VARIABILITY OF THE MOTOR EVOKED POTENTIAL

By

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Abstract

Background - The use of transcranial magnetic stimulation (TMS) to assess corticospinal excitability is troubled by trial-to-trial variability of the motor evoked potential (MEP). Many factors have been suggested to contribute to this variability including a range of methodological factors. Coil movement in the yaw axis has been studied extensively however it is unclear how the MEP is affected by coil movement in the roll and pitch axes. Moreover, setting the stimulation intensity to evoke MEPs with a 1 mV amplitude is becoming increasingly common however the reliability of measures taken using this method has yet to be investigated.

Objective – *(1)* To assess the short term (≈ 1 h) reliability of MEPs. *(2)* To investigate the effect of coil rotation in the roll and pitch axes on MEP amplitude, providing and defining the tolerance of coil movement in these two axes.

Method – Two experiments were performed. (1) Reliability of 1 mV MEPs was assessed by acquiring 8 sets of 30 stimuli, one set every 4 min, with an interstimulus interval of 4 s. Bland-Altman plots were used to assess reliability. *(2)* The effect of coil tilt in the roll and pitch axes was assessed by acquiring 25 MEPs in 3° increments over a range of 60° around each axis. 70% of peak MEP amplitude defined the range of angles in which MEP amplitude is not affected by coil tilt.

Results – (1) Bland-Altman plots revealed a consistent lack of agreement between MEP averages compared to the first set with a tendency for MEP amplitude to increase on average 0.37 ± 0.06 mV. *(2)* The range at which MEP amplitude is not affected by coil tilt is $29.59° \pm 10.02°$ for the roll axis and $17.4° \pm 4.36°$ for the pitch axis.

Conclusions – The method of setting stimulation intensity to generate 1 mV MEPs is not reliable and is therefore not recommended to be used in future TMS research. Additionally a range of angles within which coil tilt does not affect MEP amplitude has been defined. This

study has also highlighted the need for experimenters to be cautious of natural MEP variation

and attempt to control as many influential factors as possible.

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Introduction

The idea of a human motor cortex that is plastic, malleable and dynamically adapts to behavioural and environmental demands is now accepted, largely due to the advent of transcranial magnetic stimulation (TMS) allowing direct assessment of the primary motor cortex and efficacy of the corticospinal tract. TMS research has revealed the complex nature of the motor cortex which is now widely believed to play central roles in both skill learning and execution.

Since its introduction in 1985 the popularity of TMS research in neuroscience has increased, largely due to the ease with which the motor cortex and corticospinal tract can be assessed using this technique. However a commonly acknowledged problem is the variability of responses (motor evoked potential, MEP) elicited by TMS, prompting numerous investigations into the reliability of TMS measures and what can be done methodologically in order to control this variability. This has resulted in a wide range of possible methodological factors that are defined prior to the investigation, all of which can influence MEPs, which may explain why results vary across studies. In light of this, there are no strict guidelines for experimenters to follow.

The focus of this study is to address two fundamental methodological aspects of TMS studies that can influence results and therefore conclusions, and to provide recommendations regarding these methodological factors with the primary aim to improve future TMS research.

Literature Review
Introduction to Transcranial Magnetic Stimulation

Developed in 1985 (Barker et al., 1985), transcranial magnetic stimulation (TMS) made it possible to study the function of the human motor cortex, cortical physiology and motor pathways noninvasively and *in vivo*. Gaining widespread recognition since its introduction,

TMS has become an established neurostimulatory and neuromodulatory technique which continues to gain momentum. Its use in neuroscience, neurology, clinical neurophysiology and psychiatry has spread extensively, predominantly in research, though recently more focus has been drawn to clinical aims (Kobayashi and Pascual-Leone, 2003; Horvath et al., 2011). TMS has been combined with various mapping methodologies (eg, functional MRI) to study brain functions such as intra-cortical inhibition and excitation balance, cortico-cortical interactions and brain plasticity (Horvath et al., 2011).

TMS utilises electromagnetic induction as a highly effective way to painlessly generate currents in the human primary motor cortex (Rossini et al., 2015). A brief surge of current is passed through a coil held to the participant's scalp, generating a rapidly changing magnetic field which then passes into the surrounding medium, where it induces an electric field. When applied to the primary motor cortex, TMS can depolarise corticospinal tract neurons and evoke an electrical response in the contralateral target muscle, known as a motor evoked potential (MEP) (Groppa et al., 2012).

Due to the relative ease with which MEPs can be recorded through electromyography, single-pulse TMS soon emerged as a routine method to investigate the integrity of the central nervous system, known as corticospinal excitability (CSE). An MEP is a quantifiable marker, providing an assessment of the physiological state of the corticospinal tract; the principle being that the magnitude of the MEP reflects the excitability of the corticospinal system. As a result there is now a vast body of work utilising TMS to probe changes in the human corticospinal motor output system in different situations, such as in response to skill learning (Pascual-Leone et al., 1995). TMS studies have also been described in many neurological disorders, from multiple sclerosis (Hess et al., 1987) to stroke (Liepert et al., 2000), and its use is now well-established in clinical neurophysiology.

Single pulse TMS measures are regularly used to provide evidence of situation specific changes in motor cortical control or corticospinal output in patients (Rossini et al., 2015). Both simple and advanced measures have been devised to quantify damage to the pyramidal tract system and monitor disease progression or treatment effect (Wassermann et al., 2008). Basic measures such as the motor threshold, defined as the minimal stimulation intensity required to elicit a reliable MEP of minimal amplitude in the target muscle, or MEP magnitude, commonly expressed as peak-to-peak amplitude, are frequently applied due to their simplicity (Rossini et al., 2015) however other techniques give more detailed evaluations of CSE. Changes in the profile of a stimulus-response curve (Ridding and Rothwell, 1997) or the size and shape of a cortical map (Wilson et al., 1993) are both indicative of changes in corticospinal excitability. Both of these measures hinge on the use of the MEP amplitude to investigate CSE changes highlighting the importance of this simple, quantifiable measure (Ridding and Rothwell, 1997). Continued work advances our understanding of a range of neurological disorders (Kobayashi and Pascual-Leone, 2003) and generates increasingly sophisticated and widely applicable methodologies to be utilised in clinical neurophysiology (Mathias et al., 2013; van de Ruit et al., 2015).

Plasticity

Historically, there was little understanding of the role and function of the primary motor cortex (Sanes and Donoghue, 2000). Thought to be a fixed model with little or no capacity for adaptation; it was believed that the somatotopically ordered representation on the cortical space for each specific muscle was discrete and non-overlapping (Sanes and Donoghue, 2000). On the contrary, work of late has revealed that the primary motor cortex changes dynamically and is moulded throughout life; plasticity being the mechanism for learning, growth and adaptation to environmental changes (Pascual-Leone et al., 2005).

Initially, studies were carried out in animals due to the invasive techniques used; investigations revealed that the mammalian central nervous system has the capacity to reorganise itself functionally (Merzenich et al., 1983). Using highly detailed maps of the cortical representations of the hand area in owl monkeys, Merzenich et al., (1983) demonstrated that the basic features of cortical maps are dynamically maintained and suggest that these changes are experience dependent.

The advent of TMS (Barker et al., 1985) opened a new avenue of research which has allowed us to non-invasively and painlessly examine the role of plasticity in the human motor cortex and spinal cord; and the mechanisms that drive and influence these processes. Subsequent investigations utilising TMS have revealed the complex nature of the human motor cortex and how it is moulded by environmental changes, physiologic modifications and behaviour (Pascual-Leone et al., 2005); in particular the role of simple and complex skill learning (Pascual-Leone et al., 1995; Muellbacher et al., 2001).

The understanding that the central nervous system has an inherent ability to functionally reorganise with experience has very important implications for rehabilitation following damage imposed by a neurological injury, where there may be a lesion on the cortex causing functional impairment. By tailoring rehabilitation to exploit functional cortical reorganisation seen in motor learning we can promote cortical neurones to compensate and promote recovery. This has become increasingly prominent in light of the escalating prevalence of stroke occurring globally. Demographic and epidemiological shifts, particularly in low-income and middle-income countries, have seen the incidence of stroke exponentially increase due to the widespread adoption of sedentary lifestyles and an ageing world population (Strong et al., 2007; Mukherjee and Patil, 2011). Research using TMS has contributed greatly to our understanding of functional reorganisation and has been utilised as

a therapeutic modality to safely improve motor function (Hoyer and Celnik, 2011), a tool to predict recovery of function (Stinear et al., 2007) and investigate the neurophysiological effect of interventions designed to enhance recovery (Liepert et al., 2000).

These studies highlight the value of TMS and it use studying plasticity of the human primary motor cortex, providing insights into the complex role it has in learning and recovery from injury; revealing its plastic nature and how this can be harnessed to promote the best possible outcome of rehabilitation. TMS may be able to identify changes in motor cortex excitability prior to functional improvements, which is highly advantageous as this degree of sensitivity is not often achieved with the use of graded scales of functional motor recovery (Stinear et al., 2007), for example the Fugl-Meyer Motor Assessment, an assessment commonly used to monitor stroke rehabilitation. In these cases functional reorganisation, which is likely to precede overt functional improvements, may go undetected leaving both experimental and conventional rehabilitation methods alike to be deemed ineffective. Additionally, investigations involving healthy participants, in which TMS measures are still sensitive to detect changes in corticospinal excitability, provide a basis for the application of proposed therapy or training in patient groups.

The corticospinal tract and the motor evoked potential

The corticospinal tract is the most important motor pathway in the central and peripheral nervous system. It is comprised of bundles of axons, the cell bodies from which they stem arise in the primary motor cortex, decussating to the contralateral spinal cord before further descending to the muscle they project to (Schieber, 2007). Fibres maintain a somatotopic arrangement, matching the organisation of that in the primary motor cortex, prior to decussating to the contralateral spinal cord (Jang, 2011). Corticospinal tract neurones synapse
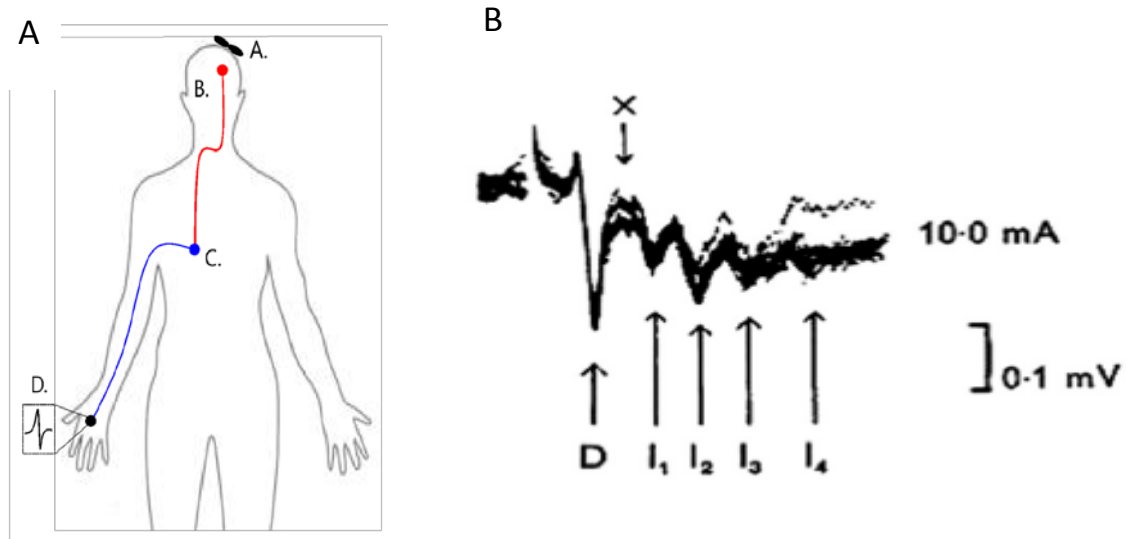
Figure 1a. When a magnetic stimulation is delivered to the motor cortex (A) pyramidal neurones are activated (B) producing action potentials which travel through to different levels of the spinal cord. These neurones synapse onto motoneurons (C). If the stimulation is strong enough to bring the motoneurons to fire, this will cause a motor response in the muscle (D). Figure 1b. Demonstration of the temporal dispersion of descending corticospinal volleys when stimulated with TMS. (Adapted from Rossini et al., 2015,)

onto lower motor neurons and interneurons to effect precise motor movements (Al Masri, 2011). The corticospinal tract is demonstrated in Figure 1a.

An MEP, elicited by a magnetic stimulus applied to the human motor cortex (Figure 2.), is comprised of a series of temporally dispersed descending corticospinal volleys which provide a quantification of CSE at the time of stimulation (Di Lazzaro et al., 2004); they are readily recorded with needle or surface electrodes placed over the target muscle (Rossini et al., 2015). Condition specific changes in the magnitude of an MEP acts as a surrogate marker for physiological changes in the corticospinal tract associated with changing circumstances. Such changes in MEP magnitude have been proposed to reflect either; changes in excitability of the same number of pyramidal tract neurons or a change in the number of pyramidal tract neurons of similar excitability activated by the TMS pulse, or a combination of both (Ridding and Rothwell, 1997).

Multiple circuits contributing to MEPs makes interpretation of changes in MEP amplitude more challenging, though a thorough appreciation of how MEPs are generated and measured allows for better use of an MEP in providing an insight into changes in the human motor system (Bestmann and Krakauer, 2015).

Conclusions related to the details of the mechanism of TMS initially had to be made indirectly through examining the form of EMG responses from target muscles. Though later, epidural recording in patients with chronically implanted spinal electrodes provided a better insight into how TMS induces corticospinal descending activity in conscious humans and confirmed previous conclusions (Di Lazzaro et al., 2004).

When the human motor cortex is stimulated with single-pulse TMS at increasing intensities, there is a paralleled increase in the number of descending corticospinal volleys recorded at the level of the cervical spinal cord (Groppa et al., 2012). These volleys are caused by highly synchronised action potentials originating from corticospinal neurons making direct connection with spinal motoneurons, seen in Figure 1a from position B to C. Distinctive descending volleys can be distinguished by their onset latency (Figure 1b.). Volleys with the shortest latency are a result of direct excitation of the corticospinal neuron at the axon hillock, known as a direct-wave or D-wave. Waves following at later intervals (1.2 – 2 ms) result from indirect, trans-synaptic corticospinal excitation known as indirect-waves or I-waves. I-waves reflect the excitation of different sets of intracortical neurons projecting onto the pyramidal neurons and occur at preferential intervals indicating a high degree on synchronisation (Di Lazzaro et al., 2004). TMS stimulation of the M1 tends to activate I-waves at lower intensities; D-waves are recruited at intensities greater than motor threshold (Di Lazzaro et al., 2004).

If the descending volleys are strong enough they will trigger action potentials in the spinal motoneurons which induce a motor response, the MEP (Figure 2.), position C to D presented in Figure 1a. The strength of the descending volleys is dependent on external (e.g. stimulation intensity) and internal (e.g. integrity of the corticospinal tract) factors. The local excitability of the spinal motoneurons coupled with the strength of the descending volleys determines the efficacy of the motoneuron excitation (Groppa et al., 2012). Therefore, spinal motor neuron excitability needs to be controlled as the excitability of the motoneuron pool can determine the size of the MEP; providing an inaccurate estimate of corticospinal integrity (Bestmann and Krakauer, 2015). For example, facilitation of MEPs when obtained during muscle contraction is thought to depend largely on an increased excitability at the spinal level.

MEPs consist of both cortical and spinal contributions and thus reflects the global excitability of cortical interneurons, fast corticospinal pathways, as well as spinal motoneurons (Cracco et al., 1999). How much each level of the pathway contributes to the resultant MEP amplitude may be difficult to dissociate as this can depend on a variety of processes (e.g. temporal dispersion) (Bestmann and Krakauer, 2015). With these caveats in mind, MEPs are still valuable measures quantifying state-changes in the human motor system. It is also important to note the differences in

Figure 2. An example MEP and its measurable features. A. The peak to peak amplitude. B. The cortical silent period. C. The MEP latency.

meanings of the terms plasticity and excitability (Ridding and Rothwell, 1997). Plasticity implies that any effects observed are due to organisational change within the primary motor

cortex itself, whereas excitability refers to changes in tonic input from other structures into the corticospinal tract (Ridding and Rothwell, 1997). Acknowledgement of the fact that an MEP represents the excitability of both the cerebral cortex and motoneuron pool allows sounder and more thorough interpretation of changes in MEP amplitude as a marker for central nervous system excitability.

An MEP has a number of different features all of which can be used as measures to assess CSE and are influenced by the integrity of the corticospinal tract. Figure 2 illustrates an example of an MEP and its measureable features. The size of an single MEP is most commonly expressed as its peak-to-peak amplitude (Rossini et al., 2015), the example in Figure 2.A shows an MEP with an amplitude of approximately 400 uV. As discussed previously, an increase in MEP amplitude is indicative of increased CSE. When an MEP is elicited during a tonic muscle contraction a period of electrical silence follows lasting up to 100 – 300 ms (Rossini et al., 2015), known as the cortical silent period (Figure 2.B.); the physiology of this phenomenon is quite complex (Rossini et al., 2015). The cortical silent period is a useful measure of changes in intracortical inhibition and excitability. The latency of the MEP, the duration between TMS delivery and the onset of an MEP (Figure 2.C.), can be used in conjunction with an additional peripheral nerve stimulation to measure central motor conduction time; reflecting the conduction between the primary motor cortex and the muscle of interest. A short latency is indicative of an efficacious motor pathway.

Factors determining the size of a motor evoked potential

MEP amplitude increases with increasing stimulus intensity, inducing a stronger descending drive which results in faster summation of cortico-motoneuronal synapses (Rossini et al., 2015); this relationship is best described by a sigmoid curve and known as the 'stimulus-response' curve, seen in Figure 3. The initial section of the curve is relatively flat

and deviates from zero at the intensity that corresponds to the motor threshold. The middle section is a linear ascending line caused by an approximately linear increase in MEP amplitude with stimulus intensity. MEP amplitude reaches a plateau at higher stimulus intensities however the MEP amplitude still varies from one stimulus intensity to the next at this plateau (Figure 3.).
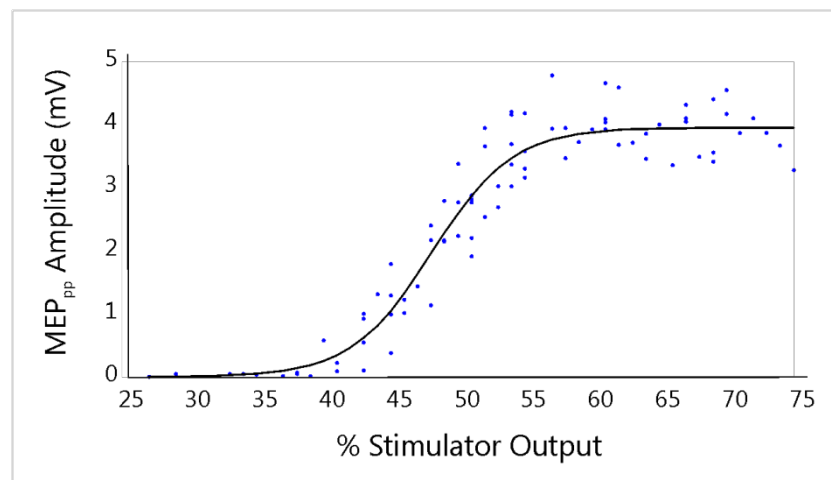
Figure 3. A scatter plot of MEP amplitude plotted against stimulus intensity with sigmoid curve fitted through data points.

The MEP can also be facilitated by a number of other mechanisms, for example through a conditioning transcranial stimulus (paired pulse) (Sommer et al., 2001), motor imagery of the target muscle (Kiers et al., 1997) and other cognitive manoeuvres (Pascual-Leone et al., 1992). The most widely reported facilitation technique is voluntary contraction of the target muscle (Rösler et al., 2002). For the hand muscles, facilitation is observed and saturates at low contraction forces (Rösler et al., 2002), for more proximal arm muscles and the lower extremities a stronger contraction is required to reach an equivalent facilitation level (Bühler et al., 2001). Facilitation is further enhanced if the stimulus is applied at the start of a contraction (ballistic) rather than during isometric contraction (Mills and Kimiskidis, 1996).

The facilitation of the MEP observed in response to voluntary contraction is caused by an increased number of spinal motoneurons brought to fire by the transcranial stimulus (Rösler et al., 2002; Z'Graggen et al., 2005).

The size of the MEP is also dependant on the localisation of the magnetic stimulus on the motor cortex and the direction of the induced electrical field. Two types of coil are predominantly used to stimulate the motor cortex; the circular and the figure-of-eight. Historically the first coil to be used, and the simplest, is the circular coil. Commonly placed on the cranial vertex, the induced current tends to be maximum near the outer edge of the coil meaning both hemispheres are stimulated simultaneously (Wassermann et al., 2008). Motor activation is considerably greater on the side in which the coil current flows from posterior to anterior across the central sulcus (Rösler et al., 1989).

When two circular coils are placed together the currents flow in the same direction at the junction, this design is known as a figure-of-eight coil. The induced current will summate and is maximum below the central segment; providing a more focused stimulation at a more definable location. Because of this, figure-of-eight coils are more commonly used in research studies (Wassermann et al., 2008). The greatest challenge with figure-of-eight coils is the need for precise and consistent placement, both position and orientation of the coil can affect the evoked response (Mills et al., 1992). Any potential differences in measures taken by the two different coils are most likely due to differences in focality of stimulation. A greater area is stimulated with a circular coil potentially exciting neurons orientated at different angles and the evoked response may spread to both sides of the body affecting reproducibility (Rösler et al., 1989; Fleming et al., 2012). A figure-of eight coil may excite other neurons though the spread will be much more restricted (Di Lazzaro et al., 2004).

Although the coil type used may have an effect on MEP amplitude, the orientation of the inducing current over the presumed motor area has found to be the most critical stimulation parameter for both coil types (Rösler et al., 1989). Using a double, figure-of-eight coil Mills et al., (1992) systematically rotated the coil around 360 degrees from a parasagittal plane over the first dorsal interosseous muscle representation in 45 degree increments. Their principal finding was that the largest MEPs were found when the coil was held 50 degrees to the parasagittal plane; an orientation that corresponds to the maximal induced current flowing approximately perpendicular to the central sulcus. More recently, Balslev et al., (2007) concluded that the optimal orientation of current direction is normally distributed around the postero-lateral orientation with a range of 63 degrees. Drawing similar conclusions, Werhahn et al., 1994 and Kaneko et al., (1996) found latero-medial stimulation elicited MEPs with a shorter onset latency than postero-anterior stimulation, due to how directly the current stimulates the corticospinal tract.

Additionally, differences in response characteristics have been observed between TMS induced currents that flow posterior-to-anterior (PA) across the central sulcus compared to current flowing in the opposite direction, anterior-to-posterior (AP). PA stimulation preferentially evokes highly synchronised corticospinal activity, whereas AP stimulation preferentially evokes less synchronised, and slightly delayed corticospinal activity (Di Lazzaro et al., 2001). Because of these differences, it is unlikely that AP and PA stimulation activate different portions of the same cortical site, but rather they activate different sites and even different populations of cortical neurons (Di Lazzaro et al., 2001).

This becomes important when considering the type of stimulator used as the direction of stimulation may differ according to the pulse configuration. A monophasic pulse consists of a strong initial current which is balanced by a dampened return current; the return current

produces no neuronal stimulation. In biphasic pulse configuration an initial rise in current is followed by a reversed and increased current. Both phases of the biphasic pulse induce physiologically significant current fluxes in the underlying brain tissue (Rossini et al., 2015). The reversal phase is longer and wider than the initial rising phase meaning the second phase induces a stronger tissue current (Groppa et al., 2012), the opposite current direction also causes stimulation of a different set of neural elements in the cortex compared to the initial rising phase. The implication of this is that monophasic and biphasic pulse configurations preferentially excite different neural elements in the cortex when using the same coil orientation (Groppa et al., 2012).

Variability of motor evoked potentials

Although the use of TMS has spread widely since its introduction there is a major practical problem of pulse-to-pulse variability of MEP amplitude. Specifically, at any given TMS strength that generates MEPs, responses vary, often quite dramatically (Kiers et al., 1993). This inter-pulse variation is of the same order as the response amplitude itself, meaning the scatter of responses can cover the full range of potential response size from microvolts to millivolts.

Due to the stochastic nature and trial-to-trial variability of MEP amplitude it is necessary for numerous, consecutive MEPs to be recorded and averaged in order to obtain a reliable estimate of CSE (Rossini et al., 2015). This becomes even more important in TMS studies in which MEP amplitude measurements are recorded several times throughout an experiment or longitudinally over multiple sessions. It has been consistently reported that reliability of TMS measures is enhanced by increasing the number of recordings taken (Bastani and Jaberzadeh, 2012; Lewis et al., 2014; Cuypers et al., 2014). However, the number of MEPs required to produce a reliable estimate is likely to change in the different situations TMS is applied.

This has led to a number of studies examining the reliability of an average of several MEPs measured from the upper arm (Kamen, 2004; Corp et al., 2015) and lower limbs (Cacchio et al., 2009, 2011; Lewis et al., 2014), though the majority of work has focused on the forearm and hand muscles (Kiers et al., 1993; Kamen, 2004; McDonnell et al., 2004; Christie et al., 2007; Bastani and Jaberzadeh, 2012). Studies have shown that reliability of MEP amplitude is muscle specific and have generally found reliability to be good to excellent (Malcolm et al., 2006) however a small number of studies have drawn contrasting conclusions (McDonnell et al., 2004).

Within-session reliability has consistently been higher in comparison to between-session reliability; it is important to establish intra-session reliability because many investigations using TMS take place over a single testing session (López-Alonso et al., 2014). However poor inter-session reliability becomes a challenge for longitudinal studies when measures are taken over the course of two or more sessions (Pascual-Leone et al., 1995). Mixed results have been found when reporting reliability of MEPs in different muscles, for example when comparing proximal arm muscles to more distal arm muscles (Brasil-Neto et al., 1992; Kamen, 2004; Malcolm et al., 2006; Corp et al., 2015). Reliability of MEP amplitude is high when measured from the biceps brachii (Brasil-Neto et al., 1992; Kamen, 2004) though whether reliability of MEP amplitude measured from the hand is higher remains unclear (Kamen, 2004; Corp et al., 2015). Differences in findings may be due to differences in study design, one comparing between session (Kamen, 2004) and one comparing within session (Corp et al., 2015).

Challenges with reliability of MEPs also arise when comparing populations with neurological conditions, particularly stroke, compared to healthy individuals (Wheaton et al., 2009; Cacchio et al., 2009, 2011; Hoonhorst et al., 2014; Lewis et al., 2014). A small number of studies have shown reliability of MEPs taken from the lesioned hemisphere of stroke

patients is comparable to the healthy population (Koski et al., 2005; Liu and Au-Yeung, 2014). However, the majority of findings support the notion that reliability is reduced in the lesioned hemisphere of stroke patients compared to the healthy hemisphere, and healthy participants, in both upper and lower limbs (Cacchio et al., 2011; Hoonhorst et al., 2014). This is clearly a limiting factor of the use of TMS to assess stroke recovery and caution should be exercised when interpreting measures taken from the paretic hemisphere.

Taken together these findings have given insights into the reliability of MEPs as a tool to assess corticospinal excitability changes. However, it becomes difficult to fully interpret the conclusions drawn from a combination of these studies as they differ considerably in methodology. For example, we need to acknowledge that between session studies will have reduced reliability and interpret these findings accordingly. There are also considerable differences in the number of MEPs recorded and evaluated upon, ranging from 4 (Lewis et al., 2014) to 20 (McDonnell et al., 2004). In light of recent findings by Cuypers et al., (2014), suggesting 30 MEPs is the minimum number necessary to obtain a reliable measure, it would seem conclusions thus far have largely been made using insufficient data. Similarly, a study has yet been conducted with a large sample size. Conclusions regarding reliability have been made with varied but generally small sample sizes (e.g. 5 participants, Kiers et al., 1993) arguably not large enough, leading to large inconsistencies in findings. Although compiling conclusions from any number of these investigations may be challenging, potential factors contributing to the trial-to-trial fluctuations in corticospinal excitability have been proposed.

Although inter-subject stimulus-response variability has been acknowledged for some time (Pitcher et al., 2003), the mechanisms that underlie the large variability in the response to an identical magnetic stimulus are not well understood. As yet, a clear contributor has yet to be elucidated meaning there could be any number of factors interacting leading to the variability

of MEPs. Of the factors that have been investigated, mixed findings and a lack of concrete evidence to show the extent to which each potential factor contributes to the trial-to-trial variability of MEP amplitude make conclusions difficult to draw.

MEP amplitude appears to vary cyclically, with a period of seconds to minutes (Wassermann et al., 2008). The basis of this cyclical variation is still unknown, however, it arises in the brain as it is not related to the cardiac (Ellaway et al., 1998) or respiratory cycles (Filippi et al., 2000). Age and gender have also been suggested to influence MEP variability. Some propose that females exhibit higher variability (Wassermann, 2002; Smith et al., 2002) due to levels of circulating ovarian hormones (Smith et al., 2002). Further, trial-to-trial variability was shown to be greater in elderly compared to young participants, specifically at low, near threshold intensities (Pitcher et al., 2003). Though contrasting findings suggest these factors are not influential (Pitcher et al., 2003; Christie et al., 2007; Cuypers et al., 2014), suggesting these may have weak associations with MEP variability.

Following findings in non-human primates, Klein-flügge et al., (2013) sought to find a link between MEP variability and the stage of action preparation. This was assessed by recording MEPs at different times during action preparation and found that prior to movement there is a temporally specific task related decline in the variability of responses; implying that trial-to-trial MEP variability tracks the state of motor preparation (Klein-flügge et al., 2013). Taking this a step further, processing emotional stimuli has been linked to action preparation and priming the motor system (van Loon et al., 2010). There is evidence that emotional stimuli or emotional state inductions modulate readiness of the action system, and by extension influence corticospinal excitability; emotional processing may fluctuate throughout a lengthy experiment, contributing the MEP variability. van Loon et al., (2010) demonstrated a typical increase in MEP amplitude as a TMS pulse was presented closer to an overt, action response

to a visual cue and this pattern was amplified by presenting both pleasant and unpleasant emotional task irrelevant stimuli compared to neutral task irrelevant stimuli.

Cortical arousal and attention may also drift throughout a laborious experiment and have also been proposed to have an impact on the variability of MEPs. Rossini et al., (1991) observed an 'idling' of the motor cortex during a relaxed, immobile state reducing MEP amplitude; thought to reflect a smaller body of cells activated by the magnetic stimuli and/or a drop in the number of spinal motoneurons brought to threshold by the descending volleys. However, increasing the attentional load either through a visual task (Kamke et al., 2012) or a mental arithmetic task (Kiers et al., 1993) does not alter MEP amplitude or variability when measured concurrently with the attentional task. Suggesting such global changes in cortical arousal cannot explain fluctuations in MEP amplitude (Kiers et al., 1993).

Any effects of attention or cortical arousal may have on MEP amplitude could be further augmented by the degrading effects of limited sleep. However, contrasting findings have been reported regarding the number of hours spent awake and the influence on CSE. De Gennaro et al., (2007) observed a significant increase in motor threshold following 40 hours of sleep deprivation, whereas Huber et al., (2013) demonstrated, using EEG and TMS, that there is a steady accumulation of excitability during prolonged wakefulness. Regardless of the influence sleep deprivation has on cortical excitability, measures rapidly return to levels observed during wakefulness following a period of sleep (Manganotti et al., 2001; Avesani et al., 2008). A study by Avesani et al., (2008) revealed changes in corticospinal excitability during different stages of sleep. Interestingly, MEP amplitudes decreased significantly in the 'sleepiness' state compared to the awakening state; suggesting drowsiness influences corticospinal excitability, progressively diminishing with the stage of sleep.

Interindividual variability and frequent oscillations in MEP amplitude were also observed during sleep (Manganotti et al., 2001), indicating no difference to consciousness. This is in line with evidence that cortical excitability shifts during neuronal oscillations in the human brain. MEPs are consistently larger when evoked during up-states of slow oscillations during sleep, compared to down states (Bergmann et al., 2012).

There is growing evidence to show that the impact of TMS on the EEG (electroencephalography) response is not only determined by the properties of the stimulus, but also by the initial state of the activated brain region (Casarotto et al., 2010). Recent studies investigating cortical excitability with EEG guided TMS have tested the hypothesis that fluctuations in neuronal activity may account for the variability in neuronal and behavioural responses to physically identical external stimuli, such as TMS (Keil et al., 2014; Ferreri et al., 2014). There is evidence to show that time-varying, spatially patterned synchronisation of brain rhythms influence cortical excitability (Ferreri et al., 2014) and evoked responses are dynamically shaped by these intrinsic neural properties at the time of stimulation (Bergmann et al., 2012). In particular, there is a correlation between the oscillatory phase in the beta-band range and MEP amplitude; MEP amplitude varies according to the phase of local beta-band activity (Keil et al., 2014).

Further to the findings that the state of the brain region stimulated impacts the response to TMS, other basic physiological mechanisms have been identified following the development of the triple stimulation technique (Magistris et al., 1998; Rösler et al., 2002). The triple stimulation technique is a collision technique using a sequence of three stimuli to the brain, ulnar nerve at the wrist and Erb's point. It has allowed the investigation of two potential causative mechanisms of trial-to-trial variability of MEPs below the level of the motor cortex:

1. Desynchronisation of evoked motoneuron discharges and 2. Repetitive discharges of motor neurons in response to a single brain stimulus (Magistris et al., 1998; Z'Graggen et al., 2005).

Findings from healthy participants indicate that a single transcranial stimulus can in fact excite virtually all motoneurons supplying a target muscle (Magistris et al., 1998). This finding combined with the observation that TMS yields responses considerably smaller than those evoked by peripheral nerve stimulation suggests the main cause of MEP size reduction is phase cancellation (positive phases of individual motor unit discharges are cancelled out by negative phases of others) of action potentials caused by desynchronisation of the motoneuron discharges (Magistris et al., 1998). Magistris et al., (1998) conclude that the reason amplitudes of MEPs vary from one stimulus to another, and between healthy participants, is primarily due to variability of the temporal dispersion of corticospinal volleys. The effect of desynchronisation also appears to be unchanged in response to differing stimulus intensities or differing amounts of facilitatory muscle contraction (Rösler et al., 2002).

Following a single brain stimulus, spinal motor neurons may fire more than once (Day et al., 1987), leading to MEPs that are less synchronised and more prolonged. By combining the triple stimulation technique with an additional nerve stimulus, Z'Graggen et al., (2005) made a number of interesting observations concerning repetitive neuronal discharges. In some participants, they will appear with a relatively high stimulus, in others, very intense stimuli in addition to facilitatory manoeuvres are necessary to produce repetitive discharges. This variability has never been quantified but appeared quite substantial in the investigation by Z'Graggen et al., (2005). Repetitive discharges also appear to have a relatively high threshold, marked amounts of repetitive discharges only occurred when over 75% of motoneurons were recruited by TMS. Repetitive discharges also vary considerably between subjects.

Aforementioned extrinsic factors such as stimulus intensity have an effect on the characteristics of an MEP. These methodological factors defined prior to data collection may also influence trial-to-trial MEP variability; other factors include voluntary muscle contraction, coil type, pulse configuration, inter-stimulus interval (ISI) and electrode size and placement.

Both longer (10 seconds) and shorter (4 second) inter-stimulus intervals (ISIs) exhibit comparable intra and inter session MEP amplitude reliability (Vaseghi et al., 2015). Similarly, the use of larger EMG electrodes does not influence the variability of MEPs (Dunnewold, 1998) and likewise, no difference in MEP amplitude was found between two different electrode placement montages (Corneal et al., 2005).

Measures made with a figure-of-eight coil have found to be generally more reliable than those made with a circular coil (Fleming et al., 2012), though contrasting findings show no significant difference in resting motor threshold between the two coil types (Badawy et al., 2011). Studies have shown measures of stimulus-response curves and cortical maps are consistent over time when using either a figure-of-eight coil (Carroll et al., 2001; Wolf et al., 2004) or circular coil (Malcolm et al., 2006) though no direct comparisons were made between the two coils in these studies.

Pulse configuration also has a significant impact on the stimulation characteristics and therefore may influence MEP variability. The complex pattern of cortical activation induced by a biphasic stimulus pulse activates a mixed combination of descending pathways, of which some will be activated by the AP and some by the PA stimulation direction (Di Lazzaro et al., 2001). Which phase preferentially activates the pathway depends on their relative threshold and the relative amplitude of the AP and PA phases. Although the second phase of the pulse is more effective, it does not consistently activate cortical neurons preferentially (Di Lazzaro et

al., 2001); meaning an unknown combination of descending pathways are activated with each biphasic stimulation.

MEP variability at the same stimulation intensity is decreased when the target muscle is tonically active compared to complete rest; voluntary muscle contraction helps to stabilise cortical and spinal excitability (Kiers et al., 1993; Carroll et al., 2001; Darling et al., 2006; Kukke et al., 2014). However, others have observed that TMS measures are equally variable when obtained in both resting and active contraction conditions (Kamen, 2004; Ngomo et al., 2012).

At high intensities when MEP response size plateaus, MEPs still vary in size trial-by-trial. However the variability of responses at higher stimulation intensities has been observed to be lower compared to intensities closer to motor threshold (Kiers et al., 1993; van der Kamp et al., 1996; Pitcher et al., 2003). This may be due to more mid-threshold neurons being recruited more consistently by a cortical stimulus of a higher intensity (Kiers et al., 1993). Conversely, van der Kamp et al., (1996) did not observe a decrease in MEP variability with increasing stimulus intensity and attributed this to the recruitment of additional motor units with successively higher thresholds; meaning equal numbers of motor units must be near their own firing threshold at both low and high intensities.

In light of this, there are no conventional guidelines regarding the most appropriate stimulation intensity that should be selected for research studies to assess corticospinal excitability. Intensity is most commonly selected as a % of resting motor threshold, usually 115 – 125% (Rossini et al., 2015) to ensure the experiment probes the MEP generated on the rising phase of the stimulus-response curve, though this is largely arbitrary. These guidelines are based on the relationship between stimulus intensity and response size, the 'stimulus-response' relationship. It is important to note that the stimulus-response relationship varies

considerably between participants (Pitcher et al., 2003), meaning if the stimulus intensity is standardised to an individual's motor threshold (e.g. 120%) the MEP amplitude reached at this intensity will differ between each participant (van der Kamp et al., 1996; Rösler et al., 2002). A study carried out 10 years ago described a method of selecting the stimulation intensity that elicits an absolute MEP amplitude (usually 1 mV) (Huang et al., 2005). However, it is not currently understood if this is an appropriate method given that the stimulus-response relationship varies so dramatically between participants; the reliability of measures obtained with the latter method are yet to be explored.

It is likely that any number of processes are occurring simultaneously to influence MEPs and their variability in size and shape from one stimulus to the next, even when the stimulus parameters are kept constant (Kiers et al., 1993; Ellaway et al., 1998). All of the above-mentioned mechanisms, physiological and physical, may contribute and it is yet unclear to what extent they interact to produce the observed trial-to-trial variability. With the current understanding of how physical, methodological factors affect the MEP and its variability, experimenters can, to some extent, control and minimise the influence they have.

The use of neuronavigation to mitigate the influence of physical factors on motor evoked potential variability

Inconsistent coil positioning within and between sessions is one of the largest sources of variability in TMS measurements (Mills et al., 1992; Brasil-Neto et al., 1992; Conforto et al., 2004). The further away from the 'hotspot' the coil is positioned, the more variable the measure will be, meaning a larger number of stimuli are required to determine representative MEP amplitudes at these suboptimal positions (Brasil-Neto et al., 1992). And as discussed previously, orientation of current direction has a profound effect on the response to magnetic stimuli (Mills et al., 1992; Balslev et al., 2007); potentially recruiting completely different groups of neurons (Di Lazzaro et al., 2001).

TMS studies are much more susceptible to physical disturbances than previously considered; with the location of stimulation the most critical parameter when assessing CSE (Julkunen et al., 2009). The positioning of the coil over the targeted cortical site has proven to be one of the most challenging aspects of TMS experimental procedures to date and has resulted in the stunted growth of TMS; as the use of any investigative tool in research relies on the minimisation of known sources of variability (Ruohonen and Karhu, 2010).

A considerable limitation with previous TMS experiments has been the lack of a method to specifically co-register the TMS coil to the brain (Wolf et al., 2004). Image-guided frameless stereotaxic neuronavigation systems have now been developed to be used with TMS to better monitor physical parameters associated with coil position and movement. These neuronavigation systems use individual structural MRI scans and a registration procedure based on facial and cranial landmarks to monitor coil position relative to a dynamic reference frame (Schönfeldt-Lecuona et al., 2005). Even with limitations, frameless stereotaxic TMS positioning is accurate in the range of millimetres (Schönfeldt-Lecuona et al., 2005; Schmidt et al., 2009). The introduction of navigation methods has shown that even minor changes in coil location and orientation significantly affect MEP amplitude (Schmidt et al., 2009), highlighting the importance of navigation systems to collect reliable data.

Navigated TMS (nTMS) systems can record, monitor and give online visual feedback of physical parameters of the TMS coil (tilt, location, and orientation) in reference to the scalp. In this way, nTMS assures a more consistent coil position held on the head. The improved spatial precision of TMS has resulted in an increased probability of eliciting responses, as well as more stable and enhanced response quality (Gugino et al., 2001; Julkunen et al., 2009). This may be due to a more precise stimulation site resulting in a more comprehensive recruitment of cortical neurons (Julkunen et al., 2009). The probability of eliciting MEPs at

lower intensities is much greater in comparison to non-nTMS when stimulating a known optimal coil location, meaning that the precision of nTMS helps to excite low threshold neuronal clusters at the lowest possible intensity. Whereas non-nTMS requires a greater intensity to achieve an equivalent response probability (Gugino et al., 2001). At higher intensities the spread of induced currents through the cortex is greater (Pascual-Leone et al., 1994) which potentially impacts the resolution of the TMS stimulation when not using a navigation system.

nTMS has not only improved the accuracy of the TMS coil and stability of responses (Gugino et al., 2001; Julkunen et al., 2009), it has improved the reproducibility of TMS coil placement within and between TMS sessions; this becomes a major advantage when applied in longitudinal studies (Schönfeldt-Lecuona et al., 2005; Julkunen et al., 2009). Within session coil stability is more repeatable than between session stability which indicates that the referencing procedure involved in frameless stereotaxy is a factor for possible imprecision when used in repeated sessions (Schönfeldt-Lecuona et al., 2005). Even with this limitation a stimulation site can be retrieved with an accuracy of 2.5mm (between session stability) and allows the investigator to maintain a stimulation site with an accuracy of 1.6mm (within session stability) (Schönfeldt-Lecuona et al., 2005). In contrast, when using non-nTMS, stimulation points have spread up to 60.2mm away from the target site over 20 repeated trials (Julkunen et al., 2009); a considerable deviation compared to nTMS and a concern for the quality of data collected using non-nTMS given measures are more variable at sub-optimal positions (Brasil-Neto et al., 1992).

It is well established that coil location and orientation are factors contributing to MEP amplitude (Schmidt et al., 2009) and variability (Brasil-Neto et al., 1992). However, no study as yet has assessed the effect of the coil tilting in the roll and pitch axes on MEP amplitude

and seems to have been overlooked as a possible factor contributing to the variability of MEPs, focus thus far has been made to the yaw axis (Mills et al., 1992; Balslev et al., 2007). This is surprising given coil tilt is monitored so readily with navigation systems and yet is difficult to maintain completely stable without; it is likely that the coil tilt will change from pulse to pulse, contributing to the pulse to pulse variability of MEPs. A recent study (Mathias et al., 2013) using nTMS retrospectively excluded samples collected if a sample was taken when the coil was held more than 2.5 mm and/or 5 degrees away from the hotspot, implying that there is a concern that even minimal tilting of the coil affects MEP response size to such an extent that samples should be removed from further analysis.

Although there seem to be obvious benefits of using nTMS, there are mixed findings regarding the reproducibility of MEP amplitude when compared to non-nTMS (Gugino et al., 2001; Julkunen et al., 2009; Jung et al., 2010; Fleming et al., 2012). Through the use of nTMS Julkunen et al., (2009) found intra-individual variation of MEP amplitude is significantly decreased in comparison to traditional non-navigated methods, this also held true for the mapped representation area of a target hand muscle which was found to be significantly more stable when using nTMS compared to non-nTMS. Although not the primary focus of the investigation, Schmidt et al., (2009) results suggest a 27% reduction in MEP amplitude variability when acquired using nTMS compared to non-nTMS. In contrast, both Jung et al., (2010) and Fleming et al., (2012) found that variability and reproducibility of MEP amplitude did not differ between nTMS and non-nTMS. Although nTMS reduced MEP variability compared to non-nTMS in one study the intra- and inter-individual variation was still very high, highlighting the need to control for other possible confounding factors (Julkunen et al., 2009).

Coil positioning is clearly a very important, controllable factor that can affect TMS measures; navigation systems help to mitigate any problems faced in maintaining a consistent coil position. However, there is clearly some ambiguity as to whether nTMS can reduce MEP amplitude variability. Nevertheless, nTMS still has a number of benefits making it a very helpful tool, namely; monitoring physical parameters on a sweep-to-sweep basis with millimetre precision, and the ability to store spatial coordinates of coil position and the quick retrieval of cortical targets.

Aims

TMS has been used extensively to assess and monitor situation specific changes in corticospinal excitability in healthy and non-healthy populations for over 30 years due to its ease of use and simplicity. However, trial-to-trial variability of MEP amplitude in response to an identical stimulus means multiple MEPs must be acquired and averaged in order to obtain a reliable estimate of corticospinal excitability. Studies attempting to elucidate the reliability of TMS measures have given some indication of how dependable TMS measures are, however methodological disparities make conclusions regarding the reliability of single MEPs difficult to determine.

The large number and variety of factors that have been proposed to influence MEPs and their trial-to-trial variability means it is important to control as many factors as possible. This study aims to investigate two methodological aspects common to TMS studies that may influence MEPs in order to provide guidelines for future TMS research.

The determination of the individual motor threshold is a fundamental procedure in all TMS studies since it provides a baseline parameter of excitability to which other TMS measurements are proportioned. Even though TMS studies have been described for 30 years there are no clear guidelines informing experimenters the most appropriate method of setting

the stimulation intensity. In a method adopted 10 years ago experimenters find the intensity that elicits an absolute MEP amplitude of 1 mV (Huang et al., 2005). This method is becoming widespread and commonly used, however the reliability of measures taken using this method has yet to be investigated. Therefore, the aim of this study was to assess the test-retest reliability of measures taken using this new method of setting the stimulation intensity. Specifically, we hypothesised that average MEP amplitude recorded with a stimulation intensity set to elicit an MEP of 1 mV will show good repeatability over eight repeated measurements.

The introduction of neuronavigation systems to monitor, record and give online feedback of physical parameters of the TMS coil has highlighted the importance of consistent coil placement on the scalp. nTMS has improved the accuracy and reproducibility of TMS coil placement and studies using navigation systems have found that even the slightest deviation from the optimum position can affect MEP amplitude; however nTMS seems to have no effect on MEP variability, remaining consistently high with or without a navigation system. Previous research into coil positioning has focused on the orientation of the stimulation in the yaw axis, for example anterior-posterior or posterior-anterior, finding that the optimal coil orientation is in a postero-lateral position. In light of this, little attention has been drawn to the effect of changing coil rotation at the scalp in the roll and pitch axes. These parameters are monitored with nTMS however not all TMS research is carried out with a neuronavigation system, it is therefore important to investigate how MEPs may be affected if the coil is not held consistently flat against the head during data collection. The present study explored the extent to which coil tilt can influence MEP amplitude with the aim to provide recommendations for future TMS studies. Specifically, we hypothesised that MEP amplitude will decrease with increasing tilt angle at the scalp.

Experiment overview

We attempted to investigate these questions through two experiments carried out in neurologically healthy participants.

To assess the reliability of 1 mV MEPs, eight sets of data was recorded with all stimulation parameters kept constant throughout. A set of MEPs was recorded once the stimulation intensity had been established and acted as a baseline to which all subsequent sets were compared. This simple test-retest experiment allowed us to examine the repeatability of measures taken using these methods. We used the recommended number of MEPs in each set and used a larger sample size than previous studies in order to get the most representative results possible.

To investigate the effect of the coil tilting on the scalp on MEP amplitude, MEPs were recorded as the coil was tilted around two axes – roll and pitch. Using a neuronavigation system, the coil was placed over the hotspot position on the scalp and tilted in one axis at a time. Using a neuronavigation system minimises any coil movement in other axes allowing us to study each axis independently. MEP amplitude was subsequently plotted against tilt angle (away from 0 – the hotspot) in order to help define a threshold at which coil tilt affects MEP amplitude.

General Methodology

Participants

A sample of convenience of 86 neurologically healthy volunteers from the University of Birmingham community were recruited for participation, with some participating in both experiments (Table 1.). Participants were screened for contra-indications to TMS using a modified version of the TMS adult safety questionnaire (Keel et al., 2001) (Appendix 1), before giving signed consent to participate in the study (Appendix 2). Participants' dominant hand was determined according to the Edinburgh handedness inventory (Oldfield, 1971) (Appendix 3). All procedures were conducted in accordance with the 2013 Declaration of Helsinki and approved by the University of Birmingham's Science, Technology, Engineering and Mathematics ethics committee.

Table 1. Key participant information for both experiments.

	Experiment 1	Experiment 2	Total
No. of Participants	26	60	86
Age (years)	20.15 (±1.46)	20.07 (±1.30)	20.11 (±1.37)
Gender (M/F)	15/11	21/39	36/50
Handedness (% Right)	96.15	87.35	91.75

Electromyography

MEPs were recorded from the First Dorsal Interosseous (FDI) muscle of the right hand using 10 mm, Ag-AgCl coated reusable surface electrodes (Digitimer Ltd, Welwyn Garden City, UK) placed in a bipolar montage (Figure 4.). The FDI is small, localised and identifiable

by palpation (Groppa et al., 2012) with a low motor threshold (Rossini et al., 2015) and therefore frequently examined in TMS research (Mathias et al., 2013; van de Ruit et al., 2015) and clinical practice (Groppa et al., 2012). By using the FDI in this experiment, the results and conclusions drawn are comparable to other work in the literature.
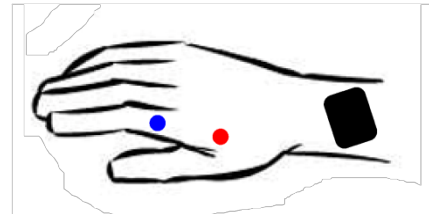
Figure 4. EMG electrode placement for the FDI.

All EMG signals were grounded using a rubber reference electrode (4.5cm x 4.5cm) placed over the head of the ulna. Signals were fed through a 50Hz noise eliminator (Humbug, Quest scientific, Vancouver, Canada) before being amplified (500 – 1k), band pass filtered (20 Hz – 1 kHz), and digitally sampled at 5 kHz using a Digitimer D360 isolated patient amplifier system. Processed signals were then passed through a NI BNC board (model 2090) and USB mass transfer onto a PC. All recorded electrophysiological signals were visualised in real-time using Mr. Kick© software (Center for Sensory-Motor Interaction [SMI], Aalborg University). Following acquisition all data was stored electronically for future offline analysis.
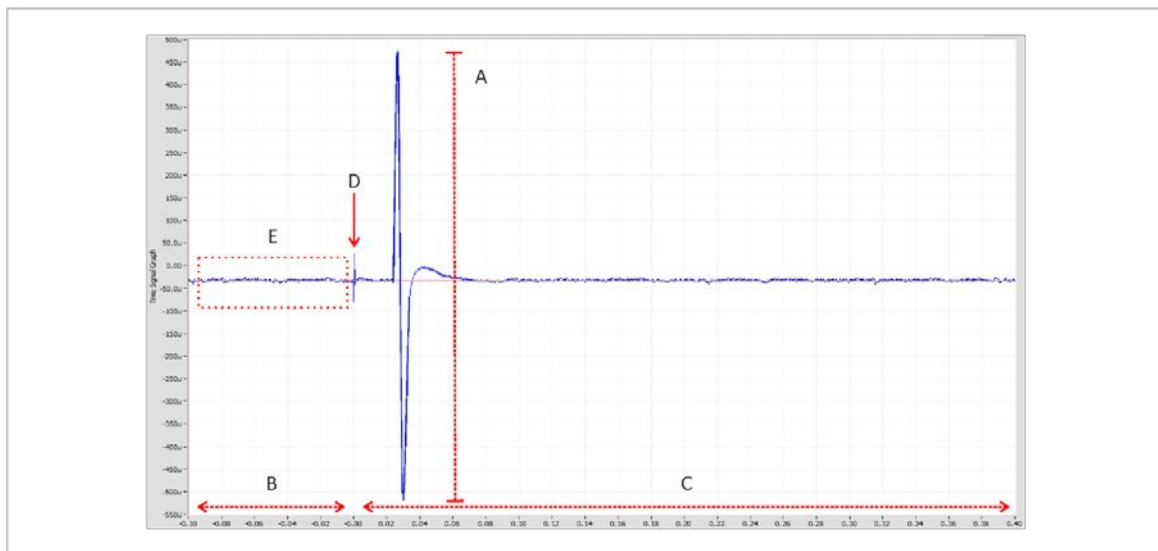
Figure 5. Mr. Kick© interface with a representative MEP. A. Represents the MEP peak-to-peak amplitude. B. Pre-trigger 0.05s. C. Post-trigger 0.45s. D. Stimulus artefact at time point zero. E. Represents background EMG. Total sweep length was 0.50 sec.

Transcranial Magnetic Stimulation

Magnetic stimuli were generated using a biphasic Magstim Rapid[2] stimulator (Magstim Ltd, Dyfed, UK) and delivered transcranially using a custom-made 90mm figure-of-eight coil, batwing design (Magstim company, Dyfed, UK) in *experiment 1* and a 70mm figure-of-eight coil, branding iron design (Magstim company, Dyfed, UK) in *experiment 2*. The use of two different coils was, in part, due to technical malfunctions at the time the experiments were carried out. Although we originally planned to use the 'batwing' coil design, the use of the 'branding iron' coil allowed the experimenters to more easily rotate the coil to greater angles; particularly in the roll axis where the curved 'batwing' coil design would have significantly impeded rotation of the coil when held on the head. It was most appropriate to use the 'batwing' coil in experiment 1 given the curved design it allowed the experimenter to better hold and maintain the coil position over the hotspot.

The coil was held at 45° 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., 1992).

Frameless Stereotaxy

Participants were seated in an adapted recumbent chair and assumed a comfortable position before placing an infrared marked elastic headband on their head (Figure 6.). The infrared reflective spheres were tracked by a Nothern Digitcal Inc.© (NDI) Polaris Vicra infrared camera and Brainsight[TM] 2 (software version 2.0.8, Rogue Research, Montreal, Canada) software enabling the real time tracking of the participants head movements. Eight pre-defined landmarks on the participants head and face were

Figure 6. Reflective infrared sphere marked headband and registration wand. In this example, 'Nose Tip' is being registered as a facial landmark.

then registered on the Brainsight^{TM} software, in reference to the marked elastic band, using an infrared marked 'wand' (Figure 6.).

The TMS coil, also marked with infrared spheres, could then be tracked in real time over the scalp in reference to the participants head position in 3D space. This allowed for accurate determination of the coil position and orientation in real time. Any deviation away from the target stimulation site on the participants' motor cortex could therefore be monitored during acquisition and regulated by the experimenter using Brainsight's^{TM} Bullseye target system (Figure 6.).

Stimulation Site and Intensity

Once registration onto the Brainsight^{TM} software was complete, the optimal site of stimulation, or 'Hotspot', was located. Single stimuli were delivered to a small area of the left motor cortex (contralateral to the right hand). The coil position was adjusted until the site which produced the greatest motor evoked potential
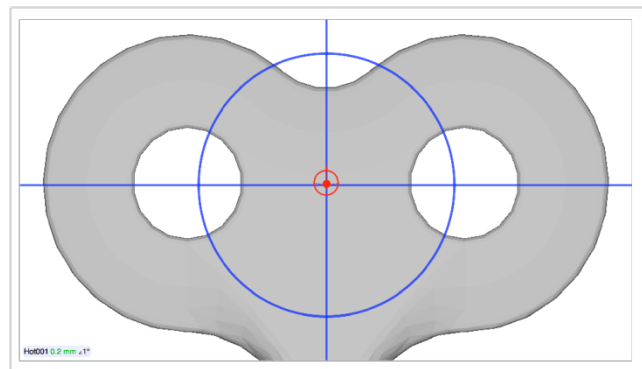
Figure 7. Brainsight^{TM} Bullseye view. Using this view the investigator can adjust the coil position so that it sits directly over the recorded hotspot stimulation site. This is achieved when the red dot and circle sit in the middle of the blue crosshairs, as illustrated here.

from the FDI was found through real-time visual inspection of the EMG trace using Mr. Kick© software. The coil position over the participants head was recorded by the Brainsight^{TM} software during each stimulation. Meaning that once the optimal position for stimulation of the FDI was located, the coils position over the scalp could be stored as a target, and later used in conjunction with the Bullseye target system during data collection.

The hotspot position was then used to determine the test stimulation intensity as; *Experiment 1.* The stimulation intensity that elicited MEPs of ~1 mV. *Experiment 2.* 120% resting motor threshold (RMT). 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 (Rossini et al., 1994).

Peripheral Nerve Stimulation

In order for MEPs to be normalised across all participants, the maximal electromyographical response of the FDI was recorded for each participant using electrical stimuli. All electrical stimuli were delivered using a constant current stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK). A bipolar probe was used to stimulate the ulnar nerve at the level of the wrist in order to elicit a response in the FDI. The current (mA) of the electrical stimuli was steadily increased from 0 until there was an apparent plateau in the peak-to-peak value of the M-wave on the EMG trace. Once the plateau was reached, the resultant value was recorded as the $M_{max;}$ and MEPs in later analysis *(Experiment 2)* are expressed as a proportion of this value to appropriately normalise results between participants.

Experiment 1. Assessing the reliability of MEPs acquired with a stimulation intensity set to generate a 1 mV response

Methodology

Design

In order to assess the variability of 1 mV MEPs, participants attended one TMS session which lasted no longer than one hour during which a total of 240 MEPs were recorded from the first dorsal interosseous of the right hand. Stimulator output was set to the intensity at which evoked an MEP peak-to-peak amplitude of 1mV. MEPs were recorded in 8 sets of 30 stimuli with an inter-stimulus interval of 4 seconds; between sets participants were given a 2 minute break (Figure 8.). The total time taken to record MEPs was 30 minutes. Coil position was held constant with online feedback using the Brainsight^{TM} Bullseye target system (Figure 8.).
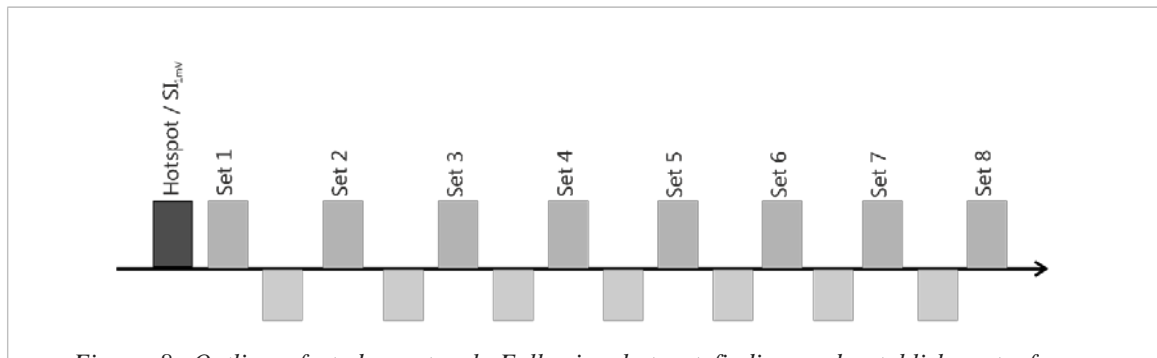
Figure 8. Outline of study protocol. Following hotspot finding and establishment of an appropriate stimulation intensity, eight sets of 30 MEPs were recorded from the hotspot with two minute breaks between sets.

To monitor background EMG in real time, a high-gain digital oscilloscope (Tektronix, Mixed Signal Oscilloscope, 2014) was connected to a PC monitor and displayed in clear view in front of the participant. Participants were instructed to keep their level of background EMG between predefined limits. If, during data collection, background EMG increased to undesirable levels, data collection was paused and only resumed once the participant had reached the required relaxed state.

Data Analysis

A bespoke MATLAB (Version 2012a, Mathworks Inc., Cambridge, UK) script was used to analyse the EMG data collected from Mr.Kick©. Individual MEP traces were excluded from further data analysis if there was any background EMG activity exceeding 20 microvolts peak-to-peak in the 50 milliseconds immediately preceding the onset of the TMS pulse. Full participant data was excluded if the first sets average MEP amplitude was not within the range of 0.7-1.3 mV, it was assumed that if the first set was outside this range the stimulus intensity was not appropriately set.

Statistical Analysis

Bland-Altman plots involve graphing the difference between samples (A-B) against the mean of the samples ([A+B]/2) (Bland and Altman, 1986). Usually applied to compare two different methods of measurement, in this case it is being applied to assess the agreement in repeated measures of the same method. The Bland–Altman method calculates the mean difference between two methods of measurement (the 'bias'), and 95% limits of agreement (LOA) as the mean difference ±1.96 SD of the differences between pairs.

The bias quantifies how much higher (i.e., positive bias) or lower (i.e., negative bias) repeated measures are. The presentation of the 95% limits of agreement is for visual judgement of how well two methods of measurement agree. The smaller the range between these two limits the better the agreement. A Bland-Altman plot makes it easier to assess the level of systematic difference, the scatter of the values, and to show whether there is a relation between the values and measurement error.

Bland-Altman plots were used illustrate the agreement between each participants average MEP amplitude from the first set, acting as a baseline, against each other set systematically (i.e. set 1 against set 2, set 1 against set 3). To create the Bland-Altman plot the average of the

two measurements was calculated and then plotted against the difference of the two measurements.

Any other statistical testing was conducted with SPSS 2012 v21.0 (IBM Corp, 2012). Results are reported as mean ± standard deviation (SD) and tests are considered significant at a Bonferroni adjusted alpha of -0.05. If the assumption of covariance matrix circularity was violated a Geisser–Greenhouse adjustment was made (denoted by GG following the F test). Descriptive statistics confirmed that the data did not violate any of the statistical tests assumptions (normally distributed and equal variances).

Results

In total, data of 52 participants were statistically analysed. Data of 8 participants were excluded. 2 participant's data was discarded as these data sets were incomplete due to equipment malfunctioning and 6 participants data sets were excluded due to the first sets average MEP amplitude being less than 0.7 or greater than 1.3 mV. Of the included data sets, with the background EMG exclusion criteria applied, 92% of the MEPs collected were included for further analysis.

In order to assess the agreement of average 1 mV MEPs between repeated measures Bland-Altman plots were examined (Figure 9.). The plots revealed a consistent lack of agreement between MEP averages compared to the first set with a tendency for MEP amplitude to increase, indicated by the bias. Repeated measures exceeded those at baseline by an average of 0.37 ± 0.06 mV, indicated by the solid line on the Bland-Altman plots. The standard deviation of the differences between repeated measures was 0.64mV. A bias of zero would indicate perfect agreement between repeated measures using the same method when analysed using Bland-Altman plots (Bland and Altman, 2003).

The presentation of the 95% LOA is for visual judgement of how well repeated measures agree. LOA indicate that for 95% of individuals, a repeated measure would be expected to be between on average 0.89 mV less and 1.64 mV greater than a measurement made at baseline. LOA also highlight the sensitivity of the method. Wide LOA indicate large differences were seen between repeated measures, therefore using this method a large change in MEP amplitude is necessary to show a meaningful change in CSE.

A box plot (Figure 10.) highlights the change in the mean MEP amplitude for group data. The initial baseline set group average MEP amplitude was 1.04 mV and increases thereafter, reaching a peak of 1.48 mV. The greatest single participant set average reached 4.14 mV and the lowest single participant set average reached 0.13 mV. A One-way repeated measures ANOVA ($F_{(4.61, 234.99)} = 5.884$, $p = 0.00^{GG}$) revealed average MEP amplitude significantly increased (Bonferroni adjusted) following baseline. Post-hoc pairwise comparisons revealed this was true for all sets following baseline (Set 1 – Set 2, p=0.00, Set 1 – Set 3 p=0.002, Set 1 – Set 4, p=0.004, Set 1 – Set 5, p=0.001, Set 1 – Set 6, p=0.001, Set 1 – Set 7, p=0.002, Set 1 – Set 8, p=0.005), demonstrated in figure 10. Average MEP amplitude variability also increased following the baseline set and stayed consistently high, demonstrated clearly by the large interquartile range observed in Figure 10. A frequency distribution (Figure 11.) including all data sets further highlights the tendency for MEP amplitude to increase above 1 mV.
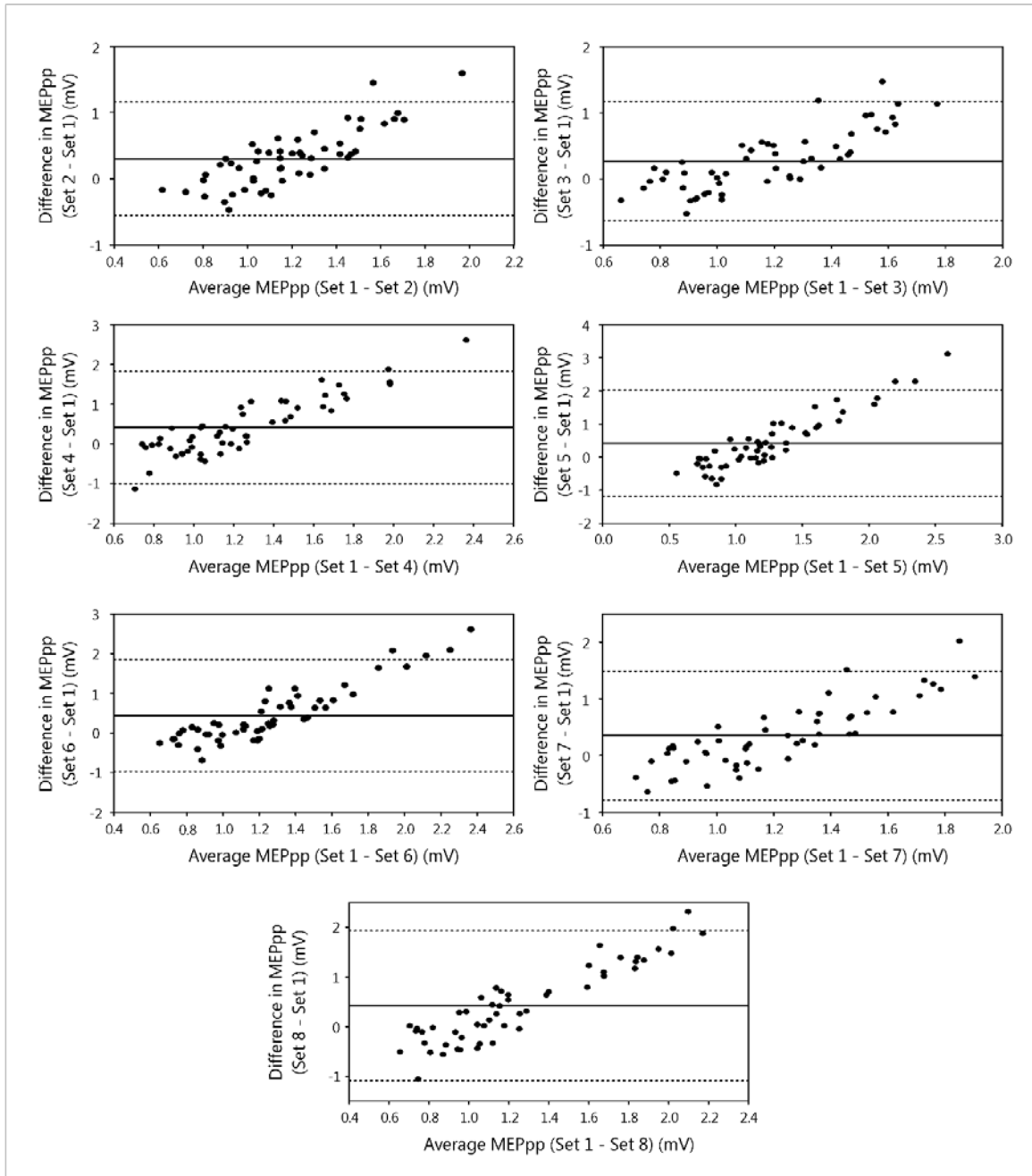
Figure 9. Bland Altman plots. The difference in average MEP amplitude (baseline minus subsequent set) plotted against the mean of the two measurements. Solid black line indicates the bias. Dotted black line indicates Limits of Agreement. Following baseline, MEP averages increased on average 0.37 ± 0.06 mV.
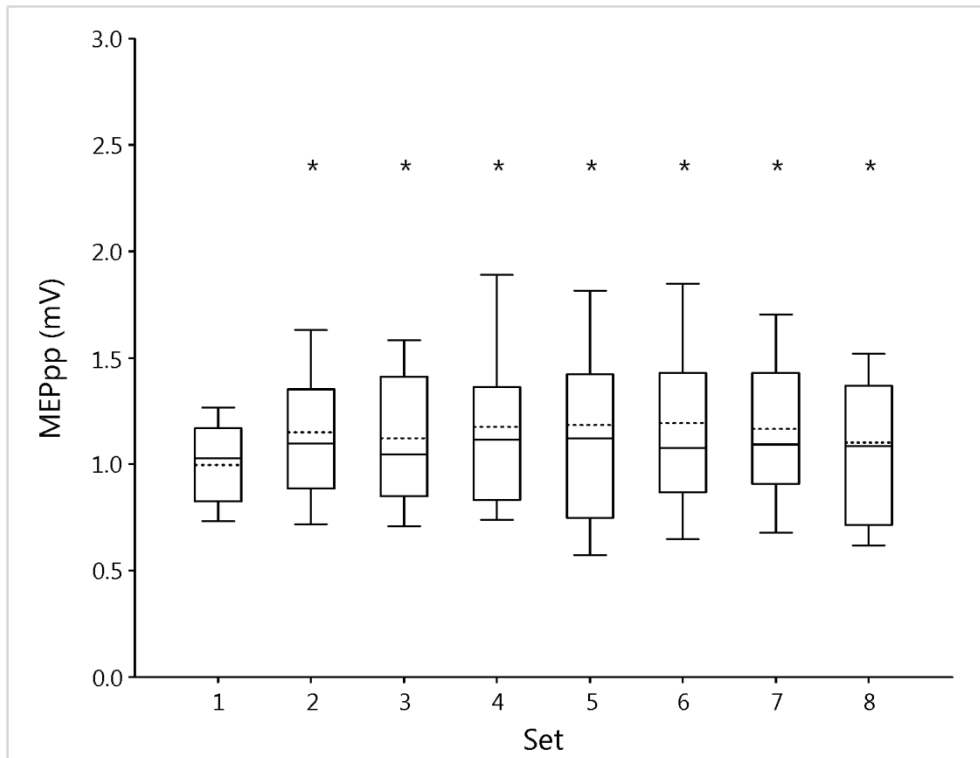
Figure 10. Group data for each set. Boxplots show the median (solid black line in box) and mean (dotted black line in box), interquartile range (IQR; box top and bottom), and 10th and 90th percentiles (error bars). * indicate a significant increase (p<0.05.) in mean MEP amplitude above the first set.

Figure 10. Group data for each set. Boxplots show the median (solid black line in box) and mean (dotted black line in box), interquartile range (IQR; box top and bottom), and 10th and 90th percentiles (error bars). * indicate a significant increase ($p<0.05$.) in mean MEP amplitude above the first set.
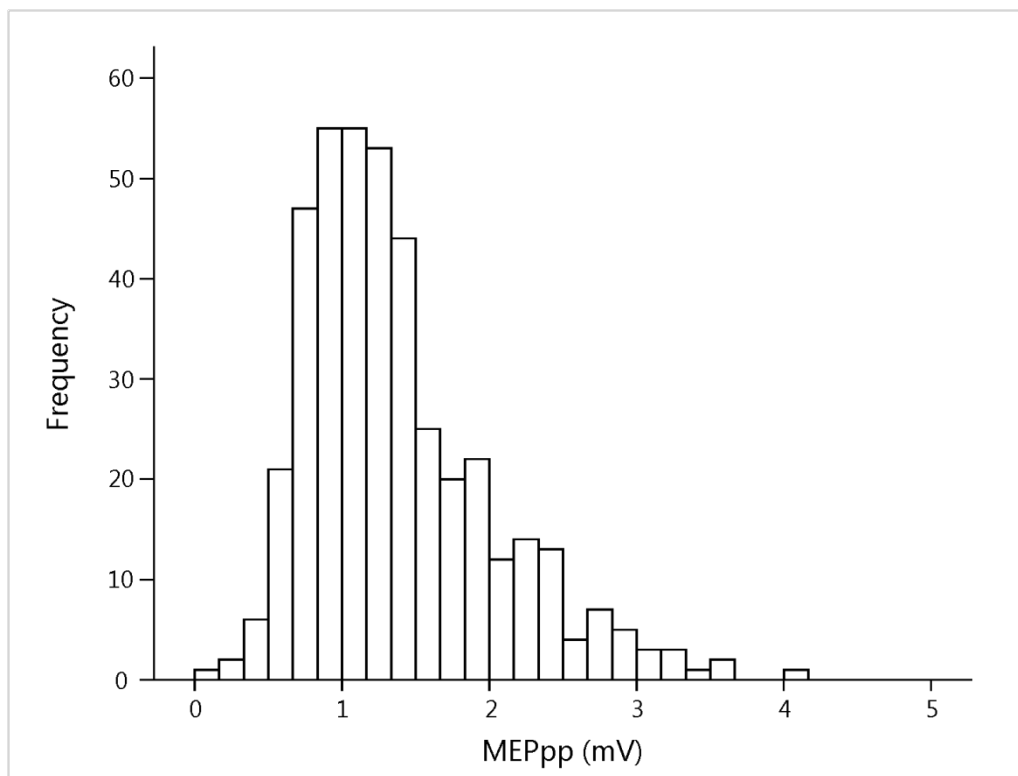
Figure 11. Frequency distribution of average MEP amplitude for all data sets included in the analysis (n = 416). A skewed right distribution highlights the tendency for MEP amplitude to increase when using the absolute method to set the stimulation intensity.

Discussion

This is the first experiment to explore the repeatability of average MEP amplitude acquired with a stimulation intensity set to evoke an absolute response size of 1mV. The reliability and agreement of repeated measures compared to a baseline measure was determined using Bland-Altman plots. We have demonstrated that average MEP amplitude is highly variable following a baseline measure; in fact, there is a tendency for average MEP amplitude to increase over repeated measures. Therefore, the method of setting the stimulation intensity to evoke a 1 mV response to assess CSE with TMS is not recommended.

Test re-test reliability of an assessment & differences in analysis

In order to be useful in clinical practice and for research purposes, measurements must be reliable. Reliability refers to the extent to which an instrument is consistent over time and free from error (Portney and Watkins, 2009). In essence, a reliable instrument is one that will perform with predictable consistency under fixed conditions.

Reliability in research is commonly determined from measurements taken from the same participant on two or more occasions; termed test-retest reliability. It refers to the consistency with which an instrument can produce similar results from repeated measurements (de Vet et al., 2006; Bialocerkowski and Bragge, 2008). If a measure is reliable, the participants score should be similar on multiple trials, the extent to which the scores vary is interpreted as measurement error; the smaller the measurement error the better the reliability.

Variation in measurements must be considered in the context of the total measurement system. Measurement errors may be attributed to three sources; 1. The individual taking the measurement. 2. The measuring instrument. 3. Variability of the characteristic being measured (Portney and Watkins, 2009). Inconsistency in which variables naturally respond over time, where there are natural fluctuations from measurement to measurement, also need

to be considered. When responses are inherently unstable, reliability becomes more challenging to assess (Portney and Watkins, 2009).

Establishing measurement test-retest reliability is paramount to good scientific practice. The reliability of a measurement is fundamental to all aspects of research, without it we simply cannot have confidence that any changes observed in the measure are due to physiological changes within the same participant, and not due to the variability in the measure itself (Portney and Watkins, 2009; Weir, 2005).

Test re-test reliability can be estimated using both relative and absolute indices (Vaz et al., 2013). Correlation coefficients, used to quantify relative reliability estimates the consistency and association of position of individuals in a group relative to others, or reflects how closely a set of paired observations follow a straight line (Portney and Watkins, 2009). Whereas absolute reliability, as evaluated by Bland-Altman plots, is concerned with variability due to random error; an absolute reliability index is affected by the degree to which measurements vary, the premise being that the less the variability, the greater the reliability. Identifying the most appropriate estimate of test re-test reliability is of great importance in studies describing reliability of research methods and instruments.

The reproducibility of TMS measures in this case has been found to be poor and differences in methods of analysis makes comparison between the present study and previous studies difficult. Studies have commonly assessed reliability using the intraclass correlation coefficient (ICC). The ICC is used frequently to calculate the correlation between two or more sets of measurements. It accounts for the consistency of performances from test-retest (within subject change), as well as changes in average performance of participants as a group over time (Vaz et al., 2013); reflecting the degree of correspondence and association between repeated measurements (Portney and Watkins, 2009).

Although the use of the ICC is common, Rankin and Stokes (1998) suggest that unless the size and attributes of the samples tested are virtually identical, comparison of reliability results between studies using the ICC is not possible. Additionally, there is ambiguity in the literature regarding the interpretation of the ICC, Portney and Watkins (2009) highlight that there are no widely accepted criteria for defining a strong, moderate or weak association and any interpretation must be based on the nature of the investigation. This makes interpretation between studies more difficult as conclusions are inconsistently drawn based on similar results. A correlation quantifies the direction and strength of the relationship between test-retest scores by estimating their linear relationship; a relative measure of reliability. The correlation coefficient is a reflection of how closely a set of paired observations follow a straight line. Bland and Altman (1986) demonstrate that even though data are highly correlated, this may conceal a considerable lack of agreement between variables. A

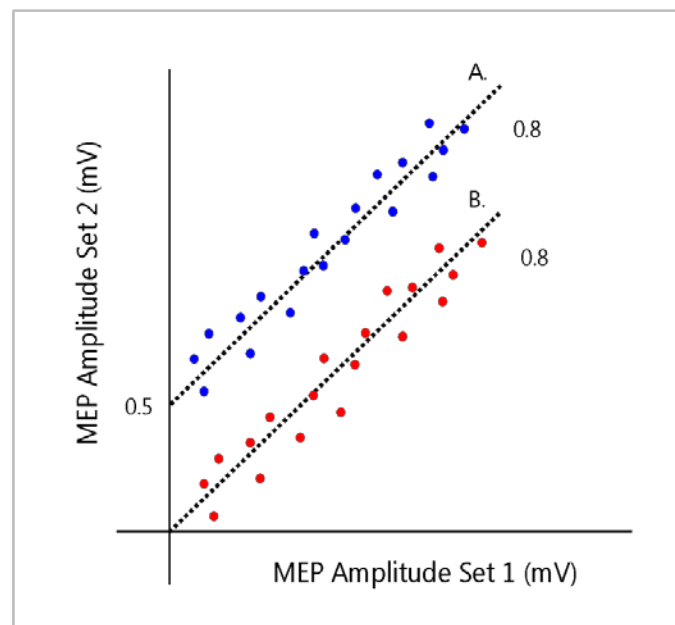*Figure 12. A graph representing two sets of hypothetical data showing the same correlation of 0.8. This would lead to the conclusion that both sets of data, for example taken from repeated measures, are as reliable as one another. However, data A shows a clear systematic increase of 0.5 mV which is not reflected in the correlation result. Leading to the incorrect conclusion that both will provide the same result.*

correlation coefficient could potentially miss bias between measurements and will thus be highly misleading (Figure 12.).

For this reason, Bland-Altman plots, an absolute measure of agreement, were used to assess the agreement of repeated measures against a baseline measure to determine if the method used in this experiment is reliable. The plots have clearly indicated that there is a consistent bias in subsequent repeated measures to be higher than baseline and a distinct lack of agreement; highlighting that 1 mV MEPs as a means of assessing CSE with TMS is not reliable.

Caveats of using an absolute response size in TMS research

In order to set a stimulus intensity many studies have previously used a predefined intensity relative to the participants resting motor threshold, commonly in the 120% - 150% resting motor threshold range (Rossini et al., 2015). Previous studies have determined the stimulus intensity as the intensity needed to obtain a baseline MEP of approximately 1mV peak to peak amplitude (Huang et al., 2005), as investigated in this experiment. The primary reasoning behind these methods is to avoid ceiling and floor effects; ideally finding an intensity that at baseline evokes an MEP amplitude equidistant between resting motor threshold and maximum amplitude, known as the I50 (50% maximum) when seen on a stimulus-response curve (Figure 13.).

By determining the test stimulus as the intensity to elicit an absolute response size we are therefore assuming that this response size is at a uniform position on the stimulus-response curve for all participants. However, it is well reported that the stimulus-response relationship differs considerably between participants, as well as between muscles within the same participant (Ziemann et al., 2004).

Pitcher et al., (2015) retrospectively examined stimulus-response curves and the resting motor threshold of 176 participants to assess the differences between the intensities that would have been used based on the absolute (1 mV) or relative (% resting motor threshold) methods. The authors reported that the absolute method of determining a stimulus intensity of approximately 1 mV substantially underestimates the I50 on the stimulus-response curve (Pitcher et al., 2015); that is, an MEP of 1 mV peak to peak amplitude is significantly smaller than that evoked at I50 (Figure 13.). This is most likely to be the reason average MEP amplitude increased following the first set in the present experiment. If the stimulation intensity has been set which substantially underestimates the I50, the probability of evoking an MEP amplitude greater than 1 mV is greatly increased, and equally the probability of evoking an MEP with an amplitude smaller than 1 mV is greatly decreased; by determining the stimulation intensity to evoked a 1 mV response size, a floor effect is clearly introduced.
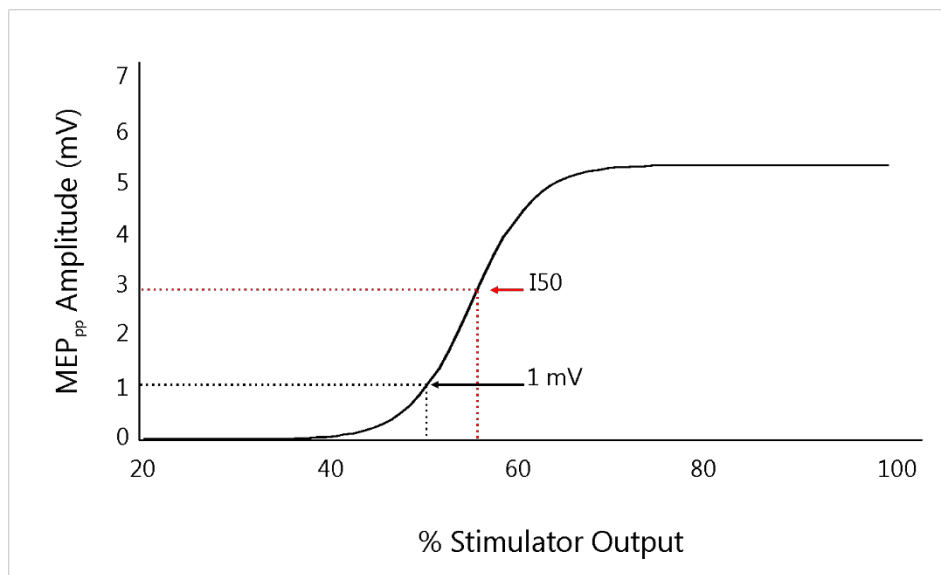

Figure 13. The I50 is the percentage of maximal stimulator output at which the MEP is mid-way between the minimum MEP amplitude and maximum MEP amplitude. It is clear from this representative stimulus response curve that a stimulus intensity set to evoke an MEP of 1 mV underestimates the intensity needed to evoke an MEP amplitude at the I50.

Pitcher et al., (2015) also found an inherently large inter-subject variability of where a target MEP size of 1 mV may be expected to occur on an individual participants SR curve. The maximum MEP amplitudes ranged from 0.44 - 12.1 mV, meaning for some, a 1 mV MEP could represent a near-maximal MEP but less than 50% of maximum for others, further highlighting the differences in responses seen between participants and confirming the principal caveat of using a stimulus intensity to elicit an absolute response size.

In terms of the relative method, the I50 consistently occurs at a mean of 127 – 128% of resting motor threshold (Pitcher et al., 2015). However, this had one standard deviation of ±11.3% which is arguably too large to approximate the I50 based on the resting motor threshold. Groppa et al., (2012) suggest stimulating at in intensity that marks the transition from the rising part of the slope to the plateau, as this evokes MEPs of maximum amplitude without causing the participant too much discomfort. However, this was suggested to be used with diagnostic TMS in patients with a neurological disorder; not to monitor corticospinal plasticity. It is therefore fair to postulate that the stimulator intensity at which the I50 is evoked is the most appropriate intensity to stimulate at when taking single pulse TMS measures, especially in terms of sensitivity and avoiding ceiling and floor effects. It is however a limitation of the current experiment that the suggested approach was not investigated and the reliability of MEPs obtained with the suggested approach is yet to be explored.

Conclusions

This experiment has highlighted and investigated a method of determining stimulation intensity in TMS studies. The primary finding is that setting the stimulation intensity to evoke a 1 mV response size is not a reliable approach to assess CSE and is therefore not recommended to be used in future TMS studies. MEP amplitude tends to increase over

repeated measures using this approach and this is due to large between-subject variability in the stimulation-response relationship, meaning a 1 mV response size is not at a uniform position in the relationship across the population; a 1 mV response size generally underestimates the I50 (50% maximum MEP) and therefore introduces a floor effect.

Experiment 2. The effect of changes in coil tilt at the scalp on motor evoked potential amplitude

Methodology

Pilot study

Background and Purpose

Given the well reported trial-to-trial variability of MEP amplitudes discussed in length in the literature review, it was necessary to conduct a pilot study to establish an appropriate protocol. The primary focus of the pilot study was to find an appropriate number of steps to take within the range of angles chosen. And to establish a suitable range of angles around the hotspot to investigate.

Pilot Design

4 participants attended one TMS session which lasted no longer than 90 minutes during which a total of 750 MEPs were recorded from the first dorsal interosseous of the right hand. Stimulator output was set to 120% of resting motor threshold and inter-stimulus interval set to 1.5 seconds.

Aided by the use of the Brainsight^{TM} Bullseye target system, the coil was rotated in the pitch and yaw axes, on the scalp, relative to the 'hotspot' target sample vector recorded on the neuronavigation system (Figure 7.). 30 MEPs were recorded at each step of 5° (over a total range of 60° in either axis). This equated to 25 steps in total as MEPs were recorded from the hotspot once.

The aims of the pilot study were to establish the range of angles in which we needed to tilt the coil and the number of steps within that range were necessary to reliably assess the effect of coil tilt on MEP amplitude.

Figure 14. Pilot Protocol. Outline of the pilot protocol. Each light grey box represents an angle at which 30 MEPs were recorded. Starting at -30° through to 30° in each direction.

Pilot Results
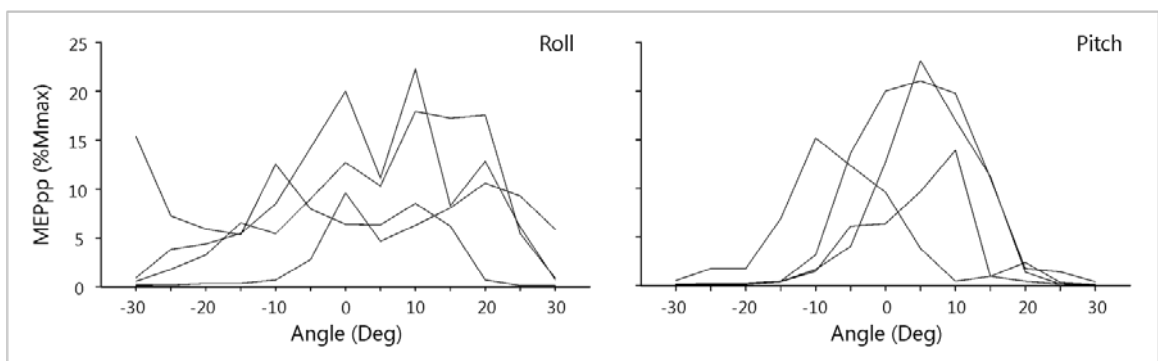
Figure 15. Pilot results. MEP amplitude plotted against coil angle for both roll and pitch axes. Each line represents single participant data. Results highlight the extent to which average MEP amplitude varies between angles, emphasising the need to reduce the steps between angles tested.

No statistical analysis was performed on the pilot data.

Implications for primary study

As expected, we found MEP amplitude varied greatly from trial-to-trial. We also observed that the average MEP amplitude at each angle also fluctuated considerably; particularly in the roll axis. It was therefore necessary to increase the number of steps between the ranges in order to validly assess coil tilt on MEP amplitude without these pronounced fluctuations affecting the interpretation of our findings.

In order to facilitate an increased number of steps within the range, the number of stimuli at each angle had to be reduced; this was seen as an 'opportunity-cost'. In light of literature showing that attention and drowsiness may drift throughout an experiment and may influence

MEP variability (Avesani et al., 2008), reducing the number of stimuli at each step meant the protocol for the primary investigation would not be too prolonged. Based on the pilot data, 25 stimuli at each angle was seen as an appropriate compromise.

The total range of angles around the hotspot used in the pilot study was 60° in either axis (pitch and roll). Pilot results show that this range of angles was not large enough for the roll axis as MEPs were still being recorded at the more extreme angles; though for pitch, MEP amplitude already reduced considerably by a deviation of 25°. Anecdotally, the coil position on the head at the more extreme angles was an extremely impractical position for the experimenter to hold and it was highly unlikely that this angle would be used or maintained in a research setting. Increasing the range would also have implications on the protocol length as discussed above.

Primary Investigation

Design

26 participants attended one TMS session which lasted no longer than 90 minutes during which a total of 1025 MEPs were recorded from the first dorsal interosseous of the right hand. Stimulator output was set to 120% of resting motor threshold and inter-stimulus interval set to 1.5 seconds.

Using the Brainsight[TM] Bullseye target system, the coil angle was changed in the pitch and yaw axes, on the scalp, relative to the 'hotspot' target sample vector recorded on the neuronavigation system (Figure 17.). 25 MEPs were recorded at each step of 3° over a total range of 60° around each axis. This equated to 41 steps in total as MEPs were recorded from the hotspot once.
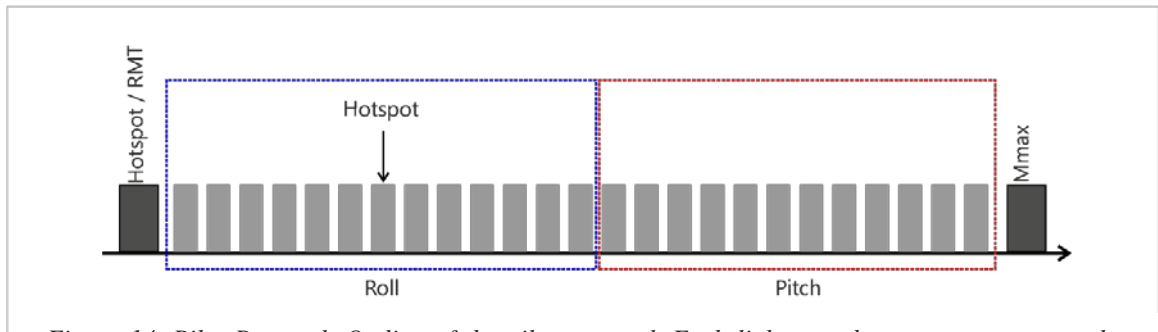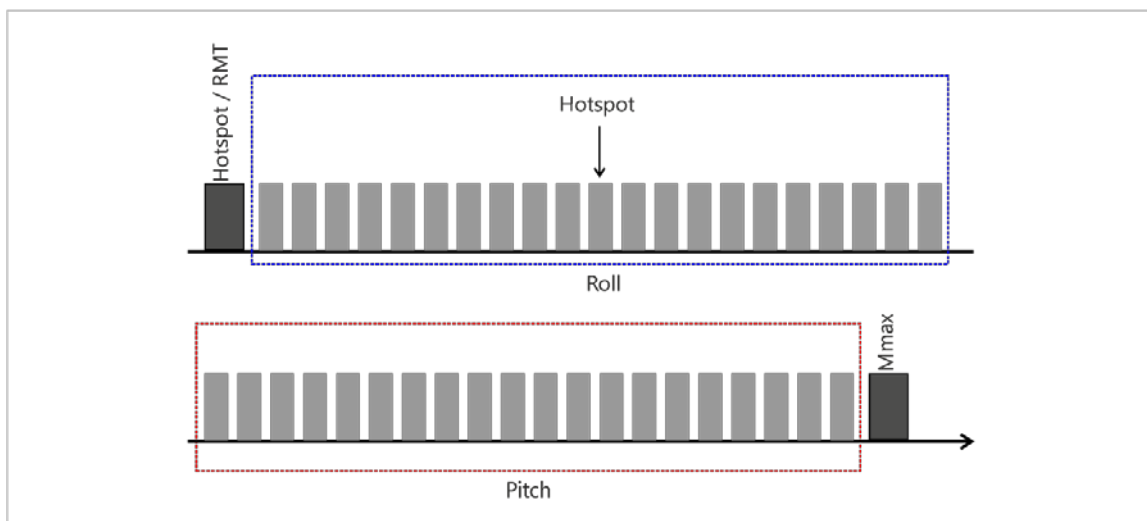
Figure 16. Primary Protocol. Outline of the primary protocol. Each light grey box represents an angle at which 25 MEPs were recorded. Starting at -30° through to 30° in each direction

Data Analysis

Data analysis was completed using a bespoke MATLAB script (MATLAB Release 2012b, Mathworks Inc., Cambridge, UK) processing both the EMG and neuronavigation data. First, the MEP was quantified by the peak-to-peak value extracted from a window 20-50 ms after stimulation. The corresponding stimulation position was extracted from the neuronavigation data.

Individual stimuli were excluded from analysis if the stimulation or corresponding MEP did not fulfil one of four conditions: 1) Background EMG. The Root Square Mean was calculated for each EMG record 50-5 ms prior to stimulation. The mean and standard deviation (SD) was calculated and EMG records were excluded based on the Root Square Mean $\pm$ 1.5 standard deviations. Participants were given online feedback during acquisition of the MEPs regarding their EMG level to limit the number of EMG records that were excluded. 2) The entry point of the stimulation vector on the scalp was greater than 3mm away from the target vector. 3) The angle deviation was greater than 3° around the desired

angle (step angle $\pm$ 1.5°). 4) Any deviation greater than 15° in yaw rotation compared to the target vector.
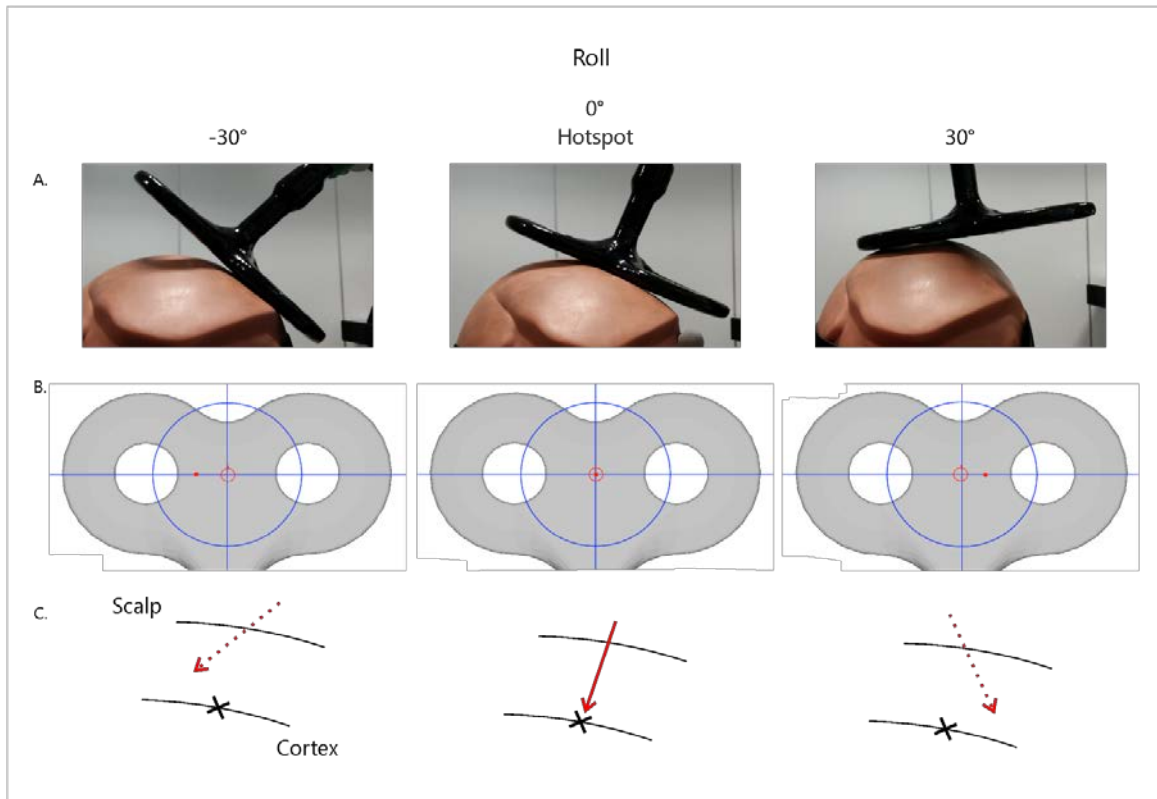
Figure 17. A step by step illustration outlining how the coil angle was changed on the scalp relative to the hotspot in the roll axis. A. Shows the position of the coil on the head at the two most extreme angles (-30° – 30°) and over the hotspot (0°) where the coil is flat to the scalp. At the extreme angles it is clear that the central segment of the coil is not in contact with the scalp. This was highlighted in the pilot study and is the primary motive for not increasing the range of angles assessed. B.The Brainsight[TM] *Bullseye target system assisted the experimenters in positioning the coil so the coil was only tilted in one axis at a time (small red dot on target), while keeping the coil positioned over the hotspot (large open circle on target). When then coil was tilted over the pitch axis, the small red dot followed the vertical guideline. C. A representation of the stimulation vector as the coil is tilted to greater angles. The black cross indicates the hotspot position on the cortex. The coil is held in the same position on the scalp meaning the position on the cortex that is stimulated changes as the angle is systematically increased.*

When data had been selected based on these exclusion criteria, MEP amplitude was plotted against coil angle (Figure 20.). In order to facilitate analysis of the data, a Gaussian curve was fitted through the mean MEP amplitude for each angle (for each participant in both the roll and pitch axes) (Figure 20.). Due to the MEP amplitude not reaching zero for the larger angles (-30° – 30°) in the roll direction, the curves were manipulated by adding null amplitudes at

extreme angles (-90° – 90°) where the MEP amplitude would be expected to be approximately zero in order to achieve a better curve fit. To be comparable, the Gaussian curves were then translated so the maximum point on the curve corresponded to zero on the x-axis. 70% of the peak MEP amplitude was employed to determine the angle at which MEP amplitude drops beyond an acceptable level.

Statistical Analysis

Statistical testing was conducted with IBM SPSS Statistics 22. Tests were considered significant at $\alpha = 0.05$. If the assumption of covariance matrix circularity was violated a Geisser–Greenhouse adjustment was made (denoted by GG following the F test). Descriptive statistics confirmed that the data did not violate any of the statistical tests assumptions (normally distributed and equal variances).

Two repeated measures ANOVA were performed on the data, one of the roll axis, one on the pitch axis. Repeated measures ANOVA were performed on the angles, -30, -21, -9, 0, +9, +21, +30 in order to assess whether coil tilt significantly affects MEP amplitude before any further analysis was undertaken.

Differences between the 70% cut-off range in the roll and pitch directions were compared using a paired samples T-test.
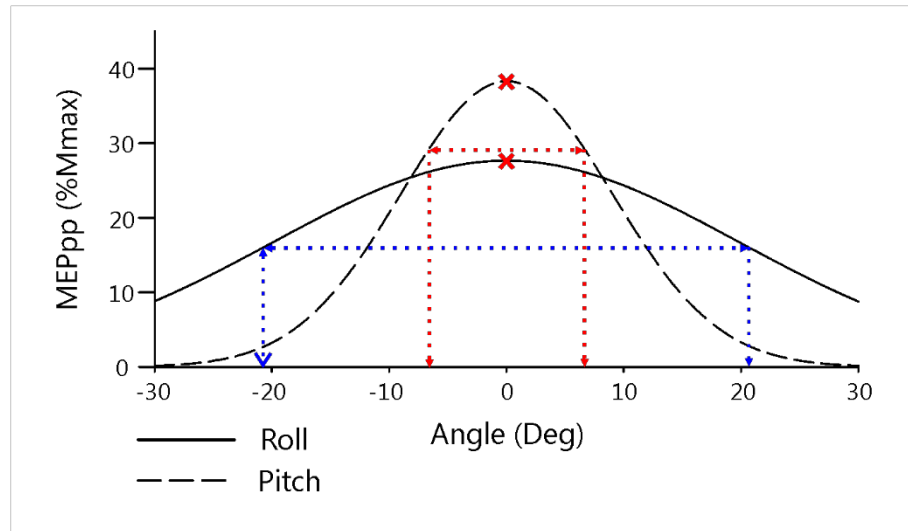
Figure 18. 70% cut-off. A representation of the 70% cut-off point on Gaussian fitted curves for a single participant. It is then possible to define the x values at which MEP amplitude is affected by coil tilt. Red crosses (x) highlight the peak of each curve.

Results

In total, data of 22 participants were analysed. Data of 4 participants were discarded as these data sets were incomplete due to equipment malfunctioning.

MEP amplitude decreases as the angle the coil is held on the scalp increases. This effect is more pronounced in the pitch direction. Two repeated measures ANOVAs were performed on all angles recorded, one on each axis to determine if coil tilt does significantly affect coil tilt. A repeated measures ANOVA performed on the roll axis ($F_{(8.367, 66.06)} = 13.337$, p = 0.00GG) revealed average MEP amplitude significantly reduced with increasing coil tilt. Post-hoc pairwise comparisons revealed this was true for angles -30 (p=0.000), +24 (p=0.004) and +30 (p=.000). P values for non-significant results are as follows, -27 p=1, - 24 p=1, -21 p=0.36, -18 p=1, -15 p=1, -12 p=0.45, -9 p=1, -6 p=1, -3 p=1, +3 p=1, +6 p=1, +9 p=1, +12 p=1, +15 p=1, +15 p=1, +18 p=1, +21 p=0.6. A One-way repeated measures ANOVA performed on the pitch axis ($F_{(5.436, 130.457)} = 35.088$, p = 0.00GG) revealed average MEP

amplitude significantly reduced with increasing coil tilt. Post-hoc pairwise comparisons revealed this was true for angles -30 (p=0.00), -27 (p=0.00), -24 (p=0.00), -21 (p=0.00), -18 (p=0.001), -15 (p=0.007), +15 (p=0.002), +18 (p=0.00), +21 (p=0.000), +24 (p=0.00), +27 (p=0.00) and +30 (p=0.00), also shown in figure 19. P values for non-significant results are as follows, -12 p=0.309, -9 p=1, -6 p=1, -3 p=1, +3 p=1, +6 p=1, +9 p=0.348, +12 p=1.

Roll

Pitch

Angle (Deg)
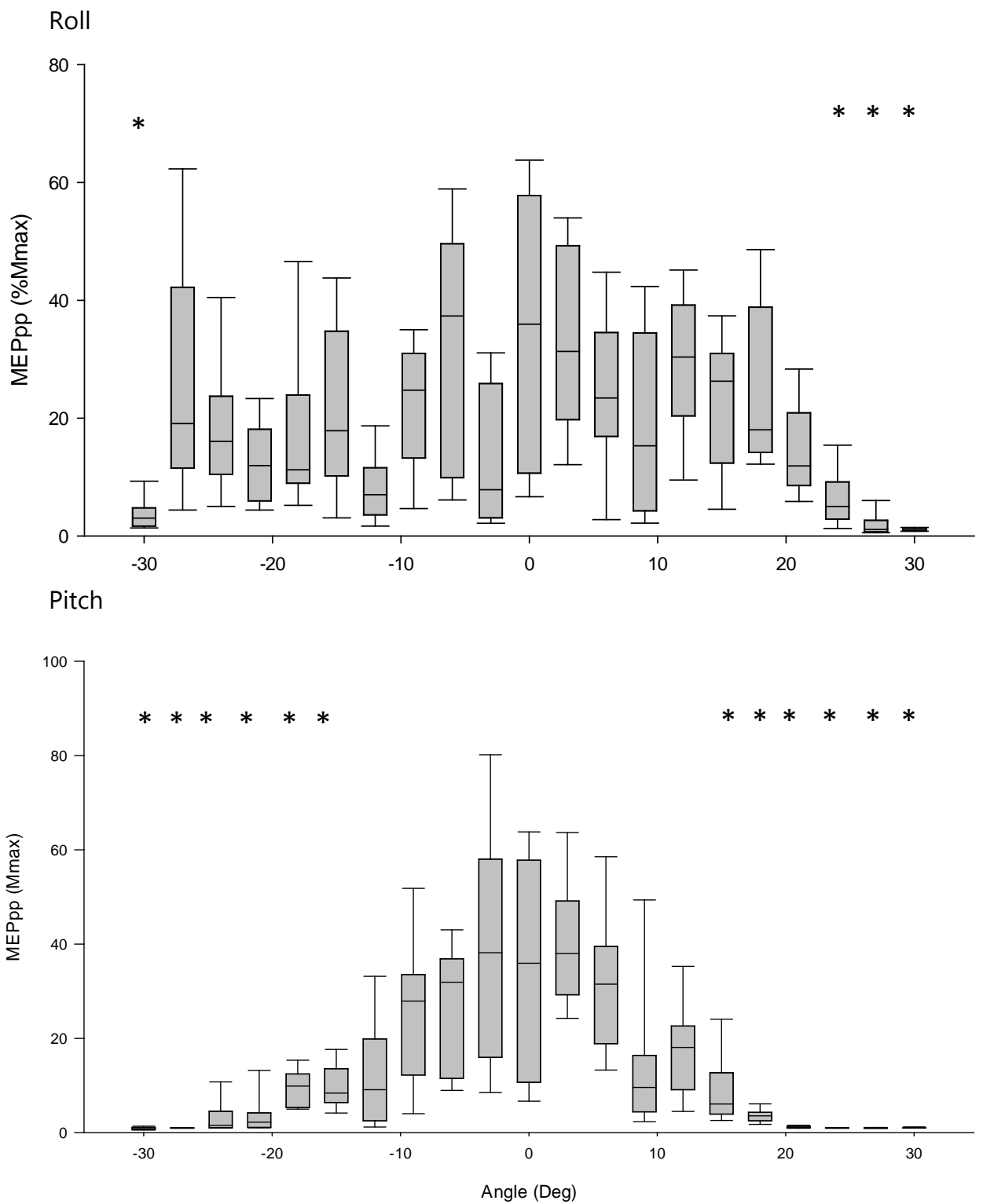
Figure 19. Group data for the angle, for both the roll and pitch axis. Boxplots show the mean (solid black line) and interquartile range (IQR; box top and bottom), and 10th and 90th percentiles (error bars). * indicates a significant decrease in mean MEP amplitude below angle 0, which corresponds to the set taken when the coil is held flat to the head. MEPs were considered significantly less than the hotspot at a p<0.05.

55

Following the results of the repeated measures ANOVA, revealing coil tilt significantly affects MEP amplitude, further analysis was performed.
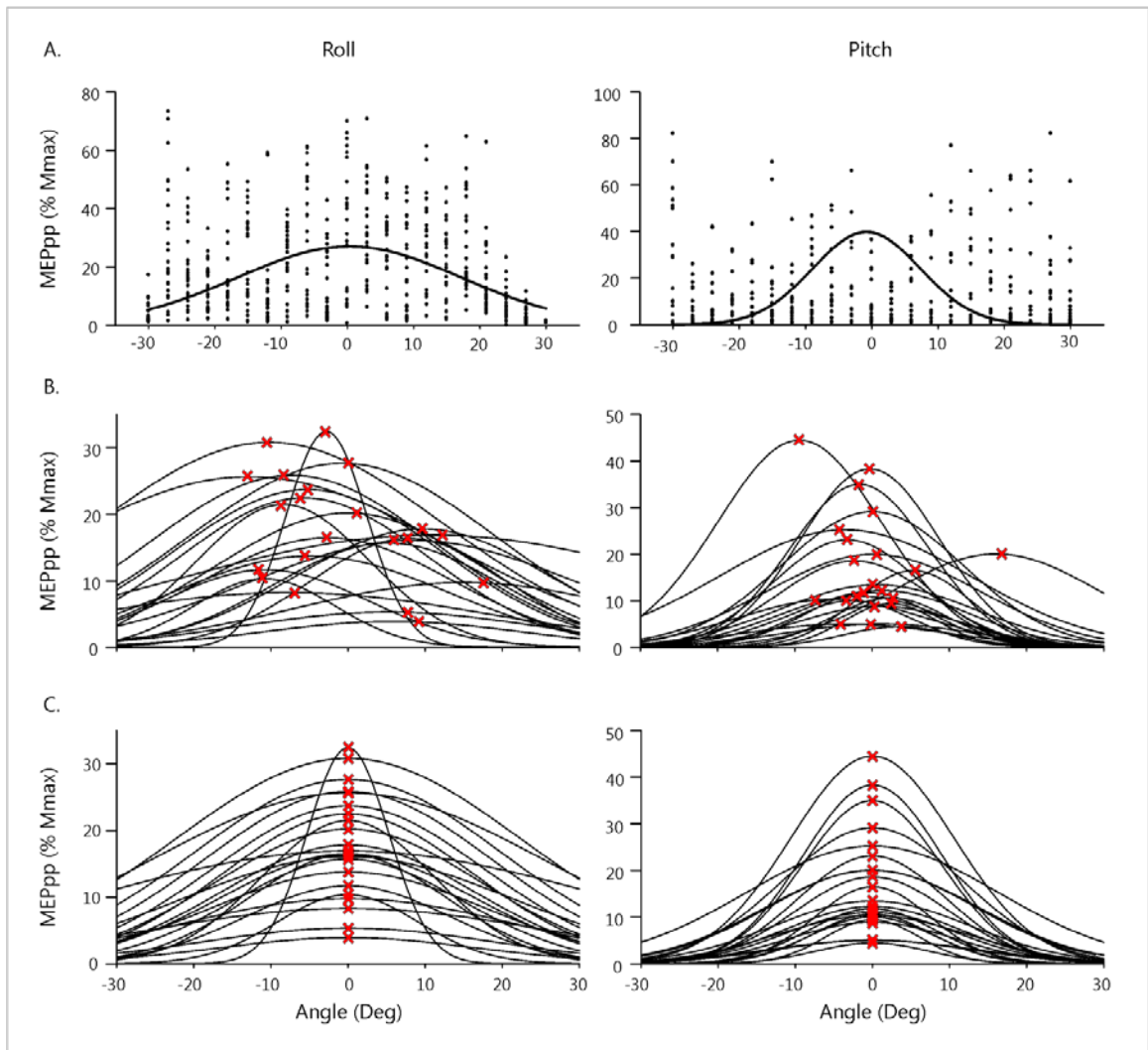
Figure 20. A step by step illustration of how curves were created and adjusted to assist analysis. A. Average MEP amplitude plotted against the corresponding angle. A Gaussian curve is fitted through the group mean MEP amplitude. B. Gaussian curves were fitted through each participants mean MEP amplitude in both the roll and pitch directions. C. The curves were translated so the maximum point was aligned with the hotspot angle (0°) in order to assist in defining angles at which MEP amplitudes are affected. Average goodness of fit (r^2) for all fitted curves was 0.76. Red crosses (x) highlight the peak of each curve.

The average range of angles around zero at which the coil angle can change without affecting MEP amplitude, defined as 70% of the peak MEP amplitude for the roll axis was 29.59° ± 10.02° and 17.4° ± 4.36° for the pitch axis. A paired samples T-test revealed a

significant difference in the range of angles at which MEP amplitude is not affected by coil angle changes in the roll and pitch directions (t(21) = 4.83, p = .000) (Figure 21.).
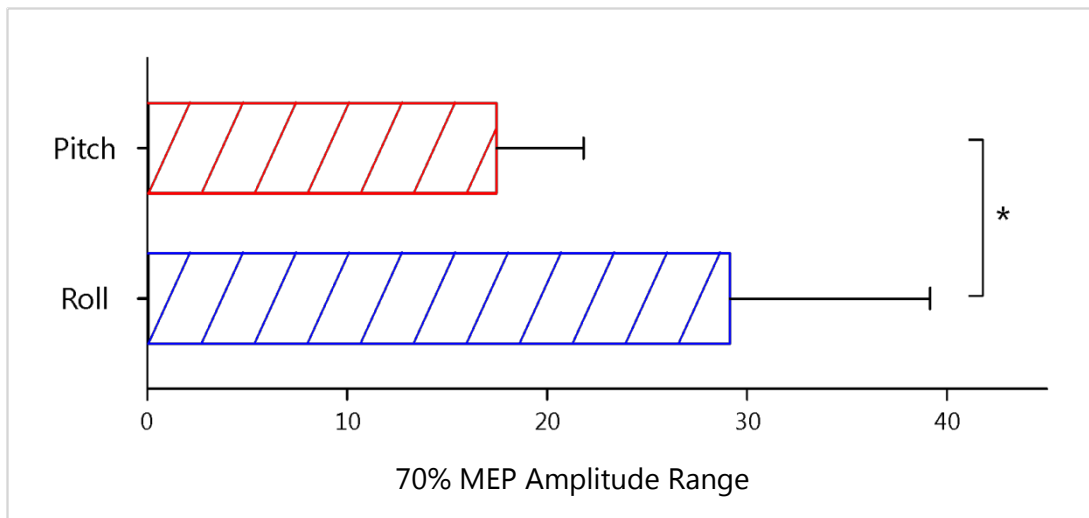
Figure 21. A bar graph to show the group average 70% cut-off range; the range of coil tilt around 0° that does not affect MEP amplitude. The cut-off range was significantly wider in the roll axis in comparison to the pitch axis. The 70% cut-off range is variable between participants, highlighted by the large error bars.

Discussion

The present experiment is the first to investigate the effect of coil tilt at the scalp on MEP amplitude. As hypothesised, MEP amplitude does decrease with increasing coil angle, as shown by the results of the repeated measures ANOVA performed on each axis. The angle at which MEP amplitude decreased beyond an acceptable level, as defined by the 70% cut-off point, was found to be quite substantial. Additionally, MEP amplitude decreased at a significantly smaller angle in the pitch axis in comparison to the roll axis.

The primary aim of the present experiment was to establish the extent to which MEP amplitude changes when the coil angle is tilted at the scalp, allowing us to investigate whether neuronavigation is necessary in TMS studies to control for this source of variability. Given the large range of angles a TMS coil can be held without affecting MEP amplitude it is reasonable to conclude that an experienced TMS experimenter will be able to maintain a coil position flat to the scalp within the limits defined in the present study, without the use of a neuronavigation system; however an inexperienced experimenter may not.

Recently, studies using nTMS have applied exclusion criteria to positional data collected with navigation systems. The finding that the cut-off range for the roll axis was significantly wider than that of the pitch axis also highlights the need for studies using nTMS to consider applying different exclusion criteria and to treat the two axes differently when analysing TMS data. Due to the large variability in cut-off range, particularly in the roll axis, it may be necessary to exercise caution when applying exclusion criteria, taking the variability of the ranges found into account.

Factors affecting the decrease in motor evoked potential amplitude with increasing coil tilt

Motor cortex anatomy

It is well established that each muscle has a representation on the M1 (Sanes and Donoghue, 2000), which is targeted when using TMS to assess CSE. During the setup of a TMS experiment the hotspot for the muscle of interest is found by stimulating a small area of the primary motor cortex until the optimal position is located. This position on the scalp is then recorded and used for subsequent measures. The present experiment revealed that it takes a considerable tilt of the coil in order to impact on MEP amplitude, shown by the large ranges assessed by the 70% cut-off point; in particular in the roll axis which was significantly wider than the pitch axis.

The anatomy of the primary motor cortex, a relatively thin strip located on the anterior wall of the central sulcus, is the primary cause for the significant difference in the 70% cut-off range for the two axes investigated in this study; roll having a wider cut-off range, meaning a greater angle is needed before MEP amplitude is meaningfully affected. In the present study the coil was held at 45° to the sagittal plane with the handle pointing in posterior direction to induce biphasic currents in the lateral-posterior to medial-anterior direction in order to achieve the optimal stimulation orientation. This means that when the coil was tilted on the scalp in the roll axis, the coil ran along the motor cortex and therefore was able to elicit MEPs at greater angles. Conversely, when the coil is tilted in the pitch axis, the coil ran across the primary motor cortex and simply had a smaller area of cortex with which it could elicit an MEP. The finding that tilt in the pitch axis affects MEP amplitude at a significantly smaller angle than the roll axis needs to be taken into account when applying exclusion criteria to neuronavigation data, as they clearly need to be treated differently.
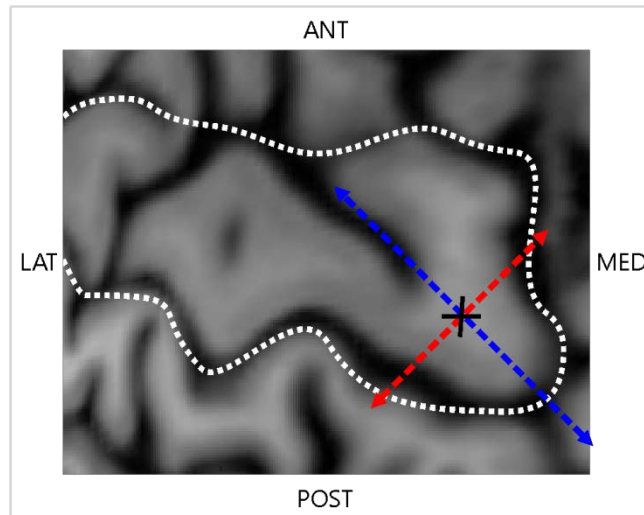
Figure 22. An illustration to show the anatomy of the primary motor cortex, highlighted by the white dotted line, and the axes about which the coil was tilted. The blue dotted line illustrates the roll axes, the red dotted line illustrates the pitch axes; the red dotted line is shorter than the blue dotted line to demonstrate the smaller range of angles the coil can tilt before MEP amplitude is affected. This illustration also highlights the reason the coil was not tilted from medial to lateral, and posterior to anterior, this is because the coil was held at 45° to the sagittal plane in order to achieve the optimal stimulation orientation. A black cross (x) highlights the hotspot position.

The wide cut-off ranges found for each angle direction also suggests that there may not be one specific hotspot position within each muscle representation, rather a small area of particularly high excitability or even multiple hotspot positions. Had the 70% cut-off range revealed a sharp decrease in MEP amplitude at small tilt angle changes it could be surmised that there is a small, specific area on the cortex that represents the 'hotspot'; however this is not the case. Inconsistent hotspot position has been noted before, the 'true hotspot' has been known to change intraindividually between days and a constant hotspot position cannot be guaranteed even within a single experimental session (Awiszus, 2014).

The focality of TMS stimulation

The gradual reduction in MEP amplitude with increasing coil tilt angle at the scalp is also because the position on the cortex that is stimulated by the most focal component of the stimulation is moving to increasingly suboptimal positions, away from the most excitable position. This finding corresponds to previous conclusions that MEP amplitude decreases

with minor changes in coil location (Schmidt et al., 2009). When stimulated at suboptimal positions individual neurons fail to become excited because their thresholds are transiently too high for effective stimulation, leading to reduced MEP amplitude (Brasil-Neto et al., 1992; Schmidt et al., 2009).

The focality of TMS stimulation was previously over-estimated from early model-based calculations and metrics, predicting regions of less than 25 mm^2 stimulated with a figure-of-eight coil (Wagner et al., 2009). More recently, however, studies have shown that although a small cortical region might be in the peak of the field, a much broader area of the cortex is affected, potentially exceeding $100 - 200$ mm^2 dependant on the coil (size, type, design) (Wagner et al., 2009).

As the coil tilt angle is progressively increased to more suboptimal angles the most focal component of the stimulation electric field will fail to stimulate the hotspot position on the cortex, leaving only the periphery of the electric field to activate the most excitable neurons; giving rise the gradual decrease in MEP amplitude with increasing coil tilt. This process is analogous to increasing or decreasing stimulation intensity. At low stimulation intensities, only the lowest threshold neurons are activated, as stimulation intensity is increased more neurons are activated as they are brought to threshold resulting in greater MEP amplitude (Rossini et al., 2015). In the case of coil tilt angle, when the coil is held in the optimal position all neurones are stimulated optimally, as even the highest threshold neurons are stimulated by the electric field. When the coil tilt angle is increased and more of the periphery of the electric field stimulates the 'hotspot' neurons, high threshold neurons will not be stimulated to fire and only the low threshold neurons will be activated; leading to a smaller MEP response.

Trajectory of the electric field

The sensitivity of MEP response size to the orientation and tilt of the TMS coil suggest that activation of neurons is related to the trajectories of the pyramidal tract neurons and the electric field direction along these columns of neurons (Ruohonen and Karhu, 2010). Increasing the length of neuronal membrane exposed to an applied current lowers the depolarisation threshold (Rushton, 1927). Further, orthodromic current (a current travelling in the normal direction of the nerve fibre) is more effective than antidromic current (a current travelling in the opposite direction of the nerve fibre), which is in turn more effective than transverse current (a current travelling across the normal direction of the nerve fibre) (Rushton, 1927). Therefore, aligning the electric field with the neuronal columns in the cortex exposes the maximum number of neurons and maximum

Figure 23. A representation of the electric field when the coil is held flat to the scalp (B) stimulating the pyramidal neuron (A) orthodromically and most optimally. As the coil is tilted through the pitch and roll axes the electric field stimulates the pyramidal neurons from less optimal transverse directions (C), leading to reduced MEP amplitude.

membrane length to the exciting stimulus (Ruohonen and Karhu, 2010).

When the TMS coil is held flat to the scalp over the hotspot, the electric field of the TMS stimulus is applied most effectively, longitudinally and orthodromically to the columns of neurons at the site of interest. As the angle at the scalp is changed, the exciting stimulus will move from the optimal stimulating direction (orthodromic) to increasingly transverse directions; resulting in the electric field becoming gradually less effective in stimulating cortical neurons and eliciting a smaller MEP. This effect becomes more accentuated as the angle of the coil held on the scalp is increased.

Individual anatomy influences the effect of coil tilt at the scalp on motor evoked potential amplitude

The orientation of current flow

The orientation of the induced current over the targeted motor area has previously been found to be the most critical stimulation parameter (Rösler et al., 1989), with different directions of current flow activating completely different populations of cortical neurons (Di Lazzaro et al., 2001). A posterior-to-anterior current flow has been found to preferentially evoke highly synchronised corticospinal activity, whereas anterior-to-posterior current flow activates slightly delayed corticospinal activity (Di Lazzaro et al., 2001).

The orientation of individual neurons within the induced electric field clearly influences the efficacy of the stimulation, therefore the degree to which the neuronal populations are stimulated will vary depending on the morphology of the neurons and the alignment of the sulci and gyri relative to the coil placement (Wagner et al., 2009). This was evidenced when Balslev et al., (2007) found that the optimal orientation of current direction is normally distributed around the postero-lateral orientation with a range of 63°. Leading to the conclusion that the coil orientation should be individually determined in order to optimise stimulation current direction (Balslev et al., 2007).

Coil orientation (in the yaw axis) was not individualised in the present experiment and held at the commonly used position of 45° to the sagittal plane with the handle pointing in posterior direction. Given the nature of the experiment it may have been beneficial to have individualised the coil position in the yaw axis. Had this have been the case, the observed 3dB 70% cut-off ranges found in this study may have increased due to the stimulation recruiting neurons more comprehensively and therefore eliciting MEPs at greater angles. Finding each individual participant's optimal orientation is not common in TMS studies but if it were to further reduce the effect of coil tilt it may be a worthwhile procedure.

Scalp to cortex distance

The stimulus-response relationship varies greatly between participants (Pitcher et al., 2003), meaning if the stimulus intensity is standardised relative an individual's motor threshold, the MEP amplitude elicited at this intensity will differ between each participant; this is also reflected in the high between-subject variation of resting motor threshold. A source of the variation in between-subject stimulus-response relationship is the variation in individual anatomy; namely the variation in scalp to cortex distance, which has been reported to range up to several millimetres (Sommer et al., 2006). This is meaningful as the induced magnetic field decreases exponentially in proportion to the distance from the stimulation coil resulting in a reduced strength of the induced electric field (Knecht et al., 2005).

It is clear from the results of the present study that there is a range of responses to changing the coil angle at the scalp across all participants; observed in the considerable variation in the shapes of the fitted curves for both roll and pitch axes seen in Figure 20, B. This may also be due to the variation in scalp to cortex distance seen across participants. In a participant with a large scalp to cortex distance, a change in tilt angle at the scalp will translate into a much larger and exaggerated change in position stimulated on the motor cortex, therefore affecting MEP amplitude to a greater extent than an individual with a small scalp to cortex distance; an equivalent change in tilt at the scalp will not culminate in such an exaggerated change in position on the motor cortex.

The effect of between-subject variation in scalp to cortex distance is also reflected in the results, particularly in the roll axis where the 70% cut-off range shows considerable variability. If the results of the present study are to be used as guidelines for future TMS studies, this variability needs to be taken into account. Given that the cut-off range at the lower bound of the standard deviation is still wide in both axis, it seems pragmatic to apply exclusion criteria with this in mind to further minimise any effect of tilt on MEP amplitude.

Interpretation of translated and non-translated curves

In the present study it was necessary to translate the fitted Gaussian curves so the peak of the curve was at zero in order to facilitate data analysis (Figure 20, B). Although the curves were manipulated to find the primary result of the study, it is also important to interpret the non-translated curves.

The need to translate the fitted curves indicates the coil was not flat to the scalp when the hotspot position was recorded on the neuronavigation system. This occurred in almost all participants and is particularly evident in the roll axis (Figure 20.). The time taken by the experimenters to ensure the coil was as flat to the participants scalp as possible was more extensive than previous studies the experimenters have conducted. Indicating that it may be more difficult than initially thought to find the optimal 'flat' coil position with the commonly used TMS methods.

The procedure used to ensure the coil was flat to the scalp was largely based on visual inspection of the coil position when held over the hotspot position on the scalp. This is due to the fact that participants did not have an MRI scan prior to TMS testing and the recorded neuronavigation positions were registered on a generic scan.

The use of a generic MRI scan and, as a result, the fact that each individuals head shape was not accounted for are two aspects of the current experiment that could have influenced the results and is therefore a limitation. Given the nature and circumstances of the experiment it was not feasible to record and utilise an MRI scan for each participant, and therefore a generic scan was used. Attempting to 'fit' each participant to this scan means the angles recorded are relative to the scan and may not show the true change in angle as the coil is rotated around each axes. Differences in head shape will have confounded this effect further as any differences between participants is not captured with an individualised MRI scan.

A navigation system can be used to locate the coil outside the head, however it will not provide information about the spread to the electric field or the intracranial location of the stimulation (Ruohonen and Karhu, 2010). Bashir et al., (2014) suggests using MRI based neuronavigated TMS which includes the systematic examination of optimal current directions relative to an individual's unique anatomy. Taking into account inter-individual differences in neuroanatomy allows more precise coil positioning in comparison to cranial landmarks and will help in positioning the coil in the most optimal position.

Conclusions

This experiment is the first to investigate the effect of coil tilt on MEP amplitude, a potential source of MEP variability in studies not using a neuronavigation system. We have demonstrated that minor coil tilt at the scalp does not influence MEP amplitude. We have concluded that an experienced TMS experimenter would be able to maintain and replace the TMS coil on the target position within the boundaries established in this experiment. The results of this study also provide guidelines for future TMS studies seeking more established exclusions criteria when analysing neuronavigation data in order to use data of the highest quality.

General Discussion

Brief summary

The experiments conducted in this study aimed to establish the influence of two methodological factors, common to TMS studies, have on MEP amplitude, with the objective to provide guidelines for future work. The first experiment sought to assess the variability of MEPs when the stimulation intensity was set to achieve a 1 mV response size, a procedure that is becoming increasingly common in TMS literature. The principle result of this experiment is that this procedure used to determine stimulation intensity yielded MEPs that were not only variable but consistently greater compared to baseline over seven repeated measures and therefore this method is not recommended to be used for future research.

The second experiment examined the effect of coil tilting at the scalp on MEP amplitude, primarily to investigate whether this could be a source of MEP variability in TMS studies that do not use neuronavigation systems. The principle finding of this study is that coil tilt only affects MEP amplitude at more extreme angles and therefore an experienced experimenter would be able to maintain or reposition the coil within the boundaries defined in this investigation; though an inexperienced one may not. We have also provided guidelines for boundaries with which exclusion criteria can be applied to neuronavigation data.

Limitations of present studies

Experiment Length

Although the evidence to suggest cortical arousal and attention influences fluctuations in MEP amplitude is not firmly established, this may have played a role in affecting the results of the experiments in present study. Due to the nature of the experiments, participants were in the laboratory for up to two hours including preparation and data collection, in some cases the length of the experiment increased if there were technical issues. Without a motor learning

paradigm or similar task within the experiment, it is likely that participant's attention and emotional state drifted throughout the experiments.

Specifically in experiment 2, the TMS coil overheating and automatically disarming was the major contributor to a prolonged single experiment. The stimulation intensity was set to 120% of the participant's motor threshold meaning in some cases the stimulator was set to high intensities. This, coupled with the short inter-stimulus interval caused the coil to overheat regularly. This occurrence of the coil regularly overheating was an unforeseeable problem, especially as there had been no such issues during pilot testing. Because of the presentation of the axis stimulated was uniform for all participants, roll axis manipulated first, the MEPs recorded when tilting the coil in the pitch axis will have been affected more by the participants drifting attention towards the end of the experiment. Future studies would benefit from randomising which axis is stimulated first; randomising the presentation of an intervention is commonplace in other research investigations.

Discrepancies in experiment methodology

Although both experiments were investigating aspects of TMS studies that could affect MEP variability, the two aspects being assessed were different, as were the aims. The most appropriate methodologies were chosen based on the specific aims of the experiments allowing us to most effectively investigate the hypotheses.

The rationale behind the methodology in experiment 1 was largely based on the findings of Cuypers et al., (2014) concluding that 30 MEPs is sufficient to obtain a reliable estimate of CSE. Eight sets of MEPs were chosen as this allowed a large number of MEPs to be collected within a one hour session; meaning attention and emotional state (van Loon et al., 2010; Kiers et al., 1993) would be less likely to influence CSE; the presentation of background EMG to the participant was used to minimise the effects of the aforementioned sources of variability.

The ISI of 4 seconds was based on findings from an investigation into the effect of ISI on MEPs, finding the ISI of 4 seconds yielded a high ICC between sets, within one session (Vaseghi et al., 2015). EMG exclusion criteria was intentionally strict to remove any effect of fluctuating background EMG as this widely reported to have the greatest influence on MEP amplitude (Rösler et al., 2002). Using these methodologies in this simple experiment, we were confident we were minimising any external sources of MEP variability.

The protocol used in experiment 2 was based primarily on two previous experiments, Mathias et al., (2013) and Vaseghi et al., (2015), and the pilot study run prior to the primary experiment. The stimulation intensity was chosen based on the finding that MEPs taken at 120% RMT yield reliable within-session MEPs (Vaseghi et al., 2015), this stimulation intensity is also commonly used in TMS research (Cuypers et al., 2014; van de Ruit et al., 2015) to assess CSE. The use of a stimulation intensity relative to the RMT also ensured that MEPs would be recorded, had the stimulation intensity been set close to the RMT, MEPs may not have been recorded, even at the hotspot, meaning the aim of the study could not be investigated. The ideal number of MEPs recorded per angle would have been 30 (Cuypers et al., 2014), however, as the pilot study showed, this had to be reduced in order to increase the number of angles investigated without prolonging the already lengthy experiment. The ISI was also chosen with the aim to reduce the length of the experiment. Mathias et al., (2013) found that is it possible to record MEPs at an ISI of 1.5s without comprising MEP amplitude, a method that has adopted in further experiments (van de Ruit et al., 2015). The EMG exclusion criteria, excluding samples based on the RMS of the background EMG, was also based on previous experiments that have used this shortened ISI (Mathias et al., 2013; van de Ruit et al., 2015). The use of the more rigorous exclusion criteria used in experiment 1 would be preferential if this experiment were to be repeated or taken further to minimise the effect of

fluctuating background EMG, however the volume of MEPs and the pace at which they were recorded may mean this is unachievable.

Given the methodologies used in each experiment were the most appropriate to investigate the aims and hypotheses, the differences in methodologies used in the two experiments means the results are not directly comparable, and as such is a limitation of this body of work.

Alternative TMS measures

As commonly used in TMS studies, both experiments in this investigation focused on averaged single MEP amplitudes recorded over a single point with a fixed intensity. Average MEP amplitude is frequently used as an assessment of CSE as it is quick and relatively simple to collect a small number of MEPs recorded from one position, the hotspot. As discussed in length in the literature review trial-to-trial variability of MEP amplitude means multiple responses need to be recorded in order to achieve a sound estimate of CSE. However a recent study by Cuypers et al., (2014) suggest that the minimum number of MEPs needed to attain a reliable estimate of CSE when averaging single MEP amplitude is 30; meaning the majority of experiments thus far have fallen short of the necessary amount of data necessary, with some using as little as 4 MEPs (Lewis et al., 2014).

Alternative TMS methods such as cortical maps or stimulus response curves may be sounder means to assess CSE as they provide more information than single MEPs such as the area of excitability found with cortical mapping. Additionally, these measures may be less susceptible to single MEP variability as a curve is fitted through data points to produce a stimulus-response curve and a 'mesh' is fitted over data points to create a cortical map.

As mentioned previously a change in the shape and size of a cortical map, and a change in the profile of a stimulus-response curve are both indicative of a change in CSE. A cortical map is characterised by the parameters: COG (the amplitude weighted mean of the map), map

area, map volume and map shape. A stimulus-response curve is characterised by the same number of parameters: the motor threshold, I50, maximum plateau and the area under the curve. All of which can be used to monitor CSE over time.

These two techniques have been commonly applied to investigate neuroplastic changes which accompany motor learning (Pascual-Leone et al., 1995; Suzuki et al., 2012). They also provide complimentary data meaning they can be used in conjunction with one another, a stimulus-response curve will always detect changes in the area of cortical maps, however only maps readily detect changes in the distribution of excitability within a cortical zone (Ridding and Rothwell, 1997).

In comparison to experiment 1 in the present study revealing a way to assess CSE with poor reliability, several studies have assessed the reliability of stimulus-response curve and cortical map parameters (Carroll et al., 2001; van de Ruit et al., 2015). Authors have reported ICCs for stimulus-response curve parameters ranging between 0.72 and 0.96 (Carroll et al., 2001) and for cortical maps ranging between 0.74 – 0.94 (van de Ruit et al., 2015), indicating that stimulus-response curves and cortical maps are both reliable means of assessing CSE.
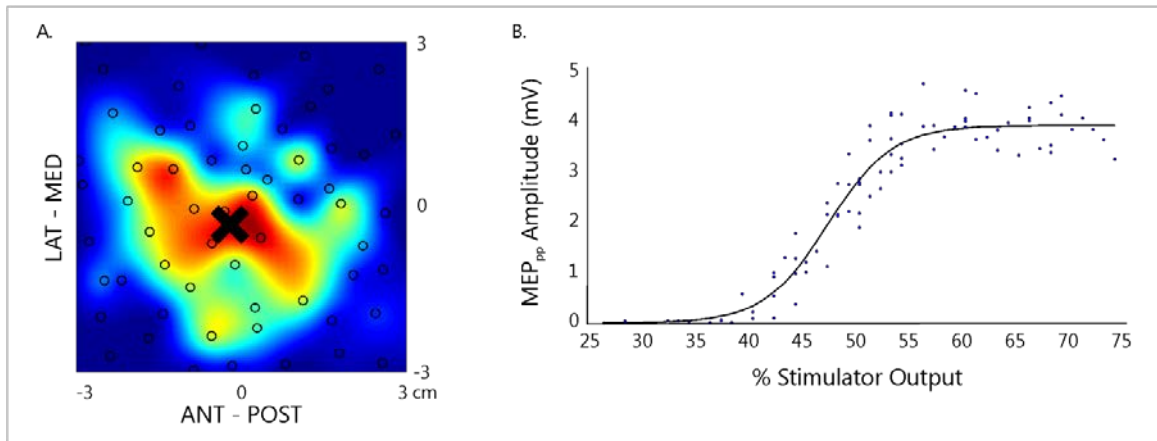
Figure 24. Representative examples of a motor map (A) and stimulus response curve (B). A motor map is constructed by plotting MEP amplitude against position data. In this example corticospinal excitability is indicated by colour, red indicates an area of high excitability, blue indicates an area of low excitability. The black cross (x) highlights the centre of gravity. A stimulus response curve is constructed by plotting MEP amplitude against the corresponding stimulation intensity, a sigmoidal curve is fitted through the data.

As this study has shown, single MEPs may not be the most reliable measure of CSE and plasticity and it might be beneficial to adopt stimulus-response curves and cortical maps in order to obtain more robust measures. The use of a stimulus-response curve negates the need to choose and set a stimulation intensity given that MEPs are recorded from the full range of intensities. As is clear from the results of the first experiment in this study, setting the stimulation intensity is not necessarily a simple process. To construct a cortical map, the cortex is stimulated in multiple positions. Previously, tilting of the coil as the experimenter moves the coil over the scalp during acquisition could have been seen as a limitation of cortical maps. The second experiment in this study has proven that minor coil tilt does not influence MEP amplitude and therefore does not pose a problem during the acquisition cortical maps.

Recent methodological advancements using both stereotaxic neuronavigation systems and considerably decreasing the inter-stimulus interval have reduced the time taken necessary to acquire a cortical map or stimulus-response curve, without relinquishing the reliability seen

with previously slower, traditional techniques (Mathias et al., 2013; van de Ruit et al., 2015). TMS researchers seem to have been reluctant to utilise stimulus-response curves and cortical maps due to the time taken to acquire them (Pitcher et al., 2015). However it is now possible to obtain these potentially more robust measures in the same time taken to collect a series of single MEPs as well as providing more information (Mathias et al., 2013; van de Ruit et al., 2015).

Further study

Experiment 1. Assessing the reliability of measures made using the absolute method of determining test stimulation intensity

This study has investigated the reliability of averaged MEP amplitude obtained using newly adopted method of setting the stimulation intensity to achieve a 1 mV response size and found it is not a reliable method. One reason this approach has been adopted by some research groups may be due to ambiguity in guidelines for TMS studies to follow when planning an experiment. Therefore more work is needed to better define the most appropriate way of determining stimulation intensity; as the method investigated here should not be used in further studies as it is not reliable. As opposed to attempting to approximate the intensity set to achieve an MEP of 50% maximum as a proportion of resting motor threshold (Pitcher et al., 2015), it would be interesting to find the optimum intensity by acquiring a stimulus-response curve initially and utilise the parameters gained, primarily the I50. The time taken to acquire a stimulus-response curve has prevented this from becoming commonplace (Pitcher et al., 2015). As discussed, recent advancements in methodology, reducing the time taken to construct a stimulus-response curve means this is now a possibility.

Experiment 2. The effect of changes in coil tilt angle at the scalp on MEP amplitude

The axes that were investigated in the second experiment of this study were only two of countless different ways in which the TMS coil can be tilted at the scalp. It is unlikely that the coil will only be tilted in one specific direction when used practically in TMS research. The effect of the coil tilting over a combination of axes could further influence MEP size, it is conceivable that as the coil is rotated over multiple axes the orientation (in the yaw axis) may rotate as a consequence, moving the coil away from its optimum position. This is speculative and cannot be inferred from the results of this study and therefore it is something that needs further investigation.

Concluding remarks

The present study has provided new information regarding previously unexplored factors that commonly affect TMS experiments. Setting an appropriate stimulation intensity and the use of a neuronavigation system are fundamental aspects of TMS studies, however there are still disparities between study methodologies despite continued work in the field; experiments in this study have expanded the current understanding of how these factors can influence the response to TMS. This body of work also highlights the need for further rigorous research into TMS methodologies in order to determine the most appropriate and optimal techniques - something that has eluded this area of research thus far - and how recent developments can facilitate this.

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Appendices

1.

Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen*

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

CIRCLE or CROSS OUT

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

Signed: ....Date: ....

Name (in block letters): ....………........................

2.

Neuroplasticity and Neurorehabilitation Laboratory

School of Sport and Exercise Sciences

INFORMED CONSENT

Participant Identification for this study:

The effects on neural excitability in chronic stroke patients following a home based mirror therapy programme assessed using Transcranial Magnetic Stimulation (TMS).

Name of Researcher:

Please initial

I confirm that I have read and understood the information sheet detailing the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reasons.

I understand that where it is relevant to my research participation, data acquired may be analysed by responsible individuals from the University of Birmingham, or from regulatory authorities. I give permission for these individuals to have access to my data records.

I agree to have transcranial magnetic stimulation (TMS) in different study sessions as outlined in the participant information sheets.

I agree to attend sessions at the Neuroplasticity and Neurorehabilitation Laboratory at the University of Birmingham's School of Sport and Exercise Sciences.

I agree to take part in the above study.

_____   _____   _____

Name of Participant   Date   Signature

_____   _____   _____

Researcher Name   Date   Signature

3.

Edinburgh Handedness Inventory[1]

Your Name:_____

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).

If you are indifferent, put one check in each column (✓ | ✓).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH − LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left Handed: R < -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right Handed: R > +40)		

[1] Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychololgia*, *9*, 97-113.