similar findings have been reported when examining the use of SR curves in the assessment of plasticity. Pitcher *et al.*, (2003) conducted a large study (n = 42) to examine, amongst other factors, the effect of ageing on the TMS SR curve. The authors note that trial to trial variability in MEP amplitudes was greatest in the elderly cohort, especially at intensities near rMT. However, there was no significant effect of age on

rMT, maximal MEP amplitudes or maximal slope of the TMS SR curve. The authors attribute these ageing related differences to either a decrease in the number of spinal alpha motor neurones being activated synchronously by the TMS pulse, or activation of similar number of spinal alpha motor neurones in a less synchronous manner which results in phase cancellation in the surface EMG. It is worth noting that the ageing related changes were greatest in the post menopausal women, suggesting these changes are a result of the loss of menstrual hormones after the menopause. Smith *et al.,* (2011) explored this gender related ageing differences further through recruiting exclusively male young and elderly cohorts. The authors examined SR curves from the left and right hands of these male cohorts and report no effects for age or hand preference or any interaction for any of the SR curve parameters. Smith *et al.,* conclude that male corticospinal SR characteristics are not altered by advancing age and that previously reported age-related changes in motor cortical excitability assessed with TMS are likely due to changes inherent in the female participants only. Future studies should consider females reproductive status when recruiting participants.

Gender and neuroplasticity

There is a wealth of evidence regarding the effect of gender on plasticity in the animal model (for review see Srivastava *et al.,* 2013) which suggests gender is potentially a powerful determinant of neuroplasticity yet there is a lack of studies in the human. There is insufficient evidence to offer a grounded opinion as to whether gender significantly influences learning induced plasticity in the healthy human. Logistic regression analysis revealed gender did not significantly influence the outcome in either

learning experiment. However, these are relatively small samples, further work with larger samples is required to explore this potentially exciting area.

Hand preference and neuroplasticity

The neurophysiological asymmetries between hands are well documented (for review see Hammond, 2002), yet these differences do not clearly translate to the functional level. Several studies have reported no significant differences in changes in CSE modulation between hands following PAS and motor learning respectively (Ridding & Flavel, 2006; Garry *et al.*, 2004; Gallasch *et al.*, 2009). Whereas, Cirillo *et al.*, (2010) observed a significantly greater increase in CSE for the non-dominant hand following training on a ballistic motor learning task. In the hand preference study presented in this thesis there was no significant difference in CSE modulation between hands. This variation in results between the present study and Cirillo *et al.*, (2010) may be due to the paradigms used in the respective studies. Visuomotor motor tracking, used in the present study, is a much more complex task involving multiple cortical regions whereas ballistic learning involves numerous repetitions of a single planar movement arising from one muscle. That said the hand preference study of this thesis did not report significant findings.

Lifestyle and neuroplasticity

This thesis will focus on three aspects of lifestyle which have shown to influence neuroplastic change sleep, diet (in particular caffeine intake) and physical activity. These factors were chosen as they have been the subject of the widest debate.

A lack of sleep has been strongly linked to difficulties with cognition as well as changes in physiological processes. Concerning plasticity, *in vitro* studies have shown that sleep deprivation alters plasticity homeostasis, making LTP induction more difficult and enhances the ease of LTD induction (Kopp *et al.*, 2006; Campbell *et al.*, 2002; McDermott *et al.*, 2003). Using TMS in man many studies point toward an increase in CSE with prolonged periods of waking (De Gennaro *et al.*, 2007; Huber *et al.*, 2013; Kreuzer *et al.*, 2011). That said, the evidence is not univocal, studies have failed to replicate these findings (Manganotti *et al.*, 2001; Manganotti *et al.*, 2006) or report conflicting results (Civardi *et al.*, 2001). Logistic regression analysis revealed that sleep deprivation did not significantly influence the outcome in either learning experiment. Due to the limitations of the TMS adult safety screen it is not possible to quantify the degree of sleep deprivation or whether participants routinely went with little sleep. Substantiating these important factors may reveal a powerful modulator of learning induced plasticity.

The evidence concerning the effect of caffeine on CSE in mixed. In order to discuss this evidence in relation to the work presented here this thesis will first tackle the effect of caffeine on the lower motor neurone and then the upper motor neurone. Concerning lower motor neurones, the effects of caffeine on lower motor neurones have commonly been studied using the H-reflex and F-wave. Caffeine is said to increase the level excitatory neurotransmitter released, in addition to lowering motor neurone activation threshold (Williams *et al.*, 1987). Two studies have report no significant modulation of H-reflex amplitude following caffeine intake (Eke-Okoro, 1982; Kalmar & Cafarelli, 1985) as well as no significant modulation of the F-wave following caffeine

consumption (Cerqueria *et al.*, 2006). Concerning upper motor neurones, TMS has commonly been employed to examine the effect of caffeine on the CNS. In a recent review, de Carvalho *et al.*, (2010) report that caffeine has no effect on MEP amplitude, central motor conduction time or motor threshold (Cerqueria *et al.*, 2006; Orth *et al.*, 2005; Kalmar & Cafarelli, 2004). However, at intensities around aMT large doses of caffeine have been shown to significantly alter the cortical silent period (a measure of inhibitory interneuron activity). In both learning experiments presented here, logistic regression revealed there was no significant relationship between caffeine intake and plastic change. That said there is no information on the dose of caffeine participants had ingested in the past previous 24 hours. To properly examine the effect of caffeine consumption on learning induced plasticity, information in greater depth around the participants' routine caffeine consumption, time of dose and since dose as well as dose of caffeine consumed is required.

There is a growing body of good evidence which suggest regular physical activity can modify an individual's capacity for plasticity (for review see Erickson & Kramer, 2009) as well as improving memory and learning (for review see van Praag, 2009). Whilst the precise mechanism behind this increase in the capacity for neuroplastic change in active individuals remains unclear, it is likely to be multi-factorial including changes in cerebral blood flow (Xiong *et al.*, 2009), angiogenesis (Swain *et al.*, 2003) as well as increased expression of neurotrophic factors (Klintsova *et al.*, 2004). Working in the healthy human Cirillo *et al.*, (2009) demonstrated that individuals who regularly undertake aerobic exercise exhibit a larger change in CSE following inhibitory PAS compared with sedentary individuals. Unfortunately there is no data on the exercise

habits of those who participated in both learning experiments, it is possible this could have influenced the outcome by shifting the homeostatic balance in synaptic plasticity in the primary motor cortex.

Attention and neuroplasticity

It is possible that the variability observed in the present study could be a result of fluctuations in attention. Attention has previously been shown to modulate CSE (Kamke *et al.,* 2012; Rosenkranz & Rothwell, 2004; Stefan *et al.,* 2004) and both learning experiments consisted of two experimental sessions each two hours. In the experiments presented in this thesis we attempted to control attention by instructing the participant to use visual feedback to ensure the coil remained over the hotspot. Another factor which could modulate attention and plasticity is time of day.

There is a large body of literature examining how time of day interacts with attention (for review see Kraemer *et al.,* 2000). Time of day has been shown to significantly modulate plasticity induced following PAS (Sale *et al.*, 2007). PAS is more effective at inducing plasticity in the afternoon or evening compared with the morning (Sale *et al.*, 2008; Sale *et al.,* 2007). The authors attribute this circadian modulation to fluctuations in cortisol levels across the day. The same team sought to determine whether time of day significantly influences plasticity induced following motor learning (Sale *et al.*, 2013). Sale *et al.,* (2013) trained 22 participants on a ballistic motor learning task at 0800 and 2000 hours in the same day and they quantified neuroplastic changes using TMS. Sale *et al.,* (2013) report a significant effect of training on MEP amplitudes, however, there was no significant effect for time of day on MEP amplitude. It is

important to highlight, to the best of my knowledge, this is the only study examining the effect of time of day on neuroplasticity induced following motor learning. It goes without saying further is warranted to substantiate this finding.

Brain Derived Neurotrophic Factor and neuroplasticity

Studies have shown that individuals with mutations of the gene controlling brain derived neurotrophic factor (BDNF) have altered responses to motor learning (Kleim *et al.*, 2006) and to TBS (Cheeran *et al.*, 2008). This thesis did not control for this particular mutation, so there is potential that it may have impacted upon the results. However, in some participants, visuomotor learning induced a plastic change for one muscle but not the other. Opening the suggestion that plasticity induction in the health human is a multi-faceted mechanism rather than a single magic bullet. Further work is required to explore this.

Prior and parallel voluntary motor activity and neuroplasticity

There has been huge interest in the therapeutic combination of learning induced and NIBS induced plasticity under the assumption that the learning induced plasticity is augmented by the NIBS paradigm; this combined therapy results in greater plastic change and a greater functional recovery.

Experiments in the animal model have shown that history of synaptic activity in the target brain region can influence the plastic response to any subsequent plasticity inducing protocols. For example, Christie & Abraham (1992) demonstrated that reliable induction of associative LTD in the dentate gyrus of the rat can be significantly

improved by priming with theta frequency stimulation. There are a wealth of studies demonstrating that priming the system with one form of stimulation can significantly enhance the effect of a second (for review see Cassidy *et al.*, 2014). A history of synaptic activity arising from voluntary behaviours has been shown to influence plasticity. Ziemann *et al.*, (2004) demonstrated that training on a ballistic motor learning task modified the subsequent response to PAS. The ballistic motor learning increased the response to inhibitory PAS and reduced the response to excitatory PAS. Similar priming effects have been reported for submaximal isometric tracking task (Stefan *et al.*, 2006). Whilst the influence of previous synaptic activity can never be truly excluded when working with humans, all participants underwent a period of compulsory before testing. It is certainly an interesting proposal, whether prior voluntary activation significantly influence learning induced plasticity and it requires further examination.

To summarise thus far, learning induced plasticity is a multi-faceted process mediated by, at least, some of the factors described. To harness the power of plasticity induction and maximise patient benefit as well as understanding the mechanism more basic science studies of factors such as Estrogens status modulates plasticity, how exercise and diet modulate plasticity and whether the CNS can be primed using NIBS to increase plasticity induction.

Did the motor task impact the result?

This section of the thesis will discuss whether the learning task used in the work presented here influenced the plastic change. Previous studies have shown that this task is capable of modulating CSE (Perez *et al.*, 2004; Lundbye-Jensen *et al.*, 2005; Cirillo

et al., 2011). Participants in the work in this thesis received the same training dose as described in Lundbye-Jensen *et al.*, (2005) who report a significant change in CSE for BB after a single session of training. So, it would be reasonable to have expected a plastic change in the present work.

In the present studies the learning task was adapted so that participants made isometric rather than actual movements to control the cursor. This adaptation was chosen for pragmatic reasons however there is a possibility it influenced the expected neuroplastic change. Whilst it is difficult to offer a grounded reason as to why this adaptation might have influenced the outcome, the potential has to be acknowledged. Further studies examining CSE changes in static and dynamic movements and well as eccentric and concentric contractions are warranted.

The visuomotor learning paradigm used in this body of work involved lots of gradual increases in force with varying rates of change, perhaps standardising the rate of force increase across all waveforms in the current experiment or adapting the paradigm so the participant has to make basic style movements would lead to results comparable to those reported in previous studies. McAllister *et al.*, (2011) standardised the maximal movement speed to 40°/S and the authors report significant neuroplastic changes as a result of training. Standardising the rate of force change brings this paradigm closer to ballistic motor learning may induce a more reliable plastic change.

The design of the learning experiment presented in this thesis lends itself to the suggestion that there was a carry-over effect between sessions which may have

influenced any neuroplastic change. There are three counter arguments to this supposition. First, there was no difference in the slope of the learning curves for each experiment. Second, in both experiments participants were randomly allocated to one of the two conditions which should prevent any ordering effect. Third, there were no statistically significant differences in the performance improvements between sessions.

Are stimulus response curves the best method to assess neuroplastic change?

There is a large body of evidence which have used SR curves to show changes in CSE following motor learning (Perez *et al.,* 2004; Lundbye-Jensen *et al.,* 2005) and NIBS (Maeda *et al.*, 2000, 2002). However, these studies are not without fault. In many of these studies SR curves are typically acquired by delivering multiple stimuli in pseudorandomised bins of stimulation intensity. A full range of stimulation intensities are delivered from just below motor threshold until either the motor evoked potential (MEP) amplitude plateaus or the maximum stimulator output (MSO) is reached. The MEP amplitudes for each stimulus intensity level are then averaged and a 3, 4 or 5 (Barsi *et al.*, 2008; Malcolm *et al.*, 2006; Pitcher *et al.*, 2003) parameter Boltzmann-like model is fit to the mean data using a nonlinear least squares algorithm to produce the SR curve (Devanne *et al.*, 1997; Valls - Solé *et al.*, 1992; Boroojerdi *et al.*, 2001a). The use of blocked acquisition protocols has been shown to influence the acquisition of SR curve. SR curves acquired using blocked decreasing stimulation intensity protocols were shifted significantly to the left suggestive of increased CSE when compared to a blocked increasing protocol, showing that it is possible to significantly alter the SR curve via the method used to acquire them (Möller *et al.,* 2009). Möller *et al.* (2009)

recommend acquiring SR curves using stimulation intensities determined randomly on a pulse-by-pulse basis.

Despite the work done in chapter three, building on the good work of Möller and colleagues, the rapid TMS SR curve acquisition protocol is not without fault. The large standard deviation in the minimum number of stimuli required for TMS SR curves has to be acknowledged as a potential limitation of this study. One could suggest that the lack of consistent plastic induction in the learning experiments was due to the SR curves being insensitive to plastic change in some participants – they had too few stimuli to detect any changes for the individual they were acquired in. Whilst this has to be acknowledged, the minimum number of stimuli determined in the first experimental chapter (figure 7 illustrates how the minimum number of stimuli was calculated) is the group mean + 1 S.D of a large dataset (108 SR curves), suggesting the SR curves should have been acquired using the minimum number of stimuli in 68% of cases. Furthermore, ICC analysis of the SR curves constructed using the minimum number of stimuli indicates a high degree of reliability.

One potential approach to overcome this weakness would be to combine TMS SR curves with TMS CSE maps. Unlike TMS SR curves, in which many different intensity stimuli are delivered over the same cortical site, CSE maps are constructed by stimulating many different cortical sites at a single intensity. The two techniques are commonly applied to investigate neuroplastic changes which accompany motor learning (Boudreau *et al.*, 2013; Suzuki *et al.*, 2012; Lundbye-Jensen *et al.*, 2005; Wassermann *et al.*, 1992). While the two techniques measure complimentary factors there are some

important differences. SR curves will detect area changes in CSE maps, they will not detect changes in the distribution of excitable elements or uneven expansion of the CSE maps (Ridding & Rothwell, 1997; Mano *et al.*, 1995; Traversa *et al.*, 1997). It is possible that any plasticity induced by the visuomotor task was expressed as a change in the distribution of excitable elements and as such, not detected by TMS SR curves.

Is variability in learning induced plasticity something we should worry about?

No. The results presented here are not entirely unexpected; studies of NIBS induced plastic change report similar levels of variability (Cheeran *et al.,* 2014, Maeda *et al.*, 2000, Muller-Dahlhaus *et al.,* 2008). Learning induced and NIBS induced neuroplastic changes have been suggested to share a common mechanism, namely LTP, and there is a growing body evidence for variability in the plastic response to NIBS (for review see Ridding and Ziemann, 2010). Understanding variability will increase the integrity of field by ensuring adequate numbers of participants, easier replication of study findings, help translate findings into larger pre-clinical and clinical trials. The work presented here offers insight into the complexity of studying LTP like induced plasticity in the waking human, and showing factors which can influence the response.

Utility of this work to future practice/ research

The technique for rapidly acquiring TMS SR curves described in this thesis steers the field in the correct direction for TMS assessments of plasticity. The technique can be easily adopted by other groups to study plastic changes in healthy young populations. Additionally, rapid TMS SR curve provides a more accurate measure of plasticity as the influence of fluctuations in attention is reduced. The technique has clinical potential – 2

minutes for to acquire the necessary data is acceptable for the patient. However it has to be noted that before the technique is used in clinical populations it requires validation. The learning experiments presented here highlight the variability in learning induced plasticity, aiding power calculations and informing investigators of the efficacy and reliability of visuomotor tracking task.

Future experiments

There are multiple studies comparing the efficacy of the different NIBS paradigms to induce neuroplastic changes (Zafar *et al.*, 2008; Goldsworthy *et al.*, 2012; Simis *et al.*, 2013). Yet, despite the variety of motor learning paradigms within the field there have been no studies comparing their efficacy to induce neuroplasticity. It would be interesting to compare the ability of some of the common motor learning paradigms such as ballistic learning, visuomotor learning and sequence, to induce neuroplastic changes in healthy volunteers. Identifying the optimal motor learning paradigm for inducing neuroplastic changes in the healthy human could serve to further our understanding of how neuroplasticity is induced and ultimately lead to therapeutic interventions.

There may be some value in comparing the neuroplastic response of two intrinsic hand muscles, where the neurophysiological asymmetries are much less, to visuomotor learning. A negative finding here – asymmetrical responses for two intrinsic hand muscles is a very interesting prospect as it opens the door to speculation over the relative weighting given to the BDNF polymorphisms in the process of plasticity induction.

Building upon the TMS SR curve work presented here there is significant value in fully automating the SR curve acquisition method. Automating SR curve acquisition is one way to overcome the variability in the minimum number of stimuli. This could be achieved by automating the code used in this work to fit the SR curve during acquisition and to stop stimulating when the curve stops deviating from 95 % CI for example. Further work is needed to identify suitable criteria to stop stimulating if SR curves are to be fully automated.

To direct the course of future experimentation one question that could be asked is whether the learning the visuomotor tracking task used in this thesis is truly a learning task. Ecologically valid forms of learning such as playing a musical instrument or learning to ride a bike take a long time to become proficient, with timescales typically in order the of weeks to years. In the present task, performance was optimised within a single session suggesting this task may reflect adaptation as opposed to learning. An alternative approach may be to use an alternative paradigm which is functionally and ecologically valid; there may be some value in examining learning to use chopsticks. In China alone, 1.4 billion people disposable chopsticks (Sprague & Stuart, 2008) making the task ecologically valid and functional. Other characteristics which make this task an appealing paradigm is that it is engaging, challenging, progressive and speaking from personal experience difficult and not a skill that can be acquired in a single session.

Concluding remarks

This thesis presents evidence that learning induced plasticity is variable in the healthy human, the level of variability is comparable to the reports of variability in NIBS induced neuroplasticity. This thesis provides a novel means, which has clinical potential, for assessing CSE changes. This thesis also highlights an important point - a single session of visuomotor tracking as described in this thesis is not sufficient to reliably induce plasticity in everyone. Further work is required to validate the rapid SR curve acquisition method in pathophysiological conditions.

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Appendix I – Transcranial Magnetic Stimulation Adult Safety Screen

Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen*

If you agree to take part in this study, please answer the following questions. The information you provide is for screening purposes only and will be kept completely confidential.

CIRCLE or CROSS OUT

Have you ever suffered from any neurological or psychiatric conditions?	YES / NO
If YES please give details (nature of condition, duration, current medication, etc)	
. . . . .	
Have you ever suffered from epilepsy, febrile convulsions in infancy or had recurrent fainting spells?	YES / NO
Does anyone in your immediate or distant family suffer from epilepsy?	YES / NO
If YES please state your relationship to the affected family member.	
. . . . .	
Do you suffer from migraine?	YES / NO
Have you ever undergone a neurosurgical procedure (including eye surgery)?	YES / NO
If YES please give details.	
. . . . .	
Do you have an implanted device such as a cardiac pacemaker, medication pump or cochlear implant?.	YES / NO
Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork?.	YES / NO
Are you currently taking any medication (prescribed or unprescribed)?	YES / NO
If YES please give details.	
. . . . .	
Are you currently undergoing anti - malarial treatment?	YES / NO
Have you ingested any alcohol in the last 24 hours?	YES / NO
Have you had any coffee or other sources of caffeine in the last hour?	YES / NO
Have you used recreational drugs in the last 24 hours?	YES / NO
Did you have very little sleep last night?	YES / NO
Have you already participated in a TMS experiment today?	YES / NO
Have you participated in more than one TMS experiment in the last 6 months?	YES / NO
Is there any chance that you could be pregnant?	YES / NO
Do you need further explanation of TMS and its associated risks?	YES / NO
Date of Birth _____/_____/_____	

Signed: .....Date: ….....

Name (in block letters): .....…….....

† For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.
* Modified TASS based on Keel JC, July 2000.