The Influence of Area 5 on the Excitation of Primary Motor Cortex

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Using functional magnetic resonance imaging in humans, Brodmann's area 5 (BA5) is observed to be activated during the suppression of motor output in the context of a NO-GO task. In monkeys, BA5 is associated with somatosensation and specifically linked with motor preparation. The goal of this thesis is to investigate BA5 influences on corticospinal excitability prior to the onset of movement, in the context of a GO/NO-GO paradigm. To achieve this goal, paired-pulse TMS is used to probe the functional connectivity between BA5 and ipsilateral primary motor cortex (M1) for a muscle specific to the hand. Three experiments are performed that investigate the differences in corticospinal output to the hand in a GO task versus a NO-GO task and the stimulation parameters that reveal such differences. Results indicate that BA5 is able to condition M1 prior to movement in a task-specific manner. Further, motor evoked potentials (MEPs) are suppressed in the context of a NO-GO task relative to a GO task, and task-specific differences rely on the intensity and direction of induced current in the cortex. In conclusion, data from this thesis contribute to our understanding of the role of BA5 in motor control.

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Tanner Mackenzie

"Embrace the variability"
-Dr. Leo Cohen, 2015

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LIST OF ABBREVIATIONS

aIPS	Anterior Intraparietal Sulcus
ANOVA	Analysis of Variance
BA5	Brodmann's Area 5
BA7	Brodmann's Area 7
cIPS	Caudal Intraparietal Sulcus
CS	Conditioning Stimulus
CNS	Central Nervous System
CSN	Corticospinal Neuron
D-wave	Direct Wave
EMG	Electromyography
FDI	First Dorsal Interosseous
IPL	Inferior Parietal Lobule
ISI	Interstimulus Interval
I-wave	Indirect Wave
LP	Lateral Posterior
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MIP	Medial Intraparietal Area
MSO	Maximum Stimulator Output
MT	Motor Threshold
PMC	Premotor Cortex
PMd	Dorsal Premotor Cortex
PMv	Ventral Premotor Cortex
PPC	Posterior Parietal Cortex
PPL	Posterior Parietal Lobe
PTN	Pyramidal Tract Neuron
RMT	Resting Motor Threshold
SI	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SMA	Supplementary Motor Area
SLF	Superior Longitudinal Fasciculus
SPL	Superior Parietal Lobule
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TS	Test Stimulus
μV	Microvolts

Chapter 1: Introduction

1.1 Overview of thesis

This thesis investigates the functional connectivity between Brodmann's area 5 (BA5) and the primary motor cortex (M1). The initial interpretation of BA5 as a solely somesthetic associative parietal region has been recently reexamined in vivo in non-human primates (Kalaska et al., 1997; Scott et al., 1997). It is now widely accepted that BA5 has a role in both motor and sensory functions, and serves a role in sensorimotor integration (Kalaska & Crammond, 1995). This knowledge allows for investigations into specific sensorimotor functionality in terms of BA5 connectivity with M1. Investigation of this interplay is accomplished using non-invasive Transcranial magnetic stimulation (TMS) in healthy young adults. The specific stimulation technique used combines two pulses of TMS, one over BA5 and the second over M1, and is known as paired-pulse TMS. How the first conditioning TMS stimulus (CS) over BA5 affects the output from M1 following the second, or test, stimulus (TS) allows the determination of BA5 causal role in modulating M1 excitability prior to movement.

1.2 Goals of the thesis

The aim of my thesis is to expand our understanding of the functional connectivity between Brodmann's area 5 (BA5) and the primary motor cortex (M1) in humans. In humans, the BA5-M1 relationship has been assessed at rest and also during the processing of somatosensory input (Ziluk et al., 2010). The control that BA5 exerts on M1 prior to movement has never been examined. I investigate whether BA5 differentially influences M1 excitability prior to movement in GO and NO-GO tasks, and the mechanisms that mediate changes in excitability.

In order to accomplish the goal of my thesis, I performed three experiments, each of which provided a novel contribution to the present understanding of the role of BA5 in humans. The effects of GO and NO-GO tasks on modulation of M1 excitability were tested in thirty-nine participants. The interstimulus interval (ISI) between stimulation of BA5 and M1 was set to 8 ms. Data was also collected for a REST condition, whereby subjects were stimulated with paired-pulse TMS outside the context of any task, to gauge BA5-M1 functional connectivity at baseline. Experiment 1 examined the effect of BA5 conditioning on M1 excitability prior to movement. In Experiment 2, the impact of strength of BA5 conditioning on M1 excitability was investigated across a spectrum of CS intensities ranging from 50 to 130% of resting motor threshold (RMT). In Experiment 3, the mechanisms which govern the BA5-M1 interaction during a GO and NO-GO task were explored by altering the coil orientation to recruit different populations of targeted neurons. Together, the experiments in my thesis represent a comprehensive investigation into the task-specific BA5 functional connectivity with ipsilateral M1 that exists prior to the onset of movement.

1.3 Significance of research

This thesis builds upon a growing number of paired-pulse TMS investigations into functional connectivity between associative regions and M1. This particular TMS technique has been used for the study of both intra- and intercortical relationships because of its ability to establish causal effects of one cortical area on another. It provides information on the nature of the functional connectivity between non-motor and motor regions, at high temporal resolution and fair spatial resolution given the focality of stimulation.

Although much of the neural activity involved in the modulation of motor responses has been localized and even associated with behaviour (Binkofski et al., 1999; Tunik et al., 2008), how BA5 affects motor cortical output during movement planning has not been investigated in humans. This is important to study, because with M1 as the cortical origin for descending motor commands, any associative area that modulates its output could be of therapeutic or diagnostic value. In stroke patients, TMS investigations have demonstrated that impaired hand function is related to activity within both ipsi- and contralesional dorsal premotor (PMd) cortices (Fridman et al., 2004; Lotze et al., 2006). Should a role for BA5 in regulating motor output prior to movement be established, a next logical step might be to examine whether BA5 activity is related to impaired function following stroke. Damage to the posterior parietal cortex through stroke or neurodegenerative diseases such as Parkinson's can cause apraxia, a deficit in motor planning. Tools for the diagnosis of apraxia currently involve qualitative measures and have been found not to correlate strongly to the patient's ability to perform activities of daily living (ADL) in a real-life setting (Vanbellingen & Bohlhalter, 2011). Assessment of BA5-M1 functional connectivity prior to movement could represent a quantitative analysis of neurophysiology useful for the diagnosis of apraxia.

This thesis has relevance to the field of motor control. Results from a historic study employing an anticipatory response inhibition (ARI) task demonstrated that reliable response inhibition is temporally sensitive. A response can no longer be inhibited when the instruction to inhibit is given at an interval too close to the response time (Slater-Hammel, 1960). These findings indicate that at some point prior to movement, a signal to move is issued which cannot be revoked, even in the presence of visual stimuli which indicate that the movement should be withheld. This thesis studies BA5 contribution to M1 excitability prior to movement using a GO/NO-GO paradigm which requires correct response inhibition. Results from the current investigations could provide a physiological basis for

previous behavioural findings related to motor control.

Novel to this thesis is the context in which the functional relationship between BA5 and M1 is being tested in humans. Paired-pulse TMS is utilized to condition M1 output with a stimulus over BA5. Results from this thesis will help establish how BA5 is involved in movement preparation. The tested relationship will be temporally specific on two levels, both with respect to the timing between CS & TS and with respect to the timing of the TS relative to the delivery of the cue to select a movement.

Chapter 2: Literature Review

2.1 Area 5

2.1.1 Area 5 in monkeys and humans

Associative areas of parietal cortex are priority targets for investigation with respect to their role in movement, with area 5 being amongst the most critical to examine. The only species which possess this area are humans and few nonhuman primates capable of dexterous hand movements including Cebus and macaque monkeys (Padberg et al., 2007). Within studied monkeys of genus Cebus, area 5 response properties demonstrated a dominance of hand and forelimb representation. Representation of the forelimb and hand was found in a mosaic somatotopic organization, similar to that in primary motor cortex (M1), promoting the thinking that this area is a primate extension of M1 (Padberg et al., 2007). This area evolved separately in both New and Old World monkeys, as it was not present in their common simian ancestor. The posterior parietal lobe, including area 5, reached its current size in hominids about 5 million years ago in genus Australopithecus, who sacrificed visual performance for associative planning compared to chimps of approximately the same size and brain size (Hyvarinen, 1982). Brodmann's anatomical definitions for humans states that its position in the cortex is fairly constant. Human BA5 runs from the caudal end of the paracentral lobule (PCL) to a position posterior to the superior postcentral sulcus (Brodmann & Gary, 2006). In lower monkeys, this area is homologous structurally to human BA5, but makes up the entire superior parietal lobule (SPL) and assumes a much greater proportion of the PCL (Brodmann & Gary, 2006).

Area 5 receives major afferent projections from the lateral posterior (LP) thalamic nucleus as

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well as other thalamic nuclei (Hyvarinen, 1982). Primary motor and sensory cortices, as well as premotor cortex (PMC) project to ipsilateral area 5, with motor cortex also projecting to contralateral area 5 (Hyvarinen, 1982). Furthermore, area 5 and nearby area 7 have reciprocal interhemispheric connections with one another. Indeed, much of area 5's connectivity is reciprocal, as it projects to ipsilateral SI and PMC as well as contralateral M1 and select thalamic nuclei including LP (Hyvarinen, 1982). Furthermore, study of macaque brain physiology has shown direct corticocortical connections between area 5 and ipsilateral M1 (Strick & Preston, 1978). Labelling techniques using horseradish peroxidase in rhesus monkeys demonstrated through retrograde transport that area 5 has direct access to arm representation in M1 (Strick & Kim, 1978). The corticocortical connectivity of area 5 is illustrated in Figure 1.

Reciprocal connectivity amongst these areas means that they are able to pass information back and forth directly. From this type of connectivity, rather than one-way transit of information, it might be intuited that these areas operate in parallel in the processing of information they receive, exchanging neural data back and forth. This parallel activation of movement-related cortical areas has been demonstrated experimentally, with parietal and frontal areas involved in the dorsal visual stream active simultaneously during reaching movements (Naranjo et al., 2007).

2.1.2 Area 5 role in movement in monkeys and humans

Area 5 reciprocal connectivity with PMC hints at its involvement in movement planning. In the macaque monkey, area 5 is involved in motor preparation, preshaping the hand prior to prehension and generating body-centered coordinates for reaching (Padberg et al., 2007). Approximately 60% of cutaneous neurons within area 5 are directionally sensitive when compared with
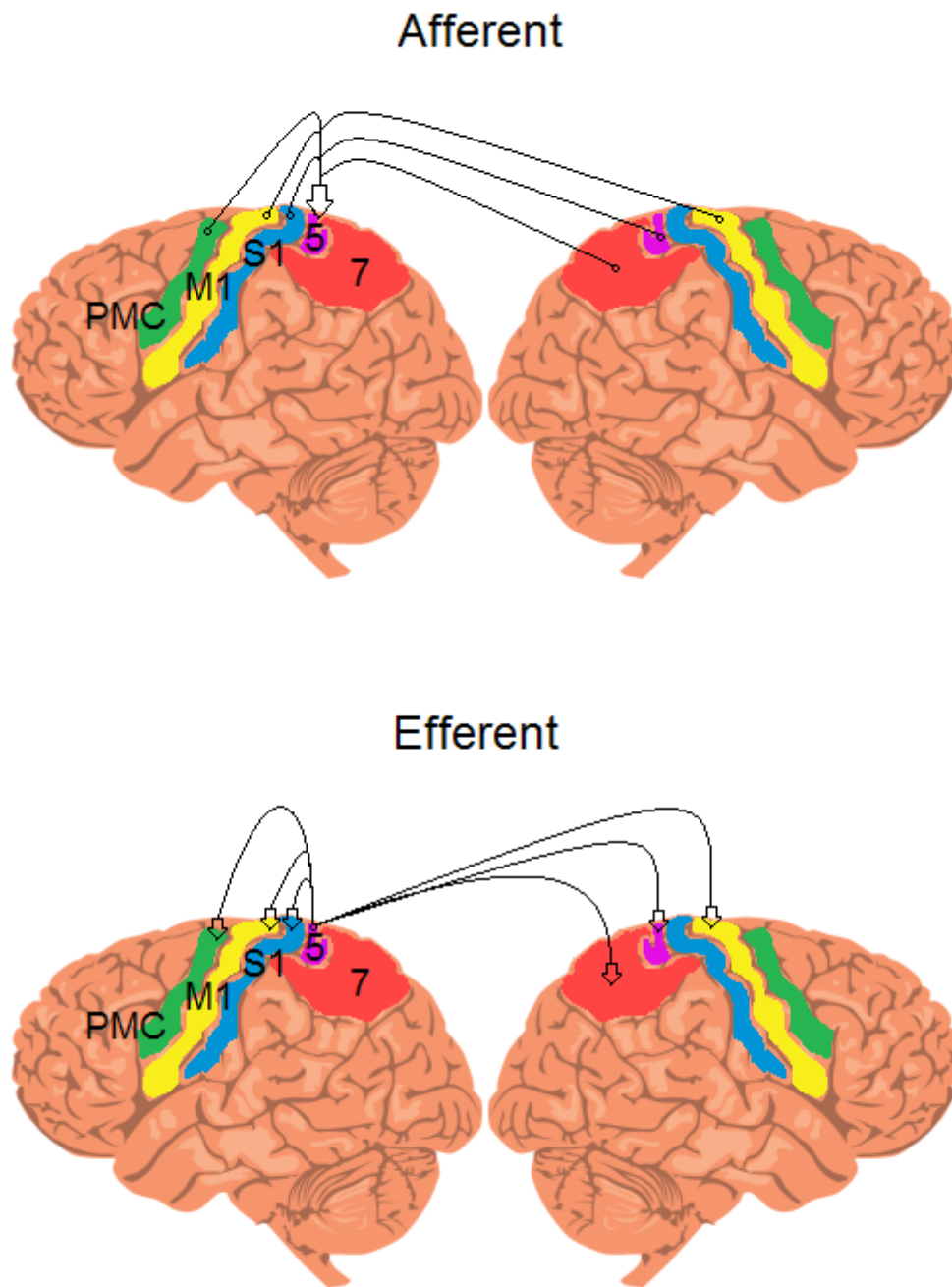
***Figure 1* - Afferent and efferent connectivity in area 5 in humans. PMC** - Premotor cortex; **M1** - Primary motor cortex; **S1** - Primary somatosensory cortex; **5** – Brodmann's area 5; **7** – Brodmann's area 7

5% of SI neurons. These neurons are also far more active during voluntary movement compared to passive movement in monkeys (Mountcastle et al., 1975). Described by Mountcastle et al. are two classes of neurons in area 5, dubbed arm-projection and hand-manipulation neurons. The former were inactive during sensory stimulation, but discharged readily with reaching movements towards a reward. The latter were similarly inactive during sensory stimulation, but discharged with manual exploration for food or other objects. Thus, area 5 seems to be critical for both accurate reaching and object manipulation.

In humans, injury to the posterior parietal lobe can generate a dizzying array of impairments. In a 1918 study of WWI veterans with bilateral PPL lesions, patients had deficits in reaching, eye movement and visual localization (Holmes, 1918). PPL lesions are able to generate what is known as Balint's syndrome, which presents as eye fixation deficits, difficulty perceiving the entire visual field and trouble reaching accurately. This last symptom, optic ataxia, is of particular interest, as it had been intuited that area 5 likely contributes to the planning of reaching movements by way of its connectivity with PMC. Interestingly enough, movements not under visual guidance are unaffected. Monkeys with lesioned PPL exhibit similar misreaching, with cooling of area 5 generating global misreaching regardless of the object's location in the visual field. Compared to cooling of area 7, which generates misreaching in the contralateral visual hemifield, area 5 is more critical for proprioception while area 7 is more important for visual control of reaching. Strong electrical stimulation of area 5 in monkeys can generate movements of the arms, legs, trunk and eyes.

2.2 Review of relevant methodology

2.2.1 Transcranial magnetic stimulation

TMS was originally developed as a less painful alternative to transcranial electrical stimulation (TES) as a method of noninvasively stimulating the brain. The technique was pioneered in 1985 by a group at the University of Sheffield including Dr. Reza Jalinous, Dr. Ian Freeston and Dr. Anthony T. Barker (Barker et al., 1985). It stimulates neurons indirectly by way of induction. This is accomplished by passing a short, high-current pulse through a coil of wire, producing a perpendicular magnetic field and in turn an electric field perpendicular to this (Hallett, 2007). The current is strongest directly underneath the wire, falling off to 0A at the center of the circle formed by the coiled wires. Different coil types exist and serve different purposes, one of the most commonly utilized being the figure-of-eight coil. Two windings of wire form two coils which meet at one point of their circumference, with the induced current being most focal under this junction.

Macroscopically, the neural tissue underneath the coil capable of being excited by a TMS pulse includes both the cortical grey matter and subcortical white matter. The aforementioned magnetic field produced by the coil falls of very quickly, such that 4 cm away from its surface the field is reduced to approximately 30% (Siebner et al., 2009). This feature of TMS means that at a given site of stimulation over the scalp, superficial cortex is easiest and most likely to be activated (Edgley et al., 1997). At the neuronal level, a combination of cell bodies and axons are being excited. However, the proportion of axons activated is likely much greater. They are more easily activated by a short duration pulse, whereas cells require a longer pulse (Siebner et al., 2009).

The experimentally determined time constant of the membrane of cell bodies is much longer than that of axonal membranes (Rothwell & Amassian, 1992), meaning a short duration electrical field is much more suited to activation of myelinated axonal membranes. With a typical monophasic pulse current rising and falling in approximately 200 μs, axonal activation is favored. As would be expected from this knowledge, in studies covered by a review of electrical stimulation of mammalian central nervous system (CNS), stimulation duration was at least twice as long when stimulating cell bodies compared to myelinated axons (Ranck, 1975). Furthermore, activation is most likely to occur where axons bend out of the coil's electric field (Maccabee et al., 1998), since efficacious activation relies on a high voltage gradient along an axon. In *Cambarus clarkii* abdominal receptors, graded neuron responses were seen with experimentally altered voltage gradient; stronger gradients resulted in greater increases in firing frequency (Terzuolo & Bullock, 1956).

When a TMS pulse excites a population of neurons, the resultant corticospinal descending activity is composed of a number of high-frequency volleys, which can be recorded from the medullary pyramid or the surface of the cervical spinal cord (Adrian & Moruzzi, 1939). The earliest of these volleys is thought to spawn from direct activation of pyramidal tract neurons (PTNs) and is termed the D-wave (Di Lazzaro et al., 2012). Subsequent waves are termed indirect or I-waves and result from synaptic activation of the same PTNs. Thus, the same individual axon of a PTN is capable of delivering both D- and I-waves (Patton & Amassian, 1954). I-waves are recruited as a function of intensity of stimulation, such that greater intensity is required to evoke longer latency I waves (Di Lazzaro et al., 2008). The time from I_n wave to I_{n+1} wave is approximately 1.5 ms, representing a PTN firing rate of 670 Hz.

A recent analysis of a great number of TMS studies concludes that these waves likely arise from

activation of synchronous clusters of excitatory and inhibitory neurons (Di Lazzaro et al., 2012). In a relevant computational modeling study, it was found that a network of sparsely connected inhibitory and excitatory neurons all receiving the same amount of inputs is capable of entering a system state whereby neurons discharge regularly (Brunel, 2000). The model made use of a leaky integrate-and-fire neuron model, shown to be fairly representative of the dynamics of neural networks employing more complex neuronal models (Bernander et al., 1991). With integrated terms accounting for refractory period and the diffusion of ions back across the membrane when the input current does not reach the firing threshold, the leaky integrate-and-fire model is a fair representation of an in vivo neuron.

There are a number of parameters which can alter the effects of TMS on the underlying neural tissue. Intensity of coil current, orientation, position, frequency and duration of stimulation can all be altered to produce different effects. Using primary motor cortex (M1) as an example site of stimulation, altering the intensity of the stimulator output will change the size of the response from the muscle whose representation underlies the induced current focus. Changing coil orientation will alter the properties of the descending motor signal with different sizes and latencies of D- and I-waves as discussed above (Di Lazzaro et al., 2008). Moving the coil to a new position over motor cortex will affect which muscle is activated by the TMS pulse. In terms of frequency, this parameter is most useful in plasticity protocols. Stimulating at low frequency (<1Hz) over motor cortex can suppress MEP sizes while high frequency ($\geq$ 1Hz) can increase them (Chen et al., 1997; Berardelli et al., 1998).

2.2.2 Motor evoked potentials

The dependent measure of many TMS studies, the motor evoked potential (MEP) is the resultant activity of the muscle whose hotspot in primary motor cortex is being stimulated by TMS.

MEPs are recorded by electromyography (EMG), with surface electrodes placed over the muscle of interest contralateral to stimulated M1. The MEP is composed of the aggregate descending volleys of corticospinal activity which excite the alpha motor neuron pool at the spinal cord and in turn the muscle. In any given subject, a greater intensity TMS pulse will yield greater peak-to-peak MEP amplitude, larger MEP area, or a combination of the two. A typical MEP as recorded by EMG can be seen in Figure 2.

The importance of the MEP as a dependent measure lies in its ability to assess corticospinal excitability. The amplitude of the MEP is representative of the level of excitability of the corticospinal tract, and can be affected by changes in both cortical and spinal components, such as M1 circuitry and the alpha motor neuron pool. When the intensity of the TMS pulse is held constant, an increase in either the amplitude or area of the MEP means that corticospinal excitability is elevated. Conversely, a reduction in amplitude or area signifies a lower level of corticospinal excitability. This measure is of use to studies employing interventions which may alter levels of excitability either in the cortex or at the spinal level.

2.2.3 Motor threshold

Motor threshold is an important measure of motor cortical excitability which has been standardized in recent years and now has a fairly rigid definition. It is defined as the minimum intensity of stimulation able to generate a small response from the target muscle (Kobayashi & Pascial-Leone, 2007). Since motor threshold can be measured both at rest and in active skeletal musculature, there are distinct measurements termed resting motor threshold (RMT) and active motor threshold (AMT).

Resting motor threshold is defined as the lowest stimulation intensity needed to elicit an MEP of at least 50μV in 5 out of 10 consecutive trials in a resting target muscle (Kobayashi & Pascual-Leone, 2003). Active motor threshold is defined as the lowest stimulation intensity needed to elicit an MEP of at least 200μV in 5 out of 10 consecutive trials in a target muscle during 10% MVC tonic contraction (Rothwell et al., 1999).
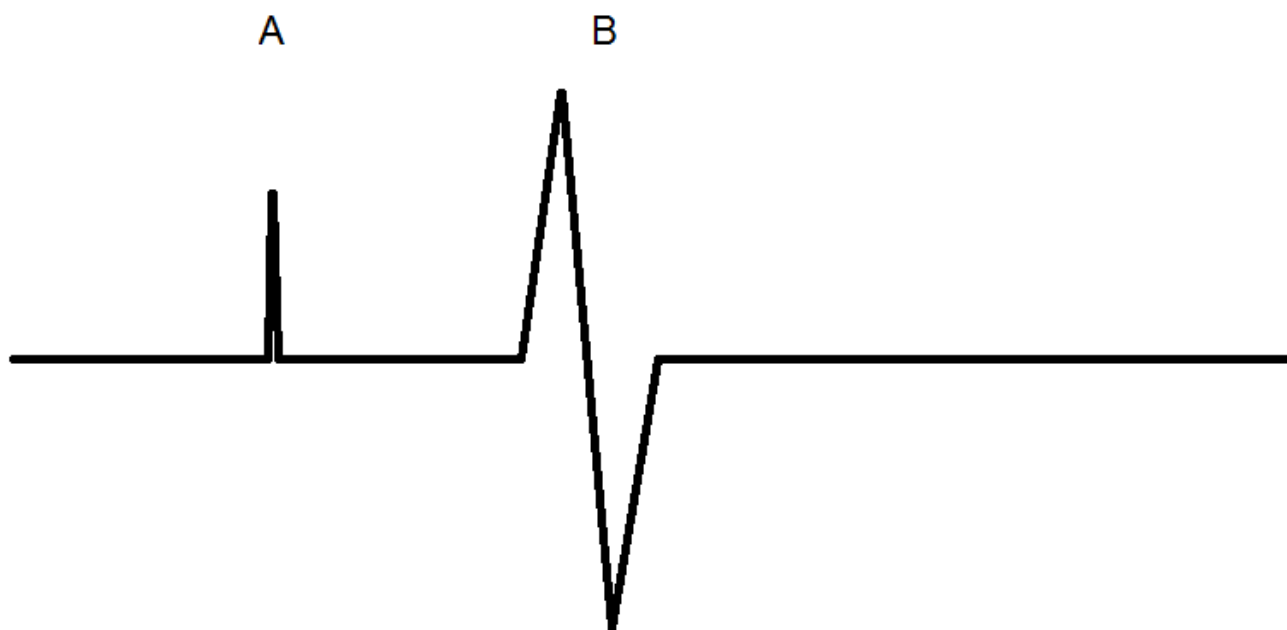
Figure 2 - **Typical MEP recorded with EMG. A** - TMS artefact; **B** - MEP

MEPs are larger in amplitude for any given stimulation intensity in active muscles compared to at rest (Rothwell, 2005). Thresholds for eliciting a motor response are also lower in active muscles, meaning AMT is lower than RMT for a given muscle being recorded. Motor threshold can be permanently decreased in patient populations, such as idiopathic epilepsy (Reutens et al., 1993). Also,

certain drugs which block sodium and calcium channels such as carbamazepine and lamotrigine can increase motor threshold (Hallett, 2007).

2.2.4 Paired-pulse TMS

This experimental technique makes use of two TMS coils simultaneously. Each is positioned over a different area of the cortex, and they fire at a short temporal offset dubbed the interstimulus interval (ISI). In paired-pulse paradigms, the first TMS pulse is called the conditioning stimulus (CS). This pulse can be delivered over a broad range of cortical areas, all of which are expected to exert some sort of influence over the excitability of M1. The second of the two pulses is delivered over M1, so as to elicit an MEP as recorded with EMG, and is called the test stimulus (TS). The main purpose of paired-pulse, or twin coil, studies is to determine the functional relationship between the area of the brain where the CS is delivered and primary motor cortex. The nature of this relationship is gauged by changes in size of MEPs elicited by the TS with the addition of preceding CS. All paired-pulse CS-TS trial MEPs are normalized to TS alone trial MEPs elicited from M1. The resultant outcome measure is a ratio between paired- and single-pulse trials that is demonstrative of the functional relationship between both areas at the specific ISI tested.

To give a straight forward example, this thesis centers around the relationship between area 5 and M1. Thus, the conditioning stimuli are delivered over putative area 5. The selected ISI between CS and TS is 8ms. As described above, test stimuli are delivered over M1, at the motor hotspot optimal for eliciting a muscle response from first dorsal interosseous (FDI). If MEPs were larger in CS-TS trials compared to TS alone trials, it could be said that in the context of this experiment, area 5 is exerting a facilitatory influence on M1 excitability. As can be seen with the use of very short ISIs, TMS is good

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for high temporal resolution, and establishing causal relationships between nonmotor and motor areas, but does not speak to their physiological connectivity (Koch & Rothwell, 2009). This is why, with any paired-pulse functional interaction, it cannot assured whether the conditioned area acted directly on M1, or whether the neural data had a rendezvous with another area before arriving.

To make this idea of direct vs. indirect connectivity more salient, consider the facilitatory relationship between posterior parietal cortex and ipsilateral M1 at rest. At 4 and 15ms CS-TS ISIs, with CS delivered over the region of caudal intraparietal sulcus (cIPS), MEPs are facilitated at rest (Koch et al., 2007). This great disparity in facilitatory ISIs was indicative to the authors that only the 4ms increase in MEPs was governed by direct connections from cIPS to M1. By 15ms, the signal will have had time to travel first to PMC, with which posterior parietal cortex has strong direct connectivity (Hyvarinen, 1982), and subsequently to M1.

The range of cortical areas studied using paired-pulse TMS is far-reaching. Inferior frontal cortex, inferior parietal lobule and dorsal premotor cortex are just three of many different areas whose functional relationships with ipsilateral M1 have been quantified (van Campen et al., 2013; Karabanov et al., 2013; Koch et al., 2009). Even within a specific cortical non-motor area, the relationship with M1 can be probed in different contexts. Area 5 relationship with ipsilateral M1 has already been probed at rest, during processing of cutaneous stimuli and in the context of motor tasks such as pinch and power grip (Ziluk et al., 2010; Ziluk, 2010).

Chapter 3: Effects of Area 5 Conditioning on Ipsilateral M1 Excitability

3.1 Introduction

Brodmann's area 5 (BA5) is located within the superior parietal cortex in primates (Hyvärinen, 1982). In the macaque, it is largely dedicated to the upper extremity whereby the shoulder, forelimb and digits occupy a disproportionate cortical territory compared to other body parts (Seelke et al., 2011). BA5 demonstrates a mosaic-like representation of body parts and provides ipsilateral projections to primary motor cortex (M1) (Strick & Preston, 1978; Strick & Kim, 1978), in support of the suggestion that it is an extension of M1 in primates (Stepniewska et al., 2005). BA5 possesses multimodal neurons, activated by kinesthetic and tactile stimuli (~ 24% of neurons tested), and directionally sensitive tactile neurons (~60% of population) indicating that complex features of somatosensation are represented (Mountcastle et al., 1975). In humans, the presence of giant pyramidal cells in layer 5 distinguishes BA5 from neighbouring loci (Scheperjans et al., 2008) and diffusion tensor imaging has revealed connectivity to M1 by way of the superior longitudinal fasciculus (SLF) I subdivision (Makris et al., 2005).

In monkeys, BA5 is involved in motor preparation (Burbaud et al., 1991; Snyder et al., 1997), pre-shaping the hand prior to prehension (Ro et al., 2000) and generating body-centered coordinates for reaching (Iriki et al., 1996; Andersen et al., 1997; Ferraina & Bianchi 1994; Lacquaniti et al., 1995). This area may have evolved with the ability to execute fine movements of the hand, such as pinch grip (Padberg et al., 2007) suggesting a key role for BA5 in motor control. Electrical stimulation of BA5 elicits complex movements of the contralateral upper limb in monkeys (Ferrier, 1876; Vogt & Vogt, 1926; Peele, 1944). In macaque, there exist BA5 neurons which are more active during volitional

versus passive movement of the limbs, and approximately 10% are classified as 'arm-projection' and 'hand-manipulation' neurons (Mountcastle et al., 1975). In rhesus monkey, BA5 activity is sustained after the presentation of a NO-GO stimulus, leading to the authors to believe that this area may hold representations of potential motor responses to stimuli (Kalaska & Crammond, 1995).

Research in humans also confirms a role for BA5 in motor control. Electrical stimulation in the vicinity of BA5 can elicit flexion of the contralateral upper limb (Foerster 1931, 1936a-c) and even movements involving the hand (Lim et al., 1994). In humans, neuroimaging studies have shown activation of BA5 during reaching movements (Culham et al., 2006), especially under visual guidance (Wenderoth et al., 2006). BA5 activation has also been observed in response to digit vibration (Francis et al., 2000; Nelson et al., 2004), and during a tactile tracking task using vibration to guide movement (Nelson et al., 2004).

Transcranial magnetic stimulation (TMS) offers the opportunity to study the role of BA5 in human motor control by probing the functional connectivity between BA5 and M1. In this paradigm, the first TMS pulse (i.e. the conditioning stimulus, CS) is delivered over BA5 followed by a second TMS pulse (i.e. the test stimulus, TS) over M1 that elicits a motor evoked potential (MEP). The effect of BA5 is shown by changes in the MEP amplitude elicited by the test pulse. This technique has revealed that BA5 facilitates M1 output during the processing of somatosensory stimuli (Ziluk et al., 2010). The present study manipulates the CS intensity, as well as CS and TS induced current direction to probe BA5-M1 connectivity in greater detail.

Previous human research has demonstrated a role of inferior parietal lobule (IPL), a neighbouring anatomical site, in regulating M1 excitability in the context of planning a reach (Koch et

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al., 2008). Monkey literature demonstrates sustained BA5 neuronal activity prior to movement, even in the context of a NO-GO task (Kalaska & Crammond, 1995). This information prompts the question of whether BA5 influences M1 excitability prior to movement in humans. BA5 has not been explored for its role prior to movement in humans. However, it is known that nearby BA7 is active during the withholding of responses to NO-GO stimuli (Watanabe et al., 2002).

The purpose of this study is to understand BA5 impact on ipsilateral M1 excitability prior to movement. BA5-M1 functional connectivity was probed with CS intensities ranging from 90-130% of RMT. It was hypothesized that BA5 would task-dependently modulate M1 output such that it would be increased during a GO vs a NO-GO task. With respect to CS intensity, it was hypothesized that a CS of 90% resting motor threshold (RMT) would show GO/NO-GO differences, based on previous studies indicating that this intensity is suitable to condition M1 at rest (Karabanov et al., 2013; Koch et al., 2007; Koch and Rothwell, 2009). The orientation of CS and TS stimuli were also experimentally manipulated to explore potential neural circuitry which governs this connectivity. With a reversal of either CS or TS, a better understanding of the pathway used to transmit information from BA5 to M1, and the motor cortical circuitry affected by BA5 conditioning can be respectively gained. Results from this study will help to show more clearly whether BA5 is involved in movement preparation in humans, and will provide insight into the mechanisms of BA5-M1 interaction.

3.2 Methods

Subjects

Participant inclusion criteria required that 1) both customized TMS coils could lay flat on the scalp of the individual, one over BA5 and the other over M1, and 2) individuals demonstrated thresholds low enough to elicit 1 mV MEPs, such that 100% of MSO was able to reliably obtain 1 mV responses. Thirty-nine individuals met the inclusion criteria (mean age = 23.5 ± 4.4 years, 20 female) of which 15, 12 and 11 participated in Experiments 1, 2 and 3, respectively. Each individual participated in one experiment only. All subjects were right-handed and none reported any history of familial epilepsy, neurological or medical illnesses, nor were taking medication as per the pre-screening questionnaire. All participants had normal or corrected-to-normal vision and wore 30 dB earplugs throughout the experiment. This study was approved by the McMaster Research Ethics Board (MREB) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to their participation.

Transcranial magnetic stimulation procedures

Electromyographic (EMG) activity was recorded from the first dorsal interosseous (FDI) muscle using 9 mm Ag-AgCl surface electrodes with the active placed over the muscle belly and reference over the metacarpophalangeal joint of the index finger of the right hand. EMG was amplified 1000 x and band pass filtered (20-1000 Hz) using an Intronix Model 2024F isolated preamplifier (Intronix Technologies Corporation, Bolton, Canada) and acquired using Signal Software and a

Cambridge Electronic device (Power 1401, Cambridge Electronic Design, Cambridge, UK).

TMS was delivered with two custom-built 50 mm diameter figure-of-eight branding coils each connected to a Magstim 200^2 stimulator (Magstim, Whitland, Dyfeld, UK). The motor hotspot was defined as the location within the left hemisphere that elicited the largest peak-to-peak MEP from the right FDI. The motor hotspot was marked by digital registration using a standard MRI template via Brainsight 2 Neuronavigation (Rogue Research, Montreal, Canada). One figure-of-eight coil was positioned over the motor hotspot at ~ 45 degrees to the mid-sagittal plane to induce a posterolateral-anteromedial current in the cortex. Resting motor threshold (RMT) was acquired at this location and defined as the minimum stimulator output needed to obtain $\geq$ 50 µV MEP in 5 of 10 consecutive trials. At this location the percentage of maximum stimulator output (%MSO) required to elicit a peak-to-peak MEP of ~ 1 mV based on the average of 15 TMS stimuli was acquired. The %MSO was used during testing as the intensity for M1 stimulation, giving ~ 1 mV unconditioned MEPs (MEP$_{TS)}$.

Left hemisphere putative BA5 was localized using the Talairach atlas (Talairach and Tournoux, 1988) with respect to the 10/20 International electrode placement system and defined 1 cm anterior and 1.5 cm lateral (Premji et al., 2011) to electrode position Pz which overlays the parietal midline. This position was digitally registered with Brainsight 2 Neuronavigation. The coil was positioned to induce a current in the posterior-to-anterior (PA) direction in the cortex and promote a voltage gradient along SLF fibres in the direction of M1 (Makris et al., 2005).

Task

All experiments employed a GO/NO-GO paradigm, whereby participants had two different

responses to two unique auditory tones named GO and NO-GO (Mallory Sonalert SC628 and

Multicomp MC25164). The GO and NO-GO tones were 60 ms in duration (van Campen et al., 2013)

and the assignment of tones as GO and NO-GO was counterbalanced across subjects. Participants were

seated comfortably in a chair with their head supported by a neck rest. A table supported the right hand

and arm. The right hand rested on a computer mouse and a point of visual fixation was positioned 2 m

away from the subject, directly ahead and at eye level. A trial schematic is shown in Figure 3.

An auditory warning cue alerted the participant to the start of each trial. The GO/NO-GO

auditory tone followed this warning cue by 2 or 3 seconds to avoid anticipatory responses (0.5

probability of each). In the GO task, participants responded with a button click. The NO-GO task

required the inhibition of the click response. Participants were asked to respond as quickly and
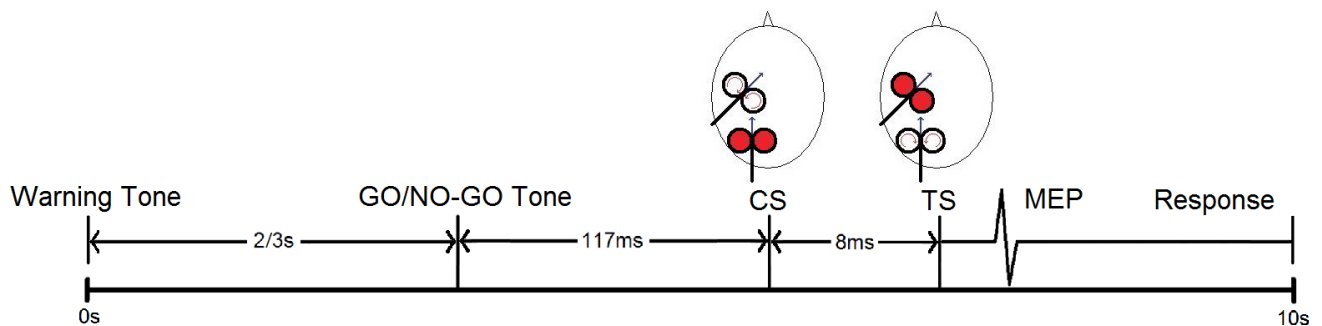
Figure 3 - **Timeline of a single trial.** The warning tone indicates the start of a trial. The GO/NO-GO tone arrives at a variable 2 or 3 s following the warning tone. **CS - conditioning stimulus:** delivered over BA5 at 117 ms following the GO/NO-GO tone. **TS - test stimulus:** delivered over M1 at 8 ms following the CS. M1 excitability is probed 125ms following GO/NO-GO tone. **MEP- motor evoked potential:** recorded from the FDI using surface EMG. Response involves mouse click and signifies participant voluntary motor output in response to the GO/NO-GO tone.

accurately as possible with the right index finger. BA5-M1 connectivity was probed following the GO/NO-GO tone but prior to response initiation. The test stimulus (TS), a TMS pulse delivered over primary motor cortex (M1), followed the GO/NO-GO cue by 125ms (Koch et al., 2007). The CS was delivered over BA5 8 ms prior to the TS (Chao et al., 2013; Karabanov et al., 2013). The ordering of GO versus NO-GO trials were pseudo-randomized within each block of trials in all experiments. To assess the BA5 – M1 connectivity in the absence of a GO/NO-GO task context, a REST condition was included. The REST condition was counterbalanced across participants to be tested before, after and between GO and NO-GO blocks, and MEP_{TS} was set to evoke ~ 1 mV. The inter-trial interval was 10s.

Importantly, pilot research and published literature (Chiu, Aron & Verbruggen, 2013; Hoshiyama et al., 1997; Leocani et al., 2000) revealed that MEP_{TS} trials evoked substantially different MEP sizes in the GO versus NO-GO trials, being much larger in the GO trial. Therefore, to allow experimenter control of the amplitude of the TS evoked MEP (MEP_{TS}) to be 1 mV and similar in the GO and NO-GO tasks, it was necessary to predefine each testing block as a 'GO' or 'NO-GO' block during which the TS was adjusted to elicit a MEP of ~ 1 mV according to that predefined block. This information was not made available to the participant. Therefore, in an experimenter determined 'NO-GO' block, all 'GO' trials were discarded since their corresponding MEP_{TS} was adjusted for 'NO-GO' trials and vice versa. All programming of the protocol was accomplished with a custom Signal configuration (Signal Version 6). Each Experiment began with practise trials without TMS (15 trials) and with TMS (15 trials) to acclimate participants to the GO/NO-GO task and the sensation of receiving TMS during the task.

Experiment 1 – Task-dependent modulation of BA5-M1 relationship

The CS and TS configuration was set to PA-PA, shown in Figure 4A, and the intensity of the CS was 90% RMT. Two blocks of 40 trials were performed. Each block was separated by 5 minutes. Within each block, 10 trials were conditioned (MEP_{CS-TS}) in the context of the GO task, 10 trials were MEP_{CS-TS} in the context of the NO-GO task, 10 trials were unconditioned (MEP_{TS}) in the context of the GO task, and 10 trials were MEP_{TS} in the context of the NO-GO task. In total, 20 trials were analyzed for each task of the GO and NO-GO tasks of which 10 were MEP_{CS-TS} and 10 were MEP_{TS}. One block of REST acquired 20 trials (10 MEP_{CS-TS}, 10 MEP_{TS}).

Experiment 2 – Influence of conditioning intensity on the BA5-M1 task-dependent relationship


This experiment tested the effects of CS intensity on the GO/NO-GO relationship observed in Experiment 1, and tested whether the change in BA-M1 connectivity was driven by changes in the GO or NO-GO tasks. The CS and TS coil orientations are shown in Figure 4A. The BA5 – M1 connectivity was tested at CS intensities of 50, 70, 90, 110 and 130% RMT during GO, NO-GO and REST tasks. GO/NO-GO trials were delivered in 2 blocks of 120. Within each block of testing, 50 trials were MEP_{CS-TS} in the context of GO (10 at each CS intensity), 50 trials were MEP_{CS-TS} in the context of NO-GO (10 at each CS intensity), 10 trials were MEP_{TS} in GO and 10 trials were MEP_{TS} in NO-GO. In total, 60 trials were analyzed for each task (GO and NO-GO), 10 of which were MEP_{TS} and 50 MEP_{CS-TS} (10 at each CS % of RMT). REST was collected with 60 trials, 10 of MEP_{TS} and 50 MEP_{CS-TS} (10 at each CS % of RMT).

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Experiment 3 – Influence of coil orientation on BA5-M1 task-dependent relationship

This experiment tested the effects of CS and TS coil orientations on BA5-M1 functional connectivity. The CS-TS orientations tested included 1) that used in Experiments 1 and 2 (Figure 4A) whereby the CS intensity was set at 90% of RMT, 2) the AP-PA CS-TS orientation shown in Figure 4B, whereby the CS induced a current in the anterior-to-posterior (AP) direction and the intensity was set to 90% of RMT, 3) the PA-AP CS-TS orientation shown in Figure 4C whereby the CS induced a PA current (set to 90% of the RMT obtained in the AP orientation) and the TS induced an AP current. All distinct sets of coil orientations were delivered in separate blocks of trials. The GO/NO-GO trials were delivered in 6 blocks of 40 (2 each in PA-PA, AP-PA and PA-AP). Within blocks of testing, 10 trials were MEP_{CS-TS} in the context of GO, 10 were MEP_{CS-TS} in the context of NO-GO, 10 were MEP_{TS} GO and 10 were MEP_{TS} NO-GO. Discarding of trials left 60 trials of each condition; 10 MEP_{TS} and 10 MEP_{CS-TS} for each orientation. Three REST blocks of trials 20 trials were also collected; one per each orientation shown in Figure 4. There were 10 trials of MEP_{CS-TS} and 10 of MEP_{TS} TMS.

3.3 Analysis

Using a custom Signal script, trials wherein the participant responded incorrectly were automatically discarded based on specific criteria: response in NO-GO trial, absence of response in GO trial, EMG activity exceeding background EMG mean at rest $\pm$ 2*SD at the time of TMS delivery and/or recording of MEP. For all remaining trials, the average of the peak-to-peak MEP amplitude was quantified for each task and each participant.
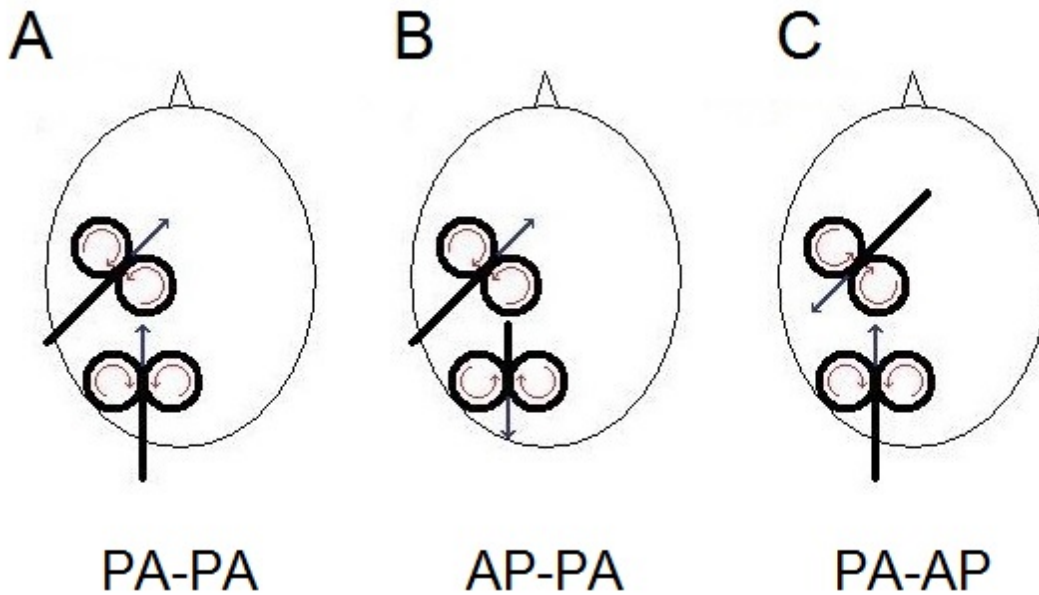
Figure 4 - **Coil orientation configurations. Figure 4A:** CS of BA5 and TS over M1 induce a current in the posterior-to-anterior (PA) direction. **Figure 4B:** CS over BA5 induces an anterior-to-posterior (AP) current while the TS over M1 induces a PA current. **Figure 4C:** CS over BA5 induces a PA current while the TS over M1 induces an AP current.

Hypotheses

Pilot data collected on a greater range of ISIs and time frames of GO/NO-GO to TS allowed for the development of the *a priori* hypothesis that BA5 would have a facilitatory effect on peak-to-peak MEP amplitudes for MEP_{CS-TS} GO trials, with respect to MEP_{CS-TS} NO-GO trials. Thus, a paired samples one-tailed t-test was used to assess task-specific differences in MEPs between GO and NO-GO trials in all experiments. In Experiment 2, this manifested as GO vs NO-GO at 90% RMT CS and in Experiment 3 as GO vs NO-GO in the PA-PA orientation. Further, in Experiment 2 it was hypothesized that MEP_{CS-TS} GO vs NO-GO differences would only persist at the previously employed CS intensity of 90% RMT (Karabanov et al., 2013; Koch et al., 2007; Koch and Rothwell, 2009). In Experiment 3, it

was hypothesized that MEP_{CS-TS} GO vs NO-GO differences would exist at the previously employed PA-PA coil configuration, owing to the information from BA5 needing to be communicated to M1 via SLF and directly via other fibre pathways travelling from posterior to anterior.

Statistical Analyses

For Experiment 1, MEPs were normalized (MEP_{CS-TS}/MEP_{TS}) and subjected to a one-way ANOVA using within-subject factor TASK (3 levels; GO, NO-GO, REST). In Experiment 2, un-normalized MEPs were compared in a two-way ANOVA using within-subject factors TASK (3 levels; GO, NO-GO, REST) and INTENSITY (6 levels; TS, 50, 70, 90, 110, 130% RMT). For Experiment 3, normalized MEPs (MEP_{CS-TS}/MEP_{TS}) from each orientation were compared in a two-way ANOVA using within-subject factors TASK (3 levels; GO, NO-GO, REST) and ORIENTATION (3 levels; PA-PA, AP-PA, PA-AP). For all three experiments , differences in MEP_{TS} across conditions were assessed with ANOVAs. Post-hoc Tukey's tests examined significance in all ANOVAs. Significance was set at $p < 0.05$. Cohen's d was used to assess effect sizes for significant findings. Where sphericity was not met, the Greenhouse-Geisser correction was used.

### 3.4 Results

Experiment 1 – Task-dependent modulation of BA5-M1 relationship

Fifteen individuals participated of whom 12 were included in subsequent analyses (mean age = 23.1 ± 2.6 years, 7 female). Three participants were removed due to the inability to experimentally constrain the MEP_{TS} between 0.5 and 1.5 mV, a necessary requirement of the study. The group-averaged

RMT was 44.6 % (± 9.0 std) MSO and the intensity required to elicit ~1 mV MEP was 54.3 % (± 9.6) MSO. Following computer automated discarding, 93.9 % (± 6.1) of trials were included in the analysis. Figure 5A displays the group-averaged MEP_{TS}. The one-way ANOVA revealed a main effect of TASK [$F(2,22) = 4.459$, $p = 0.046$] and post-hoc Tukey's revealed significantly larger MEP_{TS} in the REST versus NO-GO task ($p < 0.05$), with no difference between the GO and NO-GO tasks ($p > 0.05$). These data indicate that, although the MEP_{TS} was intended to be similar for each task, there were differences in the magnitude of the MEP_{TS} and this may impact the conditioning effect evoked by the CS pulse over BA5.

Subsequent analyses used normalized MEP data (ie. MEP_{CS-TS}/MEP_{TS}) and these data are shown in Figure 5B. One-way ANOVA revealed no effect of TASK [$F(2, 22) = 1.478$, $p = 0.250$]. The *a priori* hypothesis was confirmed such that MEPs in the GO task were significantly greater compared to the NO-GO task (one-tailed, paired t-test, $p = 0.019$). Cohen's effect size value was 0.693, indicating a medium effect size for the BA5-M1 difference between the GO and NO-GO task. Further, this effect existed despite the non-significant difference in the MEP_{TS} (Figure 5A), which would have made the GO versus NO-GO effect more difficult to observe. From the sample of 12 participants, 7 demonstrated greater MEPs during GO versus NO-GO, the opposite effect was seen in 2 individuals and there was very little change in 3 individuals (Figure 6). In summary, these data indicate that the interaction between BA5 and M1 is dependent on the task performed.
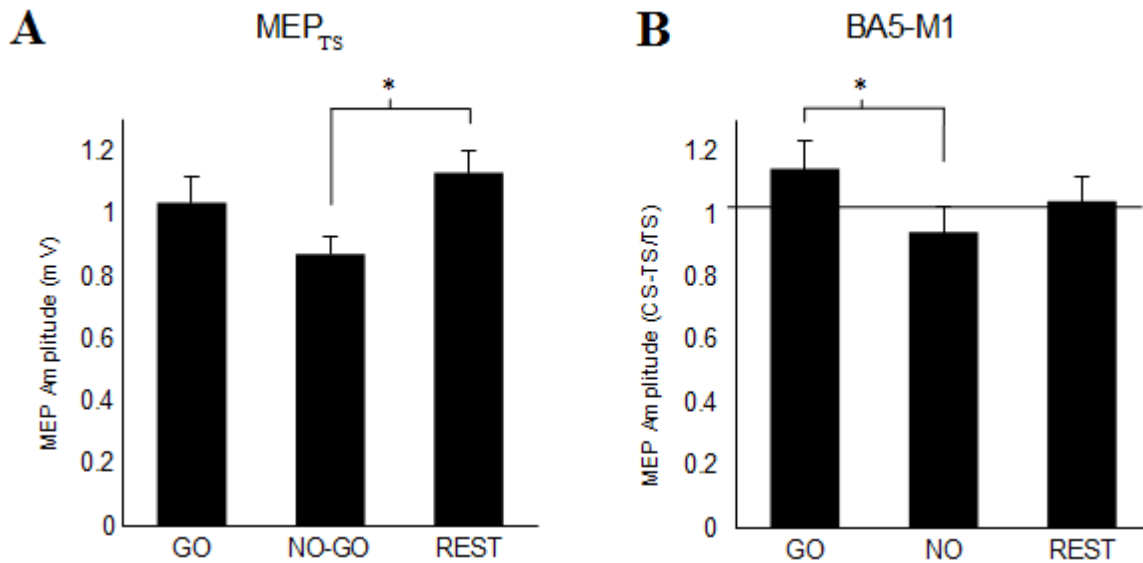
Figure 5 - **Experiment 1. Figure 5A:** Group-averaged MEP_{TS} (with standard errors) for each task. Data represent single-pulse MEP amplitude, in response to TS. MEPs were reduced in the context of NO-GO relative to REST ($p < 0.05$). **Figure 5B:** Group-averaged MEP_{CS-TS} normalized to MEP_{TS} (with standard errors). Data represent the level of BA5 conditioning on M1 excitability. Values greater than 1 on the y-axis indicate BA5 facilitation of M1 excitability. Values less than 1 indicate inhibition. BA5 differentially conditioned trials in the context of GO relative to NO-GO, such that GO normalized MEPs were larger than in NO-GO ($p < 0.05$).

Experiment 2 – Influence of conditioning intensity on the BA5-M1 task-dependent relationship

Twelve individuals participated and 11 were included in subsequent analyses (mean age = 22.4 ± 3.2 years, 7 female). The participant removed from analysis had data which was contaminated by excessive background muscle activity. Experiment 2 sought to investigate whether the GO versus NO-GO differences observed in Experiment 1 exist at other CS intensities, and whether differences between GO and NO-GO tasks are mediated by MEP changes in the GO and/or NO-GO tasks. The group-averaged RMT was 42.9 ± 9.2% MSO and intensity required to elicit ~1 mV MEP was 52.9 ± 12.2% MSO. Following computer automated discarding, 93.3 % (± 6.7) of trials were included in analyses. One-way ANOVA on the MEP_{TS} revealed no effect of TASK [$F(2,20) = 1.563$, $p = 0.234$] indicating

that the TS evoked MEP was maintained at ~1 mV for all tasks.

The group-averaged MEP data for all tasks and intensities are shown in Figure 7 and the corresponding two-way ANOVA revealed a main effect of TASK [$F_{(2, 20)} = 3.411$, $p = 0.053$] and INTENSITY [$F_{(5, 50)} = 3.206$, $p = 0.014$] without a TASK*INTENSITY interaction [$F_{(10, 100)} = 1.182$, $p = 0.312$]. In support of the hypothesis and Experiment 1, the MEP was significantly larger during GO versus NO-GO at 90% CS intensity (one-tailed, paired t-test, $p=0.020$) with a robust effect size (Cohen's $d = 1.101$). The GO versus NO-GO difference was observed in 8 out of the 11 participants while the remaining individuals showed the opposite effect or little change.

Despite the main effect of TASK, post-hoc Tukey's did not reveal significant differences between levels of task. To investigate the effect of BA5 conditioning within each task, one-way ANOVA performed independently on each task. GO and REST tasks revealed no main effect of INTENSITY [[GO:[$F_{(5, 50)} = 1.530$, $p = 0.197$], REST:[$F_{(5, 50)} = 1.458$, $p = 0.220$]]] indicating that BA5 did not condition M1 output at any intensity relative to MEP_{TS}. For the NO-GO task, one-way ANOVA revealed a significant effect of INTENSITY ([$F_{(5, 50)} = 2.865$, $p = 0.024$]) and post-hoc Tukey's indicated a significant reduction in the MEP at 90 and 110% RMT compared to MEP_{TS} ($p < 0.05$). Cohen's effect size values were 1.405 and 1.357 respectively, indicating a strong effect of BA5-M1 inhibition during the NO-GO tasks at these CS intensities. The data from Experiment 2 indicate that the difference between GO and NO-GO tasks are driven by a reduction in the MEP amplitude during NO-GO, an effect that is present only at specific CS intensities. Further, these data demonstrate the similarities in the BA5-M1 relationship during rest and within the context of the GO task, neither of which demonstrated an effect of the conditioning stimulation.
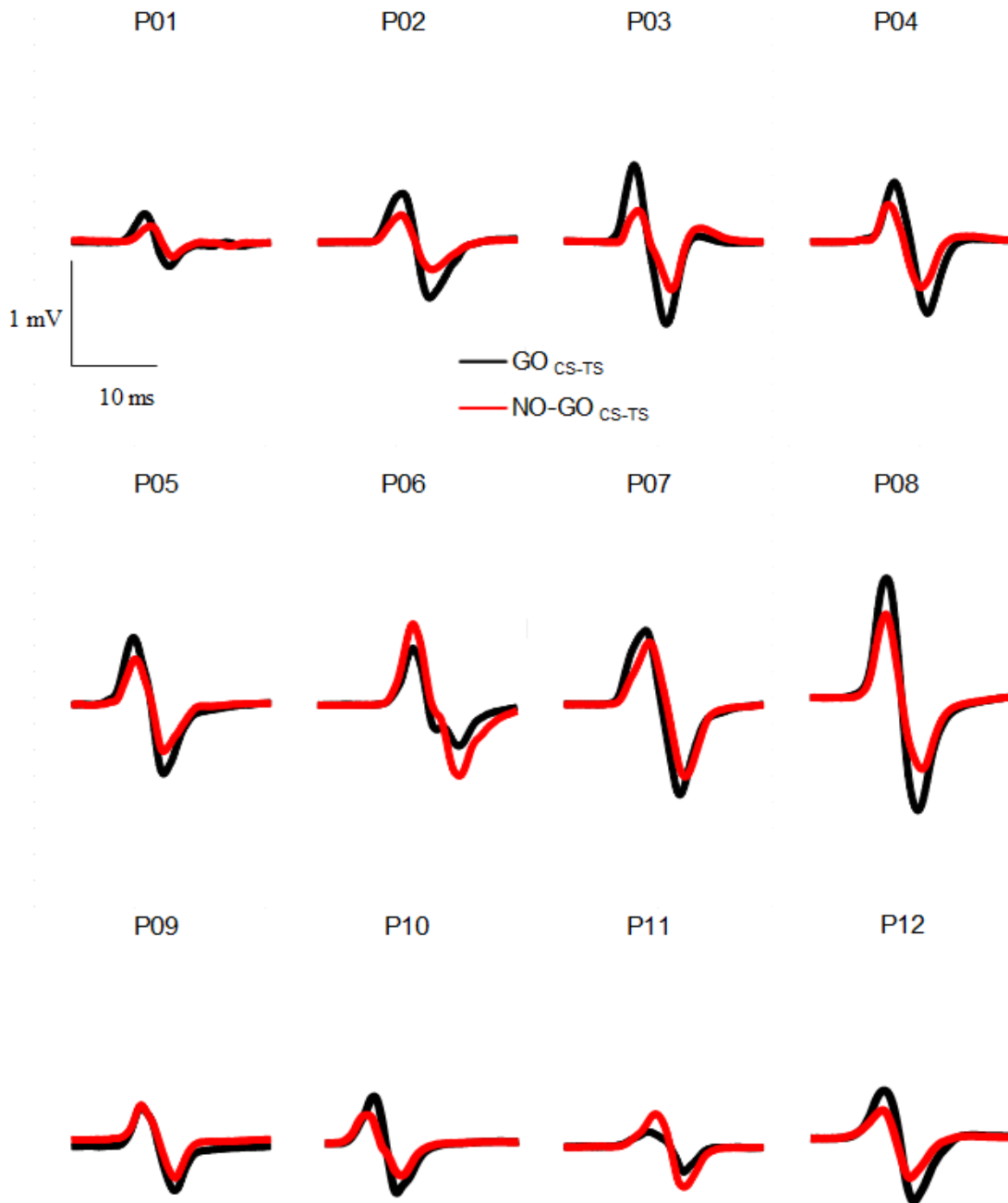
Figure 6 - **Experiment 1 Individual MEPs in GO versus NO-GO .** MEP$_{CS-TS}$ in the context of GO (black) and NO-GO (red) are shown for each participant. Seven individuals demonstrate the finding of GO > NO-GO MEPs (P02, P03, P04, P05, P08, P10, P12), 2 show the opposite effect (P06, P11) and one show little to no change (P01, P07).
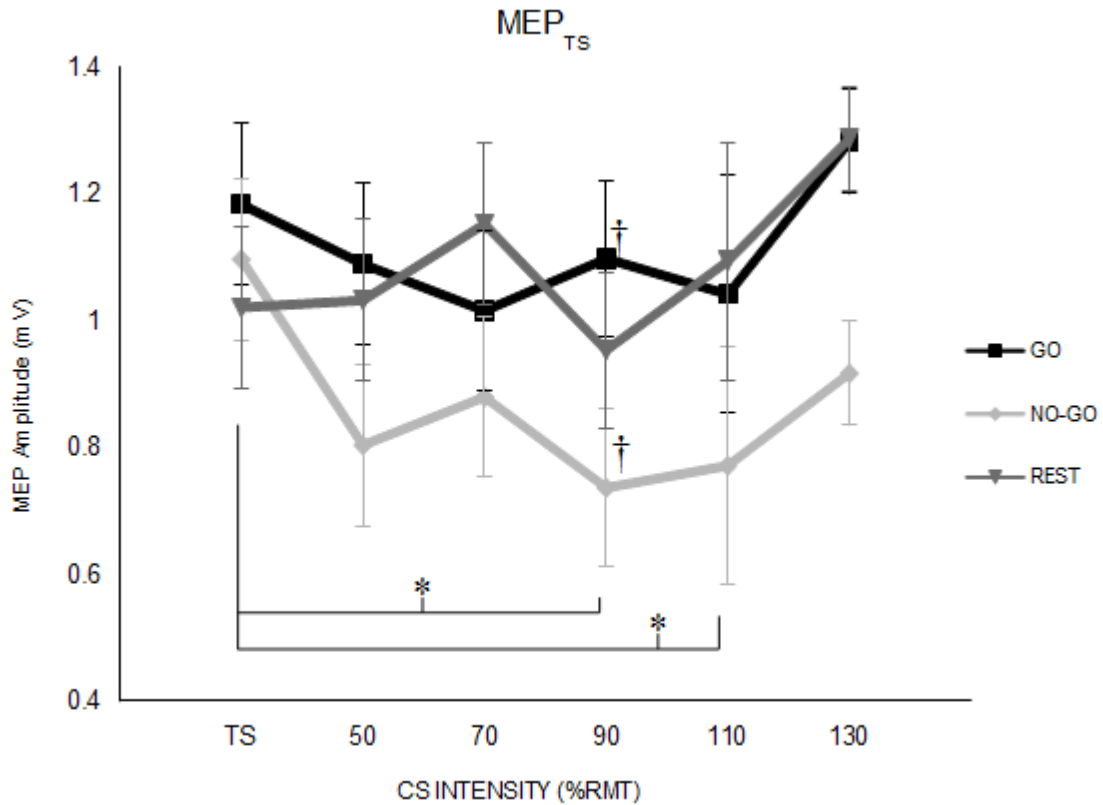
***Figure 7* - Experiment 2.** Group-averaged MEP_{TS} and MEP_{CS-TS} (with standard errors) as a function of conditioning stimulus (CS) intensity as a percentage of resting motor threshold (%RMT). Data represent changes in degree of BA5 conditioning on M1 for each task relative to CS intensity. Values for a given task that are lower than the 'TS' value of the same task have been suppressed by BA5, whilst values greater than 'TS' have been facilitated. In the context of the NO-GO task, BA5 has suppressed MEP_{CS-TS} relative to MEP_{TS} at CS intensities of 90 and 110% RMT, denoted by * in figure ($p < 0.05$). Further, the GO vs NO-GO differences observed in Experiment 1 remain, such that GO MEP_{CS-TS} are greater than NO-GO MEP_{CS-TS} at the 90% RMT CS intensity, denoted by † in figure ($p < 0.05$).

Experiment 3 – Influence of coil orientation on BA5-M1 context-dependent relationship

Experiment 3 investigated whether the GO versus NO-GO differences observed in the previous Experiments persist when 1) the CS over BA5 was positioned to induce current in the AP direction (i.e. away from M1), and 2) when the TS was positioned to evoke MEPs in the AP direction, an orientation that preferentially recruits I3 waves. In total, 11 individuals participated, of whom 9 were included in subsequent analyses (mean age = 25.8 ± 7.0 years, 5 female). One individual was removed from analysis due to excessive background muscle activity and a second individual was removed due to the inability to experimentally constrain the MEP_{TS} between 0.5 and 1.5 mV.

In the PA orientation, the RMT was 40.8 ± 5.7% MSO and the intensity required to elicit ~1 mV MEP was 47.8 ± 8.3% MSO. In the AP orientation, the RMT was 55.1 ± 11.1% MSO and the intensity required to elicit ~1 mV MEP was 63.6 ± 13.3% MSO. As anticipated, the RMT in the AP direction was greater than the PA orientation (one-tailed, paired t-test, p = 0.002). Following computer automated discarding, 92.1 % (± 7.9) of trials were included in the analyses.

The group-averaged MEP_{TS} data are shown in Figure 8A. Two-way ANOVA on the MEP_{TS} revealed no main effect of TASK [$F_{(2,16)} = 2.923$, p = 0.083]. Figure 8B displays the group-averaged normalized MEPs that revealed no effect of ORIENTATION [$F_{(2,16)} = 0.060$, p = 0.942] or TASK [$F_{(2,16)} = 0.123$, p = 0.885] and a significant ORIENTATION*TASK interaction [$F_{(4,32)} = 3.576$, p = 0.016]. In support of the hypothesis, the MEP was significantly larger during GO versus NO-GO in the PA-PA orientation (one-tailed, paired t-test, p=0.049, Cohen's d=0.766). Post hoc Tukey's test revealed that, during the NO-GO task, MEPs in the PA-PA were significantly smaller than MEPs in AP-PA and PA-AP (p < 0.05). No differences between GO and NO-GO were observed in the AP-PA or PA-AP

orientations. In summary, these data indicate that the task-dependent BA5-M1 relationship requires a specific direction of induced current flow in BA5 (i.e. PA, in the direction of M1) and in M1 (i.e. PA).

3.5 Discussion

The role of BA5 in regulating M1 excitability prior to movement in a GO and NO-GO task was explored in three experiments. Experiment 1 examined the effect of BA5 conditioning on M1 excitability. In Experiment 2, the influence of the conditioning intensity delivered over BA5 was investigated across a range of intensities. In Experiment 3, the mechanisms that govern the BA5-M1 interaction during a GO and NO-GO task were explored. The data revealed that BA5 exerts a task-dependent relationship with M1 such that MEPs are suppressed in the context of a NO-GO task. Furthermore, this relationship is dependent on the CS intensity, present only at 90% and 110% RMT CS intensities. Finally, this relationship is dependent on the direction of induced current that acts to recruit specific subsets of neurons. These findings and the potential mechanisms that underpin the BA5 – M1 relationship are discussed. I will also assess contributions of this work to animal and human neurophysiological literature, and provide indications for future directions of this research.

BA5-M1 functional connectivity is context-dependent

The most exciting and consistent finding of this thesis is the observation that the BA5-M1 functional relationship is context dependent; responses are suppressed during a NO-GO compared to a GO task. This effect was medium or greater in size according to Cohen's d and was present in all three Experiments, each of which tested a separate group of individuals. This effect is mediated by the
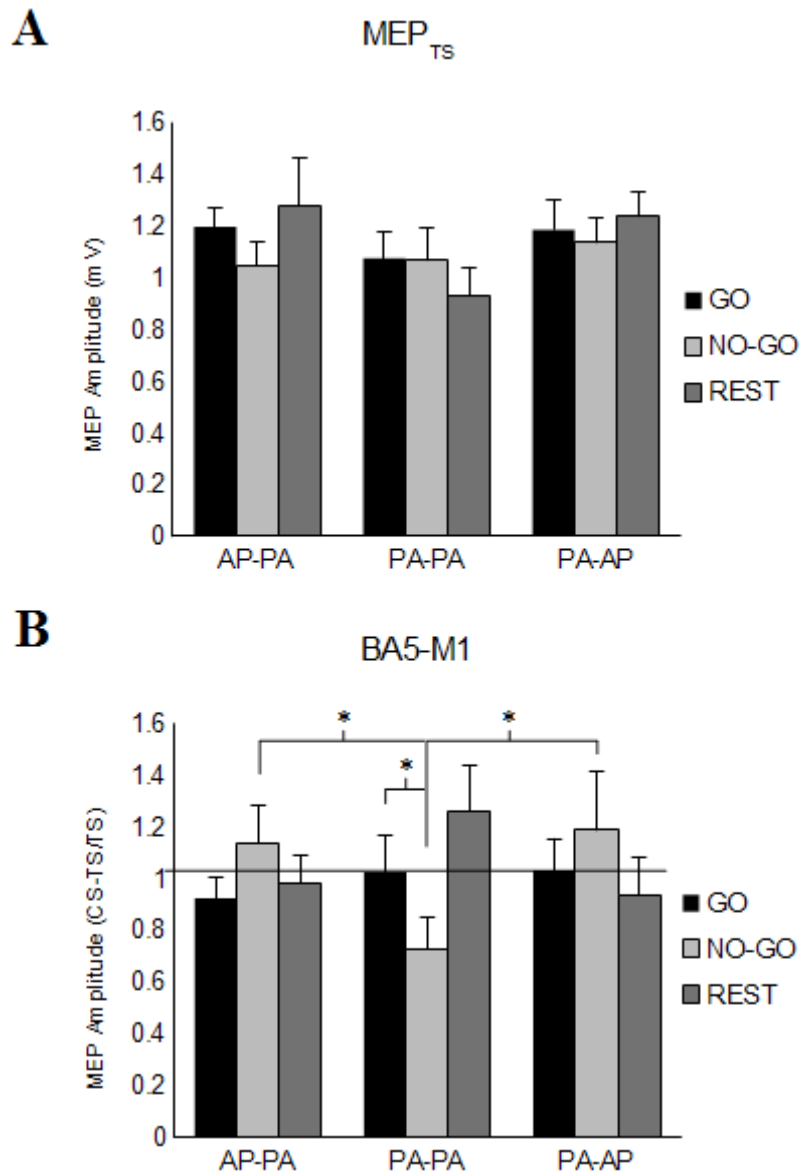
Figure 8 **- Experiment 3. Figure 8A:** Group-averaged MEP_{TS} (with standard errors) are shown. Data represent single-pulse MEP amplitude, in response to TS. **Figure 8B:** Group-averaged MEP_{CS-TS} normalized to MEP_{TS} (with standard errors) are shown. Data represent the level of BA5 conditioning on M1 excitability. Values greater than 1 on the y-axis indicate BA5 facilitation of M1 excitability. Values less than 1 indicate inhibition. The GO/NO-GO differences were observed in the PA-PA orientation ($p < 0.05$). Further, NO-GO MEP amplitudes were suppressed in the PA-PA coil configuration when compared to the AP-PA and PA-AP configurations ($p < 0.05$).

suppression of the MEP in the context of NO-GO, rather than by facilitation of the MEP in the GO task. Suppression of M1 output has been seen in other paired-pulse investigations of GO versus NO-GO differences. For example, van Campen et al. (2013) demonstrated context-dependent suppression of MEPs by conditioning the inferior frontal cortex (IFC) in NO-GO trials but only when 80% of trials were delivered as NO-GO and 20% as GO trials. In contrast, IFC facilitated NO-GO MEPs when the distribution of trial type was reversed. Functional magnetic resonance imaging (fMRI) research also supports differential effects of GO versus NO-GO tasks. Watanabe et al. (2002) observed activation of left hemisphere posterior intraparietal cortex related to NO-GO responses but not GO responses.

To determine whether it is possible to attribute GO/NO-GO differences in M1 excitability to the effects of BA5 conditioning and not just to the nature of the tasks themselves, it is important to control baseline levels of corticospinal excitability across tasks. Hoshiyama et al. (1997) report inhibition of M1 in the context of a NO-GO task 100-200 ms following the NO-GO signal. This finding is corroborated by a study which found similar inhibition of NO-GO versus GO tasks when M1 was probed 100 ms following the NO-GO signal, albeit only whilst participants were in the training phase of the study (Chiu, Aron & Verbruggen, 2013). Leocani et al., (2000) demonstrated inhibition of bilateral M1 following a NO-GO tone, with a timecourse that aligned to the mean reaction time to GO tones, approximately 250ms. Since the GO response was unimanual, this is a result which the authors interpreted as an implied active process behind suppression. In the present Experiments, TS amplitudes were experimentally manipulated to match across GO and NO-GO tasks. Across all Experiments, when GO and NO-GO MEP_{TS} were not ideally matched, NO-GO MEP_{TS} were smaller than GO MEP_{TS}, making BA5 conditioning in the directions observed ($GO_{CS-TS} >$ NO-GO_{CS-TS}) more difficult. The fact that GO vs NO-GO differences persist indicates that these task differences are truly the result of BA5 conditioning, and not attributable to changes in M1 excitability associated with each task. The ability of

BA5 to suppress NO-GO responses whilst MEP_{TS} is greater for the GO task than the NO-GO task tells of a robust functional relationship. The data from this thesis indicates that BA5 is responsible, at least in part, for the suppression of MEPs during a NO-GO task. Thus, for experiments such as those noted above, where only M1 is being probed in the context of a NO-GO task, the suppression of MEPs is due in part to BA5.

The data from the rest task did not show significant conditioning effects in any of the three experiments. Trends for facilitation were observed although these data were non-significant. This makes for an important distinction from other parietomotor paired-pulse studies of areas near to BA5 during rest, namely the intraparietal sulcus and inferior parietal lobule (IPL). Stimulation of IPL facilitates MEPs elicited from ipsilateral M1 (Koch et al., 2007). Stimulation of caudal intraparietal sulcus (cIPS) facilitates ipsilateral MEPs, while stimulation of anterior intraparietal sulcus (aIPS) inhibits ipsilateral MEPs (Koch et al., 2007; Karabanov et al., 2013). The data in this thesis is consistent with a previous investigation into BA5 – M1 connectivity that revealed no significant conditioning during rest (Ziluk et al., 2010). Importantly, no difference was observed between the GO and REST tasks. This further emphasizes the finding that GO/NO-GO differences are driven by suppression of M1 in the context of NO-GO.

BA5-M1 functional connectivity is dependent on parameters of stimulation

In Experiments 2 and 3, it was found that GO vs NO-GO differences are highly dependent on parameters of stimulation. First, the CS needs to be orientated to induce current travelling towards M1. This necessity of specific coil orientation has been found in other parietal (Koch et al., 2007) and premotor (Civardi et al., 2001) to M1 interactions, such that the CS must induce a current traveling

towards M1. The requisite of coil orientation specificity also applies to the TS whereby the GO versus NO-GO differences are observed only when the current preferentially recruits I1 waves (Di Lazzaro, Ziemann, & Lemon, 2008; Sakai et al., 1997). This suggests that suppression of NO-GO responses depends on disynaptic activation of corticospinal output cells rather than polysynaptic activation associated with I3 wave circuitry (Amassian & Cracco, 1987). This finding is in agreement with another paired-pulse study on parietomotor interactions, where stimulation of caudal intraparietal sulcus (cIPS) facilitated contralateral M1 output only when the TS coil induced current in the PA direction (Koch et al., 2009). This could be due to a differential threshold for activation of I-wave circuitry in humans, as has been observed in non-human primates whereby the I1 waves have the lowest threshold of all I-waves as determined by direct electrical stimulation of M1 (Landgren et al., 1962). Therefore, it appears that the BA5-M1 relationship is mediated largely by transmission of neural information from BA5 via the SLF I subdivision to M1, synapsing onto I1 wave circuitry. This proposed mechanism will be discussed in greater detail later in the discussion.

Another parameter of stimulation important for eliciting BA5-M1 functional connectivity is the CS intensity. As shown in Experiment 2, among the intensities tested, GO/NO-GO differences only existed at 90 and 110% RMT. The inference, ubiquitous across the literature, is that CS intensity is important for evoking functional connectivity. Other examples of the importance of CS intensity can be found in parietomotor interactions at rest, as well as intracortical interactions at rest. It has been observed that a CS intensity of 90% RMT over cIPS or IPL facilitates M1 (contralateral for cIPS) while 70% and 110% RMT result in no significant change (Koch et al., 2007; Koch et al., 2009). In a study of changes to cortical excitability following stroke, short interval intracortical inhibition (SICI) evoked by paired-pulse TMS was reduced in stroke patients relative to healthy controls, but only at higher CS intensities (Bütefisch et al., 2003). Although these studies were done at rest, they do align with the

current study, requiring a CS intensity of 90% RMT to elicit functional connectivity. There may be something special about stimulating an area just subthreshold which facilitates these interactions.

Model of BA5 influence on M1 excitability

Based on findings from all three Experiments, this study proposes a model of BA5-M1 functional connectivity which can be seen in Figure 9. Connectivity is dependent on CS coil orientation. In the model, this is illustrated by the pathway connecting BA5 with M1, the SLF. Results indicate that information must travel along the SLF to reach M1 in order that BA5 may condition M1 output. TS coil orientation is also important. Functional connectivity is only elicited with a posterior-to-anterior TS. This makes it likely that I1 wave circuitry mediates this interaction. In the model, SLF projections synapse on interneurons responsible for generation of I1 waves. Finally, CS intensity is important for BA5-M1 interaction. Only with intermediate CS intensities which are near threshold is M1 conditioned by BA5. This is accounted for in the model by different inhibitory and excitatory neuronal populations. When the CS is too low, the inhibitory population is not brought to threshold, and does not contribute any additional input to SLF. Conversely, when the CS is too high, the excitatory population is brought to threshold, attenuating the inhibitory effects. By this proposed mechanism, activity in BA5 is able to regulate tonic activity along SLF which contributes to changes in M1 excitability. Since BA5 role in motor output seems to be inhibitory, and BA5 has direct corticospinal projections, it may directly facilitate antagonist musculature, however this model does not address this possibility.
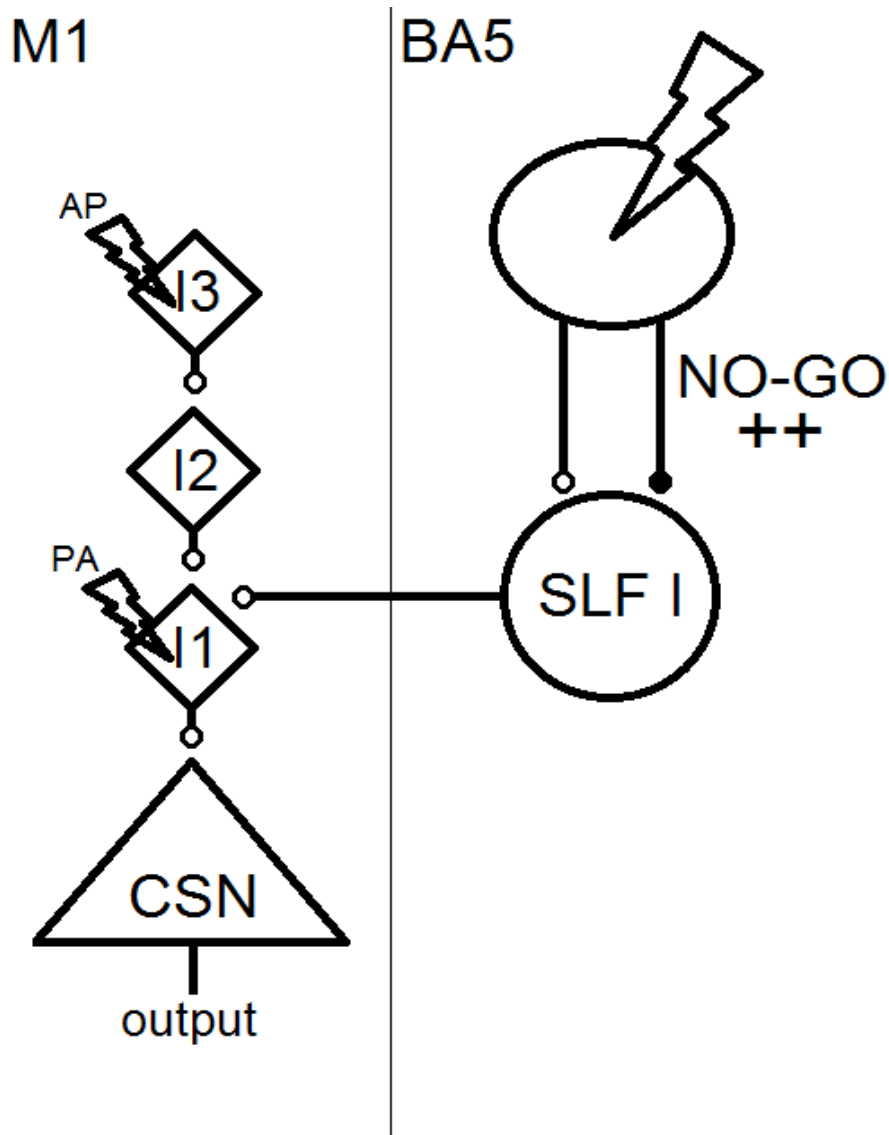
Figure 9 - **Proposed model of BA5 influence on M1 excitability.** In this schematic, **I1**, **I2** and **I3** represent excitatory interneurons within M1. The **CSN** is the corticospinal output neuron. I1 interneurons are preferentially recruited when the TS is delivered in a PA orientation. I3 interneurons are preferentially recruited when the TS is delivered in an AP orientation. A population of neurons within BA5 are activated by the CS, which is represented by the large bolt on the right of the figure. This population acts to either excite or inhibit a population of BA5 output neurons which project onto the SLF, shown in the schematic as **SLF I**. The SLF I population of neurons is thought to be sending information from BA5 to M1 constantly, exerting tonic control on M1 output. When SLF I is excited, its proposed excitatory projections onto I1 wave generating interneurons in M1 result in a facilitated motor output. Conversely, when SLF I is inhibited, the neural information traveling the same excitatory projections is reduced in amplitude, resulting in a reduction of corticospinal output. The context of a NO-GO task upregulates this inhibition. The inhibitory interneurons within BA5 are thought to have lower thresholds for activation compared to the excitatory population.

Relevance to paired-pulse literature

In this study, inhibition of M1 in the context of NO-GO was found, potentially related to disynaptic activation of the corticospinal output neuron (CSN) through early I-wave circuitry. In contrast to these findings, Koch et al., 2007 found that IPL-M1 intrahemispheric interactions relied primarily on late I-wave circuitry, although this interaction was facilitatory rather than inhibitory, and recorded while participants were at rest and not within the context of a task. The present study did not find any facilitatory effects between BA5 and M1. It is possible that BA5 facilitatory effects related to movement planning can only be found in the context of a different motor task. This was the case in a study by Koch et al. conducted in 2008 where, in the left hemisphere, IPL had a facilitatory effect on timecourse to the present study, with facilitation found at 100ms following the cue to move. With respect to interhemispheric interactions with M1, excitatory inputs from cIPS and PMd have been found to rely on early I waves (Bäumer et al., 2006; Koch et al., 2009), similar to this study.

Relevance to monkey literature

The present findings align well with data obtained in monkeys. Rhesus monkeys show sustained activity of BA5 cells when receiving a NO-GO signal, whilst this activity dissipates in PMd cells (Kalaska & Crammond, 1995). This activity could represent a reduction in excitatory input being passed to motor cortex, with the net effect of a reduction in corticospinal output. This is not to say that the neural circuitry responsible for suppressing motor output during NO-GO is isolated to BA5. For example in macaque, potentials have been recorded from prefrontal cortex linked to NO-GO responses in an auditory discrimination task (Gemba & Sasaki, 1990). The authors suggest that this area, the dorsal bank of the principal sulcus, is related to the suppression of motor execution. This study

hypothesizes BA5 connectivity with I-wave circuitry in M1, in line with monkey literature showing that intrahemispheric input to M1 synapses onto interneuronal circuitry responsible for the generation of I-waves (Shimazu et al., 2004).

Considerations

The TMS %MSO was adjusted online to give an MEP$_{TS}$ of ~1 mV. This was difficult to achieve in certain participants because their MEPs would vary greatly from trial to trial and this resulted in these individuals being excluded from analyses. A second challenge was maintaining a similar MEP$_{TS}$ for all three tasks. This was achieved by pre-defining the blocks as GO, NO-GO, or REST so that I could control the % MSO to evoke ~ 1 mV MEPs. Constraining MEP$_{TS}$ to this range is important because too low or too high of an MEP$_{TS}$ leaves no room for modulation of MEP amplitude due to floor and ceiling effects, respectively. However, this resulted in half of all trials being removed from each of the GO and NO-GO tasks for each Experiment since only one MEP$_{TS}$, specific for a single task (GO, NO-GO or rest) could be constrained to the 0.5-1.5 mV range during each block. A third limitation is that the present experiments do not probe the spinal versus cortical contribution of the NO-GO MEP suppression and it possible that spinal contributions exist due to BA5 descending corticospinal projections that may modulate muscle activity at the level of the spinal motorneuron.

Significance and future avenues

This work has added to knowledge of BA5 role in movement and, more generally, knowledge of the PPC role in motor output. Human BA5 is relatively unexplored and these findings may prompt

future research into the specific functional roles of BA5 in motor control. This study impacts future studies looking at movement preparation and motor planning, with BA5 as a new target for investigation. This study's findings represent new neuroscience knowledge made possible by advances in TMS coil technology. Specifically, small diameter customized branding coils (with the handle pointing upward) allowed the two coils to be placed on the scalp in close proximity. This research holds clinical significance in that a new functional pathway through which motor output can be modulated has been discovered. Future avenues for research include investigation into the multisensory integration properties of BA5, incorporating other sensory modalities such as vision. Clinical research informed by this thesis could use TMS to assess BA5 contributions to levels of impairment in stroke and stroke recovery. Furthermore, the relationship discovered between BA5 and M1 prior to movement could be exploited for the development of diagnostic methods for apraxia.

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