RAPID ASSESSMENT OF CORTICOSPINAL EXCITABILITY USING TRANSCRANIAL MAGNETIC STIMULATION

By

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Abstract

Human motor system plasticity can be quantified using single pulse transcranial magnetic stimulation (TMS) to measure corticospinal excitability. TMS can be used to produce excitability maps and to examine the stimulus-response (SR) relationship. The overall aims of this thesis are (1) to demonstrate that TMS mapping and SR curves can be acquired much faster than has been traditionally possible and (2) that these techniques can be used to study internally externally driven plasticity.

By modifying the TMS delivery, it is demonstrated that both the TMS map and the SR curve can be reliably produced in approximately two minutes. These techniques were then used to examine internally driven plasticity via mirror training and visuomotor tracking learning and externally driven plasticity via transcranial alternating current stimulation. Changes in corticospinal excitability were found to be variable both for internally as externally driven plasticity. Nonetheless, these studies highlight that it is possible to rapidly assess changes in corticospinal excitability.

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The following publications are a direct result of the work presented in this thesis:

Articles published

van de Ruit, M., & Grey, M. J. (2015). The TMS Map Scales with Increased Stimulation Intensity and Muscle Activation. *Brain Topography*. doi:10.1007/s10548-015-0447-1

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Articles in preparation

van de Ruit, M., Mathias, J. P., & Grey, M. J. Development of a MATLAB GUI for rapid acquisition of the Transcranial Magnetic Stimulation stimulus response curve. *In Preparation.*

van de Ruit, M., Mathias, J.P., Wright, C.W., & Grey, M. J. Inter- and intrahemispheric differences in use-dependent plasticity following visuomotor tracking learning. *In Preparation.*

van de Ruit, M., Wright, C.W., & Grey, M. J. Imagery training does not promote neuroplasticity following a single session of mirror training. *In Preparation*.

Abbreviations

AMT	Active Motor Threshold
BB	Biceps Brachii muscle
BDNF	Brain Derived Neurothropic Factor
CSE	CorticoSpinal Excitability
EDC	Extensor Digitorum Communis muscle
EEG	ElectroEncephaloGraphy
EI	External Imagery
EMG	ElectroMyoGraphy
FDI	First Dorsal Interosseous muscle
fMRI	functional Magnetic Resonance Imaging
GUI	Graphical User Interface
ICC	Intraclass Correlation Coefficient
II	Internal Imagery
ISI	InterStimulus Interval
IQR	Inter-Quartile Range
KI	Kinaesthetic Imagery
LTD	Long Term Depression
LTP	Long Term Potentiation
MEG	MagnetoEncephaloGraphy
MVC	Maximum Voluntary Contraction
NIBS	Non-Invasive Brain Stimulation
PAS	Paired Associative Stimulation
PET	Positron Emission Tomography
QCD	Quartile Coefficient of Dispersion
RMS	Root Mean Square
RMT	Resting Motor Threshold
rTMS	repetitive Transcranial Magnetic Stimulation
SD	Standard Deviation
SEM	Standard Error of the Mean
SI	Stimulation Intensity
SR	Stimulus Response
tACS	Transcranial Alternating Current Stimulation

TBS	Theta-Burst Stimulation
tDCS	Transcranial Direct Current Stimulation
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation

CHAPTER 1

Introduction

Neuroplasticity of the central nervous system (CNS) underlies our ability to learn a new skill, improve an existing skill and regain function following CNS lesions. This thesis will focus on how we can assess neuroplasticity by studying changes in corticospinal excitability using non-invasive brain stimulation.

1.1 What is plasticity of the central nervous system?

The CNS is plastic, which refers to the its ability to undergo changes, either in response to natural external stimuli or as a result of trauma. Plasticity is a never ending process that is active across the life span of every single individual (Merzenich, 2011). The term 'Plastic' is derived from the Greek word 'plastos', which means moulded. The English dictionary describes plastic as 'substances or materials that can easily be shaped or moulded'. William James (1890) was the first to use the term 'plasticity' in reference to the human nervous system and behavioural changes in the monumental text *'The Principles of Psychology'* (from Pascual-Leone et al., 2005):

"Plasticity [...] means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once. Each relatively stable phase of equilibrium in such a structure is marked by what we may call a new set of habits. Organic matter, especially nervous tissue, seems endowed with a very extraordinary degree of plasticity of this sort; so that we may without hesitation lay down as our first proposition the following, that the phenomena of habit in living beings are due to the plasticity" *(p68, James, 1890)*

Not long after James coined the term plasticity, Ramón y Cajal, Nobel prize winner of 1906, extended the notion of plasticity by suggesting its neural substrate in his search for the anatomical basis of why behaviour may change. He presented this in his widely influential work *'Textura del Sistema Nervioso del Hombre y los Vertebrados'* (from Pascual-Leone et al., 2005):

"The labour of a pianist [...] is inaccessible for the uneducated man as the acquisition of new motor skills 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 formulation of new pathways through ramification and progressive growth of the dendritic arborisation and the nervous terminals." (p296, Cajal, 1904 - English translation)

However, it took almost half a century before Konorski (1948) and Hebb (1949) provided the first evidence for CNS plasticity. They described synaptic plasticity - the persistent activity driven change in synaptic efficacy. Hebb introduced the 'Hebbian plasticity' concept, stating that the synaptic strength between two neurons increases when they fire together. The biological substrate for Hebbian plasticity was first described by Lømo (1966): long term potentiation (LTP) (Bliss and Lømo, 1973) and long term depression (LTD) (Dunwiddie and Lynch, 1978, Lynch et al., 1977). Pioneering work of Hubel and Wiesel (Wiesel and Hubel, 1965) provided the first evidence that synaptic changes drive cortical plasticity in the cortex. In short, they demonstrated that when depriving kittens from using one eye, the area receiving information from the other eye took over the visual cortical area normally related to the deprived eye (Hubel et al., 1977). For this and other work they received the Nobel prize in 1981. This was the start of a period of time in which the occurrence of structural plasticity became widely accepted. Although, synaptic plasticity, LTP and LTD are the driving forces of plasticity it is beyond the scope of this thesis to discuss them in great detail (for excellent reviews see: Lømo, 2003, Martin et al., 2000, Cooke and Bliss, 2006, Bliss and Cooke, 2011). For this thesis, focus will be on structural plasticity in the motor cortex.

The existence of a specialised motor cortical area was first shown by Fritsch and Hitzig (1870). Their motivation for exploring the exposed cortex of dogs came from observations made separately before they joined forces. Hitzig had found that non-invasive electrical stimulation of the back of the head or

ears of humans caused eye movements, whilst Fritsch accidentally discovered that irritation of the exposed brain of patients with open head wounds led to muscles twitching on the opposite side of the body (Finger, 2010). To more conclusively demonstrate their observations, they electrically stimulated a dog's cortex. Using a battery they stimulated the cortex with brief and weak electrical pulses. They discovered stimulating part of the cortex elicited twitches in contralateral muscles, whereas stimulating other adjacent parts did not. However, they also reported that stimulation of different cortical sites in close proximity elicited twitches in either the dog's forepaw, hind-paw or face (Taylor and Gross, 2003). This was the first evidence for the existence of a organised cortical motor map.

Interestingly, when publishing their work, Fritsch and Hitzig were apparently unaware off the findings of John Hughlings Jackson in epileptic patients. Jackson had previously argued different parts of the cortex must be involved in the control of different muscles. He based his idea on the typical pattern in which a seizure, that causes involuntary movements at one side of the body, develops. For example, convulsions starting around the face would propagate towards the neck, arms and torso, before affecting the leg and foot. Jackson realised a topographically organised motor cortex could best explain his observations. This also explained why small cortical lesions only led to loss of function in some body parts, but not others (Finger., 2010). Not only confirmed Fritsch and Hitzig Jackson his idea, they also demonstrated a cortical lesion indeed caused local dysfunction. By ablating part of the dog's forepaw motor cortical area, the dog lost control of its forepaw. Scotsman David Ferrier translated Fritsch and Hitzig findings to the monkey, but on top of the motor area also demonstrated the existence of a cortical sensory area (Taylor and Gross, 2003). The first to translate these findings to humans, and a pioneer in human brain stimulation, was Robert Bartholow (Zago et al., 2008). He met a patient that due to cancer had an 2-cm diameter hole in the skull through which Bartholow stimulated the exposed cortex. Like Fritsch and Hitzig, Bartholow observed distinct muscular contractions in the arm and leg contralateral to the stimulated hemisphere.

Advances in technology allowed mapping the sensorimotor cortex in greater detail using focal electrical stimulation (Beevor and Horsley, 1887, Brown and Sherrington, 1915). Eventually this led

to detailed maps of the motor and sensory cortex of a chimpanzee, gorilla and orang-utan (Leyton and Sherrington, 1917). The most significant finding of their work was that in the ape motor cortex, considerably finer movements could be elicited than reported for other species. Stimulation could be used to elicit movement in individual fingers, rather than the whole hand or paw. Later the findings of Sherrington and colleagues were translated to the human (Penfield and Rasmussen, 1950, Penfield and Boldrey, 1937). Penfield mapped both the motor and sensory cortex of epilepsy and brain tumour patients whilst they were conscious. The gross organisation of the human motor and sensory cortex appeared to be consistent with the earlier reports by e.g. Fritsch and Hitzig. The cortical areas processing sensory information or controlling movement of distinct body parts were found lying as a continuum on the cortical surface, with from medial to lateral direction the lower limb, upper limb and facial musculature. This led them to define the now famous motor and sensory homunculi. Nonetheless, once the cortex had been meticulously mapped out it became clear that there are multiple cortical sites, widely distributed and overlapping, that evoke a movement in a single body part (Kwan et al., 1978, Sessle and Wiesendanger, 1982, Donoghue et al., 1992, Jankowska et al., 1975). This distributed organisation provides a basis for structural plasticity within the sensorimotor cortex.

Structural plasticity of the brain was conclusively demonstrated in many animal studies conducted during the 1980s and 1990s. The first work demonstrating cortical reorganisation came from studies of the sensory cortex and was provided in a series of classical papers by Merzenich and colleagues. Transecting the median nerve of a monkey leads of a cortical area being deprived from sensory input. Rather than this cortical area becoming unresponsive, it gets occupied by the expansion of neighbouring cortical representations (Merzenich et al., 1983). This cortical reorganisation was also found when amputating a monkey's hand digit, with the sensory cortical area previously associated with the amputated digit taken over by the immediate adjacent digits (Merzenich et al., 1984). Comparable results were obtained following cutaneous stimulation of a limited skin area on the fingers (Jenkins et al. 1990). The observed pattern of reorganisation could be easily explain using Penfield's his sensory homunculi, but also showed its organisation is not fixed.

The evidence for plasticity within the primary motor cortex followed soon in a series of experiments using similar peripheral lesions to those used by Merzenich. Donoghue et al. (1990) transected a rat's facial motor nerve, which made the rat lose ability to control its facial whisker musculature. This led to a rapid reorganisation of the motor cortex, with expansion of the cortical areas associated with control of forelimb and eye. After amputating a rat's forelimb, cortical reorganisation of the motor cortex involved an expansion of the cortical area from which shoulder movements could be elicited (Sanes et al., 1990). An important step in demonstrating the significance of motor cortical reorganisation was taken by Randolph Nudo (Nudo et al., 1996). Nudo argued there might be an important relationship between behavioural changes and cortical physiology. To test this he trained squirrel monkeys at both a small object retrieval and key-turning task. In response to the first task, the cortical representation of the hand's digits expanded, whereas wrist and forearm representations contracted. The exact opposite was found when training the second task. Importantly, these changes were progressive but also reversible. This firmly established the important link between cortical reorganisation and behaviour (for further readings on cortical structural plasticity: Buonomano and Merzenich, 1998, Merzenich and Sameshima, 1993, Sanes and Donoghue, 2000, Kaas, 1991)

In summary, plasticity is driven by use or disuse associated with an abundance or lack of internal sensory afferent or motor efferent signalling. We will further refer to this type of plasticity as use-dependent or internally driven plasticity. Whilst the previous paragraphs suggest 'use' is the primary driver of plasticity, an expansion of movement representations may also occur following repetitive electrical stimulation for 1 - 3 h whilst the monkey is passive (Nudo et al., 1990). During the latter the signal that drives plasticity is purely external and corresponding reorganisation may be considered as externally driven plasticity. Both internally and externally driven plasticity have been studied extensively in the human brain over the last 25 years.

Studies of plasticity in the human motor system were originally restricted to neurological patients as the techniques available to study plasticity required stimulation of the exposed nervous system. Advances in neuroimaging and neurostimulation techniques made it possible to study plasticity non-

invasively in the healthy human brain. All techniques use an indirect measure to quantify cortical, spinal or corticospinal excitability (CSE). The term 'excitability' in relation to the nervous system and plasticity was first brought forward by Jerzy Konorski in his important book *'Conditioned reflexes and neuron organization'* in 1948:

"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 - English translation)*

Thus, CSE reflects the input-output transfer gain of the corticospinal tract. The transfer gain is a sum of the strength of connection between each neuronal element that action potentials originating in the motor cortex have to cross before arriving to the muscle motor units. In this thesis Transcranial Magnetic Stimulation (TMS) will be exclusively used to study CSE. When administered over the primary motor cortex it will result in a muscle twitch. Recording the muscle's electrical activity allows researchers to measure the twitch as a motor evoked potential (MEP). The MEP amplitude is used to quantify CSE, and any changes may reflect plasticity along the corticospinal tract at cortical, subcortical or spinal level. TMS to assess CSE will be extensively discussed in section 1.3.

1.2 Structural plasticity of the human brain

1.2.1 Internally driven plasticity

The first proof for cortical reorganisation within the human motor system came from amputees (Hall et al., 1991, Cohen et al., 1991a) and spinal cord injury patients (Topka et al., 1992) using TMS mapping. By systematically mapping CSE across the motor cortex TMS maps revealed a lasting reorganisation as a result of the inability to control part of the body. The inactive cortical area was innervated by the cortical representations of the muscles directly proximal to the amputation or spinal injury, similar to as had been demonstrated in animal work (section 1.1). Brasil-Neto et al. (1992c) induced an artificial peripheral injury by using an anaesthetic block of the forearm and hand, confirming the 'loss' of a body parts led to an increased MEP amplitude of muscles proximal to the nerve block. Similarly, limb immobilisation caused shrinking of cortical area associated with the muscles of the inactive body part (Liepert et al., 1995). Whilst MEP amplitude returned to baseline soon after the anaesthesia or immobilisation ended, together these results indicate that persistent use is essential for cortical organisation to remain permanently changed. This notion was substantiated when researchers, rather than studying disuse, started exploring how learning a new skill changes cortical organisation.

Plasticity of the motor cortex following learning has been investigated using many different motor tasks. The purpose of the initial experiments was to provide evidence that cortical reorganisation could be linked to skill acquisition. Grafton et al. (1992) was one of the first to study this, training participants on a visuomotor tracking task. He revealed an increased regional blood flow in motor areas using Positron Emission Topography (PET) soon after onset of training that paralleled improvement in tracking performance. His findings of rapid changes in cerebral function were supported by Pascual-Leone et al. (1994) who used TMS rather than PET. Whilst training a serial reaction time task participants developed implicit knowledge on a repeated test sequence. Their reaction times shortened, demonstrating learning, and at the same time the cortical representation associated with several hand and arm muscles expanded. However, rapidly after participants had

acquired explicit knowledge about the used sequence, the cortical representation returned to baseline. The latter made Pascual-Leone conclude that skills may become overlearned, resulting in new changes of the cortical organisation. Whereas the serial reaction time task only involved learning during a single session, in a follow-up study Pascual-Leone demonstrated changes in cortical representation persisted on a longer time scale (Pascual-Leone et al., 1995). Participants trained a finger-sequence exercise on a piano for 5 days during which the cortical representation of finger extensor and flexor muscles slowly increased every day. Karni et al. (1995) added that these changes in cortical activity persist for several months when training a rapid finger sequence movement over a longer period (4-6 weeks).

That cortical reorganisation is not restricted to learning complicated sequences was demonstrated by Classen et al. (1998b) and later Muellbacher et al. (2001). In both their experiments participants trained on a ballistic motor learning task. Classen et al. (1998b) showed that when participants were instructed to repetitively move the thumb in the direction opposite the direction of twitch elicited by TMS. After training for 15-30 min the TMS induced twitch would occur in the trained direction. Moreover, using a similar protocol but asking participants to produce brisk pinch movements every 2 s Muellbacher et al. (2001) demonstrated training resulted in increased peak pinch accelerations and MEPs after 60 min of training. Crucially, both of these elegant studies provided evidence that changes at the level of the cortical motor cortex but not spinal level are essential for observed plasticity. Repetitive TMS over the motor cortex but not other cortical areas led to disruption of retention in behavioural improvement (Muellbacher et al. 2002) and TES revealed significantly smaller changes in excitability (Classen et al. 1998b). The findings of both studies may suggest simple repetitive use can drive plasticity, but it is important to acknowledge an element of learning is essential for plasticity be present (Plautz et al., 2000, Kleim et al., 2002).

Demonstration of the significance of cortical reorganisation to human motor learning has been demonstrated in humans that mastered a skill by years of practice. For example, the somatosensory cortical area on the hemisphere associated with the hand Braille readers use, contains a larger area

exhibiting responses to sensory hand stimuli than the contralateral hemisphere. Similar findings for the motor cortex have been obtained for the hand used by experienced string musicians (Elbert et al., 1995), trained badminton and volleyball players (Pearce et al., 2000, Tyc et al., 2005).

Whereas physical practice has long been considered essential in motor learning to promote cortical reorganisation (e.g. Butefisch et al., 1995), there is evidence that also motor imagery can facilitate changes in CSE. Motor imagery is the process of mentally rehearsing a movement without creating any overt motor output, and reported to be able to lead to improved motor performance (Feltz and Landers, 1983). Pascual-Leone et al. (1995), alongside his finding that sequence learning led to progressive expansion of the cortical area related to the finger muscles, also found that learning the same task using imagery led to changes in cortical organisation. The potential of motor imagery in motor learning can be explained by the finding that the same cortical areas are active during motor execution and motor imagery (Grafton et al., 1996, Rizzolatti et al., 1996, Grezes and Decety, 2001, Jeannerod, 1994).

1.2.2 Externally driven plasticity

There are several brain stimulation techniques that can be used to trigger cortical reorganisation, with the participants being passive (Ziemann et al., 2008). The finding of Nudo et al. (1990) that repetitive invasive microstimulation to the exposed motor cortex of the rat could induce cortical reorganisation was one of the first findings suggesting sensory feedback is not essential for plasticity. Repetitive stimulation of the human motor cortex using TMS has confirmed this hypothesis. rTMS can depress or facilitate CSE dependent on the stimulation frequency, with 1 Hz stimulation depressing CSE and stimulation at frequencies > 10 Hz facilitating CSE (Chen et al., 1997, Berardelli et al., 1998, Maeda et al., 2000b). By modulating either the motor cortex or other cortical areas, rTMS is used to study processes underlying motor learning and memory (Censor and Cohen, 2011).

One downside of rTMS is that usually in excess of 1.000 stimuli are needed an therefore sessions can take up to 1 h. A special kind of repetitive stimulation, theta burst stimulation (TBS), uses short bursts

of 50 Hz stimulation repeated at 5 Hz. This has also been found effective in modulating CSE (Huang et al., 2005). The major advantage of TBS is that stimulation is only required for 40 s. Moreover, stimulation is always subthreshold and therefore causes less discomfort for the participant. A different form of TMS induced plasticity combines a cortical magnetic stimulus with an electrical peripheral nerve stimulus. Paired Associative Stimulation (PAS) results in a excitation or depression of CSE dependent on the timing of the two stimuli (Carson and Kennedy, 2013, Stefan et al., 2000), making use of the Hebbian synaptic plasticity concept 'fire together, wire together'. With the peripheral electrical stimulus administered 10 ms before the TMS pulse CSE is depressed, whilst when this interval is increased to 25 ms CSE is facilitated (Wolters et al., 2003).

Not only magnetic stimulation but also non-invasive weak (< 2 mA) current stimulation of the brain results in a facilitation or inhibition of CSE (for review: Paulus, 2011). It took almost 2 centuries following the first use of current stimulation as a treatment (Hellwag and Jacobi, 1802) before Priori reported weak DC currents (<0.5 mA) could reduce CSE. The techniques involve placing two rubber stimulating electrodes on the scalp and between the electrodes either a direct current (transcranial direct current stimulation (tDCS) - Nitsche and Paulus, 2000), alternating current (transcranial alternating current stimulation (tACS) - Antal et al., 2008) at a single frequency, or alternating current consisting of multiple frequencies is flowing (transcranial random noise stimulation (tRNS) - Terney et al., 2008). The advantage of using alternating over direct current is that it is possible to modulate natural brain rhythms underlying behaviour (Pogosyan et al., 2009, Zaehle et al., 2010, Helfrich et al., 2014). There is a plethora of literature discussing the effects of current stimulation of the brain, complicated by the fact that individual anatomy affects current pathways (Datta et al., 2012). Moreover, effects depend on stimulation duration and intensity, electrodes position, size and in which direction the current flows and, in case of tACS, the frequency of stimulation (Batsikadze et al., 2013, Moliadze et al., 2010a, Moliadze et al., 2012, Moliadze et al., 2014, Wach et al., 2013, Mehta et al., 2015, Monte-Silva et al., 2010, Nitsche et al., 2007).

One of the greatest difficulties with the non-invasive brain stimulation protocols to induce plasticity is that a canonical response does not seems to exist. Maeda et al. (2000a) was the first to report on the great interindividual variability in the response to several rTMS paradigms. This notion has been justified with many other studies showing in only one-third to two-third of the tested participants non-invasive brain stimulation alters CSE in the expected way (Wiethoff et al., 2014, Lopez-Alonso et al., 2014, Lopez-Alonso et al., 2015, Muller-Dahlhaus et al., 2008, Hamada et al., 2013). These results have recently sparked a debate of stimulation effectiveness, as a meta analysis for the effect of tDCS has shown little neurophysiologic effect beyond MEP amplitude (Horvath et al., 2014, Horvath et al., 2015). Although externally driven plasticity is possible using a wide variety of stimulation protocols, it's efficacy is debated and work is required to optimise its effects.

1.3 Assessment of plasticity using TMS

Barker et al. (1985) showed that by passing a brief pulse of current from a high-voltage capacitor through a copper coil held over the primary motor cortex, a measurable motor action potential could be evoked in a contralateral hand muscle. This meant researchers could study cortical organisation without being restricted to the damaged human brain or transcranial electrical stimulation (TES - discussed in more detail later). Other than a 'clicking' sound and the likelihood of some head or facial sensations, no discomfort is associated with TMS. TMS has become one of the most commonly used non-invasive brain stimulation techniques over the past 30 years (Rossini et al., 2015). It is important to distinguish TMS is used as a tool to both assess plasticity by measuring CSE using a single or paired pulse paradigm, or induce neuroplasticity using repetitive TMS (rTMS). This thesis will exclusively focus on TMS as a tool to assess neuroplasticity by using single pulses administered over the primary motor cortex.

Originally TMS coils were round (Figure 1-1A), but nowadays the 'figure-of-8' coil is more common due to its better focality. Triggering the TMS stimulator causes a brief pulse of current to flow through the copper coil, inducing a magnetic field. A changing magnetic field induces an electric current in a nearby conductor, flowing in the opposite direction to the direction of travel in the coil. TMS administered over the primary motor cortex will result in a twitch in a contralateral muscle that can be measured using electromyography (EMG - Figure 1-1B). The biphasic EMG response is referred to as the motor evoked potential (MEP - Day et al., 1989). An MEP is an compound response that is a result of the direct and indirect depolarisation of cortical neurons within the cortex (Di Lazzaro et al., 2004b). The peak-to-peak amplitude of the MEP is used to quantify corticospinal excitability (CSE). Any changes in the MEP amplitude may be suggestive of plasticity.

TMS is usually administered at a stimulation intensity that is defined with respect to the motor threshold. The motor threshold reflects the intensity above which MEPs can be elicited, thereby allowing standardisation between participants. The motor threshold can be defined whilst the muscle is at rest or when it is active. The most commonly used method to find the motor threshold is the relative

frequency method (Rossini et al., 1994). For the resting motor threshold (RMT) this means that at least 5 out of 10 stimuli applied need to result in an MEP amplitude > 50 μV. For the active motor threshold (AMT), the same criterion is used but MEP amplitude must be at least 200 μV, or clearly distinguishable from the background EMG (Rossini et al., 1994).

During single pulse TMS, a limited number of stimuli (< 200) is applied to the motor cortex with an interstimulus interval (ISI) > 1 s to probe CSE. The resulting MEPs are analysed and their amplitudes extracted to find an overall mean amplitude that quantifies excitability. When stimulation is performed at different intensities or at different locations of the motor cortex, one can also construct a stimulus response (SR) curve (Devanne et al., 1997) or TMS map (Wassermann et al., 1992).

1.3.1 Traditional single pulse TMS

Traditionally, CSE is quantified by taking the mean and standard deviation (*SD*) of a number of MEPs. Changes in CSE are studied by comparing the size of the MEPs before and after the intervention (e.g. Muellbacher et al., 2001). Although the method is easy to use and requires minimal post-processing of data, care should be taken when deciding how many MEPs to acquire and what stimulation intensity or ISI to use. Firstly, studies frequently acquire only 10-20 MEPs (e.g. Lopez-Alonso et al., 2014, Stefan et al., 2000, Wach et al., 2013), despite evidence that 30 MEPs are required to obtain a reliable estimate of CSE (Eisen et al., 1991, Cuypers et al., 2014). Secondly, stimulation intensity is important as different intensities have been suggested to recruit different populations of cortical neurons (Di Lazzaro et al., 2012). Traditionally, stimulation intensity is taken as a percentage of the motor threshold (Hess et al., 1987), but the stimulation intensity eliciting an 1 mV MEP_{pp} is also used (SI_{1mV}) (e.g.Wiethoff et al., 2014). The latter approach is questionable as an 1 mV MEP may be near the maximum MEP for one participant, but only 50% of the maximum for others (Burke and Pierrot-Deseilligny, 2010). Finally, due to potential alterations in CSE when stimulating at a rate equal or greater than 1 Hz (Chen et al., Berardelli et al., 1998), the ISI is also of importance. In most studies ISI is limited to about 4 s because of technical limitations, however researchers also adopt a 10 s ISI. Although ISI may be adapted when participants find stimulation uncomfortable, there seems to be no

other reason to use an ISI of 10 s rather than < 4 s when this is possible. Despite these important considerations and discrepancies in protocols, the MEP reliability is considered high for muscles in the upper and lower limbs (Corp et al., 2015, Cacchio et al., 2009, Bastani and Jaberzadeh, 2012, Kamen, 2004).

1.3.2 Stimulus Response (SR) curves

A second technique that is used to assess CSE is by constructing stimulus response (SR) curves (Devanne et al., 1997). SR curves visualise the stimulation intensity versus the amplitude of the resulting MEP (Figure 1-1C). Stimuli are applied to a single site of the primary motor cortex at different stimulation intensities from just below the RMT, up to when the MEP amplitude saturates. Usually data is acquired at 3-15 different stimulation intensities, with 3-20 stimuli administered at each intensity (e.g. Jensen et al., 2005, Ridding and Rothwell, 1997, Hamada et al., 2014, Jung et al., 2010). The relationship between stimulation intensity and MEP amplitude is modelled using the Boltzmann equation and the Levenberg-Marquart nonlinear fitting algorithm (Levenberg, 1944, Marquardt, 1963):

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

It contains 3-5 parameters (e.g. Malcolm et al., 2006, Pitcher et al., 2003, Barsi et al., 2008) which are used to quantify changes in excitability. Changes in the slope (S), the intensity at which the SR curve is halfway between the minimum and maximum plateau (I_{50}), and maximum plateau (MEP_{max}) have all been used to described changes in excitability (e.g. Houdayer et al., 2008, Jensen et al., 2005, Rosenkranz et al., 2007a). More recently, also the area under the curve has been introduced as a measure to quantify excitability using the SR curve (Carson et al., 2013). Importantly, all measures used to characterise the SR curves have been reported to be reliable (Carroll et al., 2001, Carson et al., 2013, Mathias et al., 2014).

The total time to acquire data for the SR curve may exceed 10 min, as ISI is restricted to 4-10 s. In order to speed up data acquisition it is not uncommon to see researchers reduce the number of stimulation intensities or the number of stimuli administered per stimulation intensity (e.g. Ray et al., 2002, Pearce et al., 2012, Liepert et al., 2003). It is uncertain if this affects the value of the information that input-output relationship provides. Different stimulation intensities are associated with recruitment of different neuronal populations and might therefore provide different information with respect to changes in CSE (Di Lazzaro et al., 2012). Mathias et al. (2014) tackled the problem of a long acquisition time by using a TMS stimulator that allows for rapid stimulation (ISI < 4 s) and optimising the number of stimuli. This allowed acquisition of the SR curve data within 2 - 3 min without compromising on the amount of data acquired.

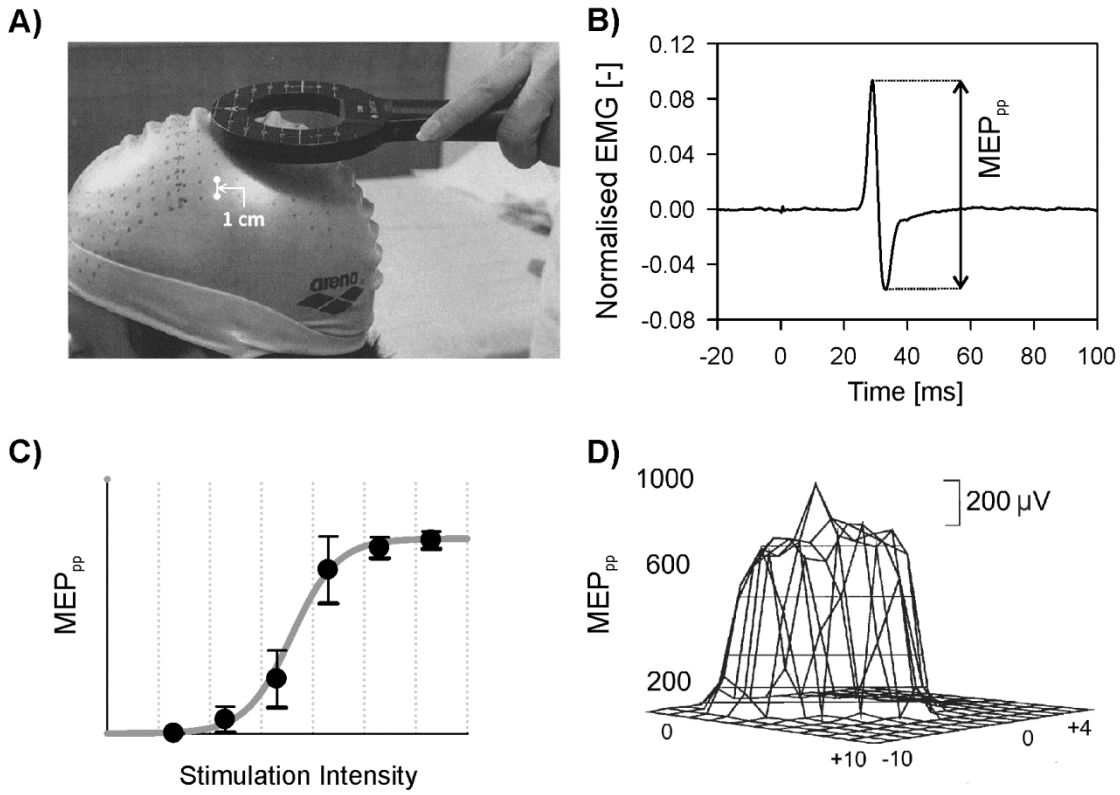

Figure 1-1: Demonstration of TMS acquisition methods. (A) Participants are fitted with a swim cap. The swim cap is used to mark the motor hotspot. When performing TMS mapping a grid is marked out, in this case 1-cm spaced (modified from Mortifee et al. 1994). (B) A motor evoked potential recorded from a muscle using EMG. Corticospinal excitability is quantified as the peak-to-peak amplitude of the MEP (MEP_{pp}). (C) Traditionally, the SR curve is constructed by stimulating the motor hotspot multiple times at different intensities, visualising the stimulation intensity versus the mean MEP_{pp} amplitude. Data is fitted with a Boltzmann sigmoid curve (grey line). (D) Stimulating at multiple different positions (1-cm spaced) over the motor cortex allows construction of a TMS map, visualising the stimulation positions versus the MEP_{pp} amplitude (modified from Uy et al. 2002).

For the SR curve, stimuli are only administered to a single site of the motor cortex and therefore changes in excitability cannot be distinguished from changes in cortical organisation (Ridding and Rothwell, 1997). To allow for detection of changes in motor cortex organisation, a third and last technique that is used to quantify changes in CSE is TMS mapping.

1.3.3 TMS mapping

In TMS mapping the motor cortex is stimulated at different sites and offline a topographical map is drawn, visualising the stimulation position with the matched MEP amplitude (Figure 1-1D). TMS mapping requires recording of the position of each stimulus in addition to the MEPs. Traditionally, the participant is fitted with a swim cap on which a 1-cm spaced grid is marked (see Figure 1-1A modified from Mortifee et al., 1994). Stimuli are then administered in a predetermined fixed sequence so each EMG recording could be linked to a single grid point. Nowadays, neuronavigation systems are used to aid coil positioning and record stimulation position (e.g. Gugino et al., 2001, Ngomo et al., 2012b). Information about cortical reorganisation can be extracted from three different parameters. First, the centre of gravity (COG) represents the amplitude weighted mean of the map and allows to quantify shifts in cortical representation. Second, the map area represents the area of the scalp that results in an MEP when stimulated. This measure quantifies changes in the size of the cortical representation. Last, map volume is used to describe the TMS map, but this measure is rarely used. Map volume is a sum of the size of MEPs and is thereby a candidate to quantify changes in excitability without a change in COG or map area. From the early 1990s the map's COG, area and to a lesser extent volume have been successfully used to study motor cortex organisation (e.g. Wassermann et al., 1992, Wilson et al., 1993) and motor cortex reorganisation following motor skill learning (e.g. Pascual-Leone et al., 1995) or as a consequence of trauma (e.g. Cohen et al., 1991a, Cohen et al., 1991b, Fuhr et al., 1992, Topka et al., 1991, Hall et al., 1990). All measures except map volume have been found to be highly reliable (Wolf et al., 2004, Malcolm et al., 2006, Mortifee et al., 1994, van de Ruit et al., 2015).

The method of acquiring data for a TMS map is critical for the obtained results and it therefore surprising to see that such a wide variety of acquisition parameters have been used to acquire TMS

maps (Table 1-1). In one of the benchmark mapping studies in healthy participants, Wilson et al. (1993) used 30-35 grid points organised in a rectangular 1-cm spaced grid. This 1-cm spaced grid was followed until no responses are visible in the EMG, after which mapping is continued in a different direction. Mapping '*until the border of the motor area had been defined*' (Wilson et al., 1993) is a typical approach. The alternative is to map out a fixed number of grid points (e.g. Classen et al., 1998a, Malcolm et al., 2006, Cicinelli et al., 2006). When using a fixed number of grid points there is a risk of the grid being too small, or alternatively administering an unnecessary amount of extra stimuli. For both approaches grid spacing is often restricted to 1-cm, despite the suggestion that a 0.5-cm spaced grid would provide a better estimate of the COG (Brasil-Neto et al., 1992b). The estimate of COG can be improved by applying at least 6-10 stimuli to each grid point (e.g. Boroojerdi et al., 1999, Wolf et al., 2004, Triggs et al., 1999). Nonetheless, only using four stimuli per site as by Wilson et al. (1993) is common, even whilst finding this leads to a further ±2 mm error in the COG (Classen et al., 1998a). One reason for not mapping using the optimal acquisition parameters is to reduce the time it takes to acquire the data for the map (explicitly stated in Cicinelli et al., 1997).

An important parameter affecting acquisition time is also the ISI, usually chosen between 4 - 15 s (Littmann et al., 2013, Guerra et al., 2015, Tyc and Boyadjian, 2011, Wilson et al., 1993). The ISI is restricted to 4 s as monophasic TMS stimulators (e.g. the Magstim 200), that are commonly used, cannot stimulate at a rate faster than once every 2-4 s (dependent on stimulation intensity). Whilst stimulating at a faster rate is achievable, this is avoided as repetitive TMS (rTMS) at 1 Hz depresses CSE (Chen et al., 1997). Nonetheless, one research group has performed mapping with an ISI of 1.1 s (Malcolm et al., 2006, Plowman-Prine et al., 2008), but mapping with such a short ISI has never been systematically compared to mapping using longer ISIs.

The TMS map is also affected by the stimulation intensity and the level of muscle activation during mapping. Both an increased stimulation intensity and muscle activation increase MEP amplitude (Hess et al., 1987, Day et al., 1989). A higher stimulation intensity will increase the strength and size of the magnetic field and thereby excite a greater number of cortical neurons. This will result in an artificial

inflation of the motor cortex representation (Thickbroom et al., 1998). The use of a fixed percentage (110-120%) with respect to the motor threshold is common (e.g. Uy et al., 2002, Guerra et al., 2015, Kesar et al., 2012). However, a fixed percentage (10-20%) of the maximal stimulator output (MSO) on top of the MT (e.g. Mortifee et al., 1994, Byrnes et al., 1999, Melgari et al., 2008) or stimulation intensity resulting in an MEP size of 0.5-1 mV has also been used (e.g. Sparing et al., 2008, Freund et al., 2011). Mapping has even been performed at maximal stimulator output (Wassermann et al., 1992, Brasil-Neto et al., 1992b). Higher stimulation intensities are associated with a greater map area (Thordstein et al., 2013), but whether map's COG, map volume and its shape are affected by these higher intensities is unclear.

There are also still open questions about what the effect of muscle activation on the map parameters is. Mapping has been performed with the muscle studied being either passive or active (e.g. Pascual-Leone et al., 1995, Wassermann et al., 1992, Byrnes et al., 1999, Wilson et al., 1993). The motor threshold is lower for a muscle that is pre-activated than when the muscle is at rest (Devanne et al., 1997). Map area is enlarged when not taking this into account (Wilson et al., 1995), but when compensating for this by adjusting stimulation intensity, the map area is unaffected (Ngomo et al., 2012b). Contradicting findings on the effect of muscle activation on COG exist, with some reporting a shift of the COG (Wilson et al., 1995) whilst others do not (Classen et al., 1998a, Ngomo et al., 2012b). Mapping has never been performed with levels of muscle activation greater than 10% of the MVC, although MEP amplitude for a small hand muscle has been reported to saturate above 10% (Helmers et al., 1989, Taylor et al., 1997).

Table 1-1: Overview of acquisition parameters during TMS mapping. This table serves to highlight the great inter-study differences, and does not provide an overview of all mapping studies to date. This table illustrates how many studies have their own unique acquisition protocol with differences across all parameters: the number of stimulation sites, stimuli administered per site, interstimulus interval (ISI), stimulation intensity (SI), grid spacing and whether maps are acquired with the muscle at rest of being active. This results in great discrepancies in time required to acquire the data for a TMS map.

Author	*# Stim sites*[1]	*# Stim per site*	*ISI [s]*	*SI*[2]	*Grid spacing [cm]*	*% MVC*	*Single map time [s]*
Wassermann et al. (1992)	U.N.R.	3	-	100% MSO	1	0	
Wilson et al. (1993), (1995)	30-35	4	10-15	+20% MSO	1	0 / 10	1200
Wassermann et al. (1993)	U.N.R.	20	-	35-45% MSO	1	10	
Mortifee et al. (1994)	U.N.R.	3	>10	+10% MSO	1	0	
Pascual-Leone et al. (1995)	25	8	3-5	110% RMT	1	0	600
Liepert et al. (1995)	U.N.R	2-5	-	130% RMT	1	0	
Wilson et al. (1996)	30-35	8	10-15	-	1	10	2400
Traversa et al. (1997)	11	4	-	+10% MSO	2	0	
Ridding and Rothwell (1997)	U.N.R.	3		120% MT	1-2	0 / 10	
Classen et al. (1998a)	49	20	~3	120% RMT	1	0	2940
Boroojerdi et al. (1999)	25	6	-	MEP 0.5-1 mV	1.5	0	
Byrnes et al. (1999)	U.N.R	4	10-15	+20% MSO	1-2	10	
Triggs et al. (1999)	81	8	3-5	120% RMT	1	0	1944
Thickbroom et al. (1999), (2004)	U.N.R.	4	5	+20% MSO	1-2	10	
Uy et al. (2002)	U.N.R.	3	-	115% RMT	1	0	
Herwig et al. (2002)	140-200	1	>2	120% MT	0.5	0	280
Foltys et al. (2003)	36	6	-	120% RMT	1.5	0	
Ferreri et al. (2003)	49	4	5-10	+10% MSO	-	0	980
Neggers et al. (2004)	25	10	4	110% RMT	0.5	0	1000
Corneal et al. (2005)	U.N.R.	10	5	+10% MSO	1	0	
Krause et al. (2006)	U.N.R.	5	-	120% RMT	1	0	
Malcolm et al. (2006)	25	5	1.1	115% RMT	1	0	138
Cicinelli et al. (2006)	19	3	10	110% RMT	1-1.5	0	570
Sparing et al. (2008)	30	6	>5	MEP 0.5-1 mV	1.5	0	900
Plowman-Prine et al. (2008)	49	6	1.1	115% RMT	1	0	323
Melgari et al. (2008)	121	4	5-10	+10% MSO	1	0	2420
Gagne et al. (2011)	15	4	4-6	120% RMT	1	0 - 10	660
Freund et al. (2011)	72	10	-	110% SI 1 mV	1	10	
Ngomo et al. (2012a), (2012b)	56	6	4-6	110% MT	1	0 / 7.5	1344
Littmann et al. (2013)	15	5	>10	120% RMT	0.5-1	0	750
Thordstein et al. (2013)	U.N.R.	1	5	100-120% RMT	Rand	0	
Guerra et al. (2015)	49	4	5-10	110% RMT	1	0	980

[1] U.N.R. - 'Until No Response'
[2] MSO - Maximal Stimulator Output & RMT - Resting Motor Threshold

1.3.4 Application in clinical practice

The many open questions and lack of consensus about how best to use TMS to assess CSE might explain its limited use in clinical practice. TMS to assess cortical reorganisation and CSE is extensively used in stroke research, providing valuable prognostic information (Byrnes et al., 1999, Chieffo et al., 2013, Hendricks et al., 2002, Liepert et al., 2000, Perez and Cohen, 2009, Talelli et al., 2006, Thickbroom et al., 2002, Traversa et al., 1997). Also for cerebral palsy (Kesar et al., 2012), dementia (Cantone et al., 2014), writer's cramp (Byrnes et al., 1998) and spinal cord injury (Topka et al., 1991, Freund et al., 2011) a clinical benefit of using TMS has been identified by monitoring cortical reorganisation, but its use is restricted to research. One exception is brain tumour mapping, where TMS has become a valuable tool to define tumour resection areas. Importantly, the use of TMS in conjunction with direct current stimulation leads to better clinical outcomes (Picht, 2014, Takahashi et al., 2013, Zdunczyk et al., 2013, Krings et al., 1997b, Krieg et al., 2014).

This raises the question why TMS has never found a strong foothold as a clinicians day-to-day assessment tool. One reason might be that the idea of stimulation of the brain might not be very appealing to patients and clinicians, preventing acceptance. Alternatively, the TMS assessments might just takes too long as data collection for a SR curve or TMS map usually takes in between 10 - 40 min (e.g. Guerra et al., 2015, Wilson et al., 1996, Littmann et al., 2013, Houdayer et al., 2008, Jensen et al., 2005). One cannot expect a patient to remain attentive and relaxed for such a long period. In order to make experiments tolerable for patients, researchers have performed TMS mapping with less grid points, fewer stimuli per grid point (e.g. Traversa et al., 1997, Littmann et al., 2013) or shorter ISIs (Plowman-Prine et al., 2008). The finding that SR curves may be acquired in 2-3 min (Mathias et al., 2014) is promising for its clinical application. A similar reduction in acquisition time would be required for TMS mapping to make this method more widely applicable.

1.3.5 Limitation of single pulse TMS assessments

A notable downside of using TMS to assess changes in CSE is that any changes in the MEP cannot be solely attributed to changes in the excitability of the cortical neurons. The MEP reflects the excitability

along the whole pathway from motor cortex to muscle. Its size is determined by the synaptic efficacy, synaptic excitability and intrinsic excitability of both neurons at the cortical and spinal level. Subcortical changes in excitability can be studied using cervicomedullary stimulation, TES or the Hoffmann reflex (H-reflex - Hoffmann, 1910). TES is the predecessor of TMS and is the first technique found to be able to non-invasively stimulate the brain through the intact scalp (Merton and Morton, 1980). Both cervicomedullary stimulation, applied between the mastoids, and TES quantify excitability of the corticospinal pathway without any influence of cortical excitability (Taylor, 2006, Di Lazzaro et al., 1998a). TES, in contrast to TMS, activates the cortical neurons directly at the axon hillock (Day et al., 1989, Burke et al., 1993). The H-reflex involves direct electrical stimulation of the Ia afferents and provides a measure of spinal excitability.

1.4 Goals and aim of this thesis

The present thesis presents the results of six different research studies which aim (1) to demonstrate that TMS mapping and SR curves can be acquired much faster than has been traditionally possible and (2) that these techniques can be used to study internally externally driven plasticity. To achieve the first aim we sought to speed up the TMS mapping method and develop a graphical user interface (GUI) to facilitate acquisition of the SR curve. To fulfil the second aim we set out to use these improved acquisition methods to demonstrate they are feasible techniques to study internally (use-dependent) and externally driven plasticity. The former was done by studying plasticity in response to mirror training augmented by imagery training and a visuomotor tracking learning task, whilst the latter was exploited by the use of tACS.

The studies in Chapter 3 and 4 focussed on optimising the TMS mapping techniques. Specifically, in Chapter 3, TMS mapping was compared administering single stimuli at pseudorandom location across a fixed size square grid to the traditional mapping method using a 1-cm spaced grid. This study aimed to optimise the ISI and number of stimuli in order to reduce the acquisition time. We tested the hypothesis that maps acquired using the pseudorandom walk method using ISIs of 1, 1.5, 2 and 3 s would be different from 4 s as evidenced by changes in COG, map area and map volume. Chapter 4 extends on these results by investigating the effect of the stimulation intensity and muscle activation for which the effect on the TMS map has not been systematically explored. In addition to the standard TMS map measures, the effect on the map's shape was also investigated. For increasing stimulation intensities we hypothesised an increase in map area and volume whilst COG and map shape remains unaffected. As the MEP response saturates when the muscle is activated above 10% MVC, for increased muscle activation we hypothesised that map area and map volume would also saturate when the muscle activity exceeds this level, with no change in COG and map shape. In Chapter 5 we move to optimising the SR curve method adding to the findings of Mathias et al. (2014), who significantly improved the acquisition time of the SR curve, by developing a GUI that allows the researcher to obtain direct feedback about the SR curve. As a great variability in the number of stimuli required to

construct a reliable SR curve was found in the Mathias study, the GUI aims to permit tailoring the number of stimuli for each individual and each acquired curve.

One of the reasons for reducing the acquisition time of TMS assessment techniques is so they can be more widely used, potentially also in clinic. Therefore, the SR curve software and novel mapping procedure were tested to demonstrate feasibility in studying both internally and externally driven plasticity. Chapter 6 aims to study changes in CSE using TMS mapping following the combination of motor imagery and mirror training in healthy participants. Mirror training was successfully introduced in the 1990s to alleviate phantom limb pain in amputees (Ramachandran and Rogers-Ramachandran, 1996) and reported to promote motor learning (Nojima et al., 2012). We hypothesised that motor imagery training in addition to mirror training would significantly increase CSE compared to mirror training alone. We also assessed the effect of a motor learning protocol, visuomotor tracking learning (Perez et al., 2004, Jensen et al., 2005), to study use-dependent plasticity (Chapter 7). We aimed to investigate intra- and interhemispheric differences in plasticity using TMS mapping, comparing learning of the dominant an non-dominant hand and learning using a proximal and distal muscle. We hypothesised to find no difference between the change in CSE following learning with the dominant and non-dominant hand or proximal and distal muscle following learning. However, we hypothesised to find less participants with an increased CSE for the dominant than the non-dominant hand and a greater number of participants with increased CSE for the distal muscle than the proximal muscle. In the last study, described in Chapter 8, externally driven plasticity was studied. The study aimed to investigate the effect of tACS on the SR curve and TMS maps, with a stimulation frequency within (20 Hz) and outside (140 Hz) the traditional beta EEG frequency band for the motor system. We picked a frequency of 140 Hz as it has been demonstrated to lead to a significant increase in CSE (Moliadze et al., 2010a). Moreover, 20 Hz was used as results regarding its effects on CSE have been equivocal (Wach et al., 2013, Feurra et al., 2011). We hypothesised that 20 Hz TMS would significantly inhibit CSE, characterised by a decreased map area and area under the SR curve. For 140 Hz we hypothesised a significant increase in CSE marked by an increase in TMS map area and the SR

area under the curve, in line with an earlier study (Moliadze et al., 2010a). Both stimulation protocols are only expected to lead to the hypothesised response in 50% of the tested individuals.

CHAPTER 2

General Methods

This chapter provides a brief background and description of the used methods in the different studies that this thesis covers. An in depth description of techniques of TMS and EMG is not given as these are well established techniques and commonly adopted methods were used. Detailed descriptions of protocols, data and statistical analysis can be found in the 'Methods' sections of the individual chapters.

2.1 Experimental Design

All studies in this thesis were reviewed and approved by the Science, Technology, Engineering and Mathematics ethics committee (University of Birmingham) and performed according to the guidelines of the Declaration of Helsinki. In Chapter 3 and 4, TMS maps were repeatedly acquired in a single session with different acquisition parameters. In all other studies measurements were taken before and after either mirror training (Chapter 6), a motor learning task (Chapter 7) or transcranial current stimulation (Chapter 8). For the studies in Chapter 7 and 8 the effect of different tasks or interventions was compared using a within-subject design, where the same subjects made repeated visits to the laboratory. To avoid carryover effects, a wash out period of one week between sessions was used. In Chapter 6 a between-subject design was used as the mirror and imagery training could only once be effectively performed by each participant. When multiple sessions in one participant were performed, each session was scheduled at the same time of the day.

2.2 Participants

Healthy males and females between 18-46y old, without a history of neurological or muscular disease, were recruited to participate. Most participants participated through the research participation scheme in the School of Sport, Exercise and Rehabilitation Sciences. Prior to commencing a study, eligibility

to participate was confirmed using a TMS adult health safety screening questionnaire to screen participants for their current and former state of health (Keel et al., 2001). Participants were given written and verbal information and had the possibility to ask questions or express concerns.

Participants were asked to provide some extra information to allow evaluation of potential confounding factors. In Chapter 6-8, participants' handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Moreover, in Chapter 6 participants were asked to provide feedback about the training task and participants' motor imagery ability was assessed using the movement imagery questionnaire (MIQ-3) (Williams et al., 2012b). Lastly, participants were asked to report any side effects as a result of the transcranial current stimulation in Chapter 8.

2.3 Behavioural measures and interventions

In Chapter 6 and 7 basic behavioural measures were taken to assess motor function and task performance. The measurements and interventions are discussed here and consisted of:

2.3.1 Mirror training

Mirror training was first introduced as a therapy to relief phantom limb pain and later also found to be successful in stroke rehabilitation and motor learning (Ramachandran and Rogers-Ramachandran, 1996, Ramachandran and Altschuler, 2009, Nojima et al., 2012). For mirror training using the hands, participants are instructed to line up both hands, with each hand on one side of the mirror (Figure 2-1A). Next, they are instructed to start moving one hand, keeping the other still, whilst watching the mirror reflection of the moving hand. This will create the visual illusion of the passive hand moving in congruence with the active hand.

Healthy participants performed 16 min (8 x 2 min, 15 s breaks) of mirror training making repetitive grasping movements. Participants were seated in front of a mirror and instructed to line up both arms, with one on each side of the mirror. The lower arms were supported in foam blocks, ensuring the hands could move freely without touching the table top or foam support. Movements were performed

only with the dominant hand, paced by a 40 bpm metronome, whilst participants watched the hand's mirror reflection and imaged both hands moving.

2.3.2 Motor imagery ability

Motor imagery ability was assessed by use of the movement imagery questionnaire (MIQ-3) (Williams et al., 2012b). Motor imagery is the mental process of performing a movement without creating an overt motor output. Imagery is traditionally defined as follows:

> *"Imagery is an experience that mimics real experience. We can be aware of 'seeing' an image, feeling movements as an image, or experiencing an image of smell, taste or sounds without experiencing the real thing. It differs from dreams in that we are awake and conscious when we form an image."* (White and Hardy, 1998)

Three imagery perspectives can be distinguished: internal visual imagery, external visual imagery and kinaesthetic imagery. Whereas visual imagery is focussed at seeing yourself performing a task from an internal or external perspective, kinaesthetic imagery focuses on the feelings associated with a movement. The ability of participants to image using these different perspectives is assessed by having them physically perform four different movements (knee raise, jump, arm movement and waist bend). Subsequently, participants image the movement using one of the imagery perspectives. As a result, each movement is performed and imaged three times. Once the participant imaged the movement he/she was asked to rate the ease of imaging on a 7-point scale (1: very hard to see/feel – 7: very easy to see/feel). The mean score of the ratings provided for each imagery perspective quantifies imagery ability.

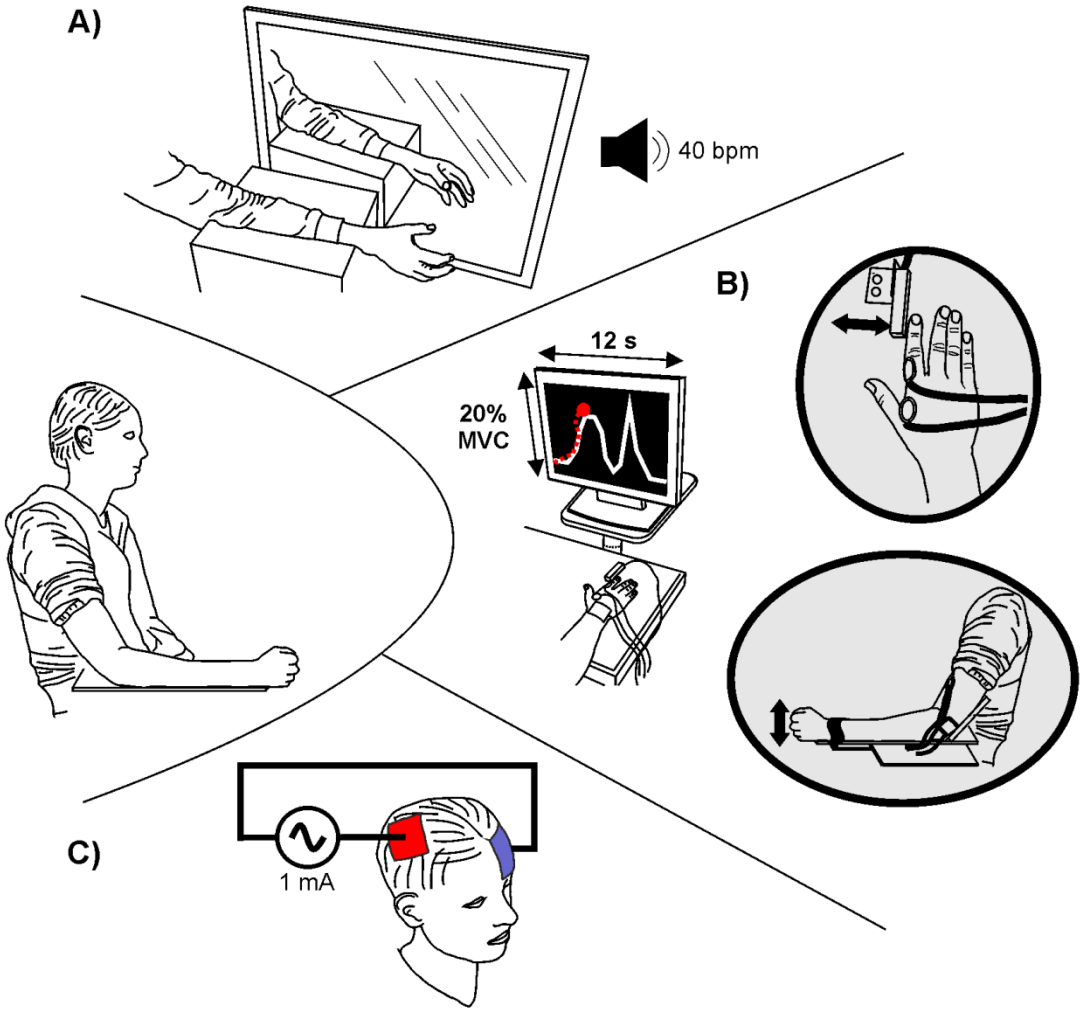

Figure 2-1: Overview of experimental setups used in this thesis. In all studies participants were comfortably seated. (A) Mirror training (Chapter 6) was performed by making repetitive grasping movements paced by a 40 bpm metronome. Hands were lined up, with one on each side of the mirror. One hand was kept passive whilst the reflection of the other, moving hand, was the point of focus. (B) Visuomotor tracking learning (Chapter 7) was performed both with the index finger as well as the biceps muscle. The waveforms to track were displayed on a monitor directly in front of the participants. Participants were instructed to track the line as close as possible by repetitively activating the first dorsal interosseous or biceps brachii muscle. (C) Transcranial alternating current stimulation (tACS) was administered (Chapter 8) to the motor cortex with a current of 1 mA. One electrode was centred over the motor hotspot, and the other over the contralateral orbit.

2.3.3 Layered stimulus response training

An imagery training protocol was included to improve participants' imagery ability and benefit mirror training. Layered Stimulus Response Training (LSRT) is an imagery training technique that was developed at the University of Birmingham (UK). LSRT was demonstrated to be effective in improving imagery ability but concurrently also motor performance (Williams et al., 2013). During LSRT, the image is built layer-by-layer by gradually including more detail. This type of imagery

training is well controlled and easy to administer making it especially well suited when only a short amount of time is available to perform the imagery training. Moreover, LSRT can be easily tailored to each individual. The imagery training was performed with the participants in the same posture as in which the mirror training was performed. Training was started asking the participant to perform the actual movement (grasping) with eyes closed, focussing on any prominent feelings and sensations whilst executing the movement. Participants were then asked to describe theses feelings and sensations, starting with the feeling or sensation most clear and/or vivid. The image was then built step-by-step using the other notable feelings and sensations, including extra detail each time. Once the participant had imaged the movement using the newly introduced feeling and/or sensations, it was confirmed the image did get clearer and more vivid based on verbal feedback. Between 3-5 layers were included before commencing the mirror training.

2.3.4 Visuomotor tracking

To study neuroplasticity in response to motor learning a visuomotor tracking learning task was used. Participants were comfortably seated with a monitor in direct line of sight, on which the target lines were presented (Figure 2-1B). All were instructed to track the target waveforms as closely as possible with a cursor by making contractions of varying degree of force. Each target line required a specific combination of contractions. The maximum contraction required to track the waveforms was 20% of the participants' MVC. The full waveform was always presented at once and the cursor moved from left to right at a fixed speed. Each waveform took 12 s to complete and 20 unique waveforms were presented in one single training block (4 min). Five blocks of training (20 min) were performed with 2 min resting breaks to prevent muscle fatigue. For each waveform tracking performance was determined by taking the root mean square (RMS) error between the followed path and the target line. Online feedback of tracking performance was provided after each waveform to encourage the participants to maintain improving over the five training blocks (Figure 2-2).

In the first experiment of Chapter 7, tracking was performed using the index finger of both the dominant and non-dominant hand. Activating the FDI muscle resulted in the cursor going up whilst

when relaxing the muscle the cursor would go down. In the second experiment, tracking was performed both with the index finger as well as the biceps muscle. When tracking using the biceps muscle, a biceps contraction movement was matched to the cursor going up whereas muscle relaxation was required for the cursor to go down. Although this visuomotor tracking task is similar to that used before (Jensen et al., 2005, Perez et al., 2004), an important difference is that in the study in this thesis tracking is force rather than position controlled. The decision to adopt force rather than position control was driven by available lab resources. At the time when this study was performed we did not have the correct goniometers to measure joint angle for both the index finger and biceps muscle.

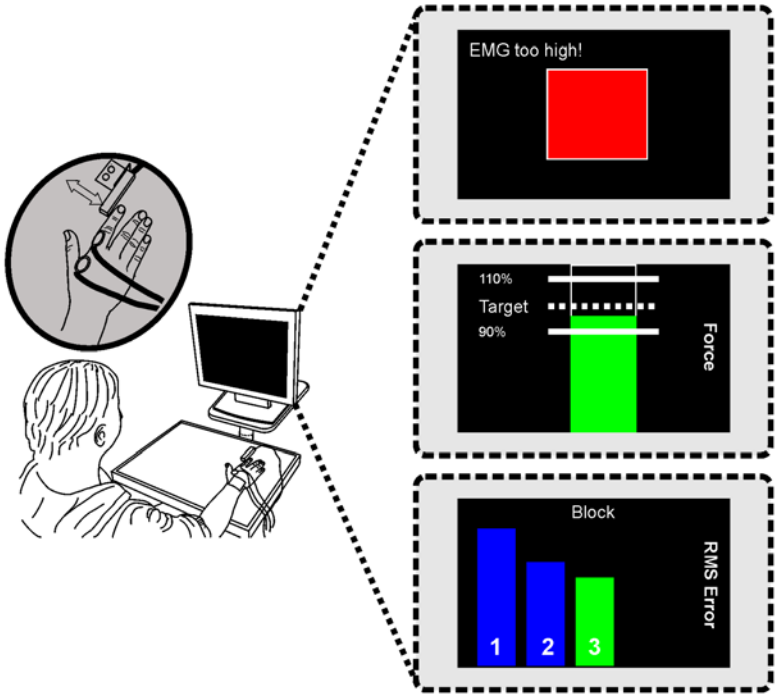

Figure 2-2: Visualisation of the feedback participants received during the different studies in this thesis. In the studies in Chapter 3,4,6 and 7 participants received feedback about their performance on a monitor directly in front of them. When a steady level of EMG was required a square, coloured either green or red, indicated whether the level of EMG was inside or outside the predefined limits (top right panel). Similarly, when participants were instructed to keep a certain level of force, multiple lines represented a target window. Whilst inside the predefined limits the bar was coloured green, whereas it was coloured red otherwise (middle right panel). Finally, during the visuomotor tracking learning direct feedback about the root mean square (RMS) tracking error was given (lower right panel). The height of the bars correlated with the tracking error, with a lower bar meaning better performance. The bar corresponding to the current training block was either coloured green or red to indicate better or worse tracking with respect to all other blocks.

2.3.5 Voluntary muscle strength

In both Chapter 4 and 7 maximal voluntary muscle strength was determined at the start of each experiment as the force exerted during a maximal voluntary isometric contraction. Participants were instructed to, after a 'GO' signal, rapidly increase their level of force to their maximum by making an isometric muscle contraction and hold this for about 2-3 s. This was repeated three times, with about 30 s rest between the maximal contractions. The maximal force reached and held steadily in the three attempts was taken as the MVC. During maximal contractions the participant was provided with online feedback about the exerted force and verbally encouraged to reach their maximum (Figure 2-2).

2.4 Electrophysiological measures and interventions

2.4.1 EMG

In all studies presented in this thesis, electromyographic (EMG) measurements were obtained from the relevant muscles. EMG was recorded using a bipolar configuration with surface electrodes. All EMG recordings were normalised to the maximal M-wave (M_{max}) to allow for comparison between participants and between different experimental sessions. The M_{max} was obtained by stimulation of the primary innervating nerve of the target muscle using a peripheral nerve stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK). Stimulation intensity was increased until the participant indicated the stimulation was too uncomfortable or the response had saturated.

Online feedback about the level of EMG was provided in Chapter 3 and 4 to assist keeping the muscle fully relaxed and focus attention (Figure 2-2). The level of EMG at rest was determined during a 30 s recording, which was divided in 1 s epochs. RMS EMG value (Mean + 1 *SD*) was calculated for the 30 epochs. During data acquisition EMG was analysed over 50 ms epochs. Each epoch's RMS value was confirmed to fall between the Mean ± 3 *SD* RMS of the baseline period. If successful, a square presented to the participant was coloured green. When coloured red, this would indicate the muscle was not fully relaxed. The colour of the square was updated at a rate of 20 Hz.

2.4.2 Transcranial Magnetic Stimulation (TMS)

d'Arsonval (1896) was the first to show that external magnetic fields could affect humans by eliciting phosphenes after having placed their heads in a coil with fluctuating magnetic fields. Nonetheless, it took almost ninety years before magnetic stimulation of the human brain was explored further. When Merton and Morton (1980) showed it was possible to stimulate the motor cortex through an intact scalp by using a brief, high voltage, electric stimulus, the associated discomfort for participants was reason for Anthony Barker and his team to explore the possibilities of magnetic stimulation. They demonstrated using magnetic stimulation it was possible to activate the corticospinal pathway when stimulating the motor cortex. This elicited a muscle twitch accompanied with a muscle action potential in a contralateral muscle (Barker et al., 1985). Later these responses were named Motor Evoked Potentials (MEPs).

Today TMS is an important tool in neurophysiology to assess human motor control, integrity of the corticospinal pathway and central nervous system plasticity (Siebner and Rothwell, 2003). Whilst TMS can also be used as a tool to modulate brain activity, in this thesis TMS has only been used as an assessment tool. Central nervous system plasticity is investigated by measuring corticospinal excitability (CSE), which is quantified by the MEP. Single pulse TMS is commonly used to draw conclusions about CSE by assessing the mean ± *SD* of the MEP amplitude of a number of MEPs. However, by applying stimulation at different stimulation intensities or over different positions of the primary motor cortex, one can also construct stimulus-response (SR) curves or TMS maps (Devanne et al., 1997, Wassermann et al., 1992). Both techniques allow for distinctive conclusions about changes in CSE and central nervous system plasticity. The three single pulse TMS techniques to study central nervous system plasticity, including measures that can be extracted, are summarised in Table 2-1.

Table 2-1: Summary of the three single pulse TMS techniques, and the measures that can be extracted, used in this thesis. Difference in the techniques relate to where and at what intensity the stimulation is applied. Dfferent measures are used to quantify changes in corticospinal excitability.

TMS method	Coil position	Stim Intensity	Measures
Traditional	Fixed	Fixed	Mean / *SD* amplitude
SR curve	Fixed	Variable	MEP_{min}, MEP_{max}, Slope, I_{50}, AuC
TMS map	Variable	Fixed	COG, Area, Volume

All three single pulse TMS techniques, traditional, SR curves and TMS maps, are used in this thesis to study CSE. In the following sections further detail will be provided on each technique, including how the data was analysed and interpreted. First some basic TMS methods, common to each of the three techniques, will be discussed.

2.4.2.1 General TMS methods

In all studies presented in this thesis MEPs were elicited by stimulation over the primary motor cortex using a biphasic Magstim Rapid^2 stimulator (Magstim Ltd, Dyfed, United Kingdom). Only in Chapter 8 a monophasic Magstim 200 stimulator was used as part of a control experiment. With both machines a custom made polyurethane coated 90 mm 'batwing' shaped figure-of-8 coil was used. The coil was always held in a 45 deg angle to the midline with the handle pointing backward, as this coil orientation has been found most effective in evoking MEPs in hand and arm muscles studied in this thesis (Brasil-Neto et al., 1992a). Each experiment was started by finding the 'motor hotspot', the stimulation site resulting in the largest MEPs. This was done by systematically mapping the motor cortex, moving the TMS coil in small steps and looking for the site that consistently elicits the greatest MEPs. Stimulation was performed at an intensity of 50-60% of the maximal stimulator output (MSO) and took 2-4 min, using about 30-50 stimuli. The hotspot was used to determine the resting motor threshold (RMT) using the relative frequency method. Starting at 60% MSO, the threshold intensity was defined by the intensity at which at least 5 out of 10 stimuli evoked MEPs with a peak-to-peak (MEP_{pp}) amplitude > 50 µV (Groppa et al., 2012, Rossini et al., 1994).

2.4.2.2 Neuronavigation

To obtain a good estimate of the motor threshold, and reliably measure MEPs, it is important that coil position and orientation over the motor hotspot can be accurately replicated. Traditionally this was done by fitting a swim cap, marking the stimulation sites with a marker pen (see e.g. Mortifee et al., 1994). In this thesis an image-guided frameless stereotaxy neuronavigation system was used (BrainSight 2, Rogue Research Inc, Montreal, Canada) to monitor and record coil position whilst performing the TMS assessments. The use of a neuronavigation system to assist TMS coil positioning is indeed advantageous in finding back previously defined stimulation sites (Gugino et al., 2001, Julkunen et al., 2009).

BrainSight makes it possible to track coil position and orientation in real-time following a calibration procedure. Participants were fitted with an elastic headband to which a set of three infrared reflective markers was attached. The position of these markers is related to the participant's head by registering eight facial landmarks, chosen on nose and ears (e.g. nose bridge and left/right preauricular point). A similar set of reflective markers is mounted to the TMS coil, with its position being calibrated with respect to the centre of the TMS coil. Accurate landmark registration and coil calibration is essential to allow for within and between session replication of stimulation sites when the headband moves or needs to be taken off. To facilitate between and within session re-registration in Chapter 7 and 8, a marker pen was used to highlight the landmarks and photos were taken.

2.4.2.3 Traditional single pulse TMS

The most common used method to assess CSE is by collecting MEPs with stimulation at a single intensity and the coil centred over the hotspot (Figure 2-3). This method was used in Chapter 8 in an attempt to replicate findings of an earlier study (Moliadze et al., 2010a). Twenty MEPs were acquired at the stimulation intensity evoking 1 mV MEPs (SI_{1mV}) and an interstimulus interval (ISI) of 4-6 s. Data was analysed by quantifying the mean ± *SD* of the MEP_{pp} amplitudes.

2.4.2.4 Stimulus Response (SR) curves

Another method of quantifying CSE is by generating stimulus-response (SR) curves. SR curves require MEPs to be acquired at multiple stimulation intensities between 0.6 - 2.0 × motor threshold with the coil centred over the hotspot (Figure 2-3). CSE is quantified by plotting the stimulation intensity versus the resulting MEP_{pp}, fitting a Boltzmann sigmoid curve (Devanne et al., 1997). In this thesis SR curves were acquired using a novel protocol, developed in our lab, to acquire the data rapidly (Mathias et al., 2014). This protocol takes advantage of the Magstim Rapid^2 stimulator, which allows stimulation with a much shorter ISI than the Magstim 200. Moreover, a graphical user interface (GUI) was developed to construct the SR curve online, control the stimulator and pseudorandomisation stimulation intensity (Chapter 5). This novel method allows acquisition of reliable SR curves with 60-80 stimuli and an ISI of 1.5-2 s. With the possibility of tailoring the acquisition protocol for each participant a single curve can be acquired in about 1-3 min.

In order to quantify CSE, the relationship between stimulation intensity and MEP_{pp} is modelled using the Boltzmann equation and the Levenberg-Marquart nonlinear fitting algorithm (Levenberg, 1944, Marquardt, 1963):

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

It contains 3-5 parameters (Malcolm et al., 2006, Pitcher et al., 2003, Barsi et al., 2008), and changes in the slope (S), the intensity at which the SR curve is halfway between the minimum and maximum plateau (I_{50}), and maximum plateau (MEP_{max}) are used to described changes in excitability (e.g. Houdayer et al., 2008). The slope of the curve reflects both the spatial distribution of excitability elements in the cortex as well as the distribution of excitability within the corticospinal pathway (Siebner and Rothwell, 2003). An extra measure, area under the curve (AuC), was recently suggested to be a valuable means of quantifying CSE for muscles proximal to the hand (Carson et al., 2013). In this thesis AuC was used as the primary measure to assess changes in the SR curve and calculated using trapezoidal numerical integration.

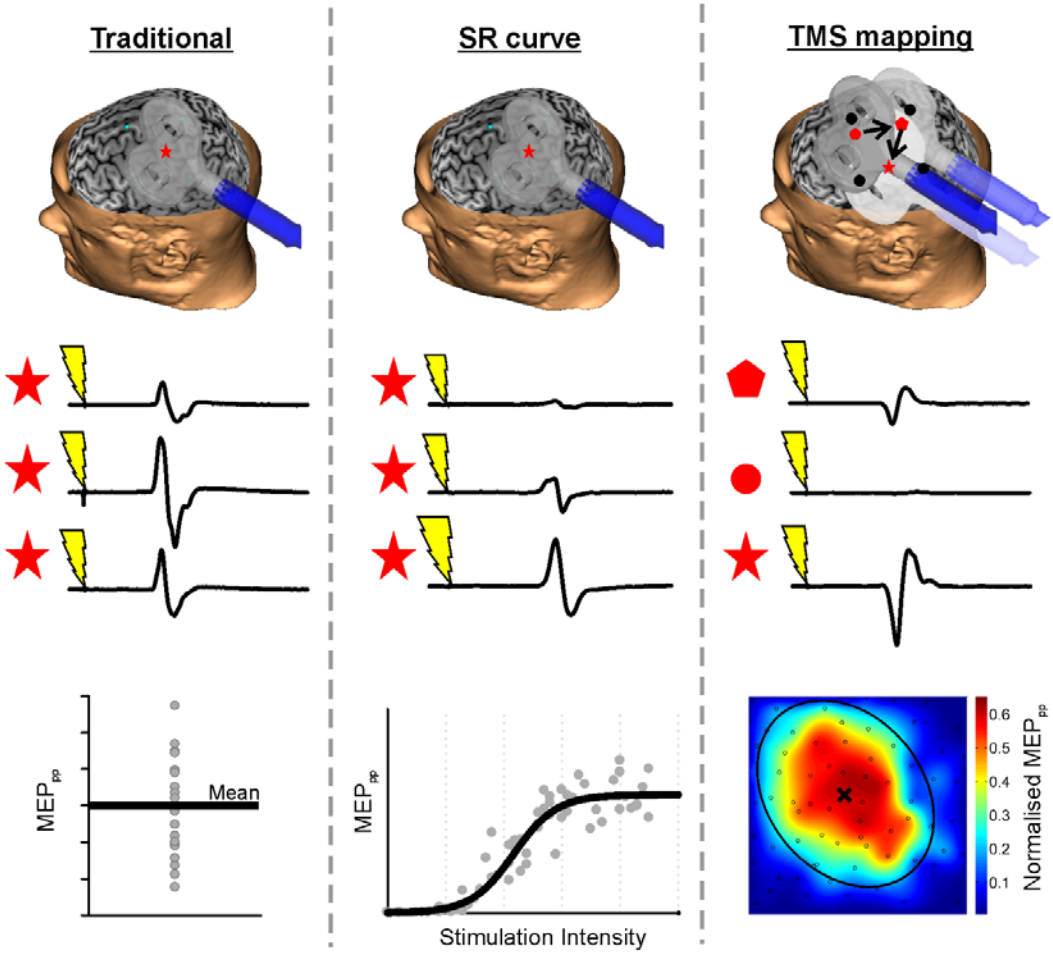

Figure 2-3: Representation of the different single pulse TMS methods used in this thesis to asses corticospinal excitability. The red symbols represent the stimulation position, whilst the size of the yellow symbols correlates with the stimulation intensity. The left panel represents the most used method quantifying corticospinal excitability, calculating the mean MEP_{pp} amplitude of 10-20 MEPs acquired by stimulation over the motor hotspot at a fixed intensity. Data for the SR curve (middle panel) is acquired by stimulating the motor hotspot 60-80 times at different stimulation intensities (pseudorandomly selected), constructing the curve by plotting the stimulation intensity against the MEP_{pp} amplitude. Finally, data for the TMS map (right panel) is acquired by stimulating at 80 pseudorandom selected locations across a 6 x 6 cm grid (marked by the black open circles) centred over the motor cortex. The map is constructed by visualising the stimulation position against the amplitude of the evoked MEP.

2.4.2.5 TMS mapping

The final method to assess CSE is by the use of TMS mapping. When assessing corticospinal excitability using a single set of MEPs or SR curves any observed changes can reflect both changes in CSE as well as changes in the cortical representation of the target muscle (Ridding and Rothwell, 1997). This is where TMS mapping becomes a valuable tool. In TMS mapping MEPs are acquired from different positions across the motor cortex and a topographical map is drawn offline visualising stimulation position versus the MEP_{pp} amplitude (Figure 2-3) (Wassermann et al., 1992, Wilson et al., 1993). TMS maps have been successfully used to demonstrate that learning or relearning a motor skill

following different types of pathophysiology lead to changes in cortical organisation (e.g. Pascual-Leone et al., 1995, Byrnes et al., 1999, Byrnes et al., 1998, Liepert et al., 1995).

The mapping method used in this thesis is presented in Chapter 1. In line with the method for acquiring the SR curve, it makes use of the ability of the Magstim Rapid^2 TMS stimulator to stimulate every 1.5 s. Moreover, by not stimulating according to a 1 to 2-cm spaced grid but administering 80 stimuli pseudorandomly in a 6 x 6 cm grid, the acquisition time was reduced to two minutes. Maps were constructed offline by combining the neuronavigation recorded stimulation positions and the MEP_{pp} amplitudes extracted from the EMG recordings. In most studies in this thesis stimuli were delivered at a stimulation intensity of 120% of the RMT both with the muscle at rest or when active. However, in Chapter 2 the effect of different stimulation intensities (110-130% RMT) and levels of muscle activation (5-40% of MVC) on the TMS map was investigated.

A custom-built MATLAB script was used to analyse the mapping data. Following transformation of all 3D positions of each stimulus to a 2D plane, a surface fitting tool ('gridfit'- D'Errico, 2005) was used to approximate a full TMS map. The approximation was performed over the 6 x 6 cm grid divided up in 2500 partitions. The approximated TMS map was used to compute the different map parameters. Parameters used to describe a map are its centre of gravity (COG), map area and the map volume. The COG is an amplitude weighted mean position of the map (Wassermann et al., 1992) and calculated as follows:

$$xCOG = \frac{\sum(x \cdot aMEP)}{\sum aMEP} \qquad yCOG = \frac{\sum(y \cdot aMEP)}{\sum aMEP}$$

where aMEP are the approximated MEP_{pp} amplitudes and (x,y) their corresponding coordinates.

Map area is defined as the section (number of partitions) of the map exceeding 10% of the maximal MEP_{pp} present in a map or, 100 µV when the maximal MEP_{pp} is smaller than 1 mV (similar as in: Uy et al., 2002, Wilson et al., 1993):

$$area = \frac{N\ (aMEP_{10\%})}{N_{total}} * area_{map}$$

Where $area_{map}$ is the total mapped area of 36 cm^2. Accordingly, map volume was the sum of all $aMEP_{10\%}$, subtracted by the 10% level. The volume was normalised to the maximum volume found in all maps acquired during a single session.

$$volume = \frac{\sum aMEP_{10\%} - 0.1 * N\ (aMEP_{10\%}) * aMEP_{max}}{MaxVolume}$$

2.4.2.6 An important note about TMS

An important limitation of TMS is that any changes observed in MEP amplitudes, potentially reflected in parameters of the SR curve and/or TMS map, cannot be exclusively attributed to changes in cortical excitability. As the MEP is a compound potential that reflects changes along the corticospinal pathway, any observed change might also be below the level of the cortex either because of subcortical effects or changes in spinal excitability. Cortical and subcortical effects can only be disentangled with TMS applied to the cervicomedullary junction, TES or peripheral nerve stimulation to study spinal reflexes.

2.4.3 Transcranial Current Stimulation (tCS)

The first treatments using transcranial current stimulation were already described in 1802 (Hellwag and Jacobi, 1802). Nowadays, we know that using direct currents to stimulate the brain non-invasively through the scalp can induce changes in CSE (Priori et al., 1998, Nitsche and Paulus, 2000). Not only transcranial direct current stimulation (tDCS) was found effective in changing corticospinal excitability, but also transcranial random noise stimulation (tRNS - Terney et al., 2008) and

transcranial alternating current stimulation (tACS - Antal et al., 2008). The latter two techniques use a sinusoidal current waveform with either a single frequency (tACS) or spectrum of frequencies (tRNS). The current stimulation is performed by passing small currents through two stimulation electrodes attached to the scalp (Figure 2-1C).

In this thesis, a common electrode configuration was used, with one electrode centred over the motor hotspot as identified using TMS, and the other electrode positioned on the contralateral orbit. This is the most commonly adopted montage, although it has recently been demonstrated electrode montage does matter for the observed after effects (Mehta et al., 2015). The duration of stimulation was limited to 10 min. This was done to ensure that participants would be able to maintain attention and drowsiness would not affect our TMS assessments. Moreover, with most studies limiting stimulation 10 min and the finding that longer stimulation might invert the effect (Monte-Silva et al., 2013), stimulation was limited to 10 min to make it easier to compare the results to other studies. For the same reason we choose a current intensity of 1 mA (peak-to-peak) (Batsikadze et al., 2013, Moliadze et al., 2012).

CHAPTER 3

TMS brain mapping in less than two minutes

3.1 Introduction

For nearly 30 years, transcranial magnetic stimulation (TMS) has been a valuable tool to study plasticity of the human primary motor cortex (M1), with the first TMS maps being documented in the early 1990s (e.g. Cohen et al., 1990a, Wassermann et al., 1992). Initially, the technique was time consuming and imprecise; however, the development of navigated brain stimulation using frameless stereoscopy (Gugino et al., 2001) improved its repeatability (Julkunen et al., 2009, Krings et al., 2001). Despite this step forward, the mapping method remains a time consuming technique and its use beyond the research environment remains limited to pre-surgical tumour mapping (Takahashi et al., 2013). The importance of reducing acquisition time is evident from the observation that corticospinal excitability fluctuates with time (Ellaway et al., 1998, Kiers et al., 1993) and attention (Rosenkranz and Rothwell, 2004, Rossini et al., 1991), and any changes following motor learning are short lasting. Moreover, in clinical practice the time available with a patient is limited. Lengthy TMS protocols are both mentally and physically demanding for the patient, thus limiting their use. As a result, numerous studies have reduced acquisition time by compromising the map quality.

Traditionally, data acquisition for a full map requires between 15-30 min (Neggers et al., 2004, Sparing et al., 2008, Ngomo et al., 2012b), and this can take up to 1 hour dependent on the protocol employed (Kleim et al., 2007). Importantly, this acquisition time does not include preparation time to set up the EMG recording, determine the most excitable scalp site (commonly referred to as the hotspot) or to determine motor thresholds. Data is typically acquired by stimulating M1 at multiple predefined sites, organised in ~1 cm spaced rows and columns (See Figure 3-1A), with 3-5 stimuli delivered at each site (e.g. Wassermann et al., 1992, Wilson et al., 1993). Offline, the position data are then matched to motor evoked potentials (MEP) acquired from the electromyographic (EMG) data to

produce a 2-dimensional contour plot (see Figure 3-1C). To reduce acquisition time many investigators now use some combination of shorter interstimulus interval, fewer stimulation sites or fewer stimuli per site.

In the literature, as few as 11 and as many as 225 stimulation sites have been reported (Cicinelli et al., 1997, Meesen et al., 2011). Sites are usually distributed in a square or rectangular grid spaced at 1–2 cm (e.g. Pascual-Leone et al., 1995). Typically, between 3–10 stimuli are administered per site (Boroojerdi et al., 1999, Corneal et al., 2005, Mortifee et al., 1994, Wassermann et al., 1992, Wilson et al., 1993) and the ISI is typically set between 3–6 s, although reports in the literature range from 1.1–15 s (Byrnes et al., 1998, Malcolm et al., 2006, Pascual-Leone et al., 1995, Plowman-Prine et al., 2008, Wilson et al., 1993). Acquisition time has been reduced to as little as 2.5–10 min (e.g. Littmann et al., 2013, Malcolm et al., 2006, Plowman-Prine et al., 2008), although this is achieved by minimising the number of stimulation sites (e.g. Littmann et al., 2013) or reducing the ISI (e.g. Malcolm et al., 2006, Plowman-Prine et al., 2008). However, the effect of reducing the number of stimuli or ISI on the TMS map has not been validated against the more traditional long mapping protocols. This observation is interesting, as compromising the mapping acquisition parameters has been observed to shift the centre of gravity (COG) of the map, and to change its area and/or volume, with respect to the 'true' values (Brasil-Neto et al., 1992b, Classen et al., 1998a). This highlights the importance of parameter selection. There is, however, no consensus in the literature about how best to optimise these parameters in order to produce a good-quality map in a short period of time.

Grey et al. (Grey et al., 2009) used frameless stereotaxy and a pseudorandom walk approach to avoid the problem of accurate coil positioning to predefined targets (see Figure 3-1A). When delivering single stimuli in a pseudorandom walk one does not need to repeatedly place the coil in a specific predefined position and orientation, thus ISI may be decreased in order to shorten the acquisition time. No statistically significant difference was observed comparing the grid system (traditional method) and pseudorandom walk method for either of the COG x-y coordinates, suggesting the two methods

are comparable. More recently Julkunen (2014) confirmed that it is not necessary to use an evenly spaced stimulus grid in order to create a reliable map.

By adopting a pseudorandom walk method the stimulation site spacing and number of stimuli per site become redundant parameters. As a result it is only necessary to consider the ISI and the number of stimuli. The aim of this study was to use the pseudorandom walk method to minimise the duration of the data acquisition (excluding preparation and data analysis) required to construct a TMS map. This minimises the effect of changing attention on corticospinal excitability and allows the method to be more feasible for motor learning and clinical assessments. Therefore, we first determined the minimum ISI at which stimuli could be delivered. Specifically, we examined five ISIs (1, 1.5, 2, 3 and 4 s) and tested the hypothesis that ISIs of 1, 1.5, 2 and 3 s would be different from 4 s (Gagne et al., 2011, Pascual-Leone et al., 1995, Tyc and Boyadjian, 2011, Neggers et al., 2004, Ngomo et al., 2012b, Wolf et al., 2004), as evidenced by changes in COG, map area and map volume. Second, we determined the minimum number of stimuli needed to create a map, therefore combining the minimum ISI and minimum number of stimuli in order to determine the time needed to create a map. Finally, to ensure validity of the method, we compared maps generated with the pseudorandom walk method to maps generated with the traditional method of data acquisition. This was achieved by comparing COG, map area and map volume between both methods. In addition, we compared within-subject reliability with both methods.

3.2 Methods

Participants

In total, 12 healthy participants were recruited for both experiments in this study (Experiment 1: 24.2 ± 7 y, range 20-46, 5 female; Experiment 2: 23.2 ± 6 y, range 18-35, 8 female), with some participating in both experiments. Participants were screened for contraindications to TMS using a modified version of the TMS adult safety questionnaire (Keel et al., 2001). The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_12-1189), and all experiments were performed in accordance with the Declaration of Helsinki.

Electromyography

Bipolar surface electrodes (Blue Sensor N, Ambu, Denmark) were used to record the electromyographic (EMG) activity of the first dorsal interosseus (FDI). All EMG signals were amplified (500-2k), band pass filtered (20-1000 Hz), and digitally sampled at 5 kHz to be stored for offline analysis.

Transcranial Magnetic Stimulation

Magnetic stimulation was delivered with a Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom), using a custom made polyurethane coated 90 mm figure-of-8 coil. The coil was held at 45 deg to the sagittal plane with the handle pointing in posterior direction to induce biphasic currents in the lateral-posterior to medial-anterior direction, optimal for exciting the area associated with hand and arm muscles (Brasil-Neto et al., 1992b, Kaneko et al., 1996a). Stimuli were delivered at a constant participant-specific intensity until the coil position over the scalp was fund that evoked the largest MEP (commonly referred to as the hotspot). The hotspot was then marked as a target with the neuronavigation system. With the coil on the hotspot, the resting motor threshold (RMT) was determined according to the definition of Rossini (Rossini et al., 1994, Groppa et al., 2012), as the threshold at which 5 out of 10 stimuli evoked an MEP with a peak-to-peak amplitude of 50 μV. In a very few number of cases, this definition could not be used due to noise in the electromyogram that just exceeded 50 μV. In these cases the threshold was determined as the intensity at which at least

5 out of 10 stimuli evoked an MEP clearly discernible from background EMG. Coil position and orientation were monitored throughout the experiment using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada). To create a map, stimuli were delivered within a rectangular 6 x 6 cm grid superimposed on a generic brain image in the Brainsight 2 software (see Figure 3-1A). The grid was placed relative to surface anatomy landmarks (e.g. vertex and ears) in an area that would encompass the hand area of the motor cortex.

Peripheral Nerve Stimulation (PNS)

MEPs were normalised to the electrically evoked maximal M-wave (M_{max}) in order to compare across different participants. To obtain the M_{max}, a bipolar probe was used to stimulate the ulnar nerve at the level of the elbow using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK).

Experimental protocol

The participants were seated comfortably in a chair with the right hand resting pronated on a table. Participants were instructed to keep the hand fully relaxed during the experiments. Online feedback of FDI EMG was provided by displaying a colour, green or red, based on the participant's root mean square EMG to ensure compliance with this instruction and to focus attention. No direct feedback of the raw EMG was provided to either the experimenter or the participant. One expert TMS experimenter performed all of the testing.

3.2.1 Experiment 1: Effect of Interstimulus Interval (ISI) and Minimum Number of Stimuli (N_{stim})

To improve the temporal resolution, this experiment was designed to investigate the effect of ISI and the number of stimuli on centre of gravity (COG), map area and map volume. This experiment was performed with 12 participants. The effect of stimulation frequency was studied using five different ISIs: 1, 1.5, 2, 3 and 4 s. A maximum ISI of 4 s was chosen because an ISI of 3-6 s is commonly reported (Gagne et al., 2011, Pascual-Leone et al., 1995, Tyc and Boyadjian, 2011, Neggers et al., 2004, Ngomo et al., 2012b, Wolf et al., 2004) and to ensure the experiment would not last longer than

2 hours. Each map was created by applying 100 stimuli at 120% RMT in the predefined grid. Stimuli were delivered to random locations within the 6 x 6 cm square. The objective was to ensure two successive stimuli were not delivered in close proximity and that that final map was populated by stimuli with a roughly equal spread across the grid (Figure 3-1A). Immediate feedback about stimuli position and orientation were provided by position markers in the neuronavigation display. Three maps were collected for each ISI, with the order of presentation randomised to avoid an ordering effect. To ensure participants would remain focussed on their task, a rest period of 1-2 min was given between the maps.

3.2.2 Experiment 2: Validation to traditional mapping protocol

This experiment, performed with 12 participants, was designed to validate if a map created using the characteristics found in Experiment 1 would compare to a map using the traditional method. For the traditional method a 6 × 6 cm grid was created from 7 rows and 7 columns with 1 cm spacing. Three stimuli were administered to each site at 120% RMT using a 1.5 s ISI. Maps acquired using the traditional method were compared to maps acquired using the pseudorandom walk method with 80 stimuli at 120% RMT and a 1.5 s ISI as determined in Experiment 1 (See Results Experiment 1). Three maps were collected for each method, with order of presentation randomised to avoid an ordering effect. Similar to Experiment 1, a 1-2 min rest period was provided between maps.

Data analysis

Figure 3-1 illustrates how the EMG and neuronavigation data were combined to construct a TMS map. Maps were created offline with a bespoke MATLAB script (MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). First, the MEP was quantified by the peak-to-peak value (MEP_{pp}) extracted from a window 20—50 ms after stimulation (Figure 3-1B). The corresponding stimulation position was extracted from the neuronavigation data and transposed into a 2D plane. An approximant based surface modelling tool (D'Errico, 2005), was used to fit a surface through the transposed data. An example of a map in both 3D and 2D are shown in Figure 3-1C. A more detailed description of the data processing may be found in Appendix A. Individual stimuli

within a map were excluded from analysis if for the stimulation or corresponding MEP: 1) the root mean square value of the background EMG (50 - 5 ms before stimulation) was outside Mean ± 2 *SD* of all stimuli; 2) stimulation fell more than 10 mm outside the grid's border; 3) MEP size was larger than Mean ± 3.5 *SD* of all MEPs in the map; 4) angle and translation of the stimulus location fell outside the 99% predication interval of all stimuli.

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4. Tests were considered significant at $\alpha = 0.05$. As the descriptive statistics showed much of the data violated the standard assumptions of normality (typical positively skewed or uniformly distributed) and equal variance, non-parametric statistics were used for the analysis.

Experiment 1: Effect of Interstimulus Interval (ISI)

COG was compared between ISIs using the Euclidean distance, hereafter referred to as distance, between each COG and the average COG of ISI = 4 s. An ISI of 4 s was chosen as the benchmark as an ISI between 3-6 s is most commonly used (Gagne et al., 2011, Pascual-Leone et al., 1995, Tyc and Boyadjian, 2011, Neggers et al., 2004, Ngomo et al., 2012b, Wolf et al., 2004). COG, area and volume were tested using the non-parametric Friedman Test across ISI. Planned post hoc comparisons were performed using the Wilcoxon Signed-Rank Test between ISI = 4 s and all other ISIs. A Bonferroni adjustment was applied to compensate for the multiple comparisons; therefore, in this case $\alpha = 0.0125$ was used for significance.

Minimum Number of Stimuli

Post processing to obtain the minimum number of stimuli (N_{stim}) was required to produce a reproducible map. Stimuli were randomly extracted from the map, the map was reconstructed and the correlation coefficient (r^2) was calculated to compare the original and reconstructed map. A map was considered significantly different if either the COG distance exceeded 3.6 mm (75^{th} percentile of COG variability – See Results – Experiment 1) or the r^2 parameter dropped below 0.9.

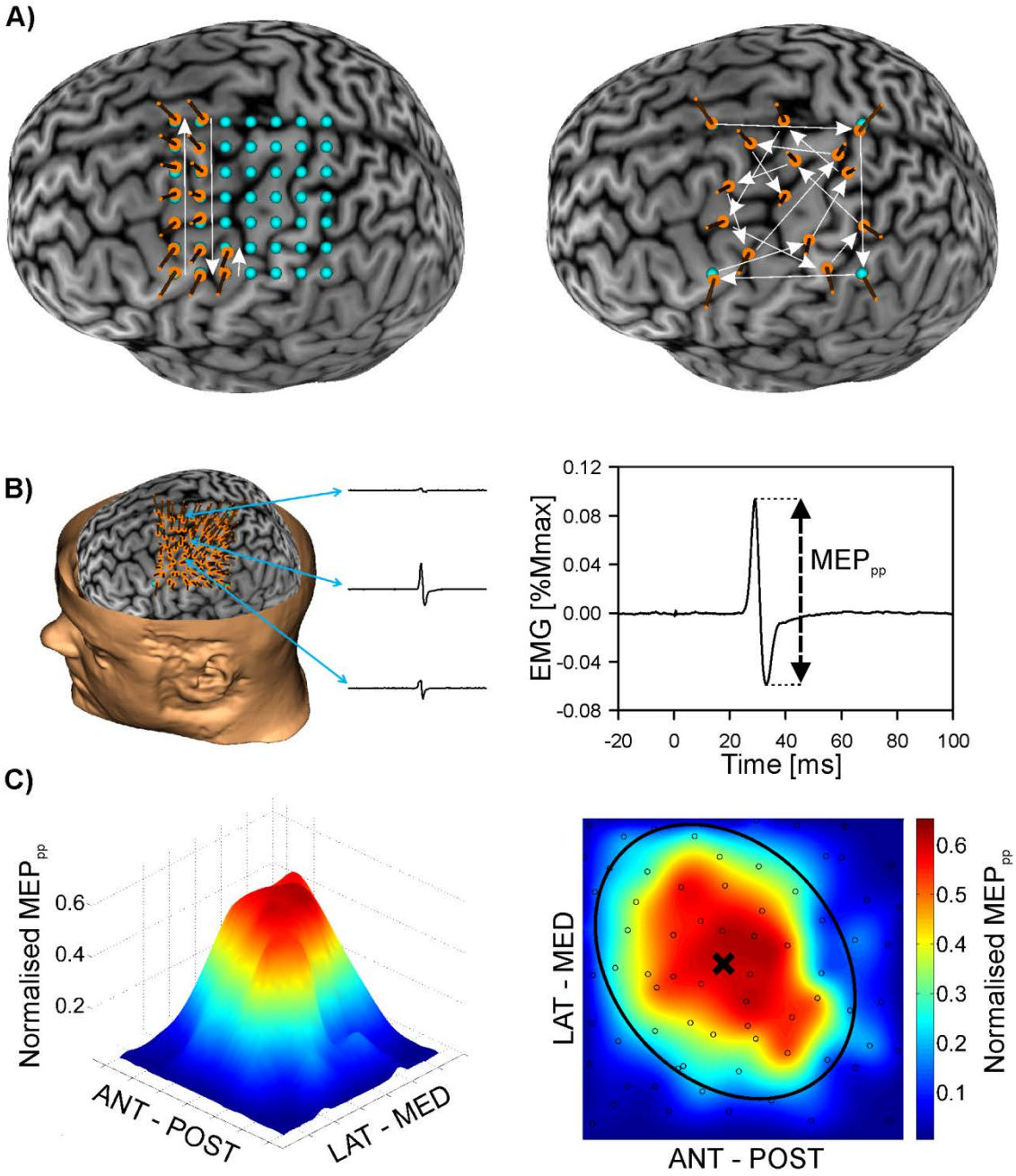

Figure 3-1: A step-by-step illustration outlining the creation of a TMS map. (A) The traditional mapping method is illustrated on the left and the pseudorandom walk method on the right. The traditional mapping method makes use of a predefined, usually 1-cm spaced grid of target locations, as indicated by the blue markers. Multiple stimuli are successively delivered to each site. In contrast, the new method uses four blue markers to define a boundary without specific targets and within which stimuli are delivered pseudorandomly. The white arrows indicate the direction in which stimuli were acquired. For clarity, these maps are as data are acquired rather than at the end of a trial. (B) A 6 x 6 cm square grid is defined in the neuronavigation software (BrainSight 2.0, Rogue Research) and each stimulation site is matched with the recorded EMG. The motor evoked potential's peak-to-peak (MEP_{pp}) value is extracted in a window between 20-50 ms after stimulation. (C) Using a bespoke MATLAB script, a surface is fitted through the 3D position data cloud to create a 2D plane. The 2D position data are then matched with the MEP_{pp} data to fit a surface map. This map can be viewed in either a 3D (left) or 2D (right) map. The colour bar represents the MEP_{pp} normalised by the maximally evoked electrical response (M_{max}).

Experiment 2: Validation to traditional mapping protocol

Mean COG of both the traditional and pseudorandom mapping method was compared using the Wilcoxon Signed-Rank Test. Area and volume were compared using the non-parametric Friedman Test. Post-hoc comparisons were performed using the Wilcoxon Signed-Rank Test. We also examined the reliability of the parameters of the map for both the traditional and the pseudorandom walk method using the intraclass correlation coefficient (ICC). Measurement reliability was defined according to the ICC, with ICC ≥ 0.75 defined as excellent reliability, ICC between 0.50 - 0.74 as moderate reliability, and ICC ≤ 0.49 as poor reliability (Portney and Watkins, 2000, McGraw and Wong, 1996).

The pseudorandom walk method was considered valid when no significant differences for the parameters between the methods were found or, if differences were found, they fell within observed variability. Moreover, the reliability of the COG and map area had to be moderate to excellent (ICC ≥ 0.50). Map volume was not considered in this assessment as findings with respect to reliability are inconclusive (Malcolm et al., 2006, Ngomo et al., 2012b, Wolf et al., 2004, Mortifee et al., 1994). In addition, to classify the between and within-subject variance the quartile coefficient of dispersion (QCD) and standard error of measurement (SEM) was calculated (Stratford and Goldsmith, 1997). SEM was calculated for all map parameters as the square root of the mean square error (MSE): $SEM = \sqrt{MSE}$. The QCD was calculated for map area and volume using: $QCD = \frac{Q_{75}-Q_{25}}{Q_{75}+Q_{25}}$, where Q_{25} and Q_{75} are the 25^{th} and 75^{th} percentile. The centre of gravity measures were excluded from the between subject analysis because we used a generic structural scan for all participants. A between participant analysis of centre of gravity was therefore not valid.

3.3 Results

Data exclusion

All participants tolerated the TMS well and completed the study. Individual stimuli were excluded based on background EMG, coil angle and translation, position relative to the grid and MEP size. In total 8.2% of all stimuli were excluded before analysing the maps (180 maps analysed). Most stimuli were excluded due to either high background EMG (4.2% of the total number of stimuli) or angle and translation of the stimulus with respect to the skull (3.3% of the total number of stimuli). On average, 8.5 (IQR: 7 ± 11) stimuli were excluded per map.

3.3.1 Experiment 1: Effect of Interstimulus Interval (ISI)

In order to study the effect of ISI on the TMS map we compared five different ISIs (1, 1.5, 2, 3 and 4 s). TMS maps collected with 1, 2 and 4 s ISI from a representative participant are shown in Figure 3-2.

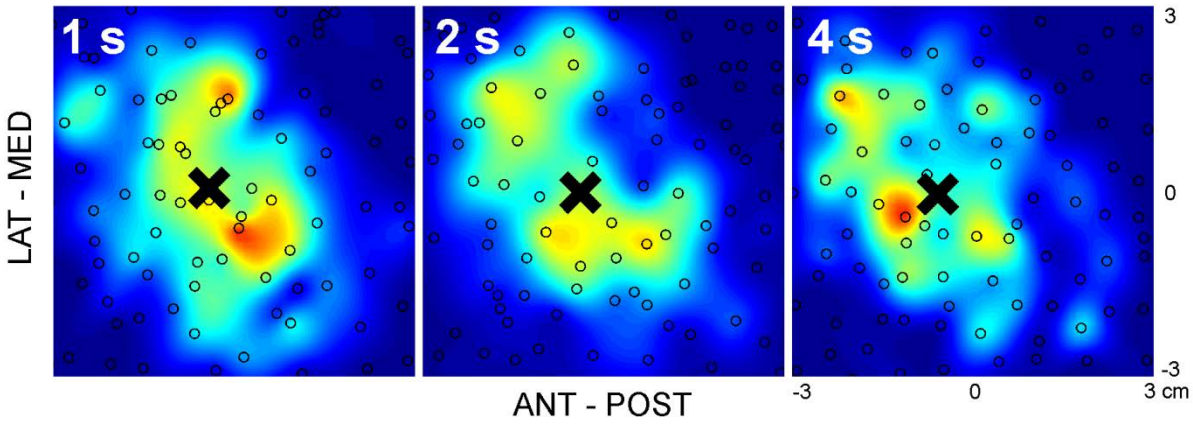

Figure 3-2: Single participant data illustrating TMS maps acquired at three interstimulus intervals (1, 2, and 4 s) using a 6 x 6 cm grid and 100 stimuli at 120% of resting motor threshold. Very similar maps were also acquired at 1.5 and 3 s, but are not shown in the figure to aid clarity. Each black open circle represents the location of a stimulus. Corticospinal excitability is indicated by colour, with blue representing lack of excitability and red representing the greatest excitability. The black cross (×) highlights the centre of gravity. In this participant, neither the centre of gravity, area or volume changed across the five ISIs.

The maps with stimuli delivered at 1 s and 2 s are very similar in shape and activity compared with the 4 s ISI map. In addition, COG is similar in all three maps across all participants, although the Freidman's test used with the group data revealed a small, but significant difference for COG between the four ISIs ($\chi^2(4) = 17.87$, $P < 0.01$). Post hoc comparisons revealed small differences between ISIs of 1.5, 2 and 3 s compared with 4 s, for the Bonferroni adjusted P-value (0.0125), whilst there was no

significant difference between ISIs 1 s and 4 s (Z = 1.56, P = 0.12, Figure 3-3A). The COGs of 4 s ISI differed less than 0.7 mm from all other ISIs. Overall, the median Euclidean distance between ISI 1, 1.5, 2 and 3 s compared with 4 s was 2.4 mm (IQR: 1.2 – 3.6 mm and 10/90th percentiles: 0.7 – 4.8 mm), with x-direction 1.3 mm (IQR: 0.6 – 2.3 mm) and in y-direction 1.1 mm (IQR: 0.5 – 2.5 mm). Neither map area nor map volume revealed significant differences with ISI (area: $\chi^2(4) = 0.47$, P = 0.98; volume: $\chi^2(4) = 1.07$, P = 0.90) (Figure 3-3B|C).

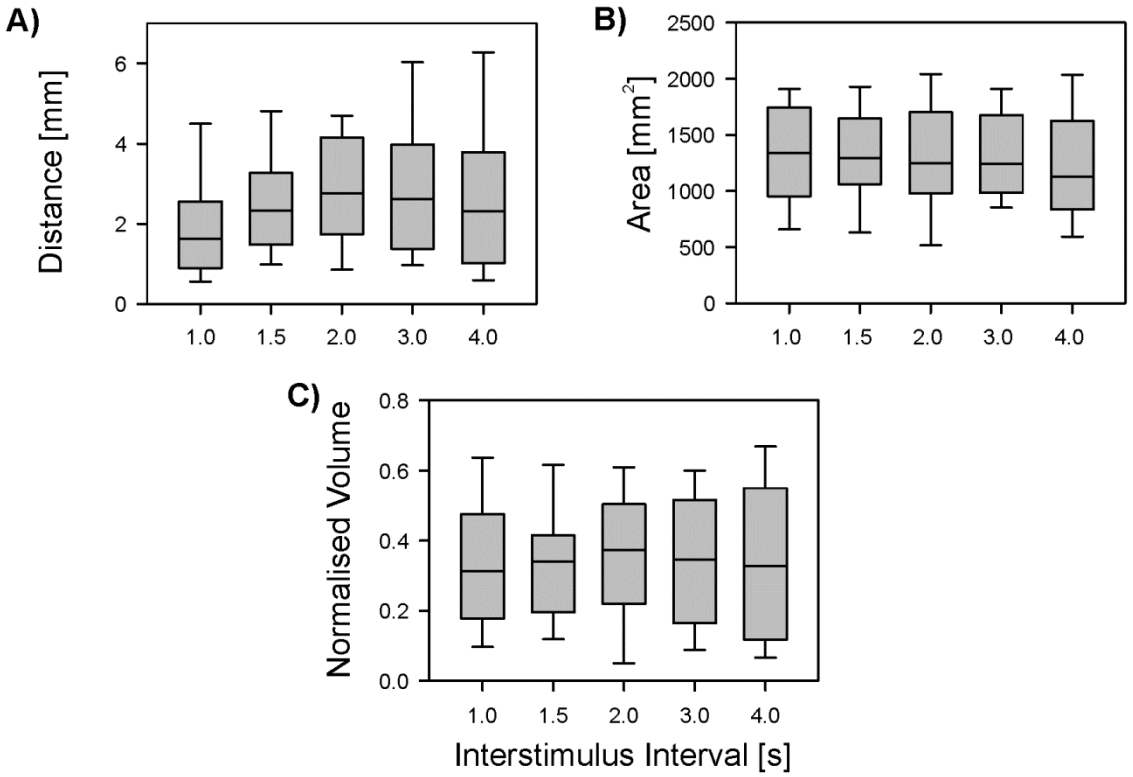

Figure 3-3: Group data for the effect of interstimulus interval on TMS maps (n = 12). All box plots show the median (black line in the box), interquartile range (IQR; box top and bottom) and 10th and 90th percentiles (error bars). Five different ISIs (1, 1.5, 2, 3 and 4 s) were compared and three maps were acquired for every ISI. All statistical testing was performed using the non-parametric Friedman test. (A) Group data of the Euclidean distance of each interstimulus interval relative to the mean centre of gravity of an interstimulus interval of 4 s. Centre of gravity was found not to be different when maps where acquired with 1 s interstimulus interval compared to 4 s. Moreover, no difference was found for (B) map area and (C) map volume between interstimulus intervals (P > 0.05).

Minimum number

All 180 data sets were analysed in order to calculate the minimum number required to produce a map. In all cases the maps with reduced stimuli were well correlated with the original map with the full complement of data until very close to the minimum cut-off, as determined by a drop in r^2 or a shift in

COG. In 95% of the cases, the minimum number was determined by r^2 crossing the 0.9 threshold rather than the COG shifting more than 3.6 mm.

Figure 3-4A is a representative example of a set of maps calculated from the same data set. In this case 6 stimuli were excluded because the background EMG exceeded the activation cut-off, leaving 94 stimuli for the full map. The correlation coefficient dropped below 0.9 after 38 stimuli were randomly removed from the analysis, leaving a minimum number for this data set of 56 stimuli. A map from this data set with 24 stimuli ($r^2 = 0.78$) and a different contour is also illustrated. The decrease of r^2 by extracting stimuli from the map is illustrated in Figure 3- 4B, dropping below 0.9 at 56 stimuli. Figure 3-5 shows the minimum number of stimuli calculated across 15 maps for each participant, sorted from participants with the highest to lowest average number of stimuli. This figure highlights the considerable spread in minimum number of stimuli needed to create a map. The median minimum number of stimuli was calculated across all participants as 63 (IQR: 46-74).

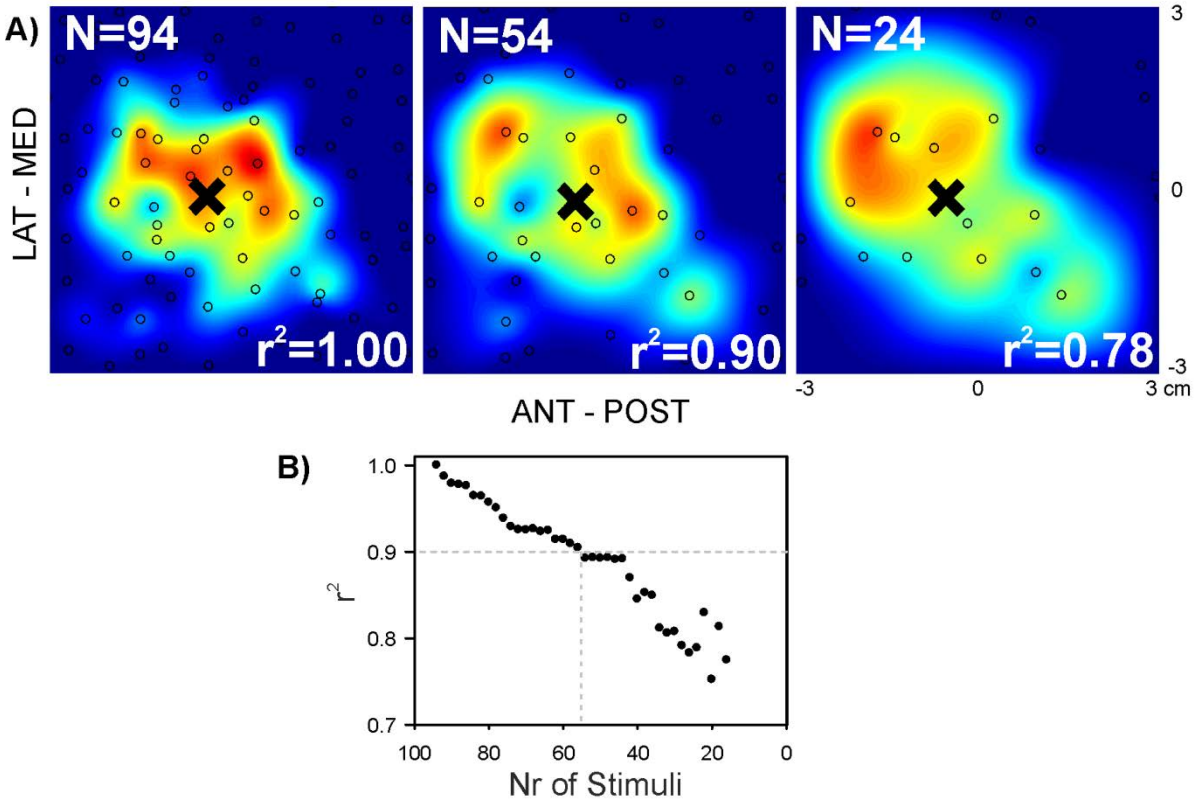

Figure 3-4: Single participant data illustrating the effect of reducing the number of stimuli on the TMS map. Minimum number of stimuli was determined by randomly extracting stimuli starting at 100 stimuli minus the stimuli removed based on criteria of background EMG, coil position and coil orientation (6 in this particular example). Stimuli were extracted at random one by one, calculating the correlation coefficient and change of centre of gravity with respect to the map containing all data. The minimum number was taken when the correlation dropped below 0.9 or the centre of gravity moved more than 3.6 mm (Euclidean distance). In this example the minimum number was taken at 56 when the correlation was 0.9. Removing more stimuli changes the map as shown when only 24 stimuli are left, while the correlation coefficient is still high (0.78). (A) The TMS maps with 94, 56 and 24 stimuli. (B) The correlation coefficient (r^2) plotted against the number of stimuli used to create the map. With 56 stimuli left r^2 dropped below 0.9 when more stimuli were removed.

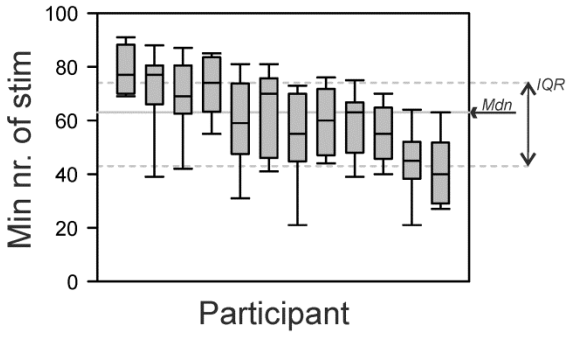

Figure 3-5: The minimum number of stimuli for each participant (n=12), as determined from 15 maps that were collected in every participant. The participants have been sorted from a high to low average minimum number. All box plots show the median (black line in the box), interquartile range (IQR; box top and bottom) and 10th and 90th percentiles (error bars). The overall median (Mdn) of 63 stimuli and interquartile range (46-74) are presented by the solid and dashed horizontal lines. The minimum number was defined as when the map's correlation with respect to a map containing all data dropped below 0.9 or the centre of gravity moved by more than 3.6 mm (Euclidean distance).

3.3.2 Experiment 2: Validation to traditional mapping protocol

To validate the pseudorandom technique, a control experiment was conducted to determine if maps collected with this method were comparable to maps acquired in the traditional manner. TMS maps with the two different methods from a representative participant are shown in Figure 3-6A. The stimulation sites are marked with black open circles.

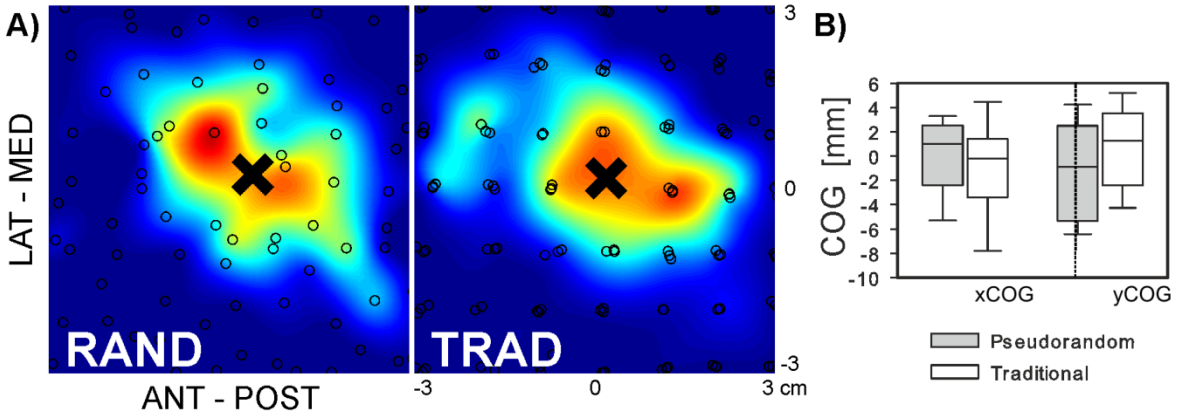

Figure 3-6: Single participant data illustrating TMS maps acquired using the traditional method and the here proposed pseudorandom walk method. (A) For the traditional method mapping was acquired from 49 stimulation sites organised in 1-cm spaced rows and columns, each stimulated three times with an interstimulus interval of 1.5 s and at 120% of resting motor threshold. For the pseudorandom method 80 stimuli were applied at random positions across the grid with an ISI of 1.5 s at 120% RMT. (B) Box plots for the group data of the x- and y-coordinate of the centre of gravity (xCOG and yCOG) for both the pseudorandom (shaded bars) and traditional method (white bars). Shown are the median (black line in the box), interquartile range (IQR; box top and bottom) and 10th and 90th percentiles (error bars). No differences were found for the xCOG, map area or map volume. However the yCOG was found to be significant between methods. Median difference for yCOG is 2.1 mm well within observed COG variability, therefore this significant change is not considered as a result of the method but rather map variability.

It can be observed that the map created using the pseudorandom method is very similar to the map created with the traditional method. No clear difference can be observed in COG and map area of the two methods. Two data sets were omitted from the analysis due excessive ambient noise in EMG recordings; therefore the analysis was performed on 10 participants. The boxplots for COG for both x and y directions are shown in Figure 3-6B. COG was significantly different between methods in Y (yCOG: $Z = 2.48$, $P = 0.01$) but not in X (xCOG: $Z = 1.89$, $P = 0.06$). However, the median xCOG and yCOG differed by only 1.2 mm and 2.1 mm, respectively, which falls within the IQR for COG variability observed in Experiment 1. Neither map area nor map volume was significantly different between methods (area: $\chi^2(1) = 0.40$, $P = 0.53$; volume $\chi^2(1) = 0.16$, $P = 0.21$).

ICCs, SEMs and QCDs for both the traditional and pseudorandom walk are listed in Table 3-1. ICCs for xCOG, yCOG and area were moderate to excellent (ICC > 0.74). However, the ICC of the volume for the pseudorandom walk method was poor (ICC = -0.63). Whist small differences in SEM for xCOG and yCOG are observed, 0.7 mm and 0.3 mm, respectively, they are within the variance reported for xCOG and yCOG in Experiment 1. For map area the SEM was 343 for the traditional method and 323 for the pseudorandom method. This difference can be considered negligible with respect to its order of magnitude. For both map area and volume, QCD was smaller for the pseudorandom method (0.2) than the traditional method (0.3 - 0.4).

Table 3-1: Intraclass correlation coefficients (ICCs), standard error of measurement (SEM) and quartile coefficient of dispersion (QCD) for both the traditional and pseudorandom walk mapping method, showing the test-retest reliability and variance of the mapping parameters. Apart for volume, correlation is good to excellent for both methods. This indicates the pseudorandom walk method is a reliable method for creating TMS maps. The small differences in SEM for both x- and y-coordinate of the centre of gravity (xCOG and yCOG) fall within 1.3 mm and 1.1 mm COG variances reported in Experiment 1. The SEM difference of 20 for map area can be considered negligible with respect to its order of magnitude. QCD is smaller for both map area and volume for the pseudorandom method compared to the traditional method.

	Method					
	Traditional			**Pseudorandom**		
	ICC	**SEM**	**QCD**	**ICC**	**SEM**	**QCD**
xCOG	0.94	1.63	x	0.82	2.30	x
yCOG	0.92	1.62	x	0.92	1.93	x
Area	0.87	343.39	0.32	0.74	323.41	0.21
Volume	0.76	0.14	0.44	-0.63	0.20	0.22

3.4 Discussion

We have demonstrated that it is possible to acquire a TMS map in less than two minutes by reducing the interstimulus interval and by taking advantage of frameless stereotaxy to deliver stimuli in a pseudorandom walk. In addition, we estimated the minimum number of stimuli required to create a TMS map was 63 (IQR: 46-74). To account for inter-participant variability in minimum number of stimuli, and stimuli excluded during data analysis (on average 7-11), we recommend using 80 stimuli. Maps created with the new method are very similar to maps created with the traditional mapping method where stimulation sites are predefined. Whilst maps can be created by acquiring data with an interstimulus interval up to 1 s, we recommend using at most 1.5 s to limit participant discomfort. As a result, maps constructed from 80 stimuli acquired with an ISI of 1.5 s can effectively reduce the acquisition time to two minutes.

How quickly can data be acquired for a TMS map?

The primary aim of the present study was to improve the acquisition time of the mapping method without reducing the quality of the map. The present study indicates the TMS map can be recorded with an ISI of 1s. Whilst significant differences in COG were observed between 1.5, 2, 3 and 4 s, they were always very small (< 0.7 mm), falling within the overall COG variability of 2.4 mm (IQR: 1.2 – 3.6 mm). The significant differences reported in this study can therefore be attributed to natural variability as caused by fluctuating corticospinal excitability. Most importantly, there was no difference in COG between maps acquired with ISIs of 1 s and 4 s. The 2.4 mm COG variability corresponds well to the 3 mm variability in COG reported by others using the traditional mapping method both within and between sessions (Classen et al., 1998a, Littmann et al., 2013, Miranda et al., 1997, Julkunen, 2014, Weiss et al., 2012) . The present study concentrated on within-session variability. We did not, however, examine between-session variability which has been shown to be larger (6 – 10 mm) (Wolf et al., 2004, Forster et al., 2014). As a result, further testing is warranted to confirm the between session variability of the COG using the pseudorandom walk method.

The observation that the map does not change with shorter ISIs is not surprising. Whilst the use of a 1 s ISI has been associated with lasting depression of excitability of the cortex when administered to a single site repetitively for 4 - 15 min (Chen et al., 1997, Maeda et al., 2000b), a number of recent observations suggest depression is unlikely to be a problem with the present method. For example, we have recently demonstrated that TMS delivered with an ISI of 1 s for 3 min to the same stimulation site does not change corticospinal excitability (Mathias et al., 2014). In addition, the use of the pseudorandom walk method ensures the same site is not repeatedly stimulated and the possibility of reduced synaptic efficiency is further reduced. However, whilst we have demonstrated in the present study that the use of 1 s ISI is technically feasible, stimulating this quickly does have some drawbacks. For example, we have observed that inexperienced users find it difficult to move the coil to a new location with only 1 s ISI. In some cases this leads to increased experimenter error. We noticed some users were not able to maintain the coil orientation correctly on the scalp at the new location because they were focusing on the neuronavigation software rather than the participant's head. More importantly, some participants reported discomfort and anxiety when the stimuli where delivered with an ISI of 1 s and had difficulty complying with the instruction to relax the target muscle. For these reasons we advocate using an ISI of at least 1.5 s when mapping with this method, however emphasize that a 1 s ISI does not affect the TMS map if an experienced TMS user performs the mapping and the participant is comfortable with the procedure.

On average the minimum number of stimuli needed to create a reproducible map was 63 (IQR: 46-74). A considerable spread in the minimum number was found between participants (Figure 3-5), highlighting the importance of acquiring sufficient data for the TMS map in order to overcome this variability. In post-processing, 7-11 stimuli were excluded from analysis. Therefore, to ensure sufficient data is collected to produce a reproducible map we suggest a minimum of 80 stimuli are required to produce a map with this method. Using an ISI of 1.5 s, a map can therefore be acquired in 2 min. It should be emphasized that this does not include setting up the EMG recording, co-registering

the participant's head to the MRI, finding the hotspot and RMT, and processing of the data to create the map.

Map variability

The within session variability of the map parameters can mainly be attributed to MEP variability, although it has been confirmed that maps can be reliably created despite this variability (Thickbroom et al., 1999). MEPs are affected by attention (Kiers et al., 1993, Rosenkranz and Rothwell, 2004, Rossini et al., 1991), asynchronous firing of motor units with phase cancellation (Magistris et al., 1998) and a variety of non-physiological factors such as coil position and coil orientation (Mills et al., 1992, Werhahn et al., 1994, Schmidt et al., 2014). In this study, we used the commonly adopted 45 degree coil angle to stimulate the motor cortex which is commonly believed to optimally excite the hand area (Groppa et al., 2012). Interestingly, it has been suggested that the optimal coil angle should be individually determined (Ruohonen and Karhu, 2010, Balslev and Miall, 2008). However the benefit is likely to be minor (Julkunen et al., 2009). Whilst individualising the coil orientation might decrease MEP variability it would also increase the mapping time, which is not beneficial for clinical application.. In addition the use of electrical field estimates as opposed to RMT has been advocated as a more reliable measure (Danner et al., 2008, Schmidt et al., 2014), however this is not common practice. MEP variability also depends on the muscle studied and the stimulation site, with proximal muscles usually reported to have more variable MEPs than distal muscles. and variability increasing as the coil is moved away from the hotspot (Brasil-Neto et al., 1992b). Map reliability has also been argued to be sensitive to experimenter error (Wolf et al., 2004, Zdunczyk et al., 2013). In an attempt to reduce these sources of variability and improve the quality of the map we took several precautions both during data acquisition and in post-processing.

First, to ensure attention was maintained during data acquisition, participants were provided with continuous feedback about the level of EMG which they were instructed to keep between predefined boundaries. In general, participants reported this task as being easy to achieve but also that it required continuous focus to successfully perform. Whereas this task minimized and stabilised background

EMG, any trials with increased background EMG were excluded to further minimize MEP variability. Second, the neuronavigation data was scrutinised offline to ensure coil orientation was consistent throughout the session. Furthermore, the TMS map was made less sensitive to MEP variability by smoothing the data with a Matlab surface fitting tool called 'gridfit' (D'Errico, 2005). Full details are available in Appendix A. Briefly, local variability in the surface fit was filtered by setting the compliance of the fit with a stiffness setting in the gridfit tool. This setting was determined through extensive pilot testing and maintained constant for all maps analysed in this study. This filtering is especially beneficial in the periphery of the map, where variability in the smaller MEPs has been argued to be source of reduced reliability of the map parameters (Mortifee et al., 1994). As a result, the quality of the map is improved and the number of stimuli needed to construct a map is reduced without compromising information content.

For both the pseudorandom as the traditional method we found the greatest ICCs for xCOG and yCOG. In general most literature supports the notion that COG is a more reliable parameter than either area or volume (Malcolm et al., 2006, Mortifee et al., 1994, Ngomo et al., 2012b, Wolf et al., 2004). We confirmed for the pseudorandom walk method that also area is a reliable measure but this does not hold for volume. The difference in reliability of the map volume between the methods is in line with the equivocal reports earlier (Mortifee et al., 1994, Ngomo et al., 2012b) and is unlikely to be a consequence of the method. Therefore, we recommend focusing on COG and area when analysing TMS maps.

Further considerations

It is interesting to note the increased use of TMS mapping in neurosurgery as a tool for brain tumour localisation. This contrasts to its use in studying motor system plasticity and motor rehabilitation, where the technique remains confined to research studies. The present study indicates it may be possible to use a shorter ISI for pre-surgical mapping, where a 4 s ISI is common practise (Takahashi et al., 2013). However, it must be emphasised that further study in this area is warranted and that the

computational method should be validated against existing methods to determine corticomotor representation size (Julkunen, 2014).

The method to create a TMS map presented here makes it possible to assess cortical organisation in less than 2 minutes. We recommend using at least 80 stimuli to take account for variability. Whilst it is possible to use fewer stimuli and an ISI of 1 s to produce a map in as little as 1 min, maps produced in this manner will be subject to greater error. To tackle the observed variability in the minimum number of data required to produce a map, a potential next step is to develop a system whereby maps are generated online as the data are acquired to provide the researcher direct feedback about the map. Such a method could, for example, use a parameter estimation algorithm (PEST) as has recently been used in this field for threshold tracking (Silbert et al., 2013). This would negate the need for a minimum number of stimuli as data could be acquired until a robust map is achieved. This would also give the opportunity to improve spatial resolution in areas of interest such as the area in the immediate proximity of the hotspot.

CHAPTER 4

The TMS map scales with increased stimulation intensity and muscle activation

4.1 Introduction

Transcranial Magnetic Stimulation (TMS) maps of the primary motor cortex have been used to non-invasively study brain organisation and brain topography. The TMS map is created by stimulating at different sites across the motor cortex, combining the position of every stimulus with the size of the recorded motor evoked potentials (MEPs) (Wassermann et al., 1992, Wilson et al., 1993). Recently, we presented a method to acquire data for the TMS maps that reduces acquisition time to 2 minutes (van de Ruit et al., 2015). Whilst the MEP increases with a higher stimulation intensity and greater muscle activation (Day et al. 1989; Hess et al. 1987; Kiers et al. 1993; Rothwell et al. 1991), it is unknown what happens with the TMS map's centre of gravity (COG), map area and map volume.

In one of the early studies using TMS mapping, Wasserman et al. (1992) used a stimulation intensity of 100% of the maximum stimulator output (MSO). Although 100% MSO may be required in clinical studies where MEPs are small, a stimulation intensity of 110-120% of resting motor threshold (RMT) is more commonly used in healthy participants (e.g. Classen et al., 1998a, Pascual-Leone et al., 1995, Uy et al., 2002). Higher stimulation intensities are associated with stronger magnetic fields; thereby stimulating a greater area of the cortex including deeper lying structures (Day et al., 1989). Whereas MEP amplitude increases with higher stimulation intensities, the amplitude saturates when the intensity is high enough. This can be clearly observed when constructing recruitment curves, plotting the stimulation intensity versus MEP amplitude (Devanne et al., 1997). Nonetheless, higher stimulation intensities are associated with a greater area of the cortex resulting in MEPs (Thordstein et al., 2013), but it remains unclear how stimulation intensity affects the COG and map volume.

Not only stimulation intensity but also muscle activation at the time of administering TMS is correlated with MEP magnitude (Hess et al., 1987, Kiers et al., 1993). In contrast to the effect of stimulation intensity, for which its effect on the TMS map has not been systematically examined, it has been documented that muscle activation leads to a greater map area and translation of the COG compared with a map acquired when the muscle is relaxed (Wilson et al., 1995). However, not all groups report that COG moves when the muscle is activated (Classen et al., 1998a, Ngomo et al., 2012b). Moreover, when acquiring the map at a stimulation intensity relative to active motor threshold instead of resting motor threshold, the map area does not change with muscle activation (Ngomo et al., 2012b). Mostly, TMS maps are created either when the muscle is at rest (e.g. Pascual-Leone et al., 1995, Wassermann et al., 1992) or slightly active, usually between 5-10% of the maximum voluntary contraction (MVC) (Byrnes et al., 1999, Wilson et al., 1993). At voluntary muscle activation greater than 10% of MVC, MEP amplitude for a small hand muscle has been reported to saturate (Helmers et al., 1989, Taylor et al., 1997). However, no study has investigated the effect of different levels of muscle activity on the TMS map when muscle activation exceeds 10% of MVC.

Frequently, the map is elongated along the main coil axis (Wilson et al., 1993) but it is unclear if the map's shape remains unchanged when stimulating at higher intensities or when the cortex is more excitable during muscle activation. Quantifying the map's shape might be of interest when brain reorganisation is studied, and has never been explored. Therefore, in this study the map shape was used as a novel measure to quantify the TMS map.

The aim of this study was to describe the effects of stimulation intensity and different levels of muscle activation on map outcome parameters: COG, map area, map volume and map shape. As the stimulated cortical area scales with stimulation intensity (Thielscher and Kammer, 2004), we hypothesized an increase in map area and volume whilst COG and map shape remains unaffected. As the MEP response saturates when the muscle is activated above 10% MVC, we hypothesized that map area and map volume would also saturate when the muscle activity exceeds this level, with no change in COG and map shape.

4.2 Methods

Participants

In total, 16 healthy participants were recruited for the study with some participating in both experiments (Experiment 1; 12 participants: 23 ± 3 y, range 20-29, 6 female; Experiment 2; 12 participants: 23 ± 3 y, range 20-28, 3 female). Participants were screened for contraindications to TMS using a modified version of the TMS adult safety questionnaire originally suggested by Keel et al. (2001). All participants provided written informed consent. The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_12-1189), and all experiments were performed in accordance with the Declaration of Helsinki.

Electromyography

Bipolar surface electrodes (Blue Sensor N, Ambu, Denmark) were used to record the electromyographic (EMG) activity of the first dorsal interosseus (FDI). All EMG signals were amplified (500-2k), band pass filtered (20-1000 Hz), and digitally sampled at 5 kHz to be stored for offline analysis.

Transcranial Magnetic Stimulation

Magnetic stimulation was delivered with a Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom) and a custom made polyurethane coated 90 mm figure-of-8 coil (type: batwing; type no. 15411). The coil was held tangentially to the scalp and orientated at 45 deg to the midline with the handle pointing posteriorly (Brasil-Neto et al., 1992a). The stimulation site evoking the largest MEP, was found by repeated stimulation approximately every 2 s during which the EMG was visually inspected. Whilst holding the coil over the hotspot, resting motor threshold (RMT) was determined as the intensity at which at least 5 out of 10 stimuli evoked MEPs with a peak-to-peak amplitude of greater than 50 μV (Rossini et al., 1994, Groppa et al., 2012). Coil position and orientation were monitored throughout the experiment using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada).

Experimental protocol

The participants were seated comfortably in a chair with the right hand resting pronated on a table and the distal phalanx of the index finger fixed to a force transducer. Each TMS map was created from 80 stimuli using an interstimulus interval of 1.5 s pseudorandomly applied in a 6 x 6 cm grid using the rapid mapping technique described by van de Ruit et al. (2015). Excitability maps were constructed and analysed offline. Map COG, area, volume, and shape were calculated (see *Data Analysis* below).

4.2.1 Experiment 1: Effect of Stimulation Intensity

To study the effect of stimulation intensity, maps were created from 12 participants at 110%, 120% and 130% of resting motor threshold (RMT). The participants were instructed to keep their hand fully relaxed during the experiment. Online feedback of FDI EMG was provided to ensure compliance with this instruction and to focus their attention as the stimuli were being delivered. Three maps were acquired at each stimulation intensity, with the order of presentation randomised.

4.2.2 Experiment 2: Effect of muscle activation

To study the effect of muscle activation , maps were created from 12 participants with the FDI muscle activated at 5%, 10%, 20% or 40% of their MVC and when relaxed. TMS maps were constructed at each level of muscle activation using a stimulation intensity of 120% RMT. The force exerted by activation of the FDI was measured by a cantilever beam load cell (NL 62 – 50 kg, Digitimer Ltd, Welwyn Garden City, UK). The participant's MVC force was determined during three consecutive trials, with a 30 s rest period between trials. The force feedback signal was low-pass filtered at 1 Hz with a second order Butterworth filter. The participant was instructed to maintain a steady force throughout the mapping procedure. Visual feedback of the force signal was provided on a monitor in direct line of sight of the participant. A single bar was presented with a horizontal target line and two additional horizontal lines to denote a window that was 10% of the target force. Whenever the force was outside this target window, the bar turned red to indicate the force exerted was not in the target window. Three maps were collected for each level of muscle activation, with order of presentation randomised. To prevent muscle fatigue, a rest period of at least 2 min was used between each map.

Data analysis

Creating the map

Figure 4-1 illustrates how the EMG and neuronavigation data were used to construct a TMS map. The stimulation position was extracted from the neuronavigation data and transposed into a 2D plane. The corresponding MEP observed in the EMG was quantified by its peak-to-peak value (MEP_{pp}), which was extracted from a window between 20—50 ms after stimulation. All MEPs were normalised to the electrically evoked maximal M-wave (M_{max}). To obtain the M_{max}, a bipolar probe was used to stimulate the ulnar nerve at the level of the elbow using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK).

Analysis was performed offline with a bespoke MATLAB script **(**MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States) to create a full 2D surface TMS map, using an approximant fitting function ('gridfit" - D'Errico, 2005). Individual stimuli within a map were excluded from analysis if for the stimulation or corresponding MEP: 1) the root mean square value of the background EMG (50 - 5 ms before stimulation) was outside Mean ± 2 *SD* of all stimuli; 2) stimulation fell more than 10 mm outside the grid's border; 3) MEP size was larger than Mean ± 3.5 *SD* of all MEPs in the map; 4) angle and translation of the stimulus location fell outside the 99% predication interval of all stimuli. All maps were created with the same colour axis, so differences could be easily observed.

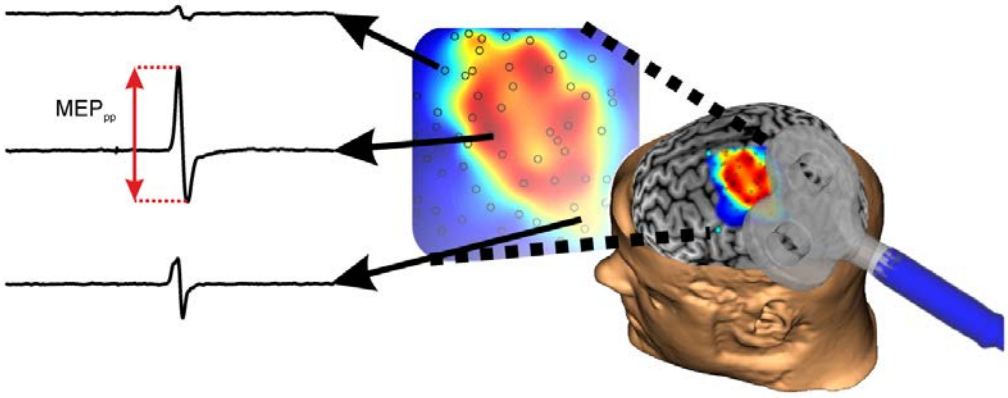

Figure 4-1: An illustration outlining the creation of a TMS map. A 6 x 6 cm square grid is defined in the neuronavigation software (BrainSight 2.0, Rogue Research) and each stimulation site is matched with the recorded EMG. The motor evoked potential's peak-to-peak (MEP_{pp}) value is extracted from each EMG recording. Using a bespoke MATLAB script, the 3D position data are then matched with the MEP_{pp} data to fit a surface and visualise the resulting TMS map in a 2D plane.

Map parameters

Maps were characterised by COG, map area, map volume and map shape. The map area was defined as the part of the map where the MEP_{pp} exceeded a predefined threshold. In Experiment 1 this threshold was set to 10% of the maximum MEP_{pp} as recorded in the 110% RMT condition. For Experiment 2 the threshold was chosen as 10% of the maximum MEP_{pp} for the maps created in the 5% of MVC condition. These thresholds were chosen based on the lowest stimulation intensity condition (110% RMT) and muscle activation condition (5% of MVC) to enable appropriate characterisation of the effect of increasing stimulation intensity or greater muscle activation on the map. The stimulation points and their corresponding MEP_{pp} values were used to approximate a 6 x 6 cm grid composed of 2500 pixels using MATLAB's 'gridfit' function (D'Errico, 2005). Next, the number of pixels with an approximated MEP_{pp} amplitude greater than the 10% threshold was calculated, and expressed as total map area (in mm^2). The map volume was determined by the sum of all MEP_{pp} exceeding the same threshold, normalised to the maximum volume of all maps in a session. The maps COG x- and y-coordinate was calculated by using the MEP_{pp} amplitude and its position on the map, creating an amplitude weighted mean of the map. Full details of this process are described in van de Ruit et al. (2015). Finally, in Experiment 2, we quantified the order of COG translation as a

result of muscle activation by calculating the Euclidian distance between the COGs during all active conditions with the mean COG in the resting condition.

In addition to these traditional measures, we defined an extra measure to quantify the map shape: the aspect ratio. The aspect ratio is characterised by the ratio of the major and minor axes of a fitted ellipse and was defined to describe the expansion of the excitable area. The ellipse was fitted through the points that defined the positions where the MEP_{pp} amplitude fell below the 10% threshold. By choosing the 10% cut-off, the ellipse roughly outlines an area similar to the area parameter. The cut-off was increased to 30% for Experiment 2 because the increased muscle activation produced much larger MEPs and, in many cases, the 10% cut-off resulted in an inability to fit an ellipse because it would fall outside the border of the map.

Statistical Analysis

Statistical testing was conducted with NCSS 2007 v07.1.4. Tests were considered significant at $\alpha = 0.05$. As the descriptive statistics showed much of the data violated the standard assumptions of normality (typical positively skewed or uniformly distributed) and equal variance, all statistical tests were conducted with non-parametric tests.

Experiment 1: Effect of Stimulation Intensity

All parameters (area, volume, xCOG, yCOG and aspect ratio) were compared between stimulation intensities using the non-parametric Friedman Test. Post-hoc comparisons were performed using the Wilcoxon Signed-Rank Test. A Bonferroni adjustment was applied to compensate for the multiple comparisons; therefore, in this case $\alpha = 0.017$ (3 comparisons) was used for significance.

Experiment 2: Effect of muscle activation

All parameters (area, volume, xCOG, yCOG and aspect ratio) were compared using the non-parametric Friedman Test across all conditions with muscle activity. Post-hoc comparisons were performed using the Wilcoxon Signed-Rank Test. A Bonferroni adjustment was applied to compensate

for the multiple comparisons; therefore, in this case $\alpha = 0.0083$ (6 comparisons) was used for significance.

4.3 Results

Data exclusion

All participants tolerated the TMS well and completed the study. Stimuli were excluded from the analysis based on high background EMG, or incorrect coil position and/or orientation relative to the grid. In total 8.0% of all stimuli were excluded before analysing the maps (285 maps analysed). Most stimuli were excluded based on a high background EMG (4.2%) or angle and translation of the stimulus with respect to the skull (3.3%). On average, a median number of 6 stimuli were excluded for each participant (inter quartile range: 5-8).

4.3.1 Experiment 1: Effect of Stimulation Intensity

Three different stimulation intensities (110%, 120% and 130% of RMT) were used to examine the effect of the stimulation intensity on the excitability maps. Data from a representative participant are shown in Figure 4-2. In this case it can be clearly observed that the cortical representation scales with stimulation intensity, whilst the COG and aspect ratio were unaffected.

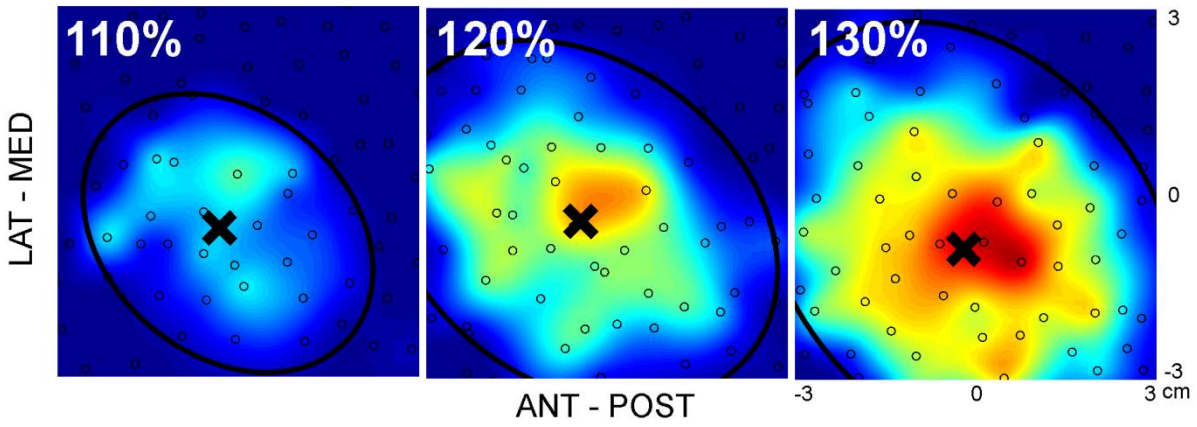

Figure 4-2: Single participant data illustrating TMS maps acquired at three different stimulation intensities (110%, 120% and 130% of resting motor threshold) using a 6 x 6 cm grid and 80 stimuli with a 1.5 s interstimulus interval. Each black open circle represents one stimulus. The size of the approximated MEPpp is indicated by the colour, with blue representing a small MEPpp and red representing the greatest MEPpp. The black cross (×) highlights the centre of gravity. In this participant, stimulation intensity was found not to affect the x- or y-coordinate of the centre of gravity, however map area and volume significantly increased with stimulation intensity. An ellipse was fitted through the data points representing 10% of the maximum MEP within the 110% maps and used to study changes in the shape of excitable area of the map. No change in the shape of the ellipse was found.

Across all participants, no difference was observed for either the x- or y-coordinate of the COG (xCOG: $\chi^2(2) = 1.17$, $P = 0.56$; yCOG: $\chi^2(2) = 0.50$, $P = 0.79$; Figure 4-3A|B). Map area and volume were both significantly increased with stimulation intensity (area: $\chi^2(2) = 22.17$, $P < 0.01$; volume: $\chi^2(2) = 24.00$, $P < 0.01$). For both area and volume, post-hoc testing showed all pairwise comparisons were significantly different using the Bonferroni adjusted P-value (0.017) (Figure 4-3C|D). Finally, the aspect ratio was analysed. No significant effect of stimulation intensity on the aspect ratio was found ($\chi^2(2) = 0.17$, $P = 0.92$; Figure 4-3E). Therefore, it can be concluded that the map area increased with stimulation intensity without affecting its shape.

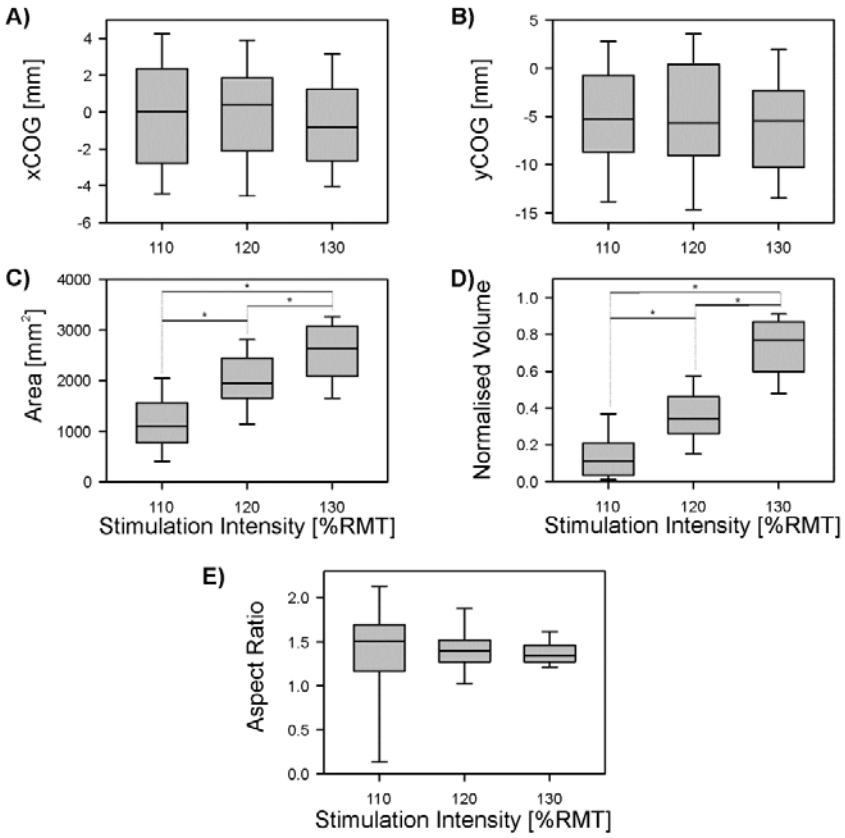

Figure 4-3: Group data for the effect of stimulation intensity on TMS maps (n = 12). Three different stimulation intensities (110%, 120% and 130% of resting motor threshold) were compared. All statistical testing was performed using the non-parametric Friedman test and any significant difference were further explored using the Wilcoxon Signed-Rank Test. Statistical significance between pairs was declared when P<0.017 (Bonferroni adjusted) and is indicated by *. (A-B) Group data for both x- and y-coordinate of the centre of gravity. No effect was found for stimulation intensity. (C-E) Group data for the effect of stimulation intensity on map area, map volume and aspect ratio. A significant effect of stimulation intensity on map area and volume was found with all pairs being significantly different. No effect was found for stimulation intensity on the aspect ratio.

4.3.2 Experiment 2: Effect of muscle activation

The effect of muscle activation was studied for four different levels of activity (5%, 10%, 20% and 40% of MVC). One data set had to be discarded as the 5% MVC data was missing, and therefore, the analysis was performed on 11 participants. Maps for all levels of muscle activation from a representative participant are shown in Figure 4-4. In this case an increase in the map area can be observed from both 5%-10% as well as 10%-40% of MVC. There is no clear difference in COG between the different levels of muscle activation. Although the excitable area is increased, its shape seems to be unaffected by muscle activation.

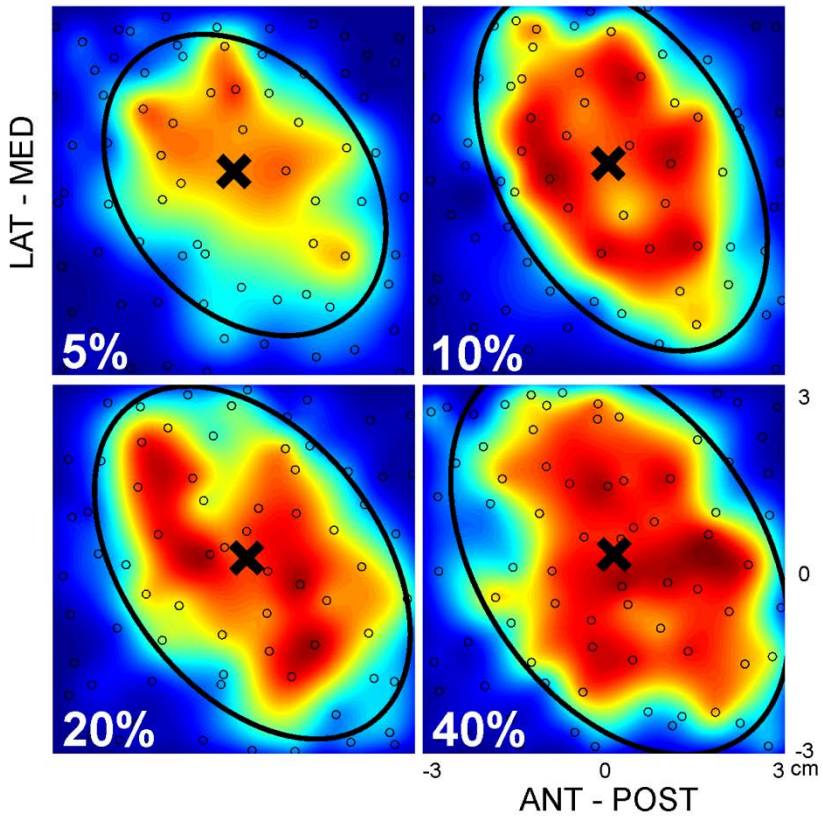

Figure 4-4: Single participant data illustrating TMS maps acquired at all levels of muscle activation (5%, 10%, 20% and 40% of maximum voluntary contraction (MVC)) using a 6 x 6 cm grid, 80 stimuli with an interstimulus interval of 1.5 s at 120% of resting motor threshold. Each black open circle represents one stimulus. The size of the approximated MEPpp is indicated by the colour, with blue representing a small MEPpp and red representing the greatest MEPpp. The black cross (×) highlights the centre of gravity. In this participant muscle activation was found not to affect the x- and y-coordinate of the centre of gravity, however map area and volume significantly increased with muscle activation. An ellipse was fitted through the data points representing 30% of the maximum MEP within the 5% MVC maps and used to study changes in the shape of the excitable area of the map. No change in the shape of the ellipse was found.

No significant effect was shown for level of muscle activation on the COG (xCOG: $\chi^2(3) = 3.55$, P = 0.31; yCOG: $\chi^2(3) = 1.58$, P = 0.66; Figure 4-5A|B). Both map area and volume significantly increased with level of muscle activation (area: $\chi^2(3) = 31.91$, P < 0.01; volume $\chi^2(3) = 25.47$, P < 0.01). Post-hoc testing showed a significant difference between all pairs for area and volume, for the Bonferroni adjusted P-value (0.0083; Figure 4-5C|D). Finally, aspect ratio was found to be unaffected by condition ($\chi^2(4) = 6.38$, P = 0.09; Figure 4-5E). The Euclidian distance characterising the distance between all COGs off all active conditions and repetitions compared to the mean COG of the resting condition revealed no effect of condition ($\chi^2(4) = 7.49$, P = 0.11; Figure 4-5F).

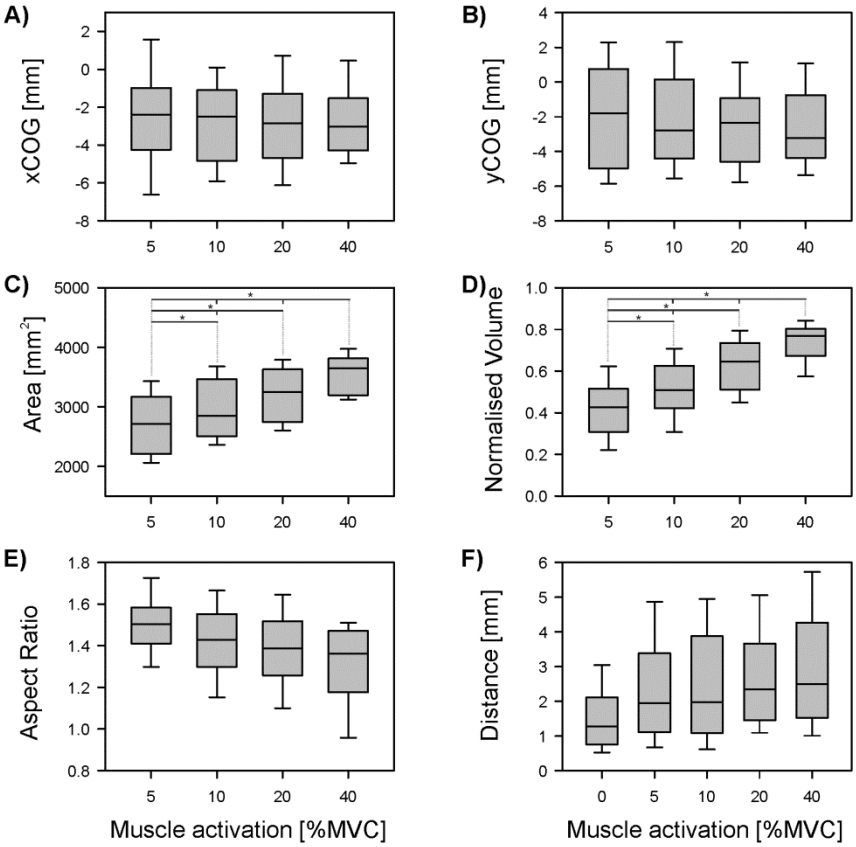

Figure 4-5: Group data for the effect of muscle activation on TMS maps (n = 11). Four levels of muscle activation, 5% 10%, 20% and 40% of maximum voluntary contraction (MVC), were compared. All statistical testing was performed using the non-parametric Friedman test and any significant difference were further explored using the Wilcoxon Signed-Rank Test. Statistical significance between pairs was declared when P<0.0083 (Bonferroni adjusted) and is indicated by *. (a-b) Group data for both x- and y-coordinate of the centre of gravity. No effect was found for muscle activation. (c-d) Group data for the effect muscle activation on map area and map volume. A significant effect of muscle activation was found on both map area and map volume. All pairs were found to be significantly different for both the map area and map volume. (e) The maps aspect ratios for different levels of muscle activation. No effect for muscle activation on aspect ratio was found. (f) Group data of the Euclidian distance of each level of muscle activity versus the resting condition.

4.4 Discussion

In this study we demonstrated that map area and volume increased with stimulation intensity and muscle activation, but centre of gravity and shape were unaffected. For both an increased stimulation intensity and higher level of muscle activation, we confirmed the hypothesis that the increased map area reflects a simple scaling of the map.

The effect of stimulation intensity on the TMS map

The effect of stimulation intensity on the map's COG has never been systematically explored. In line with previous studies, area and volume were observed to increase with stimulation intensity (Thordstein et al., 2013) In the present study, both central tendency (COG) and shape (aspect ratio) were invariant to stimulation intensity. It has been suggested that the area of a TMS map is primarily determined by the extent to which the current spreads in the motor cortex (Thickbroom et al., 1998) Therefore, the increase in map area with stimulation intensity might simply be explained by greater activation of the motoneuron pool. With increasing stimulation intensity the increased motoneuron pool activation together with the constant aspect ratio and stable COG, suggests the hand area of the motor cortex is activated symmetrically about the major and minor axes of the stimulation coil.

Stimulating at 130% of RMT might have induced D-waves by direct activation of the axon hillock (Di Lazzaro et al., 1998a, Di Lazzaro et al., 2003). In this study it is difficult to unequivocally determine if D-wave recruitment has been present because single stimuli were administered to multiple sites close to, but not specifically over, the motor hotspot and we used a biphasic TMS stimulator which has been reported to result in a less consistent cortical output (Di Lazzaro et al., 2001). Moreover, all recordings at the three different stimulation intensities were performed at rest whilst muscle activation might be needed to evoke a D-wave. This makes it difficult to use the current data to conclude on D-wave recruitment. Nonetheless, it is likely that in some participants we have elicited D-waves during the mapping.

In this study we investigated the cortical representation of the FDI muscle. It is not straightforward that the results presented here do directly translate to the TMS maps of other muscles. Thordstein et al. (2013) reported differences in the effect of stimulation intensity on the map area of the abductor pollicis brevis (APB), the extensor digitorum communis (EDC), the biceps brachii (BB) and the tibialis anterior (TA) muscle, but also highlighted great interindividual differences. Our findings combined with those of Thordstein et al. (2013) highlight that stimulation intensity is an important parameter in TMS mapping and should be carefully considered based on the aim of the mapping procedure and the muscle studied.

The effect of muscle activity on the TMS map

In the present study, TMS maps were acquired at four different levels of muscle activation. Whilst it is well documented that MEPs are larger for a muscle that is active compared with a muscle at rest (Hess et al., 1987, Kiers et al., 1993), MEP size does not increase substantially when the muscle is activated above 10% MVC (Taylor et al., 1997, Helmers et al., 1989). Nonetheless, we found a progressive increase in map area with muscle activation, which contrasted our hypothesis. When comparing a resting and slightly active muscle, the increased excitability is mainly attributed to changes in excitability at the spinal level. This followed from the observation that with muscle activation, stimulation at a level below the cortex did enhance the response amplitudes to a same extent as cortical stimulation (Ugawa et al., 1995, Maertens de Noordhout et al., 1992). These findings have been supported by epidural recordings (Kaneko et al., 1996b, Kaneko et al., 1996c, Di Lazzaro et al., 1998b). An increase in cortical excitability has also been argued when comparing a resting and slightly active muscle (Mazzocchio et al., 1994). Di Lazzaro et al. (1998b) suggested that an increase in the corticospinal volley might be primarily important when the muscle is contracted at different levels, which is supported by the findings of Ugawa et al. (1995). Nonetheless, based on our results we cannot say if the increased map area is a result of increased spinal or cortical excitability, or a combination of both. However, the contrasting finding of a saturating MEP size and an increased map area does suggest the saturating MEP response might just be a result of the inability of the maximal

magnetic stimulus to recruit all cortical neurons to generate greater descending volleys. The progressively increasing map area found in this study shows a greater cortical area is sensitive to eliciting an MEP when the muscle is active. The dissociation between a saturating MEP and increased map area might be explained by TMS directly recruiting additional connections (e.g. from the ventral premotor cortex) when the muscle is active. Because this activity will likely be small and temporally dispersed, it might not be readily observable when recording D- and I-waves epidural (Di Lazzaro et al., 1998b).

However, not only greater cortical area with increased excitability can explain the increased map area, as it could also be a result of the stimulation intensity used. Here the approach of Wilson et al. (1995), was adopted maintaining the stimulation intensity at 120% RMT for all levels of muscle activation. One could argue that because of the 8-10% reduction in motor threshold and increase in MEP_{pp} amplitude observed for an active muscle versus a muscle at rest (Wassermann, 2002, Devanne et al., 1997), it would be straightforward to think map area increases as well. Therefore, the observed increase in map area might be a result of the reduction in motor threshold rather than the cortical excitable area expanding. This viewpoint is supported by the findings of Ngomo et al. (2012b), who compensated for the 10% MSO difference between resting and active muscle motor threshold, and failed to find a difference in the map area between a resting and active muscle. However, in this study we only directly compared the map area at different levels of muscle activation, rather than comparing the map area when the muscle is at rest and active. A minimal change in threshold has been reported when muscle activation exceeds 10% MVC (Devanne et al., 1997). Therefore, it is unlikely that adjusting the stimulation intensity relative to threshold at every level of muscle activation would have provided different results as those presented here.

When it was first investigated, Wilson et al. (1995) observed a 6 mm mediolateral shift of COG when maps were acquired when the muscle was at rest and activated at 10% of MVC. However, this was not observed in later studies employing a similar paradigm (Classen et al., 1998a, Ngomo et al., 2012b). Previously, we reported the COG variability of the adopted mapping method at ±2.4 mm (van de Ruit

et al., 2015), which is consistent with other studies where the traditional mapping method was employed (3 mm; Classen et al., 1998a, Littmann et al., 2013, Miranda et al., 1997). The statistically insignificant difference of 1 mm in COG between maps acquired with the muscle at rest and all active conditions is an order of magnitude below the inherent variability of the map. Therefore, it can be concluded that in the present study no translation of COG was observed between maps constructed with the muscle at rest or when active.

Lastly, it was observed that the map's aspect ratio, which was used to define the map's shape, is indifferent to muscle activation. Combined with the finding of no translation in COG, this suggests a simple scaling of the TMS map area and implies cortical neurons at are equally excitable along the perimeter of the muscle's cortical representation. Whilst, not statistically significant, Figure 4-5E suggests the aspect ratio may decrease with muscle activation. This trend is likely just a consequence of the restricted area that was mapped. The major axis of the ellipse was usually found to be orientated about 45 deg relative to the anterior-posterior axis, in line with the coil orientation during stimulation. Combined with the notion that the magnetic field is elongated in line with the coil (e.g. Wilson et al., 1993, Roth et al., 1991) the major axis most often covered the full diagonal of the map. Therefore, with increasing muscle activation the major axis could not lengthen, in contrast to the minor axis. As the aspect ratio was calculated by dividing the length of the major axis by the length of the minor axis, this likely explains the decreasing trend.

Limitations

The mapping method used in the present study uses 80 stimuli delivered pseudorandomly to different locations in a 6 x 6 cm grid with an ISI of 1.5 s (van de Ruit et al., 2015). Using this method, the acquisition time for each map was less than 2 minutes. As a result, the method allows direct comparison of TMS maps at multiple stimulation intensities and levels of muscle activation whilst keeping the duration of a single session within 2 hours. It is unlikely that the use of this method, as compared to a more traditional method using multiple stimuli applied to sites organised on a 1-cm fixed space grid, has affected our results. By adopting the pseudorandom walk method we also

minimised any effects of fluctuating corticospinal excitability with time (Ellaway et al., 1998, Kiers et al., 1993) and attention (Rosenkranz and Rothwell, 2004, Rossini et al., 1991). As we stimulate with and ISI of 1.5 s, it might be argued that motor cortex excitability might be reduced as is well known to happen with 1 Hz repetitive TMS (Chen et al., 1997). However, the protocols used to reduce excitability deliver at least 5 times the number of stimuli than are used in the present study. We have recently demonstrated that short trains (180 stimuli) delivered at 1 Hz do not alter motor cortex excitability (Mathias et al., 2014). The likelihood of affecting excitability using an ISI of 1.5 s is further reduced by the fact that stimuli are applied at different sites across the 6 x 6 grid, and the distance between these sites is maximised during the mapping.

The use of a fixed 6 x 6 cm might have affected our results as previous studies have shown map area might exceed 36 cm^2 (Thordstein et al., 2013, Wilson et al., 1995). The grid size was limited to 6 x 6 cm as we found that when using a larger grid, stimuli would be administered close to and on the temple and ear which caused significant discomfort for the participants. However, in future it would be beneficial to base the grid size on the participant's head size, to ensure all cortical sites that evoke MEPs are mapped. It is unlikely that the adopted grid size has affected our results as the map area was calculated without the map's fringe and sites that would evoke an MEP smaller than 10% of the maximum MEP.

Implications

As the map area significantly increases with muscle activation and stimulation intensity but the COG and map shape remain the same, this study highlights the importance of choosing experimental conditions and TMS stimulation parameters carefully. This becomes of great importance when using TMS mapping to study brain plasticity in a clinical population (Liepert et al., 1999, Byrnes et al., 1999, Guerra et al., 2015), where fatigue and discomfort are a significant confounding issue. Inadequate parameter selection might lead to the inability to observe a difference in studies investigating changes in corticospinal excitability but also unnecessary participant discomfort. As a

result, care should be taken when selecting the parameters for TMS motor mapping and better standardisation of protocols is warranted.

CHAPTER 5

A MATLAB GUI for rapid acquisition of the Transcranial Magnetic Stimulation stimulus-response curve

5.1 Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive and painless brain stimulation technique that is used to quantify corticospinal excitability (CSE - Rothwell et al., 1991). A motor evoked potential (MEP) is evoked when TMS is administered over the primary motor cortex, and used as a measure of excitability that quantifies the strength of the brain-muscle connection (Rossini et al., 2015). When stimulating at multiple different stimulation intensities one can construct a TMS stimulus response (SR) curve to describe the relationship between the stimulation intensity and the size of the resulting MEP (Devanne et al., 1997, Carroll et al., 2001).

Typically, data for the SR curve is acquired at 3-10 different stimulation intensities with respect to the motor threshold. Between 3-20 stimuli are administered at every stimulation intensity and the mean MEP amplitude at every intensity is used to construct the SR curve (e.g. Jensen et al., 2005, Ridding and Rothwell, 1997, Moliadze et al., 2010a). With inter stimulus intervals (ISIs) limited to 3 - 10 s, acquisition time often exceeds 10 min, which is undesirable given the variable nature of the MEP that is affected by attention, drowsiness and time (Rosenkranz and Rothwell, 2004, Kamke et al., 2012, Kiers et al., 1993, Tormos et al., 1997). To date, SR curves have not been acquired at a faster rate, which can be attributed to several different factors.

Firstly, it is documented that stimuli delivered at rates equal to or faster than 1 per second (1 Hz), CSE can be either depressed or facilitated (Chen et al., 1997, Berardelli et al., 1998), exceeding the time of stimulation. However, these effects on MEP amplitude are only visible after several hundred stimuli, as the reported depressive effect elicited by 1 Hz TMS is absent when only 180-240 stimuli are delivered (Maeda et al., 2000b, Mathias et al., 2014). Further, in all these cases, stimuli are delivered

at a fixed sub- or suprathreshold intensity. This is not the case when acquiring data for the SR curve, for which stimulation at different intensities is required. Secondly, although stimulating at a rate of faster than one stimulus every 3 s does not affect excitability, one of the most commonly used TMS stimulators is unable to provide stimuli at an ISI shorter than 2-4 s (Magstim 200). This technical limitation does become less relevant considering stimulation intensity has to be adjusted by hand on a pulse-by-pulse basis when acquiring data for the SR curve in many TMS systems. The importance of setting TMS stimulation intensity on a pulse-by-pulse basis has been shown by Möller et al. (2009), as any other method could produce a systematic bias, shifting the SR curve along the intensity axis. Finally, prior to each experiment a decision has to be made about how much data will be acquired, as data can only be analysed offline. It is likely that in many cases fewer stimuli are needed in order to fit a SR curve. It would be beneficial to have a platform in which the acquired data is analysed online. The resulting SR curve would be directly fed back to the experimenter so an - on the spot - decision can be made about how much data is required.

The traditional problem of a limited ISI can now be circumvented by using systems that are commonly used for repetitive TMS (e.g. Magstim Rapid^2), which can stimulate at a higher rate. Moreover, the latest Magstim systems are provided with a communication protocol and a RS-232 interface port to trigger the system externally and set stimulation intensity automatically on a pulse-by-pulse basis. We recently demonstrated that using this system it is possible to acquire reliable SR curve data in 2 - 3 min but also highlighted a great variability in the number of stimuli needed to construct a reliable SR curve (Mathias et al., 2014). To facilitate rapid acquisition of the SR curve a platform is required to control the TMS stimulator and provide online feedback about the acquired data allowing cessation of stimulation when sufficient data is acquired. This reduces the risk of acquiring insufficient or too much data. In this article the development of a simple graphical user interface (GUI) is described. The GUI should allow communication with a TMS stimulator and retrieve direct feedback about the data acquired for the TMS SR curve. Evidence is provided that the presented GUI can be used to rapidly acquire a SR curve.

5.2 Methods

The primary aim of developing this software was to provide users of the TMS SR curve a platform to rapidly acquire TMS data and obtain online feedback about the resulting SR curve. In order to achieve best acquisition performance, minimal data processing was performed online. A separate GUI was developed for more detailed data analysis and post processing of data. All data acquisition and data processing was performed by a GUI developed in the MATLAB environment **(**MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States)**.**

Requirement analysis

A requirement analysis was performed with four experienced users of TMS SR curves before commencing the project. This resulted in the following key needs of the GUI:

(1) Design of GUI should provide ability to:

- Start / stop data acquisition
- Customise range of stimulation intensity
- View EMG recordings
- Visualise the SR curve plotting stimulation intensity vs MEP_{pp}

(2) Data acquisition and processing should provide ability to:

- Externally trigger TMS stimulator based on set ISI
- Perform basic EMG processing and MEP_{pp} detection
- Randomise stimulation intensities online
- Provide Boltzmann sigmoid curve fit which is updated with each stimulus

(1) Designing a graphical user interface for data acquisition

The GUI is presented in Figure 5-1. The primary function of the GUI is to provide the user with direct feedback about the EMG recordings, the SR curve and the ability to interact with the TMS stimulator. For feedback purposes, the GUI contains two graphs: one to display the acquired electromyographic (EMG) response, and one plotting the stimulation intensity versus the detected MEP_{pp}. Pushbuttons allow the user to start / stop stimulation, provide a calibration stimulation and save acquired data. A calibration stimulation can be administered to define the detection window for the MEP. To set the range of stimulation intensities two slider bars were implemented which are used to define the minimum and maximum stimulation intensity during stimulation.

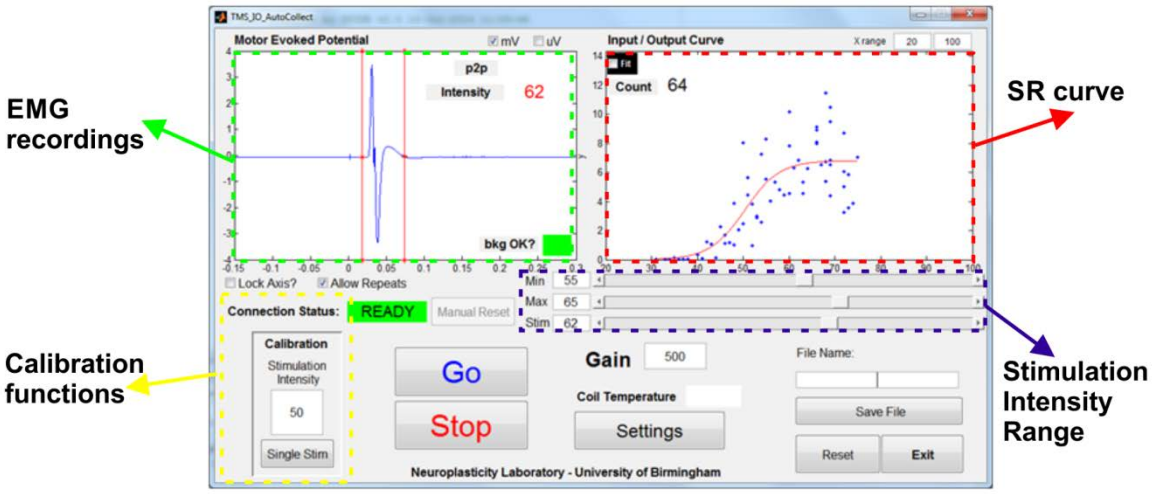

Figure 5-1: The main GUI for rapid acquisition of the SR curve. The top left window (green outline) provides direct feedback of each EMG recording following every stimulation. The two red vertical cursor lines allow the user to define the time window from which the peak-to-peak amplitude is extracted. The top right window (red outline) is where the SR curve is plotted (stimulation intensity vs MEP_{pp}). Each blue dot represents one stimulation. The red line is the Boltzmann curve fit which is updated online following each stimulation. Below the SR curve plot are the slider bars that allow the user to set the minimum and maximum stimulation intensity, effectively setting the range of stimulation intensities used during stimulation (blue outline). The bottom left window (yellow outline) provides the ability to do one or more single calibration stimulus, used to e.g. set the time window from which the MEP_{pp} is extracted or perform 'hotspot' finding.

(2) Data acquisition and processing

EMG data acquisition and communication with the TMS stimulator was performed with a National Instruments (NI) BNC board (BNC-2090A) connected to a mass transfer unit (USB-6259) (National Instruments Corporation, Austin, Texas, United States). EMG data was acquired from 100 ms before to 300 ms after the TMS stimulator was triggered to stimulate, and sampled with 5000 Hz. A 5V TTL trigger is sent over a digital output channel to externally trigger the TMS stimulator. All settings can be defined by the user and can be accessed through the main GUI.

The GUI was designed to communicate with a Magstim Rapid^2 stimulator (Magstim Ltd, Dyfed, United Kingdom). Combining a Magstim provided communication protocol and a communication toolbox freely available (http://www.psych.usyd.edu.au/tmslab/rapid2andrept.html, TMS laboratory, School of Psychology, University of Sydney), there is the ability to arm, disarm, trigger, read system and coil temperature and set the stimulation intensity of the stimulator. Communication is performed over a 9-pin RS-232 serial port which was connected to a custom-built trigger box. The trigger box contains two BNC female connectors, one to relay a trigger signal to the stimulator originating from the NI board, and another one to relay a trigger signal back from the stimulator to the NI board confirming successful stimulation. This box was hooked up to the TMS stimulator by a 26-pin male connector. To allow the GUI to catch up and facilitate smooth operation of serial communication between the PC and TMS stimulator the baseline loop time, without stimulating, was about 1.5 s. For the setup, see Figure 5-2.

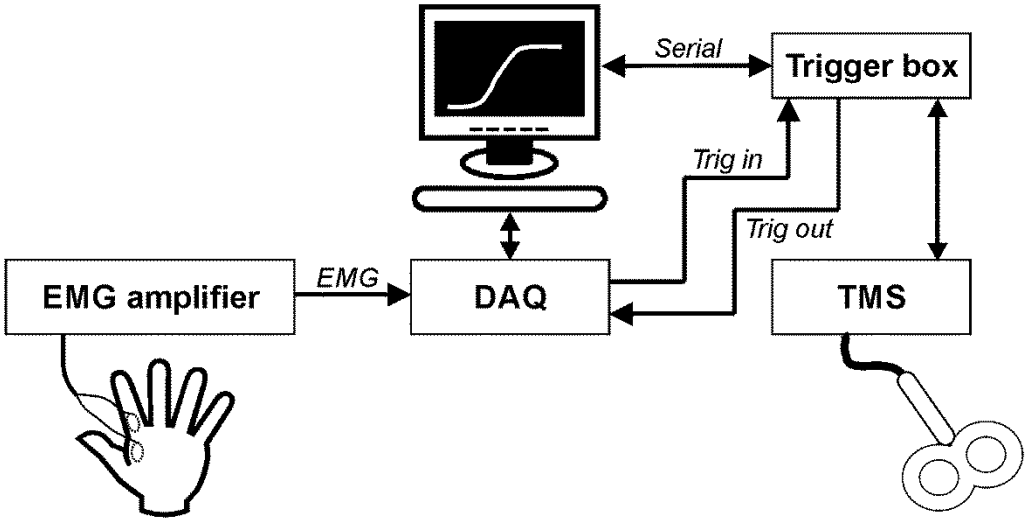

Figure 5-2: The experimental setup during data acquisition of the SR curve using the MATLAB GUI. The National Instruments data acquisition board is connected to the PC (USB) to allow acquisition of EMG data and sending and receiving a trigger signal to/from the TMS stimulator. The custom-built trigger box serves as a relay of the trigger signal to / from the TMS stimulator through BNC connectors. In addition, stimulator settings (e.g. stimulation intensity) can be defined using a serial port (RS232).

Preparing the system for acquisition

Before starting data acquisition for the SR curve, three conditions have to be met: (1) Communication between stimulator and computer has to be established; (2) stimulator must be disarmed; and (3) the time window to extract the MEP_{pp} from has to be predefined. The first two of these conditions must be met before the software runs and are therefore checked during initialisation of the GUI. A stimulus at 10% of the maximal stimulator output (MSO) is performed to confirm the stimulator has been disarmed, checking if a trigger is returned from the stimulator. If successful, the buttons to start data acquisition will be enabled, else the setup has to be checked and a reset of the communication performed. Once communication has been successfully established and the system is disarmed, a calibration stimulation can be performed. The calibration stimulation is administered to define the time window from which the peak-to-peak amplitude needs to be extracted. The intensity of this calibration stimulation can be set manually in a text box. After obtaining a single MEP recording, two vertical red cursor lines are visible in the window displaying the EMG recording (Figure 5-1 - bottom left). These red lines mark the time window from which the MEP_{pp} amplitude is extracted. By default these are set to mark a time window 20 - 50 ms after stimulation.

Online EMG processing

Online EMG processing is conducted to allow the experimenter to obtain some information about the level of background EMG and visualise the SR curve. Only analogue filtering is performed on the EMG signal (20-1000 Hz). To extract the level of background EMG the peak-to-peak amplitude was extracted in the time window 100 to 10 ms prior to the TMS stimulus. A colour indicator is displayed to alert the operator when background EMG peak-to-peak amplitude exceeds predefined limit (e.g. in case of a resting muscle: 30 μV). As a result, the operator can easily see when the muscle is not relaxed and provide verbal feedback to the participant to reduce the number of exclusions when analysing the data. For the MEP_{pp} the peak-to-peak amplitude is extracted from the time window defined by the user following the calibration stimulation and plotted against the stimulation intensity. No further EMG processing is performed or exclusion criteria predefined for online exclusion to avoid any unnecessary delays that limit the ability of reducing the ISI.

Randomising stimulation intensities

Technical limitations make it impossible to truly randomise stimulation intensities when short ISIs are desired. The biphasic Magstim Rapid^2 has been designed to enable use of short ISIs by ensuring there is a residual voltage on the capacitor following stimulation, reducing the charging time. However, when pseudo-randomising stimulation intensity on a pulse-by-pulse basis the capacitor needs to be discharged in case the intensity is dropped. Discharging the capacitor takes time and is dependent on the last stimulation intensity at which a stimulus was administered. The higher the stimulation intensity, the more the stimulation intensity can be dropped in a short space of time. Ignoring this limitation can lead to repetitive misfirings, i.e. a trigger is sent whilst the stimulator is not ready to stimulate. In contrast to dropping the stimulation intensity, increasing the stimulation intensity does not provide technical challenges and is does not restrict ISI.

In order to determine the maximal drop in intensity that could be achieved in a predefined time interval a trial-and-error test was performed. Three different time intervals (wait time) were tested - 100, 300 and 500 ms. These wait times signify the time in between setting the new stimulation

intensity and sending a trigger to stimulate. A minimal wait time of 100 ms was adopted to allow the system to drop the intensity by at least 1% at 10% of the MSO. The maximal wait time of 500 ms was chosen to keep the total ISI in between 1.5 and 2 s. By systematically dropping the stimulation intensity with 1% MSO steps from every stimulation intensity between 10 - 100% MSO the maximal allowable drop was established for each wait time. The relationship between the stimulation intensity, wait time and maximal intensity drop ($\Delta MaxStimInt_{drop}$) is shown in Figure 5-3A. $\Delta MaxStimInt_{drop}$ is both a function of the wait time as well as the start intensity (PrevInt) from which the drop has to be achieved. It would be easy to restrict the drop in intensity based on $\Delta MaxStimInt_{drop}$ and start intensity at a wait time of 500 ms. However, to optimise GUI performance and reduce the ability of the participant to predict when a stimulation is coming, the wait time was determined between every pair of stimuli based on PrevInt and desired drop ($\Delta StimInt_{drop}$).

The linear relationship between the wait time and maximal intensity drop at 100% MSO was determined, to allow maximal change in intensity (Figure 5-3B). The relationship between wait time and stimulation intensity is:

$$\Delta MaxStimInt_{drop} = \frac{14}{400} \cdot t + 12.25$$

where t is the required time to wait in milliseconds. However, this function for $\Delta MaxStimInt_{drop}$ is only valid at 100% and needs to be extended to all potential starting intensities (PrevInt). Therefore, also $\Delta StimInt_{drop}$ was determined as a function of $\Delta MaxStimInt_{drop}$ and PrevInt for a 100 ms wait time to counteract the poor behaviour at low intensities:

$$\Delta StimInt_{drop} = \frac{(\Delta MaxStimInt_{drop} - 1)}{90} \cdot PrevInt - \frac{2}{3}$$

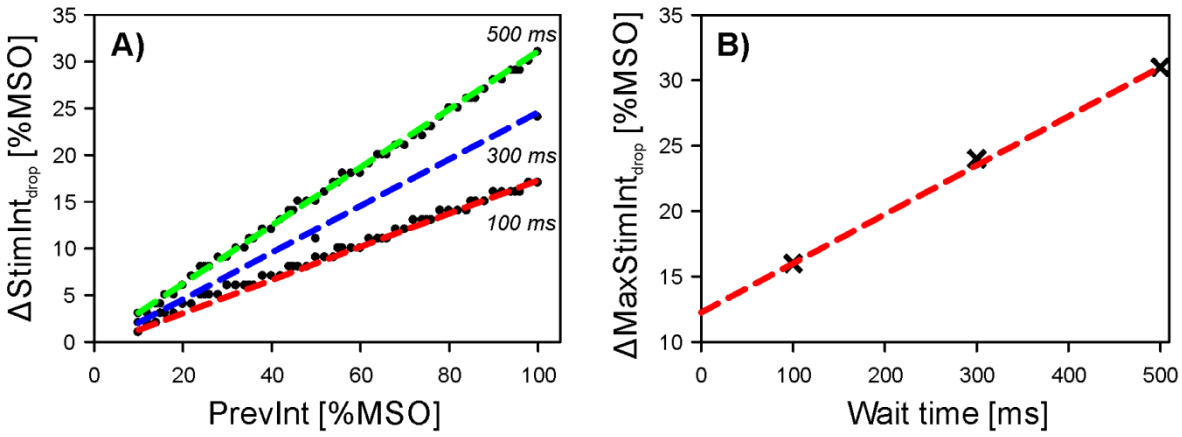

Figure 5-3: The relationship between starting stimulation intensity (PrevInt), wait time and acceptable drop in intensity ($\Delta StimInt_{drop}$) for a Magstim Rapid^2 stimulator. (A) For two different wait time (i.e. the time in between setting the new stimulation intensity and triggering the stimulator) of 100 and 500 ms, the maximal intensity drop was determined for every intensity between 10 and 100% of the maximal stimulator output (MSO) (block dots). A linear fit was made for both 100 ms (red dashed line) and 500 ms (green dashed line). For 300 ms the maximal drop was determined for only a few intensities and a linear fit made (blue dashed line). (B) The relationship between the wait time and maximal drop in intensity ($\Delta MaxStimInt_{drop}$) at 100% MSO.

Substituting $\Delta MaxStimInt_{drop}$:

$$\Delta MaxStimInt_{drop} - 1 = \frac{90 \cdot \left(\Delta StimInt_{drop} + \frac{2}{3}\right)}{PrevInt}$$

$$\frac{14}{400} \cdot t + 11.25 = \frac{90 \cdot \left(\Delta StimInt_{drop} + \frac{2}{3}\right)}{PrevInt}$$

$$Wait\ Time = \frac{\left(\frac{90 \cdot \left(\Delta StimInt_{drop} + \frac{2}{3}\right)}{PrevInt}\right) - 11.25}{\left({}^{14}/_{400}\right)}$$

This relationship is visualised in Figure 5-4. In MATLAB this function was implemented to determine the necessary wait time based on the intensity of every last stimulation. First, the maximal possible drop was calculated, for a maximal wait time of 500 ms. Subsequently, this drop was reduced by 5% MSO to reduce the chance of a misfiring. This number, together with a maximum increase in intensity of 30% MSO, was then used to create an array of intensities from PrevInt-MaxDrop to PrevInt + 30% MSO. From this array, an intensity was chosen at random. Accordingly this intensity was used to determine the actual wait time required. By default all stimulation intensities could only be presented once. Only when at all intensities one stimulation had been administered, repeating previously administered intensities was allowed. Repetition of stimulation intensity can be easily enabled if desired.

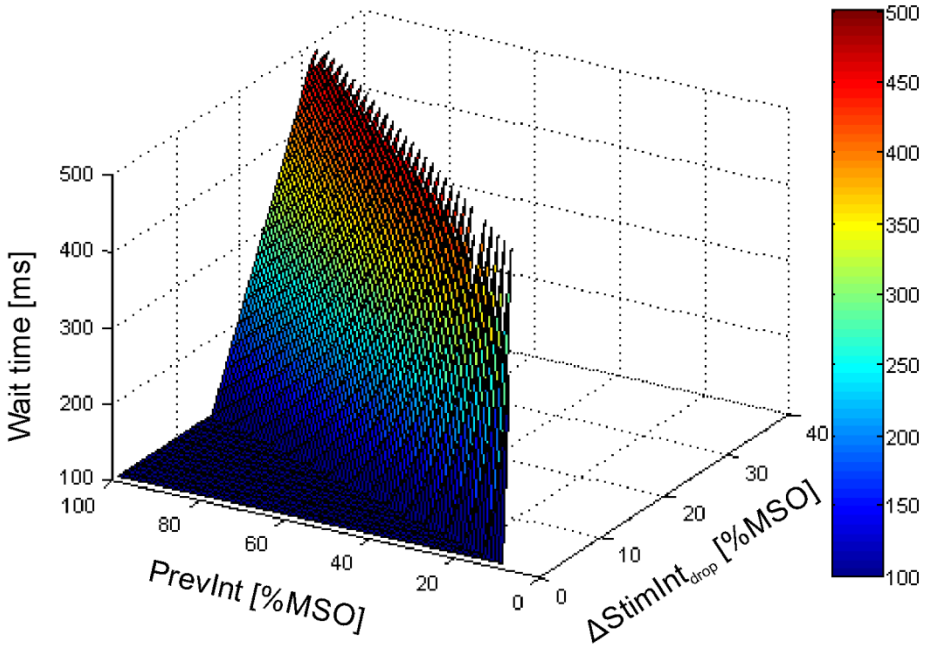

Figure 5-4: Surfaceplot representing how the wait time between two TMS stimuli is dependent on the drop in stimulation intensity and the last stimulation intensity used. Because wait time was limited to 500 ms, only a limited drop ($\Delta StimInt_{drop}$) can be performed at each intensity, from 32% MSO when the last used stimulation intensity (PrevInt) was 100% MSO to only 1% MSO when PrevInt was 10% MSO.

During acquisition and post processing - Curve fitting

During data acquisition and offline post processing, the MEP_{pp} values are determined and plotted against the stimulation intensity. A curve fit is performed online, similar to how it is done traditionally, to support the decision if sufficient data has been acquired to create a good fit.

A four-parameter Boltzmann sigmoid curve is fitted to the data using the following equation:

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

where the parameters MEP_{min}, MEP_{max}, I_{50} and S are extracted. MEP_{min} and MEP_{max} indicate the minimum and maximum horizontal asymptote of the function, I_{50} is the stimulation intensity mid-way between MEP_{min} and MEP_{max} and S indicates the slope of the relationship at I_{50}.

To find the best fit a non-linear least squares Levenberg-Marquardt algorithm (Levenberg, 1944, Marquardt, 1963) is used with a maximum of 1,000 successive iterations. The start point for the different parameters was provided as follows: MEP_{min}: min(MEP(I)), MEP_{max}: max(MEP(I)), I_{50} = 50% MSO, S = 1. To minimise the effect of outliers a robust least squares regression was included using bisquare weights. For online curve fitting no robust regression method was included to avoid incorrect fitting when no data for a full range of intensities had been acquired.

Post processing

A separate GUI was developed to post process the acquired SR curve data and exclude any EMG trials as a result of high background EMG or noise (Figure 5-5). The GUI was designed to load the acquired data and provide the possibility to exclude recordings trial-by-trial. The user can scroll through the EMG recordings one-by-one whilst mean and root mean square (RMS) value of the background EMG in a window from 100-10 ms prior to the stimulation is displayed. Any trials with a mean background EMG > 30 µV or the RMS exceeding Mean + 3 *SD* of the mean RMS over all recordings are automatically excluded from the curve fitting procedure. Moreover, it is ensured that the trigger actually occurred at time = 0 as system jitter sometimes delayed the system triggering by several

milliseconds. If this behaviour was observed, the whole recording was shifted to ensure stimulation occurred at time = 0 (see Results). Once the user finishes excluding individual trials, the MEP_{pp} values are determined and the curve fit is performed as described previously. After a first curve fit, additional outliers are determined based on a predefined prediction interval of the curve fit (usually 95%). All results, the curve parameters, raw EMG recording, and data used for the fit (stimulation intensities with extracted MEP_{pp} values) can be exported for any additional data analysis.

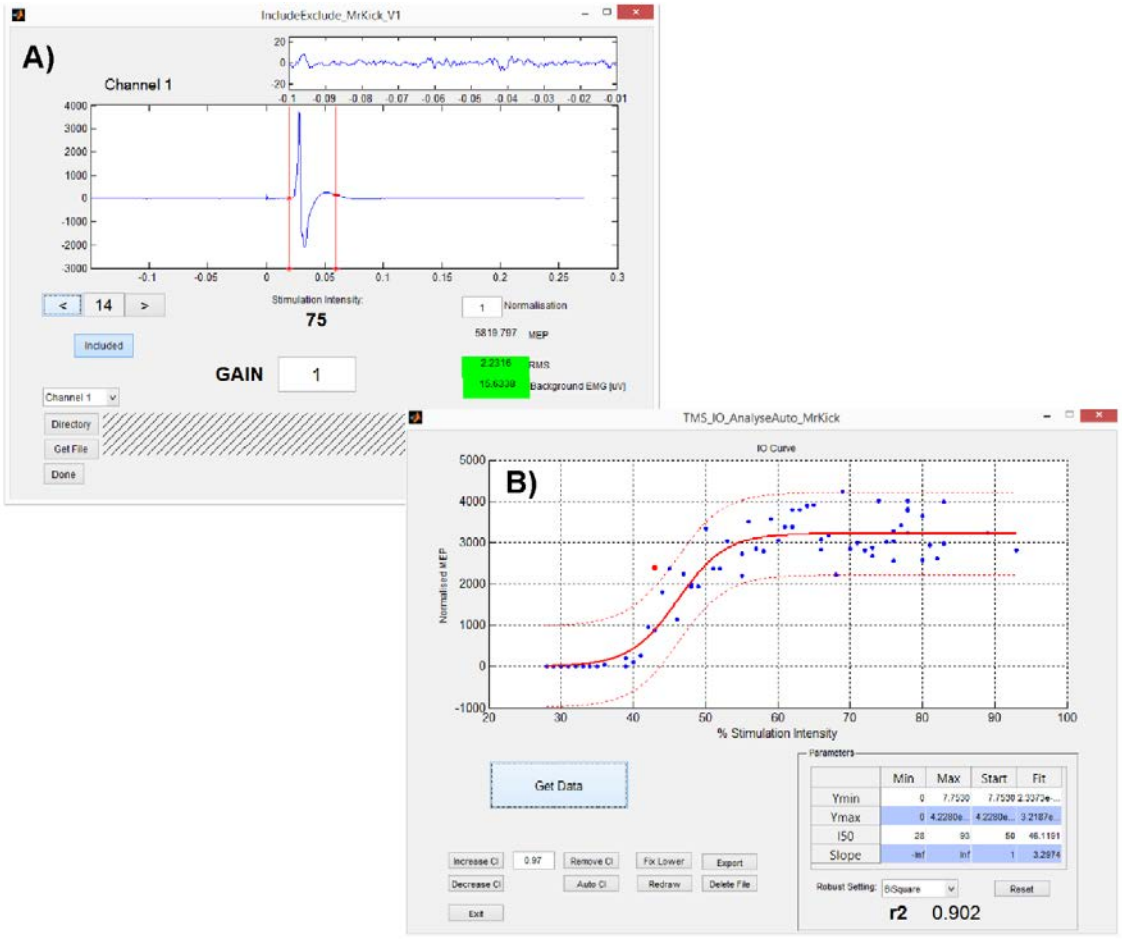

Figure 5-5: Post processing GUI for SR curve data. (A) EMG recordings can be studied trial-by-trial and excluded if necessary. Trials with high RMS or peak-to-peak value of background EMG (> 30 µV) are excluded automatically. A separate panel provides a zoom-in of 90 ms background EMG. (B) Once data has been screened a Boltzmann sigmoid curve is fitted using a Levenberg-Marquardt algorithm. Additional outliers are defined based on the 95% prediction intervals (red dashed lines and red dots). All curve parameters and goodness of fit (r^2) are provided and can be exported.

5.3 Results and Discussion

Data acquisition was performed and consequently post processed to obtain the final curve fit parameters and test the developed GUI.

Example data sets

EMG recordings were obtained during a ongoing study using the SR curves to quantify changes in CSE and a as part of the study by Mathias et al. (2014). These studies were approved by the University of Birmingham Science, Technology, Engineering and Mathematics ethics committee (ERN 11-0444 and ERN 14-0950). All participants completed a TMS adult safety screening and had to sign a consent form before participating.

EMG was recorded from the first dorsal interosseous using a Digitimer D360 EMG amplifier (Digitimer Ltd, Welwyn Garden City, UK). The EMG signal was amplified (500-1k) and filtered (20-1000 Hz) before being sampled (5000 Hz) and stored on a PC. A Magstim Rapid^2 TMS stimulator was used to stimulate over the primary motor cortex using a figure-of-8 coil. Coil position was continuously monitored using frameless stereotaxy and the BrainSight 2 neuronavigation software (Rogue Research Inc, Montreal, Canada). TMS was administered to the motor hotspot, which was determined by visual inspection of the EMG looking for the site eliciting the biggest MEPs. Acquisition of the SR curve was started with the minimum and maximum intensity set to 30% and 70% of MSO respectively.

SR curves were also acquired following the traditional method of data acquisition, to provide evidence that acquiring the data rapidly using this GUI does not affect the curve. Resting motor threshold (RMT) was determined at the motor hotspot as the intensity at which 5 out of 10 stimuli had an MEP_{pp} amplitude > 50 μV (Rossini et al., 1994, Rossini et al., 2015). Stimuli were then administered at six different intensities from 10% below RMT to 40% of MSO above RMT. Ten stimuli were administered at each intensity and each intensity was presented in pseudorandom order.

Validation of communication between PC and TMS stimulator

Successful communication between the PC and TMS stimulator must meet two criteria: (1) a working serial communication to arm, disarm and set intensity and (2) successfully trigger the TMS stimulator. By implementing a low intensity dummy stimulation before the GUI starts and checking if a trigger is received back from the stimulator successful communication is confirmed. Every time the GUI is run, the system needs about 5 s to establish communication after which the test trigger is sent. This functionality was successfully tested.

Any delay between sending the trigger signal and the time of stimulation was confirmed by recording both the signal that was sent out and the signal generated by the TMS stimulator when a stimulation is delivered. This data showed a variable delay of 2-5 ms between sending the trigger out and receiving a trigger back (Figure 5-6A). This delay was removed before post-processing by shifting the full EMG recording and is present but comparable across different testing sessions (Figure 5-6B). Unfortunately, this variable delay could not be corrected for in online detection of the MEP_{pp} value. By making the time window for MEP_{pp} detection cover a time window sufficiently wide (e.g. 10-50 ms), any problems are avoided. The cause of this jitter is likely related to PC background processes, that we were unable to control.

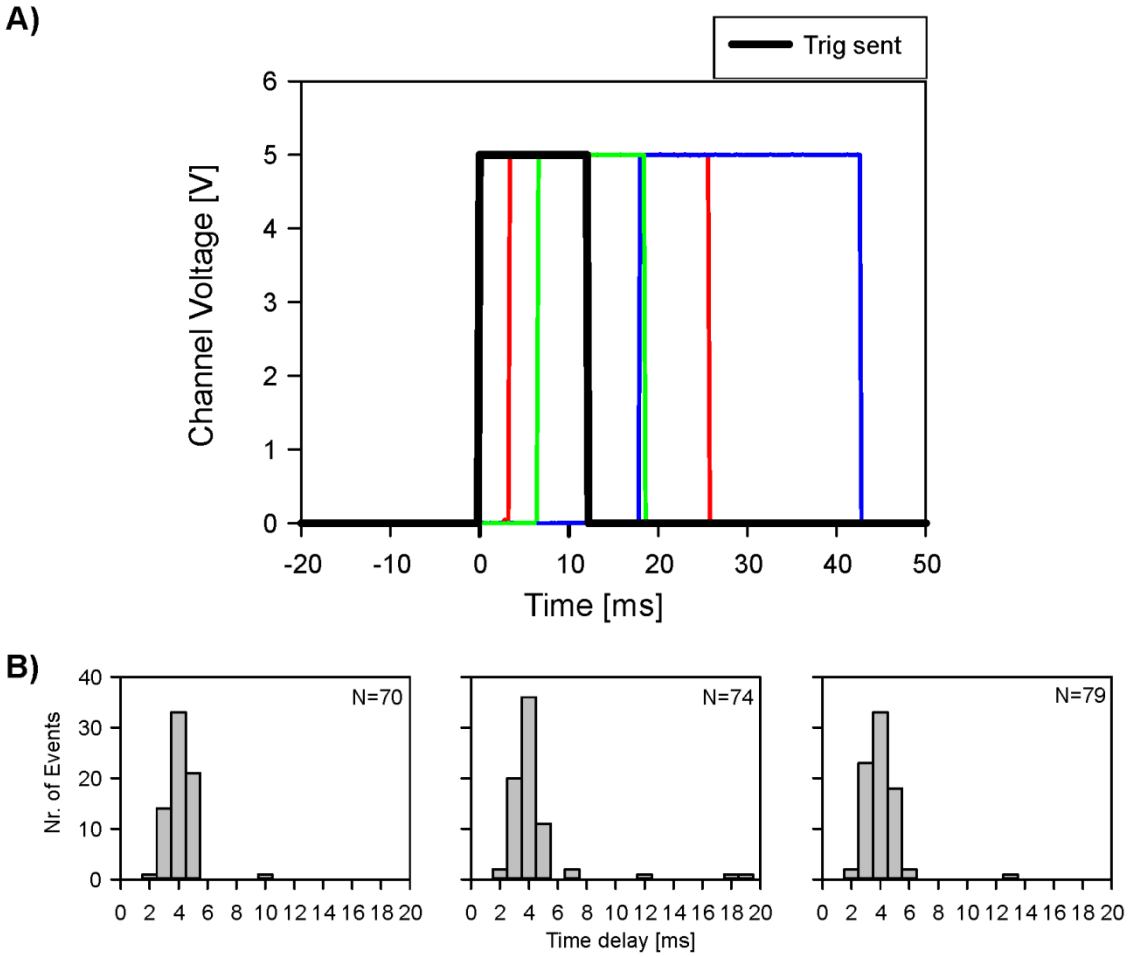

Figure 5-6: Demonstration of the variable delay between sending and receiving a trigger confirming succesful TMS stimulation.(A) Three randomly selected triggers recorded following triggering the TMS stimulator at t=0 (black solid line). A delay of 3 ms (red line) and 6 ms (green line) can be observed. Rarely a 20 ms delay (blue line) was also found. (B) Histograms showing the distribution of trigger delays of three data sets consisting of 70-79 stimuli. A delay of 2-5 ms is most common, with occasional outliers up to 19 ms.

Testing in healthy participants and post-processing software

Figure 5-7A shows one data set acquired using the here presented GUI alongside the corresponding data acquired without the GUI. No differences were found for a SR curve acquired using the two methods with the means at the different intensities closely matching the curve fit. Three more data sets with the curve fit are presented in Figure 5-7B. In each case a different number of stimuli was needed to construct the curve. The curve fits provided here were performed using the post processing software, correcting any delay as a consequence of jitter and removing any trials with background activity. Also some stimuli were excluded because they fell outside the 95% prediction interval of the curve fit.

No misfiring occurred during any of the trials, proving the reliability of the communication and GUI. This reinforces that the established relationship between the change in stimulation intensity and required wait time is correct. Nonetheless, occasionally the system required a longer time than the preset ISI during stimulation. The extra delay resulted in temporary loss of communication between computer and stimulator, possibly due to any uncontrollable Windows background processes or a problem in the serial communication. Whilst rare, the issue does not present any major challenges as communication is restored within 10 s.

Further developments

The software presented in this chapter was fully developed in the MATLAB environment. Whilst this enables the user to tailor the software to their individual needs, there are also limitations with respect to its use of computational resources. Implementation in a different programming language, generating an executable, and integrating in existing software packages used for recording electrophysiological data (e.g. Spike or Signal, Cambridge Electronic Design Ltd.) would greatly enhance its potential. Moreover, the software would be greatly improved by the ability to control stimulation by an external signal. Examples of this include the ability to trigger a stimulation when the participant holds a certain force, or temporarily cease stimulation when the coil is outside some predefined limits with respect to the motor hotspot or the muscle is not fully relaxed. Some initial tests have been performed to confirm that these applications are feasible. Lastly, it would benefit the GUI if there are objective criteria to determine if the curve fit does not change anymore. Currently it is up to the user to decide when stimulation is ceased.

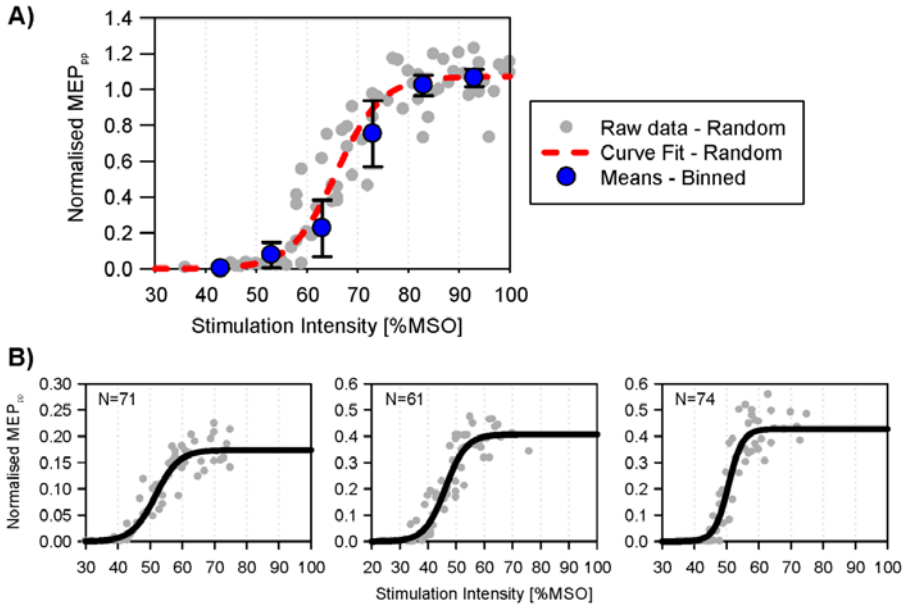

Figure 5-7: Comparison of SR curves acquired the traditional way and by the method used in the GUI. (A) For the pseudorandom method in this case 76 stimuli were administered with in interstimulus interval of 1.5-2 s. For the traditional ways 6 bins were chosen, and 10 stimuli performed at each intensity. Bins of stimuli were presented pseudorandomly. For all 71 stimuli included in the curve fitting the gray dots represent the MEP_{pp} value at each intensity. The Boltzmann sigmoid curve fit had a good fit to the data ($r^2 = 0.91$) (red dashed line). The means and standard deviations for every 10 stimuli at each of the 6 stimulation intensities for the binned method are represented by the blue circles with error bars. The data acquired using the traditional method fits the sigmoid curve fit well. (B) Three representative examples of data acquired and the corresponding curve fits using the here presented MATLAB GUIs. Hence, stimulation intensity was limited here to ~80% MSO as the maximum MEP_{pp} was reached. The number of stimuli included for the curve fit is indicated in the top right corner.

Conclusions

In this report we have introduced software to rapidly acquire data for the SR curve using a Magstim Rapid^2 stimulator. The software records EMG data and communicates with the TMS stimulator to stimulate across a range of different intensities. During data acquisition the acquired data is fitted with a Boltzmann sigmoid curve which provides the user a platform to tailor data acquisition to the individual and stop data acquisition once the curve fit does not change anymore. This negates the need for a fixed number of stimuli as presented by Mathias et al. (2014) and reduces the chance of administering too few or too many stimuli. The data presented provides evidence it is possible to acquire SR curves using a simple GUI. With its potential to be integrated in exiting software packages that allow electrophysiological recordings it provides a valuable tool for researcher using the SR curve to rapidly quantify changes in excitability without the need for lengthy data acquisition protocols or post processing steps to obtain the results.

CHAPTER 6

Imagery training does not promote neuroplasticity following a single session of mirror training

6.1 Introduction

Mirror training was successfully introduced in the 1990s to alleviate phantom limb pain in amputees (Ramachandran and Rogers-Ramachandran, 1996). In mirror training a mirror is lined up in front of the participant, parallel to the sagittal plane, and each limb (e.g. hand or foot) is positioned on one side of the mirror. When one limb is being moved whilst watching its mirror reflection, the visual illusion is created that the passive limb behind the mirror is moving. With repetitive practice this has been found to lead to neuroplasticity in the hemisphere associated with the passive limb (Ramachandran and Altschuler, 2009). In healthy participants changes in corticospinal excitability (CSE) following mirror training were linked to improved motor performance of the non-trained hand after just 10 sets of 30 s on a ball rotation task (Nojima et al., 2012).

The positive outcomes associated with mirror training are attributed to its close correspondence to motor imagery and action observation (Stevens and Stoykov, 2003, Vogt et al., 2013). Motor imagery is the process of mentally rehearsing a movement without creating any overt motor output, and has been reported to be able to lead to improved motor performance (Feltz and Landers, 1983). Similarly for action observation, motor performance has been found to improve by observing a movement performed by another individual (Vogt and Thomaschke, 2007, Vogt, 1995). Whereas physical practice has long been considered essential in motor learning and rehabilitation (e.g. Butefisch et al., 1995), these findings suggest otherwise.

The potential of motor imagery and action observation in motor learning and rehabilitation can be explained by the finding of shared neural representations between motor execution, motor imagery and action observation (Grafton et al., 1996, Rizzolatti et al., 1996, Grezes and Decety, 2001, Jeannerod,

1994). These findings have been supported by the observations that motor imagery as well as action observation increase CSE as assessed using transcranial magnetic stimulation (TMS), suggesting cortical involvement during these passive processes (Fadiga et al., 1995, Kasai et al., 1997). These studies reported an increase in CSE specific for the imagined muscle and in time with the imagined or observed movement. Moreover, training a sequence learning task using motor imagery was shown to result in a expanding cortical representation (Pascual-Leone et al., 1995).

Increased CSE has also been found following or during mirror training (Garry et al., 2005, Fukumura et al., 2007, Funase et al., 2007, Kang et al., 2011, Nojima et al., 2012). This would suggest that if motor imagery and action observation play an important role in mirror training, either could be used to promote neuroplasticity following mirror training. This idea is supported by the finding that imagery ability affects the degree of change in excitability (Williams et al., 2012a). Moreover, improved imagery ability will benefit the learning process by recruitment of different brain regions (Guillot et al., 2008). Recently, imagery training by gradually building the complexity of the image, Layered Stimulus and Response Training (LSRT), has been found to be an effective way of improving imagery ability and motor performance (Williams et al., 2013). LSRT introduces detail to an image using a layered approach based on the participants feedback. It allows researchers to effectively improve imagery ability in a short space of time. This study aimed to assess if a single session of LSRT in addition to mirror training could induce significantly greater changes in CSE than mirror training alone as assessed by TMS maps. We hypothesised that motor imagery training in addition to mirror training would significantly increase CSE compared to mirror training alone.

6.2 Methods

In total, 44 healthy participants (21 ± 4 y, range 18-35, 28 female) were recruited for the study. Participants were screened for contra-indications to TMS using a modified version of the TMS adult safety questionnaire originally suggested by Keel et al. (2001). The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_13-0701), and all experiments were performed in accordance with the Declaration of Helsinki.

Electromyography

Bipolar surface electrodes (Blue Sensor N, Ambu, Denmark) were used to record the electromyographic (EMG) activity from the finger extensor muscle, extensor digitorum communis (EDC), which was the primary muscle of interest. At the same time EMG was recorded from the finger flexor muscle, flexor digitorum superficialis (FDS). All EMG signals were amplified (EDC; 2k, FDS; 5k), band pass filtered (20-1000 Hz), and digitally sampled at 5 kHz to be stored for offline analysis.

Transcranial Magnetic Stimulation

Magnetic stimulation was delivered by a Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom) with a custom made polyurethane coated 90 mm 'batwing' shaped figure-of-8 coil. The coil was always held in a 45 deg angle to the midline with the handle pointing backward. The 'hotspot', or stimulation site resulting in the largest motor evoked potentials (MEPs), was found by visual inspection of the EMG recorded from the EDC muscle. The hotspot was used to determine the resting motor threshold (RMT). Starting at 60% MSO, the threshold intensity was defined by the intensity at which at least 5 out of 10 stimuli evoked MEPs with a peak-to-peak amplitude > 50 μV (Groppa et al., 2012, Rossini et al., 1994). Coil position and orientation were monitored using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada).

Peripheral Nerve Stimulation (PNS)

MEPs were normalised to the electrically evoked maximal M-wave (M_{max}) to ensure valid comparison between participants. To obtain the M_{max} a bipolar probe was used to stimulate the deep radial nerve (EDC) and ulnar nerve (FDS) at the level of the elbow using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK).

Experimental protocol

In all experiments participants were seated comfortably in a custom built chair with their non-dominant arm resting pronated on a height-adjustable stool. Participants' handedness was assessed using a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971). The score runs from -100 to +100, indicating purely left or right handed respectively, with participants with a score between -40 - 40 classified as ambidextrous. The non-dominant hand was studied as the non-dominant hemisphere has been reported to be more sensitive to imagery and action observation induced changes in CSE (Bianco et al., 2012). Three TMS maps were acquired before and directly after mirror training with the muscles at rest. Another three TMS maps were acquired 20 min after the mirror training had finished, as some consolidation time has been shown to be important before remodelling of neural pathways can be identified (Pascual-Leone et al., 2005). Before the start of the experiment, every participant was randomly assigned to the control or imagery training group. In both groups imagery ability was assessed using the movement imagery questionnaire (MIQ-3). Following the assessment of imagery ability, the imagery training group received about 10 min of LSRT including explanation of the mirror training and imagery concepts. The control group only received brief explanation about the mirror training and how to perform the task before the training commenced. We ensured the time between baseline TMS assessment and mirror training was equal for both groups. All participants received 8 blocks of 2 min mirror training separated by 15 s rest breaks. Figure 6-1 provides an overview of the experimental setup and protocol.

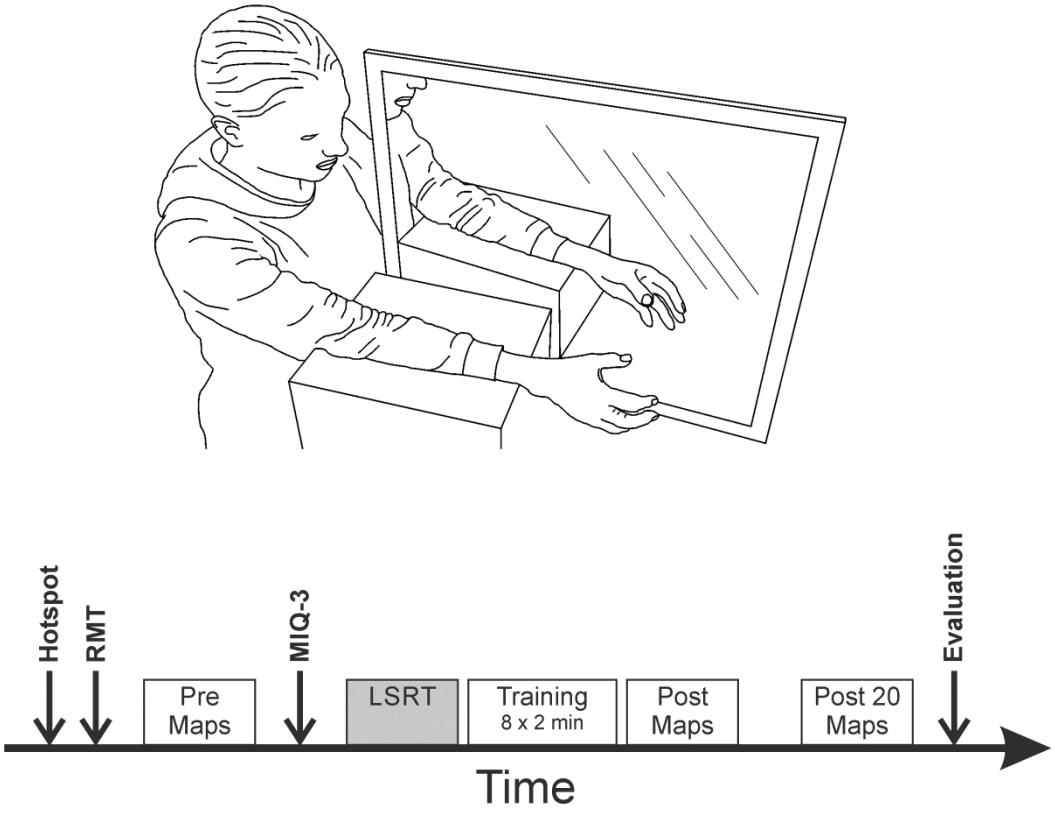

Figure 6-1: Overview of the experimental set-up and protocol during mirror training. (top) Participants were seated comfortably with their arms supported by foam blocks and lined up, one on each side of the mirror. (bottom) Each session was started with finding the hotspot and resting motor threshold (RMT) for the EDC muscle. Then data for three TMS maps was collected. Imagery ability was assessed of all participants using the MIQ-3 questionnaire. Participants assigned to the imagery group received ~10 min of layered stimulus response training (LSRT) while the control group went straight through to the mirror training. Mirror training was done in 8 blocks of 2 min with 15 s breaks. After mirror training data for three TMS maps was collected directly and 20 min after end of the training. The session was finished with an evaluation questionnaire.

Creating the TMS map

For the TMS maps eighty stimuli were provided in a 6 x 6 cm grid at pseudorandom locations at 120% of EDC RMT and a 1.5 s interstimulus interval (ISI). The TMS map was constructed offline (van de Ruit et al., 2015).

Movement imagery ability

General motor imagery ability of simple movements was assessed using the Movement Imagery Questionnaire (MIQ-3; Williams et al., 2012b). The MIQ-3 assesses the participants' visual and kinaesthetic imagery (KI) ability. The visual imagery ability is divided up in the ability to image in an internal (first person; II) and external (third person; EI) perspective. Four movements (knee raise,

jump, arm movement and waist bend) are performed and imaged three times, once for each imagery perspective. First, the movement is clearly explained and physically performed before the participant is instructed to image the movement from either a visual internal, visual external or kinaesthetic perspective. When the participants finished the imagery exercise, they were asked to rate the ease of imaging on a 7-point scale (1: very hard to see/feel – 7: very easy to see/feel).

Layered Stimulus Response Training

Participants assigned to the Imagery training group received ~10 min of Layered Stimulus Response Training (LSRT) to make their image as realistic and vivid as possible. This training took place in the same environment and with the same body posture and arm position as in which the mirror training took place. Participants rested one arm on each side of the mirror in a foam support. Hands remained in a neutral position with the thumbs up throughout training. With their eyes closed, participants were first instructed to perform repetitive grasping movements with both hands; gently opening and closing the hand without making a tight fist or over-extending the fingers. These movements were performed for 30 s and were paced by a 40 bpm metronome; the hand being either fully opened or closed at every beat. After this initial practice, participants were asked to describe the feelings in their hands and arms as detailed as possible. Subsequently, participants were asked to keep both hands still and use the sensation that was easiest to identify during imaging the non-dominant hand making repetitive grasping movements. The image was then built by including more detail step-by-step, making sure to incorporate sufficient detail to benefit the imagery process but without overloading the participant with instructions (Williams et al., 2013). In the next step, movement of the dominant hand was introduced, now using the mirror reflection to further facilitate the imagery. Following, the introduction of movement with the dominant hand, vividness of the imagery was checked, performing extra training following the LSRT format in case the imagery quality had deteriorated, which was based on verbal feedback by the participants

.

Mirror Training

In total 16 minutes of mirror training was performed, divided in 8 blocks of 2 min and separated by 15 s rest breaks. The participant was instructed to keep the non-dominant hand still whilst making the repetitive grasping movement with just the dominant hand. Participants were encouraged to imagine the non-dominant hand moving, using the mirror visual feedback to strengthen the illusion. Participants in the imagery group were encouraged to use the feelings and sensations practiced, thereby emphasizing using kinaesthetic imagery:

> *"Now, imagine your right/left hand moving while watching the mirror reflection of your repetitively grasping right/left hand. Keep the hand behind the mirror still but imagine it is moving; really using the mirror reflection to help you feeling and seeing your left/right hand making the grasping movements."*

During the breaks participant were requested to keep both hands relaxed and still, and to remain focussed on the mirror reflection. In addition, participants were reminded to focus on the feelings and sensations whilst imaging.

Post training evaluation questionnaire

In order to assess the task compliance and engagement of all participants, they were asked to fill out a task evaluation questionnaire. To check task compliance, participants were asked to what extent they were engaged in the imagery process, if their imagery did get better throughout the training blocks and to what extent they performed the task as instructed and practiced. Moreover, participants were asked how easy they found it to image the feelings associated with the grasping movement of the non-dominant hand and how clear and vivid the image was that they could create. Finally, it was confirmed if participants had been aware of any physical movement in the non-dominant arm throughout the training. The first question was rated on a percentage scale (0%: not engaged, 100%; fully engaged) while all other questions were rated on a 7-point scale ranging from 1 (not at all/very hard/ no image at all) to 7 (considerably / exactly / very easy / perfectly clear and vivid / full grasping).

Data Analysis

TMS maps

To create the TMS map data was analysed offline with a bespoke MATLAB script (MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). All stimulation positions were projected in a 2D plane. Accordingly, each position was matched with its corresponding MEP peak-to-peak (MEP_{pp}) value as extracted from the EMG, 20-50 ms after stimulation.

Maps were quantified by the map area and centre of gravity (COG). MEP_{pp} values were used to approximate a 6 x 6 cm grid composed of 2500 pixels using MATLAB's 'gridfit' function (D'Errico, 2005). The number of pixels with an approximated MEP_{pp} amplitude greater than 10% of the maximum MEP_{pp} value, or > 100 µV peak-to-peak if the 10% threshold was smaller than this, was calculated and expressed as total map area (in mm^2). The maps COG x- and y-coordinate was calculated by using the MEP_{pp} amplitude and its position on the map, creating an amplitude weighted mean of the map. To quantify changes in the position of the COG, the x- and yCOG translation was calculated by subtracting the x- and y-position of the baseline map from map COG after mirror training (xDisp and yDisp). In this way a negative value would indicate a shift in anterior (x) or lateral (y) direction and a positive value a shift posteriorly (x) or medially (y).

The absolute displacement was calculated by taking the Euclidian Distance (ED) between COGs of the median maps before and after the mirror training :

$$ED = \sqrt{\left(yCOG_{pre} - yCOG_{post}\right)^2 + \left(xCOG_{pre} - xCOG_{post}\right)^2}$$

Full details of this process are provided in van de Ruit et al. (2015).

Imagery ability and post training evaluation questionnaire

Imagery ability is quantified for the different perspectives by taking the mean of the scores provided for each movement imaged using a internal, external or kinaesthetic imagery perspective.

Statistical analysis

Statistical testing was conducted with IBM SPSS Statistics 21. Tests were considered significant at $\alpha = 0.05$. It was confirmed that the data did not violate any of the statistical tests assumptions. When the assumption of covariance matrix circularity was violated a Geisser–Greenhouse adjustment was made (denoted by GG following the *F* test). Data are reported as Mean ± 1 *SD* unless otherwise noted.

TMS maps

To assess learning induced changes in excitability the effect of learning on the TMS map area was studied. First the map with the median map area at each time point (Pre, Post, Post20) was selected. In case only two baseline measures were considered, e.g. data was missing for one map, the map with the lowest mean background EMG was taken as the median. Subsequently, the difference in map area between the pre and post measurement was taken ($AreaDiff = Area_{POST} - Area_{PRE}$). A negative AreaDiff would thereby indicate a decrease in map area, and a positive difference an increase. The AreaDiff data was then tested for statistical significance with respect to the fixed value of 0 using a one sample t-test for both time points (Post, Post20) and groups (Control, Imagery), which would highlight an effect of mirror training on TMS map area. Further, an mixed design ANOVA (within factor: time point, between factor: group) was used to reveal any effects between groups and an effect of the consolidation time following training. The same analysis was performed for the translation of the COG (xDisp and yDisp).

On a descriptive basis responders and non-responders to the mirror training were quantified. To quantify the response rate to mirror training three groups of responders were defined. Participants were classified as positive responders if they exhibited a increase in map area > 1 *SD* of the baseline variance in map area. In contrast, negative responders were classed as participants with a decreased map < -1 *SD* of baseline variance in map area. All other participants were classified as non-responders. Baseline variance in map area was calculated by calculating the standard deviation of the AreaDiff with respect to the two other baseline maps, not selected as the median baseline map, for all participants.

Imagery ability and post training evaluation questionnaire

Imagery ability and scores to each question of the evaluation questionnaire were compared between the groups using an independent samples t-test.

Predicting factors of response to mirror training

A two-way multivariate analysis of covariance (2w-MANCOVA), with the dependent variables TMS map area at time points Post and Post20, was used to investigate whether time of the day the experiment was performed, baseline imagery ability and participants' experiences during the mirror training could predict participants' their response, i.e. change in map area. Group and Time of the day were used as fixed factors whilst as covariates imagery ability (KI, II and EI) and participants' responses to the post evaluation questionnaires (5 questions on 7-point Likert Scale) were included. The time of the day the experiment was performed was classified as 1 to 4 dependent on if the experiment was performed between 08.00-11.00 (1), 11.00-14.00 (2), 14.00-17.00 (3) and 17.00-20.00 (4).

6.3 Results

In total, data of 40 participants was analysed, 20 in both groups. Data of four participants, two in both groups, had to be discarded as these data sets were incomplete as consequence of equipment malfunctioning. Scores for the Edinburgh Handedness Inventory indicated 36 participants were right handed (score: 90 ± 28). All other participants were left handed (score: -79 ± 22). Data for the FDS muscle was not analysed, as for most participants the stimulation intensity used to acquire the data for the TMS maps was below motor threshold.

General Imagery Ability

The baseline imagery ability for all three perspectives (external visual imagery, internal visual imagery and kinaesthetic imagery) for both groups is presented in Figure 6-2. Baseline imagery ability was found similar for both groups for each imagery perspective (KI: $t(38) = 0.13$, $p = 0.90$; II: $t(38) = -0.70$, $p = 0.49$; EI: $t(38) = 0.91$, $p = 0.37$), usually rated between 5 and 6 (somewhat easy to feel/see – easy to feel/see).

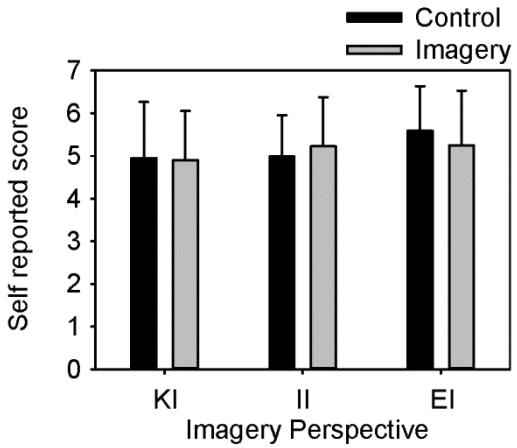

Figure 6-2: Mean self-reported imagery ability score for the three perspectives, kinaesthetic imagery (KI), internal imagery (II) and external imagery (EI) for both the control and imagery group. Ratings were provided on a 7-point scale. No significant differences were found between the groups for any of the imagery perspectives.

Layered Stimulus Response Training

Following the initial practice of the movement with eyes closed, most participants reported specific feelings and sensations involving hand and arm movements. Seven participants could not identify any

specific feeling and/or sensations. The most common feelings described were: 'the muscles in the forearm contracting', 'the finger tips touching the palm when closing the hand' and 'stretching of the skin between the fingers when opening the hand'. As these three feelings were commonly described, they were used in most participants to build the image. Most participants needed two or three practice runs to build the image of the closing and opening hand behind the mirror whilst keeping the eyes closed and both hands still. Another two or three practice runs were needed using the mirror reflection of the moving, dominant, hand to further improve the imagery. Participants reported improvement of image quality throughout the imagery training. Many participants reported that introduction of movement of the dominant hand and using the mirror visual feedback did lead to difficulties with the imaging process. Participants reported verbally how imagery improved with extra practice runs, of which usually two or three were required.

TMS maps

In order to study the effect of mirror training with or without LSRT on CSE the COG and area of the TMS map were examined. The maps included in the analysis for all sessions were constructed out of 73 ± 2 stimuli. The *SD* of the AreaDiff in the baseline recordings (with respect to the median map) was 265 mm^2 for the imagery group and 281 mm^2 for the control group. Therefore a participant was a positive responder if AreaDiff > 281 mm^2 (control group) and AreaDiff > 265 mm^2 (imagery group), negative responder if AreaDiff < 281 mm^2 (control group) and AreaDiff < 265 mm^2 (imagery group) and else a non-responder.

Figure 6-3 shows single participant data from both the control and imagery group. In this figure, one TMS map is shown for each time point and three different participants, two from the control group (top and middle row), and one from the imagery group (bottom row). The data from the control (top row) and imagery group (bottom row) represents a positive responder, with an increase in map area as evident from the greater presence of larger MEPs (more red). In contrast, also a non-responder (middle row) is shown, with no change in map area. In all cases, the COG is unaffected by the training.

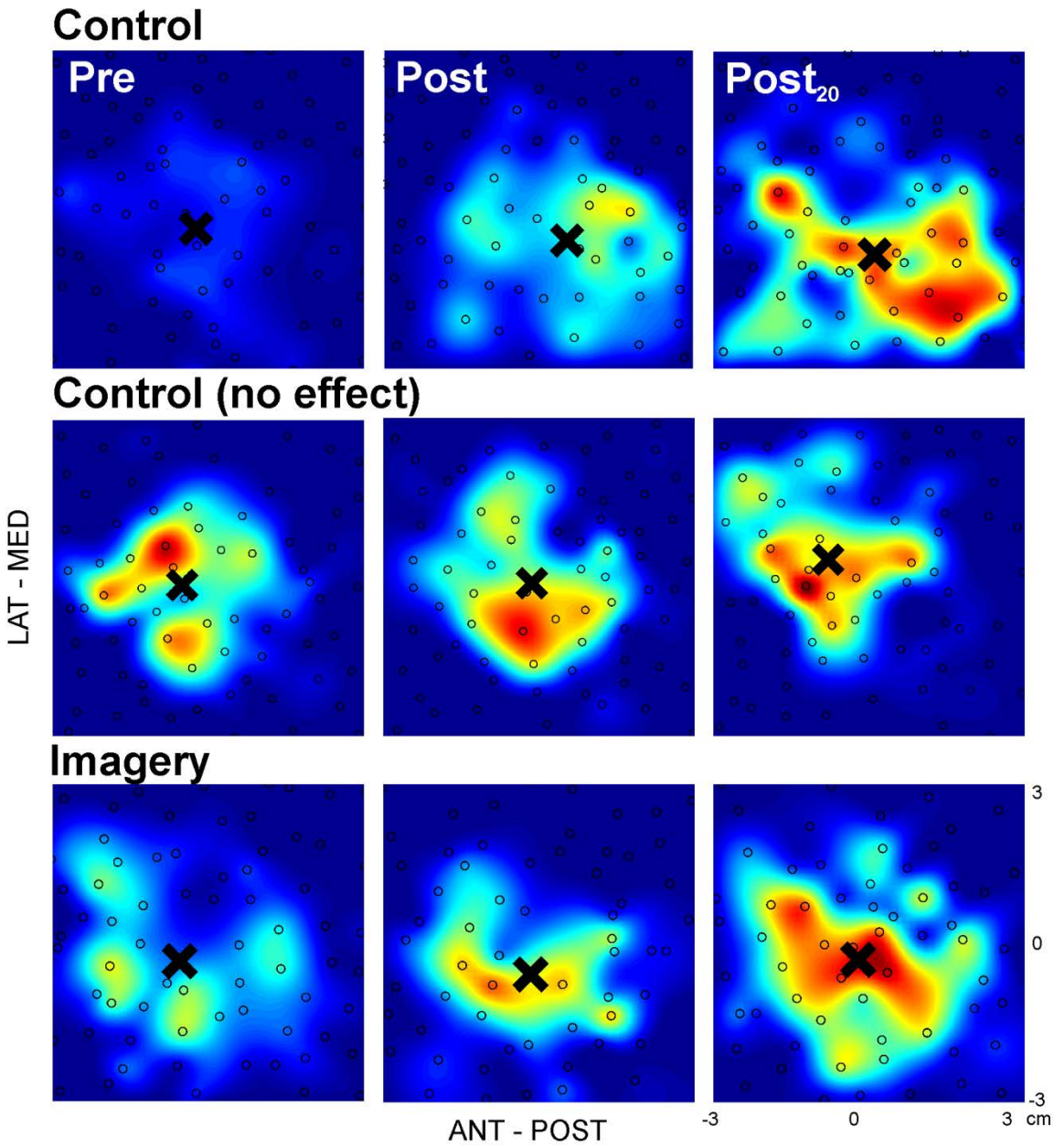

Figure 6-3: TMS maps before and after mirror training for representative participants in both the control and imagery group. The size of the approximated MEP_{pp} is indicated by the colour, with blue representing no or small MEPs and red representing the greatest MEPs. In each map the black open circles mark one stimulation and the black cross highlights the COG (×). (top row) Participant from the control group with an increased map area from before to 20 min after training; (middle row) Participant from the control group with no change in map area from before to 20 min after training; (bottom row) Participant from the imagery group with an increased map area from before to 20 min after training. No systematic shifts in COG can be observed for any participant in these examples.

A fixed one sample t-test for AreaDiff, compared to 0, revealed no effect of mirror training for any time point or group following training (Control group: Post: t(19) = 0.27, P = 0.79; Post20: t(19) = 1.16, P = 0.26; Imagery group: Post: t(19) = -0.99, P = 0.34; Post20: t(19) = - 1.22, P = 0.24). Comparing map area following training between the groups, a two-way repeated measures ANOVA (within factor: time point, between factor: group) revealed a non-significant effect (F(38,1) = 2.01, p = 0.17). Moreover, no effect for the map area was found for training (F(38,1) = 0.67, p = 0.42) or the training x group interaction (F(38,1) = 1.15, p = 0.29). Group data for map area is shown in Figure 6-4.

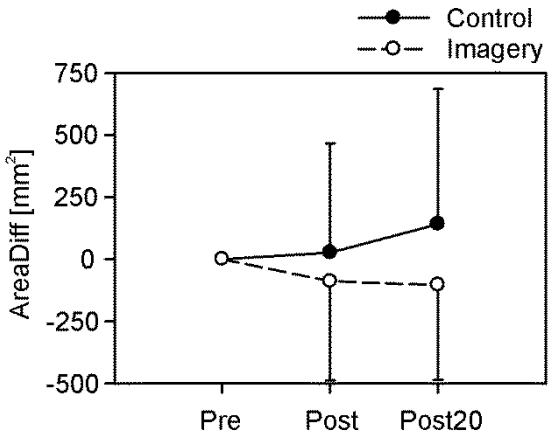

Figure 6-4: Group data for the map area for both the control and imagery group at all time points (Mean ± 1 *SD*). No significant differences were found from before to after mirror training for any group.

No effect of mirror training was found on the COG for the control group (xDisp: Post: t(19) = 0.68, P = 0.50; Post20: t(19) = 0.26, P = 0.80; yDisp: Post: t(19) = 1.06, P = 0.31; Post20: t(19) = 1.47, P = 0.16) or imagery group (xDisp: Post: t(19) = -0.09, P = 0.93; Post20: t(19) = -0.69, P = 0.50; yDisp: Post: t(19) = 1.40, P = 0.18; Post20: t(19) = 1.12, P = 0.28). In addition, a two-way repeated measures ANOVA revealed no differences between the groups, time points after training or time point × group interaction (xDisp: time point: F(38,1) = 1.07, P = 0.31, group: F(38,1) = 0.42, P = 0.52, group*time point: F(38,1) = 0.54, P = 0.70) (yDisp: time point: F(38,1) = 0.85, P = 0.36, group: F(38,1) = 0.47, P = 0.59, group*time point: F(38,1) = 1.74, P = 0.19).

These inconclusive results can be partly explained by the great differences in response to the mirror training. Figure 6-5 shows the changes in TMS map area following mirror training for each participant in the control and imagery group. Participants show either a strong increase, strong decrease, or no change in the map area. Classifying participants as positive-, negative-, or non-responder based on the change in map area revealed that for the control group, 10 participants were classified as positive responder, 6 as negative responders and 4 as non-responder. In contrast in the imagery group, there were 5 positive, 5 negative, and 10 non-responders.

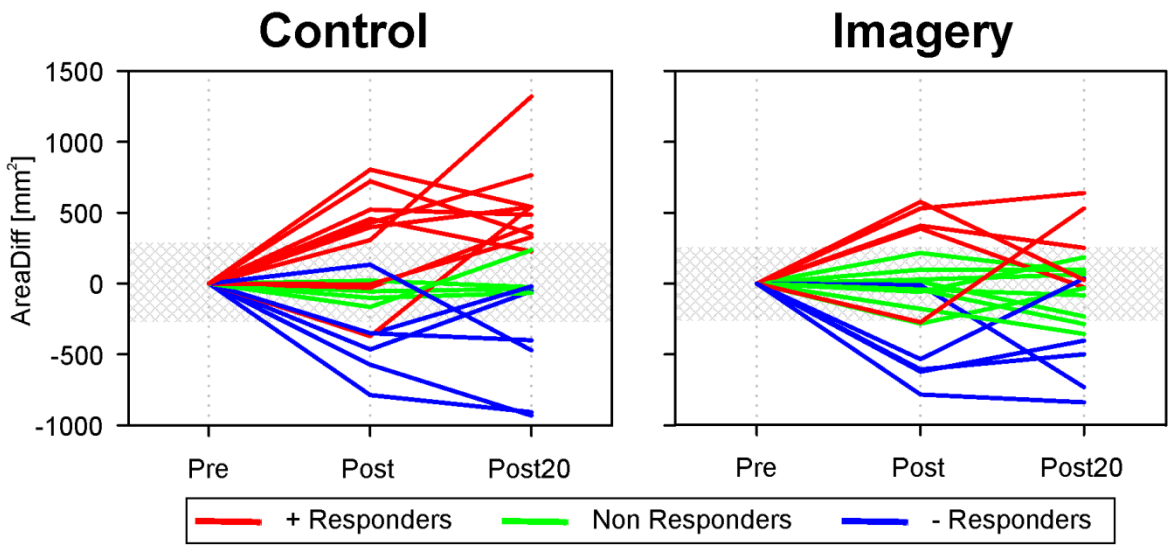

Figure 6-5: Map area before and after mirror training for all participants individually in both groups. Variability in the response to the training can be observed in both the control and imagery group. Participants were either classified as a positive responder (+ Responder - red lines) or negative responder (- Responder - blue lines) when the increase or decrease in map area was greater than the baseline variability (shaded area). For the control group, 10 participants were classified as positive responder, 6 as negative responders and 4 as non-responder. In contrast in the imagery group, there were 5 positive, 5 negative, and 10 non-responders.

Post training evaluation questionnaire

During the mirror training participants reported that on average they were engaged 80% of the time. No difference was found between the groups (control: 80% ± 12%; imagery: 78% ± 14%: $t = -0.97$, $p = 0.34$). Ratings for all other questions can be found in Table 6-1. For most participants imagery did get better throughout the 8 training blocks, and they fulfilled the task as instructed and practiced. Participants in the imagery group rated the question if they imaged as instructed and practiced significantly higher than the control group ($t = -2.55$, $p = 0.02$), showing the

imagery training did benefit the participants. For the other questions no significant differences were found between the groups. In general, participants reported finding it easy to attribute the feelings of a grasping movement to the non-dominant passive hand, with the image being fairly clear and vivid. Although subjective, people in the imagery group reported feelings like twitches or tingling in the passive hand more often than participants from the control group. One participant in the imagery group reported the feeling of her fingernails digging into her palm, which became progressively stronger with training.

Table 6-1: Results of the task evaluation questionnaire for both groups (** indicates a significant difference between the groups). All questions were rated on a 7-point scale. A significant difference was found for the ability to image as instructed and practiced, showing successfulness of the applied imagery training. No other significant differences were found.

	Control (n=20)		**Imagery (n=20)**	
Did you….	M	*SD*	M	*SD*
…feel your imagery improved? *(1: not at all, 7: considerably)*	5.45	1.10	5.80	0.77
…image as instructed and practiced? ** *(1: not at all, 7: exactly)*	5.65	0.67	6.20	0.70
…notice any movement in the non-dominant hand? *(1:not at all, 7: full grasping)*	3.70	1.08	3.32	1.25
How…				
…easy to image the feelings? *(1: very hard, 7: very easy)*	5.00	1.41	5.20	1.01
…clear and vivid was the imagery? *(1: no image at all, 7: perfectly clear/vivid)*	5.40	0.94	5.45	0.76

Predicting factors of response to mirror training

A 2w-MANCOVA revealed no significant main effects for either group (F (2, 23) = 0.66, P = 0.53; Wilk's Λ = 0.95) , Time or the day (F (6, 46) = 1.90, P = 0.10; Wilk's Λ = 0.64) or group × time of the day interaction (F (6, 46) = 1.55, P = 0.18; Wilk's Λ = 0.69). Two of the eight included covariates were found significantly related to the TMS map area: vividness of the imagery (F (2, 23) = 10.01, P < 0.01; Wilk's Λ = 0.54) and internal imagery ability (F (2, 23) = 3.85, P = 0.04; Wilk's Λ = 0.75). Further analysis of the parameters of the estimates regression equations showed that participants better at internal imagery and rating their imagery as being very vivid and clear tended to have a smaller increase (or greater decrease) in TMS map area.

6.4 Discussion

In this study a brief period of motor imagery training preceding mirror training was used to enhance changes in CSE in healthy people. With motor imagery and mirror training suggested to be closely linked (Stevens and Stoykov, 2003), we hypothesised that mirror training augmented by motor imagery would increase CSE significantly compared to mirror training alone. The results from the study did not support the hypothesis and revealed no effect of either the mirror training alone or mirror training enhanced by imagery on CSE as studied using TMS mapping.

This study presents the somewhat surprising finding that neither mirror training alone nor motor imagery augmented mirror training led to changes in CSE. This despite Nojima et al. (2012) providing solid evidence of increased CSE following mirror training in healthy participants. Other studies also report increases in CSE associated with the passive hand behind the mirror but assessed CSE whilst the mirror training was performed (Fukumura et al., 2007, Funase et al., 2007, Garry et al., 2005). Similar findings have been reported when motor imagery and action observation are performed (Kasai et al., 1997, Rossini et al., 1999, Strafella and Paus, 2000, Fadiga et al., 1995). Therefore, motor imagery was expected to enhance mirror training induced changes in CSE. The findings may be a result of the use of healthy participants, rather than patients, for which neuroplasticity following mirror training has been extensively reported (e.g. Yang et al., 1994, Kang et al., 2012). We will now critically discuss our findings in light of two important conditions for use-dependent plasticity in both healthy people as well as patients: repetition (Karni et al., 1995) and attention (Stefan et al., 2004).

Considerations of factors affecting use-dependent plasticity following mirror training?

A total of 16 min repetitive mirror training was performed in this study, which should suffice to induce changes in CSE. Nojima et al. (2012) studied mirror training in healthy participants and demonstrated an increase in CSE of the non-trained hand following only 5 min of a ball rotation task. In contrast, another mirror training study had participants perform 10 min of a ballistic finger tapping task with the left hand, without finding any changes in CSE (Avanzino et al., 2014). In motor skill learning experiments, both ballistic and visuomotor learning, training is usually in the order of 12-32 minutes

(Jensen et al., 2005, Willerslev-Olsen et al., 2011, Classen et al., 1998b, Perez et al., 2004, McAllister et al., 2011). Collating this evidence suggests 16 min of training should have been enough to find changes in CSE.

The type of training rather than the amount of training may also explain the contrasting findings between Avanzino et al. (2014), our study and study by Nojima et al. (2012). Nojima et al. (2012) had participants perform a task during which three cork balls had to be rotated as quickly as possible in one hand. In our study and the study by Avanzino et al. (2014) either a repetitive grasping or finger tapping task was performed. Whilst repetition is important, reshaping of the neural pathways has only been found in combination with repetitive learning of a task requiring skill, and not with repetitive use alone (Plautz et al., 2000, Nudo et al., 1996). It is plausible that the grasping movement in this study did not involve an element of skill and therefore not resulted in any changes in CSE. The repetitive grasping movement performed in this study was adopted for its potential application in motor rehabilitation of hand grasp, as mirror training is ultimately aimed to be applied in clinical practice. We argued that repetitive grasping paced with a 40 bpm metronome would require skill learning as grasping usually involves few and slow movements.

If the mirror training did not involve an element of learning, participants may not have conceived the task as challenging and lost attention. The cork ball used by Nojima et al. (2012) during mirror training may have made the task challenging and promoted plasticity. Indeed, greater changes in CSE were found when observing a target directed grasping movement than a grasping movement alone (Enticott et al., 2010). The grasping task in this study was not performed using an object. We conducted pilot testing during which we explored the benefits of introducing an object in mirror training. Participants reported that as a result of the greater proprioceptive input associated with handling an object, it was more difficult to see and feel the hand behind the mirror performing the same action. This could cause an internal conflict between visual and proprioceptive information and for this reason no object was used during task execution.

The notion that internal conflict may have contributed to the lack of changes in CSE is supported by two findings. Firstly, several participants reported a feeling of numbness and cold in the non-moving arm. This signifies their proprioceptive awareness, which conflicts with the visual feedback. Consequently, a conflict may have existed between what is imaged or observed and the proprioceptive information. By emphasizing the imagery strategy in the imagery group a larger conflict might have been created than in the control group. Secondly, the 2w-MANCOVA revealed the change in map area was smaller for participants with better internal imagery ability and for those that the imagery was more clear and vivid. This does suggest that in this study imagery training has worked counterproductive, as better imagery ability leads to smaller changes in CSE. It is not unthinkable that the better imagery ability has drawn attention of the participant to the existing conflict between proprioceptive and visual feedback, reducing the effect of the mirror training. Hence, the existing conflict may also have affected attention, which has been shown to affect changes in CSE and primary motor cortex activation (Rosenkranz and Rothwell, 2006, Binkofski et al., 2002).

Participants may adopt different strategies to perform the mirror training when they have difficulties to remain focused and receive conflicting sensory information. Emphasis could be put either on observing or imaging. It is likely participants will unconsciously determine strategy based on the instructions provided. In this study, participants were explicitly instructed to both feel (kinaesthetic motor imagery) and see (action observation and visual imagery) the hand behind the mirror making the repetitive grasping movement. Participants in the imagery group received 10-15 min of imagery training and, therefore, it is likely that these participants will have adopted an imagery focused strategy during mirror training. In contrast, as no specific practice was performed, participants in the control group could only rely on their interpretation of the instructions. Participants may have adopted a strategy that was easiest for them, either focussing on feeling or seeing the hand move. Since motor imagery is an active process which, especially when performed repetitively over 16 min, is mentally demanding, participants may have unconsciously shifted strategy throughout training when having

difficulties to remain focussed. As the instructions given to the participants are rarely reported, it is difficult to compare the outcome of different studies based on the likely adopted strategy.

Whereas different studies report changes in CSE following mirror training, these studies assessed CSE before and directly after mirror training. Pascual-Leone et al. (1995) demonstrated consolidation is important and changes can only be observed 20 min after training. A subsequent review from Pascual-Leone et al. (2005) mentioned 20-30 min of consolidation time, suggesting a longer consolidation period may be required. Therefore, in this study CSE was assessed both directly as well as 20 min after the mirror training. CSE did change from directly to 20 min after the training and there was no consistent trend indicating increasing CSE with time.

Variability in plasticity and motor imagery ability

Although the used methods or adopted mirror training strategy by participants in this study might have affected the outcome, it is not uncommon for use-dependent and non-invasive brain stimulation induced plasticity to only be present in a subset of the participants tested (Chapter 7 and 8, Wiethoff et al., 2014, Hamada et al., 2013, Lopez-Alonso et al., 2014, Muller-Dahlhaus et al., 2008). In the mirror group, 10 participants responded with an increased CSE (50%), which matches the earlier reported response rates. However, only 5 out of 20 (25%) of the participants responded with an increased CSE when the imagery training was introduced. Internal conflict and attention might well have mediated this reduced response rate. Because of the between subject design adopted in this study the possibility this has been a result of the different participants tested in each group cannot be excluded.

To enable better quantification of whether imagery ability affects plasticity it would be beneficial for future studies to incorporate several different measures of imagery ability as suggested by Guillot and Collet (2005). These involve mental chronometry, skin conductance measurement and mental rotation tasks. The assessment of imagery ability using the MIQ-3 questionnaire is subjective and did not allow us to exclude any participants based on their imagery ability. Based on the MIQ-3 score, most of the participants in our study were good at imagery. As imagery ability is known to affect CSE changes

and neuronal activation (Williams et al., 2012a, Guillot et al., 2008), additional measures might allow for a better insight in participants imagery ability and ultimately reduce the variability in the response to mirror training.

Conclusion

In this study motor imagery was used to augment changes in CSE following mirror training. Whereas no changes in CSE where observed this does not mean these techniques are not efficacious in promoting plasticity. This study performed in a patient group, e.g. stroke survivors, rather than healthy participants, might reveal that imagery training can be an important addition to mirror training. In stroke patients many challenges faced when training healthy people are not present, e.g. internal conflict won't be a problem as they are explicitly instructed to perform the training task bilaterally (Stoykov and Corcos, 2009). Moreover, imagery and mirror training may need to be combined on a longer time scale to allow the imagery to benefit the mirror training. As there are many methodological confounding factors it is important that authors clarify their methodology (e.g. task instruction) so studies can be better compared. This might be a first step into further disentangling the role of imagery and action observation in mirror training so that mirror training can be further tailored to the individual according to their abilities.

CHAPTER 7

Inter- and intrahemispheric differences in use-dependent plasticity following visuomotor tracking learning

7.1 Introduction

Learning or re-learning a motor skill has been linked with neuroplasticity within the human motor system (Sanes and Donoghue, 2000). Use-dependent plasticity has been studied using a vast amount of learning tasks including but not limited to serial motor reaction time tests (Pascual-Leone et al., 1994), sequence learning (Pascual-Leone et al., 1995), ballistic motor learning (Muellbacher et al., 2001, Classen et al., 1998b) and visuomotor tracking learning (Jensen et al., 2005, Perez et al., 2004). Transcranial magnetic stimulation (TMS) over the motor cortex is commonly used to quantify use-dependent plasticity by studying the motor evoked potential (MEP). Motor learning has traditionally been linked to changes in MEP amplitude (Ziemann et al., 2001, Muellbacher et al., 2001), with long term potentiation (LTP) like plasticity in the motor cortex being suggested as the underlying mechanism (Ziemann, 2004, Jung and Ziemann, 2009). However, more recently the relationship between motor learning and associated changes in MEP amplitude has been challenged, with differences found between the dominant and non-dominant hand but also for different muscles in the arm (Cirillo et al., 2010, Krutky and Perreault, 2007).

Anatomical and physiological asymmetries between the right and left motor regions of the brain, together with a suggested discrepancy in the role of both, could explain the dissociation between learning and MEP facilitation for the dominant and non-dominant hand (Hammond, 2002, Serrien and Spape, 2009, Schambra et al., 2011). Hemispheric asymmetries of the motor system include a larger cortical muscle representation (Krings et al., 1997a), lower stimulation threshold (Triggs et al., 1994) and deeper central sulcus (Amunts et al., 1996) in the hemisphere associated with the dominant hand. Cirillo et al. (2010) reported a greater increase in MEP amplitude for the non-dominant hand than with

the dominant hand, despite a greater improvement for the dominant hand, when a ballistic motor learning task was performed. In contrast, Hammond and Vallence (2006) reported similar improvement for both hands, but found greater MEP changes for the dominant hand. Others did not find an asymmetry in MEP facilitation following learning (Garry et al., 2004, Gallasch et al., 2009). Differences in the results of these studies may be caused by the different learning tasks employed e.g. Cirillo et al. (2010) used ballistic learning, Gallasch et al. (2009) goal-directed learning and Garry et al. (2004) a fine motor control task. Task complexity has been linked with differences in cortical activity (Datta et al., 1989) and may thereby affect use-dependent plasticity.

There is equal lack of consensus about potential differences in use-dependent plasticity of distal versus proximal muscles. It is well documented that there are anatomical differences, with proximal muscles having smaller representations (Wassermann et al., 1992, Penfield and Boldrey, 1937), and a smaller number of direct cortical projections from the motor cortex (Palmer and Ashby, 1992). Moreover, these direct pathways may only play a small role in the control of movement of proximal muscles, whereas they were found to have a big contribution in the control of distal muscles (Turton and Lemon, 1999). Several researchers have studied changes in corticospinal excitability (CSE) associated with motor learning of a proximal muscle. Ziemann et al. (2001) reported small or no changes in MEP amplitude following a ballistic learning task using the biceps muscle. This contrast a later study that found significant changes in MEP amplitude following a visuomotor tracking task (Jensen et al., 2005). Nonetheless, Ziemann's findings were supported by a study from Krutky and Perreault (2007) that compared changes in CSE following ballistic learning of a hand, wrist and upper arm muscle. They reported greatest increases in MEP amplitude for the hand muscle, with a progressively smaller increase for the wrist and upper arm muscle. Similar to studies demonstrating hemispheric asymmetries in use-dependent plasticity, the differences between adopted learning tasks could explain the contrasting findings.

Plasticity is affected by the extent to which the skilled hand is used (Rosenkranz et al., 2007b), visual and spatial attention (Kamke et al., 2012, Kamke et al., 2014) and the history of synaptic activity

together with many other factors (for review: Ridding and Ziemann, 2010). Nonetheless, interindividual variability has received limited attention in literature where TMS is used to quantify changes in CSE following motor learning. Evidence for a non-canonical response induced using non-invasive brain stimulation has accumulated lately, with usually only between a third to two-third of the tested participants expressing the 'expected' changes in CSE (Wiethoff et al., 2014, Muller-Dahlhaus et al., 2008, Hamada et al., 2013, Lopez-Alonso et al., 2014). Therefore, the differences in use-dependent plasticity observed between both dominant and non-dominant hand muscles, as well as proximal and distal muscles may not be a result of a limited ability to undergo plasticity or task complexity. Instead, a difference in the number of participants showing increased CSE following learning, may result in a reduced overall main effect when grouping data.

This study aims to investigate inter- and intrahemispheric differences in use-dependent plasticity following a visuomotor tracking learning task. In the first experiment, interhemispheric differences in changes of CSE were studied by comparing learning with the dominant and non-dominant hand using TMS mapping. We hypothesised to find no difference between the change in CSE following learning with the dominant and non-dominant hand. However, we hypothesised to find less participants with an increased CSE for the dominant than the non-dominant hand. To explore intrahemispheric differences in changes of CSE, learning with a proximal and distal muscle was compared in a second experiment. We hypothesised to find no difference in the increase in CSE for the proximal and distal muscle following learning. Nonetheless, we hypothesised a greater number of participants with increased CSE for the distal muscle than the proximal muscle.

7.2 Methods

Participants

In total, 40 healthy participants (Experiment 1: 20 participants - 22 ± 3 y, range 18-29, 12 female; Experiment 2: 20 participants - 22 ± 5 y, range 18-37, 13 female) were recruited for the study. Participants were screened for contraindications to TMS using a modified version of the TMS adult safety questionnaire originally suggested by Keel et al. (2001). The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_11-0444), and all experiments were performed in accordance with the Declaration of Helsinki.

Electromyography

Bipolar surface electrodes (Blue Sensor N, Ambu, Denmark) were used to record the electromyographic (EMG) activity of the first dorsal interosseus (FDI) and biceps brachii (BB). All EMG signals were amplified (FDI: 1k; BB: 2k), band pass filtered (20-1000 Hz), and digitally sampled at 5 kHz to be stored for offline analysis.

Transcranial Magnetic Stimulation (TMS)

A custom made polyurethane coated 90 mm figure-of-8 coil (type: batwing; type no. 15411) was used to magnetically stimulate the primary motor cortex using a Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom). The coil was held at an 45 deg angle to the sagittal plane, with the handle in the posterior direction, to induce biphasic currents in the lateral-posterior to medial-anterior direction. The site evoking the largest MEPs, the 'hotspot', was found by visual inspection of the EMG. Subsequently, resting motor threshold (RMT) was determined as the threshold intensity at which at least 5 out of 10 stimuli evoked MEPs when stimulating at the hotspot with a peak-to-peak amplitude >50 μV (Rossini et al., 1994). Coil position and orientation were monitored throughout the experiment using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada).

Assessing corticospinal excitability: TMS maps

Changes in CSE were quantified by TMS maps. Each TMS map was created with 80 stimuli administered at pseudorandom locations in a 6 x 6 cm grid and with an interstimulus interval of 1.5 s. The grid was defined in the neuronavigation software. Further detail on the method of acquiring data for the TMS map can be found in van de Ruit et al. (2015).

In Experiment 1, MEPs were normalised to the electrically evoked maximal M-wave (M_{max}) in order to compare across different participants. To obtain the M_{max}, a bipolar probe was used to stimulate the ulnar nerve at the level of the elbow using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK). In Experiment 2, MEPs were normalised to the mean maximal MEP (MEP_{max}) amplitude in response to 10 stimuli at maximal stimulator output over the motor hotspot. Finding M_{max} in the proximal BB muscle was found too uncomfortable for the participants.

Visuomotor tracking task

The visuomotor tracking learning task used in this study has been successfully applied by others (e.g. Perez et al., 2004, Jensen et al., 2005). Participants were verbally instructed to track a jagged waveform as close as possible by making isometric contractions of varying degrees of force.

Participants were presented with 5 different waveforms to familiarise them with the task. Each waveform was 12 s long, including a 1 s rest period at the start and end. The familiarisation period was followed by 5 training blocks consisting of 20 waveforms, making each training block take 4 min. After each training block, participants were allowed a 2 min break to minimise the possibility of muscle fatigue. The same 20 waveforms were presented in each training block; however, the order of presentation was randomised. The waveforms were scaled between $0 - 20\%$ of the participants maximal voluntary contraction (MVC), determined before the start of the learning task. Directly after each waveform, a bar chart was displayed showing the absolute tracking error of that waveform in all blocks executed. The online feedback on performance served to keep participants motivated to reduce their tracking error and stabilise attention.

Experimental protocol

Two experiment were performed, one to compare the response to visuomotor tracking learning of the FDI muscle of both hands (Experiment 1) and the other to compare a distal (FDI) to a proximal muscle (BB) (Experiment 2). Fort both experiments the testing of both muscles was done in two different sessions, separated by at least 7 days. Three TMS maps were acquired before and after the visuomotor tracking learning task. At the end of every session either M_{max} or MEP_{max} was determined.

Figure 7-1 shows the experimental setup for both experiments. Participants were seated comfortably, with a monitor in their direct line of sight. When the FDI muscle was tested, participants were asked to rest the hand that was tested palm down on a table 20 cm in front of them. The distal phalanx of the index finger was lined up with a force trans*d*ucer (NL 62 – 50 kg, Digitimer Ltd, Welwyn Garden City, UK). To ensure an isolated contraction of the FDI muscle, the wrist and other finger were immobilised using Velcro straps. During the learning task, participants repetitively activated the FDI muscle to track the presented waveforms.

Whilst BB was tested, participants rested their dominant arm in a custom built rig. The rig was height-adjustable to prevent any strain in the shoulder and neck. The elbow joint was rested in the rig at an angle of approximately 120 deg. The arm was immobilised by a Velcro strap at the level of the wrist and just below the elbow. Participants were instructed to always keep the hand in a neutral position with the thumb up. A torsion bar attached to the Velcro strap was used to record the forces exerted by a biceps contraction. During the learning task, participants repetitively activated the BB muscle to track the presented waveforms. In both experiments the force signal was high pass filtered by 30 Hz, amplified by 500-2k and sampled at 4 kHz to be stored for offline analysis.

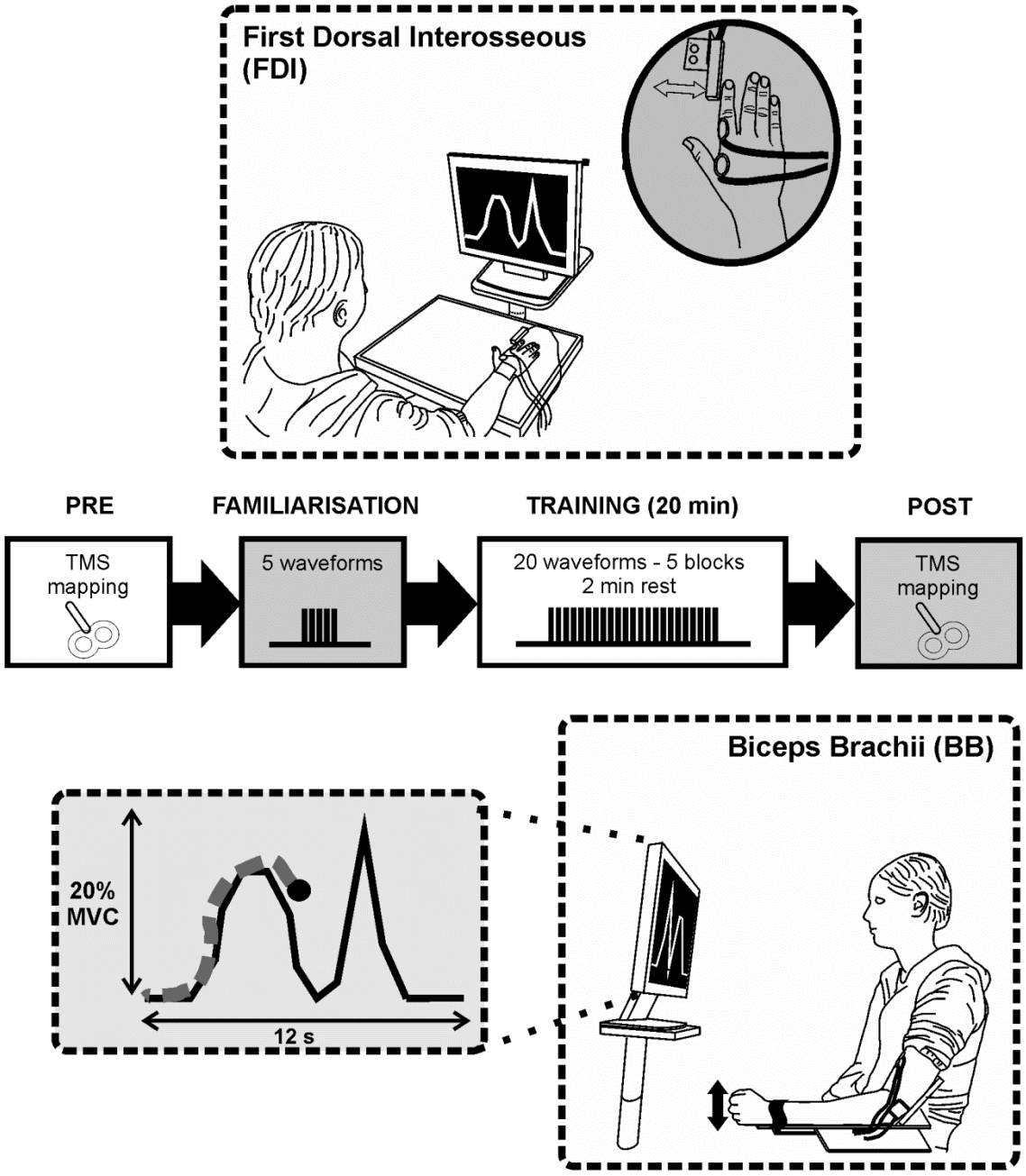

Figure 7-1: Experimental protocol and setup during the visuomotor tracking learning task. Participants were seated with a monitor in direct line of sight on which the waveforms were presented. The task was performed by either activating and relaxing the first dorsal interosseous muscle (top panel) or biceps brachii muscle (bottom right panel). Training was performed for a total of 20 min (5 blocks of 4 min) with 2 min rest between each block. TMS was used to probe changes in excitability using TMS mapping of the FDI or BB muscle before and after training.

Data analysis

Visuomotor tracking performance

Tracking performance was quantified by the root mean square (RMS) error between the target waveform and the tracked path. The effect of the training was examined by comparing the mean RMS error over all training blocks. A lower RMS would be indicative for increased performance and a decreased tracking error with respect to the target waveform.

TMS maps

To create the TMS map, data was analysed offline with a bespoke MATLAB script (MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). All stimulation positions were projected in a 2D plane. Accordingly, each position was matched with its corresponding MEP peak-to-peak (MEP_{pp}) value extracted from the EMG 20-50 ms after stimulation. All EMG recordings with a background EMG over 30 µV (peak-to-peak) between 50-5 ms before stimulation were excluded.

Maps were quantified by the map area and centre of gravity (COG). MEP_{pp} values are used to approximate a 6 x 6 cm grid composed of 2500 pixels using MATLAB's 'gridfit' function (D'Errico, 2005). The number of pixels with an approximated MEP_{pp} amplitude greater than 10% of the maximum MEP_{pp} value, or > 100 µV peak-to-peak if the 10% threshold was smaller than this, was calculated and expressed as total map area (in mm^2). The maps COG x- and y-coordinate was calculated by using the MEP_{pp} amplitude and its position on the map, creating an amplitude weighted mean of the map. To quantify changes in the position of the COG, the x- and yCOG translation was calculated by subtracting the x- and y-position of the baseline map from map COG after the visuomotor tracking learning task (xDisp and yDisp). In this way a negative value would indicate a shift in anterior (x) or lateral (y) direction and a positive value a shift posteriorly (x) or medially (y).

The absolute displacement was calculated by taking the Euclidian Distance (ED) between COG of the median TMS map before and after learning:

$$ED = \sqrt{\left(yCOG_{pre} - yCOG_{post}\right)^2 + \left(xCOG_{pre} - xCOG_{post}\right)^2}$$

Full details of this process are described in van de Ruit et al. (2015).

Statistical Analysis

Statistical testing was conducted with IBM SPSS Statistics 21. Tests were considered significant at $\alpha = 0.05$. It was confirmed that the data did not violate any of the statistical test assumptions. When the assumption of covariance matrix circularity was violated a Geisser–Greenhouse adjustment was made (denoted by GG following the *F* test). Data are reported as Mean ± 1 *SD* unless otherwise noted.

Learning

To examine visuomotor tracking learning performance during the training a three-way repeated measures analysis of variance (3w-rmANOVA) was used with factors MUSCLE (*left*, *right*) × BLOCK *(block 1 to 5)* × WAVEFORM *(Waveform 1 to 20).* This statistical analysis was performed on the tracking performance data. The factor WAVEFORM was included to test for heterogeneity waveforms difficulty.

TMS maps

To assess learning induced changes in CSE the effect of learning on the TMS map area was studied. First, the map with the median map area of the three maps acquired before and after learning was selected. In case only two baseline measures were considered, for example when data was missing for one map, the map with the lowest mean background EMG was used. Subsequently, the difference in map area between the pre and post measurement was taken ($AreaDiff = Area_{POST} - Area_{PRE}$). A negative AreaDiff would thereby indicate a decrease in map area, and a positive difference an increase. The AreaDiff data was then tested for statistical significance with respect to the fixed value of 0 using a one sample t-test. A paired t-test was used to study any differences in learning induced

changes in map area between the two muscles used in each experiment. The same analysis was performed for the translation of the COG (xDisp and yDisp).

To quantify the response rate to visuomotor tracking learning three groups of responders were defined. Baseline variance was calculated by calculating AreaDiff of the median map with respect to the two other baseline maps. The standard deviation of the baseline variance of all participants was used to classify participants as either positive responders, non-responders or negative responders. Participants were classified as positive responders if AreaDiff of the median map before and after learning > 1 *SD* of the baseline variance in map area. In contrast, negative responders were classed as participants with a decreased map area greater than -1 *SD* of baseline map variance. All other participants were classified as non-responders.

Relationship between motor learning and changes in corticospinal excitability

A linear regression was performed on the tracking performance data and changes in map area to determine if a greater improvement in tracking performance could predict the change in map area. To quantify the improvement in tracking performance the percentage improvement between different blocks was calculated. Based on the found results of the training, linear regression was performed between the improvement from Block 1 to 3, the early learning phase, and Block 3 to Block 5, the late learning stage, separately with the change in map area. Finally, the Pearson Correlation was used to determine if there was any association between the changes in map area (AreaDiff) in the dominant and non-dominant hand (Experiment 1) or the proximal and distal muscle (Experiment 2).

7.3 Results

All participants that completed the study tolerated TMS well and none of the participants reported any muscle fatigue following visuomotor tracking learning. For all participants sessions were separated by 7 days and performed at the same time of the day

7.3.1 Experiment 1

In total 19 of the 20 recruited participants completed both sessions of the experiment. The participant that did not complete the study failed to show up at the second experimental session.

Visuomotor tracking learning

For analysis of the visuomotor tracking performance data, 17 of the 19 available data sets were included. Two data sets had to be omitted because of missing force data or force amplifier malfunctioning.

All participants successfully completed 20 min of visuomotor tracking learning. Figure 7-2A displays tracking performance in the first and last block of training with the dominant hand for one single waveform. Here, tracking has become much more controlled following learning. Overall, a progressive decrease in tracking error over the 5 blocks for both the dominant and non-dominant hand was found (Figure 7-2B). This was confirmed by the three way repeated measures ANOVA (within factors: MUSCLE, BLOCK and WAVEFORM), that showed a significant effect for BLOCK ($p = 0.03$). Bonferroni post-hoc testing indicated significant differences between Block 1 and 2 and Block 1 and 3, but not any of the other blocks. Moreover, a significant main effect for WAVEFORM ($p < 0.01$) and BLOCK*WAVEFORM interaction was found ($p < 0.01$), indicating there was a different learning rate for the different waveforms, some being more difficult than others.

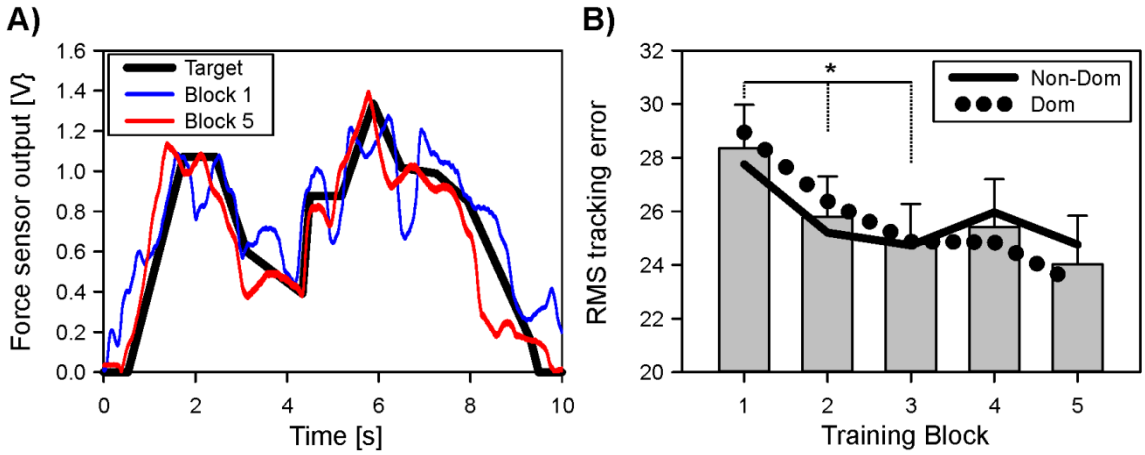

Figure 7-2: Effect of training a visuomotor tracking task with the FDI muscle of either the dominant and non-dominant hand on tracking performance. (A) Example of a single waveform for which tracking performance improved from the first block (blue line) to last block (red line) with respect to the target waveform (black line). (B) Overall, tracking performance improved over 20 waveforms and 5 blocks (shaded bars), for either the dominant (solid line) and non-dominant index finger (dotted line). Tracking performance significantly improved with training (* - $p<0.05$) from block 1 to 3, after which performance stabilised.

TMS mapping

Analysis of the TMS map data was performed on 17 participants. Two data sets had to be discarded as a result of measurement noise. The maps included in the analysis for all sessions were constructed out of 66 ± 12 stimuli.

There was no significant difference for RMT between the dominant and non-dominant hand ($t(16) = 0.75$, $p = 0.47$). The *SD* of the AreaDiff in the baseline recordings (with respect to the median map) was 343 mm^2 for the dominant hand and 330 mm^2 for the non-dominant hand. Based on 1 *SD* of the baseline AreaDiff, a positive responder was defined as AreaDiff > 343 mm^2 (dominant) or > 330 mm^2 (non-dominant), negative responder if AreaDiff < 343 mm^2 (dominant) or < 330 mm^2 (non-dominant) and else a non-responder.

Figure 7-3 shows TMS maps before and after training for the dominant and non-dominant hand. In this example the map area increased for the dominant hand whereas there is no change in map area for the non-dominant hand.

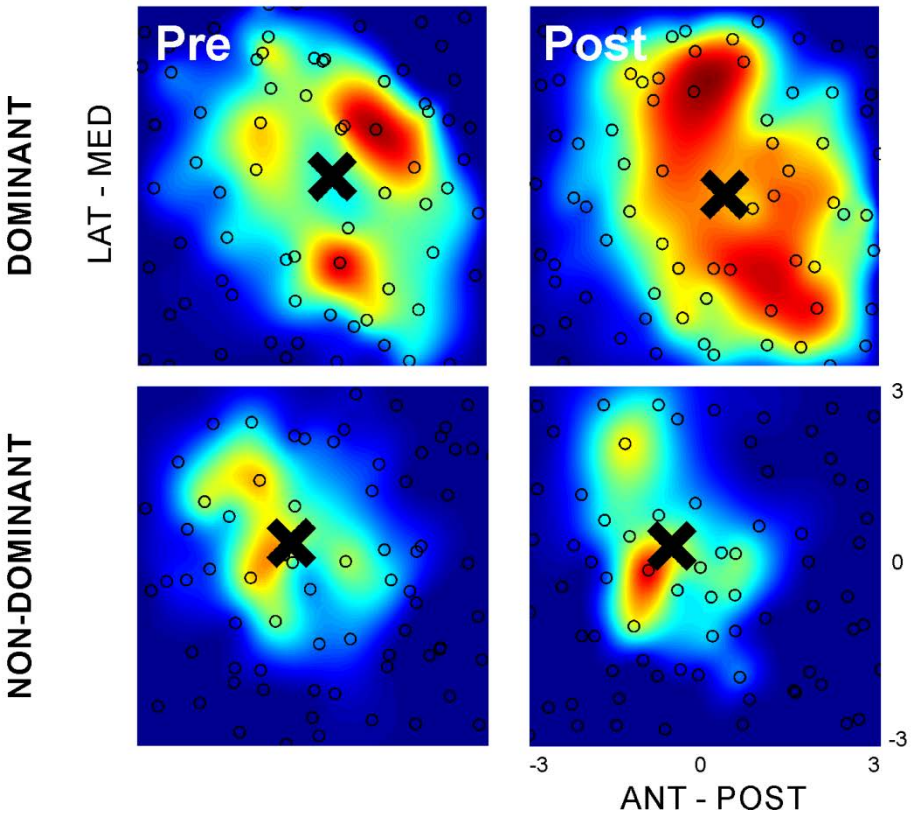

Figure 7-3: Individual examples of TMS maps before and after learning for both the dominant (top row) and non-dominant hand (bottom row).In this case, the top row represents a positive responder (increase in map area), whilst the bottom row represents a non-responder following the visuomotor tracking learning task. All black open circles represent one stimulation and the black cross (×) highlights the COG. Red indicates the biggest MEPs, in contrast to small or no MEPs which are indicated by blue.

The maps provided are representative for the results, with some participants exhibiting an increase in map area following learning, for some participants map area remained the same and in others it decreased. The mixed response was accentuated by a non-significant effect for both the dominant and non-dominant hand when comparing the difference in map area before and after training to 0 using a student t-test (Dominant: $t(16) = 0.84$, $p = 0.41$; non-dominant: $t(16) = 0.21$, $p = 0.84$) (Figure 7-4A). Moreover, a paired t-test indicated no difference in the response size between the two hands ($t(16) = 0.64$, $p = 0.54$). Classifying responders and non-responders based on map area revealed 6 positive responders after training with the dominant hand and 3 after training with the non-dominant hand. Results are summarised in Table 7-1.

Similarly no significant displacement of the COG was found for either the x- or y-coordinate in both hands (Dominant - xDisp: $t(16) = 0.62$, $p = 0.54$ - yDisp: $t(16) = 0.91$, $p = 0.38$; non-dominant - xDisp:

t(16) = 0.50, p = 0.62 - yDisp: t(16) = 1.63, p = 0.12) (Figure 7-4B). The COG displacement following learning was for the dominant hand 5.5 ± 2.5 mm and the non-dominant hand 5.6 ± 2.7 mm.

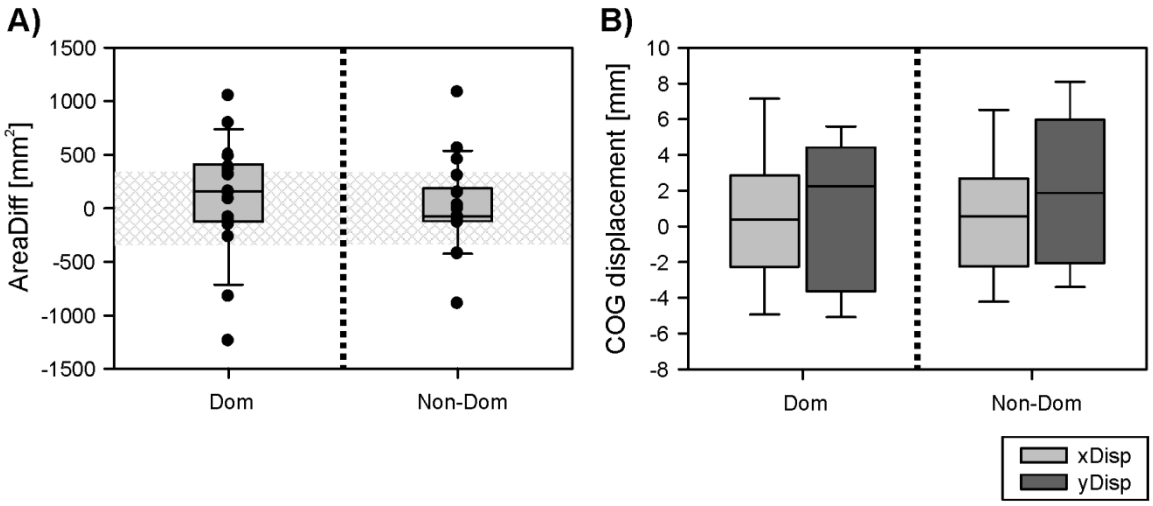

Figure 7-4: Effect of training a visuomotor tracking task with the FDI muscle of either the dominant and non-dominant hand on TMS map area and COG.(A) Change in map area (AreaDiff = Post - Pre map area) was not significantly different from 0 for either hand, however there are great interindividual differences with some participants showing an increase, decrease or no change in map area (black dots). Based on baseline variability of the map area, all participants with a change in map area within the range as marked by the shaded rectangle were classified as non-responders. (B) No change was found for either the displacement of the x- or y-coordinate of the COG for any hand.

Table 7-1: The number of positive (+), negative (-) and non-responders following a visuomotor tracking task with a finger muscle of the dominant and non-dominant hand.

TMS MAP AREA	Dominant	Non-Dominant
+ Responder	6	3
Non-Responder	9	11
- Responder	2	3

Relationship between learning and change in map area

As there was no significant improvement in performance during the training after block 3, the training data was split up by analysing the improvement from block 1 to 3 and 3 to 5 separately (early and late learning). The relationship between the improvement in tracking performance and the change in map area was assessed using a linear regression (Figure 7-5A). For block 1 to 3 and both hands, improvement in tracking performance and change in MEP area tended to be positively correlated

(Dominant: r = 0.09; Non-Dominant: r = 0.20), however, these regressions were non-significant (Dominant: p = 0.76; Non-Dominant: p = 0.47). In addition, the regression analysis for both hand and block 3 to 5 revealed no significance for either the dominant (r = 0.03, p =0.93) or non-dominant hand (r = 0.41, p = 0.13). Moreover, there was no association between changes in map area of the dominant and non-dominant hand (Pearson correlation: r = 0.31, p = 0.23) (Figure 7-5B).

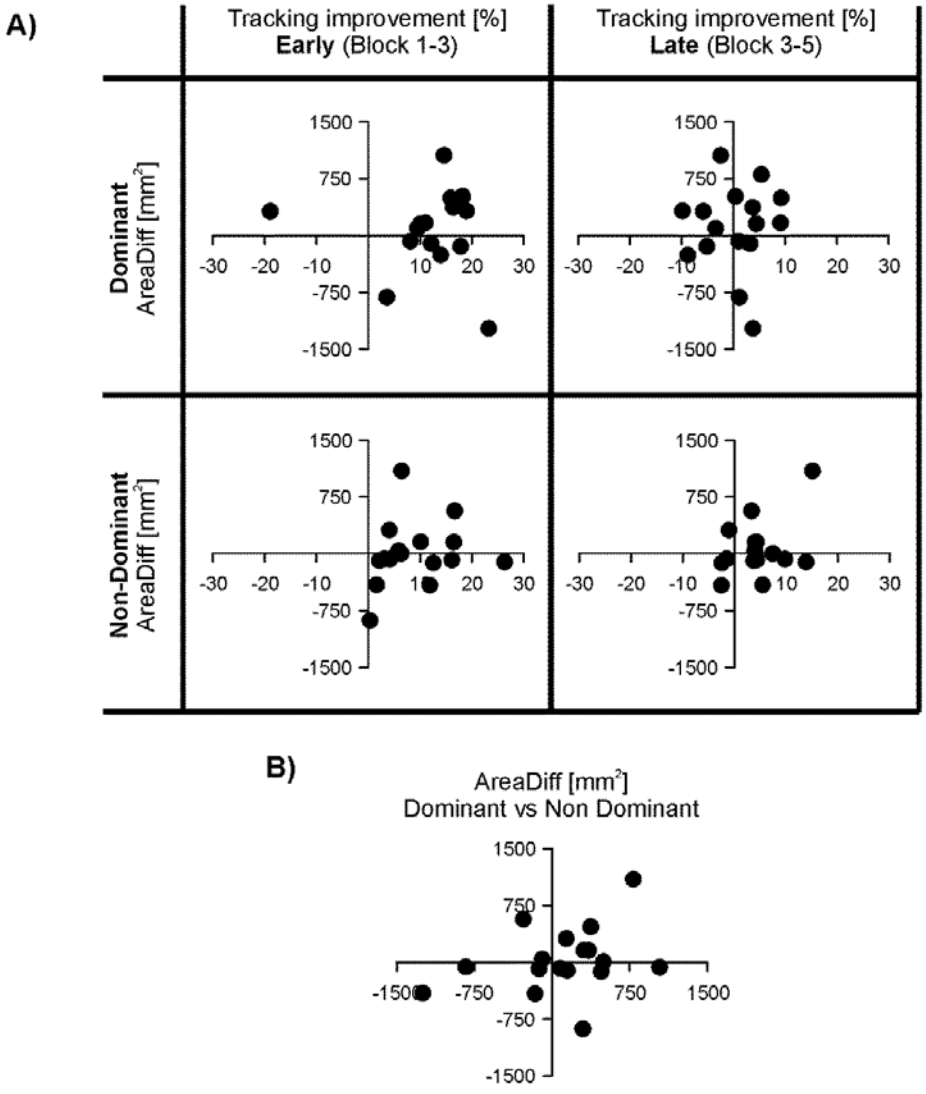

Figure 7-5: Results of the linear regression performed to investigate whether improvement in tracking performance could predict change in TMS map area. A correlation analysis was performed to find out if changes in map area of both hand are correlated. (A) Linear regressions were performed to see if the improvement in tracking error (expressed as % improvement) during either the early (block 1 - 3) or late (block 3 - 5) phase of training could predict change in map area, as overall performance was found to not improve significantly after block 3. The improvement in tracking error in either the early or late phase of motor learning could not predict change in map area for either the dominant or non-dominant hand. (B) A correlation analysis revealed changes in map area for the dominant hand could not predict change in map area of the non-dominant hand, or vice versa.

7.3.2 Experiment 2

All 20 participants completed both sessions of the experiment.

Visuomotor tracking learning

For analysis of the visuomotor tracking performance data of all available data sets was included. One data set had to be omitted because of missing force data.

All participants successfully completed 20 min of visuomotor tracking learning. Figure 7-6A displays tracking performance in the first and last block of training for one single waveform when training with the BB. Tracking has become more accurate following learning. Overall, a progressive decrease in tracking error over the 5 blocks for both the distal and proximal muscle was found (Figure 7-6B). This was confirmed by the three way repeated measures ANOVA (within factors: MUSCLE, BLOCK and WAVEFORM), that showed a significant effect for BLOCK ($p = 0.04$). Bonferroni post-hoc testing indicated significant differences between Block 1 and 2 and Block 1 and 3, but not any of the other blocks. Moreover, a significant main effect for WAVEFORM was found ($p < 0.01$), indicating some waveforms were more difficult than others.

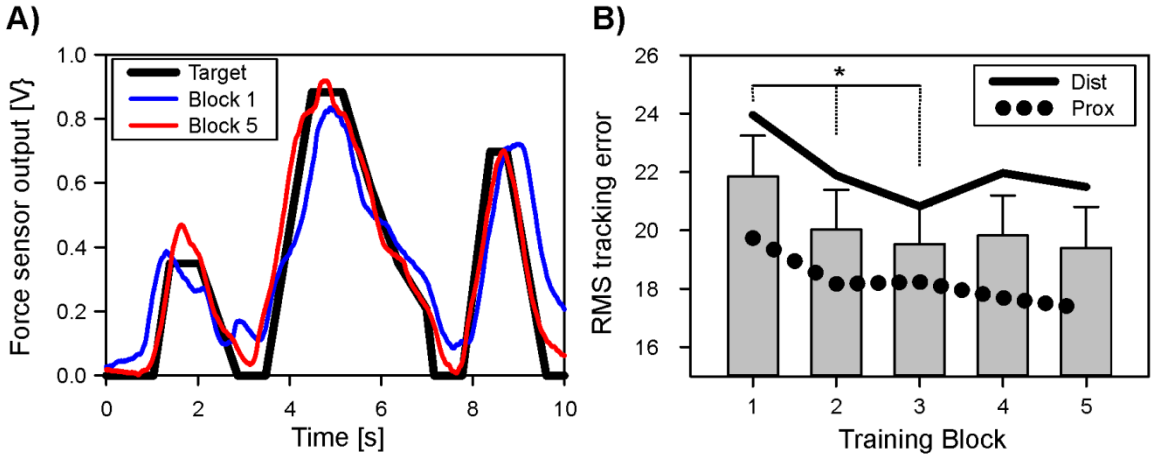

Figure 7-6: Effect of training a visuomotor tracking task with the distal FDI muscle or proximal BB muscle on tracking performance. (A) Example of a single waveform for which tracking performance improved from the first block (blue line) to last block (red line) with respect to the target waveform (black line). (B) Overall, tracking performance improved over 20 waveforms and 5 blocks (shaded bars), and for either the distal (solid line) and proximal muscle (dotted line). Tracking performance significantly improved with training (* - $p<0.05$) from block 1 to 3, after which performance stabilised.

TMS mapping

Analysis of the TMS map data was performed on 17 participants. Two data sets had to be discarded as a result of missing BrainSight data and one data set because of noisy EMG recordings. The maps included in the analysis for all sessions were constructed out of 73 ± 4 stimuli.

There was a significant higher RMT for the proximal than the distal muscle ($t(16) = 4.8$, $p < 0.01$). The *SD* of the AreaDiff in the baseline recordings (with respect to the median map) was 239 mm^2 for the proximal muscle and 254 mm^2 for the distal muscle. Therefore a participant was a positive responder if AreaDiff > 239 mm^2 (proximal) or > 254 mm^2 (distal), negative responder if AreaDiff < 239 mm^2 (proximal) or < 254 mm^2 (distal) and else a non-responder.

Figure 7-7 shows TMS maps before and after training for both muscles. In this example the map area decreased for the distal muscle whereas a increase in map area for the proximal muscle is present.

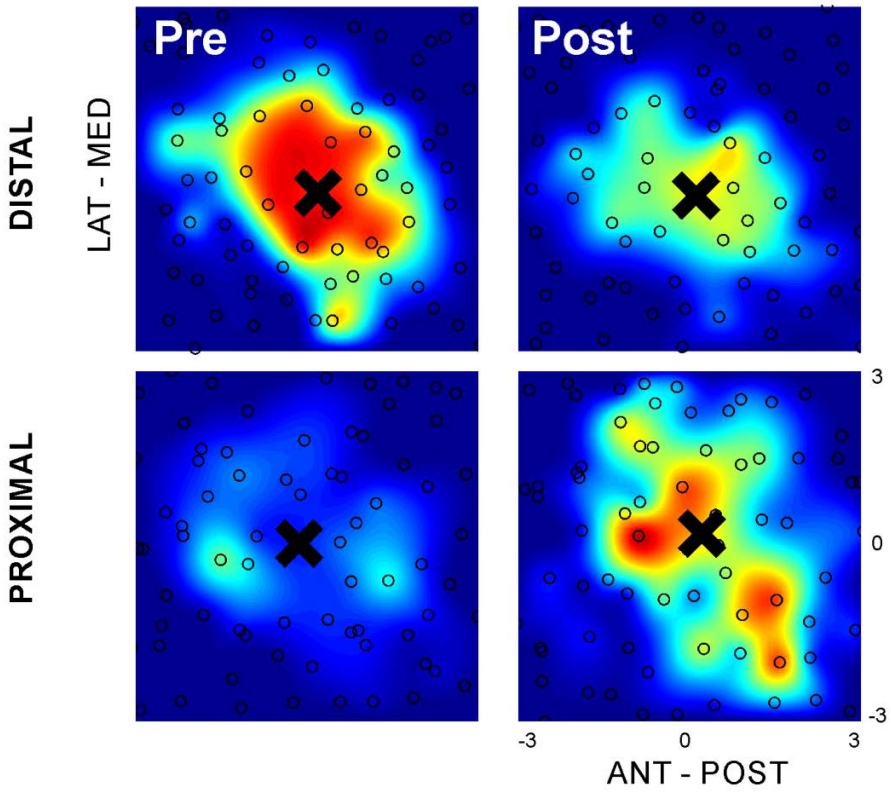

Figure 7-7: Individual examples of TMS maps before and after learning for both a distal muscle (top row) and a proximal muscle (bottom row).In this case, the top row represents a negative responder (decrease in map area), whilst the bottom row represents a positive responder following the visuomotor tracking learning task (increase in map area). All black open circles represent one stimulation and the black cross (×) highlights the COG. Red indicates big MEPs, in contrast to small MEPs which are indicated by blue.

The maps provided are representative for the results, with some participants exhibiting an increase in map area following learning, but also in some participants map area remained the same whilst in others it decreased. The mixed response was accentuated by a non-significant effect for both the proximal and distal muscle when comparing the difference in map area before and after training to 0 using a student t-test (Distal: $t(16) = 1.04$, $p = 0.32$; Proximal: $t(16) = 0.74$, $p = 0.47$) (Figure 7-8A).

Moreover, a paired t-test indicated no difference in the response size between the two muscles ($t(16) = -0.27$, $p = 0.79$). Classifying responders and non-responders based on map area revealed 6 positive responders after training with the distal muscle and 5 after training with the proximal muscle. Results are summarized in Table 7-2.

No significant displacement of the COG was found for the x-coordinate in the distal muscle and x-coordinate of the proximal muscle (Distal - xDisp: $t(16) = 0.20$, $p = 0.84$ - yDisp: $t(16) = -1.04$, $p = 0.31$; Proximal $t(16) = 0.14$ $p = 0.89$). However, a significant displacement in the COGs y-coordinate was found for the proximal muscle ($t(16) = 2.44$, $p = 0.03$) (Figure 7-8B). The COG displacement following learning was for the distal muscle was 4.1 ± 3.0 mm and the proximal muscle 3.8 ± 2.3 mm.

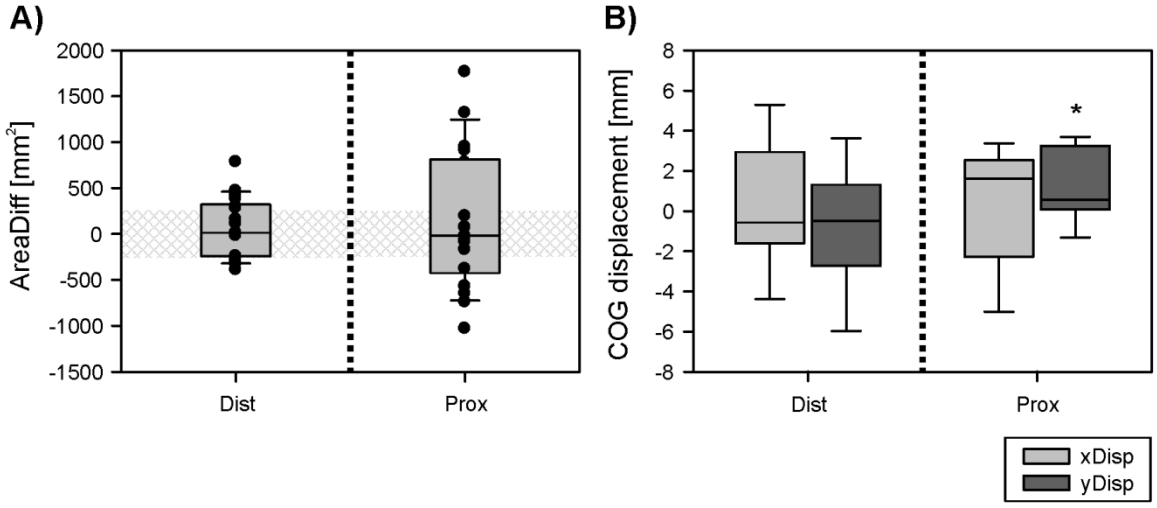

Figure 7-8: Effect of training a visuomotor tracking task with a distal (FDI) and proximal (BB) muscle on TMS map area and COG.(A) Change in map area (AreaDiff = Post - Pre map area) was not significantly different from 0 for either muscle, however there are great interindividual differences with some participants showing an increase, decrease or no change in map area (black dots). Based on baseline variability of the map area, all participants with a change in map area within the range as marked by the shaded rectangle were classified as non-responders. (B) No change was found in x-coordinate of the COG for any muscle, whilst a significant displacement of the y-coordinate for the proximal muscle was found but not the distal muscle.

Table 7-2: The number of positive (+), negative (-) and non-responders following a visuomotor tracking task with a proximal and distal muscle.

TMS MAP AREA	Proximal	Distal
+ Responder	5	6
Non-Responder	7	8
- Responder	5	3

Relationship between learning and change in map area

As there was no significant improvement in performance during the training after block 3 the training data was split up by analysing the improvement from block 1 to 3 and 3 to 5 separately (early and late learning). The relationship between the improvement in tracking performance and the change in map area was assessed using a linear regression (Figure 7-9A). For both the proximal and distal muscle, there was a poor relationship between performance improvement in block 1 to 3 and change in map area. Therefore, both regressions were found not to be statistically significant (Proximal: $r = 0.36$, $p = 0.19$; Distal: $r = 0.08$, $p = 0.78$). In addition, the regression analysis for both hand and block 3 to 5 revealed no significance for either the proximal ($r = 0.04$, $p = 0.89$) or distal muscle ($r = 0.21$, $p = 0.47$). There was no association between changes in map area of the proximal and distal muscle (Pearson correlation: $r = -0.04$, $p = 0.89$) (Figure 7-9B).

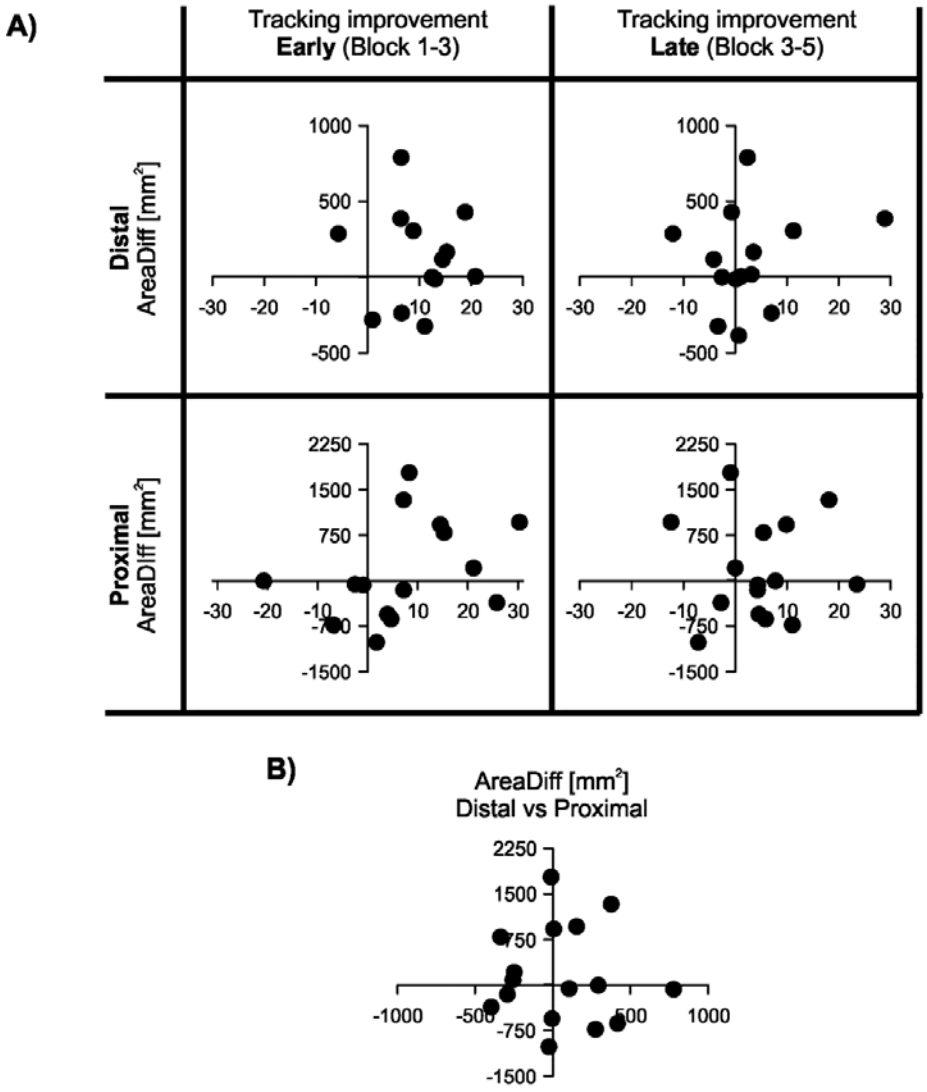

Figure 7-9: Results of the linear regression performed to investigate whether improvement in tracking performance could predict change in TMS map area. A correlation analysis was performed to find out if changes in map area of the proximal and distal muscle are correlated. (A) Linear regressions were performed to see if the improvement in tracking error (expressed as % improvement) during either the early (block 1 - 3) or late (block 3 - 5) phase of training could predict change in map area, as overall performance was found to not improve significantly after block 3. The improvement in tracking error in either the early or late phase of motor learning could not predict change in map area for either the dominant or non-dominant hand. (B) A correlation analysis revealed change in map area for the dominant hand could not predict change in map area of the non-dominant hand, or vice versa.

7.4 Discussion

In this study TMS mapping was used to investigate inter- and intrahemispheric differences in use-dependent plasticity following 20 min of visuomotor tracking learning. In the first experiment learning was performed using the FDI muscle of both the dominant and non-dominant hand to study intrahemispheric differences in use-dependent plasticity. Similar to what we hypothesised, no difference in changes in CSE between the dominant and non-dominant hand were found. Whereas it was hypothesised that a greater number of responders would be found following training with the non-dominant hand rather than the dominant hand, the opposite was found (3 vs 6). To follow up on these results, in the second experiment intrahemispheric differences in use-dependent plasticity were investigated comparing learning with a proximal (BB) and distal (FDI) muscle. In line with our hypothesis no difference in change in CSE was found following learning with the proximal and distal muscle. In contrast to our hypothesis, the number of responders for the distal (6) and proximal muscle (5) were similar. Overall, only between 18% - 36% of the participants revealed an increase in CSE following visuomotor tracking learning, despite a significant increase in tracking performance.

Do inter- and intrahemispheric differences in use-dependent plasticity exist?

In Experiment 1 changes in CSE following a visuomotor tracking learning task were compared for the dominant and non-dominant hand using TMS mapping. No difference was found in learning of the dominant and non-dominant hand. Likewise, no difference in the change in CSE was found. Despite similar learning and change in CSE a higher number of positive responders were found for the dominant hand (6) than the non-dominant hand (3). Hemispheric asymmetries of the motor system have been suggested to exist (Krings et al., 1997a, Amunts et al., 1996, Triggs et al., 1994), and be the cause of differences in use-dependent plasticity. Results of studies comparing plasticity of the hemispheres associated with the dominant and non-dominant hand are equivocal. Whilst Cirillo et al. (2010) found a greater improvement in thumb peak acceleration for the dominant hand, Ridding and Flavel (2006) report a larger improvement for the non-dominant hand. Furthermore, these hemispheric differences in learning have been linked to a larger increase in MEP size in the non-dominant

hemisphere (Cirillo et al., 2010) or no difference in MEP changes when induced by paired associative stimulation (Ridding and Flavel, 2006). In another study, training a ballistic pinch movement led to similar behavioural gains but greater MEP changes for the dominant motor cortex (Hammond and Vallence, 2006). Similar changes in MEP amplitude for both hands were found following motor learning of more complex tasks (goal-directed movement task or Purdue pegboard task) (Gallasch et al., 2009, Garry et al., 2004). A visuomotor tracking learning task as performed by the participants in this study can be classified as a complex learning task, as continuous feedback is required to perform the task successfully. Therefore, it can be concluded that the result of this study supports the findings of Garry et al. (2004) and Gallasch et al. (2009), and suggests similar capacity for use-dependent plasticity for both hemispheres.

In Experiment 2 changes in CSE following a visuomotor tracking motor learning task were compared for a distal (FDI) and proximal (BB) muscle. Tracking error improved significantly for both muscles following learning. This resulted in an increased CSE in a similar number of participants for both muscle (proximal: 5; distal; 6). Previous research would suggest that proximal and distal muscles have different capacities to undergo use-dependent plasticity. Both Krutky and Perreault (2007) and Ziemann et al. (2001) reported no or minimal changes in peak acceleration following a ballistic motor learning task with the upper arm. In contrast, for the same task, great and long lasting changes in peak acceleration and movement direction were reported for finger muscles (Muellbacher et al., 2001, Krutky and Perreault, 2007). The findings that proximal muscles have a smaller cortical representation and less monosynaptic connections than distal muscles (Wassermann et al., 1992, Palmer and Ashby, 1992, Penfield and Boldrey, 1937) were used to explain the results. Whereas this might suggest motor learning does not occur when more proximal musculature is trained, this is not supported by findings that learning occurs in a force-field adaptation task (Shadmehr and Mussa-Ivaldi, 1994) and a similar visuomotor tracking task as performed here (Jensen et al., 2005). In the study presented here, changes in CSE did not differ significantly between the proximal and distal muscles, despite significant learning with both.

Whereas anatomical and physiological inter- and intrahemispheric differences have been reported to explain differences in use-dependent plasticity this suggests the changes in CSE observed are primarily a result of changes in cortical excitability. Changes in CSE can be a result of alterations in excitability of any neuronal element along the corticospinal pathway both at cortical and spinal level. Perez et al. (2004) used transcranial electrical stimulation, activating corticospinal cells directly (Di Lazzaro et al., 2001), to show that changes in CSE following visuomotor learning likely originate at a cortical level. This was backed up by neuroimaging studies that demonstrated plasticity of the motor cortex is associated with motor learning (Karni et al., 1995, Ungerleider et al., 2002). At the same time, Muellbacher et al. (2002) demonstrated that using repetitive TMS over the primary motor cortex, retention of motor learning could be blocked. This highlights the essential role of the motor cortex. Although changes in spinal excitability cannot be fully excluded, these results suggest changes in cortical excitability are responsible for the observed effects

Visuomotor tracking learning

The finding that fewer than 30% of the 20 participants exhibited an increase in CSE following visuomotor tracking learning raises the question whether there was sufficient training. Between 12 and 32 min of training has been performed (Jensen et al., 2005, Perez et al., 2004, McAllister et al., 2011, Willerslev-Olsen et al., 2011) and linked with changes in CSE. In this study, training within a single session was limited to 5 blocks and a total of 20 min to prevent overlearning but allow us to quantify changes in CSE following the fast phase of learning (Luft and Buitrago, 2005, Floyer-Lea and Matthews, 2005). The significant improvement only up to block 3, matched the findings of Floyer-Lea and Matthews (2005) for single session learning but not those of Jensen et al. (2005), who reports continuous improvement over all blocks in the first training session. Our findings suggest there was no further improvement in performance after block 3, potentially as a result of a lack of focus or that the skill had been mastered. Overlearning was first reported by Muellbacher et al. (2001) who found that after an initial learning stage associated with rapid MEP facilitation, with further learning the MEP size returned to baseline. To ensure the great variety in changes in CSE following learning was not

mediated by participants rate of learning a linear regression between the improvement in performance from block 3 to block 5 and the change in map area was performed. As this regression came out non-significant it confirms that improvement during the last training blocks could not predict the change in map area. However, with only this result and the fact that CSE was not measured after block 3 the possibility that overlearning has affected our results cannot be excluded.

A next question is whether visuomotor tracking learning is a learning task that sufficiently engages the primary motor cortex. A ballistic motor task involves primarily feedforward control whereas in a visuomotor tracking task feedback control plays a major role as well. Findings of Baraduc et al. (2004) that rTMS over the motor cortex can disrupt ballistic but not learning of a more dynamic task highlight that whereas the motor cortex is essential in ballistic learning it might not be in more complex tasks. Evidence for widely distributed cortical activity during visuomotor tracking learning was also provided by Floyer-Lea and Matthews (2004). In conclusion, in more complex learning tasks, learning induced plasticity might be present outside the primary motor cortex, which we are unable to detect using TMS. This might explain the low response rate found in this study.

One primary difference between the current study and others using visuomotor learning is that in this study the visuomotor tracking was force controlled, rather than position controlled. Therefore, proprioceptive feedback was likely dominated by feedback from golgi tendon organs. In contrast, in a position controlled visuomotor task as performed in Jensen et al. (2005) proprioceptive feedback will primarily originate from muscle spindles. Ziemann et al. (2001) showed proprioceptive feedback as a result of voluntary muscle activation is essential for plasticity, but whether proprioceptive feedback from either golgi tendon organs or muscle spindles is more important is unclear. A direct comparison of a force controlled and position controlled visuomotor tracking learning task is warranted to confirm if this would affect use-dependent plasticity.

Concluding remarks

There is ample evidence that not every participant will display similar responses to a non-invasive brain stimulation protocol that aims to enhance or suppress CSE (Wiethoff et al., 2014, Hamada et al., 2013, Lopez-Alonso et al., 2014, Muller-Dahlhaus et al., 2008). The predicted response only occurs for about a third to two-third of the healthy participants. The response is mediated by a plethora of factors like age, gender, attention, history of synaptic activity together with many other factors (for review: Ridding and Ziemann, 2010). Even so, very few motor learning studies report on inter-individual variability in use-dependent plasticity. Only a study by Vallence et al. (2013) demonstrates increased MEP amplitudes in 75% of the participants following a ballistic thumb abduction task. Moreover, they demonstrated no associations between plasticity induced following motor learning or non-invasive brain stimulation protocols. This signifies the importance of inter-individual variability, as common mechanisms are thought to underlie both types of plasticity induction. This study accentuates variability of use-dependent plasticity for learning a visuomotor tracking task, with only 18-36% of the participants showing an increased CSE despite significant tracking improvement. A dissociation exists between changes in CSE and improvement in tracking performance in both experiments, confirming other reports (Delvendahl et al., 2011, Bologna et al., 2015, Jung and Ziemann, 2009). In conclusion, this study does not support intra- or interhemispheric differences in use-dependent plasticity following visuomotor tracking learning and highlights the complex relationship between different motor learning tasks and changes in TMS measures.

CHAPTER 8

Stimulation frequency dependent changes in corticospinal excitability following transcranial alternating current stimulation

8.1 Introduction

In healthy human participants stimulating the brain non-invasively through the scalp by weak direct currents changes corticospinal excitability (CSE) assessed using transcranial magnetic stimulation (TMS) (Priori et al., 1998, Nitsche and Paulus, 2000). The discovery that transcranial direct current stimulation (tDCS) could modulate excitability revived the use of current stimulation, first described in 1802 (Hellwag and Jacobi, 1802). In addition to tDCS, researchers started to explore the possibilities of using different current waveforms to modulate brain activity, from which transcranial random noise stimulation (tRNS) (Terney et al., 2008) and transcranial alternating current stimulation (tACS) (Antal et al., 2008) are the most common studied. Both use a sinusoidal current waveform either with a single (tACS) or spectrum of different frequencies (tRNS).

Whereas tDCS has been suggested to polarise a group of neurons, tACS might be able to entrain neuronal oscillations by matching the frequency content of the stimulation with frequencies found in the electroencephalography (EEG) of the brain (Antal and Paulus, 2013, Herrmann et al., 2013). By combining EEG and tACS, tACS has been reported to entrain cortical rhythms at the applied oscillatory frequency (Pogosyan et al., 2009, Zaehle et al., 2010, Helfrich et al., 2014). This has been suggested to result in behavioural changes. For example, Pogosyan and colleagues used a stimulation frequency of 20 Hz, a prominent beta band oscillatory frequency found in the motor system (Baker, 2007), to study the effect of tACS on movement speed. By applying brief periods of 20 Hz tACS they demonstrated it was possible to modulate the temporal patterning of brain activity by demonstrating an increased coherence between the electromyographic (EMG) and EEG activity. The modulation of

brain activity in the motor system was linked to slowing of voluntary movement, which was later replicated by others (Wach et al., 2013). Although Wach et al. (2013) did report slowing of movement following 20 Hz tACS, this was found not to be correlated with a change in CSE. This is surprising given that tACS induced after effects have been ascribed to changes in synaptic plasticity (Zaehle et al., 2010, Antal and Paulus, 2012). Another study from Feurra et al. (2011) did find a significant increase in MEP size, when assessed during 20 Hz tACS application, contrasting Wach et al. (2013) lack of change when comparing MEPs directly before and after 20 Hz tACS.

Not only 20 Hz tACS was found ineffective in modulating CSE. The first study comparing changes in CSE before and after tACS studied five different frequencies between 1-45 Hz and found none to modulate CSE (Antal et al., 2008). However, later studies did indicate that the lack of change in CSE might have been a result of the short time of stimulation and low stimulation intensity. Only Zaghi and colleagues reported a significant decrease in MEP size when 15 Hz tACS was applied for 20 minutes at 1 mA (Zaghi et al., 2010). Whereas stimulation frequencies part of the traditional EEG frequency bands produce equivocal results, frequencies > 100 Hz have been shown more successful. Moliadze demonstrated that 140 Hz tACS is able to enhance excitability for up to 1h after stimulation (Moliadze et al., 2010a, Moliadze et al., 2012). Similar results were reported when stimulating at 1, 2 and 5 kHz (Chaieb et al., 2011).

All studies to date assessing CSE following tACS have made use of single pulse TMS at one intensity over the motor hotspot. The use of this method might not reveal any shifts in the corticospinal representation or excitability changes of different neuronal populations recruited at different stimulation intensities (Di Lazzaro et al., 2012). To obtain better insight in the tACS induced changes in CSE, one could acquire stimulus response (SR) curves (Devanne et al., 1997) and TMS maps (Wassermann et al., 1992). Recent studies have shown it is possible to acquire one TMS map or SR curve in two minutes, allowing characterisation of the time course of changes in excitability following tACS (Mathias et al., 2014, van de Ruit et al., 2015). Moreover, it remains yet to be established what the interindividual variability is in the response to various tACS protocols. It is well known that

generally only 50% of all people respond in a canonical manner to various types of non-invasive brain stimulation (e.g. Maeda et al., 2000b, Wiethoff et al., 2014, Lopez-Alonso et al., 2014).

This study aimed to investigate the effect of tACS on CSE using the SR curve and TMS maps, with a stimulation frequency within (20 Hz) and outside (140 Hz) the traditional beta EEG frequency band for the motor system. In addition we aimed to show that excitability changes in response to tACS have similar variability as the response to other non-invasive brain stimulation techniques. As increased beta power impairs motor processing and slows voluntary movement (Baker, 2007, Wach et al., 2013), we hypothesised that 20 Hz TMS would significantly inhibit CSE, characterised by a decreased map area and area under the SR curve. For 140 Hz we hypothesised a significant increase in CSE marked by an increase in TMS map area and the SR area under the curve, in line with an earlier study (Moliadze et al., 2010a). Both stimulation protocols are only expected to lead to the hypothesised response in 50% of the tested individuals.

8.2 Methods

Participants

Thirty healthy participants were recruited to take part in this study (Experiment 1: 18 participants - 19.8 ± 1.3 y, 12 female; Experiment 2: 12 participants – 20.5 ± 2.6 y, 8 female). All participants were screened for any contra indications to brain stimulation using a modified version of the TMS safety screening for adults, as presented by Keel et al. (2001). The study was approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee (ERN_14-0950). All experiments were performed in accordance with the Declaration of Helsinki.

Electromyography (EMG)

In all experiments electromyographic (EMG) activity of the first dorsal interosseus (FDI) was recorded using Ag-AgCl cup electrodes and the Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, UK). The EMG signal was filtered (20 - 1000 Hz) and amplified (250 - 1k), before being digitally sampled at 5 kHz to be stored for offline analysis.

8.2.1 Experiment 1

Transcranial Magnetic Stimulation (TMS)

A Magstim Rapid^2 (Magstim Ltd, Dyfed, United Kingdom), generating a biphasic pulse waveform, with a custom made polyurethane coated 90 mm figure-of-8 coil (type: batwing; type no. 15411) was used to stimulate the hand area of the primary motor cortex. The position and orientation of the stimulation coil were monitored throughout the experiment using frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada), for which eight fixed landmarks were registered. During TMS, the coil handle was always pointing postero-laterally and held at approximately 45 deg to the parasagittal plane. Using this coil orientation the site eliciting the largest motor evoked potentials (MEPs), referred to as 'hotspot', was found by visual inspection of the EMG. Consequently, with the coil held over the hotspot and changing the stimulator output with steps of 1 or 2%, the resting motor threshold (RMT) was determined as the intensity at which at least 5 out of 10 evoked MEPs with a peak-to-peak amplitude of 50 µV (Groppa et al., 2012, Rossini et al., 1994).

Transcranial alternating current stimulation (tACS)

tACS was delivered by a battery driven stimulator (NeuroConn GmbH, Ilmenau, Germany). All tACS protocols used a sinusoidal stimulation waveform without DC offset and 1 mA (peak-to-peak) stimulation intensity. Stimulation was performed using two conductive 4 x 4 cm rubber electrodes (current density: 0.63 A/m^2) in saline-soaked sponges. One electrode was placed over the TMS motor hotspot whilst the other electrode was placed over the contralateral orbit (Figure 8-1). Before commencing stimulation it was ensured that the impedance was < 10 kΩ, by performing a light abrasion of the scalp and wetting the hair using saline solution at the site of the electrode.

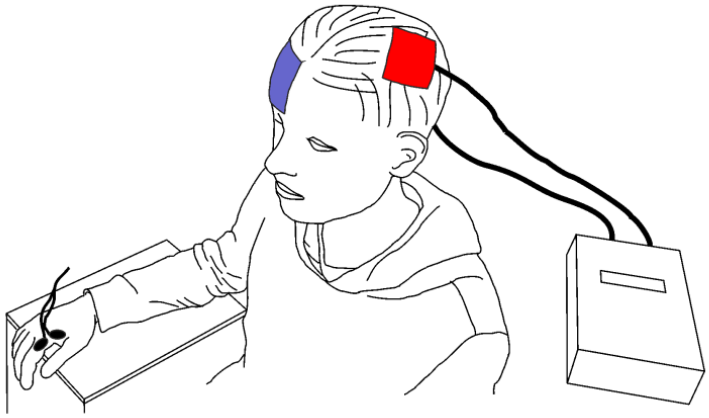

Figure 8-1: The experimental setup during tACS stimulation. Participants received 10 min of tACS stimulation with a peak-to-peak amplitude of 1 mA. Stimulation was either sham, or at a frequency of 20 or 140 Hz. The anode (red electrode) was placed over the motor hotspot as found with transcranial magnetic stimulation, whilst the cathode (blue electrode) was placed over the contralateral orbit.

Experimental Protocol

All participants received three different stimulation protocols (Sham, 20 and 140 Hz) over three different sessions. Sessions were separated by at least 3 days and performed the same time of the day. The first session started with registering the eight facial landmarks. Photos were taken to ensure correct landmark registration in follow up sessions. Subsequently, the hotspot and RMT were found. In the second and third session only the RMT was determined using the hotspot identified in the first session. tACS was applied to the left primary motor cortex for 10 min at one of three stimulation frequency conditions: sham, 20 or 140 Hz. For 20 and 140 Hz the current was ramped up and down

the first and last 5 and 0.7 s respectively. The frequency conditions were pseudo-randomised across the three sessions. Every tACS stimulation period was started by a 2 min familiarisation period in which all tACS conditions were pseudorandomly applied for 20 s. We used this period to ensure participants were comfortable with the applied stimulation and blind the participants for the stimulation versus sham condition. Before and after tACS application, CSE was assessed using TMS stimulus response (SR) curves and maps. To establish baseline excitability three SR curves and three maps were acquired. Following tACS, every 5 min, data for either a SR curve or TMS map was acquired. In total four SR curves and four TMS maps were acquired, therefore CSE was studied up to 40 min after tACS. During both tACS and TMS application participants were instructed to remain fully relaxed whilst they watched nature documentaries to maintain attentive. Documentaries were pre-screened by the experimenters to ensure not to contain any scenes that could elicit emotional responses. Figure 8-2A provides a comprehensive overview of the experimental protocol for Experiment 1.

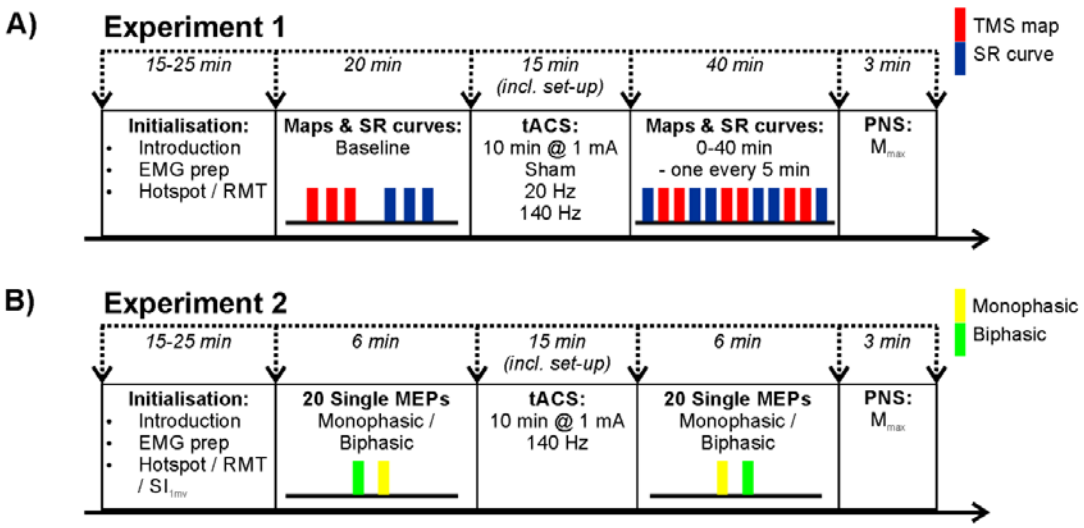

Figure 8-2: Timeline providing an overview of the experimental protocol for both Experiment 1 (A) and Experiment 2 (B).

SR curves

Data for each SR curve was acquired in about 2 min using a recently proposed protocol in which stimulation intensities are selected pseudo-randomly and stimuli administered every 1.5-2 s (Mathias et al., 2014). In general, about 50-80 stimuli were administered to construct a SR curve. During data collection online feedback was available about the SR curve, and acquisition was ceased once the curve fit did not change anymore.

TMS mapping

For the TMS maps 80 stimuli were provided in a 6 x 6 cm grid at pseudorandom locations at 120% of RMT and a 1.5 s interstimulus interval. The TMS map was constructed offline (van de Ruit et al., 2015).

Participant feedback

After every session participants filled out a questionnaire based on the recommendations by Brunoni et al. (2011) to identify any side effects as a result of the tACS.

8.2.2 Experiment 2

To investigate if the findings of Experiment 1 could be explained by the used TMS assessments and in an attempt to replicate the findings of Moliadze et al. (2010a), this second experiment was performed.

Transcranial Magnetic Stimulation & tACS

TMS assessment was performed a stimulator eliciting monophasic (Magstim 200) and biphasic (Magstim Rapid^2) waveform magnetic pulses. All other TMS and tACS procedures were performed as described in Experiment 1.

Experimental Protocol

After finding the hotspot and RMT, the intensity at which MEPs with a 1 mV peak-to-peak amplitude were elicited (SI_{1mV}) was determined for both TMS machines. Using this intensity 20 MEPs were acquired with an interstimulus interval between 4 - 6 s, before and directly after tACS. Ten minutes of tACS was applied at a frequency of 140 Hz. During TMS and tACS, participants were instructed to

keep the muscle fully relaxed. To aid keeping the muscle relaxed, direct feedback of the EMG level was provided on an oscilloscope positioned directly in front of them. Figure 8-2B provides a comprehensive overview of the experimental protocol for Experiment 2.

Data analysis

For all TMS recordings, the MEP peak-to-peak (MEP_{pp}) amplitude was extracted from the EMG recording between 20-60 ms following the magnetic stimulation. Individual MEPs were excluded from TMS maps or SR curves if the corresponding root mean square (RMS) EMG 50-5 ms before the stimulation was greater than twice the mean RMS of the data set. We also confirmed that the mean background EMG in this time window did not exceed 30 µV. If so, the recording was excluded from further analysis.

Experiment 1

In Experiment 1 all MEPs were normalised to the electrically maximal evoked M-wave (M_{max}) using the DS7A peripheral nerve stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK) by stimulating the ulnar nerve at the level of the wrist.

Stimulus response (SR) curve

After MEP_{pp} amplitudes were extracted and plotted against the stimulation intensity, data was fitted with a four parameter Boltzmann sigmoid function using a Levenberg-Marquardt nonlinear least mean square algorithm.

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

The four parameters optimised are the MEP_{min}, MEP_{max}, I_{50} and S. MEP_{min} and MEP_{max} define the minimal and maximal horizontal asymptotes of the curve, the I_{50} defines the stimulation intensity at which the MEP_{pp} is halfway between the two asymptotes and S defines the slope of the curve at I_{50}. As a primary measure the area under the curve (AuC) was used which was calculated by using trapezoidal

numerical integration (Carson et al., 2013). Secondary, I_{50}, MEP_{max} and the slope of the curve were studied. Individual curves were excluded from the analysis if their goodness of fit (r^2) was below 0.7 or less than 20 stimuli remained after exclusions.

TMS maps

To construct the TMS map, the MEP_{pp} amplitudes were matched with the position data obtained from the neuronavigation system. The Matlab function 'gridfit' (D'Errico, 2005) was used to fit a surface map through the data which was transformed to be projected in a 2D plane. Individual stimuli were excluded when the coil angle or translation with respect to an estimated scalp surface exceeded the 95% prediction intervals of all data in a dataset. The maps were quantified with two measures: the map's centre of gravity (COG) and the map area. The COG represents an amplitude weighted mean position in the map and is calculated as follows:

$$xCOG = \frac{\sum(x \cdot aMEP)}{\sum aMEP} \qquad yCOG = \frac{\sum(y \cdot aMEP)}{\sum aMEP}$$

The area of the map was defined by all approximated MEP_{pp} values that exceeded 10% of the maximum MEP_{pp} within a map. To quantify a change in the COG two measures were calculated. The x and yCOG translation was calculated by subtracting the x and y position of the baseline map from each maps COG after tACS (xDisp and yDisp). In this way a negative value would indicate a shift in anterior (x) or lateral (y) direction and a positive value a shift posteriorly (x) or medially (y). This measure provides a measure for the direction of the COG shift and not the absolute displacement. The absolute displacement was calculated by taking the Euclidian Distance (ED) between COGs of all maps after tACS and the baseline map $ED = \sqrt{(y_{post} - y_{pre})^2 + (x_{post} - x_{pre})^2}$

Experiment 2

Mean MEP_{pp} amplitude was determined of the 20 EMG recordings before and after tACS, for both the mono- and biphasic TMS assessment. In addition mean background EMG was compared to ensure stable background activity throughout assessments.

Statistical Analysis

Statistical testing was conducted with IBM SPSS Statistics 21. Tests were considered significant at $\alpha = 0.05$. It was confirmed that the data did not violate any of the statistical tests assumptions. When the assumption of covariance matrix circularity was violated a Geisser–Greenhouse adjustment was made (denoted by GG following the *F* test). Data are reported as Mean ± 1 *SD* unless otherwise noted.

Experiment 1

To assess tACS induced changes in excitability the effect on the TMS map area was studied. The map with the median map area or SR curve with median AuC from the three baseline measures was selected. Subsequently, this median map area was subtracted from all the map area of all the time points after tACS (AreaDiff = $Area_{POST}$ - $Area_{PRE}$), and similar for the AuC (AuCDiff = AuC_{POST} - AuC_{PRE}). A negative AreaDiff or AucDiff would thereby indicate a decrease, and a positive difference an increase, in map area or AuC. In case only two baseline measures were considered, when for example a SR curve had to be excluded if $r^2 < 0.7$, the measure with the lowest mean background EMG was taken as the baseline recording. To study the effect of the different tACS conditions on AreaDiff and AuCDiff, a repeated measures ANOVA with two within subject factors, FREQUENCY (sham, 20 and 140 Hz) and TIME (Post 1, Post 2 , Post 3, Post 4) was performed. The same statistical test was performed for our secondary measures to characterise changes in the TMS map (xDisp and yDisp) and the SR curves: I_{50}, MEP_{max} and slope (S). A one way repeated measures ANOVA was performed to confirm RMT and M_{max} were not affected by FREQUENCY. For all ANOVA tests any significant differences were further explored using a Bonferroni post hoc test.

Responders or non-responders to the tACS stimulation were quantified based on the baseline variance of AreaDiff and AuCDiff. After picking the median baseline map or curve, the variance of the baseline measures was established by subtracting out the median map area or median AuC from the map area or AuC of the other baseline measures. The median map area of AuC was also subtracted from all measures after stimulation, and the average change over all time points was calculated. For the TMS maps a baseline variance in map area of 292 mm^2 was found and as a result a participant with an

average AreaDiff > 292 mm^2 was qualified as a facilitatory responder and < -292 mm^2 as a inhibitory responder. Similarly, for the AuC for the SR curve a baseline variance of 1.2 was found and therefore participants with a AuCDiff > 1.2 were quantified facilitatory and with a change < -1.2 as inhibitory responders. All other participants were considered non-responders.

Experiment 2

To study changes in excitability mean MEP_{pp} amplitude before and after tACS application were compared. A repeated measures ANOVA with two within subject factors, ASSESSMENT TYPE (mono- and biphasic) and TIME (Pre and Post tACS) was performed to compare the effects of tACS on CSE when assessed using two different TMS stimulus waveforms. The same test was performed on the mean level of background EMG.

8.3 Results

8.3.1 Experiment 1

In total, 17 out of the 18 recruited participants, completed all three sessions of the study. Sessions for all but one participant were separated by exactly one week and performed the same time of the day. For one participant two sessions were performed separated by 4 days. One participant had to be withdrawn from the study after the 2nd session, after falling asleep whilst performing the TMS assessments. All participants tolerated the TMS and tACS well. A repeated measures ANOVA revealed no differences for the RMT or M_{max} between the different tACS conditions (RMT: $F(2,32) = 0.55$, $P = 0.58$; M_{max}: $F(2,32) = 0.82$, $P = 0.45$). Re-registration of participants for correct motor hotspot and map localisation between sessions using photos was found successful with registration error for all landmarks and dimensions on average between 1-3 mm.

TMS maps

Out of the 17 participants that completed all three sessions and for which their data was analysed, 11 full data sets were used to perform the statistical analysis on map area and COG. In total six data sets were excluded due to: failed re-registration (registration error > 10 mm) (two participants), MEP_{pp} amplitude at maximum stimulator output less than 1 mV (two participants) and due to excessive number of trials with muscle background activity (two participants). The maps included in the analysis (median baseline map and four post maps) for all sessions were constructed out of 71 ± 5 stimuli.

Effect of tACS

The *SD* in AreaDiff of the baseline maps was 292 mm^2. Based in this a participant was classified a facilitatory responder when the AreaDiff > + 1 *SD* and an inhibitory responder when AreaDiff < - 1 *SD*. Figure 8-3 shows an example of the effect of tACS on the TMS map for all conditions. The area with greater responses (yellow / red colour) is reduced following 20 Hz tACS and increased following 140 Hz tACS. For the sham condition the map area is similar before and after tACS. Small displacements in the COG can be observed.

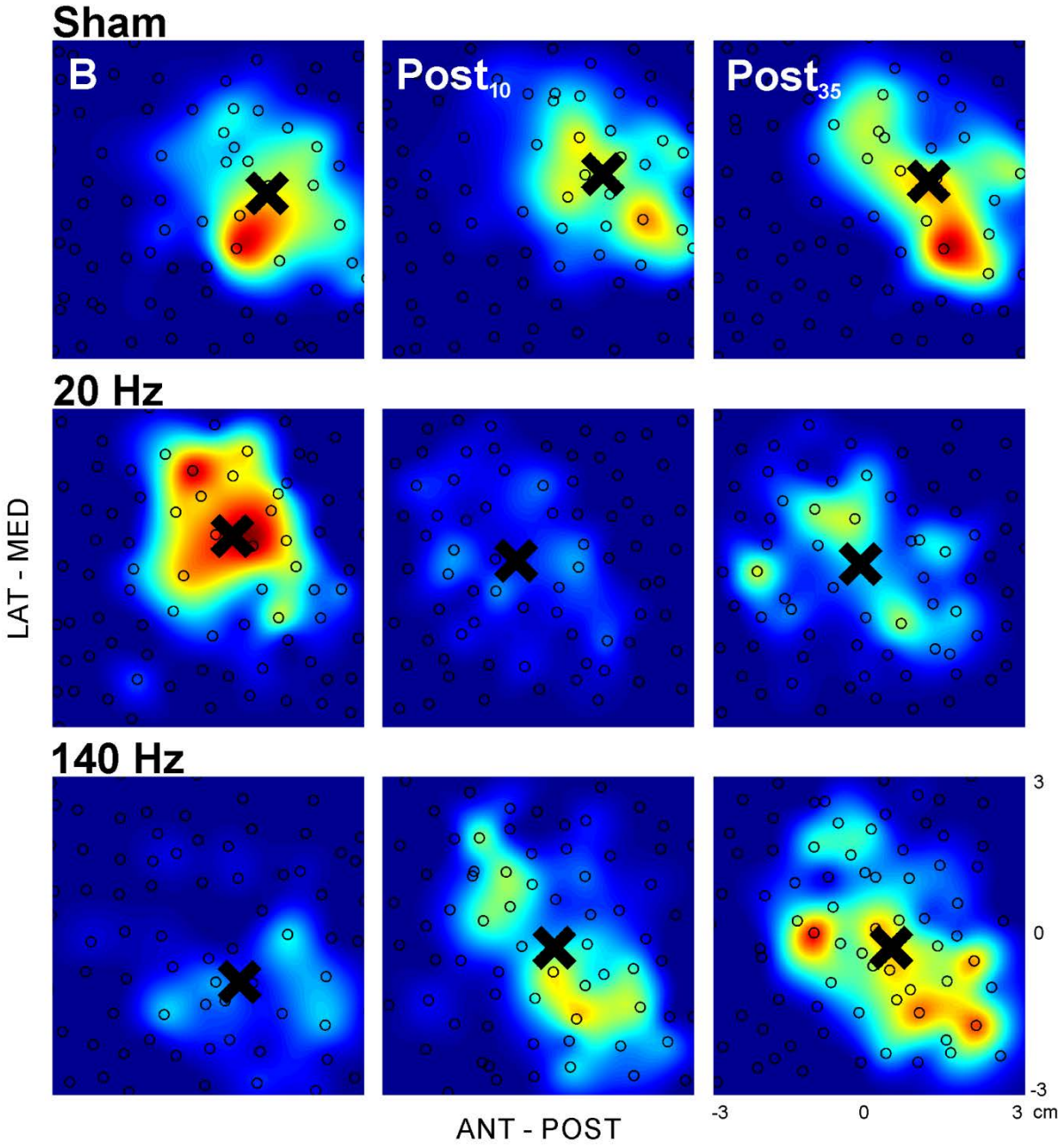

Figure 8-3: The effect of 10 min of tACS on the TMS map for three conditions: sham, 20 Hz and 140 Hz. For each condition presented a different participant was taken. In each map the different stimuli are indicated by the black open circles, whereas the black cross marks the centre of gravity (COG). The colour indicates either no or small MEPs (blue) with progressively larger MEPs when towards red. Whereas for the sham condition the area of the map that provides equal sized responses is stable, MEPs are smaller after 20 Hz tACS and bigger following 140 Hz tACS. Small displacements in COG can be observed, likely caused by re-registration errors.

For AreaDiff the group data is shown in Figure 8-4A. On average, map area after 140 Hz tACS is increased (red line) and decreased after 20 Hz (blue line). A repeated measures ANOVA (within subject factors: FREQUENCY and TIME) revealed no significant effect for either FREQUENCY ($F(2,20) = 3.126$, $P = 0.07$), TIME ($F(3,30) = 0.25$, $P = 0.86$) or a FREQUENCY*TIME interaction ($F(6, 60) = 1.152$, $P = 0.34$). The number of facilitatory, inhibitory and non-responders based on the map area are summarised in Table 8-1 and individual responses to tACS are displayed in Figure 8-4B. Five participants showed increased map area following 140 Hz tACS, and following 20 Hz tACS map area decreased for three participants.

On average COG were displaced 5.2 ± 3.0 mm in the post maps compared to the baseline map. However, COG were not systematically displaced in either the x (xDisp: FREQUENCY: $F(2,20) = 1.029$, $P = 0.38$, TIME: $F(3,30) = 0.29$, $P = 0.83$, FREQUENCY*TIME: $F(6,60) = 0.47$, $P = 0.83$) or y direction (yDisp: FREQUENCY: $F(2,20) = 1.578$, $P = 0.23$, TIME: $F(3,30) = 0.08$, $P = 0.97$, FREQUENCY*TIME: $F(6,60) = 0.30$, $P = 0.94$).

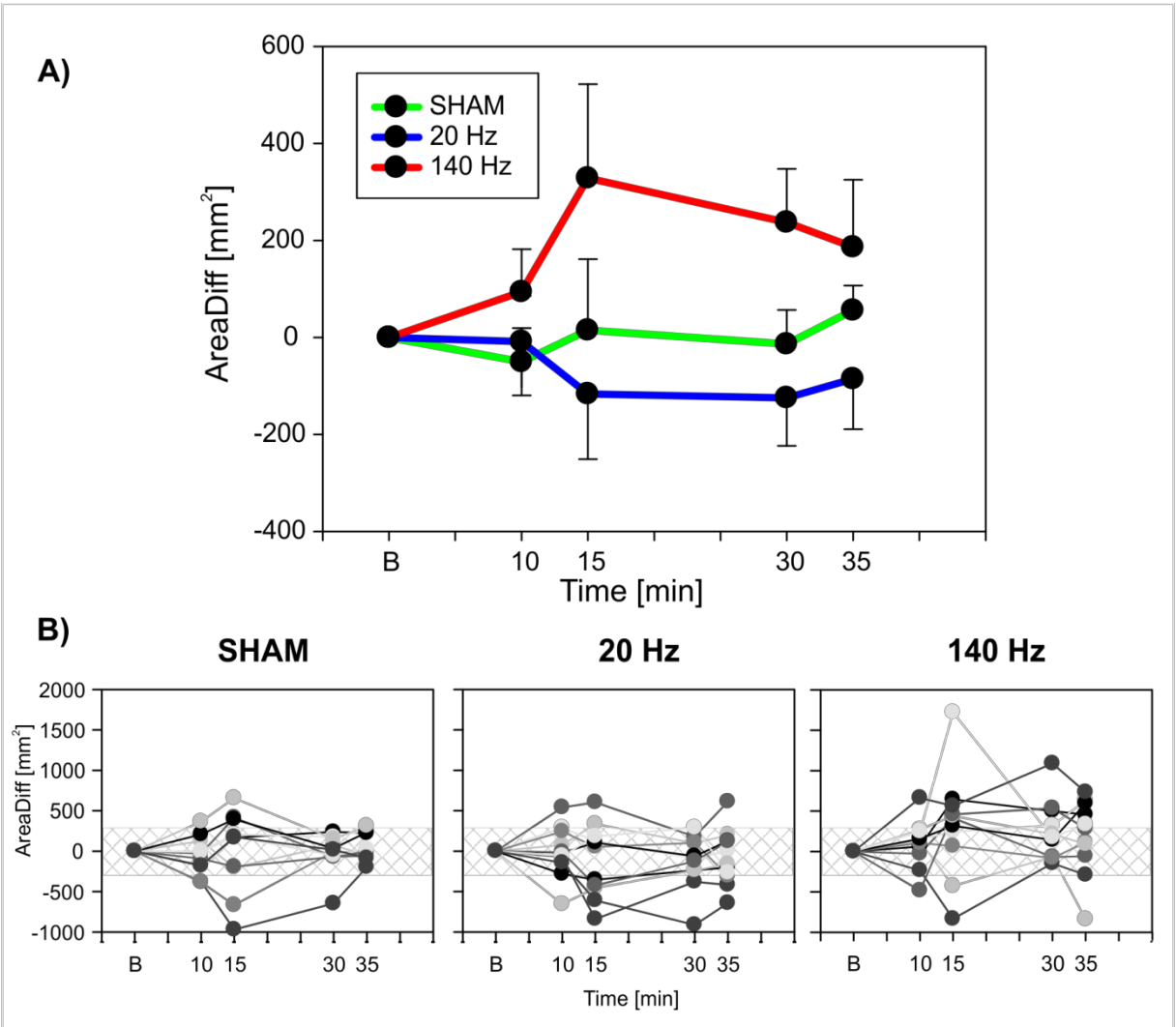

Figure 8-4: Group and individual data of effect of tACS on map area. (A) Group data (Mean ± 1 *SE*) of the difference in map area before and after stimulation for all tACS conditions: sham (green), 20 Hz (blue) and 140 Hz (red). All measured time points are displayed: baseline - B - and 10, 15, 30 and 35 min after tACS application. No significant effects were found for either time or frequency. There is a tendency for 20 Hz tACS to inhibit map area, whereas 140 Hz facilitates map area. (B). The individual responses to the three tACS conditions highlighting the great variability in the response. The shaded area marks the zone in which a participant would be considered a non-responder.

Table 8-1: The number of participants showing no, a facilitatory or a inhibitory response in response to sham stimulation, or 10 min tACS stimulation at 20 or 140 Hz based on TMS map area.

Condition	Facilitatory	Non	Inhibitory
Sham	1	9	1
20 Hz	1	7	3
140 Hz	5	5	2

SR curves

Out of the 17 participants that completed all three sessions and for which their data was analysed, 7 full data sets were used to perform the statistical analysis on AuC, I_{50}, MEP_{max} and Slope.

Six data sets were omitted for the same reasons as mentioned with the TMS maps. A further 4 data sets had to be discarded because of poor curve fits ($r^2 < 0.7$ for more than 1 curve in a session), or low number of stimuli left after exclusions (number of stimuli < 20). The SR curves included in the analysis (median baseline curve and four post curves) for all sessions were constructed out of 66 ± 12 stimuli.

Effect of tACS

The *SD* of AuCDiff of the baseline curves was 1.2. Based on this a facilitatory responder was defined as AuCDiff > + 1 *SD* and a inhibitory responder as < - 1 *SD*. Figure 8-5 shows a representative example of the effect of tACS on the SR curve for all conditions. There is little change in curve parameters for any of the conditions. Only an increase in the maximum MEP amplitude can be observed following 140 Hz tACS.

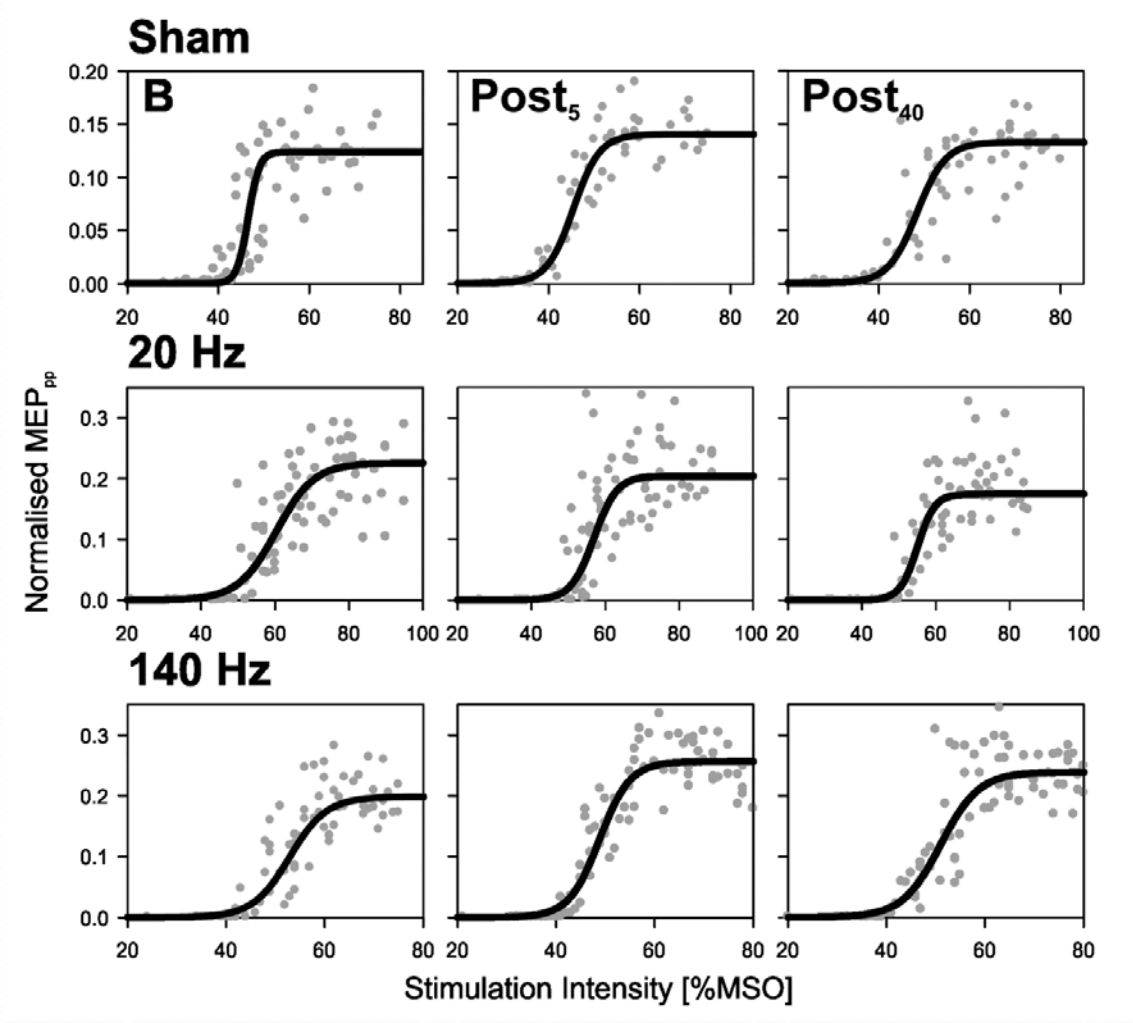

Figure 8-5: The effect of 10 min of tACS on the SR curve for three conditions: sham, 20 Hz and 140 Hz. For each condition presented a different participant was taken. In each SR curve the different stimuli to construct the curve are the indicated by the grey dots, whereas the solid black line marks the Boltzmann sigmoid curve fit. In the examples shown here, little difference is found between the curves from baseline (B) to after stimulation (Post10 and Post40). Only after 140 Hz tACS there is an increase in maximum MEP amplitude.

For AuCDiff the group data is shown in Figure 8-6A. A repeated measures ANOVA (within subject factors: FREQUENCY and TIME) revealed no effect for either FREQUENCY ($F(2,12) = 0.67$, $P = 0.53$), TIME ($F(3,18) = 0.78$, $P = 0.52$) or a FREQUENCY*TIME interaction ($F(6,36) = 1.144$, $P = 0.36$). The number of facilitatory, inhibitory and non-responders based on the map area are summarised in Table 8-2 and individual responses to tACS are displayed in Figure 8-6B. No participant responded with decreased AuC following 20 Hz, and only one with increased AuC following 140 Hz tACS. Likewise, no effect was found for any of the other measures used to quantify the SR curve (MEP_{max}, I_{50} and Slope), summarised in Table 8-3.

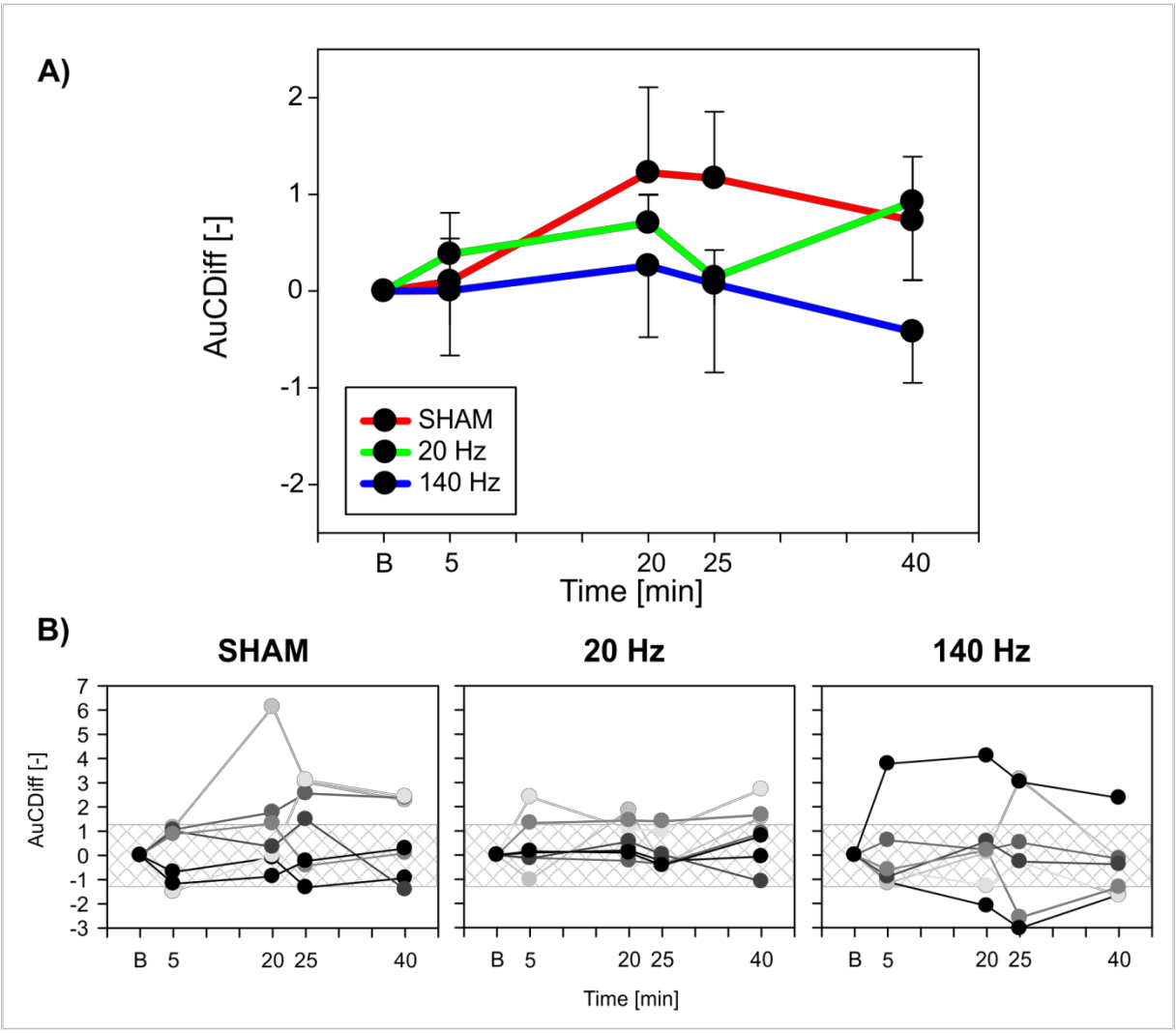

Figure 8-6: Group and individual data for the effect of tACS on area under the curve (AuC).(A) Group data (Mean ± 1 *SE*) of the difference in AuC before and after stimulation for all tACS conditions: sham (green), 20 Hz (blue) and 140 Hz (red). All measured time points are displayed: baseline and 5, 20, 25 and 40 min after tACS application. No significant effects were found for either time or tACS condition. (B). The individual responses to the three tACS conditions highlighting the great variability in the response. The shaded area marks the zone in which a participant would be considered a non-responder.

Table 8-2: The number of participants showing no, a facilitatory or a inhibitory response in response to sham stimulation, or 10 min tACS stimulation at 20 or 140 Hz based on the SR area under the curve (AuC).

Condition	Facilitatory	Non	Inhibitory
Sham	2	5	0
20 Hz	2	5	0
140 Hz	1	5	1

Table 8-3: Statistical results of the effect of tACS on the secondary SR curve measures used to quantify changes in excitability. No significant effect was found for FREQUENCY or TIME or an interaction for any of the parameters.

Factor	FREQUENCY	TIME	FREQUENCY*TIME
MEP_{max}	F(2,12) = 0.13, P=0.88	F(3,18) = 0.41, P=0.75	F(6,36) = 0.11, P=0.30
I_{50}	F(2,12) = 3.02, P=0.09	F(3,18) = 2.74, P=0.74	F(6,36) = 0.50, P=0.80
Slope	F(2,12) = 1.07, P=0.38	F(3,18) = 1.32, P=0.30	F(6,36) = 1.41, P=0.24

Participant feedback

All 17 participants provided feedback about side effects experienced during and after tACS application. From the 17 participants, 9 correctly identified the sham session. Headache, neck pain, concentration, fatigue, nausea and a burning sensation on the head or scalp were reported, but only for 1 to 6 of the participants, with no link to any condition. Likewise, sleepiness was reported for 11 or 12 participants, regardless of stimulation condition. An itchy feeling was reported by 8 participants, but more prevalent in the stimulation conditions (20 and 140 Hz, 7 and 5 participants respectively) than in the sham condition (3 participants). Phosphenes were reported by 16 participants, however reported as only occurring at the beginning or once in the sham condition or when stimulating at 140 Hz, whereas only 3 participants reported phosphenes occurring occasionally. In contrast, for 20 Hz stimulation the phosphenes were reported to occur occasionally or be present throughout by 8 participants. Eleven participants reported muscle twitches, but reports of intensity and frequency of these were similar for the sham and stimulation condition. Participants have likely reported on the TMS induced muscle twitches.

8.3.2 Experiment 2

For this experiment 12 participants were recruited who had not participated in Experiment 1. All participants successfully completed this single session experiment, tolerating the TMS and tACS well.

Effect of 140 Hz tACS on 1 mV MEPs

After MEPs were excluded based on background EMG, 93% of the MEPs were included for analysis. Mean MEP_{pp} was calculated of all included MEPs before and after the tACS when assessed with both a monophasic and biphasic TMS stimulator. In Figure 8-7A, mean MEP_{pp} amplitudes are shown for every participant and both assessment types. This revealed that 9 of the 12 participants showed a similar response regardless of assessment type. In three participants the response was contradicting for both assessment types.

Group data (Figure 8-7B) revealed no difference in mean MEP amplitude before and after tACS or a difference for the different assessment types. A repeated measures ANOVA revealed no effect of TIME ($F(1,11) = 0.56$, $P = 0.47$), ASSESSMENT TYPE ($F(1,11) = 1.19$, $P = 0.30$), or a TIME*ASSESSMENT TYPE interaction ($F(1,11) = 0.46$, $P = 0.51$). No significant differences for either TIME or ASSESSMENT TYPE in the level of background EMG were found.

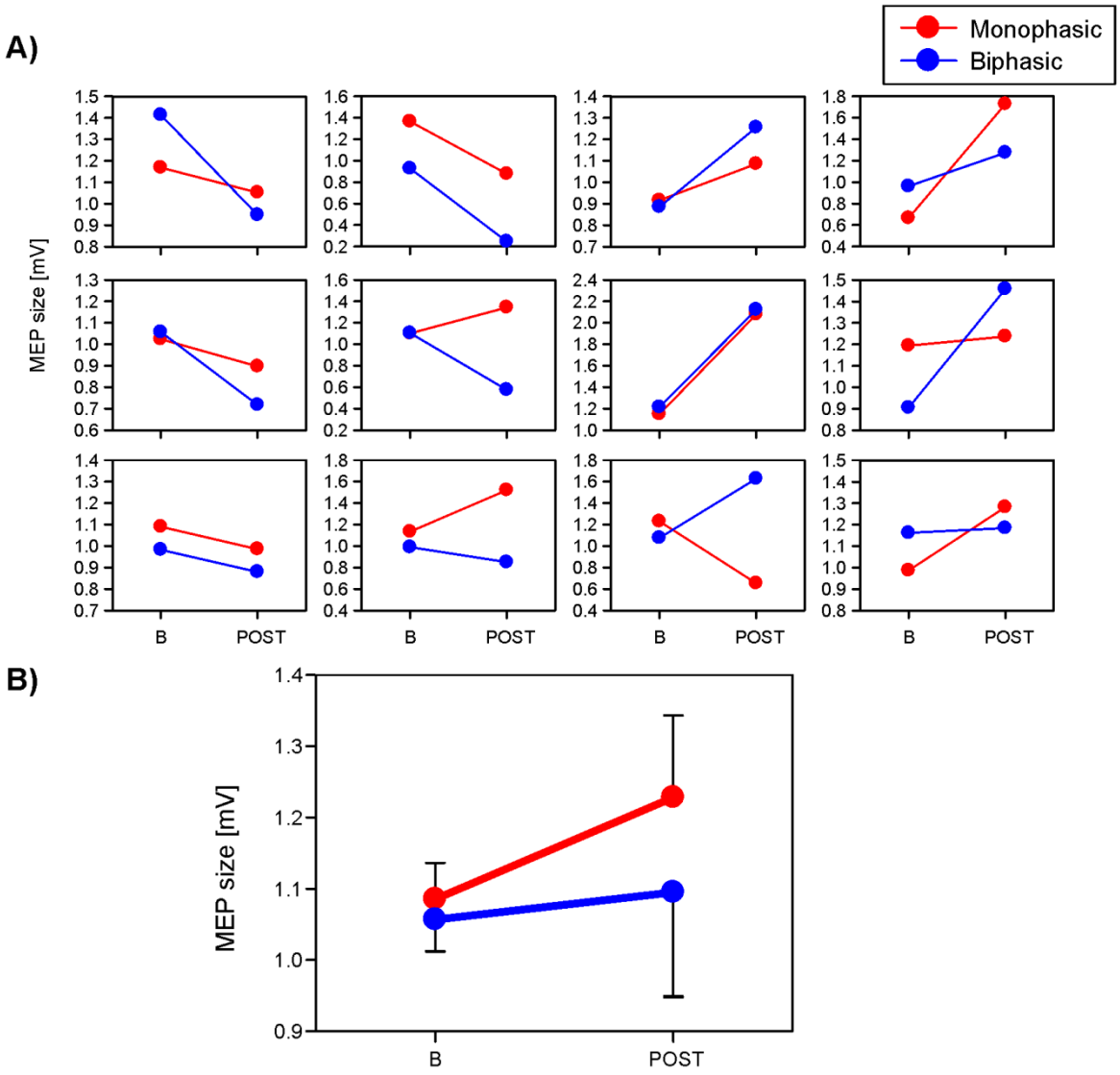

Figure 8-7: Effect of 10 min tACS at 140 Hz on MEP size when assessed using using mono- and biphasic TMS. (A). Mean MEP_{pp} amplitude before and after tACS application for both assessment types in all 12 participants. This highlights the great variability in response to tACS whilst similar effects are observed when assessed using either TMS pulse waveform. (B) Group data (Mean ± 1 *SE*) for both assessment types before and after tACS. No effect of time or significant interaction between the two types of assessments was found.

8.4 Discussion

This study aimed to investigate the effect of tACS applied at two different frequencies, 20 and 140 Hz, on CSE as assessed by SR curves and TMS maps. Moreover, we aimed to provide an insight into the variability of the tACS response. No main effect of tACS was found on CSE, either for TMS map area or area under the SR curve. Nonetheless, the data suggests that 10 min of 20 Hz stimulation inhibits excitability and 140 Hz stimulation facilitates excitability, in line with our hypothesis. Noticeably, this effect is primarily observed in the TMS maps and not in the SR curves. Only a third to a half of the participants responded in the hypothesised way, highlighting that the response to tACS is equally variable as to other non-invasive brain stimulation techniques promoting changes in CSE.

The effect of 20 and 140 Hz tACS on corticospinal excitability

This is the first study that used both TMS maps and SR curves to quantify changes in CSE following tACS. Our results point towards an inhibition of excitability following 20 Hz tACS and a facilitation following 140 Hz tACS. This trend is observed in the TMS map area, but not the SR curve data. Increased excitability following 140 Hz tACS was earlier reported by Moliadze et al. (2010a) who found a 50% increase in MEP amplitude. Oscillations in the brain in a frequency range of 80-200 Hz have been described as 'ripples' and have been characterised in the hippocampus (Buzsaki et al., 1992, Buzsaki and Draguhn, 2004). Though, the mechanisms and relevance of these oscillations to drive changes in CSE within the motor system are unclear.

In contrast, the frequency of 20 Hz is a prominent beta band frequency in the motor system (Baker, 2007). The tendency for an inhibition following 20 Hz tACS does contradict the findings by Feurra et al. (2011), who reported larger MEPs when assessed during short bursts of tACS. Our result suggests that after 10 min of tACS, the initial potentiating effect is reversed to inhibit excitability. This effect has been observed for tDCS previously (Batsikadze et al., 2013, Monte-Silva et al., 2010). It has also been suggested that greater beta peak power is linked to increased cortical inhibition (Baker and Baker, 2003). As tACS has been suggested to increase EEG power at the frequency of stimulation, this potentially explains the observed tendency.

The mixed results reported here do not differ from the unequivocal results of others, suggesting either a clear canonical response (Moliadze et al., 2010a), or no response at all (Antal et al., 2008, Wach et al., 2013). The inconsistency of our and the results of others highlight three important topics: (1) interindividual variability in the response to tACS (2) the method to assess changes in CSE, and (3) the used tACS method.

Interindividual variability in response to tACS

Our findings highlight that tACS does not lead to the canonical response in every single participant. When considering the change in CSE for all participants individually, for 20 and 140 Hz, 36% and 54% responded in the hypothesised way respectively. Experiment 2 confirmed this variability with half the participants showing an increase in mean MEP_{pp} amplitude by more than 10% of the baseline, when 140 Hz tACS was administered. This response rate of about one-third to a half is similar to other forms of non-invasive brain stimulation (Wiethoff et al., 2014, Maeda et al., 2000b, Hamada et al., 2013, Muller-Dahlhaus et al., 2008). For tACS, such data has not been explicitly reported. Chaieb et al. (2011) were the only that have provided individual responses; supporting our findings that the tACS response is highly variable. Nonetheless, only a small sample of participants was tested here, and more high quality data needs to be collected to confirm these response rates.

Assessing changes in corticospinal excitability using TMS

The data on changes in CSE provides some discrepancies in the responses to tACS when assessed using either TMS maps, SR curves and single MEPs at one fixed intensity. Especially interesting is the dissociation between TMS maps and SR curves which have been argued to provide similar information (Ridding and Rothwell, 1997). Two major differences exist between TMS maps and SR curves. Whereas in the former the cortex is stimulated at multiple sites, in the latter only the motor hotspot is stimulated. Moreover, whilst SR curves are constructed stimulating at different intensities, TMS maps are constructed stimulating with just a single intensity. There are two reasons for why in this study TMS maps and SR curves may provide conflicting results.

Firstly, it has been argued that with different stimulation intensities, different neuronal populations are recruited both spatially distributed as well as originating in different cortical layers (Di Lazzaro et al., 2012). This effectively means that the SR curve potentially captures the effect on multiple different neuronal populations, whereas the TMS maps only quantifies changes in a subset of those. In light of this, not finding any changes in the SR curves might be a consequence of the Boltzmann sigmoid fitting algorithm not well quantifying the different changes that occur in the SR relationship (Goetz et al., 2014). This is further supported by the finding that 4 of 12 participants showed an increase >50% of baseline MEP size in Experiment 2 whilst only 1 participant showed an increased AuC in Experiment 1. Secondly, it has been argued that with transcranial current stimulation protocols the current concentrates at the edges of the stimulating electrodes (Miranda et al., 2006, Miranda et al., 2009) and maximum current lies somewhere in between the two electrodes (Datta et al., 2012). In the latter study, in which the same electrode configuration was used as in this study, maximal current was most commonly frontal to the motor cortex electrode. However, this effect varies between individuals. Nonetheless if it would be assumed that plasticity would be greatest where current is maximal these findings would suggest greatest plasticity would be observed away from the motor hotspot and therefore these changes are easier to quantify using TMS maps which stimulates a big area of the motor cortex.

In general, the contrasting findings between this and previous studies may be caused by the use of a biphasic TMS stimulator to assess CSE using TMS map and SR curve. TMS stimulators either produce monophasic or biphasic stimulation pulse waveforms, which have been reported to recruit different neuronal populations (Sommer et al., 2006, Salvador et al., 2011, Di Lazzaro et al., 2012). tACS might modulate the neuronal elements in such a way that it is detectable by only one of the stimulation pulse waveform. Moliadze et al. (2010a) and others reporting on excitability following tACS (Feurra et al., 2011, Antal et al., 2008, Chaieb et al., 2011) used a TMS stimulator generating monophasic pulse waveforms. In line with our results, Wach et al. (2013) failed to find any changes in excitability when using a biphasic TMS stimulator. The second experiment was performed to ensure

the results of Experiment 1 were not a result of the TMS stimulator used. We were unable to find a difference in the effect of 140 Hz tACS on the observed effect when excitability was assessed by both a monophasic and biphasic TMS stimulator, as indicated by the lack of an ASSESSMENT TYPE * TIME interaction. This supports the notion that the type of pulse waveforms used to assess CSE does not change the observed effects.

Confounding factors in mediating tACS induced plasticity

The tACS effect is mediated by a multitude of variables related to the stimulation characteristics (duration, intensity, frequency) and method of application (electrode size, electrode position). In most tACS studies to date, that aimed to induce changes in excitability, stimulation was applied for 10 min (Chaieb et al., 2011, Moliadze et al., 2010a, Moliadze et al., 2012, Wach et al., 2013), with the intensity and frequency determining the direction of change (Zaghi et al., 2010, Moliadze et al., 2010a). In this study the most commonly used stimulation protocol of 10 min with stimulation at 1 mA peak-to-peak was adopted. tACS was applied by two 4 x 4 cm electrodes: one over the motor hotspot and one over the contralateral orbit. Electrode size and position has been found to mediate both the tACS and tDCS effect (Bastani and Jaberzadeh, 2013, Moliadze et al., 2010b, Neuling et al., 2012), but again the most commonly used electrode size and configuration was adopted to allow best comparison to studies published to date.

During TMS and tACS stimulation, a video was played to the participants in an attempt to stabilise and maintain their attention. It has been argued that spatial attention plays an important role in mediating stimulation induced plasticity, with greatest change observed when participants attend to the hand stimulated (Kamke et al., 2014, Stefan et al., 2004). In Experiment 1 participants likely attended to the video displayed, which may have reduced the plasticity effect. It may be argued this is unlikely given the similar results of Experiment 1 and 2. In Experiment 2, participants were more focused on the stimulated hand as direct feedback on the level of EMG was provided, and no video was shown during either tACS or TMS. Another issue of showing videos may have been increased cognitive load, found to abolish plasticity (Kamke et al., 2012). To minimise the cognitive load, participants watched

the videos without instructions or questions to be answered,. Further, the videos were selected to avoid eliciting strong emotional positive or negative emotional responses that may affect CSE during TMS or tACS (Giovannelli et al., 2013).

There are many other confounding factors which might mediate the effect of tACS (for review: Ridding and Ziemann, 2010). These include but are not limited to: the time of the day the participant is tested (Sale et al., 2007, Sale et al., 2008), participant gender (Kuo et al., 2006, Galea et al., 2006) phase of the menstrual cycle (Inghilleri et al., 2004) and genetic profile (Kleim et al., 2006, Cheeran et al., 2008). Participants were always tested at the same time of the day in this study. As about two-third of our participants were female, their phase in the menstrual cycle might have affected our results. However, attempting to control for menstrual cycle was unfeasible in this study. Nonetheless, a study with more participants than this study would be able to run a covariate analysis to see if any of these factors predict the tACS response.

Concluding remarks

This study provides first evidence that tACS changes the TMS maps for both 20 and 140 Hz tACS. Moreover, it highlights the variability of the response to tACS stimulation in line with the reported variability of other non-invasive brain stimulation techniques. As a first step more data needs to be acquired to confirm the reported trends.

CHAPTER 9

Discussion

9.1 Summary of the work in this thesis

The aims of this thesis were (1) to improve existing techniques to study CNS plasticity (SR curves and TMS maps) and (2) demonstrate these can be used to study internally and externally driven plasticity. To achieve this in Chapter 3-5 we exploited a novel method to perform TMS mapping and developed a GUI to facilitate rapid acquisition of the SR curve. TMS maps can be acquired within two minutes by stimulating pseudorandomly in a square grid, reducing the ISI and optimising the number of stimuli. The GUI to facilitate SR curves acquisition provides online feedback about the SR curve during data acquisition. Together these findings allow further optimisation of the data acquisition procedure to quantify CSE using TMS maps or SR curves. In Chapter 6-8 we demonstrated these techniques may be successfully used to study internally and externally driven plasticity. Both rapid TMS mapping and acquisition of SR curves were used to study plasticity following mirror training augmented by imagery training, a visuomotor tracking learning task (both internally driven plasticity) and tACS (externally driven plasticity).

9.2 The assessment of central nervous system plasticity using TMS

In the introduction of this thesis the three TMS methods to assess CSE and cortical organisation were presented: traditional single pulse TMS, SR curves and mapping. The work in this thesis and of Mathias et al. (2014) highlights one can acquire valuable information about CSE using SR curves and TMS maps in 2 min.

For the SR curve, work in this thesis presents the development of a GUI that is used to obtain direct online feedback about the SR curve. The GUI was successfully used in Chapter 8 to study externally driven plasticity. By obtaining direct feedback about the SR curve, the experimenter can make an on

the spot decision whether sufficient data has been collected and cease stimulation. The need for this was evident from Mathias et al. (2014), who for the FDI muscle claimed 61 stimuli were required to obtain a reliable SR curve, but this number exhibited a large interindividual variability (*SD* = 18 stimuli). In addition, the GUI allows the experimenter to online adapt the range of stimulation intensities used. This prevents the use of stimulation intensities far below or above the point where the MEP saturates, resulting in unnecessary extra stimuli and discomfort for the participant. The study in Chapter 8 proves successful use of the GUI, with a great variance in number of stimuli acquired in each individual (66 ± 12 stimuli). One downside of the present GUI is that the decision when sufficient data has been acquired is entirely up to the experimenter. This may lead to data acquisition being stopped too early. To prevent this happening a next step in optimising the SR curve method is to develop objective criteria that ensure sufficient data is acquired in each participant. These criteria should include the goodness of fit of the Boltzmann sigmoid curve and a convergence test making use of the rate of change of the curve parameters with each additional data point.

For TMS mapping. the first study in this thesis demonstrated it is possible to acquire the data to construct a reliable TMS map within 2 min. This is a significant improvement from the 10 - 40 min that researchers traditionally needed to acquire sufficient data to construct a TMS map (e.g. Guerra et al., 2015, Wilson et al., 1996, Littmann et al., 2013). However, to allow doing this several design decisions had to be made to find a balance between speed of acquisition and quality of the TMS map measures. All TMS map measures (COG, map area and map volume) will be discussed below, focussing on their reliability and factors that may affect their usefulness in demonstrating plasticity.

The COG provides information about the position of the cortical representation of a muscle and allows to detect changes in its shape. Variability of the COG is generally considered to be in the range ±3 mm (Classen et al., 1998a, Littmann et al., 2013, Miranda et al., 1997, Julkunen, 2014, Weiss et al., 2012), which isn't any different when TMS maps are acquired in 2 min. Therefore, shifts in COG must at least exceed ~5 mm (Mean + 1 *SD*) in order to be meaningful (Byrnes et al., 1998, Thickbroom et al., 2004, Liepert and Neveling, 2009, Liepert et al., 2000). Care should be taken in longitudinal studies,

where registration errors might increase variability in the COG. Interpretation of shifts in COG is complicated by differences in location of cortical representation in different animals from one primate species (e.g. Huang et al., 1988). Therefore, COG may shift in different directions across tested participant (e.g. Byrnes et al., 1999, Ridding et al., 2001). To avoid this making results difficult to interpret both the direction and size of the displacement should be considered when analysing changes in COG. In none of the studies included in this thesis a significant change in COG was found. This is likely caused by the use of only healthy participants for which changes in COG have rarely been reported (for exception see e.g. Liepert et al., 1999). Shifts in COG are more commonly observed in patients (e.g. Byrnes et al., 1998, Thickbroom et al., 2004, Liepert et al., 1998, Liepert et al., 2000).

The TMS map area represents the area on the scalp resulting in an MEP. Classen et al. (1998a) estimated the size of the cortical representation would in the human brain be about 970-1210 mm^2 for a forearm muscle. In Chapter 3 the TMS map area for the FDI was larger, on average ~1300 mm^2, primarily as a result of the current spread (Thickbroom et al., 1998). Whilst traditionally map area was expressed as the 'number of excitable grid points', it is now more common to provide more accurate estimates of map area (in mm^2) for which different computational methods are available (Julkunen, 2014). Based on the findings in this thesis we may conclude the variability of the map area is about 250-300 mm^2 when the TMS map is acquired in 2 min. This means the coefficient of variation is about 20%, in line with the findings of others (Wolf et al., 2004, Mortifee et al., 1994). In order to reduce variability in map area, the fringe of the map area is usually defined as those positions were the size of the MEP drops below a predefined percentage of the maximum MEP in the map (in this thesis 10%). This is to avoid the variable MEPs in the periphery making the area less reliable (Brasil-Neto et al., 1992b). Whilst using a fixed percentage with respect to the maximal MEP to define the representations fringe is common (e.g. Wilson et al., 1993, Uy et al., 2002, Plowman-Prine et al., 2008), it also poses a risk. When the maximal MEP is smaller than 500 µV, and a 10% cut-off is used, parts of the map were the MEP is smaller than 50 µV will be considered part of the map area. However, 50 µV is commonly considered the threshold for defining an MEP when the muscle is at rest (Rossini et al., 1994).

Therefore, one needs to take care when using this approach. Alternatively, one can define the map area as the part of the map were MEPs are greater than for example 100 µV (as in Chapter 6 and 7).

The TMS map area turned out to be good marker of neuroplasticity in Chapter 6-8. Unfortunately, results were not univocal, with some participants exhibiting an increase in map area following a plasticity inducing protocol, whilst for others map area decreased. Although, the adopted acquisition parameters for TMS mapping may explain the great heterogeneity in the response, this seems unlikely. Firstly, the ISI or grid spacing could have inflated or disrupted cortical reorganisation. To induce lasting changes in excitability in excess of 1.000 stimuli are required to a single site at ISIs shorter than 1 s (Chen et al., 1997, Berardelli et al., 1998). For TMS mapping only 80 stimuli are administered and, as a precaution, spacing between two consecutive stimuli is maximised during acquisition whilst only single stimuli are administered at each position. Secondly, there is a remote possibility that the number of stimuli was insufficient to accurately capture the cortical representation

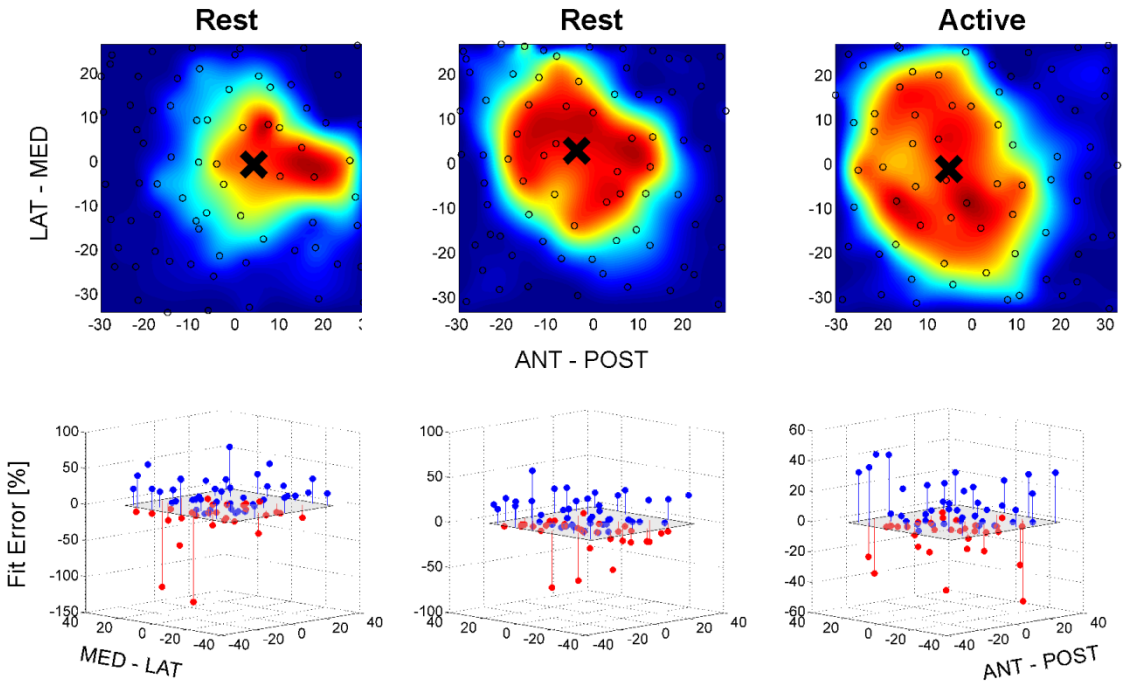

Figure 9-1: Depiction of the goodness of fit of the TMS map.For each map (top row) the fitting error for each acquired MEP is displayed (bottom row). In the TMS map the size of the MEP is colour coded with small or no MEP represented by blue and the biggest MEPs in red. The map's COG is marked by the black cross (×). A red stem indicates the actual MEP_{pp} was lower than the fitted MEP_{pp}. In contrast, the blue stems represent MEPs for which the actual MEP was higher than the fitted MEP_{pp}. The fit error is expressed as a percentage of the actual MEP_{pp} at each site. Fit error was generally <10% close to the COG The fit is worse near the fringe of the map as a result of increased MEP variability.

in some participants. Chapter 3 highlighted a great variability in the number of stimuli required to construct a map, with the IQR indicating 46-74 stimuli are required. Therefore, not sufficient stimuli might have been administered in approximately 20% of the maps. This suggests in 1 out of 5 participants we did not obtain an accurate measure of the TMS map area, which may have resulted in a poor quantification of any changes in CSE. A similar issue arises for the grid size. A 6 x 6 cm grid was used during TMS mapping, which when mapping a muscle at rest is sufficient see e.g. Malcolm et al. (2006 and results in Chapter 3). However, when the muscle is mapped during a small precontraction the map area enlarges and simply scales (Chapter 4), exceeding 36 cm^2 (Wilson et al., 1995, Thordstein et al., 2013). A larger grid size was not used as pilot testing revealed this creates discomfort by stimulating close to, and over, the temple, resulting in twitches of the facial musculature in a significant number of participant. As all mapping to study cortical reorganisation was undertaken when the muscle was at rest this cannot have affected our results.

The map volume might add valuable information to the COG and map area and inform about changes in CSE without a change in map area or COG. Unfortunately, in Chapter 3 map volume was found an unreliable measure and therefore not included in Chapter 6-8. The possibility that our method of fitting the data caused the poor reliability of map volume cannot be excluded. One may question how well the surface that is fitted, fits the data points. This hasn't been systematically explored but the examples in Figure 9-1 illustrate the surface fitted the data well. Greater fitting errors are observed near the fringe of the cortical representation, than close to the COG. These fitting errors are primarily caused by the variability of the MEP, which is greater in the periphery of the map (Brasil-Neto et al., 1992b). Cuypers et al. (2014) argued an average of at least 30 MEPs is required to obtain a reliable measure of CSE. Consequently, single MEPs spread across the grid do not provide a reliable estimate of CSE which may translate to map volume. Increasing the number of stimuli per site does not simply result in a more reliable map volume, as shown by Ngomo et al. (2012b) who administered six stimuli per site but also found a poor reliability of map volume. To counteract the time-variant behaviour of the MEP, a surface stiffness was chosen in order to reduce the effect of big differences of MEPs close proximity

but at the same time prevent smoothing out any variation. Further optimisation of the surface stiffness may be required to make map volume a more reliable measure.

The assessment of CSE using TMS maps or SR curves may be affected by the intensity and shape of the magnetic pulse. From the SR curve we know that a higher stimulation intensity leads to bigger MEP. For the TMS map we demonstrated the area simply scales with stimulation intensity (Chapter 4), which is a result of current spread (Thickbroom et al., 1998). Stimulating at a different stimulation intensity means different neurons are recruited, resulting in different descending volleys (Di Lazzaro et al., 2004a), although this did not show in a difference in COG. Thus, stimulating at one intensity might not reveal CSE changes, whereas another does. This is where the SR curve becomes valuable (Goetz et al., 2014). In all studies in this thesis stimulation intensity was set to 120% RMT during TMS mapping, which for most participants will be on the lower slopes of the SR curve (Pitcher et al., 2015) and has been used frequently before (see Table 1-1 in Chapter 1). Whether this adopted stimulation intensity has affected our ability to detect plasticity is hard to say given so few studies use TMS mapping to study plasticity following motor learning or non-invasive brain stimulation

Most researchers use a TMS stimulator (e.g. the Magstim 200) eliciting monophasic current waveforms to assess CSE. However, the stimulator used to allow rapid acquisition of the TMS measures in this thesis uses biphasic pulses, of which the 2nd and 3rd cycle of the pulse have been suggested to contribute to the induced effect (Di Lazzaro et al., 2001). A lower RMT when CSE is assessed using biphasic than monophasic pulses suggests different interneurons are recruited or interneurons are excited at a different site (Di Lazzaro et al., 2004a, Sommer et al., 2006, Kammer et al., 2001). This may have led to a recruitment of neurons that did not express plasticity and inflated variability following our plasticity inducing protocols. To confirm this, in chapter 8 a control experiment was conducted to compare observed changes in CSE using the traditional single pulse approach of a mono- and biphasic stimulator. We found no difference in the effect of tACS on CSE assessed using the two pulse waveforms. It is unlikely the biphasic nature of our assessment techniques has affected our results.

9.3 TMS mapping versus SR curves

The previous section briefly touched on one advantage of the SR curve with respect to the TMS map. The SR curve provides information about CSE at multiple different intensities, whereas the TMS map is only acquired at one fixed intensity. Stimulating at different intensities recruits multiple different populations of interneurons (Di Lazzaro et al., 2004a). However, SR curves can only detect changes in the area of the TMS map but cannot detect changes in the distribution of the excitable elements or uneven expansion of the cortical area (Ridding and Rothwell, 1997). In Chapter 8 of this thesis both methods were used to quantify changes in cortical organisation following tACS applied at two different frequencies. The data suggests an increase in the FDI TMS map area following 140 Hz tACS, in line with previous finding of increased CSE (Moliadze et al., 2010a), and a decrease in area following 20 Hz tACS. This trend was only visible in the TMS mapping data and was not found in the SR curve data. Notably, this is the first study that has been able to acquire data for both the TMS maps and SR curve in a short period of time in the same participants following a plasticity inducing protocol.

It would be odd that changes in the TMS map area would not be reflected at any point in the SR curve. Ridding and Rothwell (1997) claim SR curves and TMS maps provide similar information. They conclusively demonstrate that changes in TMS map area are reflected in the SR curve. To quantify changes in the SR curve the AuC was used in this thesis, a measure that is highly reliable for both proximal and distal muscles (Carson et al., 2013). The AuC is a measure of global excitability which cannot quantify any subtle changes in a specific intensity range. The same holds true for the slope of the SR curve. Recently, it has been questioned how good the Boltzmann sigmoid curve and extracted parameters are for quantifying CSE (Goetz et al., 2014). In contrast, Ridding and Rothwell (1997) did not make use of a Boltzmann sigmoid curve to model the SR curve but used a multiple analysis of variance and found an increase in MEP_{pp} at different intensities. Possibly, when discrepancies in the results between SR curves and TMS maps are found this means only specific subset of interneurons

was modulated. The parameters extracted from the Boltzmann sigmoid curve are not able to quantify this subtle change whereas the TMS map may.

Notably the parameters of slope, I_{50} and MEP_{max} as extracted from the fitted Boltzman sigmoid curve do not have a strong statistical basis, e.g. a high interindividual variability, to detect changes on a group level. This might explain why those have not been frequently used to detect quantify changes in excitability despite their high reliability (Carroll et al., 2001). Researchers seem to fall back on using repeated measures analysis on the individual intensities stimulated at and use the curve parameters on a descriptive basis (e.g. Suzuki et al., 2012, Gangitano et al., 2002) or fit the SR curve based on a linear regression line (Rosenkranz et al., 2007b). Despite these issues, which warrant further investigation, the reduction in acquisition time for both the SR curve (Mathias et al., 2014) and the TMS map makes it appealing to use the two techniques simultaneously or select the appropriate technique based on the working hypothesis. One could also consider use of the SR curve to determine I_{50}, and perform mapping at this intensity to allow an equal amount of inhibition and facilitation. This prevents recruitment of different sets of interneurons in each participant when using a fixed percentage of the threshold, possibly reducing interindividual variability of the TMS mapping results.

9.4 Variable response to plasticity inducing protocols

It deserves attention that the response to any plasticity inducing protocol seems to be highly variable. In chapter 6-8 only 20-50% of the tested participants responded in the hypothesised way, with either an increase, decrease or lack of change in map area. There is a large body of literature that tries to explain the variability in the response to externally driven plasticity (for excellent review: Ridding and Ziemann, 2010), which likely also holds for internally driven plasticity. Here, the most important factors related to the findings in this thesis will be discussed.

Plasticity has been suggested to be affected by various participant characteristics of which age, gender, hand preference, physical activity and genetic profile are those mostly discussed. It shouldn't come as a surprise that ageing is associated with impairments in memory and learning, and that the capacity to

undergo synaptic plasticity reduces (for review: Barnes, 2003). This has been linked to a reduced capacity to undergo plasticity following PAS (Muller-Dahlhaus et al., 2008, Tecchio et al., 2008, Fathi et al., 2010) and rTMS (Todd et al., 2010). Motor learning is also declined as reported for mice (Tennant et al., 2012) and human participants (Cirillo et al., 2010, Cirillo et al., 2011, Rogasch et al., 2009), although results are not univocal. Participants in this thesis were almost exclusively undergraduate students aged between 18-25y old and therefore the effect of age can be considered negligible.

It is well documented that there are differences in cortical processes underlying plasticity in males and females (Galea et al., 2006, McEwen, 1994). Two tDCS studies suggest greater changes (both facilitatory and inhibitory) in female than male participants (Kuo et al., 2006, Chaieb et al., 2008). This might be related to the phase of the menstrual cycle female participants are in when tested (Inghilleri et al., 2004, Smith et al., 1999). In our studies the majority of participants were female (59%). No screening was performed for their phase in the menstrual cycle and therefore the possibility this has affected our results cannot be excluded. With the small sample sizes it was deemed irrelevant to perform regression analysis to quantify any differences in plasticity between gender.

Hand preference has also been suggested to affect plasticity with greater plasticity associated with training of the non-dominant hand (Cirillo et al., 2010). This is not supported by the findings in this thesis (Chapter 7) and several other studies (Ridding and Flavel, 2006, Garry et al., 2004, Gallasch et al., 2009). For convenience, the right hand was used in all participants and all other studies. In 90% of the participants this involved the dominant hand and given the equivocal results it is unlikely this has negatively contributed to the observed variability. More important than hand preference might be the genetic profile of the participant, which has been suggested to mediate plasticity. The brain derived neurothropic factor (BDNF) gene is the most studied genetic marker and has a significant role in promoting changes in synaptic efficacy (Bramham, 2008). A single mutation of BDNF has been demonstrated to result in reduced internally and externally driven plasticity (Kleim et al., 2006, Cheeran et al., 2008). Finally, active individuals have been found to show greater changes in CSE than

sedentary individuals following PAS (Cirillo et al., 2009). Almost all participants in the study in this thesis were students of the School of Sport, Exercise and Rehabilitation sciences, and therefore a significant part of our sample may be considered to be physically active. In conclusion, young adults that are physically active, as used in this thesis, seem ideal candidates to study plasticity.

Other factors that have been associated with variability in plasticity are related to the experimental conditions and involve the attentional focus of the participant and time of the day the participant is tested. There are several studies reporting a greater degree of plasticity when attention is focussed on the stimulated hand following PAS or muscle vibration (Stefan et al., 2004, Rosenkranz and Rothwell, 2004), or following TBS when a task is performed simultaneously without creating a high attentional load (Kamke et al., 2012). Experiments in this thesis were undertaken with the participants instructed to remain relaxed and focus either on the neuronavigation software or watch a neutral content video (Chapter 8). In general participants were found to attend to what they had been asked to attend to, without getting drowsy. With respect to attentiveness also the time of the day the experiment is performed has been found to be an important mediator of plasticity. This was only the case for PAS induced plasticity (Sale et al., 2007, Sale et al., 2008), where PAS was more effective in the afternoon and evening, but not plasticity following a ballistic motor learning task (Sale et al., 2013). This difference may be linked to the participant being passive during PAS and active during motor learning, keeping the participant more engaged and attentive, thereby promoting plasticity. All experiments in this thesis were performed same time of the day, when multiple sessions were involved, to reduce within subject variability.

9.5 The use of TMS assessments in clinical practice

The previous section highlighted that central nervous system plasticity can be altered by many intrinsic and extrinsic factors in healthy participants. It also raises an important point, which is whether we can translate our findings to the lesioned brain. As mentioned in the introduction of this thesis, TMS can be a valuable tool in assessing plasticity in relation to motor recovery (Byrnes et al., 2001, Liepert et al., 2000, Chieffo et al., 2013), disability (Kesar et al., 2012, Freund et al., 2011) or cortical degeneration (Cantone et al., 2014). One of the reasons TMS has not made its way into the treatment rooms of physiotherapists and neurologists is the time it requires to obtain data to construct a TMS map or SR curve. This is well illustrated in two papers where it is explicitly stated that the number of stimulation sites and number of stimuli was limited to *'achieve an acceptable compromise between the duration of the examination and a clinically suitable test*' (Cicinelli et al., 1997, Traversa et al., 1997). The lengthy acquisition procedure associated with TMS mapping makes researcher often prefer to use SR curves, for which the acquisition time can be reduced more easily whilst still providing reliable measures of CSE (e.g. Pomeroy et al., 2014). In this thesis we have demonstrated it is possible to acquire the data for a TMS map in 2 min.

Now that TMS mapping can be done in two minutes, there is ample opportunity to use TMS mapping especially in a patient population. In particular in the lesioned or malfunctioning brain, TMS mapping can provide invaluable additional information to SR curves as the cortical representation might be distributed through the cortex or expand unevenly. TMS mapping has been reported to enable accurate localisation of the cortical representation and enable quantification of motor cortex plasticity when validated against fMRI (Boroojerdi et al., 1999, Krings et al., 1997a, Lotze et al., 2003). TMS mapping can be used during rehabilitation for prognosis, monitoring of progress and to guide the rehabilitation process (Stinear et al., 2007). For example, in stroke rehabilitation where the patient gets regularly seen by a physiotherapist TMS mapping can now be performed in about 15 min (including set-up). This allows monitoring plasticity on a weekly basis, and using the results to guide and optimise the rehabilitation process based on the neuroplastic response. Another application of rapid

TMS mapping could be to assess efficacy of botulinum toxin injection to reduce spasticity in patients with spastic paraparesis or cerebral palsy. The benefits associated with these injections have been suggested to be a result of cortical reorganisation (Pauri et al., 2000, Redman et al., 2008). This is a procedure often performed in children where it is advantageous not to have a lengthy boring procedure. Moreover, it will provide clinicians with an easy to obtain neurophysiological measure aside standard spasticity and functional measures. Unfortunately, we have not been able to test the rapid TMS mapping method in a patient population. Stimulation may have to be administered at a slower rate in patients, but even when the ISI is increased to 4 s, data can be acquired in about 20 min.

It deserves attention that TMS mapping is recognised as a feasible and cost effective tool in neurosurgery to define resection areas for tumours in the motor region (Picht, 2014, Sanai and Berger, 2010). Multiple studies have demonstrated that TMS can be reliably used to guide surgical decision making and treatment (for review: Takahashi et al., 2013). Importantly, the results of using TMS are reliable and correlate well to those obtained using direct electrical stimulation of the cortex, a method that was long considered the golden standard (Krings et al., 1997b, Zdunczyk et al., 2013). It has even been argued that pre-operative TMS mapping in addition to direct electrical stimulation leads to more extensive resections and better functional outcome (Krieg et al., 2014). Mapping of brain tumours is commonly performed with a 4 s ISI (Takahashi et al., 2013). In Chapter 3 we have shown mapping can be performed with an ISI of 1 s without affecting the TMS map. Whereas the map is unaffected by ISI, the study described in Chapter 4 demonstrated stimulation intensity affects the TMS map area mainly as a result of current spread. This means the choice of stimulation intensity is a critical part of the mapping procedure to define the resectable brain area. Intensities closer to the motor threshold will minimise the effect of current spread on the defined TMS map area but also result in the MEP variability being increased. The latter may complicate accurate definition of the tumour fringe and therefore a trade-off between an accurate characterisation of the tumour fringe and MEP variability at intensities near threshold needs to be found. It is this reason that TMS can never be used exclusively to guide the surgical procedure, and direct electrical stimulation will always be required to explore

critical areas near the tumour fringe. The choice of intensity is complicated by the definition of the motor threshold, which may also fluctuate from time-to-time. Accurate localisation of the tumour fringe not only relies on stimulation intensity but also accurate registration of the patients with respect to the obtained MRI scan. Registration errors are commonly in the order of 1-2 mm, which becomes critical when resecting brain tissue. However, with the reduction in acquisition time, TMS mapping may now be performed directly prior to surgery rather than in a separate session. This prevents registration errors and benefits both the patient, surgeons and hospital as the time the patient needs to be hospitalised is reduced.

9.6 Recommendations for future work

The ultimate aim of improving the TMS mapping and SR curve method is to get the techniques used on a day-to-day basis to assess neuroplasticity in the clinic. Throughout the time of my PhD I have been so lucky to work with our Italian partners to validate the rapid SR curve acquisition method in a small group of stroke survivors. These tests gave me confidence that rapid acquisition of TMS measures is feasible in a patient population, however both for TMS mapping and the SR curves this has to be validated. This process could be facilitated by the development of a direct feedback system for the TMS maps, similar to what was done for the rapid SR curve acquisition method in Chapter 5 of this thesis. The major advantage of direct online feedback about the curve or map is that the number of stimuli acquired can be tailored to the individual, thereby circumventing the great interindividual variability in the number of stimuli required to construct a reliable map or curve. This would not only allow further optimisation of the number of stimuli needed and thereby the acquisition time, but in case of the mapping also allow distribution of the stimuli in such a manner that the fringe of the map is accurately mapped (especially important in neurosurgery). Brasil-Neto et al. (1992) reported that the MEPs do get more variable when stimulating away from the motor hotspot. As a result, not the same density of stimuli is required near the motor hotspot as in the periphery of the map to get a good estimate of CSE. In order to get the best estimate of the cortical representation using direct online feedback, more stimuli could be administered close to the fringe of the representation, whereas near

the motor hotspot only a limited number of stimuli is required. Moreover, it would allow for mapping without a predefined fixed grid reducing the chance of unnecessarily stimulating a big area of the cortex or finding the cortical representation extended beyond the predefined grid.

Further to the improvement of the TMS assessment techniques it is critical that more understanding is gained about what drives and affects neuroplasticity. Although the variability of plasticity induced by different non-invasive brain stimulation techniques is now widely accepted, there is limited evidence on the variability of internally driven plasticity. The findings in this thesis highlight that plasticity in response to motor learning is probably as variable, however, the possibility that this has been caused by the used learning tasks cannot be fully excluded. It would be good to see a study performed in which different form of learning are compared, e.g. ballistic motor learning, visuomotor learning, sequence learning and also a more ecological valid form of learning like learning to play darts. This would allow us to further untangle what explains the variability in internally driven plasticity. It might be the various task demands but also the participants personal skill set the affects the plastic response. At the same time the possibility should not be excluded that limitations of the TMS assessment techniques cause the observed variability, leading us to suggest that a canonical response seems not to exist. It would be valuable to assess plasticity with multiple imaging modalities, combining strength of multiple imaging techniques (e.g. fMRI and TMS). These studies may provide an explanation for the disassociation between participants. In addition, better standardisation of TMS assessment techniques; especially closer examination of the effects of stimulation intensity and pulse waveform on the recruitment of different interneuronal circuits, is required to improve selectivity of the corticospinal circuitry assessed.

9.7 Concluding remarks

This thesis presents improvements on studying neuroplasticity using TMS maps and SR curves. It allows for rapid acquisition of a great number of measures that can quantify changes in CSE. These improvements may bring TMS a step closer to being used in clinical practice on a daily basis, for which it has been proven to provide valuable insights for assessment, prognosis and treatment. It was demonstrated that both TMS mapping and SR curves are a feasible way of assessing changes in CSE in response to internally and externally driven plasticity in healthy participants.

Appendix A

Behind the map

Data acquisition: Collecting the EMG and neuronavigation data

Data acquisition for the TMS maps is started after determining the hotspot and motor threshold. Frameless stereotaxy (BrainSight 2, Rogue Research Inc, Montreal, Canada) was used to define a 6 x 6 cm grid as indicated by blue markers (see Figure 1-1A – right panel).The position and trajectory of each stimulus was illustrated on the display immediately after it was acquired. Experimenters were instructed to use this feedback to adjust coil position and orientation whilst stimuli were delivered at a constant interstimulus interval (typically 1.5 s). Moreover, experimenters were instructed to attempt to ensure the stimuli were equally spread across the grid, and not too stimulate twice in close proximity. The resulting grid of data was most consistent if the first four stimuli were delivered close to the blue corner markers of the grid. Thereafter, the procedure continued by pseudorandomly stimulating across the 6 x 6 cm square, with the location of successive stimuli determined by the experimenter.

Data analysis: How the map is created

Figure 1-1 in Chapter 1 illustrates how the EMG and neuronavigation data are used to construct a corticospinal excitability map. Maps were created offline with a bespoke MATLAB script (MATLAB Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). For all EMG recordings the MEP was quantified by its peak-to-peak (MEP_{pp}) value, which was extracted from a window 20—50 ms after the stimulation (Figure 1-1A). The corresponding stimulation position in 3D space was extracted from the neuronavigation data. BrainSight makes use of the Polaris Vicra optical tracking system (NDI Medical, Ontario, Canada), which has an accuracy of 0.5 mm.

Three different coordinate systems were defined enabling transformation of the data from MRI coordinates to real world coordinates. The output data from the neuronavigation system includes a transformation matrix relating the orientation and position of every stimulation site to a global, MRI based, reference coordinate system (CSref).

$$BrainSight_{out} = \begin{bmatrix} X_{ref} & X \cdot x & X \cdot y & X \cdot z \\ Y_{ref} & Y \cdot x & Y \cdot y & Y \cdot z \\ Z_{ref} & Z \cdot x & Z \cdot y & Z \cdot z \end{bmatrix} \quad (1)$$

Stimulation position (X_{ref}, Y_{ref}, Z_{ref}) is expressed relative to the origin of CSref (x, y, z) located in the bottom left corner of the MRI (frontal view). Thereby, the x-axis runs parallel to the mediolateral axis, the y-axis parallel to the dorsoventral axis and the z-axis parallel to the superoinferior axis. A coil-based local coordinate system (CScoil; X, Y, Z) was used to determine the orientation of each stimulus. The stimulus position is given in millimetres while the orientations are expressed as direction cosines (in radians) representing the angles between the different axes. A third coordinate system generated from the cloud of position data represents the orientation of a plane fitted through all stimulation positions (CSFit) (Figure S A|B).

CSFit was determined by fitting a rectangular plane through the cloud of 3D position data. Using the assumption that every z-coordinate is functionally dependent on its respective x and y-coordinate (x, y, f(x,y)), the fitting function is defined as:

$$\hat{Z}_{ref} = AX_{ref} + BY_{ref} + C \quad (2)$$

The plane fit was created using a least squares algorithm optimising a three parameter (A, B, C) error function:

$$Plane\_Fit(A,B,C) = \sum_{i=1}^{NrStim} \left[\left(AX_{ref,i} + BY_{ref,i} + C\right) - Z_{ref,i}\right]^2 \quad (3)$$

This hyperparaboloid function is solved by finding the combination of parameters (A,B,C) which give the minimum error between $\hat{Z}_{ref}$ and Z_{ref}. This corresponds to the combination of parameters where the integrated error function leads to a zero gradient in x, y and z:

$$\nabla E = \begin{bmatrix}0\\0\\0\end{bmatrix} = 2\sum_{i=1}^{NrStim} \left[\left(AX_{ref,i} + BY_{ref,i} + C\right) - Z_{ref,i}\right] \begin{bmatrix}X_{ref,i}\\Y_{ref,i}\\1\end{bmatrix} \quad (4)$$

Written in matrix form, the equation becomes:

$$\begin{bmatrix} \sum {X_{ref,i}}^2 & \sum X_{ref,i} \cdot Y_{ref,i} & \sum X_{ref,i} \\ \sum X_{ref,i} \cdot Y_{ref,i} & \sum {Y_{ref,i}}^2 & \sum Y_{ref,i} \\ \sum X_{ref,i} & \sum Y_{ref,i} & 1 \end{bmatrix} \begin{bmatrix}A\\B\\C\end{bmatrix} = \begin{bmatrix} \sum X_{ref,i} \cdot Z_{ref,i} \\ \sum Y_{ref,i} \cdot Z_{ref,i} \\ \sum Z_{ref,i} \end{bmatrix} \quad (5)$$

This is an easily solvable three parameter (A, B, C) equation. The best fit plane is then solved by inputting the resulting parameters A, B and C input to equation 2 (Figure SC). These parameters were only determined once for each mapping session, using the first map data collected. Consequently, CSFit was expressed as the direction cosines matrix to CSref and used to define the orientation of the fitted plane. All position data were then transformed from 3D space to a 2D plane centred on the origin of CSref. An extra rotation was performed if the sides of the grid were not aligned with the X and Y axes of CSref (Figure S D).

Triangular linear interpolation was used to calculate an approximant that was subsequently used to create a full surface map within the transformed plane. This was calculated using the 'gridfit' MATLAB function (D'Errico, 2005). This function uses a plane that is deformed using non-linear least

squares methods to best fit the data. Two settings determine how this plane is transformed to best fit the data. The sensitivity (stiffness) of the plane defines how sensitive it is to rapid changes. The gridfit function allows for sensitivity range between 1-10. Using pilot data, we chose to use a sensitivity value of 2 as this afforded high sensitivity for rapid changes without over smoothing the variability. In addition, the function uses an interpolation density (step size) that defines the number of points with which the fitted value is approximated based on the acquired data. The grid was divided into 2500 partitions (50×50), with each point being assigned an approximated MEP value (aMEP) based on the nearest acquired MEP data (Figure SE). The result is a 2D representation of the corticospinal excitability akin to a contour plot (Figure 1-1B). A 3D corticospinal excitability map is also created using aMEP on the Z-axis (Figure 1-1B). In order to compare maps between participants, the colour bar was normalised to the minimum and maximum MEP value within a session.

Exclusion criteria

Before the data was fitted with the rectangular plane and transformed to the origin of the CSref coordinate system, individual stimuli within a map were excluded based on four predefined criteria:

RMS of background EMG: RMS value of 45 ms EMG (50 – 5 ms preceding stimulation) was calculated for each individual EMG record. Mean and *SD* of all RMS values were then calculated and used to exclude EMG recordings exceeding mean + 2 *SD*. To limit the amount of data excluded by excessive background EMG, feedback was provided to the participant about their level of EMG during the experiment.

Position in 3D and 2D: As the plane fit (Equation 3) was needed to transform the data from 3D to 2D, any outliers would worsen the fit and result in an inaccurate transformation. Therefore, to avoid stimuli outside the predefined grid affecting the plane fitted through the stimuli positions an initial transformation from 3D to 2D in CSref was calculated using the grid's orientation matrix as derived from the output of the neuronavigation software (Equation 1: $\text{BrainSight}_{\text{out}}$). Subsequently, all stimulation positions exceeding the sides of the grid by more than 20 mm in either X or Y when

transformed to the origin were excluded from further analysis. This value was chosen based on pilot testing. Next, all data were transformed back to 3D to determine the plane fit according to Equation 3. After transformation to a 2D plane using the fitted plane, any stimuli exceeding the sides by more than 10 mm away were also excluded. In this case, 10 mm was used as it was found that stimuli delivered near the border of the grid as observed in BrainSight were usually found just outside the predefined grid when projected in a 2D plane. Accordingly, stimuli outside the grid but within 10 mm were included and the grid enlarged. However, the same grid size was used for all maps in a participant; therefore grid sizes differed slightly between, but not within, participants.

Extreme MEP outliers: MEP values exceeding mean + 3.5 *SD* of all MEP values within a map were excluded to avoid skewing the map based on a single MEP. As this criteria might be closely correlated with background EMG it was checked how many stimuli of the stimuli excluded on this criteria were also excluded based in the background EMG criteria. In total 55% of the stimuli excluded based on this criteria was also excluded based on a too high background EMG.

Angle and translation relative to skull surface: The positioning of the TMS coil relative to the scalp is important to reduce MEP variability (Werhahn et al., 1994, Mills et al., 1992). Therefore the coil angle and translation relative to the scalp were used for exclusion. A single quadratic 3D surface was fitted through obtained neuronavigation data, to represent the skull. Best fit was determined for the transformed data in CSref:

$$\hat{Z} = A_1 + A_2 X_{ref} + A_3 Y_{ref} + A_4 {X_{ref}}^2 + A_5 {Y_{ref}}^2 + A_6 X_{ref} Y_{ref} \quad (6)$$

Translation and angle of each stimulus was determined relative to the fitted surface. Translation was expressed as the distance between the fitted surface Z-coordinate ($\hat{Z}$) and the actual stimulus Z-coordinate (Z_{ref}). The angle was calculated using $BrainSight_{out}$ to extract the CScoil. Thereby the

direction of each axis of the coil is known (X_{coil}, $Y_{coil,}$ Z_{coil}). We also calculated the perpendicular axis (Z_{scalp}) to the derivatives in x and y direction of CSref at the stimulation location (X_{ref},Y_{ref}) of the quadratic 3D surface fit. Calculating the angle between Z_{scalp} and Z_{coil} gives a comparable measure for coil orientation relative to the scalp. Exclusion was based on the translation or angle falling outside the 99 % prediction interval.

In addition to taking precautions to reduce map variability, the TMS map was made less sensitive to MEP variability by the algorithm used to create the map. It has been suggested that the relative variability of MEPs near the border of the map is larger than the variability associated with MEPs recorded closer to the hotspot, and that this is the main source of the observed COG variability (Miranda et al., 1997, Uy et al., 2002). Moreover, Brasil-Neto et al. (1992b) suggested more stimuli should be delivered at positions further away from the hotspot in order to achieve equal maximum error in determining the MEP_{pp} value at these positions. Both problems are reduced by the adopted method of creating a map. A plane is fitted through all acquired data; with a stiffness setting that determines the flexibility of the surface. The stiffness setting of the fitted surface prevents skewing of the fitted plane as a result of greater variability in the periphery and thereby reduces the sensitivity of the map parameters to this local variability. In addition, in contrast to Brasil-Neto et al. (1992b) we suggest that using this method of creating the map it is possible to use fewer stimuli in the periphery and more near the 'hotspot', in order to achieve a higher spatial resolution in this most excitable area.

In total 8.2% of all stimuli were excluded before analysing the maps (180 maps analysed). Most stimuli were excluded due to high background EMG (4.2%) or angle and translation of the stimulus with respect to the skull (3.3%). For each map between 5 – 11 (8 ± 3) stimuli were excluded based on these predefined criteria.

Map parameters

Traditionally, the map area is defined by the number of excitable scalp sites and their distribution, typically a 1-cm spaced grid, with multiple stimuli per site (Wassermann et al., 1992). In the present study, a map was created using a fixed grid size and by stimulating at random positions. A map was constructed from the grid position and EMG records by approximating the MEP size for 2500 partitions within the 6 x 6 cm grid. The map area was calculated by taking the ratio of the number of approximated partitions where the approximated MEP exceeded 10% of maximum approximated MEP ($aMEP_{10\%}$) relative to all partitions (N_{total} = 2500). This method is based on Uy et al. (2002), who demonstrated that the 10% cutoff reduces the variability of the area by excluding the small variable MEPs near the boundaries of the map.

$$area = \frac{N\ (aMEP_{10\%})}{N_{total}} \times area_{map}$$

Where $area_{map}$ is the total mapped area of 36 cm^2.

Accordingly, map volume was the sum of all $aMEP_{10\%}$, subtracted by the 10% level. The volume was normalised to the maximum volume found in all maps acquired during a single session.

$$volume = \frac{\sum aMEP_{10\%} - 0.1 \times N\ (aMEP_{10\%}) \times aMEP_{max}}{MaxVolume}$$

COG is an amplitude weighted mean position of the map (Wassermann et al., 1992).

$$xCOG = \frac{\sum(x\ \cdot aMEP)}{\sum aMEP}$$

$$yCOG = \frac{\sum(y\ \cdot aMEP)}{\sum aMEP}$$

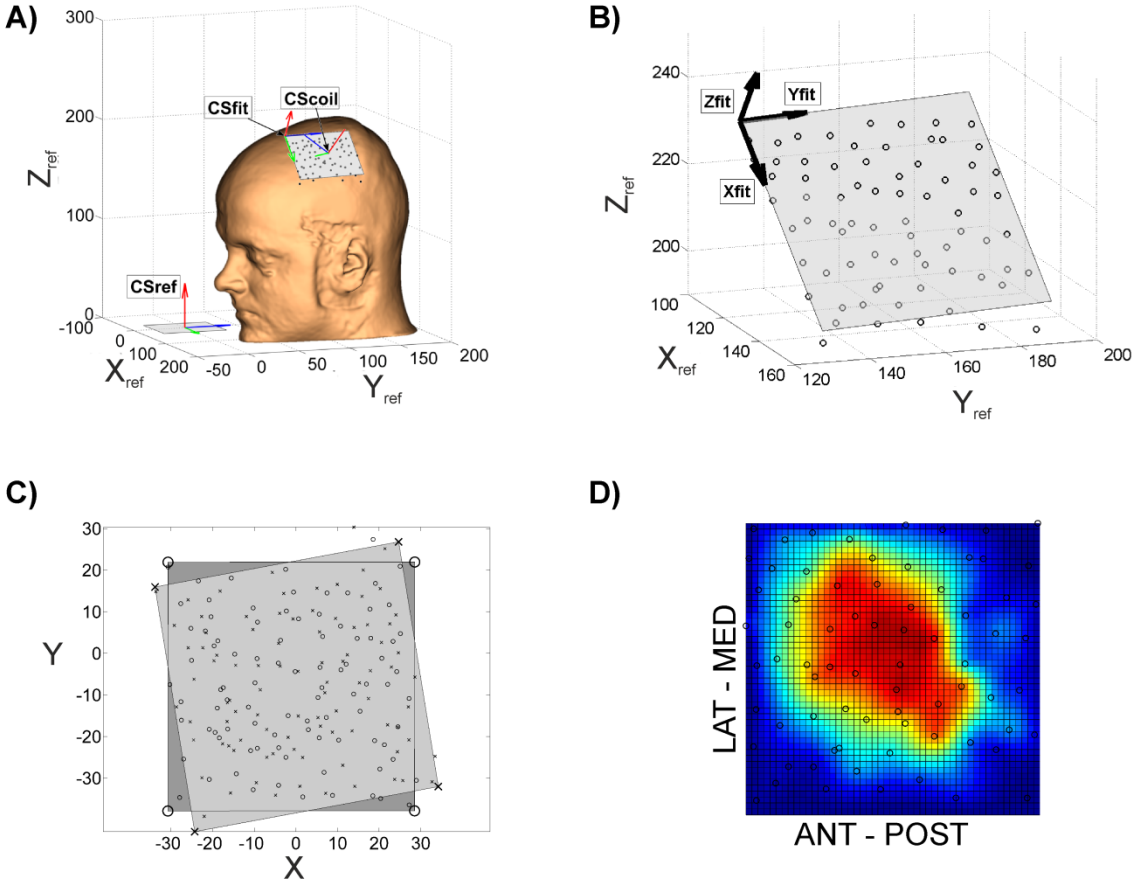

Figure S: This figure highlights how the neuronavigation data is processed to create a 2D TMS map. (A) Three coordinate systems are used with x, y and z direction indicated by the green, blue and red arrow respectively. First, a global MRI based coordinate system (CSref) wherein all stimulation position is defined. Two local coordinate systems are used, one coil based (CScoil) to determine coil orientation and (B) one calculated (CSFit) based on a rectangular plane fitted through the data that contains the position of each stimulation administered. This plane fit is used to transform all neuronavigation from 3D to a 2D plane. (C) To align the grid with the X and Y axis of CSref an extra rotation of the transformed fitted plane is performed. Subsequently, every stimulus is matched with the from the EMG extracted peak-to-peak value of the MEP (D) To create the map an approximant is used to fill all 2500 (50 x 50) partitions of the grid based on the nearest acquired MEP data.

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