THE FACTORS INFLUENCING TMS MAPPING

By

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ABSTRACT

The functions of different areas of the human brain has fascinated academics for centuries, but it is only within the last century that a viable, non-surgical method to assess cortical plasticity and corticomotor pathways has become available, in the form Transcranial Magnetic Stimulation (TMS) (Groppa et al., 2012). TMS has wide ranging practical applications, including assessing alterations in cortical plasticity during rehabilitation or after specialised training using a technique known as TMS mapping. Nonetheless, there are a number of confounding factors that may influence the outcome of the technique, including the pulse type used, the orientation of the stimulating coil and muscle activity. The aims of this thesis is to investigate the influence of current direction, pulse type and muscle activity on TMS mapping outcomes.

Utilising the mapping protocol developed by van de Ruit et al., (2015) these factors were assessed within this thesis, primarily looking at their influence upon the size (map area) and the centre of gravity (COG) of TMS maps, as well as other minor parameters. The four experiments consisted of neuronavigated TMS mapping of the first dorsal interosseous of the left hand, with the orientation of the TMS coil (and therefore the current direction) altered. The protocols were performed under both active and passive conditions, and participants received between 750 and 1500 TMS pulses.

This thesis confirms that map area is highly susceptible to changes as a result of alterations in current direction, pulse type and muscle activity. It was shown that sub-optimal current directions reduced the map area, half-sine pulses generally produced smaller maps than biphasic pulses and muscle activation increased map area. This thesis also shows that COG is largely robust to changes in the same factors. The findings from these experiments have important implications for future TMS practice in both a scientific and healthcare setting.

CONTENTS

ADM	Abductor Digiti Minimi
AP	Anteroposterior
APB	Abductor Pollicis Brevis
AMT	Active Motor Threshold
BB	Biceps Brachii Muscle
COG	Centre of Gravity
CMAP	Compound Muscle Action Potential
Cz	Cranial Vertex
CSE	Corticospinal Excitability
EDC	Extensor Digitorum Communis muscle
EEG	Electrencephalography
EMG	Electromyography
EPSP	Excitatory Postsynaptic Potential
FDI	First Dorsal Interosseous muscle
(f)MRI	(functional) Magnetic Resonance Imaging
GUI	Graphical User Interface
ISI	Interstimulus Interval
IQR	Inter-Quartile Range
LM	Lateromedial
MEP	Motor Evoked Potential
MEP_{pp}	Motor Evoked Potential peak-to-peak amplitude
MVC	Maximum Voluntary Contraction
PA	Posteroanterior
PET	Positron Emission Tomography
RMT	Resting Motor Threshold
rTMS	(repetitive) Transcranial Magnetic Stimulation
SI	Stimulation Intensity
SR	Stimulus Response
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation

CHAPTER 1

Introduction

1.1 Background

In the early 1800s, neuroscience was a fledgling discipline with few methods available capable of assessing brain function. Nonetheless, the field was inspired by various isolated case studies. Most notably the case of Phineas Gage in 1848, who famously suffered from an iron bar through the skull whilst working laying train tracks in North America. Gage miraculously survived, however it was later noted that he had suffered some severe changes in both his intelligence and personality (Harlow, 1848; Damasio et al., 1994). This suggested that damage to particular areas of the brain could elicit specific alterations in an individual's ability to think. Indeed, this was corroborated some years later by Broca (1861), who discovered a patient suffering with aphasia had a lesion in the posterior inferior frontal gyrus. Broca deduced that this lesion was likely resulting in his speech impediment, thus giving rise to the notion that specific areas of the cortex performed specific functions. Building on this work, Fritsch and Hitzig (1870) performed a series of investigations upon live canines, removing the skulls and administering electrical stimulation to the cortex. They discovered that performing electrical stimulation to one side of the cortex elicited movements of limbs on the contralateral side to stimulation. This work suggested the existence of the motor cortex, a specific area in the brain responsible for controlling, or at least eliciting movement. In the first study in humans, Bartholow (1874), elicited a motor response in a patient with a scapular ulcer, via stimulation with needle electrodes. The patient underwent a number of uncoordinated movements of the limbs, but nonetheless, this rather crude investigation indicated that stimulating the cortex using electricity could elicit a motor response. Over the coming years, this method of stimulation was refined, firstly with Ferrier (1886) and his seminal work assessing, mapping the motor cortex of macaque monkeys using specific electrical stimulation. The early investigations by Penfield (1937), utilised electrical stimulation to create motor and sensory maps of the cortex in humans

under local anaesthesia. This seminal work not only allowed development of the notion that particular areas of the cortex have specific functions in both a sensory and motor contex, but also resulted in the creation of the famous sensory and motor homunculi that are so integral to our understanding of the cortex, even today. Nonetheless, the reality seems that there is not a well-defined muscular map within the cortex, and instead a rather more complex reality exists where cortical representation of muscles overlap in particular areas of the cortex, where only broad areas of representation can be grouped together (Sanes and Donoghue, 2000; Graziano et al., 2002). More recently, further recent scientific progress has brought these theories into question, and work by Graziano and colleagues (2002). The investigators stimulated areas of the primary motor and premotor cortex of monkeys for 100-500ms. The investigations showed that one could elicit particular final postures by stimulating precise areas of the cortex. Movements were traced using high-speed cameras and it was determined that final limb positions could be elicited by stimulating particular areas of the cortex. It was also noted that although the original position of the limb was of little consequence, but the final location of the limb was highly consistent when stimulating the same area of the cortex. This suggests that not only broad areas of muscular representation can be confined to particular areas of the motor cortex, but also specific postures can be elicited (and therefore are represented) in particular areas of the cortex..

Despite the obvious potential of stimulating the motor cortex to investigate function, one obvious flaw remains; the stimulatory techniques were restricted by a reliance on an open skull to access the cortex for electrode implantation. It was not until the latter part of the 20th century that Merton and Morton (1980) applied silver-cup electroencephalogram electrodes above the motor cortex. With a very high voltage (2000v) and small discharge time constant (<10μs), twitch responses were elicited in the contralateral fingers and foot, in accordance with the placement of the electrodes over the corresponding areas of the motor cortex. This investigation meant that one could investigate the motor cortex in an intact subject in a way that was not

previously possible. In the following years, investigations looking into myriad diseases, primarily assessing the integrity of corticomotor pathways (Groppa, 2012). This was achieved in a number of ways, such as the investigation in multiple sclerosis (Cowan 1984), who had increased central conduction time between cortical stimulation and muscular contractions when compared to healthy individuals. Nonetheless, similar increases in conduction time were not seen in patients with Parkinson's disease (Dick 1984), thus showing that this technique can help demonstrate the pathophysiology and associated corticomotor abnormalities that arise from these diseases. Nonetheless, the main disadvantage with this form of stimulation is that the high resistance of the skull and the need to use high voltages to elicit a response meant that participants were placed in considerable discomfort. In order to perform longer investigations to access cortical function in greater detail, a more practical, and less painful method was required.

1.2 TMS and Physiology

A solution to the aforementioned practical issues came from via Barker and colleagues (1985) in the form of TMS. The investigators noted that using a coil (attached to a magnetic stimulator) over the motor cortex, movement of contralateral limbs can be elicited without pain. This gave investigators the means to assess wide ranges of cortical motor function without distressing participants. Subsequently, in the following 30 years, TMS has evolved into a vital tool in neurophysiology, allowing non-invasive investigation into the plasticity of the human cortex and the function of the cortex during disease and following injury.

TMS consists of an electromagnetic coil attached to a high voltage, high current discharge system (Jalinous, 1991). Upon discharge, a transient, magnetic field is produced at right angles to the stimulating coil (Groppa et al., 2012).

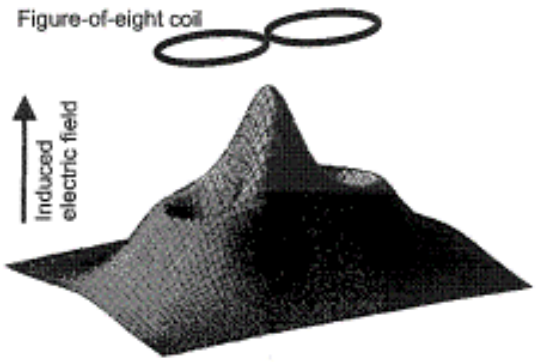

Figure 1: Adapted from Ilmoniemi et al. (1999). Electric field strength below a figure-of-eight TMS coil. The electric field is produced at right angles to the plane of the stimulating coil.

When a stimulating coil is placed on the scalp, and the stimulator is discharged, the magnetic field produced passes through the high-resistance skull and creates a secondary current flow within the conductive brain tissue (Cowey, 2005). The current produced within the brain tissue is small, but has the ability to produce action potentials providing there is sufficient ion flow across the membrane in the cortical axons that enables depolarisation of the membrane. These action potentials can move trans-synaptically, and down the corticospinal tract to be recorded in contralateral target muscles as MEPs (Groppa et al., 2012). The corticospinal tract is the major pathway comprised of axonal bundles, along which motor signals can pass to the target muscle. The pathway runs from the upper motor neurons (pyramidal/Betz cells) of the primary motor cortex (M1). Nonetheless the pathway continues to the medullary pyramid prior to decussating contralaterally, and continuing to the lower motor neurons and peripheral musculature (Purves et al., 2004; Schieber et al., 2007).

With magnetic stimulation of a sufficiently high intensity, a muscle twitch can be elicited, and MEPs (see *Figure 2*) can be recorded via surface electrodes. An MEP is described as the brief, somewhat synchronous muscle response to the descending volleys (see *Figure 2***A**) that can be detected via electromyography (Day et al., 1989; Di Lazzaro et al., 2004a; Hallet, 2007). When a TMS pulse is delivered to the primary motor cortex, a number of descending volleys are produced, which can be recorded within the corticospinal tract (Patton and Amassian, 1954). These descending volleys temporo-spatially summate at the cortico-motorneuronal synapses, to produce an action potential and resulting in an MEP (see *Figure 2*) (Groppa et al., 2012).

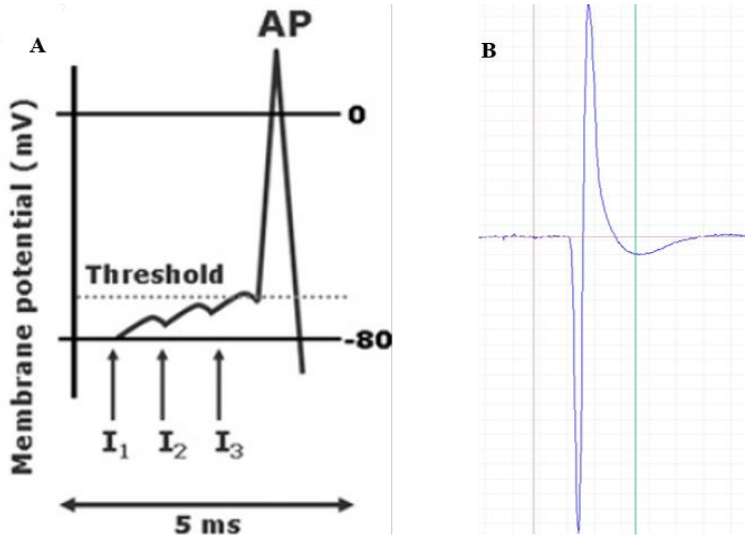

Figure 2. Temporal=spatial summation at cortico-motoneuronal synapses to produce an action potential, adapted from Groppa et al. (2012) in **A**. *This produces an MEP, as shown by the raw single MEP obtained from Mr. Kick™ software in* **B**

The descending volleys produced differ in terms of their latency, and were initially detected by Patton and Amassian (1954), via direct electrical stimulation to the exposed cortex of cats and monkeys. Two distinct volley-types were found upon recording the responses in the cervical corticospinal tract; Direct- or D-waves and Indirect- or I-waves. The terms for the volley-types were coined as a result of the activation of different neurons in the cortex. So called, 'D-waves' were suggested to be produced via direct stimulation of the pyramidal tract neurons, whereas the I-waves were said to be produced via synaptic activation of the pyramidal tract (Di-Lazzaro et al., 2004b).

The development of TMS by Barker et al. (1985) allowed further assessment of these descending volleys. The earliest studies combined both TMS and TES (Transcranial Electrical Stimulation), in patients who were under anaesthesia (Berardelli et al., 1990; Burke et al., 1993; Thompson et al., 1991b). Indeed it was confirmed that these two volley types were separated temporally, with I-waves occurring with a latency of approximately 1.5ms (Berardelli et al., 1990). Nonetheless, these initial investigations were limited by the effect of anaesthesia on the I-waves produced in these subjects (Burke et al., 1993). The usage of conscious patients with implanted epidural electrodes removed the influence of anaesthesia (Di Lazzaro et al., 2004a). Thus, it was determined that D- and I-waves were produced by both forms of stimulation, it became apparent that although TMS could elicit D-waves at sufficiently high intensities, on the whole TMS recruited D-waves and TES recruited I-waves (Kaneko et al., 1996c).

1.3 Cortical Plasticity

The concept of a 'plastic' cortex emerged some time ago (Lashley, 1923), with the suggestion that the organisation of the motor cortex could be 'temporary'. This bought into question the long-standing belief that the cortex changed little upon reaching adulthood. Despite this early indication, it is not until more recently that this notion has become well established.

In their seminal papers, Merzinich and colleagues (1984) investigated the alterations in the somatosensory cortex in owl monkeys following digital amputation, using microelectrode mapping techniques. It was known that each digit had its own cortical representation in the somatosensory cortex, however, amputation of a digit resulted in the loss of the cortical representation of that finger. Instead, the cortical neurons that initially responded to the now amputated digit, now responded to stimulation of the adjacent digits. Thus suggesting that the somatosensory cortex is plastic and dynamically maintained with experience, and that inputs to the cortex could be in use-dependent competition with one-another (Kaas et al., 1983). This research was corroborated by Allard et al. (1991) where the 3rd and 4th digits of owl monkeys were connected to form a syndactyly. Indeed, months later, the microelectrode maps revealed

that there was near-simultaneous input from the surfaces of the connected fingers, and the normally separate cortical representations of the two adjacent fingers was removed.

This alteration of the cortex was also seen after peripheral nerve injury in rats (Donoghue & Sanes, 1987). The organisation of the motor cortex was studied in rats that had the right forelimb amputated on the day of birth. When compared to normal counterparts via intracortical microstimulation, the experimental rats had more robust connections with muscles proximal to the site of amputation and greater cortical representations of these muscles, thus demonstrating the motor cortex can be altered during development by peripheral injuries.

This plasticity of the cortex in response to peripheral injuries has also been demonstrated in humans, most notably in the seminal studies by Ramanchandran and colleagues (1992). Here, the investigators examined the localisation of touch sensations after amputations of the upper limb, by applying light touch or deep pressure to the body surface, and mapping the receptive fields. Individuals reported the sensations on their amputated limbs when touch stimulation was applied to the face or upper arm. Thus suggesting cortical reorganisation of adjacent somatosensory areas in the cortex and neurons that once responded to peripheral limb stimulation now respond to facial stimulation. It was further ascertained that these alterations could be detected just 24 hours following arm amputation, suggesting rapid somatosensory reorganisation. Similar observations regarding the cortex have been observed in blind humans where the visual cortex responds to auditory stimulation (Weeks et al., 2000) and tactile stimulation via Braille reading (Buchel et al., 1998), and in deaf individuals whose auditory cortex responds visual stimuli (Finney et al., 2001).

Notably, plasticity of the cortex is not a response that is limited to disease and injury, it is a phenomenon that is associated with experience and motor learning. Jenkins et al. (1990), demonstrated this in owl monkeys, where hand-use was modified behaviourally. Over a period of several days, the monkeys were trained to make contact with a rotating disk, with alternating

raised and lowered surfaces, increasing tactile stimulation to digits 2, 3 and 4. Indeed, post-intervention, microelectrode mapping revealed cortical remodelling via increased representations of the fingertips of digits 2-4 within the somatosensory cortex.

This plasticity as a result of experience has repeatedly been demonstrated in humans, notably after performing skill-based training. This has been evidenced by Karni et al. (1995) who trained individuals to perform rapid sequences of finger movements over a period of several weeks, with daily practice sessions. The extent of cortical activation during fMRI, when performing the trained sequence was enlarged versus an unpractised sequence. This increased cortical activation was maintained for several months, indicating experience-driven reorganisation of the motor cortex, thus providing a possible mechanism to understand the ability to learn, and retain new motor skills. Further, Lundbye-Jensen et al. (2005), who assessed alterations in corticospinal excitability following skill-based training. Individuals, performed elbow flexion and extension to move an on-screen target, with the goal of tracing a pre-determined path. After several sessions of training, there was an upward and leftward shift in the stimulus-response curve indicating increased corticospinal excitability, and thus plastic changes in the corticospinal pathway, as a result of the skill-based training. Despite the clear indication that cortical plasticity occurs with skill-based training, plasticity does not occur with strength training.

The use of TMS to assess cortical plasticity in humans has become increasingly widespread in the literature, most notably within the last 20 years. Indeed, Pascual-Leone et al. (1995) assessed alterations in map area after one-handed piano exercising. Map area refers to the expanse of the skull surface that can be stimulated to activate a particular muscle and produce an MEP. It was ascertained that after several weeks of training, cortical map area of the finger flexor and extensor muscles was increased.

The importance of aforementioned studies is two-fold. Firstly, there is plenty of empirical evidence to suggest that the human motor cortex is plastic and is involved in motor learning, an idea that had been at the subject of much debate until recently. Secondly, and perhaps an area that has been somewhat negated, is the notion that TMS can be used as a powerful tool to assess cortical plasticity safely and effectively within humans. Perhaps most notably, Pascual-Leone's study (1995) suggests that the motor cortex can be mapped over several time periods allowing examination of alterations in cortical plasticity over time. This highlights TMS as a potentially invaluable measure for a number of different areas, from assessing changes in cortical plasticity in both healthy and clinical populations.

1.4 TMS Pulse Types

Currently, there are three predominant forms of single-pulse TMS, more specifically, three forms of stimulus wave form that are used: monophasic pulses, half-sine pulses and biphasic pulses. Traditionally, early stimulators were only capable of delivering monophasic TMS. In these stimulators, the discharge current through the coil produces a monophasic magnetic field, with the magnetic field peaking in strength around 50μs (see *Figure 3*). Although there is a dampened return current, this return current cannot produce action potentials. Thus, in the case of monophasic stimulation, the initial current flow is the physiologically relevant stimulus (see *Figure 4*) (Kammer et al., 2001; Groppa et al., 2012). More recently, stimulators have been developed that are capable of delivering biphasic stimuli.

Biphasic stimulation that produces a wave form similar to that of a cosine wave, with two clear reversals in current flow (*see Figure 3)*. The initial rise in current flow, then falls below zero, to then reversal back up to zero. With biphasic waveforms, both positive and negative sections of the waveform can produce stimuli that are physiologically relevant. Notably though, the fact that the reversal phase (i.e. $3^{rd}/4^{th}$ quarter of the biphasic pulse) has considerably greater amplitude and duration means that the reversal phase is the more physiologically relevant phase (Di Lazzaro et al., 2001a; Cowey et al., 2005; Sommer et al., 2006; Groppa et al., 2012).

Finally, the half-sine pulse is highly similar to that of a biphasic pulse, but is terminated after the second quarter cycle of the cosine wave (Sommer et al., 2006). Thus, it lies somewhere in between a monophasic and biphasic pulse as it does not have the two reversals in current direction that signify a biphasic pulse, yet it does not taper off like a monophasic pulse (Sommer et al., 2006) (see *Figure 3)*.

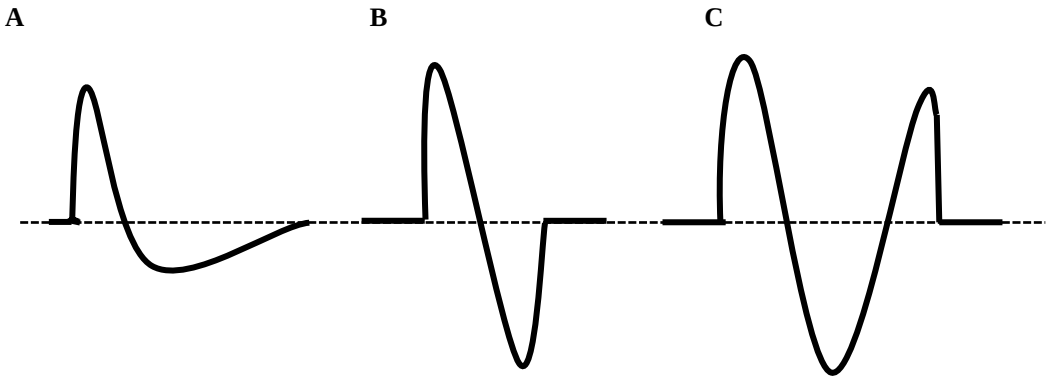

Figure 3. *Currents induced in a probe coil when recording onto an oscilloscope from each of the three common stimulator types (Adapted from Sommer et al., 2006).* **A** *monophasic pulse,* **B** *half-sine pulse,* **C** *biphasic pulse.*

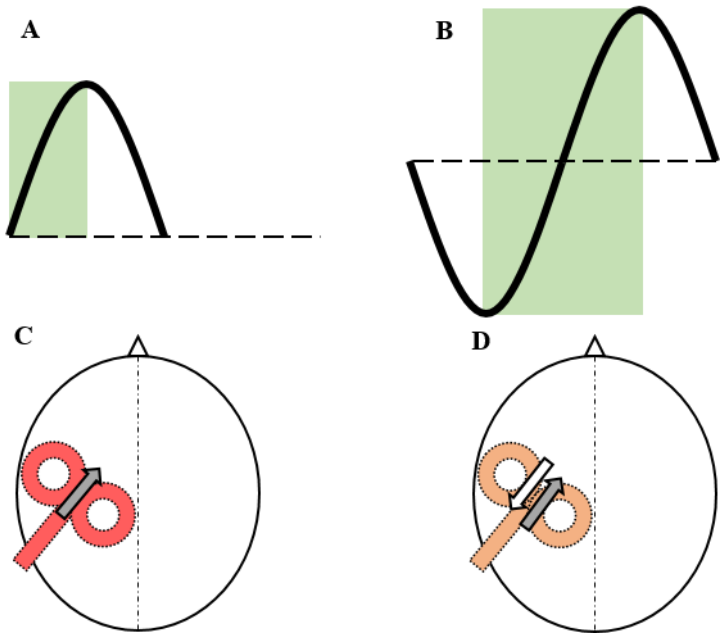

Figure 4. *The physiologically most relevant phase of the pulse is differs when comparing the two pulse types. A graphic representation of the physiologically most relevant phases of:* **A**, *a half-sine and monophasic pulses and* **B**, *biphasic pulse, can be seen in the shaded areas. During the biphasic pulse, the initial negative component can produce a stimuli sufficient to produce a response, however, the positive phase is more likely to produce a physiologically relevant stimuli, owed to the greater amplitude and longer duration of this phase (Groppa et al., 2012). For instance in the Dantec Magpro stimulator used by Kammer et al. (2001),* **C**, represents a *monophasic pulse, whereas* **D** *represents a biphasic pulse. The filled arrows represent the physiologically most relevant phase, however in* **D**, *this phase is the second phase (i.e. 3rd and 4th quarter) of the biphasic pulse.*

The three stimulators are still frequently used, in different circumstances in accordance with the required data. Biphasic stimulation is becoming increasingly more common due to its superior effectiveness at producing a response from a muscle (Kammer et al., 2001; Arai et al., 2005; Sommer et al., 2006). Half-sine pulses are becoming frequently more commonplace as one can make assessments using the same stimulators to deliver biphasic and half-sine pulses (Sommer et al., 2006; Jung et al., 2012), thus potentially reducing data acquisition time and preventing the need to switch stimulators for data collection, which may lead to difficulties comparing results due to different power outputs, pulse widths and current directions. Nonetheless, monophasic stimulation is still preferred by some investigators as the pulses are more focal (Brasil-Neto et al., 1992b), utility in repetitive TMS (rTMS) (Arai et al., 2005) and the effects of each pulse are potentially easier to analyse. These differences are suggested to be as a result of the different neuronal populations that each pulse type excites. Monophasic pulses may activate populations of neurons oriented in one particular direction, whereas biphasic

pulses may activate several different populations of neurons (Arai et al., 2005) which is often cited as an important consideration for repetitive TMS, and may have relevance for single pulse TMS, but this notion needs investigation.

1.5 TMS Mapping of the Motor Cortex

Mapping of the motor cortex has become a well-established technique for TMS, allowing investigation of alterations in cortical organisation and excitability in response to both therapeutic and exercise interventions (Littmann et al., 2013). TMS mapping involves altering the position of a stimulator coil around the motor cortex, with a set number of focal stimuli, and subsequently assessing the motor response in particular muscles. This allows the creation of a 'motor map', in other words, a map highlighting the representation of a given muscle in the motor cortex. In their series of experiments, Nudo and colleagues performed mapping studies in squirrel monkeys. These experiments involved mapping of the motor cortex using intracortical microstimulation, and could demonstrate motor cortex reorganisation, following an induced legion in the finger area of the motor cortex – alongside recovered motor function (Nudo and Milliken, 1996a), or alternatively, following learning of new motor skills (Nudo et al., 1996b).

Wasserman and colleagues (1992) were the first to use mapping methodology in humans. These investigations elicited the parameters commonly used to assess outcomes from mapping procedures, COG and map area. The COG of a map represents the amplitude-weighted centre of the motor map (Wasserman et al., 1992) which is usually used to show functional alterations in the cortical representations of a muscle after skill acquisitions or interventions (Liepert et al., 2000a; Uy et al., 2002). Map area refers to the expanse of the skull surface that can be stimulated to activate a particular muscle and elicit an MEP (Wasserman et al., 1992).

The practical applications of mapping are wide-ranging, and are useful in both healthy and diseased or injured patients. A large proportion of the literature focuses on the reorganisation of the cortex following a stroke. The importance of developing the technique cannot be

underestimated, given the projection that there will be 7.8 million deaths worldwide due to stroke by 2030 (Strong et al., 2007).

Currently, TMS has two major functions for assessing stroke patients. Firstly, TMS can be used to assess the cortical reorganisation transiently following a stroke. Byrnes et al. (1999) hypothesised that functional reorganisation of the motor cortex was a potential mechanism for recovery in stroke patients. Thus, the investigators used TMS to map the representation of the abductor pollicis brevis muscle, alongside other physiological measures, including motor threshold and corticospinal conduction velocities. Indeed, the severity of the motor deficits could be ascertained by assessing the shifts in the cortical motor maps, alongside the peak-to-peak amplitude of the MEPs and motor thresholds versus the unaffected hemisphere in these patients. Moreover, this finding was concomitant to previous work that found differences between the affected and unaffected hemispheres in stroke patients, with a significant reduction in cortical motor area, increased MEP latency and motor thresholds (Traversa et al., 1997).

Stinear et al. (2007) used TMS mapping of the extensor carpi radialis in conjunction with MRI data to determine factors that could predict the extent functional improvement after a stroke, in accordance with the neurophysiological data obtained at baseline and following a 30-day programme of motor practice involving gross motor skills of the affected arm. Indeed, if patients exhibited very few or no distinct MEPs during baseline testing, their capacity for functional improvement was limited, especially if fractional anisotropy revealed a significant disruption to the corticospinal tract. Similarly Koski et al. (2004), showed that both immediate and long-term reductions in MEP asymmetry and motor thresholds, alongside increased map volumes between the affected and unaffected hemispheres, were correlated with greater functional improvement.

These studies highlight the notion that neurophysiological data obtained from TMS mapping can thus be useful tool to track the progress of rehabilitation in stroke patients. Liepert et al.

(2000b) detected transient improvements in motor function and an enlargement in the cortical representation of the APB after a single session of intensive physiotherapy. In the longer term, responses from the affected hemisphere improved significantly during the recovery period, assuming continuous rehabilitation (Rossini et al., 2003). Liepert et al. (2000a) assessed the efficacy of constraint-induced movement therapy and forced-use therapy (Liepert et al., 2001), detecting alterations in the size of cortical representation and the COG, or amplitude weighted mean of a map (Liepert et al., 2000a) of the target muscle, akin to other investigations using MRI (Kopp et al., 1999; Levy et al., 2001).

Nonetheless, the utility of TMS mapping is not restricted to stroke, indeed cortical reorganisation and plasticity has been assessed in patients with chronic writer's cramp (Byrnes et al., 1998, following upper (Cohen et al., 1991) and lower (Fuhr et al., 1992) limb amputations, ankle joint immobilisation (Liepert et al., 1995) and the cortical representation of the reading finger in Braille readers (Pascual-Leone and Torres, 1993). Further, mapping has been used to assess changes in cortical representation outside of disease and illness, including specific skill training in elite volleyball players (Tyc et al., 2005) and skilled racquet players (Pearce et al., 2000), detecting increased MEP amplitudes, reductions in motor thresholds, and shifts in the cortical motor maps (Pearce et al., 2000) and increased map areas (Tyc et al., 2005) after training in the dominant hand.

1.6 MEP Variability

Despite the clear utility of TMS and mapping in a number of settings, there appears to be some reluctance for widespread use of the tool, particularly within a clinical environment. There could be numerous reasons for this, including (but not limited to): the expense of the equipment, the technical expertise required to produce reliable data. Even so, one of the most profound contributors to this hesitancy is likely to be the inherent variability associated with TMS, particularly when considering that TMS mapping can often be a time consuming assessment. For instance, when measuring MEP_{pp} outputs, Lissens and Vanderstraeten (1996), Kiers et al.

(1993) and Julkenen et al. (2009) noted significant variation around the mean, showing mean figures of 2.39 ± 1.99mV, 1.91 ± 0.91mV and 1.74 ± 1.05mV respectively. This phenomenon has been extremely well documented, with a number of different sources of variability having an influence on motor output and physiological data obtained from the technique. For instance, potential sources of variability include: follicular phase (Smith et al., 2002), time of day (Ellaway et al., 1998; Kiers et al., 1993; Koski et al., 2005; Sale et al., 2007) and cortisol levels (Sale et al., 2008). Even a parameter as simple as electrode placement has elicited alterations in MEP and map characteristics. In a seminal study on the subject of TMS variability, Kiers et al. (1993) analysed the variance within MEP amplitude, altering the state of pre-stimulus muscle contraction, mental alertness, stimulus intensity and coil size. Indeed, all conditions had some influence, but MEP variability was significantly reduced with pre-stimulus muscle contraction and increased stimulation intensity. Moreover Corneal et al. (2005) evaluated the variability in motor map characteristics with 'conventional' and 'close-spaced' surface electrode placement. Indeed, the investigators detected significant differences in the normalised map volume and resting motor thresholds between the two electrode configurations.

These findings have two important implications. Firstly, there is a need to assess and verify numerous potential sources of variability, and secondly, attempt to negate the influence of these sources of variability, either by taking particular care when administering TMS, or alternatively moving towards a 'standardised' protocol for TMS mapping.

1.7 Techniques used to Reduce TMS Variability

The techniques used to perform mapping of the motor cortex vary considerably, with alterations in the number of stimuli administered, the locations of these stimuli, the inter-stimulus intervals and aids to ensure that the correct location on the scalp is located. Early investigations mapping the human motor cortex were performed both electrically (Cohen et al., 1988) or magnetically (Cohen et al., 1990b), however studies by Wasserman et al. (1992) and Wilson et al. (1993) set the basic protocol for many of the future mapping studies.

Recording from surface electrodes placed on proximal and distal arm musculature, the investigators marked grid positions 1 cm apart on the scalp, and delivered 3 or 4 stimuli to each of the 30-35 grid positions using a figure-of-eight monophasic stimulator. The order of direction of movement through the stimulator sites was randomised between subjects and between sessions. Stimulation of successive positions along the scalp continued until an area of MEP activity was surrounded by an area of inactivity. The maps were successfully produced and allowed the determination of the map area, volume and COG. However, perhaps the greatest utility of these investigations was the protocol for mapping. A method had finally been determined for focal TMS to systematically stimulate locations on the scalp to elicit a motor map. Notably, the aforementioned influence of coil orientation may have an effect with these protocols, particularly as the use of a skull-cap with grid markings solely enables the correct location to be stimulated, but not the optimal orientation. This variance in coil orientation may occur through yaw, pitch and roll, all of which may have an influence on MEP output. Notably Schmidt et al. (2015) suggest that alterations in stimulus orientation of >2 mm, whether this is via rotation of the coil through yaw or tilting the coil through roll and pitch and the propensity to significantly increase MEP variability.

Therefore, one may argue that the development of a more robust technique to determine the stimulation locations and orientations would be beneficial. Indeed, later investigations have utilised a 3D digitiser (Miranda et al., 1997) and MRI-based neuronavigation (Julkenen et al., 2009; Weiss et al., 2012) in an attempt to improve mapping reliability between stimulations, sessions and participants. Essentially, these techniques use the position of anatomical landmarks to ascertain the size of the participants skull, which can then be computed and tracking software can then be utilised to allow determination of coil position and orientation on the head to a high degree of accuracy. Miranda et al. (1997) showed that across sessions, COG position was found to be reproducible within ± 3 mm, map area and normalised volume were reproducible to within ± 20%, using a 3D digitiser, results that were further reproduced using

MRI-based neuronavigation (Weiss et al., 2012). Indeed, when comparing the use of non-navigated and navigated TMS, Julkenen et al. (2009) found that MEPs "exhibit significant differences depending on whether navigation is used". Thus neuronavigation allows the determination of the optimal coil angle for a given muscle, and just as importantly, the maintenance of this coil orientation during mapping procedures. Bashir et al. (2013) took the opportunity to determine the optimal coil orientation through yaw for a number of different muscles, including the FDI, and mirrored previous findings (Brasil-Neto et al., 1992b; Mills et al., 1992) that the optimal stimulus orientation for this muscle is in a segment 45^{o} to the midline, and thus can be maintained by neuronavigated TMS.

The second issue with the protocols used by Wasserman et al. (1992) and Wilson et al., (1993) is the significant amount of time that data acquisitions requires. A single mapping session required up to 3-4 hours of maintained alertness from the participant. Even if alertness was maintained, other aforementioned uncontrollable sources of variability could contribute to the results of the investigation; most notably diurnal fluctuations in corticospinal excitability (Ellaway et al., 1998; Kiers et al., 1993; Koski et al., 2005; Sale et al., 2007) and cortisol levels (Sale et al., 2008). An investigation by Grey et al. (2009) sought to address these temporal issues, using neuronavigation to apply stimulations to the cortex, however, the stimulations were applied using the pseudorandom walk approach. In this approach, one pseudorandomly stimulates a location on the scalp without necessitating the following stimulation to be a specific distance away from the previous. The only requirement is that by the completion of mapping acquisition, one has covered a sufficient area of the cortex. Indeed, there were no differences between this method of map acquisition on the COG and the traditional methods suggested by Wasserman et al. (1992) and Wilson et al. (1993).

This study has two important implications, firstly, one does not need to be concerned about precise stimulation site, as long as, upon completion of the mapping procedure, a suitable area of the motor cortex has received stimulation. Secondly, one can potentially reduce the inter-

stimulus intervals to reduce total acquisition time. In a recent study, Van de Ruit et al. (2015) used neuronavigation to acquire a number of maps within a 6 x 6 cm grid, with interstimulus intervals varying between 1 – 4 s. The purpose of the investigation was not only to ascertain the ISI required to produce a reliable map, but also the minimum number of stimuli required. The investigators showed that reliable maps could be produced with just 63 stimulations with a 1 s ISI, with no alterations in COG and area when comparing both maps with longer ISIs and maps acquired with the traditional method.

With such short ISIs and small number of stimulations, a reliable map was produced in under 2 minutes, a significant improvement on previous studies using the traditional approach (Wasserman et al., 1992; Wilson et al., 1993), and other pseudorandom approaches (Classen et al., 1998). This consequently transforms mapping into a far more viable technique to use in both a clinical and healthy population.

1.8 Methodological Factors Influencing TMS Measures

1.8.1 Coil Position and Coil Orientation

Original studies assessing MEP variability investigated the influence of coil position on the latencies of the MEPs. Fuhr et al. (1991) demonstrated this by recording MEPs from shoulder (deltoid), arm (biceps brachii and flexor carpi radialis) and hand (abductor pollicis brevis) muscles whilst altering the position of the stimulating coil along the scalp in small (1 cm) increments along the coronal axis using monophasic pulses. As expected, the further away from the hotspot that one moved the stimulating coil, into "sub-optimal" positions, the greater the mean latencies of the MEPs for that particular muscle. Indeed, movements of just 2 cm away from the hotspot, had the delayed the onset latency by 4 ms, but the mean latency for stimulating "sub-optimal" scalp locations was 1ms longer than that of optimal scalp locations.

The effect of stimulation position is not solely restricted to MEP latencies, indeed Brasil-Neto et al. (1992) used similar methodology to Fuhr et al. (1991), investigating the alterations in

MEP amplitude when moving the stimulating coil along the coronal axis in 0.5-1.0 cm increments. The investigators detected significant increases in MEP amplitude variability when "sub-optimal" scalp locations (i.e. away from the hotspot) were stimulated with monophasic stimulation. It was suggested that when stimulating these "sub-optimal" locations, MEPs are occasionally not elicited as the stimulation ineffectively excites the cortical motor neurons, leading to increased variability in MEP amplitude.

Logically, if stimulating position has an influence on MEP amplitude and latencies, then the orientation of the stimulating coil will also have an effect on these parameters. This notion was examined by Mills et al. (1992) by investigating the effect of orientation of the stimulation coil on MEP variability (as seen in compound muscle action potentials) by rotating the double coil clockwise through yaw, above the hotspot of the target muscle, in increments of 45^{o}, where 0^{o} was defined as parallel to the parasagittal line (i.e. the midline). The greatest amplitudes in CMAPs with monophasic stimulation were seen at a stimulus orientation of 45^{o}, with the amplitudes of the CMAPs reducing dramatically when deviating away from this orientation. This suggests that the orientation, and thus the direction of current flow is critical in exciting the neural elements below the scalp. It is suggested that the current induced flows at approximately right angles to the central sulcus, where the horizontal neural elements are aligned in this direction, and are thus most excited by currents in this orientation.

Futher, Werhahn et al. (1994) investigated the influence of current direction on the latencies of the surface EMG, and single motor unit responses to monophasic TMS. The investigators used two different orientations, with the current flowing along the midline in a postero-anterior (PA) direction, or the current flowing perpendicular to the midline in a latero-medial direction (LM). The latency produced in LM stimulation was shorter than that of PA stimulation, with a mean latency difference of 1.0 ms. It was noted that LM stimulation produced an early peak in the single motor unit responses, corresponding to the D-waves seen with electrical stimulation, as confirmed by Kaneko et al. (1996b), who performed a similar investigation with monophasic

TMS, but recorded evoked spinal cord potentials (ESCPs). Indeed, the investigators showed the shortest onset latency MEPs were induced by LM stimulation, followed by PA stimulation, suggesting that LM stimulation preferentially directly activated the corticospinal tract to produce D-waves, and PA stimulation activates the tract synaptically, producing I-waves.

This was further corroborated by Wilson et al. (1996) who assessed the latency components with medio-lateral (ML), AP and PA directed currents, stimulating a number of sites over the cortex to generate maps using monophasic TMS. Indeed, the shortest latencies were observed with ML directed current, with increasingly long latencies with AP and PA directed currents respectively. It was also noted that some stimulation sites yielded short latency responses (likely near the hotspot), and some stimulation sites produced longer latency responses, similar to the findings of Fuhr et al. (1991).

Sakai et al. (1997) elicited similar data to Werhahn et al. (1994), Kaneko et al. (1996b) and Wilson et al. (1996), but moved the coil through yaw, over the hotspot, through 8 orientations, in increments of 45^{o}. Indeed, the two orientations that produced the shortest latencies were medially (M) and antero-medially (AM) directed. Notably, this study did not detect D-waves, only early and late I-waves, most likely as the stimulation intensities used were considerably lower than that of the aforementioned investigations (Bashir et al., 2013). Di Lazzaro et al. (2001b) assessed the effect of 3 coil orientations on latencies: AP, LM and PA. Indeed, it was shown that there were differences in the latencies detected through the orientations during monophasic stimulation, with LM predominantly recruiting D-waves, PA and AP recruiting predominantly I_1-waves and I_3-waves. Nonetheless, all of these studies further corroborate the notion that the descending volleys, and thus MEPs produced by TMS can be influenced by the orientation of the coil, and thus the direction of the induced current, hence contributing to the variability of TMS, preventing its use as a tool in a wider context.

This idea that coil orientation alters TMS output, was further corroborated by Balslev et al. (2007), who investigated the influence of coil angle on the motor threshold of TMS, however, these investigators utilised biphasic stimulation. It was determined that the lowest stimulation intensity required to deduce the motor thresholds – and thus likely the most effective stimulus – were obtained when the coil was orientated so that the first wave of the biphasic current flowed in a postero-lateral direction, and the second, and most physiologically relevant (Di Lazzaro et al., 2001a; Cowey et al., 2005; Groppa et al., 2012) wave flowed in an antero-medial direction. Again, when the coil was not at the optimum orientation, the stimulation intensity at the motor threshold was considerably higher. Stephani et al. (2016) stipulated that posteriorly directed (the direction of the most relevant phase) pulses may induce a current flow from cortical layers VI to I, whereas the reverse may be true for anteriorly directed pulses. This is important as the latter may induce a greater spread of activation, whereas the former may excite axons directly and close to the axon's origin. Thus, this may lead to alterations in threshold levels, MEP amplitudes and indeed mapping characteristics.

Logically, one may deduce that if thresholds, MEP amplitude and latencies are influenced by coil orientation, then TMS mapping may also be influenced. Indeed, Wilson et al. (1993) investigated the effect of coil orientation on monophasic TMS mapping characteristics from the APB and ADM, orienting the coil medio-laterally or postero-anteriorly, stimulating at sites across the scalp in 1 cm increments. In conjunction with Brasil-Neto et al. (1992b), the MEPs with the greatest amplitude were located nearest to the hotspot. There was no change in the relative position, or area of the TMS map produced, however the maps were elongated and medially shifted when oriented medio-laterally versus postero-anteriorly. Furthermore, there was a significant medial shift in the location of the COG in both muscles after rotating the coil into a medio-lateral position. This alteration in COG is key, as many studies have reported the inherent stability of COG over a number of sessions (Uy et al., 2002; Wolf et al., 2004; Malcolm et al., 2006), and thus is often used to assess changes over a rehabilitation or training period.

Notably, the study by Malcolm et al. (2006), reported good test-retest reliability for the COG in regard to its lateral position relative to the Cz (cranial vertex), but poor test-retest reliability when looking at the COGs anterior/posterior location. Thus, if the COG is susceptible to change then could its utility as a suitable mapping parameter to be assessed across sessions be bought into question? Importantly, these data suggest that some TMS mapping characteristics may be influenced by coil orientation, but more work is required to confirm this.

1.8.2 Muscle Activity

As part of their larger investigation, Kiers et al. (1993) compared the MEP variability in a muscle undergoing a slight contraction, at 5% of MVC, and a larger contraction at 30% of MVC, versus the same muscle at rest during monophasic stimulation. Indeed, it was found that the MEP variability at the same stimulation intensity was significantly reduced whilst performing a slight contraction, in comparison to the resting condition. Furthermore, the variability of the MEP was attenuated further with increased pre-stimulus contraction force. The notion that MEP amplitude variability with pre-stimulus contraction, was corroborated by Taylor et al. (1997) the influence of a wide-range (0-75% MVC) of muscle contraction intensities on MEP variability, in the biceps brachii and adductor pollicis during monophasic stimulation. In the adductor pollicis, the MEPs increased in amplitude at with a slight contraction (5%), but plateaued after this point. Conversely, in the biceps brachii MEP amplitude continued increasing with higher pre-stimulus muscle contraction, up to 50% MVC. This increase in MEP amplitude with pre-stimulus muscle contraction, has been since been frequently confirmed across TMS literature (Devanne et al., 1997; Buccolieri et al., 2004; Darling et al., 2006).

Alterations in other MEP characteristics were also found by Kaneko et al. (1996a), who recorded spinal cord potentials during TMS, in patients with implanted epidural electrodes, measuring ESCPs resulting from monophasic stimulation at rest and during 20% MVC of the ADM. The onset latency of the MEPs recorded by the surface electrodes was reduced by a mean

of 2.3 ± 0.8ms, the threshold required to elicit and MEP was reduced and the amplitude of the MEPs was increased by up to 500% when the muscle was an active versus resting state.

Again, it can be suggested that if MEPs are influenced by muscle contraction, then surely TMS mapping characteristics can also be influenced. This idea was investigated by Wilson and colleagues (1995), assessing a variety of TMS variables, including: motor map area, map volume, and COGs during monophasic stimulation. The authors detected an alteration in the COG when comparing active and resting conditions, indeed the COG of the APB was shifted by an average of 6 mm medially as a result of the pre-stimulus muscle contraction, suggesting alterations in the "spatial properties of corticomotor excitability" during a voluntary muscle contraction. However, a more recent investigation contradicts this notion, as Ngomo et al. (2012) performed a similar study, utilising neuronavigation software to ensure reliable locations of stimulations across conditions and found no such alterations in COGs in resting and active conditions, using biphasic stimulation. The influence of prestimulus muscle contraction on mapping outputs still requires further investigation.

1.8.3 Pulse Type

Surprisingly, it is not until recently that the effect of pulse type on physiological outputs of TMS has been investigated. Given the variable nature of TMS, in order to compare the results of different investigations to one another, the influence of pulse type should not be ignored. One of the earliest studies assessing the differences between the monophasic and biphasic pulses was conducted by Maccabee and colleagues (1998). The investigators recorded CMAPs from the right thenar muscle, after direct stimulation of the median nerve with either monophasic or biphasic pulses. Indeed, it was discovered that the reverse phase of a biphasic (hyperpolarising-depolarising) pulse was more effective at stimulating a peripheral nerve axons than a monophasic pulse of the same intensity. The investigators also elude to the notion that coil/current direction of the biphasic pulse could be a significant factor when administering TMS.

These findings have since been replicated using stimuli applied to the cranium, biphasic stimulation has been shown to be more effective at producing a response versus the equivalent monophasic pulse. This was shown by differences in threshold intensities for the APB across the pulse types, even after normalising to the maximal stored energy of the stimulator types (Kammer et al., 2001). Furthermore, the most effective direction of stimulation for monophasic pulses was the opposite to that of biphasic pulses (Kammer et al., 2001). Indeed the lowest thresholds were observed if the first phase of the biphasic pulse was oriented in an AP direction, but oriented in a PA direction for a monophasic pulse.

These results have potentially significant implications for mapping, but surprisingly, very little research has focused on the effect of pulse type on mapping outputs. Stephani et al. (2016) altered current direction and pulse type through AP and PA stimulations, assessing the motor maps from hand musculature. It was elucidated that current direction had an influence on the COG in a posterior-anterior (cranio-caudal) direction, but not latero-medially, but there were no significant differences detected in the shape of the map, or indeed any differences as a result of pulse type. A criticism of this investigation could be that as neuronavigation was not used, administration of stimuli to create motor maps may not have been sufficiently accurate. Given the variability associated with not utilising neuronavigation, perhaps the range of MEPpp would be reduced using neuronavigation (Julkenen et al., 2009; Weiss et al., 2012), hence yielding different results? Further, we have seen that activity has an influence on MEP values (Taylor et al., 1997), yet this requires further study not considered within this investigation. Nonetheless, this study does again show that current direction does indeed have an influence on motor mapping.

1.9 Literature Summary

As seen in the literature, there are a number of confounding factors that need to be considered when performing TMS, some of which have been studied thoroughly, whilst others have been somewhat negated. Indeed, as TMS mapping is becoming a more widely used technique to

investigate alterations in cortical plasticity, it is vital that the factors that may confound the technique are understood and can be minimised to produce consistent and verifiable data.

1.10 Aims and Hypotheses

The experiments performed in this thesis will attempt to clarify the factors that affect the outcomes of TMS mapping. More specifically the following elements will be assed: the effect of current direction, the effect of pulse type and the effect of muscle activity. These factors will be assessed whilst performing a recently-developed (van de Ruit et al., 2015; van de Ruit et al., 2016) mapping technique, either with the stimulation intensity held at a constant level, or altered in line with current direction and pulse type. In line with the outcomes in the investigations by van de Ruit, two main mapping outputs will be assessed: map area and centre of gravity (COG).

Firstly, as map area is influenced by the effectiveness of TMS stimuli to produce a response, and small responses are elicited when the TMS coil is in a sub-optimal orientation (Brasil-Neto et al., 1992b; Kammer et al., 2001). Moreover, investigations have shown that when compared to rest, TMS maps increase in size with muscle activity (van de Ruit et al., 2016). Furthermore, alongside the reported increased effectiveness of biphasic pulses when compared to half-sine pulses (Sommer et al., 2006), it is hypothesised that:

1. Map area will be significantly influenced by current direction, pulse type and muscle activity

Secondly, as it has been reported that when performing TMS with the assistance of neuronavigation, there are few alterations in terms of COG as a result of current direction, pulse type (Stephani et al., 2016) and muscle activity (van de Ruit et al., 2016). Therefore it is hypothesised that:

2. COG will remain the same with changes in current direction, pulse type and muscle activity

CHAPTER 2 - Methodology

2.1 General Methodology

2.1.0 Experiment Overview

Experiment 1a assessed the effect of three coil orientations (i.e. current direction) on maps produced during rest. *Experiment 1b* assessed the effect of three coil orientations and muscle activity on maps produced in active (10% of MVC) and resting (passive) conditions. *Experiment 2* assessed the effect of pulse-type (biphasic vs. half-sine) and two coil orientations on maps, with stimulation intensity normalised to 1mV for each *pulse type*. *Experiment 3* assessed the effect of pulse-type and two coil orientations on maps produced, with stimulation intensity altered according to *current direction* and *pulse type.*

2.1.1 Participants

For the 3 investigations, healthy volunteers were recruited from a sample of convenience via email communication from the University of Birmingham, totalling 48 participants (including 3 participants that took part in both *Experiment 2* and *3*). The key participant information for each investigation is in *Table 1*. Upon recruitment, participants were provided with a full explanation of the investigation's purpose, including an electronic version of the protocol and risks associated with TMS, alongside a consent form that required signing. Upon arrival into the laboratory, the protocol was explained in full, written consent was obtained (see *Appendix **A**)*, handedness was ascertained via the Edinburgh Handedness inventory, (Oldfield, 1971) (see *Appendix **B**)* and the participant was screened for any contraindications to TMS using a modified version of the TMS Adult Safety Screen (TASS) (Keel et al., 2001) (see *Appendix **C**)*. The investigation was approved by the University of Birmingham STEM Ethical Review Committee (ERN 12-1189) in accordance with the Declaration of Helsinki.

Table 1. *Participant information across all experiments.*

	Experiment 1a	*Experiment 1b*	*Experiment 2*	*Experiment 3*
Number	24	14	9	14
Sex	11 M, 13 F	9 M, 5 F	6 M, 3 F	5 M, 9 F
Age (S.D.)	20.08 (± 1.67)	20.64 (± 1.91)	21.78 (± 0.67)	20.92 (±1.44)
% Right Handed	75	71	89	93

2.1.2 Electromyography

The participant was comfortably seated in a chair, with their right arm placed onto an adjacent stool and supported with a cushion. The skin above the FDI was prepared with 3M™ Red Dot Trace Prep (3M United Kingdom plc, Berkshire, UK). For each investigation, either reusable silver coated (Ag-AgCl) electrodes (Digitimer Ltd, Welwyn Garden City, UK) (*Experiment 1*) or bipolar disposable surface electrodes (Blue Sensor N, Ambu, Denmark) (*Experiment 2 and 3*) were placed above the FDI of the right hand in a belly-tendon montage (in accordance with SENIAM guidelines), and secured with tape. A 4.5 x 4.5cm reference electrode was placed onto the styloid process of the ulna. All electrodes were connected to a Digitimer D360 Patient Amplifier (Digitimer Ltd, Welwyn Garden City, UK), for EMG signal amplification. All electrodes and wiring were fixed in place with tape (see *Figure 5*). The EMG data was sampled at 5 kHz, with a gain of 500 and band-pass filtering of 20-1000 Hz, having passed through a 50Hz noise eliminator (Humbug, Quest Scientific, North Vancouver, Canada). After processing via a National Instruments™ BNC board (model 2090) (National Instruments Corporation, Newbury, UK) and mass transfer onto a PC, data was retained for offline analysis. EMG signals were available for online viewing via Mr. Kick software (SMI, Aalborg University, Denmark).

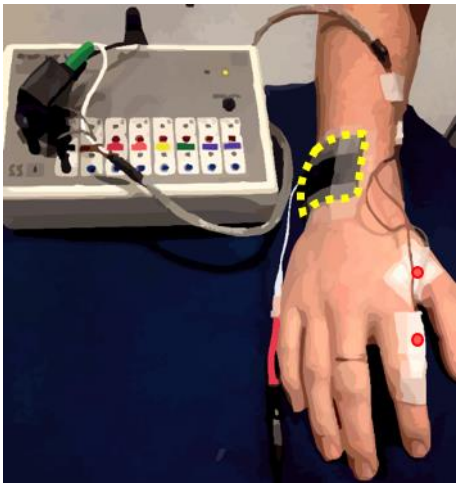

Figure 5. Ag-AgCl EMG electrode (red circles) and ground electrode (yellow dashes) placement for the target muscle, FDI. A similar configuration was used with the disposable electrodes for Experiments 2 and 3

2.1.3 Neuronavigation

The use of BrainSight™ (BrainSight 2, Rogue Research Inc, Montreal, Canada) software for navigated TMS (nTMS) allowed real-time tracking of both the TMS coil and the head of the participant. This was achieved using a Polaris Vicra optical tracking system (Northern Digital Inc, Ontario, Canada), an elasticated headband, specialised infrared pointer and a fixture for the TMS coil. These tools use spherical, reflective markers to reflect infrared light emitted by the tracking system, to ascertain the 3D coordinates of each piece of equipment and allow real-time tracking. In order to track the participant, a 'template' MRI scan from a previous investigation was uploaded into the software, and participants could be calibrated to the template using eight pre-defined landmarks on the head via the infrared pointer (*Figure 6*).

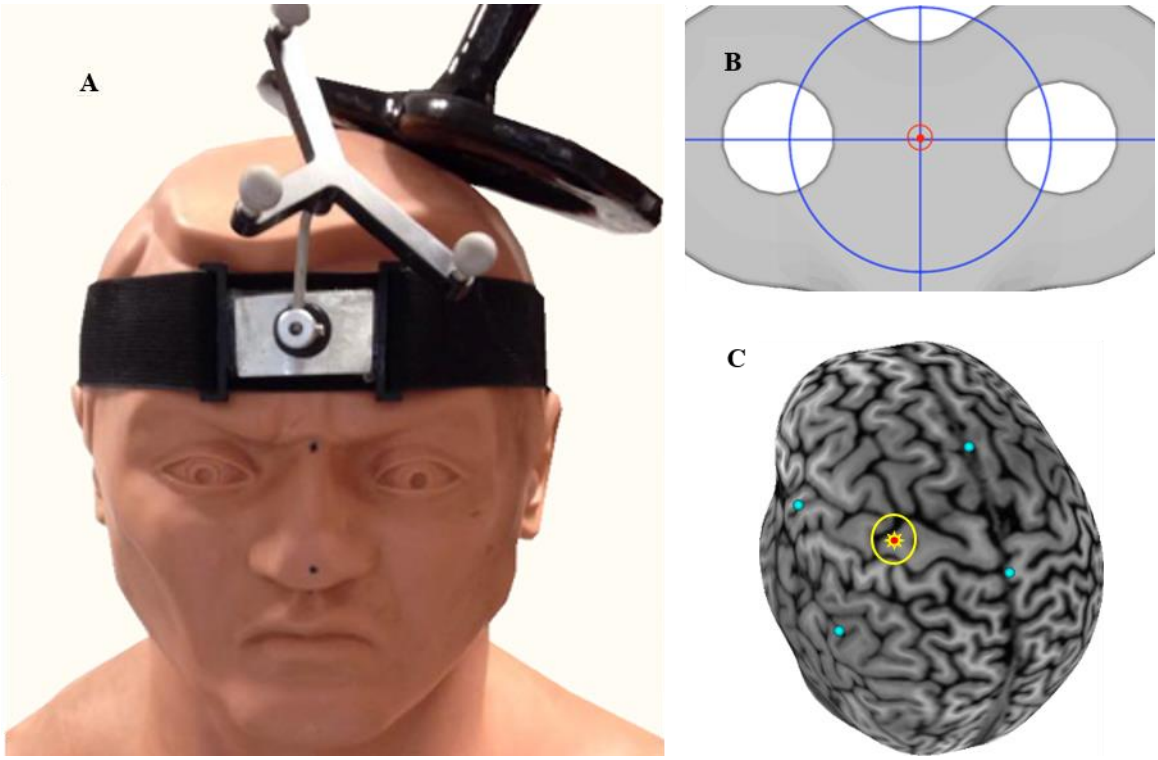

Figure 6. ***A*** *shows the TMS coil held over the hotspot and the elasticated headband with reflective markers.* ***B*** *and* ***C*** *indicate the hotspot on Brainsight, with* ***B*** *highlighting the identified hotspot location for accurate threshold determination, and* ***C*** *showing the hotspot location prior to mapping*

2.1.4 Transcranial Magnetic Stimulation

In *experiment 1* (*a:* investigating coil orientation, *b:* investigating coil orientation and activity), biphasic stimulation was delivered by a Magstim Rapid^2 stimulator (Magstim Ltd, Dyfed, United Kingdom) using a 70mm figure-of-eight of branding iron design. In *experiment 2* and *experiment 3*, both half-sine and biphasic stimuli were delivered by the PowerMAG ANT 100

(ANT Neuro, Enschede, Netherlands) using a passive cooled (Double coil PMD70-pCool) 70mm figure-of-eight coil (MAG & More GmbH, Munich, Germany).

2.1.5 Hotspot identification and Mapping parameters

'Hotspots' are commonly defined as the position of the coil on the scalp that elicits the largest MEP in response to TMS for a particular muscle (Rossini, 1994). Thus, the hotspot for the FDI was located on the participant, using nTMS to apply stimulation to the primary motor cortex. This was achieved by altering the stimulus intensity and location of the stimulating coil around the left cerebral hemisphere, until the strongest FDI MEP_{pp} (see *Figure 7*) was detected on EMG output within the Mr. Kick software. This identified hotspot location is marked as a target on the BrainSight™ software, to act as a reference point throughout the investigation. This hotspot location could then be used to ascertain the resting motor threshold (RMT) and thus the stimulation intensity to be used through the investigation (see *Methodology* section for each experiment).

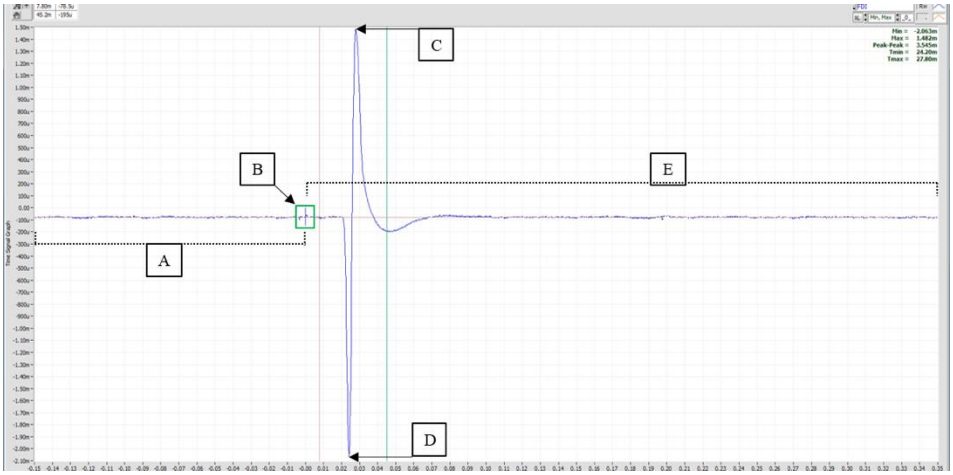

*Figure 7. Raw single MEP obtained offline from the Mr. Kick software. The total sweep length is 500ms, comprised of a pre-trigger of 150 ms (**A**) and a 350 ms post-trigger (**E**). The value of greatest import to both experiments is the peak-to-peak amplitude, obtained by measuring between points **C** and **D**. The stimulus artefact, can be seen at 0 ms (**B**).*

For all participants, a fixed 60mm x 80mm grid could be created within the Brainsight™ software and aligned in accordance with the Cz and hotspot, to allow pseudorandom mapping of the FDI within a constant area, similar to the investigation by van de Ruit et al. (2015). Further, the neuronavigation ensured that TMS could be delivered with the coil held at 45° to

the midline, in accordance with the optimal current direction for exciting hand musculature (Mills et al., 1992; Brasil-Neto et al., 1992b). In this investigation, two different stimulator types were used, in order to assess the effect of pulse type. The PowerMAG stimulator could not be used for *Experiment 1,* as we only had access to the stimulator for a limited time-period. Utilising two different stimulators presented a challenge, as the coils deliver their pulses in alternate ways. As can be seen in *Table* 2, using the 'same' coil orientation between experiments actually produces currents in different directions when comparing the stimulator. Thus, for this investigation, the coil orientations will be referred to by the most relevant current direction produced in the cortex (Brasil-Neto et al., 1992b; Sommer et al., 2006).

Table 2. *The different coil orientations, and current directions in both experiments. For ease of determination, each condition will be referred to by the pulse-type and the most relevant current direction within the cortex. The current induced in the cortex are in an opposite direction when comparing the biphasic pulses from the different stimulators. Note the PowerMAG can produce currents in both directions in the half-sine mode Coil orientations are referred to over current directions as these are more easy to understand in a practical context.*

Stimulator	*Pulse Type*	*Coil Direction (Handle-Coil)*	*Most Relevant Current Direction (Cortex)*
Magstim Rapid^2	Biphasic	Postero-anterior	Antero-posterior
Magstim Rapid^2	Biphasic	Antero-posterior	Postero-anterior
Magstim Rapid^2	Biphasic	Latero-medial	Medio-lateral
PowerMAG	Biphasic	Postero-anterior	Postero-anterior
PowerMAG	Biphasic	Antero-posterior	Antero-posterior
PowerMAG	Half-sine	Postero-anterior	Postero-anterior
PowerMAG	Half-sine	Postero-anterior	Antero-posterior

To determine the optimal mapping grid size for all participants, initial, unrecorded pilot testing was undertaken on individuals within the laboratory group. Prior to map acquisition, the distance between the participant's vertex (Cz) and preauricular point (anterior to the tragus) was measured using a tape measure. Single maps were then obtained within each of the possible coil orientations across both investigations, to assess the practicalities of various grid sizes.

The grid is aligned with the Cz and the size was altered from 60 mm x 60mm as used by van de Ruit et al. (2015), and laterally enlarged by 10 mm increments, to 60 mm x 90 mm. For

individuals with smaller heads, large grid sizes (>80 mm) were often inappropriate, as the coil would venture too far laterally down the side of the skull, leading to unwanted facial cranial nerve stimulation. Equally, smaller maps (<70 mm) were not appropriate for individuals for larger heads as sufficient stimulation along the motor strip may not be achieved. Thus, it was determined that a grid size of 60mm x 80mm would be appropriate, to ensure sufficient stimulations along the motor strip whilst avoiding facial cranial nerve stimulation.

For all conditions (see *Methodology* section for within each separate experiment for each of the conditions), maps were obtained using the pseudorandom method outlined by van de Ruit et al. (2015) (see *Figure 8*). Maps were created by applying between stimuli to the scalp of an individual, with a 1.2s (*Experiment 1*)/ 1.25s (*Experiment 2* and *3*) inter-stimulus interval. The ISIs are slightly different as a 1.2s ISI could not be achieved with the PowerMAG through Mr. Kick, so the stored settings on the stimulator were utilised instead. Within this grid, 110 stimuli were applied pseudorandomly, allowing a sufficient section of the motor cortex to be represented (Classen et al., 1998). Between each map, the participant was allowed a short rest period to ensure the participant remained attentive during data acquisition (throughout) and enable cooling of the TMS coil (in *experiment 1*).

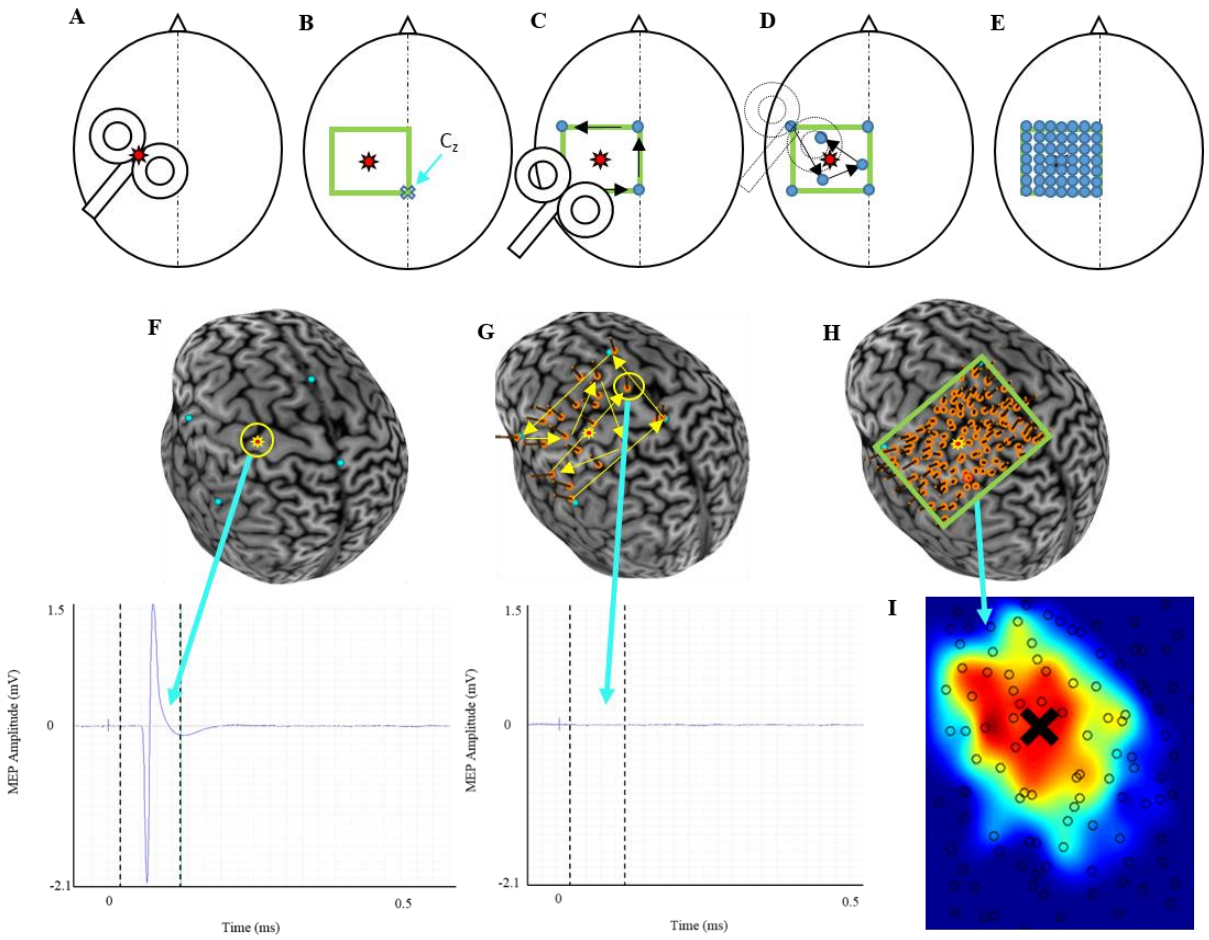

Figure 8. The mapping procedure utilised throughout both experiments. **A** *shows the location of the hotspot, and* **B** *shows how the grid is set up, by alignment with both the hotspot and vertex (C_z). Mapping commences by moving the stimulating coil to the corners of the created grid (***C***), followed by pseudorandomly altering the position of the coil to ensure no two consecutive stimuli are adjacent (***D & G***). Upon completion, a grid of 110 stimuli will be formed (***E & H***), allowing the creation of a 2D map (***I***).*

2.1.6 Peripheral Nerve Stimulation

The M_{max} was used obtained to normalise the data, thus accounting for variance in stimulation threshold, enabling comparison between-participants. Thus, prior to mapping, the M_{max} (i.e. maximal output from the FDI) was established via direct electrical stimulation to the ulnar nerve at the motor point on the anterior, ulnar aspect of the wrist, using a Constant Current Stimulator (Digitimer DS7A, Digitimer Ltd, Welwyn Garden City, UK). The M_{max} was determined by stimulating the ulnar nerve with increasing stimulus intensities until there was a plateau in the MEP_{pp} value.

2.1.7 Muscle Activation

Since maps would be obtained whilst the participant's FDI was either active or passive, biofeedback via a mixed signal oscilloscope (MSO 2014, Tektronix, Beaverton, United States) was provided to the participants to ensure that appropriate muscular activation was maintained. The participants performed three maximal voluntary abductions of the index finger onto a force transducer (NL62 – 5kg, Digitimer Ltd, Welwyn Garden City, UK), utilising wrist and finger restraints to ensure that the FDI was isolated during contraction. During MVCs, participants were verbally encouraged to perform the strongest possible contraction (Boe et al. 2007). Between MVCs, participants were given a minutes rest to minimise fatigue. For following experimentation, the participants would be asked to maintain a contraction equivalent to 10% of their MVC, as used by Wilson et al. (1993) and Hamada et al. (2014). The oscilloscope provides a voltage reading for the contraction, thus 10% of the voltage produced during a maximal contraction represents 10% of their MVC. Online feedback was provided via a screen to ensure that a constant 10% contraction was held throughout the relevant maps (*Figure 9*). To prevent muscle fatigue influencing the data, a 2 minute rest period was taken between maps.

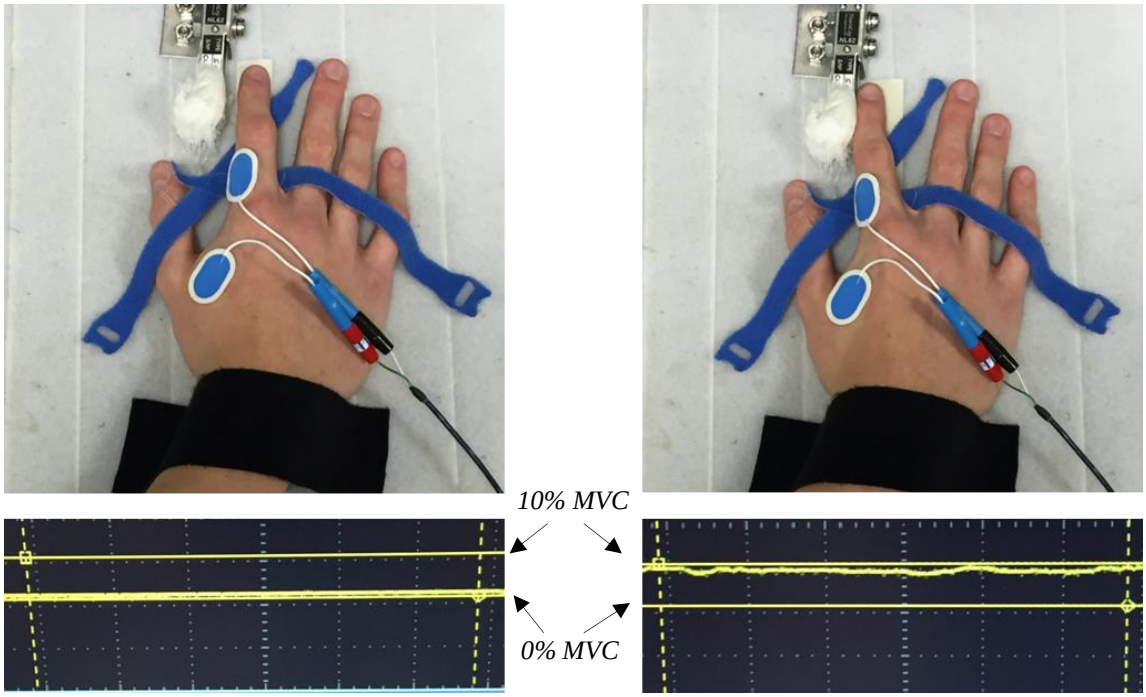

Figure 9. An illustration of the oscilloscope output during passive (left) and active (conditions). The participant is instructed to maintain a consistent force during the active condition, equivalent to 10% of their MVC. Online feedback is thus provided to the participant regarding their level of contraction. Furthermore, online feedback is provided via Mr. Kick during the passive condition from the experimenter, to ensure that background EMG is minimised

2.1.8 Map Analysis

2.1.8.1 Map Creation

Upon completion of the protocol, all EMG and neuronavigation data were stored for offline analysis. Maps were created and analysed using a custom-built MATLAB script (MATLAB Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States), as used by van de Ruit et al. (2015). The script obtains the 3D coordinates of each stimulus in space according to a reference coordinate system, obtained from the neuronavigation software. The script then transforms these 3D positions onto 2D plane, and subsequently approximates a full TMS map. Thus, 2D a grid is created with 2500 partitions and each partition is assigned an MEP_{pp} value using interpolations from MEPs surrounding the grid point. This allows the creation of a coloured contour map, such as those seen in *Figure 8* and *Figure 11*.

2.1.8.2 Exclusion Criteria and MEP preparation

A number of exclusion criteria were applied to the data. The need to exclude large amounts of data was attenuated thanks to online feedback to the participant concerning the level of background EMG. Nonetheless, data was excluded if the RMS of the background EMG, extracted from EMG pre-trigger data, 100ms prior to stimulation, was 2 standard deviations higher than the mean of all RMS values. Further, MEPs that exceeded the mean + 3.5 standard deviations were also excluded to avoid outliers having an undue influence on the maps. Moreover, individual MEP data were excluded if the stimulation occurred more than 10mm outside of the pre-defined grid border, to ensure that all stimuli included within the mapping analysis consisted of only stimuli that were delivered with the TMS coil perpendicular to the skull. Finally, MEPs were excluded if the angle or translation of the stimulation coil fell outside of the 99% prediction interval of all stimuli, thus ensuring that stimuli in the maps were delivered in the optimal orientation at 45^{o} to the midline in an effort to attenuate variability in MEP values where possible (Mills et al., 1992; Brasil-Neto et al., 1992b).

Prior to creating the maps, the MATLAB script prompts the user to provide an area in which peak-to-peak measurements can be made of the MEPs (see *Figure 10*, which is an illustration

of the prompt and the defined areas). These are defined by red cursors and ensures that only true MEP_{pp} values are obtained, and MEPs with irregular or anomalous shapes can be removed. Further, the user is prompted to remove any MEPs that are clearly anomalous and that the mapping script may have negated to remove. For example, in *Figure 10***B**, it can be seen that there are several MEPs where there is significant noise both before and after the stimuli, which may skew the map. These MEPs can be marked (in red) and are not included in the map creation and analysis. Further, MEPs marked in cyan have been excluded by the MATLAB script due to excess background noise.

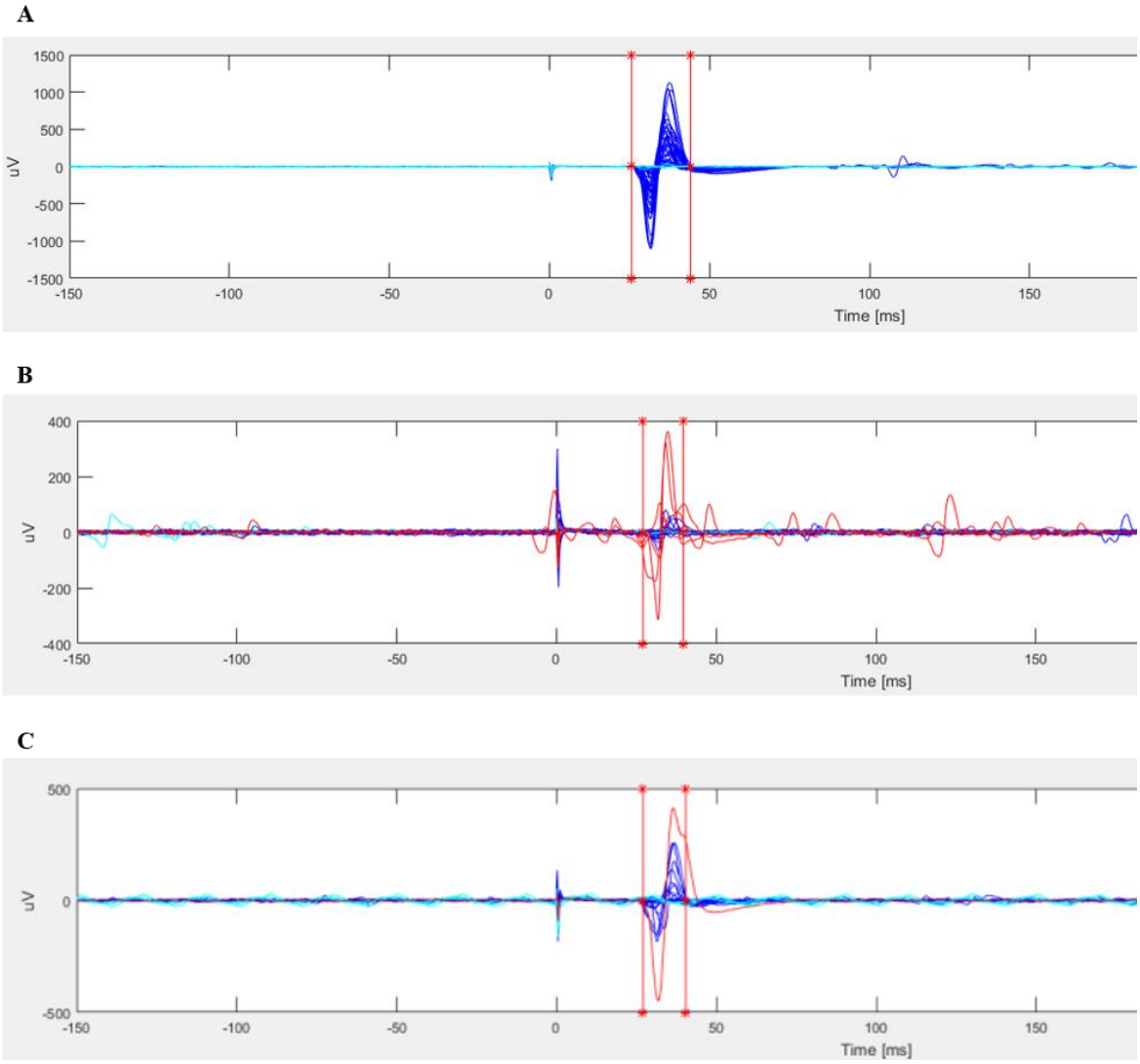

*Figure 10. Pre-map analysis MEP preparation exclusion and cursor placement with PA (***A***), ML (***B***) and AP (***C***) directed most physiologically relevant currents. The red cursors are placed either side of the MEP to ensure accurate measurement of the peak-to-peak values. The red MEPs in* **B** *and* **C** *indicate MEPs that have been excluded manually due to excess noise or abnormal shape, whereas cyan MEPs are those excluded within the MATLAB script due to excess background noise.*

2.1.8.3 Map Parameters Assessed

During this investigation, the main parameter that is used to determine map characteristics is the MEP_{pp} values (*Figure 7*). After processing these MEP data into maps, 3 map characteristics were selected to be assessed; COG, map area and the map aspect ratio (see *Figure 11*). As previously stated, the COG of a map represents the amplitude-weighted centre of the motor map (Wasserman et al., 1992) which is usually used to show functional alterations in the cortical representations of a muscle after skill acquisitions or interventions (Liepert et al., 2000a; Uy et al., 2002). Secondly, map area refers to the expanse of the skull surface that can be stimulated to activate a particular muscle and elicit an MEP. Again, like COGs, it has been suggested that the map area can change following a stroke (Traversa et al., 1997; Byrnes et al., 1999), rehabilitation (Stinear et al., 2007), or after a period of training (Pascual-Leone et al., 1995). Thus, it is important to assess the influence of both pulse type, muscle activity and coil orientation on these parameters, as conclusions in many previous investigations have been based upon the assumption that firstly, COG is an inherently reliable and stable (Ridding et al., 2001; Wolf et al., 2004; Z'Graggen et al., 2009; Weiss et al., 2012; Littmann et al., 2013) mapping parameter, and secondly that the alterations in map area are purely down to the disease or intervention. If altering the coil orientation and/or pulse type does indeed have an influence on map area and COGs, then the stipulations made by the authors may need to be revisited. Resultant COG was measured rather than separate values for COG in the different directions, as the resultant will still show changes in COG, but in a given direction – importantly with a single figure that can be easily assessed in a real-life context. The current system of several COG values seems redundant, especially when only large changes are of any significant interest. Finally, the aspect ratio of the map will be calculated in order to determine any changes in the shape of the maps across the different conditions. Indeed, the aspect ratio is the ratio of the major and minor axes of a fitted ellipse which surrounds the area of excitability in a map (van de Ruit et al., 2016). Again, like COG, it has been suggested that the shape of the map is inherently stable, and does not significantly change with alterations in stimulation intensity and

muscle activation (van de Ruit et al., 2016), could alterations in pulse type and coil orientation question the stability of this parameter.

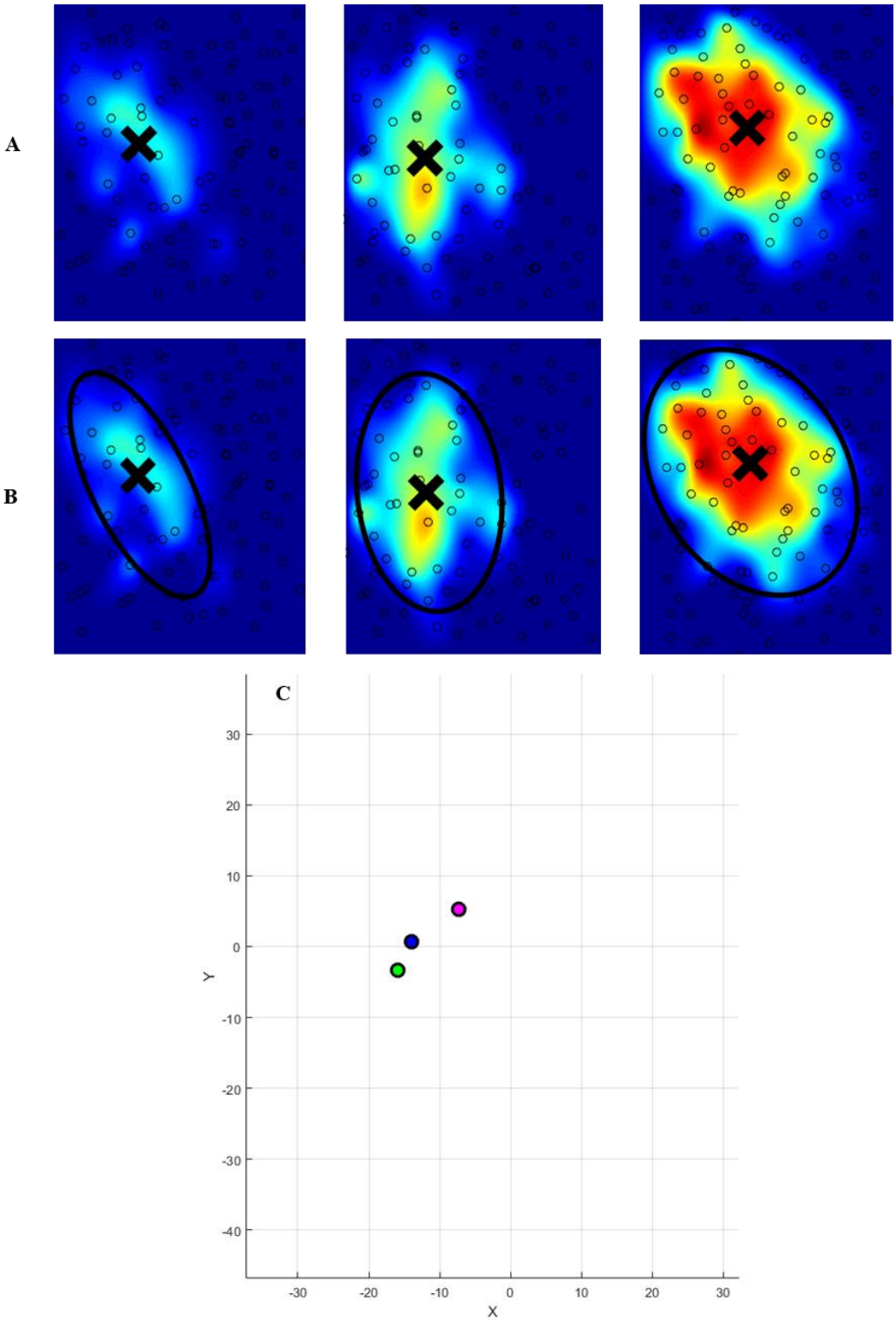

*Figure 11. Example maps (**A**), fitted ellipses (**B**) and COGs (**C**) through the three coil orientations during rest in Experiment 1. The conditions from left to right in **A**, **B** and **C** are PA, ML, AP respectively. The map area is defined as the number of partitions on the map exceeding 10% of the maximal MEPpp (as used by van de Ruit et al., 2015). The ellipse is then fitted around this area of excitability, allowing calculation of the aspect ratio. For COG, the amplitude weighted mean position of the map is positioned on the 60 x 80mm grid shown in **C**, thus allowing determination of X and Y coordinates relative to the grid.*

2.2 Methodology – Experiment 1a and 1b

2.2.1 Design – Experiment 1a

The purpose of *Experiment 1a* was to assess the effect of three coil orientations on maps produced during rest. The participant was seated comfortably in a chair and instructed to place their pronated right arm into a comfortable, relaxed position onto a cushioned platform adjacent to the seat. The platform included the force transducer, connected to a mixed signal oscilloscope, allowing simple transference between the relaxed and active conditions. Prior to TMS, the M_{max} was determined using the constant current stimulator. Thereafter, using the procedure outlined previously (see *General Methodology*), the hot-spot and RMT were determined. The stimulation intensity was set at 10% higher than the stimulator intensity that elicited a 50μV signal in 5 out of 10 stimuli, with the coil held in the latero-medial direction (Ziemann et al., 1996). Then, the participants received stimulations to create maps with the coil in the postero-anterior, latero-medial and antero-posterior orientation, eliciting a more relevant antero-posteriorly (AP), medio-lateral and (ML) and postero-anteriorly (PA) respectively (Kammer et al., 2001; Sommer et al., 2006) (see *Table 2* and *Figure 12*) and will subsequently be referred to by this convention. To account for any potential order effects, participants were randomly assigned to either AP or ML stimulation using a random number generator. However, as administering accurate stimuli in the PA orientation, the infrared tracker had to be rotated, and re-registered. Therefore, PA stimulation was always performed last.

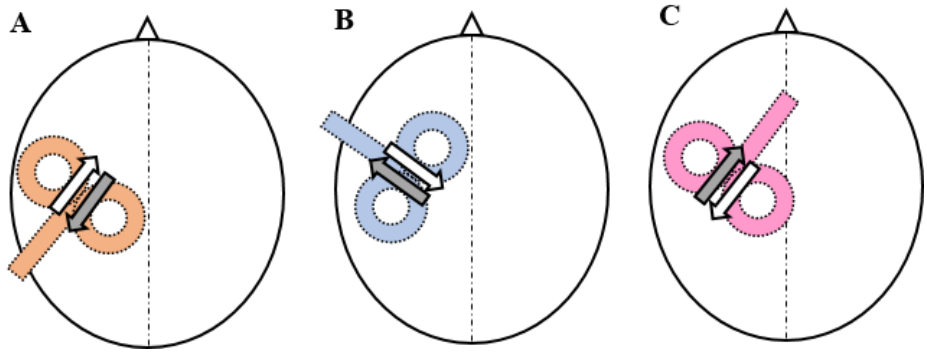

Figure 12. The different coil orientations utilised throughout Experiment 1, and the filled arrows indicate direction of the physiologically most relevant phase of the pulse (Kammer et al., 2001; Groppa et al., 2012). **A**, **B** *and* **C** are in the orientations AP, ML and PA respectively. *The mapping in this experiment is performed with biphasic pulses.*

Each map was obtained using the pseudorandom method outlined by van de Ruit et al. (2015) (*Figure 8*). Maps were created by applying between stimuli to the scalp of an individual, with a 1.2 s inter-stimulus interval. Using nTMS, one could restrict stimulations to a pre-determined grid, thus prevent unnecessary stimulations to areas of the cortex that would not yield MEPs and/or may cause discomfort. Within this grid, 110 stimuli were applied pseudorandomly (*Figure 13*), ensuring a sufficient section of the motor cortex to be represented. Therefore, each participant would undergo stimulations needed to create 3 maps in total, thus, including hotspot and threshold determination, one receives roughly 750 stimuli. Between each map, the participant was allowed a short rest period to ensure the participant remained attentive during data acquisition and enable cooling of the TMS coil. The maps were subsequently stored for offline analysis.

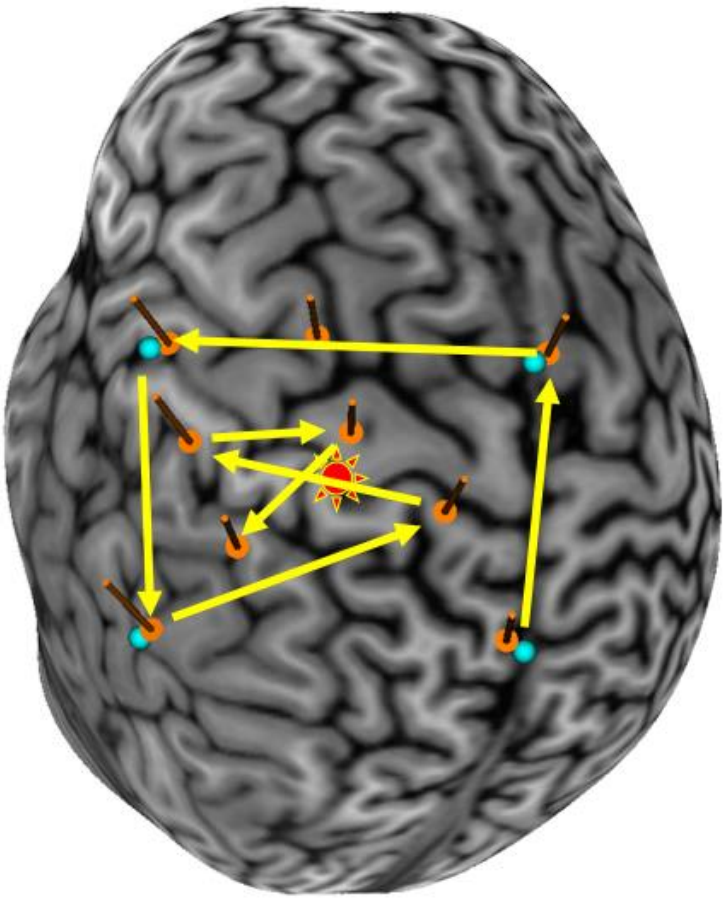

Figure 13. The mapping procedure utilised throughout Experiment 1, where one begins stimulation by traversing the perimeter of the grid prior to pseudorandom mapping of the cortex. The hotspot can be seen in the centre of the grid, and 110 stimuli are delivered per map

2.2.2 Design – Experiment 1b

The purpose of *Experiment 1b was to* assess the effect of three coil orientations and muscle activity on maps produced in active (10% of MVC) and resting (passive) conditions. In Experiment 1b, the participants followed the same protocol as outlined above, in *Experiment 1a*. However, in this experiment, participants also underwent maps whilst performing a small muscle contraction, equating to 10% of their MVC. Participants in this experiment also took part in *Experiment 1a*, thus their data is shared across both experiments. In this experiment, 2 maps would be obtained for each current direction (AP, ML & ML), 1 passive and 1 with muscle activation, with the order of the conditions assigned randomly via a random number generator. Therefore, each participant would undergo stimuli needed to create 6 maps in total, thus, including hotspot and threshold determination, one receives roughly 1,000 stimuli. Between each map, the participant was allowed a short rest period to ensure the participant remained attentive during data acquisition and enable cooling of the TMS coil. The maps were subsequently stored for offline analysis.

2.2.3 Map Analysis

The details regarding map creation, parameters assessed and exclusion criteria are outlined in *General Methodology*. Within this experiment, corresponding Brainsight samples (neuronavigation coordinates) and MEPs within Mr. Kick were exported from the respective software packages prior to analysis through the bespoke MATLAB script. The MEPs and Brainsight samples are matched so that any given MEP can be located relative to the skull surface, using the neuronavigation software. The maps were then created for each participant, with maps analysed separately within resting and active conditions. In *Experiment 1a*, 3 maps were created for each participant across the three current directions. The key parameters assessed within this experiment were resultant COGs and map area. Whereas, in *Experiment 1b* two sets of three maps were created for each participant, with three active maps within each current direction, and three resting maps. The key parameters assessed were resultant COGs, map area and aspect ratio.

2.2.4 Statistical Analysis – *Experiment 1a*

Statistical tests were performed with IBM SPSS Statistics 22 software. In order to retain as much of the data as possible, for individual data sets that were missing, the Expectation-Maximisation function was utilised on SPSS, to ensure full statistical analysis could be completed upon the missing data. In all cases, Little's MCAR test was not significant ($p > .05$) indicating the data were missing at random. Descriptive statistics confirmed that the data was normally distributed. For data that violated the sphericity assumption, a Greenhouse-Geisser adjustment was used (indicated by $^{G\text{-}G}$). Where appropriate, the Bonferroni correction was used to compensate for multiple comparisons. One-way RM ANOVAs were performed separately for map area and COG assessing the effect of the current directions on these parameters.

2.2.5 Statistical Analysis – *Experiment 1b*

Statistical tests were performed with IBM SPSS Statistics 22 software. As before, the Expectation-Maximisation function was utilised, and in all cases, Little's MCAR test was not significant ($p > .05$) indicating the data were missing at random. Descriptive statistics confirmed that the data met the assumptions of parametric testing (normal distribution and equal variances). Where appropriate, the Bonferroni correction was used to compensate for multiple comparisons. Descriptive statistics confirmed that the data was normally distributed and had equal variances. 2 x 3 RM ANOVAs were performed for Area, COG and AR respectively assessing the effect of two activity conditions and three current directions.

2.3 Methodology – Experiment 2

2.3.1 Design

The purpose of *Experiment 2* was to assess the effect of pulse-type (biphasic vs. half-sine) and two coil orientations on maps, with stimulation intensity normalised to 1mV in each *pulse type*. The participant set up (electromyography, M_{max} etc.) for this experiment is the same as that used in *Experiment 1* and *2*. Thereafter, using the procedure outlined previously (see *General Methodology*), the hot-spot and RMT to elicit both 50µV and 1mV responses, were determined. The stimulation intensity was set at 10% higher than the threshold to elicit a 5 out of 10 MEPs at a 1 mV peak-to-peak amplitude with the most relevant current induced in the *postero-anterior* orientation for *each pulse type*. Then, the participants received stimulations to create maps with the coil in the postero-anterior, and antero-posterior orientation (*Figure 14*). Subsequently 2 maps would be obtained for each orientation, (with slight muscle activation - 10% of MVC) with either biphasic pulse type, or a half-sine pulse-type. Thus, 4 maps would be produced in total for each participant. Again, these stimuli will be referred to by the most relevant current induced in the cortex as outlined in *Table 2*, matching the coil orientations as outlined above.

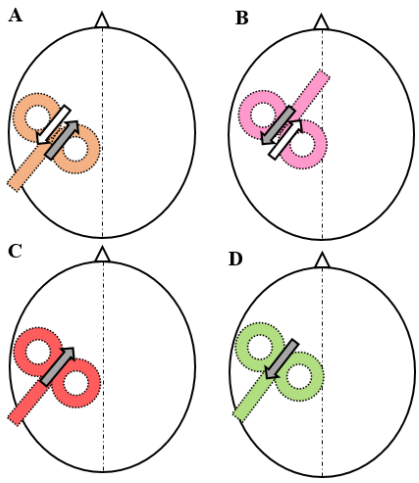

Figure 14. The different coil orientations utilised in Experiment 2, and the filled arrows indicate direction of the physiologically most relevant phase of the pulse (Kammer et al., 2001; Groppa et al., 2012). **A, C** *and* **D** are held in the PA orientation, whereas **B** is in the AP orientation. **A** *and* **B** *are performed with biphasic pulses, whilst* **C** *and* **D** *are half-sine. Note that* **C** *and* **D** *are performed in the same orientation, but the polarity of the half-sine pulse reverses after every stimulation.*

Each map was obtained using the pseudorandom method outlined by van de Ruit et al. (2015). Maps were created by applying between stimuli to the scalp of an individual, with a 1.25 s inter-stimulus interval. Using nTMS, one could restrict stimulations to a pre-determined grid, thus prevent unnecessary stimulations to areas of the cortex that would not yield useful MEPs and/or may cause discomfort. Within this grid, 110 stimuli were applied pseudorandomly, allowing a sufficient section of the motor cortex to be represented. As the polarity reverses every stimulation with the PowerMAG ANT 100 stimulator during half-sine pulses, the coil was still moved pseudorandomly, however, in each stimulation location, two pulses were delivered at the aforementioned ISI to ensure that the influence of both polarities could be ascertained (see *Figure 13* and *Figure 16* for the differences in mapping procedure*)*. Therefore, each participant would undergo stimulations needed to create 4 maps in total (2 half-sine, 2 biphasic), thus, including hotspot and threshold determination, one receives roughly 1,000 stimuli. Between each map, the participants were allowed a short rest period to ensure the participant remained attentive during data acquisition. The maps were subsequently stored for offline analysis.

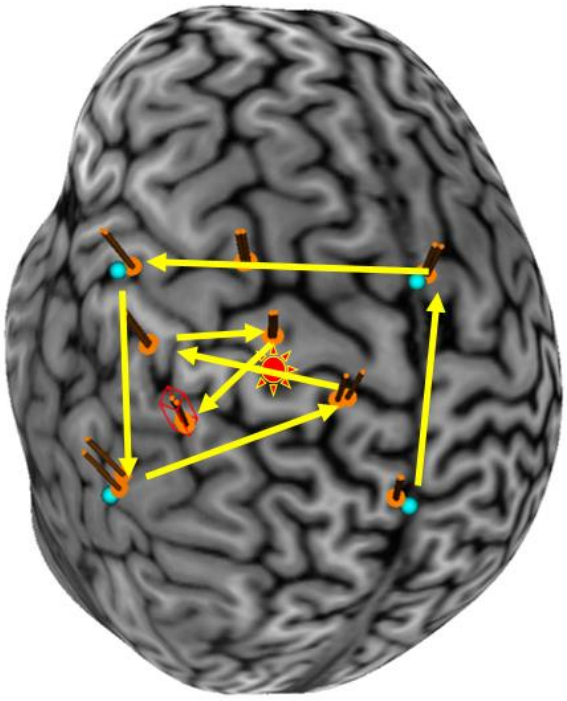

Figure 15. The mapping procedure utilised in Experiment 2 and Experiment 3. The procedure is identical to that of Experiment 1, where one begins stimulation by traversing the perimeter of the grid prior to pseudorandom mapping of the cortex. However, at each stimulus location, ***two*** *stimuli are delivered to ensure that both the positive and negative components of the half-sine stimuli can be assessed. 110 stimuli are delivered per map.*

2.3.2 Map Analysis

The details regarding map creation, parameters assessed and exclusion criteria are outlined in *General Methodology*. Within this experiment, corresponding Brainsight samples (neuronavigation coordinates) and MEPs within Mr. Kick were exported from the respective software packages prior to analysis through the bespoke MATLAB script. The MEPs and Brainsight samples are matched so that any given MEP can be located relative to the skull surface, using the neuronavigation software. As previously mentioned, the PowerMAG ANT 100 stimulator reverses the current direction every stimulation when using the half-sine pulse setting. This means that care must be taken when matching the Brainsight samples to the corresponding MEP. Thus half-sine MEP data files were appended in each of the activity conditions, to produce files with 220 stimuli. The individual MEPs were then excluded or included within these files to ensure that the current direction was correct within each 110 sample map. These MEPs were then matched to their corresponding neuronavigation coordinates from Brainsight. Therefore, each participant would undergo stimulations needed to create 4 maps in total (2 half-sine, 2 biphasic), thus, including hotspot and threshold determination, one receives roughly 1,000 stimuli. Between each map, the participants were allowed a short rest period to ensure the participant remained attentive during data acquisition. The maps were stored for offline analysis.

2.3.3 Statistical Analysis

Statistical tests were performed with IBM SPSS Statistics 22 software. As before, the Expectation-Maximisation function was utilised, and in all cases, Little's MCAR test was not significant ($p > .05$) indicating the data were missing at random. Descriptive statistics confirmed that the data met the assumptions of parametric testing (normal distribution and equal variances). Where appropriate, the Bonferroni correction was used to compensate for multiple comparisons. Descriptive statistics confirmed that the data was normally distributed. 2 x 2 RM ANOVAs were performed for map area and COG assessing the effect of two pulse types (biphasic and half-sine) conditions and two coil current directions.

2.4 Methodology – Experiment 3

2.4.1 Design

The results of *Experiment 2* led to the design of *Experiment 3* assessing the effect of pulse type and two coil orientations on maps where the stimulation intensity was set at 10% higher than the 1mV RMT in *each respective current direction* and *pulse type* as opposed to just normalising to pulse type. This most accurately reflects the current procedures accepted as 'standard practice' in TMS protocols and thus demonstrates the potential effects of alterations to pulse type and current direction in a 'true-to-life' context. The participant set up (electromyography etc.) for this experiment is the same as that used in *Experiment 1*. Thereafter, using the procedure outlined previously (see *General Methodology*), the hot-spot and RMT to elicit both 50μV and 1mV responses, were determined. Then, the participants received stimulations to create maps with the coil in the postero-anterior, and antero-posterior orientation (*Figure 16*). Subsequently 2 maps would be obtained for each orientation, (with slight muscle activation - 10% of MVC) with either biphasic pulse type, or a half-sine pulse-type. Thus, 4 maps would be produced in total for each participant.

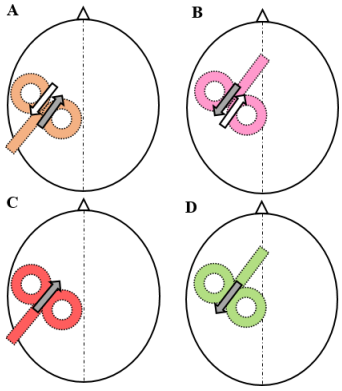

Figure 16. The coil orientations in Experiment 3. The filled arrows indicate direction of the physiologically most relevant phase of the pulse (Kammer et al., 2001). **A** *&* **C** *are held in the PA orientation, whereas* **B** *and* **D** *are held in the AP orientation.* **A** *&* **B** *are performed with biphasic pulses, whilst* **C** *and* **D** *are half-sine.*

Each map was obtained using the pseudorandom method outlined by van de Ruit et al. (2015) (see *Figure 15*). Maps were created by applying between stimuli to the scalp of an individual, with a 1.25 s inter-stimulus interval. Using nTMS, one could restrict stimulations to a pre-determined grid, thus prevent unnecessary stimulations to areas of the cortex that would not yield useful MEPs and/or may cause discomfort. Within this grid, 110 stimuli were applied

pseudorandomly, allowing a sufficient section of the motor cortex to be represented. The maps were subsequently stored for offline analysis.

2.4.2 Map Analysis

The details regarding map creation, parameters assessed and exclusion criteria are outlined in *General Methodology*. Within this experiment, corresponding Brainsight samples (neuronavigation coordinates) and MEPs within Mr. Kick were exported from the respective software packages prior to analysis through the bespoke MATLAB script. The MEPs and Brainsight samples are matched so that any given MEP can be located relative to the skull surface, using the neuronavigation. As previously mentioned, the PowerMAG ANT 100 stimulator reverses the current direction with every stimulation when using the half-sine setting. Thus half-sine MEP data files were appended in each of the activity conditions, to produce files with 220 stimuli. The individual MEPs were then excluded or included within these files to ensure that the current direction was correct within each 110 sample map. These MEPs were then matched to their corresponding neuronavigation coordinates from Brainsight. The maps were then created for each participant, with maps analysed separately within resting and active conditions. Therefore, two sets of four maps were created for each participant, with four active maps, and four resting maps. The key parameters assessed were resultant COGs, map area and aspect ratio.

2.4.3 Statistical Analysis

Statistical tests were performed with IBM SPSS Statistics 22 software. As before, the Expectation-Maximisation function was utilised, and in all cases, Little's MCAR test was not significant ($p > .05$) indicating the data were missing at random. Descriptive statistics confirmed that the data met the assumptions of parametric testing (normal distribution and equal variances). Where appropriate, the Bonferroni correction was used to compensate for multiple comparisons. Descriptive statistics confirmed that the data was normally distributed. 2 x 2 RM ANOVAs were performed for map area and COG assessing the effect of two pulse types (biphasic and half-sine) conditions and two coil current directions.

CHAPTER 3 – Results

3.1 General Results

All but one participant completed the session with no complaints during and following the investigation. The single participant felt 'light headed' upon commencing low-intensity stimulation, so the session was ceased. Upon consultation with the individual, this was likely due to a combination of tiredness and the participant had not consumed any food prior to testing. The symptoms quickly subsided and the participant reported no further ill-effects. This participant is not included within *Table 1*. Following exclusions of participant data as a result of the exclusion criteria outlined in *Methodology*, and individual data sets as a result of further exclusion criteria outlined in the *Results* section for each experiment (for example a map was not produced by the MATLAB script due to low MEP_{pp} values or an individual data set had excessively high background noise), the total number of data sets remaining for statistical analysis is outlined in *Table 3* below. For the remaining participants, Friedman's tests were performed to assess any differences in background activity between the conditions, across all experiments. No significant differences were found between the conditions in any experiment. Moreover, mapping data was assessed to ensure that rotating the coil had no influence upon the results produced by the mapping script. This was achieved by performing stimuli in the corners of the pre-defined grid, and on the hotspot with the coil held in PA and LM orientations. Indeed, altering the orientation had no influence on the COG produced by the mapping script, thus any alterations in COG detected would reflect the effect of the current direction and excitability, rather than the effect of rotating the coil on the mapping script.

Table 3. *Data Sets excluded across the investigations, the final row indicates the % of the total possible number of data sets if no data sets were removed. Data removed primarily due to excess background activity or insufficient data to form a map*

Experiment	*1a*	*1b*	*2*	*3*
Number of Participants Analysed	21	12	9	13
Total Possible Data Sets	72	84	36	56
Total after Full Exclusion	63	72	36	52
Total after Excluding Individual Data Sets	59	70	33	46
% of Total Possible Data Sets	**82**	**83**	**92**	**82**

3.2 Results - Experiment 1a

3.2.1 Data Collection and Analysis

In this experiment, all 24 participants completed the study and all participants tolerated the TMS well. Out of the 24 participants that completed the study, 21 participants had their data analysed assessing map area and COG, with 1 participant excluded due to excess muscle background activity, and two excluded as only one map out of three was produced. Example individual participant data can be seen in *Figure 17*.

Moreover, 2 participants had individual data sets excluded due to excess muscle background activity, and 2 participants had data sets removed as maps were not created by the analysis.

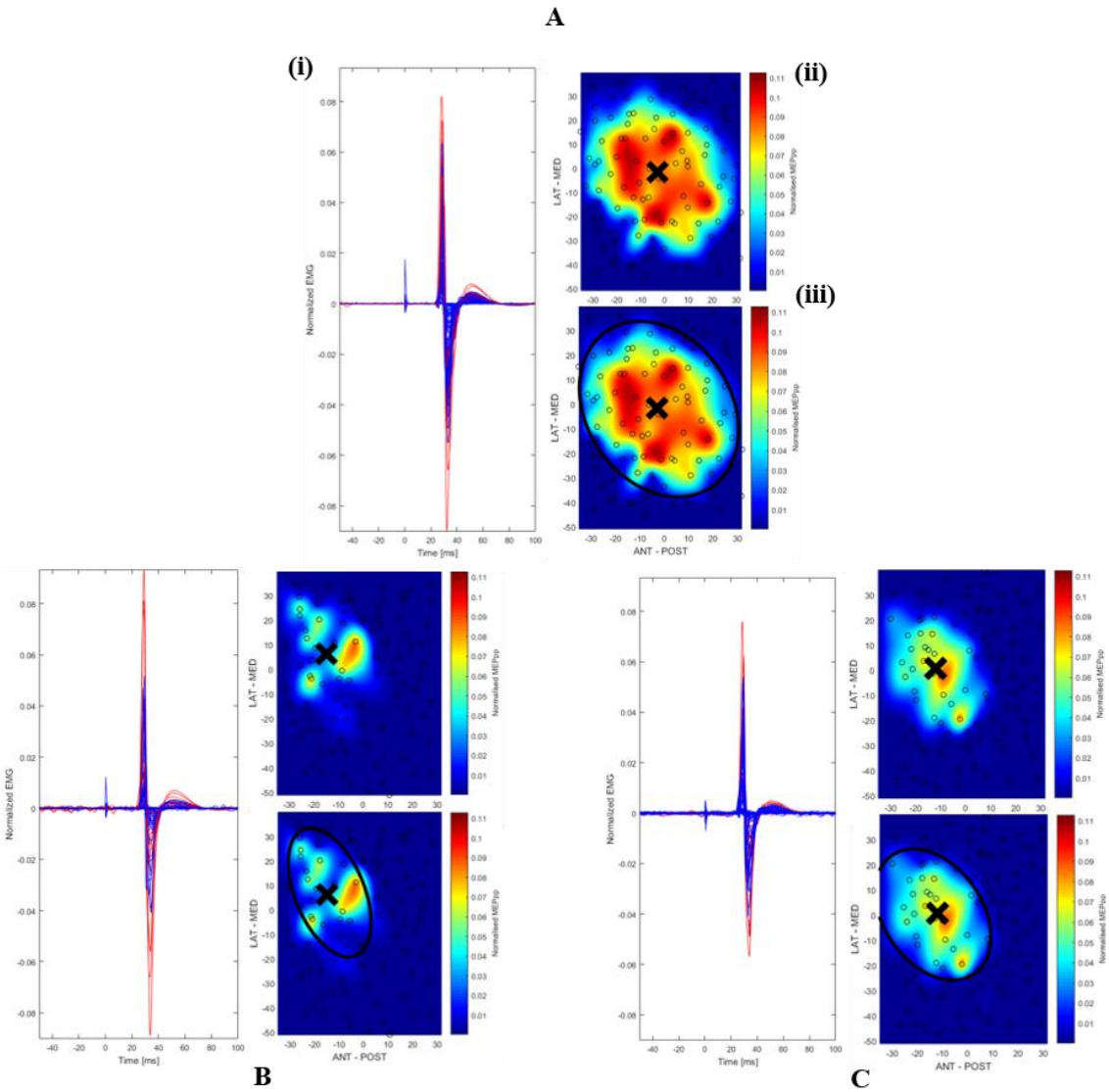

*Figure 17. Example active data from a single participant, across PA (**A**), LM (**B**) and AP (**C**). (i) Shows the grouped MEPs from each of the conditions. (ii) Shows the map produced by the bespoke MATLAB script, whilst (iii) shows the map with the ellipse fitted, which is utilised to calculate AR.*

3.2.2 Statistics – Effect of Current Direction on COG

There was found to be no significant effect of CURRENT DIRECTION on COG (see *Figure 18*) in this investigation, $F(1.350,40) = 1.700$ ($p = 0.205^{G-G}$). There were no significant post-hoc pairwise comparisons.

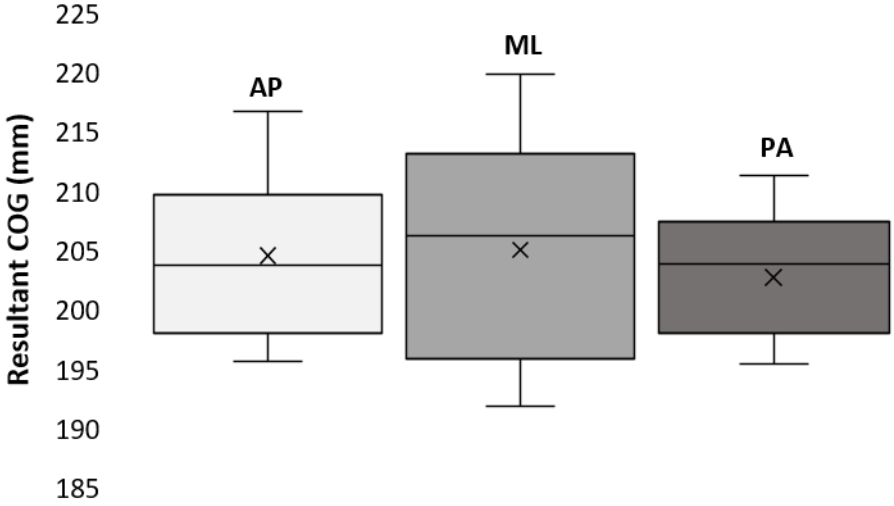

*Figure 18. Box and whisker plot for **COG**. There is no significant main effect of current direction, and there are no significant post-hoc pairwise-comparisons*

3.2.3 Statistics – Effect of Current Direction on Map Area

There was found to be a significant main effect of CURRENT DIRECTION upon map area (see *Figure 19)*, $F(2,44) = 80.752$ ($p < 0.01$), with significant ($p < 0.05$) post-hoc pairwise-comparisons between both AP (1157 ± 636 mm^2) and PA (2452 ± 620 mm^2), as well ML (855 ± 369 mm^2) and PA (2452 ± 620 mm^2), indicating map areas are largest in the PA current direction. No difference was found between AP (1157 ± 636 mm^2) and ML (855 ± 369 mm^2).

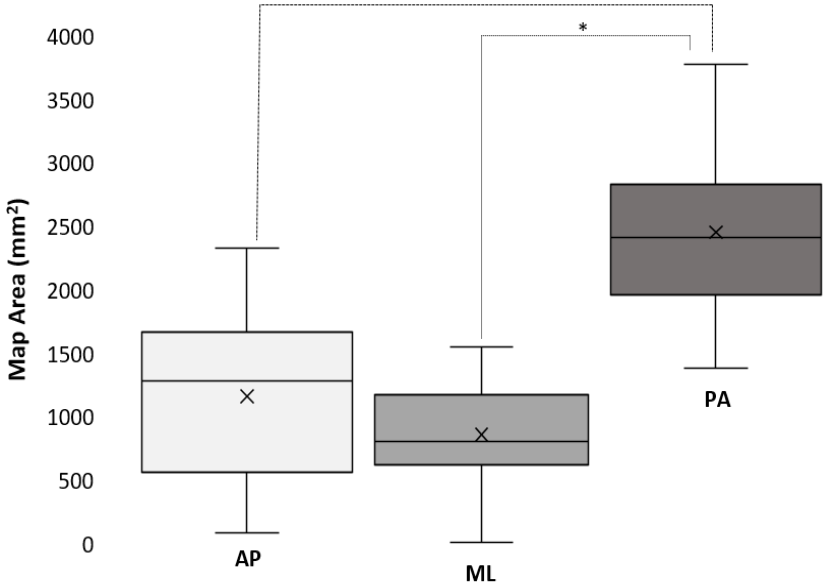

*Figure 19. Box and whisker plot for **map area**, showing the means (indicated by X), interquartile range and medians across each of the three current directions: AP (light), ML (medium) and PA (dark). There is a significant main effect of orientation ($p < 0.05$) (not shown). The significant ($p < 0.01$) post-hoc pairwise-comparisons are indicated on the chart (*)*

3.3 Results – Experiment 1b

3.3.1 Data Collection and Analysis

In this experiment, all 14 participants completed the study and all participants tolerated the TMS well. Out of the 14 participants that completed the study, 12 participants had their data analysed assessing map area, AR and COG (see *Table 3*). Two participants were excluded as only 1 resting map was produced, as a result of insufficient numbers of MEPs. Moreover, 1 participant had individual data sets excluded due to excess muscle background activity. Further, 1 participant had individual data sets removed as a map was not created by the analysis and 5 participants had individual AR data excluded or the data was missing as the mapping analysis was unable to fit an AR, both due to small numbers of MEPs within a given map.

3.3.2 Statistics – Effect of Current Direction and Activity on AR

There was a significant main effect of ACTIVITY: $F(1,11) = 5.171$ ($p = 0.044$) on AR (see *Figure 20).* Indeed, during post-hoc pairwise-comparisons, Active was shown to be significantly different to Rest in the ML current direction. However, there was no significant main effect of CURRENT DIRECTION: $F(2,22) = .528$ ($p = 0.597$) on AR (see *Figure 20),* neither was there a significant interaction effect $F(2,22) = 2.311$ ($p = 0.123$). There were no other significant post-hoc pairwise-comparisons.

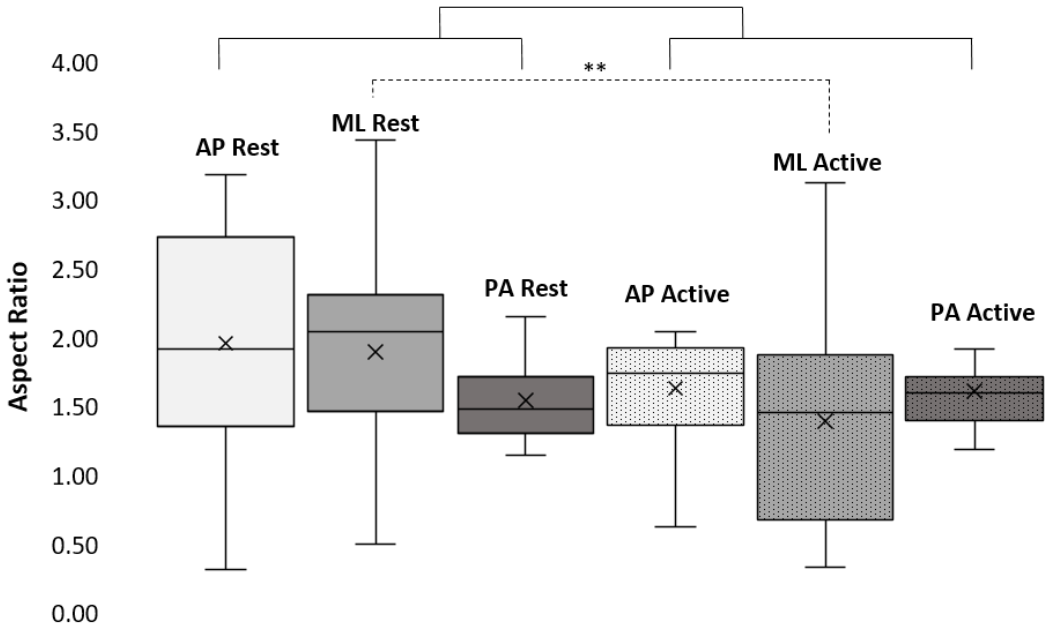

*Figure 20. Box and whisker plot for **AR** across each of the three current directions and activity levels: AP (light), ML (medium), PA (dark), Active (patterned) and rest (unpatterned). There is a significant main effect of activity ($p < 0.044$*), indicated by the solid lines and the significant ($p < 0.001$**) post-hoc pairwise-comparisons (after Bonferroni adjustments) are indicated on the chart by dashed lines.*

3.3.3 Statistics – Effect of Current Direction and Activity on COG

There were no significant main effects of ACTIVITY $F(1,11) = 1.041$ ($p = 0.330$) or CURRENT DIRECTION $F(2,22) = .167$ ($p = 0.847$) on COG, and there was no significant interaction effect $F(2,22) = 1.912$ ($p = 0.172$) (see *Figure 21)*. There were no significant post-hoc pairwise-comparisons. These results suggest that COG remains stable with changes in current direction and with muscle activation

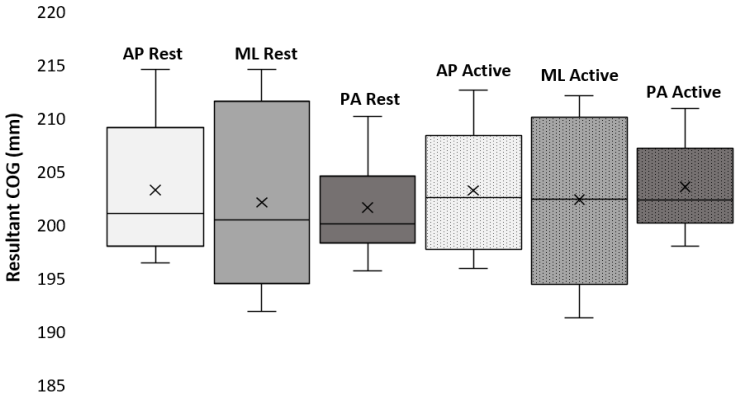

*Figure 21. Box and whisker plot for **COG** across each of the current directions and activity levels. There are no significant main effects or post-hoc pairwise-comparisons*

3.3.4 Statistics – Effect of Current Direction and Activity on Map Area

There was a significant effect of ACTIVITY on map area: $F(1,11) = 61.226$ ($p < 0.001$) and significant effect of CURRENT DIRECTION on map area: $F(2,22) = 67.764$ ($p < 0.001$) (see *Figure 22)*. There was a significant interaction of ACTIVITY*CURRENT DIRECTION on area, $F(2,22) = 4.491$ ($p = 0.023$). This once again suggests that PA current directions produce larger maps, alongside increases in map size with muscle activation.

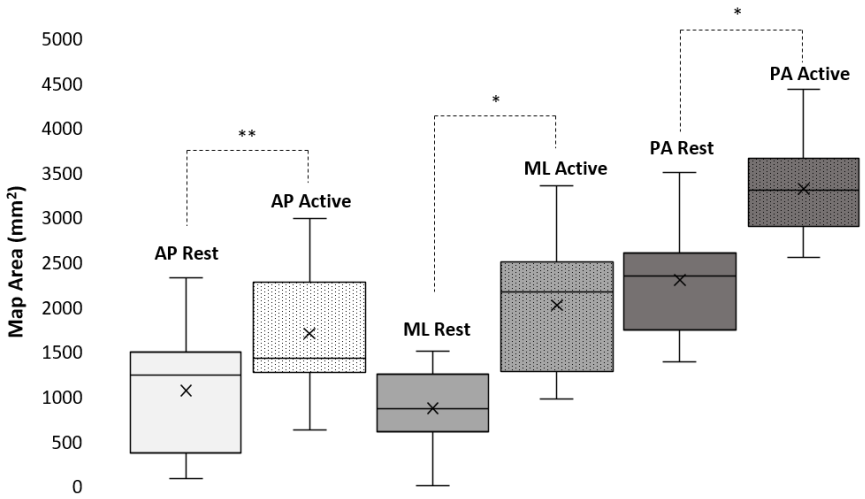

*Figure 22. Box and whisker plot for **map area** across each of the current directions and activity levels. Significant post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated on the chart, with significant differences across all current directions between active and rest ($p = 0.002$*, $p < 0.001$**). The significant main effect of activity is not indicated*

Post-hoc pairwise comparisons showed rest had significantly smaller map area compared Active across all current directions (see *Figure 23)*. Further, across both active and rest, both AP and ML had a smaller map area compared to PA, but AP and ML were not significantly different.

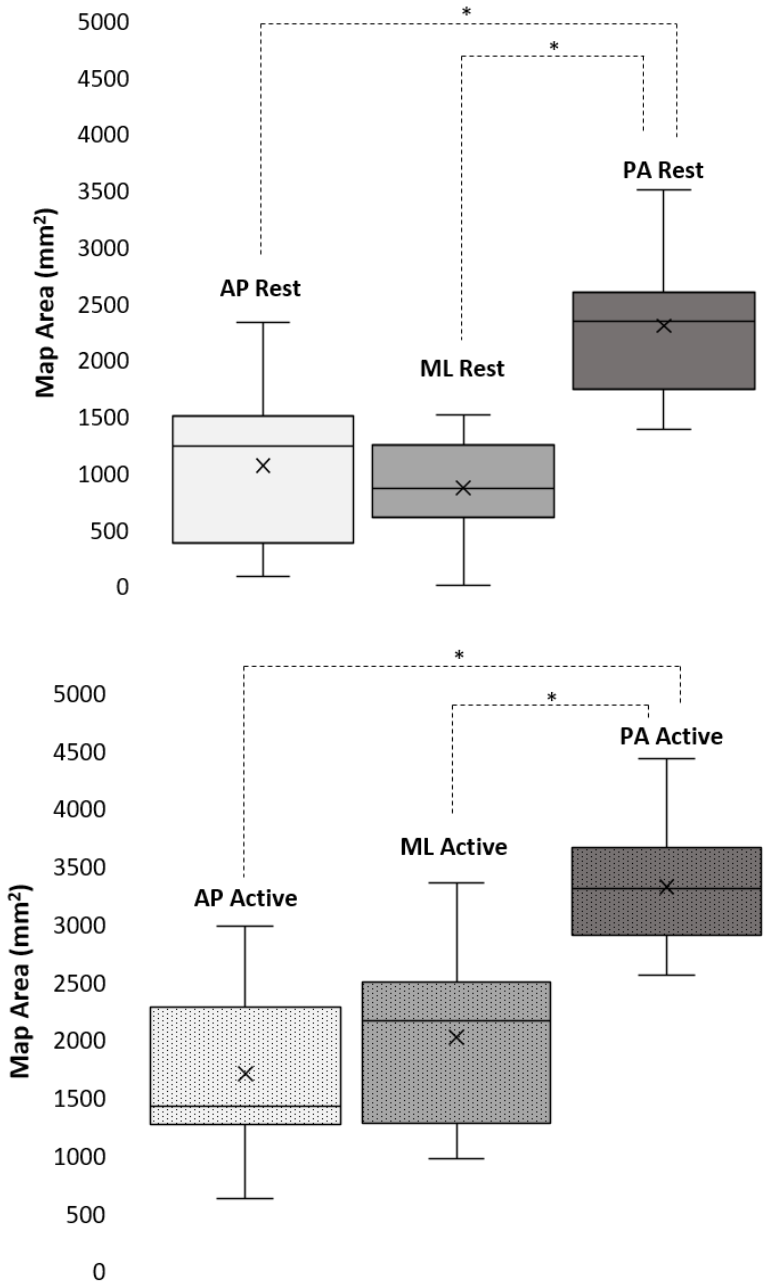

*Figure 23. Box and whisker plot for **map area** across each of the three current directions and activity levels (Rest = top, Active = bottom). Significant (p < 0.001*) post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated on the chart, with significant differences seen between AP & PA and ML & PA across both activity conditions*

3.4 Results – Experiment 2

3.4.1 Data Collection and Analysis

In this experiment, all 9 participants completed the study and all participants tolerated the TMS well. Out of the 9 participants that completed the study, all 9 participants had their data analysed assessing map area and COG. Nonetheless, 1 participant had two data sets excluded, and another participant had a single data set excluded as maps were not created by the analysis, likely due to an insufficient number of viable MEPs elicited by stimulation. Example individual participant data can be seen in *Figure 24*.

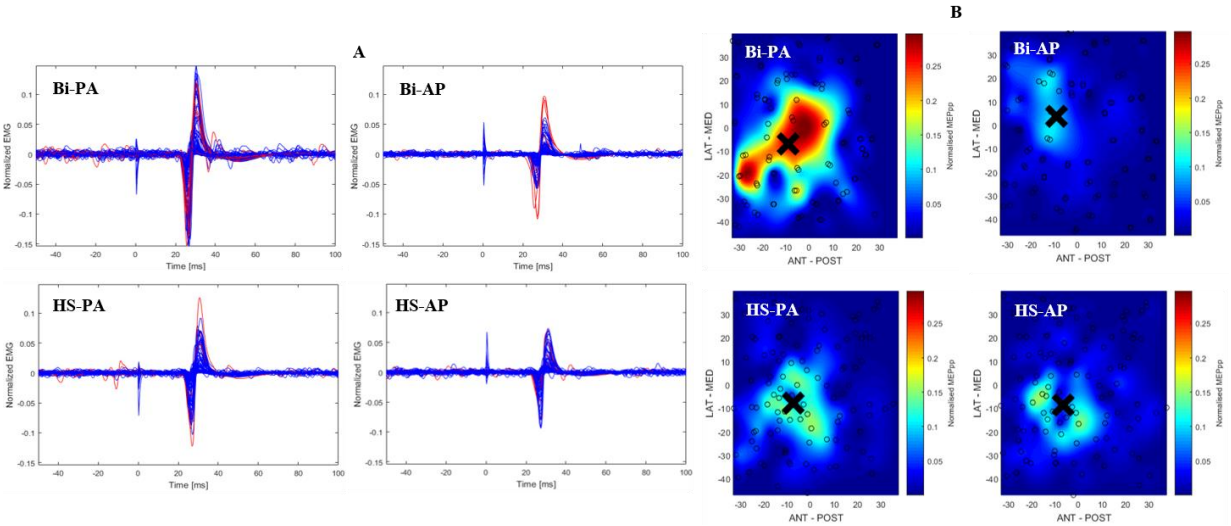

*Figure 24. Example data from a single participant, across both pulse types (***Bi** *&* **HS***) and both current directions (***PA** *and* **AP***).* (**A**) *Shows the grouped MEPs from each of the conditions. (***B***) Shows the maps produced by the bespoke MATLAB script*

3.4.2 Statistics – Effect of Pulse Type and Current Direction on COG

There was a significant main effect of PULSE on COG: F(1,8) = 10.623 (p = 0.012) (see *Figure 25*), but no significant main effect of CURRENT DIRECTION on COG: F(1,8) = .122 (p = 0.736). There was no significant interaction effect of PULSE*CURRENT DIRECTION: F(1,8) = .219 (p = 0.652). Bonferroni adjusted post-hoc pairwise-comparisons revealed a significant different in the COG between Bi and HS in the PA orientation (p = 0.005) (see *Figure 26*). There were no further significant post-hoc pairwise-comparisons.

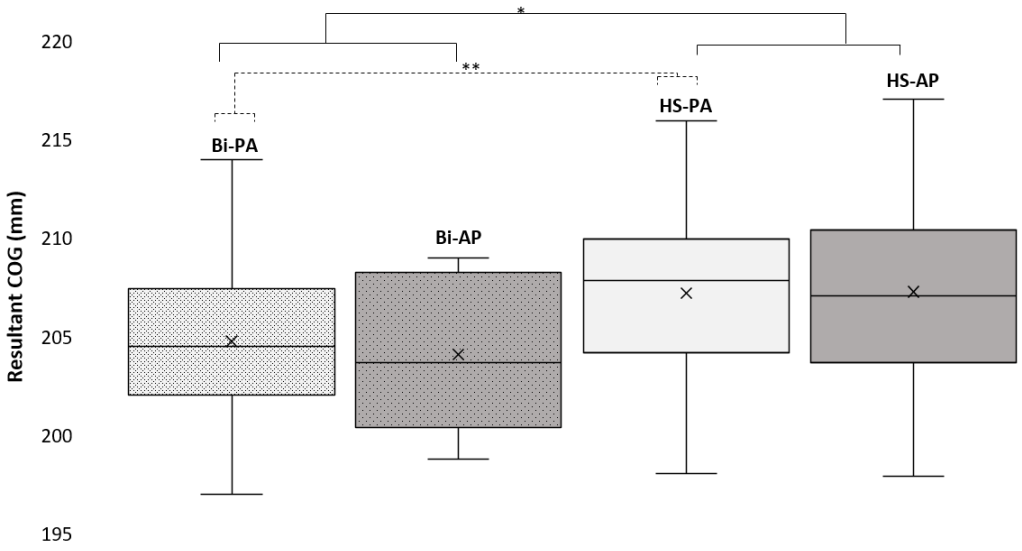

*Figure 25. Box and whisker plot for **COG** across both directions: AP (Dark) and PA (light) and pulse types Biphasic (patterned) and half-sine (unpatterned). Significant main effects (p = 0.012*) of pulse type is indicated by the solid lines. Significant (p = 0.005**) post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated on the chart by the dashed lines.*

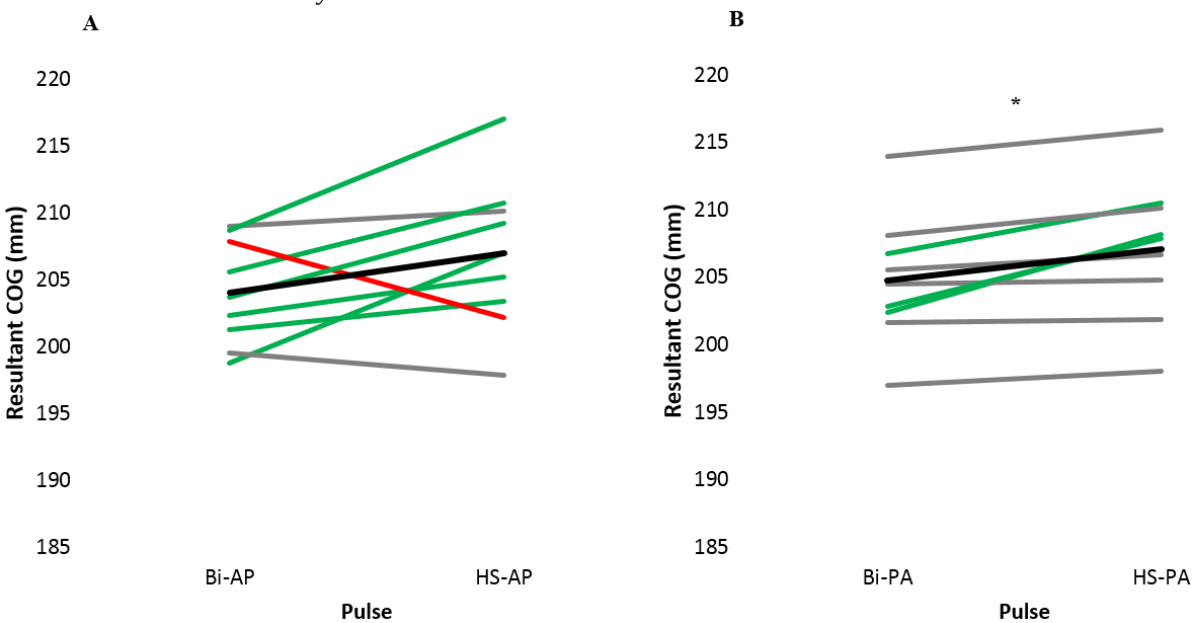

*Figure 26. Individual data for **COG** comparing **pulse type**, across **A** AP and **B** PA current directions. The group mean is shown by the black line. Significant post-hoc pairwise comparisons (Bonferroni adjusted) are indicated (p < 0.001*).*

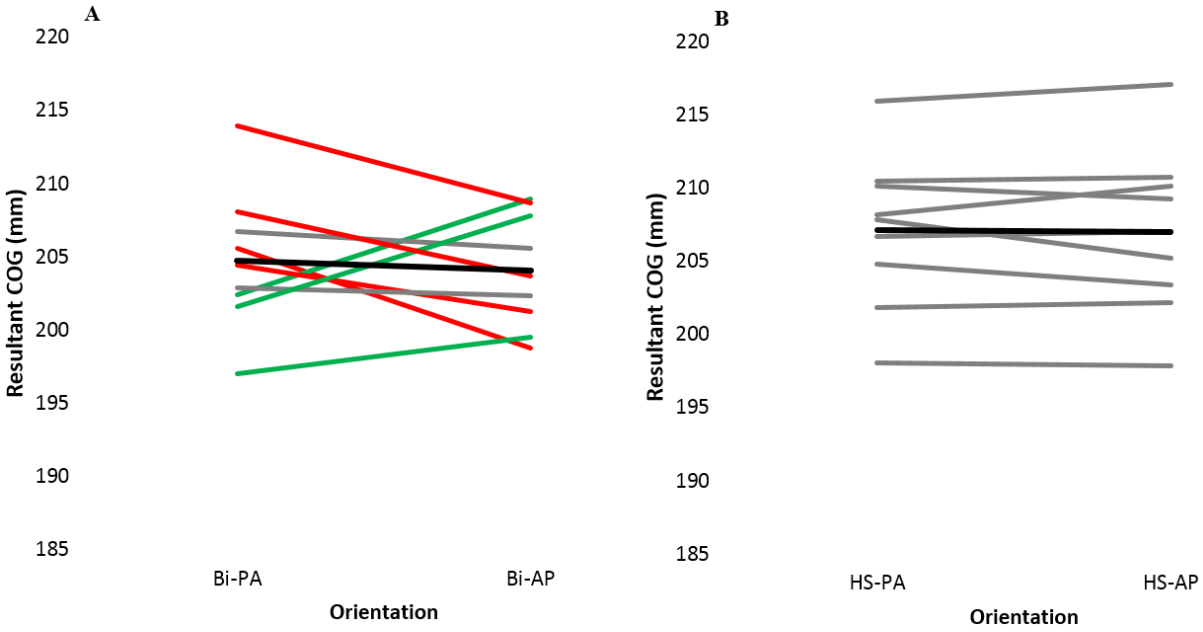

*Figure 27. Individual data for **COG** comparing **current direction**, across **A** AP and **B** PA current directions. The group mean is shown by the black line. There are no significant post-hoc pairwise-comparisons*

3.4.3 Statistics – Effect of Pulse Type and Current Direction on Map Area

There was both a significant main effect of PULSE on map area: $F(1,8) = 13.008$ ($p < 0.01$) as well as a significant main effect of CURRENT DIRECTION: $F(1,8) = 130.91$ ($p < 0.01$). There was also a significant interaction effect of PULSE*CURRENT DIRECTION: $F(1,8) = 6.198$ ($p = 0.038$) (see *Figure 28*). Bonferroni adjusted post-hoc pairwise-comparisons revealed a significant different in the map area between Bi and HS in the PA direction ($p = 0.005$) (see *Figure 29*). There was no significant difference between Bi and HS in the AP direction ($p = 0.057$). Furthermore, there was a significant difference in map area between AP and PA with a HS pulse ($p = 0.003$) and with a Bi pulse ($p < 0.001$) (see *Figure 30*). These results suggest that biphasic pulses produce larger maps than half sine pulses, whilst in a similar vein to Experiment 1, stimulation in the PA current direction produces larger maps than in the AP direction

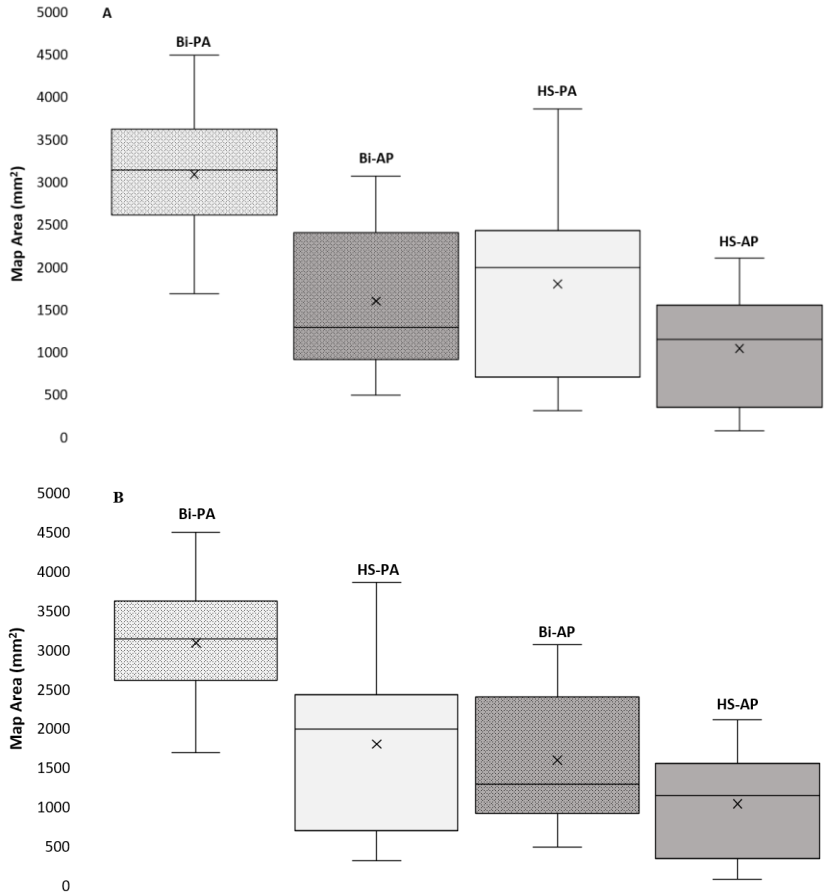

*Figure 28. Box and whisker plot for **map area** across both pulse types current directions. **A** is sorted by pulse type, **B** is sorted by current direction. Significant main effects are not indicated on this chart.*

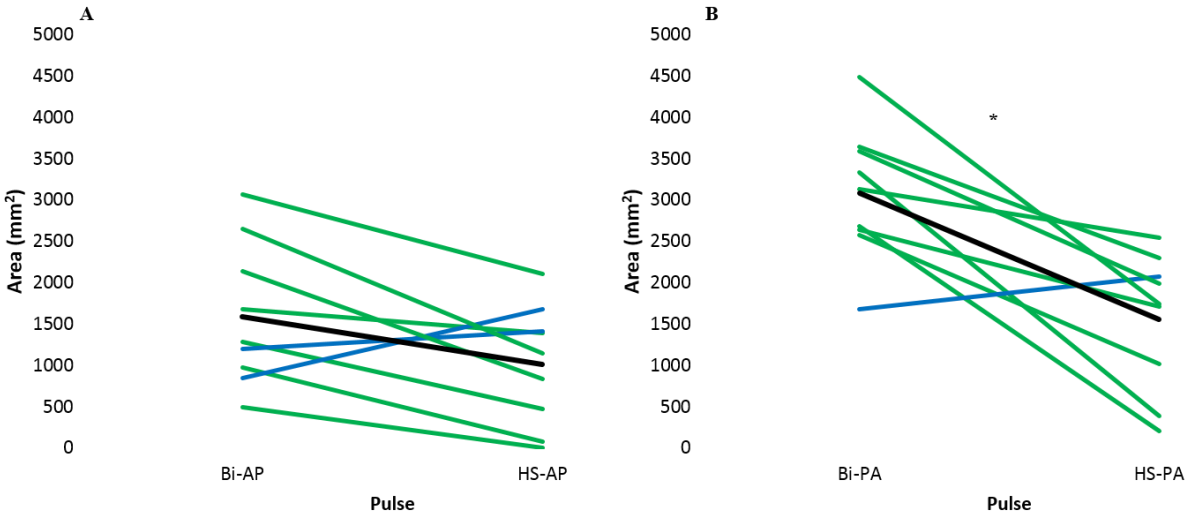

*Figure 29. Individual data for **map area** comparing **pulse type**, across **A** AP and **B** PA current directions. The group mean is shown by the black line. Significant post-hoc pairwise comparisons (Bonferroni adjusted) are indicated (p = 0.005*). Coloured lines are used to illustrate the direction of change*

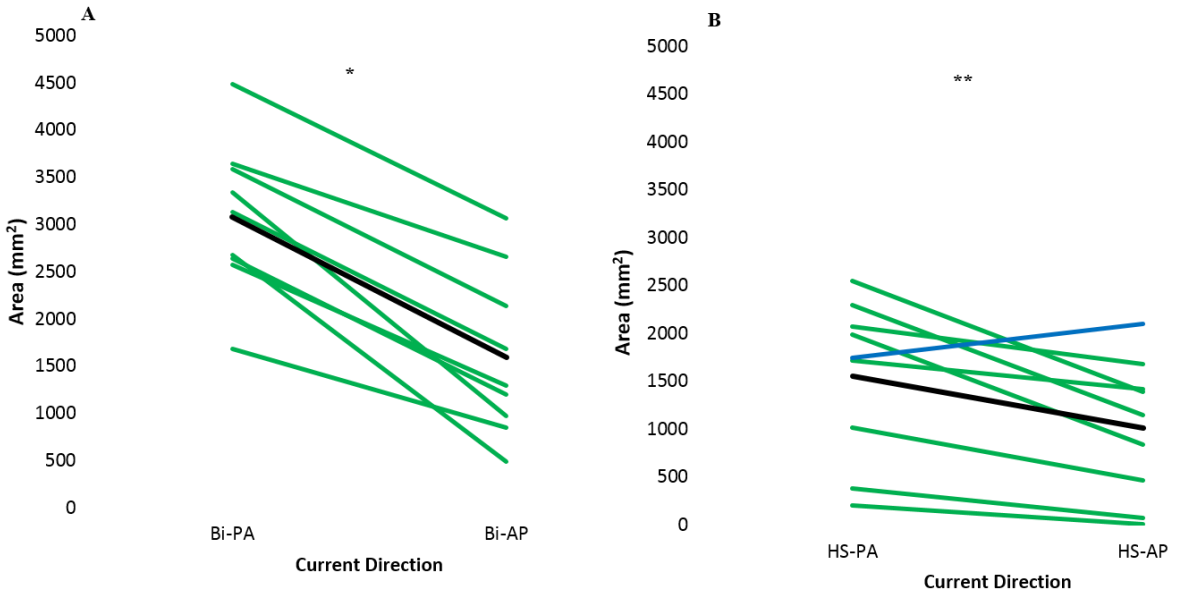

*Figure 30. Individual data for map area comparing **current direction**, across **A** biphasic and **B** half-sine pulse types. The group mean is shown by the black line. Significant post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated (p < 0.001*, p = 0.003**). Coloured lines are used to illustrate the direction of change*

3.5 Results – Experiment 3

3.5.1 Data Collection and Analysis

In this experiment, all 14 participants completed the study and all participants tolerated the TMS well. Out of the 14 participants that completed the study, 13 participants had their data analysed assessing map area and COG, with 1 participant excluded due to excess muscle background activity. Moreover, 3 participants had a single data set excluded due to excess muscle background activity, whilst 4 participants had individual data sets removed as a map was not created by the analysis. Example individual participant data can be seen in *Figure 31*.

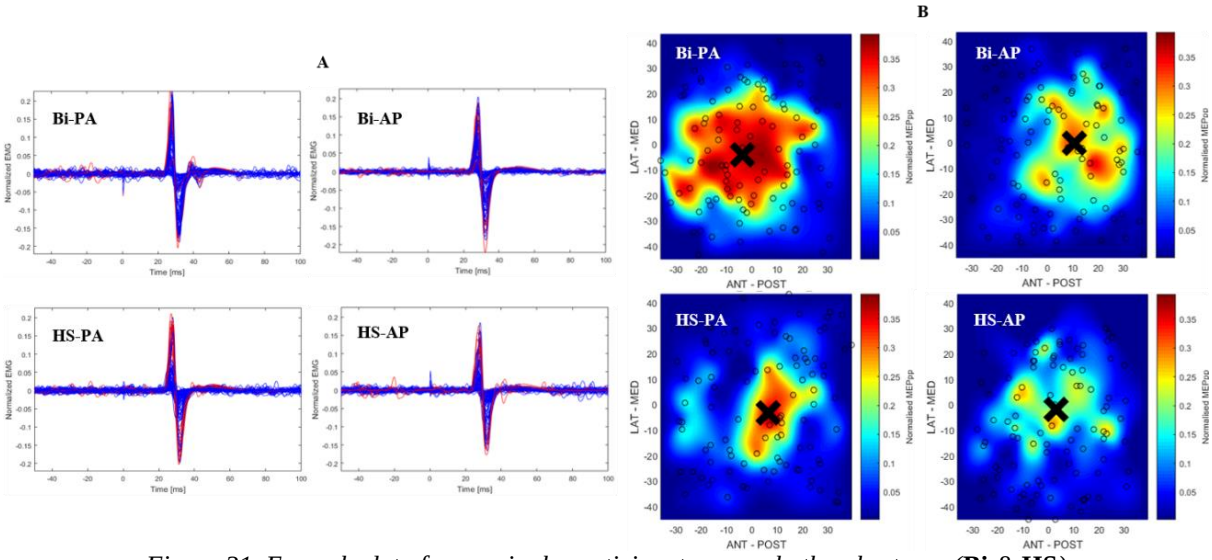

*Figure 31. Example data from a single participant, across both pulse types (***Bi** *&* **HS***) and both current directions (***PA** *and* **AP***).* (**A**) *Shows the grouped MEPs from each of the conditions. (***B***) Shows the maps produced by the bespoke MATLAB script*

3.5.2 Statistics – Effect of Pulse Type and Current Direction on COG

There was a significant main effect of PULSE on COG: F(1,12) = 5.571 (p = 0.036), but no significant main effect of CURRENT DIRECTION detected on COG: F(1,12) = 1.665 (p = 0.221). There was a significant interaction effect of PULSE*CURRENT DIRECTION: F(1,12) = 6.893 (p = 0.022) (see *Figure 32*). Bonferroni adjusted post-hoc pairwise-comparisons revealed a significant different in the COG between Bi and HS in the AP direction (p = 0.007) (see *Figure 33*) and between AP and PA with a Bi pulse (p = 0.048) (see *Figure 34*). There were no further significant post-hoc pairwise-comparisons.

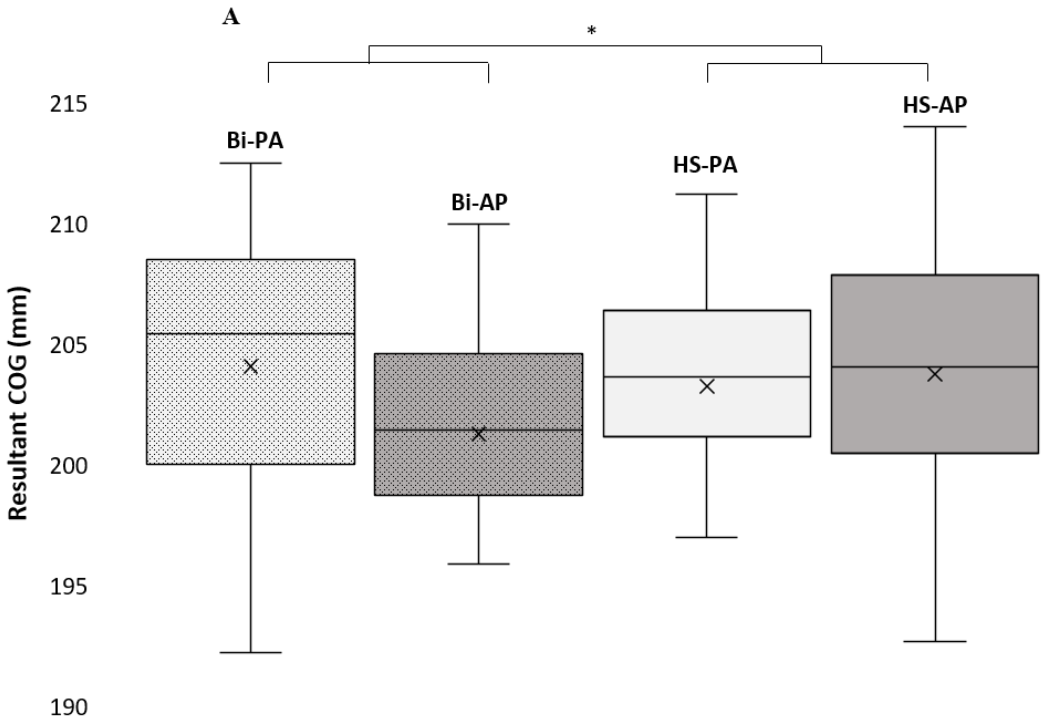

*Figure 32. Box and whisker plot for **COG** across both current directions: AP (Dark) and PA (light) and pulse types: biphasic (patterned) and half-sine (unpatterned). The figure demonstrates the data, sorted by pulse type. A significant main effect of pulse type (p = 0.036*) is indicated by the solid lines.*

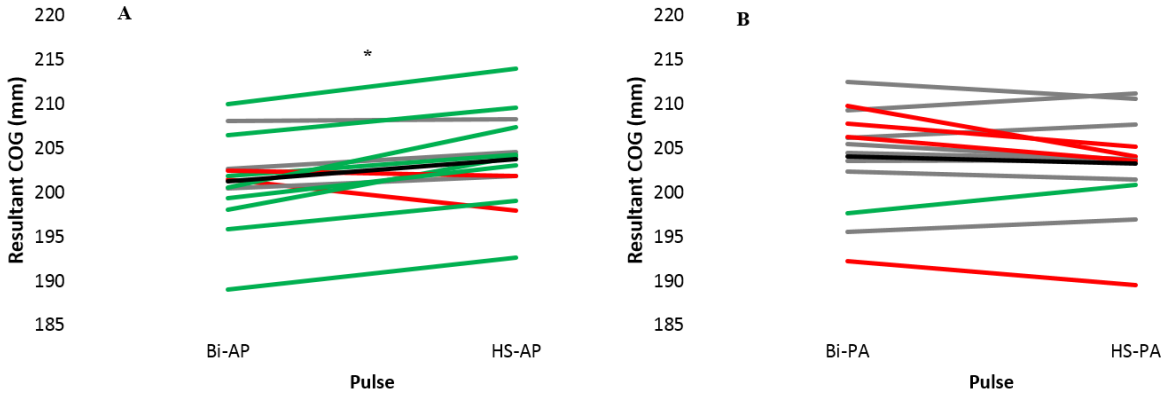

*Figure 33. Individual data for **COG** comparing **pulse type**, across **A** AP and **B** PA current directions. The group mean is indicated by the black line. Significant post-hoc pairwise comparisons (Bonferroni adjusted) are indicated (p = 0.007*).*

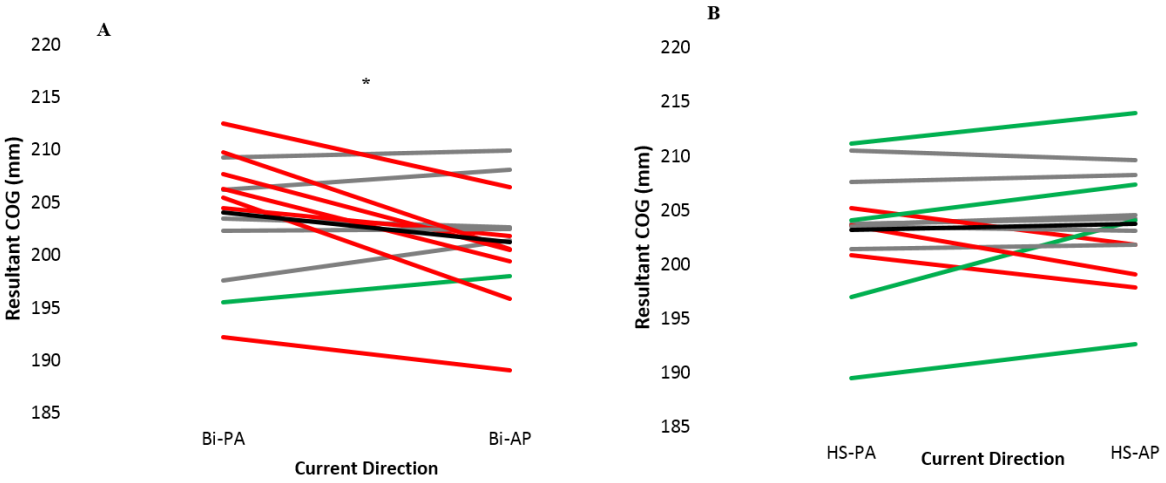

*Figure 34. Individual data for **COG** comparing **current direction**, across **A** biphasic and **B** half-sine pulse types. The group mean is shown by the black line. Significant post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated (p = 0.048*).*

3.5.3 Statistics – Effect of Pulse Type and Current Direction on Map Area

There was a significant main effect of PULSE on map area: $F(1,12) = 29.306$ ($p < 0.001$), as but no significant main effect of CURRENT DIRECTION on map area: $F(1,12) = 0.697$ ($p = 0.420$). There was a significant interaction effect of PULSE*CURRENT DIRECTION: $F(1,12) = 24.228$ ($p < 0.001$) (see *Figure 35*). Bonferroni adjusted post-hoc pairwise-comparisons revealed a significant difference in the map area between Bi and HS in both the AP ($p < 0.001$) and PA ($p < 0.001$) direction (see *Figure 36*). Furthermore, there was a significant difference in map area between AP and PA with a HS pulse ($p = 0.003$), but this was not seen with a Bi pulse ($p = 0.133$) (see *Figure 37*). This suggests that normalising the stimulating intensity can negate the changes in map size seen in *Experiment 1* and *2*. Nonetheless, biphasic stimuli produce larger maps when compared to half-sine pulses

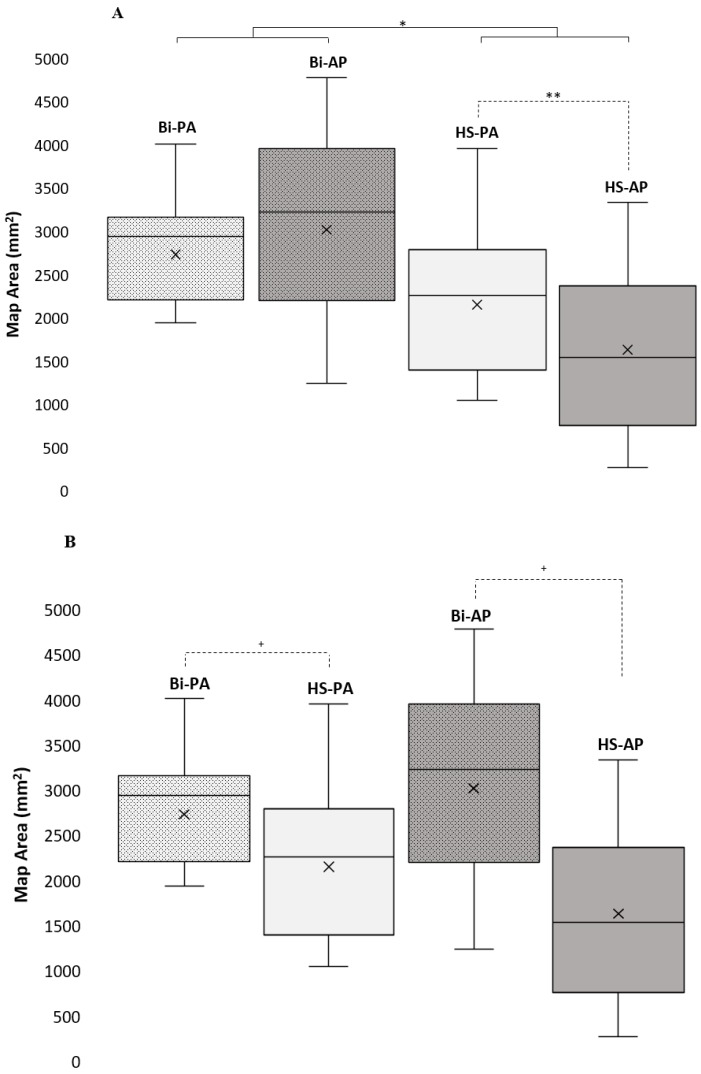

*Figure 35. Box and whisker plot for **map area** across both current directions and pulse types. A significant main effect of pulse type (**A**) ($p < 0.001$*) is indicated by the solid lines.*

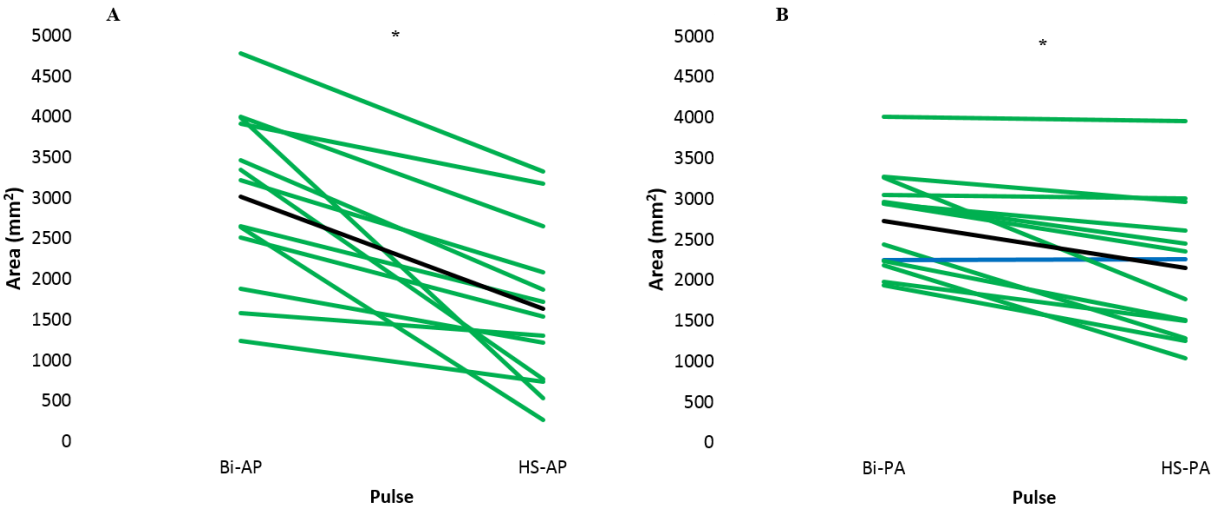

*Figure 36. Individual data for **map area** comparing **pulse type**, across **A** AP and **B** PA current directions. The group mean is shown by the black line. Significant post-hoc pairwise comparisons (Bonferroni adjusted) are indicated (p < 0.001*). Coloured lines are used to illustrate the direction of change*

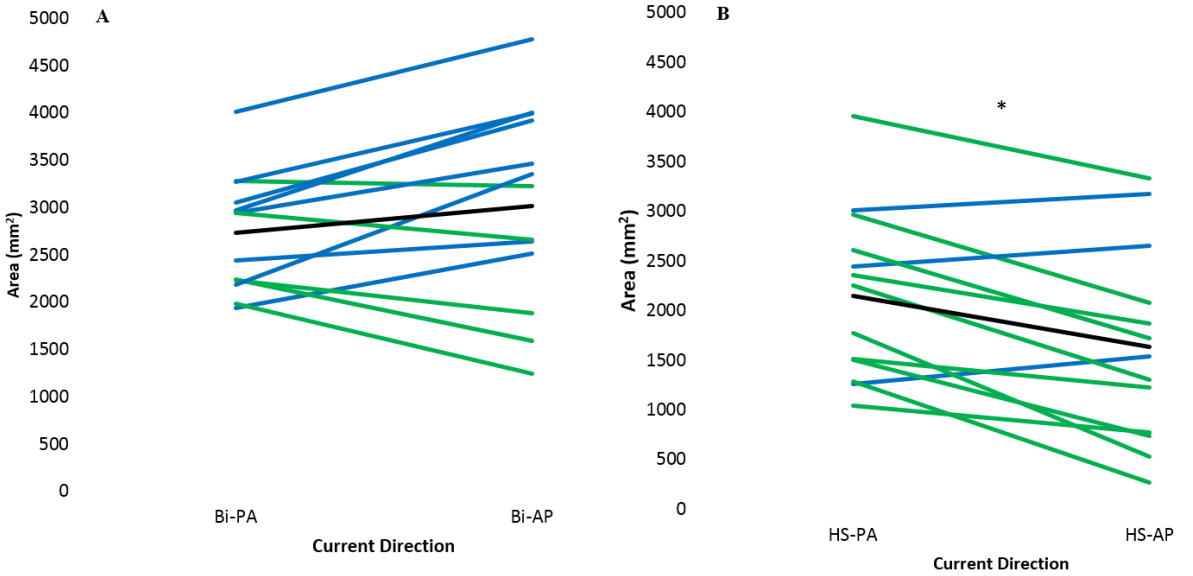

*Figure 37. Individual data for COG comparing **current direction**, across **A** biphasic and **B** half-sine pulse types. The group mean is shown by the black line. Significant post-hoc pairwise-comparisons (Bonferroni adjusted) are indicated (p = 0.003*). Coloured lines are used to illustrate the direction of change*

CHAPTER 4 – Discussion

4.1.1 Overview of Findings

The first part (*a*) of *Experiment 1* was to explore the effect of TMS current direction on map area and COG, during biphasic TMS with a fixed stimulation intensity. It was demonstrated that current direction had an effect on map area, with the largest maps generated with the most physiologically relevant phase of the biphasic TMS pulse (Brasil-Neto et al., 1992a; Sommer et al., 2006) oriented in a posteroanterior current direction on the cortex. Conversely, it was also determined that altering the current direction had little effect on the COG between maps, perhaps highlighting the inherent stability (Uy et al., 2002; Ngomo et al., 2012) of this measure. The second part (*b*) of *Experiment 1* was to explore the effect of both TMS current direction and different states of muscle activity (active and passive) on map area, map shape (AR) and COG, during biphasic TMS with a fixed stimulation intensity. It was shown that activity had an effect on map area and AR, but no influence on COG. Further, current direction had no effect on AR and COG.

The purpose of *Experiment 2* was to explore the effect of TMS current direction and pulse type on map area and COG, during biphasic and half-sine TMS with a fixed stimulation intensity for a *given pulse type*. It was demonstrated that current direction had an effect on map area, with the largest maps generated with the most physiologically relevant phase of both the biphasic and half-sine TMS pulses (Sommer et al., 2006) oriented in a PA current direction on the cortex. Conversely, it was also determined that altering the current direction had little effect on the COG, perhaps highlighting the previously mentioned stability (Uy et al., 2002; Ngomo et al., 2012) of this parameter. The other variable investigated was pulse type, and it was determined that pulse type had an effect on map area, with larger maps generated with a biphasic TMS pulse. Furthermore, it was found that pulse type had an influence on COG, therefore further considerations maybe needed when using different pulse types to perform TMS mapping.

The purpose of *Experiment 3* was to explore the effect of TMS current direction and pulse type on map area and COG, during both biphasic and half-sine TMS with stimulation intensity altered in accordance with current direction and pulse type. This experiment has yielded some surprising results which will be examined further. Firstly, it was determined that there was a significant main effect of pulse type on both map area and COG, whilst there was no main effect of current direction. Importantly though, an interaction effect of current direction and pulse type were detected for both map area and COG.

4.1.2 The Influence of Current Direction on Mapping Parameters

4.1.2.1 Effect of Current Direction on Map Area

Both parts *Experiment 1*, and *Experiment 2* investigation showed that map area has the propensity to be influenced by current direction when the stimulation intensity remains constant (for a given current direction). This is largely unsurprising given that previous literature also points to this conclusion. The clearest finding of this investigation into map area highlights that PA (*Experiment 1* and *2*) directed currents produced the largest maps, when compared to ML (*Experiment* 1) and AP (*Experiment 1* and *2*) currents. This corresponds to a number of findings (Mills et al., 1992; Brasil-Neto et al., 1992b), who found that the greatest MEPs were elicited when the coil was held at 45^{o} relative to the parasagittal plane (corresponding to PA in this investigation). However, in *Experiment 3*, where stimulation intensity was changed in accordance with current direction, no alterations in map area were detected. The potential reasons for these findings are explored in this chapter.

Preferential Activation of Different Cortical Neurons

A compelling argument for explaining changes in map area between the current directions could be that each particular current direction preferentially activates different sites of cortical neurones (Di Lazzaro et al., 2001b). It has been well documented that stimulation preferentially occurs at particular sites along neurones and TMS could be maximally initiated at axonal boundaries, or perhaps most notably, at axon bends (Macabee et al., 1993; Wagner et al., 2009). However, this preferential stimulation will only occur if the stimulating current can induce an outward current flow at the bend (Di Lazzaro et al., 2001b). Therefore, some neurones are likely to be optimally activated by currents induced in one direction, whereas others are more likely to be preferentially activated by currents in another direction (Di Lazzaro et al., 2001b). It could

be the case, that the neurones responsible for the FDI are preferentially activated in a PA current direction, perhaps explaining why the AP (*Experiment 1* and *2*) and ML (*Experiment 1*) current directions are considerably less effective at generating MEPs, and thus reduced map areas.

This notion can also be extended into explaining the lack of a difference in map area between ML and AP (*Experiment 1*) oriented currents in both parts of the investigation. Whilst preferential activation of neurones at particular sites and in particular current directions has been discussed, differences between participants in cerebral architecture may also be important when distinguishing the effects of ML versus AP oriented currents (Wagner et al., 2009). For example, it can be seen (*Figure 19*) that in *Experiment 1a* there is considerable variation in the map areas between participants in the AP oriented currents, which is not mirrored in ML oriented currents. In their investigation Balslev et al. (2007) suggest that the ideal coil orientation for a muscle could lie within a range around 45^{o} to the parasagittal plane. Indeed, this idea could be extended when the current flow is in the opposite direction (AP). It could be suggested that differences in cerebral architecture means that the bends in axons may have been activated for some participants with an optimally oriented AP current, but not in others. Perhaps if all participants had the most optimal current direction for AP stimulated, these maps may have been larger than the maps produced in the ML orientation. Nonetheless, whilst this could be seen as ideal for research and clinical purposes, due to obvious expense and temporal restraints, perhaps this approach is not practical. It is important to acknowledge that some may argue that smaller maps may actually more accurately reflect the true representation in the cortex. Whilst this is a fair argument, in both the context of this investigation and in a clinical setting, it is likely that producing larger maps is more suitable. This is the case because the largest possible representation may be needed in individuals who have suffered from a cortical disease, such as a stroke. In these situations, the individual may have reduced representations of a particular muscle. Therefore, in order to ensure that the full representation of the muscle is obtained in these individuals, it is of benefit to obtain large maps. This would allow full

assessment of baseline measurements with TMS, as well as any alterations to the cortex that result4 from rehabilitation. If sub-optimal TMS techniques are used in these situations, TMS mapping may not be appropriate.

The subtle differences between ML and PA (*Experiment 1*) can be explained more simply. The maps produced with PA current directions include a number of stimuli which are administered at locations which are on the fringes of excitability, and thus produce small MEPs. It could be argued that with an ML oriented current, the current flow is not suitable for depolarising axons at the fringes of excitability as it does not produce an outward flow at the axon bend (Di Lazzaro et al., 2001b). Thus in the locations where PA directed currents may have produced small MEPs, in the ML current direction an MEP is not produced. This gives an explanation as to why the maps produced in the ML current direction are smaller than PA. Furthermore, this logic can be extended to providing reasoning for both a lack of differences between AP and ML map sizes and the variation seen in AP maps. In some individuals, the stimulation intensity may not have been high enough to depolarise axons at the fringe of excitability in AP as well as ML. In these cases, there would be no difference in map size when comparing ML and AP. However, in other cases, some individuals may have had a greater propensity for stimuli at the fringes of excitability to depolarise axons, whether this is because the stimulation intensity was just high enough for these individuals, or subtle differences in cerebral architecture allow stimuli to be more effective. Thus, there were a range of map sizes in AP, which were not mirrored in ML. It therefore can be suggested that in some individuals, AP directed currents would produce significantly larger maps than ML, but this is not the case in all participants. This once again adds further credence to the notion that correct threshold determination is crucial to obtaining reliable maps, and thus offset any potential alterations in current direction.

In *Experiment 3*, where the stimulation intensity was changed in accordance with current direction in an attempt to reduce the influence of current direction on map area. Indeed, in this circumstance there was no significant effect of current direction upon map area. This could be

because the stimulation intensity was high enough in the AP current direction to effectively elicit a response from the FDI, despite the sub-optimal alignment of the coil. Therefore, appropriate threshold and stimulation intensity determination can be seen as a key factor in mitigating the effect of changes in current direction, whether as a result of a rotation of the coil through the yaw axes by 1 or 2 degrees, or indeed through differences between stimulator types.

The Effect of a Constant Stimulation Intensity

The differences in map area seen in *Experiment 1* and *2* can be directly related to the constant stimulation intensity used within these experiments. It has been regularly cited that altering the coil orientation has an effect on the stimulation intensity required to generate a particular response. Indeed, Kammer et al., 2001 found that the orientation with the lowest threshold corresponds to the PA current direction in the present study. Moreover, Balslev et al. (2007) corroborate the results from this investigation further, with the PA current direction (PL in Balslev) producing the lowest average threshold, and with AP and ML (L and AM in Balslev) with strikingly similar average thresholds (56% and 55% of machine output respectively). Thus, it is unsurprising that given a constant stimulation intensity, maps obtained in a PA direction are larger than those in an ML or AP direction. To obtain the same level output from a given muscle, one must increase the stimulation intensity if administering stimuli in a non-optimal current direction (AP). Perhaps with appropriate threshold determination and, as a consequence, suitably high stimulation intensities, the influence of preferential activation in particular current directions can be negated (Di Lazzaro et al., 2001b).

Induced Current Flow in the Cortex

Another potential consideration for future study could be concerning the influence of current direction and the induced current flow in the cortex. Stephani et al. (2016) suggest that different coil orientations may induce current flows in opposite directions, either from cortical layers VI to I or from I to VI. The former, are thought to be activated by AP stimuli, whereas the latter

are thought to be activated by PA stimuli (Sommer et al. 2013). When the induced current flow is from layer VI to I (AP), a greater spread of activation in the cortex maybe induced, which is not focal. Whereas, the PA stimuli inducing current flows from I to VI may be able to excite axons directly (Stephani et al., 2016). If this is the case, as the axons are stimulated directly, it could be suggested that it is more likely that PA stimuli will lead to depolarisation of the neurons and subsequent production of MEPs (and larger maps) in comparison with AP stimuli which are less effective at stimulating the axons of interest within the central sulcus, thus producing smaller maps – as seen in *Experiment 1* and *2*. However, in *Experiment 3* we have shown no differences in map size when the stimulation intensity is adjusted in accordance with current direction. Perhaps, the reduced efficacy of AP stimuli could be negated by utilising higher stimulation intensities and by appropriately determining the threshold, to produce maps of similar areas to PA stimuli.

4.1.2.2 Effect of Current Direction on COG

Experiment 1

The findings from *Experiment 1a and 1b* add credence to the notion that COG is a fairly robust measure, as despite observed changes in map area in different current directions with a constant stimulation intensity, the COG remained largely unchanged. Indeed, across both experiments there were no significant differences in the mean resultant COG as a result of altering either the current direction. As previously mentioned, COG is commonly defined as the position of a motor map that yields the highest amplitude weighted response to stimulation (Wasserman et al., 1992). COG corresponds with an area of high excitability of corticomotor neurons and thus is closely associated with the optimal stimulus site of the muscle being mapped (Thickbroom et al., 1999; Wilson et al., 1993). Therefore, it may be a little surprising that there were no significant differences in COG in either *Experiment 1a* or *1b*, especially considering a constant stimulus intensity was used. This is particularly true as some studies have indicated that COG is susceptible to changes as a result of altering the coil orientation. Indeed, Wilson et al. (1993)

detected significant medial shifts in the location of the COG of over 5 degrees in both the APB and ADM when rotating the coil mediolaterally versus a PA oriented coil. More recently, Stephani et al. (2016) detected differences in COG location in the y-axis as a result of the current direction. Nonetheless, these alterations were only detected after taking into account when analysing every stimulation location during mapping. When the analysis was restricted to the original "37 inner locations", there were no significant differences in COG location in any current direction or axis.

Perhaps then COG can be stipulated to be an inherently stable parameter in healthy individuals, and can be further utilised in TMS practice. Uy et al. (2002) reported that COG is stable over intervals of up to 2 weeks, moving an average of 4mm over that time period. Furthermore, Malcolm et al. (2006) reported good test-retest reliability in the lateral position of the COG, with slightly reduced reliability in the anterior/posterior location of the COG. The stability of COG is further enhanced when utilising neuronavigational techniques. Miranda et al. (1997) showed that across sessions, COG location was found to be reproducible within ± 3 mm when using a 3D digitiser, results that were further reproduced using MRI-based neuronavigation (Weiss et al., 2012). Ngomo et al. (2012) showed that COG measures showed good to excellent long and short term reliability, in the posteroanterior axis. However, their investigation stated that COG location in the mediolateral axis was poor. As stated by the authors, this could be attributed to the fact that the ICC may have been influenced by the noticeable between-subject variance. Indeed our investigation also showed this variation, as can be seen from *Figures 18* and *21* there was substantial between-subject variation in the location of the COG. This could be as a result of small differences in the location of the hotspot within the pre-determined stimulating grid, defined at the start of the investigation, or indeed differences in individual cerebral architecture as previously mentioned in this section (Balslev et al., 2007; Bashir et al., 2013).

Experiment 2

In similarity to the data observed in *Experiment 1*, it has been determined once again that the mean resultant COG was unchanged by altering the current direction of the administered TMS pulse. This once again adds further evidence to the idea that COG is a fairly robust measure, as once again, despite not changing the stimulation intensity between current directions, the mean resultant COG remains largely unaffected. Finally, *Experiment 3* showed that in terms of COG, there were no changes elicited as a result of current direction with a normalised stimulus intensity to a 1 mV threshold. Once again, this result is largely unsurprising given that COG corresponds with an area of high excitability of corticomotor neurons and thus is closely associated with the optimal stimulus site of the muscle being mapped (Thickbroom et al., 1999; Wilson et al., 1993).

In a similar study to ours, Stephani et al. (2016) assessed the effect of current direction and pulse type using monophasic (rather than half-sine) and biphasic pulses. In their investigation, they found that current direction had an effect on COG, which is in contrast to the current study. A reason for the difference between these two studies could be that the present study utilises neuronavigation to aid both hotspot determination and the mapping procedure. As previously mentioned, studies have shown that effective neuronavigation has the propensity to enhance the reliability of COG measures (Miranda et al., 1997). Furthermore, the use of neuronavigation has a secondary effect of allowing the user to possibly produce higher resolution maps than traditional methods involving skull caps may allow. Using neuronavigation allows the technique by van de Ruit et al. (2015) to be used, thus numerous separate locations on the cortex can be stimulated, without the need for multiple stimuli at the same location. This means that MEP values can be obtained for multiple sites within the pre-defined grid area, as opposed to the 'fixed' sites utilised by Stephani and colleagues. Perhaps the higher resolution of the maps is improved by the stimulation of many loci, as seen in this investigation and van de Ruit et al., 2015, and therefore in combination with the enhanced accuracy associated with utilising

neuronavigation, a more reliable COG can be found, when compared with the methods used by Stephani et al. (2016).

Moreover, the present investigation assessed COG by obtaining the mean resultant COG. This was not the case with Stephani et al. (2016) where the COG location in both the x- and y-axes were assessed separately. Indeed, the investigators only found a difference in COG location in the y-axis. Perhaps if a similar approach was taken in this investigation, differences in COG across the different axes may have been elucidated, but as previously mentioned, only larger changes observed by alterations in the mean resultant COG would be of relevance. However, it should be noted that Stephani et al. (2016) only found this difference as a result of current direction when utilising the full matrix of stimuli locations, when assessing the 37 inner locations no differences in COG were found, corroborating the present investigation Although, it could be suggested that the utilisation of resultant COG in these investigations may hide small, but important changes as a result of changing various mapping techniques. Indeed, future research could assess the efficacy of utilising resultant COG in a practical context, when compared to assessing several COG values. It is our belief that assessing resultant COGs rather than several values will facilitate usage in a practical context, in clinical and academic settings.

An important difference in this study is that the stimulation intensities utilised were higher than in *Experiment 1*, in order to achieve a 1mV response. This meant that differences between various stimuli locations became increasingly clear when confirming the position of the hotspot prior to mapping (even though this position was altered between current directions). The location of the hotspot then determined the position of the map for each individual, with the centre of the map corresponding to the identified hotspot location. This method of confirming the location of the hotspot, alongside the fact that the mapping protocol used in this investigation is capable of producing high resolution maps (van de Ruit et al., 2015), it is likely that the identified hotspot location and therefore the position of a motor map that yields the

highest amplitude weighted response to stimulation (Wasserman et al., 1992) should correspond fairly well. Therefore, accurate hotspot determination, high resolution maps, the use of resultant COG and the fact that pulses are administered along the same cortical axis in both current directions in *Experiment 2*, it could be suggested that COG is unlikely to change for the FDI when changing current direction.

The findings that current direction has little influence over COG and map area when the stimulation intensity is adjusted accordingly does have some important implications relating to common practice of TMS. As previously discussed, current direction is an extremely important factor to consider in TMS practice, particularly for mapping, as ascertained by the influence upon MEPpp even with small deviations from the optimal current direction (Brasil-Neto et al., 1992b; Werhahn et al., 1994; Kammer et al., 2001). Nonetheless, one could suggest that with appropriate threshold determination, many of the potential issues related to current direction can be largely negated. As it has been shown in this investigation, by adjusting the stimulation intensity in accordance with the current direction, the potential alterations to a map that may be elicited (map area and COG) can be minimised, even though the method used has scope for improvement. Perhaps then a pragmatic future for TMS mapping could lie in the rapid acquisition of SR curves pioneered by Mathias et al. (2014) in conjunction with the mapping techniques applied in this investigation. This would elicit a scenario in which an appropriate threshold can be ascertained and maps can be produced in a short amount of time, therefore negating many of the issues that have previously blighted TMS mapping, including attention (Rossini et al., 1991; Conte et al., 2007), time of day (Sale et al., 2007) and cortisol levels (Sale et al., 2008).

4.1.2.3 Effect of Current Direction on AR

Experiment 1 showed that current direction had little influence on the shape of the map, as indicated by the map's aspect ratio. This lack of statistical significance could be seen as a fairly surprising result, given that it has been reported that map shape mirrors the pattern of induced current flow along the axis of the coil (Wilson et al., 1993; Malcolm et al., 2006). Perhaps it could be perceived that there is unlikely to be a difference between AP and PA directed currents given that these two are simply the reverse initial directions of each other, and ultimately stimulate along the same axis. Nonetheless, one may expect the ML directed currents would produce maps of a different shape. Whilst statistical significance is not reached in this investigation, there were cases where the ellipses in the ML oriented maps were rotated by roughly 90^{o} to the axes observed in both the PA and AP maps. However, this was not always the case. The lack of a difference can thus be possibly explained by two factors.

Firstly, a number of the maps produced (particularly during rest) in the ML current direction were fairly small, and thus the shape were near-circular (giving an AR of 1), or alternatively an AR could not be produced. Perhaps in these circumstances, had a higher stimulation intensity been used (such as 120% of RMT in ML direction), then the influence of current direction on map shape may be more easily elucidated.

Secondly, from briefly assessing the box plots seen in *Figure 20*, it can be deduced that looking solely at the statistical differences between the means maybe inappropriate. One can see that across both activity levels there is substantial variation around the mean in the ARs obtained. These substantial variations could perhaps be masking any potential differences in map shape, particularly in the ML current direction. It is perhaps surprising that there is such marked variation in the AP current direction that this is not mirrored in the PA direction. Perhaps this variance could be as a result of an insufficiently high stimulation intensity to produce reliable aspect ratios in some individuals. Alternatively, the aforementioned effects of individual

cerebral architecture (Balslev et al., 2007), could have an influence here as some individuals may not have received optimum currents for eliciting responses in that particular current direction producing small maps (and thus unreliable ARs), whereas others did, producing larger maps with more reliable ARs.

4.1.3 The Effect of Pulse Type on Mapping Parameters

The fact that pulse type was found to have an effect on COG and map area in both *Experiment 2* and *3*, may be surprising given that efforts were made to normalise the stimulation intensity to a 1mV threshold in both pulse types. The possible reasons for an effect of pulse type on COG and map area are explored below.

Suitability of a 1mV Threshold

The first area to be explored should be the suitability of utilising a '1mV threshold' as the basis for setting the stimulation intensity. Previous unpublished research within the laboratory (Wright, van de Ruit and Grey) suggests that setting a stimulation intensity to achieve a 1mV is not recommended, most notably as the absolute MEP_{pp} elicited varies substantially, even with a given stimulation intensity. Indeed, Pitcher et al. (2015) reported that using the 1mV method consistently underestimates the I_{50} (i.e. the % of maximal stimulator output at which the MEP_{pp} is halfway between the maximum and minimum MEP_{pp}) and thus is could be seen as ineffective. Perhaps, a more reliable approach would be to perform an SR curve (i.e. perform numerous, pseudorandomised) stimulations of varying intensity prior to TMS mapping, and thus the I_{50} and appropriate stimulation intensity can be ascertained. However, performing a full SR curve greatly increase the time to perform TMS mapping study, thus potentially negating efforts to reduce acquisition time (Mathias et al., 2014; van de Ruit et al., 2015). Furthermore, difficulties were faced when attempting to find the 1mV threshold for half-sine measurements. When the PowerMAG stimulator is in 'half-sine' mode, the current direction is reversed every pulse. This presents a difficulty when determining the 1mV threshold both in practical terms as the current direction had to be accounted for, as well as any possible cross over effects between the two current directions. Once again it may be appropriate to suggest that using the SR curve method of determining stimulator intensity and threshold to produce more robust and reliable data, particularly in terms of map area.

The influence of Current Spread

Alternatively, this alteration in map size with pulse type could be attributed to current spread. Indeed, Thickbroom et al. (1999) stated that the surface area of TMS maps is greatly influenced by current spread, and in experiments where stimulation intensity is altered between conditions current spread is potentially a major mediating factor for any changes in map size (van de Ruit et al., 2016). Again, there was an effort to negate the effect of current spread by normalising the stimulus intensity to a 1mV threshold, nonetheless current spread was likely still a factor in this investigation. Indeed, this can be seen in the results with larger maps seen when maps are elicited with biphasic pulses as opposed to half-sine pulses. It has been regularly cited that the biphasic pulses are longer in duration than half-sine pulses (Sommer et al., 2006), and if this is the case, it could be argued that the excitable area of the cortex could be extended to loci that may not be excited by shorter stimuli. For instance, the longer duration biphasic pulse may excite an area of the cortex distant to the location of the hotspot, whereas this may not be the case when stimuli with shorter durations are used. Moreover, biphasic TMS is reportedly less focal than its half-sine or monophasic counterparts (Brasil-Neto et al., 1992b). Therefore, it could be argued that through facilitating connections between neurons, the extent of the cortex excited by the cortex maybe increased, even if only a small area of the cortex is truly effectively excited by a given TMS input (Wagner et al., 2009) (See *Figure 38)*.

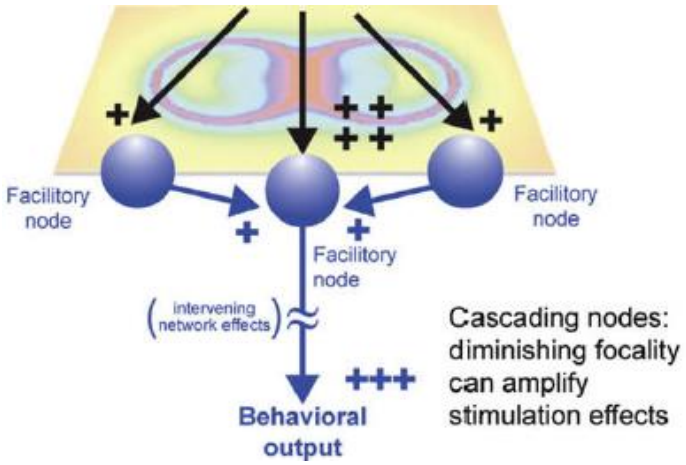

Figure 38. Adapted from Wagner et al., 2009. A situation where a stimulus of limited focality may artificially enhance the amplitude of a given response, where in fact the true excitability of the area is less pronounced. With the reduced focality of a biphasic pulse, the area of a map may be extended beyond the true limits of excitability if this phenomenon repeatedly occurs during map acquisition.

Perhaps then it could be argued that when performing mapping protocols, half-sine stimuli could be seen as preferential thanks to their increased focality reduced propensity to induce current spread. This reduced current spread diminishes the likelihood of providing stimuli to neurons with facilitatory inputs to the final descending volley, but do not reflect the underlying excitability of the motor cortex.

Characteristics and Efficacy of Pulse Types

Sommer et al. (2006) assessed the effect of waveform (biphasic, half-sine and monophasic) on thresholds and input/output curves. It was shown that the effect of the half-sine pulses on these parameters lay somewhere in between that of monophasic and biphasic waveforms – higher stimulation intensities were required to reach a threshold when comparing half-sine to biphasic waveforms, however the difference was not as marked as between monophasic and biphasic pulses. The asymmetrical monophasic waveform is substantially different to that of a biphasic pulse, however half-sine waveforms differ to biphasic in that they lack the $3^{rd}/4^{th}$ quarter cycle. Thus, there could be two reasons as to why threshold may be lower in biphasic pulses when compared to half-sine pulses. Firstly, the $3^{rd}/4^{th}$ quarter of the cycle present in the biphasic pulse acts to lower the motor threshold. Secondly, the longer duration of a biphasic pulse may be more effectively at depolarising cortical neurons and thus generating responses (Sommer et al., 2006). The lowering of the motor threshold should be counteracted by the alteration in stimulation intensity between pulse types. However, the logic that the longer duration of the biphasic pulse more effectively activates cortical neurons can also be applied to mapping, as in both instances a more effective pulse could potentially extend the stimulation locations that are excitable to a biphasic pulse further along the surface of the cortex, where a less effective pulse would not produce a response. This logic provides a possible explanation for the difference in map area as a result of pulse type.

In terms of COG, it has been shown (Brasil-Neto et al., 1992b) that for high intensity biphasic pulses the location of the optimal MEP amplitude (which could be extended to COG position) had less marked directional specificity, with high amplitude MEPs elicited in the opposite (180^{o}) current direction. Perhaps then, it could be suggested that for monophasic or half-sine, the stimuli provided are more focal (Brasil-Neto et al., 1992b) and are potentially more influenced by characteristics associated with variance in MEP amplitude, such as coil angle, coil position and current direction. Therefore, when mapping with half-sine or monophasic waveforms, the enhanced susceptibility to alterations of the coil may alter the position of the COG more greatly when compared to biphasic waveforms. Therefore, one could say that the COG position is fairly robust when utilising a biphasic waveform, but perhaps is less reliable when using other TMS waveforms. This is corroborated by the fact that the half-sine condition produced mean resultant COGs with greater variance around the mean, when compared to biphasic. The magnitude of this variance is similar to that seen in previous studies where differences in COG were not detected when comparing pulse types (Stephani et al., 2016).

However, one should note that the differences in mean resultant COG position are not substantial (roughly 2.5mm in the AP orientation and 3.5mm in the PA orientation), and are similar to the values seen in previous investigations assessing the reliability of COG as a measure (Miranda et al., 1997; Weiss et al., 2012). Furthermore, the values seen in this investigation (particularly using half-sine stimuli) should be taken with caution, given that the expectation-maximisation function (EM) on SPSS was utilised to account for missing data. Perhaps the utilisation of EM may have had an influence upon the results of this experiment. Perhaps the true variance of COG may have been masked by the fact values were added using EM. For instance, the alterations in COG may have been overestimated as a result of utilising the EM. Perhaps, if all the values were successfully obtained experimentally, different results may have been detected. However, EM was used as it is a powerful way of replacing missing data, particularly in a small sample size.

Preferential Activation of Different Cortical Neurons

A further possible explanation for the differences in COG with pulse type could be linked with the suggestion that different structures and sites within the motor cortex maybe activated with different pulse waveforms (Di Lazzaro et al., 2001b; Sommer et al., 2006). Di Lazzaro and colleagues (2001b) showed that different pulse types (biphasic and monophasic) could produce different iterations of descending volleys, in accordance with the initial current direction. Therefore, it could be suggested that waveforms may activate different sites of cortical neurons preferentially when compared to one another. Considering that the COG is associated with an area of high excitability of corticomotor neurons (Thickbroom et al., 1999; Wilson et al., 1993), perhaps then a shift in the optimal stimulus site according to the waveform used may induce a slight alteration in the location of the COG. Therefore, one could suggest that these data suggesting that COG is affected by pulse type.

4.1.4 The Interactions between Current Direction and Pulse Type on Mapping Parameters

Finally, it is important to assess the how both pulse type and current direction may interact to have an influence upon map area. The finding that current direction had an influence upon map area with an unchanged stimulation intensity (*Experiment 1* and *2*) is not surprising. The difference in *Experiment 2* investigation is that we used two different waveforms in our assessment: biphasic and half-sine. This means that it is important to assess the influence of the interaction between pulse type and current direction on map area. The characteristics of each of the pulse types lend themselves to having a more physiologically relevant stimulus at different phases of the pulse type. Indeed, the half-sine pulse behaves like a monophasic pulse and has its most physiologically relevant phase in one direction (i.e. the same direction that the coil is held) (Sommer et al., 2006). Conversely, with biphasic pulses, it is argued that the fact that the reversal phase has a considerably larger amplitude and duration, this means that the reversal phase is more physiologically relevant – the direction of which varies according to manufacturer (Di Lazzaro et al., 2001b; Cowey et al., 2005; Sommer et al., 2006; Groppa et al., 2012). Kammer et al. (2001) show results that corroborate with this idea, that when the most relevant phase of the corresponding pulse type flows in a PA direction in the cortex, then the thresholds elicited are lower than in the opposite direction. Across both waveforms in *Experiment 2*, the maps were larger when the physiologically most relevant phase of the stimulus was oriented in a PA direction. Therefore, it is important to assess the effect of current direction and pulse type together, as they may both interact to alter map area to a greater extent than either in isolation.

Furthermore, the idea that different structures may be activated by different waveforms and current directions maybe an appropriate avenue of exploration when assessing map area. Several epidural studies have assessed the influence of current direction and pulse type on the

induced descending volleys, and a complex picture is painted. It has been suggested that PA directed monophasic pulses induce early I-waves (I_1) (Di Lazzaro et al., 1998a; Salvador et al., 2011), whereas monophasic pulses in the opposite current direction produce both later I-waves (I_2 and I_3) and some D-waves (Di Lazzaro et al., 2001b). This pattern of recruitment is mirrored by PA (early I-waves) and AP (late I-waves/D-waves) directed biphasic pulses respectively. Possibly then these complex patterns of recruitment have an influence upon map area, with early I-waves potentially being more conducive to producing larger maps. Epidural studies focusing on the half-sine pulse type and patterns of recruitment have not been performed to the same capacity, and requires further study into the matter.

In terms of the significant interaction observed in *Experiment 3* between current direction and pulse type upon both map area the potential influence of current spread cannot be ignored. In this investigation, higher intensities were usually utilised to achieve the 1mV threshold in both the AP orientation. In these cases, it would not be unreasonable to expect that when solely looking at the influence of current direction upon map area, the higher intensities utilised to obtain a 1 mV threshold in the AP orientation, may increase the level of current spread during mapping in this orientation. In reality it could be the case that if current spread could be negated, there would be a difference in map area between the PA and AP current directions. Furthermore, in combination with this the reportedly greater duration of a biphasic pulse when compared to a half-sine pulse (Di Lazzaro et al., 2001a; Sommer et al., 2006) the current spread may be exacerbated when comparing pulse types. Therefore, it is the combination of both current direction and pulse type that may reveal differences in map area, rather than focussing on current direction alone.

In terms of the somewhat surprising finding of an interaction effect between pulse type and current direction on COG, a different possible explanation must be provided. Brasil-Neto et al. (1992b) demonstrated that the position of the COG (location of optimal MEP amplitude) had reduced directional specificity when utilising high intensity *biphasic* pulses. In their

experiment, high amplitude MEPs were elicited in similar locations whether the coil was oriented normally (PA) or rotated by 180^{o} (AP). One could suggest that as high intensity biphasic pulses that were utilised in the current investigation to reach the 1 mV threshold, reduced directional specificity of the COG may have occurred. The relative stability of the COG location during *biphasic* pulses across both current directions contributed to the lack of main effect as a result of current direction. However, in the case of the interaction, one could suggest the addition of half-sine pulses into the equation, where no such reduced directional specificity or stability at high intensities has been reported, to have an influence upon the COG location. Perhaps the reportedly more focal aspect of half-sine and monophasic pulses (Brasil-Neto et al., 1992b) may enhance the susceptibility for the location of the COG to change with alterations in current direction. Therefore, when solely assessing pulse type and current direction, alteration in COG location may be missed, but when assessing both factor simultaneously, changes to mapping outcomes may be elucidated.

4.1.5 The Influence of Muscle Activity on Mapping Parameters

Effect of Muscle Activity on Map Area

The results from *Experiment 1b* indicate that muscle activity has an influence upon both map area, when stimulations are given at a constant intensity. Indeed, maps in all orientations were considerably larger in the active condition than in the resting condition. These results are not surprising and follow in-line with our hypotheses, but there are multiple possible mechanisms for this change which should be explored.

Firstly, it can be suggested that the differences in map area between resting and active conditions are unlikely to be due to alterations in current spread (Thickbroom et al., 1999). Indeed, as the same stimulation intensity was used for all conditions, the magnitude of current spread should be consistent and thus the current spread over the cortex is unlikely to change significantly throughout the conditions. Indeed, in experiments where stimulation intensity was changed, the primary factor for changes in map area was cited to be increased current spread with increased stimulation intensity (van de Ruit et al., 2016).

Nonetheless, another important factor elucidated by van de Ruit and colleagues (2016) is the fact that active muscles have been shown to require a lower stimulation intensity to reach the motor threshold than resting muscles. Although the exact figure is not known, Ngomo et al. (2012) suggested that the stimulation intensity required to reach the motor threshold is reduced by around 8%. Indeed, in their investigation, after adjusting the stimulation intensity between active and resting conditions no differences were detected in MEP amplitudes or map area. This argument is compelling and represents the most likely reasoning for the increased map areas observed with muscle activation in the present experiment. However, as the changes across the

orientations are not consistent in magnitude (i.e. ML area increases far more than PA area), other explanations must be explored.

Another well-documented reason for changes in MEP amplitude and map size with active versus resting muscles is alterations in corticospinal excitability (Wilson et al., 1993; Mazzocchio et al., 1994, Ugawa et al., 1995; Di Lazzaro et al., 1999). The exact location for the increased excitability is debated. Early investigations suggested that increased spinal motorneuron excitability during muscle activation lowers motorneuron thresholds, thus increasing the likelihood that both the motorneurons will discharge upon the arrival of a descending volley (Thompson et al., 1991a) and target muscle will be activated (Wilson et al., 1993). Nonetheless, an investigation by Mazzocchio et al. (1994) showed that the threshold to produce H-reflex facilitation was lowered by tonic contractions (by around 6% of stimulus intensity), and proposed that a voluntary contraction increased cortical excitability when compared with resting conditions, producing larger corticospinal volleys. As evidenced from the literature, it is difficult to deduce the exact location of increased excitability as a result of tonic muscle contraction during TMS. However, this experiment does perhaps add credence to the notion that voluntary contraction may increase the size and number of descending volleys, as evidenced by the increased map areas observed in this investigation (Di Lazzaro et al. 1998c).

Perhaps then, the important of this part of the investigation lies not only in the fact that map area increased, but perhaps that they were more stable. One could suggest that by utilising the ability of the participant to perform a small muscle contraction during data collection may lead to maps being produced with stable characteristics. For example, as previously mentioned, some data was missing in resting conditions because there were insufficient MEPs to produce a map. With even a small voluntary contraction, this issue was largely negated. Further, as a result of the increased probability that descending volleys will activate a target muscle (Thompson et al. 1991a), it has been suggested that using facilitated muscles the map area achieved during mapping protocols may better demonstrate the *full* extent of the excitable area

of the muscle of interest (Wilson et al. 1993). This should be taken with some caution though, as there may be circumstances, particularly in stroke research where the participant may be unable to perform at the intensity outlined in this investigation or maintain a voluntary contraction for as long as is necessary to produce a map. Nonetheless, in mapping research in healthy participants, it can be suggested that performing tonic contractions during the mapping protocol may produce maps of a higher quality.

Effect of Muscle Activity on AR

The results from *Experiment 1b* showed that muscle activity influenced the shape (AR) of the map when comparing the FDI in a resting and active state. This is once again a somewhat surprising result, given the lack of alterations in map shape as a consequence of current direction. Arguably, map shape should be less influenced by muscle activity than by current direction, given that reports that map shape mirrors current flow along the axis of the coil (Wilson et al., 1993; Malcolm et al., 2006). Moreover, an investigation by van de Ruit et al. (2016) showed that across several different levels of muscle activation, a motor map scales with muscle intensity. The implication made by this study is that cortical neurons are equally excitable along the perimeter of the muscle's cortical representation.

Indeed, from this investigation, this notion must not be dismissed, and an explanation for activity influencing map shape can be provided. When assessing the post-hoc pairwise-comparisons, it is seen that there is only a significant difference in the ML current direction when comparing the muscle in its active and resting states. As previously mentioned, few MEPs were reliably produced in this current direction, so the maps were either small, thus producing near-circular ellipses (thus giving an AR of 1), or no maps were produced, so the expectation-maximisation function was used. This issue was not so marked with active data, as there were more viable MEPs as a result of the activity, thus producing more and larger maps. Here perhaps, when the muscle was in its resting state, maps produced in the ML current direction

had a tendency to have a shape mirroring the current flow along the axis of the coil (Wilson et al., 1993; Malcolm et al., 2006), but this result was hidden by the relatively small number of maps that were successfully produced in comparison remaining current directions. In contrast, the maps in the ML current direction produced during the *active* condition were had large enough areas to allow detection of a lateral alteration in current direction, thus producing a significant result for activity, whereas in reality, current direction is the most probable cause of the change in map shape. This perhaps adds credence to the notion addressed in the *Effect of Muscle Activity on Map Area* that maps produced during tonic contraction may be of a higher quality and more accurately reflect the excitable area of interest (Wilson et al., 1993). Therefore, in future, it may be pertinent to perform mapping studies with tonic contraction.

Effect of Muscle Activity on COG

There has been fairly limited study into the influence of activity upon COG location, but early studies by Wilson and colleagues (1993) showed that activity may have an effect upon the COG, but had fairly small sample sizes and did not use neuronavigation. Indeed, more recent studies (Gagné et al., 2011; Ngomo et al., 2012) revealed no alterations in COG location between active and resting conditions, mirroring the present study.

The lack of alterations observed within resultant COG across the three tested current directions and two activity levels in *Experiment 1*, adds further support to previous literature (Uy et al., 2002; Wolf et al., 2004; Malcolm et al., 2006, Ngomo et al., 2012) that COG could be a suitable parameter to use in future clinical and research settings.

4.1.6 Summary and Impact Upon Future TMS Practice

The three investigations performed within this thesis have sought to determine some of the factors that will have an influence upon TMS mapping outcomes. The major findings are primarily associated with map area and COG. We have determined that map area is susceptible to alterations in current direction, pulse type and muscle activity. Furthermore, this investigation has shown that, in line with previous literature, COG is a reliable measure, with little change in resultant COG with changes in current direction, pulse type and muscle activity.

In terms of current direction, the methodology used in this investigation is fairly extreme, with current directions either differing by 90^{o} from each other (i.e. ML vs PA/AP), or by 180^{o} (PA vs AP). It is therefore unlikely that TMS in the hands of a well-trained user would be performed with such 'inappropriate' coil orientations. Nonetheless, the findings from these investigations do raise some important questions. Most notably, previous studies (e.g. Brasil-Neto et al., 1992b; Kammer et al., 2001) have shown that changes in coil orientation during TMS can have a dramatic influence upon the results. For instance, if the user was a few degrees out from the optimal angle of stimulation, many of the elements mentioned in this thesis would apply, but most notably the preferential activation of different cortical neurons in accordance with current direction. In terms of pulse type, it has been shown that it would be inappropriate to compare the different pulse types, without accounting for stimulation intensity.

Therefore, the most important factor to consider whilst performing TMS mapping in a practical setting is elucidating an accurate threshold in order to set an appropriate stimulation intensity. Whilst notoriously difficult to do, the most pragmatic approach would likely be to perform an SR curve prior to mapping protocols, similar to that of Mathias et al. (2014). This would act to negate the potential effects of current direction (alongside neuronavigation) and may allow comparisons to be made between investigations where different pulse types are used.

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Appendices

Appendix A – Participant Consent Form

INFORMED CONSENT

Participant Identification for this study:

The Effect of Pulse Type, Current Direction and Muscle Activity on TMS Mapping

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

Appendix B – Edinburgh Handedness Inventory (Oldfield, 1971)

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. *Neuropsycholoigia*, *9*, 97-113.

Appendix C – Modified TMS Adult Safety Screen (Keel et al., 2001)

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

CIRCLE or CROSS OUT

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

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

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

† For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.

* Modified TASS based on Keel JC, July 2000.