Influence of area 5 on primary motor cortex: a paired-pulse TMS investigation in healthy adults

by

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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.

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Abstract

The neural correlates that underpin fine motor control of the hand and their connections with the primary motor cortex (M1) require further investigation. Brodmann's area 5 located in the superior parietal lobule (SPL) is suggested to be an important cortical area involved in the processing of somatosensory input important for precision movements. Area 5 is present in monkey species capable of opposable thumb movements and it is proposed that this area evolved with the ability to execute manual behaviours such as pinch grip. Further, area 5 is dominated by the representation of the hand and forelimb, and has direct connectivity with M1 implicating its role in the control of hand movements. Few studies have investigated the function of area 5 in humans and none have examined the connectivity between area 5 and ipsilateral M1. This thesis presents a novel approach to study the influence of area 5 on M1 output in healthy and awake humans during the processing of somatosensory inputs and during performance of motor tasks involving the hand. Using paired pulse transcranial magnetic stimulation over left area 5 and ipsilateral M1, the connections between the two cortical loci was probed. It was hypothesized that area 5 would facilitate M1 output at short and long latencies during the processing of tactile inputs and during the performance of motor tasks compared to rest. The current results demonstrate that changes in M1 output are task and temporally specific. Facilitation of the motor evoked potential (MEP) was present at short latency of 6 ms during the processing of somatosensory input whereas inhibition was present during conditions where the hand was performing a task with the thumb and index finger. Further, an inhibitory effect was seen at 40 ms during cutaneous stimulation. In experiments 1 and 2, there was no net influence of area 5 on M1 output observed at rest. The findings presented may have revealed a novel path with which to alter the motor output, and possibly movement of hand muscles.

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LIST OF ABBREVIATIONS

AMT	Active Motor Threshold
ANOVA	Analysis of Variance
APB	Abductor Pollicis Brevis
BA5	Brodmanns Area 5
BA 7	Brodmanns Area 7
CS	Conditioning Stimulus
CSN	Cortical Spinal Neuron
DT-MRI	Diffusion Tensor Magnetic Resonance Imaging
D-wave	Direct Wave
PMd	Dorsal Premotor Cortex
PMv	Ventral Premotor Cortex
EMG	Electromyography
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resonance Image
GABA	Gamma (γ) Amino Butyric Acid
HRP	Horseradish Peroxidase
IHI	Interhemispheric Inhibition
IPL	Inferior Parietal Lobule
ISI	Interstimulus Interval
I-wave	Indirect Wave
LICI	Long-Interval Intracortical Inhibition
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MRI	Magnetic Resonance Imaging
MT	Motor Threshold
PPC	Posterior Parietal Cortex
RMT	Resting Motor Threshold
S1	Primary Somatosensory Cortex
S2	Secondary Somatosensory Cortex
SICI	Short-Interval Intracortical Inhibition
SMA	Supplementary Motor Area
SLF	Superior Longitudinal Fasciculus
SPL	Superior Parietal Lobule
TMS	Transcranial Magnetic Stimulation
TS	Test Stimulus
μV	Microvolts

Chapter 1: Introduction

1.1 Overview of thesis

Humans and few nonhuman primates have the ability to execute precision grip and opposable thumb movements, a complex feat involving a combination of peripheral and central networks (Graziano, 2001). Sensory information in the form of proprioceptive inputs is delivered to the cortex by muscles, joints and cutaneous afferents where they are integrated (Radovanovic *et al.*, 2002). One cortical area that appears to be a candidate for the integration of somatosensory input is Brodmann's area 5 (BA 5). BA 5 is present in species capable of opposable thumb movements and it is proposed that this area evolved with the ability to execute manual behaviours such as pinch grip and goal-directed tool use (Padberg *et al.*, 2007). Area 5 is largely dedicated to the representation of the hand and forelimb with little territory devoted to other body parts (Padberg *et al.*, 2007). In addition to its role in processing somatosensory input, BA 5 has been shown to be a major source of intrahemispheric input to the arm and hand area within the primary motor cortex (Strick & Preston, 1978). Collectively, these findings suggest that neural processing within area 5 may be important for the control of hand movements.

Paired-pulse transcranial magnetic stimulation (TMS) involves probing inputs to M1 from an area of interest by first delivering a conditioning stimulus (CS) to a target area followed by a test stimulus (TS) to M1. This technique permits the investigation of the timecourse[A1] and nature of the projection between area 5 and M1. The interaction can be probed during a number of states in awake and behaving humans.

The goal of the research thesis is to examine how area 5 modulates the output from M1 during rest, passively applied cutaneous inputs and during the performance of motor tasks involving the hand. To achieve this goal, paired-pulse TMS was applied to left area 5 and ipsilateral M1 at various interstimulus [A2]intervals (ISI) during rest and during the performance of motor tasks involving the hand. There were two experiments performed.

1.2 Summary of experiments

Paired-pulse TMS was used to investigate the influence of left hemisphere area 5 on the output of ipsilateral M1 in healthy humans. This neural interaction was probed with and without the demands of processing somatosensory input. Electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the right hand for each experiment in right hand dominant participants.

The purpose of Experiment 1 was to investigate the nature and timing of the area 5 to M1 influence. This was achieved by testing two conditions in 13 participants; rest, whereby no task was imposed and the individual was required to relax the hand muscles targeted by TMS, and vibration, whereby the index finger and thumb received vibrotactile stimulation with the hand relaxed. The data was analyzed for the rest condition only, and also by comparing the rest and vibration conditions. The interaction between area 5 and M1 was probed at both short and long ISIs which included 6, 8, 10, 12, 30, 40 and 50 ms.

The purpose of Experiment 2 was to investigate how area 5 modulates M1 output during the performance of motor tasks involving the hand. To achieve this purpose, three motor tasks of the right hand were tested. Each task was hypothesized to engage neural processing within area 5. Twelve individuals were tested during these tasks and the interaction between area 5 and M1 was probed at ISIs of 4, 6, and 15 ms.

1.3 Significance of research

Paired-pulse TMS is particularly useful for revealing the causal interactions between two areas; a conditioning TMS pulse is delivered to an area of interest (i.e. area 5) and the effect on a distinct area (i.e. ipsilateral M1) is measured. The output from M1 is compared with and without conditioning area 5. In this way, we can learn about whether the conditioned area interacts with M1, and whether this interaction is excitatory or inhibitory, the timing that the interaction occurs (i.e. short intervals versus long intervals), and what tasks selectively modulate the interaction.

Novel to this thesis is the attempt to investigate the interaction of area 5 with M1. Results from the two studies presented have provided new information on the nature and timing of the area 5 to M1 influence and the specific tasks that modulate this interaction. Specifically, the data show that the connectivity between area 5 and M1 is altered during isometric contraction and pinch grip and during cutaneous inputs delivered passively. The inference from these findings is that area 5 is involved in the processing of both tactile and motor tasks that require activation of muscles in the thumb and index finger.

The experiments presented in this thesis were performed in healthy adults. Applying TMS to the cortex of healthy individuals can be used to investigate the functional role of area 5 without having to test patients whom often have large and complex lesions that may complicate the interpretation of behavioural results (Koch & Rothwell, 2009). However, the data obtained from the present research can be compared with data collected in patients who show impairments in hand control.

Chapters 3 and 4 in this manuscript include detailed descriptions of each experiment. In the following chapter, a review of the literature most relevant to the thesis topic will be discussed, specifically focusing on the anatomy, function, connectivity of area 5 and properties of neurons as they relate to hand function. The second half of chapter two will focus on the technique of TMS and related topics including; motor evoked potentials, motor thresholds and the applications of paired-pulse paradigms.

Chapter 2: Literature review

2.1 Area 5

2.1.1 Anatomy of area 5 in humans

In humans, area 5 is located in the superior parietal lobule (SPL) between M1, somatosensory (areas 3b and 2), cingulate (area 23c) and superior parietal association cortex (area 7). Scheperjans and colleagues (2005a) used regional and quantitative receptor autoradiography to reveal that area 5 was a heterogeneous cortical region comprised of three sub-areas that varied in cytoarchitecture and distribution of binding site densities. These regions included area 5ci (located around the cingulate region), medial area 5M on the mesial cortical surface near the vertex and lateral area 5L situated on the lateral convexity. The subdivisions were found to show receptor expression patterns similar to adjoining higher-order somatosensory, multi-modal parietal, or cingulate regions (Scheperjans *et al.*, 2005b) providing strong evidence that area 5 is a higher-order cortical area distinct from the primary somatosensory cortex (S1) and M1. This finding may also suggest multiple roles for area 5 in incorporating both sensory and motor functions.

2.1.2 Anatomy of area 5 in monkeys

Recent electrophysiological and anatomical studies have revealed that the area defines the middle of the rostral bank of the inferior parietal sulcus (IPS) and folds around to spread onto adjacent postcentral gyri (Pons & Kaas, 1985). Anterograde and retrograde transport methods have been used to study the functional anatomy of area 5, S1 and motor regions. Jones and colleagues (1978) reported extreme difficulty in distinguishing a clear cut division between primary somatosensory area 2 and area 5 because of the similarities in architecture and variable borders found within adjacent sections of both regions. Despite the difficulty in defining each region, identifiable differences were found. Neurons in area 5 were characterized by the presence of

relatively large pyramidal cells in layer V and in the deeper layers of layer III (Jones *et al.*, 1978;Scheperjans *et al.*, 2005a). Layers IV and V were also found to consist of higher neuron densities than in comparable layers in area 2. Though neurons believed to be involved in hand-manipulation were segregated to deeper layers of area 5, cutaneous and joint neuron types were generally present in any layer from II to VI (Mountcastle *et al.*, 1975).

2.1.3 Connectivity with M1 in monkeys and humans

The precentral gyrus, M1, [A3]plays a key role in the voluntary execution of fractionated voluntary movements of the digits in monkeys (Leichnetz, 1986). Early studies in the rhesus monkey have confirmed that descending pathways from the cerebral cortex to the spinal cord arise primarily in the precentral and postcentral gyri (Pandya & Kuypers, 1969) and these areas may influence ascending pathways by means of intercortical connections. Investigators have identified a dorsolateral region of the gyrus where movements of the hand and arm could be evoked (Strick & Preston, 1978). Afferent and efferent connections of M1 for the control of the upper extremity in the macaque have been examined using retrograde and anterograde labelling techniques. A possible relationship between area 5 and M1 was first suggested by Strick & Preston (1978) who found that area 5 projects and receives fibers from M1 in the macaque monkey. Consequent studies in macaques have confirmed the existence of heavy corticocortical connections between the dorsolateral precentral cortex and area 5 (Leichnetz, 1986). Other connections by authors have been found between area 5 and premotor cortex, supplementary motor area (SMA), secondary somatosensory cortex (S2), visual areas, 7b, and the cingulate cortex (Padberg *et al.*, 2005). [A4] There was notably less retrograde and anterograde labelling found in primary somatosensory cortex (S1), specifically area 1 and 2, compared to higher-order somatosensory regions. This finding suggests that an area 5 influence on M1 hand area is more critical to the output of M1 than inputs from the S1 region (Leichnetz, 1986).

Anatomical studies in old world cebus monkeys (Jones *et al.*, 1978;Strick & Kim, 1978) have demonstrated a substantial direct input to the arm area of the primate motor cortex originating from area 5. Area 5 neurons, which were labeled following horseradish peroxidase (HRP) injections into M1, were found within layer III and adjacent granular layer IV as well as small neurons in layer V within area 5. The presence of these labeled neurons indicates that area 5 may have direct access to the descending motor pathways with M1 through corticocortical connections.

In humans, the existence of pathways connecting non-motor cortical regions and M1 has been explored in vivo with diffusion tensor magnetic resonance imaging (DT-MRI) and indirectly using paired-pulse TMS. Makris and colleagues (2005), used DTI in humans to identify and segment the four subdivisions of the superior longitudinal fasciculus (SLF); the main fiber tract connecting parietal with frontal regions of the brain (Dejerine J., 1895). This study revealed the existence of the SLF I in humans, a white matter tract situated within the SPL and the postcentral and precentral gyrus (areas 4, 5 and 7). Koch and colleagues (2007) used paired-pulse TMS technology to investigate the nature of corticocortical connections between the posterior parietal cortex (PPC) and ipsilateral M1. Results of their study suggested that the direct connectivity between PPC and M1 was contingent on TMS intensity, ISI and coil direction. These findings revealed the potential for applying paired pulse TMS to probe connections mediated by the SPL in humans.

2.1.4 Role of area 5 in sensation

Sensory inputs from cutaneous receptors in glabrous skin deliver information necessary for exploration and manipulation of small objects. The cortical areas responsible for this processing and its interactions with M1 are not clearly understood (Francis et al., 2000). Further, debates surrounding the cutaneous contributions to kinaesthesia by receptors in the hand have existed for over a century (for review see Collins et al., 2005).

Human studies have revealed that stimulation of cutaneous receptors of the hand produce illusions of finger movements (Collins *et al.*, 2005) and patients with median nerve sensory deficits have emphasized motor problems rather than sensory impairments when describing their injury (Johansson & Westling, 1984). Studies applying local anaesthesia to the thumb and index finger have demonstrated changes in the ability of the digits to manipulate objects (Monzee *et al.*, 2003). It appears that cutaneous afferents signalling contact with a target object play an important role in triggering and regulating finger muscle activity during grasping. The loss of tactile sensation is associated with a significant increase in the overall grip force applied to the object during grasping, lifting as well as during static holding (Collins *et al.*, 1999). Studies in patients with lesions restricted to somatosensory cortex have also demonstrated impairments in hand movement (Corkin *et al.*, 1970). Following neurological injury, alterations are seen in somatosensory processing that may result in dysfunctions in object exploration and pinch grip. Collectively, these studies imply that hand movement is very dependent on the integrity of sensory receptors within the hand.

Research on BA 5 response properties in monkeys and the anatomical location within the cortex suggest that the area 5 is tightly linked to sensation (Duffy & Burchfiel, 1971;Mountcastle *et al.*, 1975;Iwamura *et al.*, 2002). Removal of areas 5 and 7 was shown to impair tactile form discrimination contralateral to the side of lesion (Moffett & Ettlinger, 1970). In humans, fMRI revealed that cutaneous vibration delivered to the digits via a piezoelectric device evoked activity within contralateral area 5 (Francis *et al.*, 2000). It has also been shown that the right SPL is involved in tactile object discrimination while the left is predominantly involved in information maintenance (Stoeckel *et al.,* 2004). Other fMRI studies have shown that the SPL is involved in discrimination of spatial features (Mountcastle *et al.*, 1975). Responses of area 5 neurons to various forms of cutaneous inputs applied to the forelimbs of old world monkeys have been previously examined (Mountcastle *et al.*, 1975;Sakata *et al.*, 1973;Iwamura *et al.*, 2002). Results have suggested that populations of neurons sensitive to cutaneous inputs fire during the

application of appropriate stimuli. Though authors have demonstrated difficulty in recording consistent responses from these neurons (Mountcastle *et al.*, 1975;Sakata *et al.*, 1973;Duffy & Burchfiel, 1971), conclusions regarding their response properties have readily shown that area 5 neurons demonstrate a preference for inputs delivered to the hand such as light mechanical stimulation of the skin, stationary pressure and vibration delivered to the hand (Mountcastle *et al.*, 1975;Sakata *et al.*, 1973). An fMRI study in humans (Nelson *et al.*, 2004) revealed that the SPL was activated during high-amplitude vibration to the digit tip of the index finger and also during an attention-demanding tactile tracking task whereby changes in vibration amplitude were used to guide motor behaviour.

2.1.4 Role of area 5 in motor control

The direct corticocortical projection from area 5 to M1 suggests a key role for area 5 in the execution of forelimb movements in monkeys. Similarities between topographic maps of area 5 and M1 in the cebus monkey have also supported this view; both maps are fragmented and consist of multiple representations of the forelimb and portions of the hand (Padberg *et al.,* 2007). Area 5 neurons demonstrate continuously-graded changes in activity during unloaded arm movements in different directions (Kalaska *et al.*, 1983) which resembles several aspects of M1 neurons (Georgopoulos *et al.*, 1982). Though the above authors argue that the posterior parietal cortex is an extension of the motor cortex, evidence suggests that its role in the execution of hand movements is different than that of M1. In a study conducted by Kalaska and colleagues (1990), apparent similarities in arm movement related activity between the two cortical areas was investigated. Area 5 demonstrated a role in the kinematics of movement defined by the spatial parameters (position, direction, displacement) and rates of change of these attributes, while M1 reflected movement dynamics (forces, torques and muscle activity) of a produced movement. This conclusion was based on the finding that area 5 cells are found to be less sensitive to muscle loads than those of M1 cells.

Previous studies using retrograde HRP labelling in the spinal cord of the monkey revealed that layer V within area 5 gave rise to spinal projections that have a major contribution to the corticospinal tract (Murray & Coulter, 1981). According to these authors, area 5 contained 5.1% of the total labeled cells projecting to spinal enlargements in four animals. Retrograde tracers originating in area 5, were confined to the dorsal root of the spinal grey matter (Murray & Coulter, 1981;Coulter & Jones, 1977).

In summary, area 5 may play some role in the control of hand movements, though its contributions and mechanisms of action area unclear. There are likely two possible mechanisms by which area 5 influences the motor output of the hand. These possibilities include a corticocortical influence or directly through corticospinal connections. It is however more likely that the former is a greater contributing factor to hand control. Evidence for this claim is supported by studies in monkeys using retrograde labelling. Injections into the dorsal horn of the spinal cord revealed labelled neurons within area 5. This result was not found when injections were confined to the ventral horn. This suggests that projections to the spinal cord from area 5 have not been shown to directly activate a pathway necessary for the execution of a movement.

2.2 Review of relevant methodology

2.2.1. Transcranial magnetic stimulation

TMS was first introduced by Anthony Barker in 1985 as a non-invasive and painless method of activating motor pathways and evaluating the integrity of the corticospinal tract in awake humans (Kobayashi & Pascual-Leone, 2003). The technique is based on the principle of electromagnetic induction that is described by Faraday's Law. In TMS, a high-current pulse is produced in a coil of wire that then generates a magnetic field that passes perpendicular to the plane of the coil and penetrates the scalp and skull (Ruohonen & Illmoniemi, 2005). A secondary current is then induced in the brain tissue, tangential to the skull and in the opposite direction from the current in the coil. The site of stimulation of a nerve fibre occurs at the point where the current is strong

enough to cause depolarization of its membrane (Hallett, 2007). Since pyramidal tract neurons are aligned perpendicular to the surface of the brain, they are optimally stimulated with a radial, not tangential current flow (Tofts, 1990). Afferent axons and horizontally oriented interneurons however, are most effectively activated by the tangential current spread induced by TMS (Rothwell, 2005). Therefore, TMS may activate pyramidal cells directly, but more commonly this technique recruits them indirectly by synaptic connections via tangentially oriented interneurons (Rothwell, 2005).

The population of neurons activated with TMS depends on a number of factors many of which can be controlled by an operator. These factors are described below and include the strength of the applied electric field, the orientation of the coil with respect to the skull (and corresponding neurons beneath), the frequency of stimulation and the coil type used. The intensity of stimulation controls the magnitude of the magnetic field and capacity for the induced secondary electric field to activate neural tissue. Increasing stimulus intensity results in a greater spread of current within the cortex that may result in greater neural activation (Rothwell, 2005). Coil orientation is a critical factor in determining the direction of current flow since any neuron is best excited by a potential difference along the length of the axon (Kobayashi & Pascual-Leone, 2003).

The shape of the coil determines the spatial refinement of the magnetic field. Two of the most frequent types used in TMS are the circular and figure-of-eight shaped coils. The region activated by the former is found under the circumference of the coil. These coils stimulate a relatively large area and generate a diffuse electric field that makes them useful when there is uncertainty about the exact location of a target region (Kobayashi & Pascual-Leone, 2003). The figure-of-eight coil has its magnetic field concentrated under the centre where the two wings are joined. This coil has the advantage of producing a focal magnetic field and is optimal for targeting specific cortical areas such as studies of cortical mapping.

A single pulse of stimulation delivered with TMS is believed to recruit corticospinal neurons in two different ways (Kobayashi & Pascual-Leone, 2003). The cell can be directly stimulated through depolarization of its initial axon segment resulting in a short latency direct wave (D-wave), or indirectly through synaptic connections that result in multiple longer latency components known as indirect waves (I-wave) that begin 1.5 ms after the initial D-wave and continue at these regular intervals (Rothwell, 2005). The number of D and I-waves depends on the configuration of the stimulating electrodes as well as the intensity of stimulation (Reis *et al.*, 2008).

Evaluating the excitability of the motor cortex is most efficiently achieved by inducing the greatest proportion of I-wave volleys as possible because they are synaptically induced. Since D-waves are a result of stimulation at the axon of the pyramidal tract neurons, they are not greatly influenced by changes of excitability within the grey matter of the cerebral cortex (Di Lazzaro *et al.*, 1998).

2.2.2 Motor evoked potentials

When a single TMS pulse is delivered over motor cortex, a motor evoked potential (MEP) in the form of a muscle twitch can be recorded from a contralateral muscle of interest using EMG. MEPs are typically measured from surface electrodes positioned on the skin overlying a target muscle in the hand. Corticospinal recordings of MEPs represent the summed activity of all active fibers within the tract. Each volley reflects synchronous activity in many single fibers since asynchronous potentials are cancelled and therefore not present in the population discharge (Rothwell, 2005). Factors affecting the MEP recorded from a muscle such as onset latency, amplitude and threshold intensity are influenced by whether the subject is relaxed (at rest) or active (engaging in a voluntary contraction). These differences are described in the section below.

2.2.3 Motor threshold

Motor threshold (MT) refers to the lowest single-pulse stimulation intensity required to evoke an MEP of minimal size at rest and during voluntary muscle contraction (Curra *et al.*, 2002). In most TMS studies, resting motor threshold (RMT) is defined as the lowest intensity necessary to elicit an MEP of at least 50 μV peak-to-peak amplitude in a minimum of 50% of successive trials in a resting target muscle (Kobayashi & Pascual-Leone, 2003). For active motor thresholds (AMT), the minimal peak-to-peak MEP is defined as 200 μV in at least 50% of successive trials in a target muscle during a 10% voluntary muscle contraction (Rothwell *et al.*, 1999). An oscilloscope is normally used to ensure a tonic muscle contraction is maintained.

Threshold values are believed to reflect membrane excitability of corticospinal neurons in the motor cortex as well as the excitability of motor neurons in the spinal cord (Kobayashi & Pascual-Leone, 2003). The different onset latencies between rest and active states reflect the time required for excitatory post synaptic potentials (EPSPs) to depolarize a motor neuron to its firing threshold. At rest, a single EPSP produced by the arrival of a D-wave volley may not bring a motor neuron to its firing threshold. This delays discharge since the motor neuron will have to await the arrival of the first indirect wave 1.5 ms later. During a voluntary contraction however, there are typically motor neurons near firing threshold that discharge at the arrival of the first EPSP. Thus, MEPs have the shortest latency when evoked in an active muscle and longer latencies during resting states. A similar explanation describes the differences in MEP amplitudes existing between rest and active states. Increased excitability of spinal motor neurons during contraction results in larger MEPs at any given stimulus intensity since descending excitation causes more spinal motor neurons to discharge when compared to rest (Rothwell, 2005). This explanation is extended to explain the variation in threshold values for RMT and AMT. RMT is typically obtained at higher stimulus intensities because resting cortical and spinal motor neurons require more excitation to reach discharge threshold than during contraction.

During contraction, there is a higher level of activity within the motor neuron pool, therefore a single TMS pulse can easily provoke an increase in activation in corticospinal neurons that are already highly excitable through muscle contraction.

2.2.4 Paired-pulse TMS

Though the technique of TMS was initially developed as a tool for evaluating the integrity of the corticospinal tract in humans, advancements in paired-pulse protocols have allowed for the investigation of inhibitory and excitatory interactions of various motor and non-motor regions within the cortex. These applications have provided valuable information on the relationship between physiological processes and the anatomical organization of specific brain areas and connected pathways (Reis et al., 2008). Using paired-pulse protocols, the influence of non-primary motor areas on primary motor cortical output has been studied (Reis *et al.*, 2008).

The paired pulse paradigm involves activating putative pathways to M1 by first delivering a conditioning stimulus (CS) over an area of interest, followed by a second pulse, known as a test stimulus (TS) to M1 a few milliseconds later. Changes in M1 excitability attributable to the CS can be quantified by calculating the ratio [CS-TS MEP amplitude/TS alone MEP amplitude]. Depending on the intensity of the CS and the ISI, both facilitation and inhibition may be detected in M1, ipsilateral or contralateral to the site of conditioning. Various studies have confirmed the existence of pathways between cortical regions by delivering a CS over the contralateral M1 (Hanajima *et al.*, 2002), the cerebellum (Fierro *et al.*, 2007), the posterior parietal cortex (Koch *et al.*, 2007) and the premotor cortex (Davare *et al., 2009)*.

Chapter 3: Functional connectivity from area 5 to primary motor cortex via paired-pulse transcranial magnetic stimulation

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Abstract: In non-human primates area 5 is dominated by the representation of the hand and forelimb, and has direct connectivity with primary motor cortex (M1) implicating its role in the control of hand movements. To date, few studies have investigated the function of area 5 in humans or its connectivity with M1. Using paired-pulse TMS, the present study investigates the functional connectivity between putative area 5 within the medial superior parietal lobule and ipsilateral M1 in humans. Specifically, the motor evoked potential (MEP) from the first dorsal interosseous muscle of the right hand was quantified with and without conditioning TMS stimuli applied to left-hemisphere area 5. The timecourse of functional connectivity was examined during cutaneous stimulation applied to the thumb and index finger and also during rest whereby no somatosensory processing demands were imposed. Results indicate that area 5 facilitates and inhibits the MEP at 6 and 40 ms, respectively, during somatosensory processing. No net influence of area 5 on M1 output was observed during rest. We conclude that area 5 has a task-dependent and temporally specific influence on M1 output, and suggest that the interaction between these areas presents a novel path with which to alter the motor output, and possibly movement of hand muscles.

3.1 Introduction

The classic cytoarchitectural studies of Brodmann recognized the biological importance of cortical area 5 (Brodmann, 1999), yet many uncertainties surrounding the functional roles assigned to this region remain (Mountcastle, 2005). In humans and monkeys, area 5 is located in the superior parietal lobule (SPL) (Brodmann, 1999;Scheperjans *et al.*, 2008;Scheperjans *et al.*,

2005a) and projects to primary motor cortex (M1) via the superior longitudinal fasciculus (SLF) (Dejerine J., 1895;Makris *et al.*, 2005) and in monkeys, is a major source of intrahemispheric input to the arm and hand area within M1 (Strick & Kim, 1978). In monkeys, area 5 is dominated by the representation of the hand and forelimb with little territory devoted to other body parts (Padberg *et al.*, 2007) and is present in species capable of opposable thumb movements suggesting its involvement in manual behaviours such as pinch grip (Padberg *et al.*, 2007). Further, neurons within this area in non-human primates receive input from somatosensory areas 3b, 1 and 2 (Jones *et al.*, 1978;Burchfiel & Duffy, 1972;Mountcastle, 2005), and respond to cutaneous or proprioceptive input and in some instances, both (Padberg *et al.*, 2007) integrating across somatic sub-modalities (Duffy & Burchfiel, 1971;Sakata *et al.*, 1973;Mountcastle *et al.*, 1975).

Less is known about human area 5 located within the medial SPL (Mountcastle, 2005). Functional magnetic resonance imaging (fMRI) activation within the SPL is enhanced during tactile object discrimination (Stoeckel *et al.*, 2004b), congruent visual and tactile motion processing (Nakashita *et al.*, 2008) and during movement preparation (Astafiev *et al.*, 2003). The findings in non-human primates suggest that the neural projection from area 5 to M1 may be important for the control of hand movements yet little is known about this neural path in humans. Understanding the functional connectivity between area 5 and M1 may reveal a novel and alternative path to modify motor cortical output and possibly influence hand movement.

Using paired-pulse transcranial magnetic stimulation (TMS), the functional connectivity between non-motor cortical areas and M1 may be probed. In this paradigm, a conditioning TMS (CS) is delivered to the non-motor site and is followed by a test TMS (TS) applied to M1. The amplitude of the resulting motor evoked potential (MEP) is compared with and without conditioning stimuli. This technique has been used to investigate the influence of the posterior parietal cortex (Koch *et al.*, 2007;Koch *et al.*, 2008), ventral (Davare *et al.*, 2008) and dorsal (Koch *et al.*, 2006;O'Shea *et al.*, 2007) premotor cortex on M1 output, and the interaction

between M1 bilaterally (Ferbert *et al.*, 1992;Nelson *et al.*, 2009). This technique has also exposed context-dependent modulations in functional connectivity (Koch *et al.*, 2008;Davare *et al.*, 2009) and can be used to reveal changes in connectivity during altered task demands.

In the present study we investigated the functional connectivity between area 5 and M1 using paired-pulse TMS. We investigated the neural interaction at rest and also investigated the issue of context-dependency by introducing a condition that involved somatosensory processing. In the latter case, cutaneous stimulation delivered to the thumb and forefinger was expected to engage neurons in early and higher-order somatic loci such as area 5. We hypothesized that area 5 would facilitate M1 output at short latencies, similar to that observed from the inferior parietal lobule (IPL) region (Koch *et al.*, 2007). To our knowledge, this is the first investigation of the functional connectivity between area 5 and M1 in humans.

3.2 Methods

3.2.1 Participants

Thirteen right-handed subjects (5 males, mean = 25 years, SD = 5 years) participated. Right-handedness was determined using a subset of the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects gave informed written consent. The study was approved by the Office of Research Ethics at the University of Waterloo and conformed to the Declaration of Helsinki.

3.2.2 Experimental approach

EMG recording

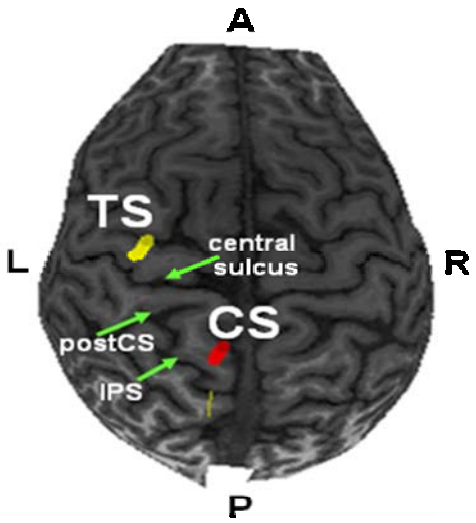
Surface EMG was recorded from the first dorsal interosseous (FDI) of the right hand with 9 mm diameter Ag-AgCl surface electrodes. The active electrode was placed over the muscle belly and the reference electrode was placed over the metacarpophalangeal joint of the index finger. EMG was amplified 1000 x, band-pass filtered between 2 Hz to 2.5 kHz (Intronix Technologies

Corporation Model 2024F, Canada), digitized at 5 kHz by an analog-to-digital interface (Micro1401, Cambridge Electronics Design, UK) and stored on a computer for off-line analysis.

Neuronavigation and transcranial magnetic stimulation

TMS was performed using two customized 50 mm inner diameter figure-of-eight branding coils with Magstim 200^2 stimulator (Magstim, Whitland, UK). The motor hotspot was defined as the optimal location within left-hemisphere precentral gyrus to elicit a MEP in the right FDI muscle with the coil oriented 45 degrees to the mid-sagittal line. From the motor hotspot, rest motor threshold (RMT) was determined as the lowest intensity that evoked a peak-to-peak response of at least 50 μV in a series of 10 consecutive stimuli in the relaxed right FDI muscle (Orth & Rothwell, 2009). The test stimulus (TS) coil was positioned over the motor hotspot and the intensity was adjusted to evoke a MEP of ~1 mV peak-to-peak in the relaxed right FDI. The conditioning stimulus (CS) coil targeted putative area 5 within the medial superior parietal lobule (Figure 3.1A) and was positioned to induce a current flowing posterior to anterior in the underlying tissue. The intensity of the CS stimulus was set at 90% of RMT (Koch *et al.*, 2007). The interstimulus interval (ISI) between the CS and TS was tested at 6, 8, 10, 12, 30, 40 and 50 ms. Ten responses were collected for TS alone and each ISI for a total of 80 trials in each condition (below). A six second interval separated trials. In all subjects, magnetic resonance imaging (MRI) guided Brainsight Software (Rogue Research, Canada) was used to verify and monitor the position of both coils with respect to the motor cortex and area 5 throughout the experiment. MRI was conducted on a 3T GE scanner (172 images) with 3 dimensional fast spoiled gradient recalled inversion recovery sequences using a 20 cm field of view (256 x 256). The mean Euclidean distance between the CS and TS coil locations was 80 ± 16 mm (mean ± SD).

3.1A



3.1B

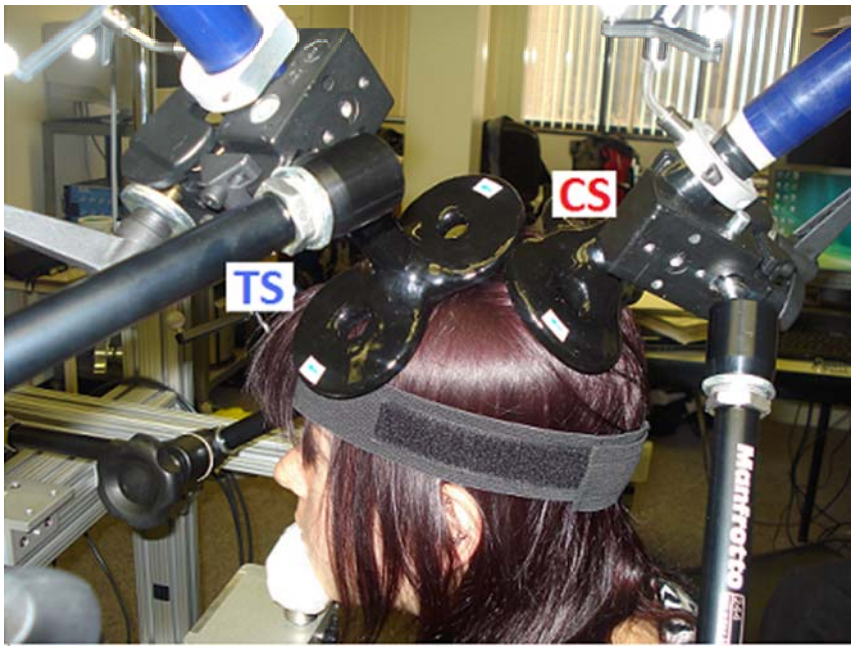


Figure 3.1 **Neuronavigation and positioning of TMS coil**. **A)** Location of the CS and TS targets in one participant. Area 5 was identified as the medial and anterior part of the superior parietal lobule using the anatomical MRI for each participant. **CS**- conditioning stimulus; **TS**- test stimulus; **A**- anterior; **P**- posterior; **L**- left-hemisphere; **R**- right-hemisphere; **IPS**- intraparietal sulcus and **postCS**- postcentral sulcus. **B)** Photograph of a participant with coils positioned over left hemisphere area 5 (red) and ipsilateral M1 (blue).

Conditions

Two conditions were tested. The rest condition required the right hand to be placed within a customized vibrotactile device without any stimulation delivered. The cutaneous vibration condition involved simultaneous stimulation to the volar digit tips of the thumb and index finger on the right hand, contralateral to the CS and TS. Vibration was delivered at 23 Hz using piezoelectric bimorph benders (Noliac, Denmark) via a 3 mm diameter plastic post that drove outward through a hole in an acrylic platform upon which the fingertips rested. Sinusoid waveforms were generated by Labview software using a 5 V output sent to a high voltage linear amplifier (15 x) that displaced the bimorph ~ 70 microns (Nelson *et al.*, 2007). Cutaneous vibration was played for 8 seconds blocks followed by 2 seconds of 'no vibration'. TMS pulses were delivered at least one second following the onset of cutaneous vibration. The order of each condition was randomized across participants. RMT and the intensity to achieve a ~1 mV MEP were determined separately for each condition. Data from our pilot study indicated that the TMS intensity to achieve RMT and ~1 mV MEP was not different between rest and cutaneous vibration conditions. Therefore, the CS and TS intensities in the present study were derived from the rest condition only and used for testing both conditions.

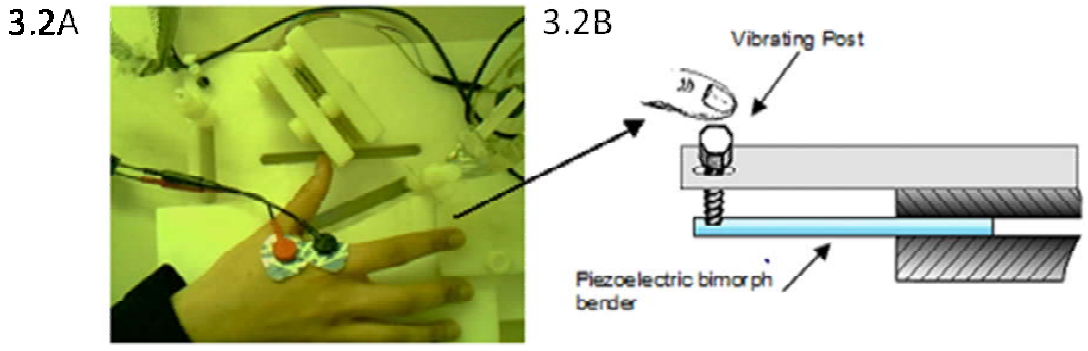


Figure 3.2 Vibrotactile device **A)** Photograph of actual device used to deliver 23 Hz vibration to the digit tips of the thumb and index. Note the orientation of the piezos for the index finger and thumb are oriented according to the plane of the digit. [A5]**B)** Schematic of index finger positioned over acrylic vibrating post with piezo attached underneath.

3.2.3 Data analysis

The peak-to-peak MEP amplitude was measured offline. To determine whether area 5 alters the MEP during rest, a one-way repeated measures ANOVA was performed with within-subject factor *ISI* (8 levels; TS alone, 6, 8, 10, 12, 30, 40, 50 ms). MEP amplitude was not normalized to TS alone for this analysis. To compare the timecourse during rest versus cutaneous vibration, the MEP amplitude was expressed as a ratio of the mean unconditioned MEP amplitude (TS alone) for each participant. Normalized MEP amplitudes were subsequently used in a two-way repeated measures ANOVA with within-subject factors *condition* (2 levels; rest, cutaneous vibration) and *ISI* (7 levels; 6, 8, 10, 12, 30, 40, 50 ms). *Post-hoc Tukey's* tests were used to identify significant differences between conditions. Ratios below one represent inhibition and ratios above one represent facilitation. To test for differences in the RMT and the TMS output intensities to achieve ~1 mV MEP between the two conditions, separate two-tailed paired t-tests were performed. Statistical significance was set at $p \leq 0.05$.

3.3 Results

All participants successfully completed the experiment. RMT was not different between the rest and cutaneous vibration conditions (t-test, $p = 0.71$: rest, 48.2 ± 6.35 (SD); cutaneous vibration, 47.8 ± 5.7 (SD). Similarly, the TMS intensity to achieve ~1 mV MEP output was not different between conditions (t-test, $p = 0.21$: rest, 52.1 ± 7.38 (SD); cutaneous vibration, 50.8 ± 5.81(SD). To test whether area 5 influences M1 output during rest a one-way ANOVA was conducted and revealed a non-significant main effect of *ISI* ($F_{(7,84)} = 1.4$, $p = 0.21$). Figure 3.3 plots the timecourse of the neural interaction during rest. These data indicate that area 5 does not significantly facilitate or inhibit M1 output during rest for the ISIs tested.

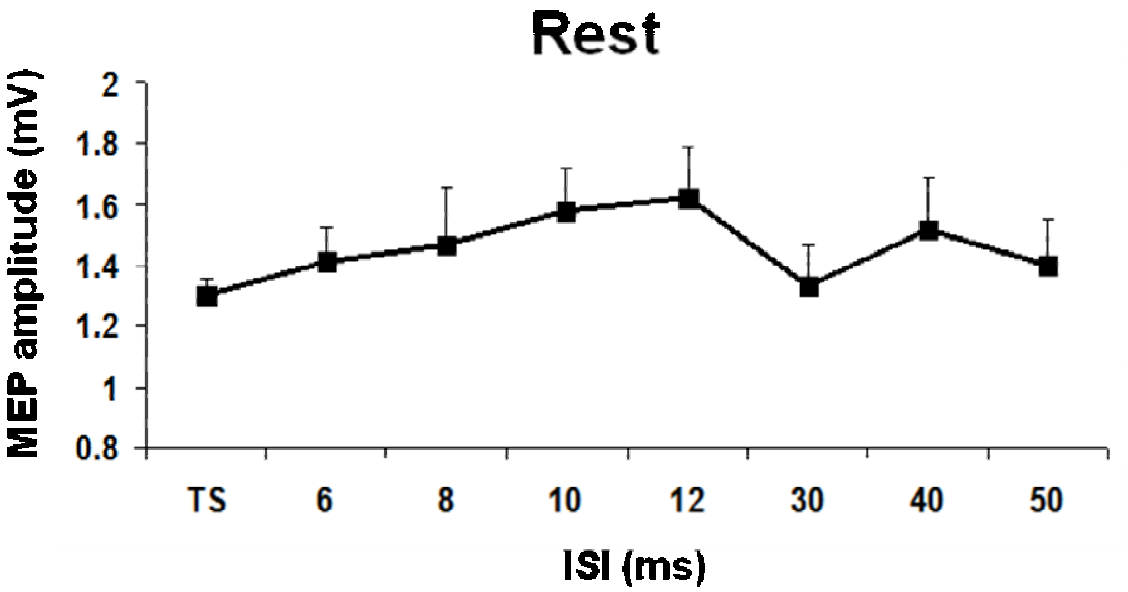


Figure 3.3 **Area 5 influence on M1 output during rest.** Group-averaged (n=13) MEP amplitude (mV) with standard errors for each ISI including TS alone during rest. MEP amplitudes are not normalized to TS alone.

To compare the functional connectivity during rest versus digit vibration MEPs for each ISI were normalized to TS alone for each condition. Two-way ANOVA revealed a significant main effect of *ISI* ($F_{(6,72)} = 2.23$; $p = 0.049$) but not *condition* ($F_{(1,12)} = 0.19$; $p = 0.67$), and a significant interaction between *ISI* and *condition* ($F_{(6,72)} = 2.82$; $p = 0.016$). *Post hoc Tukey's test* revealed significant differences between rest and digit stimulation at 6 ms ($p = 0.007$) and 40 ms ($p = 0.02$). Figure 3 displays the group-averaged data (n= 13) during rest and cutaneous vibration for each ISI. During cutaneous vibration, output from M1 was facilitated at 6 ms and inhibited a 40 ms.

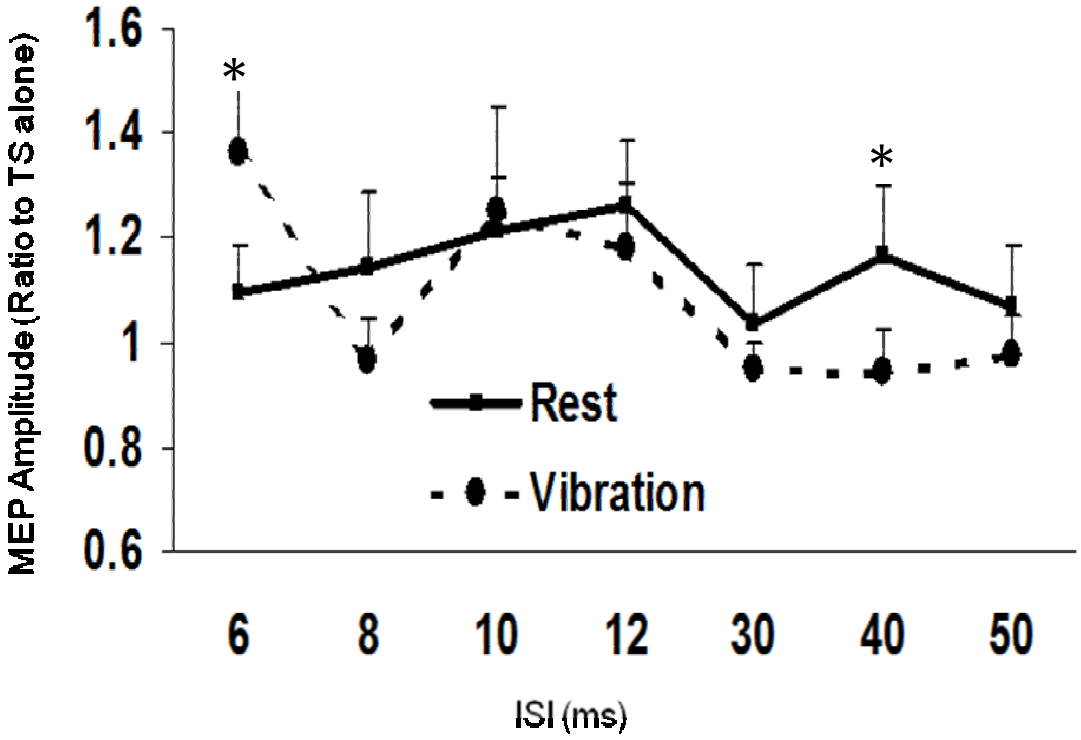


Figure 3.4 **Functional connectivity during rest versus cutaneous vibration.** Group-averaged (n=13) MEP amplitude normalized to TS alone for each condition. Post-hoc Tukey's test revealed significant facilitation at 6 ms and inhibition at 40 ms during cutaneous vibration. * $p \leq 0.05$

3.4 Discussion

Using paired-pulse TMS, we investigated the functional connectivity from area 5 to ipsilateral M1 in healthy subjects. With the hand at rest, we found no evidence of a net facilitatory or inhibitory influence from area 5 on M1. Compared to rest, cutaneous vibration of the digits leads to early facilitation of M1 at 6 ms and late inhibition at 40 ms. The data demonstrate the context-dependent and temporally-specific influence from area 5 on M1 output, and reveal an opportunity to alter motor output from a higher order somatic area.

At rest, the functional connectivity between area 5 and M1 was not different than the unconditioned responses from M1 (TS alone). This finding is unlike those from the IPL region whereby at rest, conditioning the caudal versus anterior intraparietal sulcus (IPS) facilitates or

suppresses the MEP, respectively (Koch *et al.*, 2007). The robust effects reported elsewhere (Koch *et al.*, 2007) may reflect different divisions of the SLF that interconnect these lobules with M1. Evidence suggests that M1 is connected to SPL and IPL via division I and II, respectively (Makris *et al.*, 2005). However, it is noteworthy that we did observe a modest facilitation of the MEP at several ISIs (Figure 3.4) similar to the findings from caudal IPS (Koch *et al.*, 2007) though the effects were not statistically greater than TS alone.

Cutaneous vibration applied to digits 1 and 2 was expected to engage area 5 neurons since responses in monkeys are driven by tactile input (Mountcastle *et al.*, 1975) and the area is dominated by the representation of these particular digits (Padberg *et al.*, 2007). During cutaneous vibration, we observed alterations in the functional connectivity between area 5 and M1; net facilitation was observed at 6 ms and net inhibition resulted at 40 ms. The early facilitation may result from direct glutamatergic projections (Dingledine *et al.*, 1999a) from the SLF synapsing onto lamina II/III excitatory interneurons that synapse on corticospinal neurons in M1 (Weiler *et al.*, 2008a). [A6]The later inhibition may be mediated by the activity of GABA acting on $GABA_B$ receptors on corticospinal neurons or interneurons within M1 similar to the mechanisms thought to mediate long-latency interhemispheric inhibition in humans (Ni *et al.*, 2009;Nelson *et al.*, 2009). Alternatively, the inhibition at 40 ms may be mediated via an influence from area 5 on spinal motor neurons. In monkeys, corticospinal neurons originating in area 5 (Jones & Wise, 1977;Murray & Coulter, 1981) terminate within the dorsal spinal gray matter (Jones *et al.*, 1978;Darian-Smith *et al.*, 1996;Galea & Darian-Smith, 1997;Murray & Coulter, 1981) and may alter the output of ventral horn cells via di- or polysynaptic circuitry within the spinal gray matter. One additional possibility is that facilitation at 6 ms and inhibition at 40 ms could be explained by in-field excitation and inhibition, respectively, if neural mechanisms within M1 are analogous to those in SI (Gardner & Costanzo, 1980b;Gardner & Costanzo, 1980a).

The present study probed functional connectivity during passively applied tactile inputs. However, it is well-known that functions of area 5 are also linked to hand movement. In monkeys, area 5 neurons fire during pre-shaping of the hand prior to object contact (Gardner *et al.*, 2007;Debowy *et al.*, 2001), and during contact with rates that reflect object properties (Gardner *et al.*, 2007), preferred hand postures (Gardner *et al.*, 2007) and movement kinematics (Kalaska *et al.*, 1990)[A7]. Area 5 is largely absent in species lacking opposable thumbs (Padberg *et al.*, 2007) suggesting a parallel emergence of this loci with skilled manipulation involving the thumb (Padberg *et al.*, 2007;Krubitzer, 2007). In humans, surgical stimulation of area 5 may evoke movements, some involving the hand (Lim *et al.*, 1994) and fMRI activation occurs during imagined finger movement (Hanakawa *et al.*, 2003). It is likely that tasks probing the motor aspects of area 5 will demonstrate alterations in functional connectivity, though it will be interesting if the timecourse and direction of changes (i.e. facilitation at 6 and inhibition at 40 ms) are similar to passively applied cutaneous input.

The present study identified the influence of a higher-order somatic region, area 5, to alter the motor output of one muscle of the hand. The effects were only present during a task that involved somatosensory processing and likely engaged areas responsive to such inputs. These findings indicate that targeting neural function within area 5 modifies M1 output, in support of the functional connectivity reported in monkeys (Zarzecki *et al.*, 1978b). It has yet to be determined whether input from area 5 will alter hand movements or the quality of hand control. Targeting the activity of neuronal populations within area 5 may yield novel therapeutic approaches to improving hand control in patient populations. For example, neural injury as a result of stroke or insult may render representations of the hand in primary sensorimotor cortex severely damaged. Targeting an intact alternate path to M1 important for hand control may provide a means to promote recovery of hand function. Alternatively, damage to neuronal processing in area 5 or its interaction with M1 may yield impairments specific to hand function. In one case, stroke affecting left SPL specifically resulted in writing impairments without other

deficits (Otsuki *et al.*, 1999). Future studies in patients with movement disorders affecting hand function such as focal hand dystonia may reveal abnormalities within area 5 and/or in its interaction with M1.

ACKNOWLEDGEMENTS
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Chapter 4- Paired-pulse TMS investigation of the neural influence of area 5 on M1 output during the performance of hand motor tasks

4.1 Introduction

The functions of area 5 are linked to the control of upper limb movement including the hand [humans; (Lim *et al.*, 1994;Hanakawa *et al.*, 2003), monkeys; (Gardner *et al.*, 2007;Georgopoulos *et al.*, 1982;Kalaska *et al.*, 1990;Debowy *et al.*, 2001)]. In monkeys, precision grip, object grasp, or simple isometric contractions of the hand recruit neurons within area 5 (Sakata *et al.*, 1973;Mountcastle *et al.*, 1975;Kalaska *et al.*, 1983) that are thought to be integral to the performance of these tasks (Mountcastle, 2005;Hyvarinen & Poranen, 1978). Anatomical (Strick & Preston, 1978;Leichnetz, 1986) and electrophysiological studies in monkeys have revealed direct connectivity between area 5 and M1, and diffusion tensor imaging in humans suggests connectivity between these areas (Makris *et al.*, 2005). However, in humans, the role of area 5 in the control of hand movements remains unclear (Padberg *et al.*, 2007). Using paired-pulse TMS the role of area 5 in hand control may be investigated whereby a conditioning stimulus (CS) is applied over area 5 and is followed by a test stimulus (TS) over M1. A change in the magnitude of the M1 output of the CS-TS compared to the TS alone suggests an influence of area 5 on M1 output that occurs during a particular motor task. The nature and investigation of early versus late latencies within this neural interaction may be probed to reveal an inhibitory versus excitatory interaction from area 5 to M1. In the present study, the influence of area 5 on M1 output is investigated during motor tasks involving the hand using paired-pulse TMS.

Power grip refers to a palmar opposition grasp in which all digits are flexed around an object such as a tennis ball or water bottle (Ehrsson *et al.*, 2000a). In addition to greater skin-object contact surfaces, power grip also engages a greater number of hand muscles compared to pinch grip. Gripping an object in the hand is an essential component of haptic manipulation and several human imaging studies have revealed activation within medial SPL during such tasks (Stoeckel *et al.*, 2004a;Hinkley *et al.*, 2009b). However, the act of power grip does not always activate area 5

(Ehrsson *et al.*, 2000), results that may reflect the smaller cortical territory devoted to the hand compared to the thumb and index finger (Padberg *et al.*, 2007) or the inability of fMRI to detect such neural activity (Logothetis, 2008). In monkeys, electrophysiological data reveal that some area 5 neurons have receptive fields that encompass the entire hand (Padberg *et al.*, 2007) or both hands (Sakata *et al.*, 1973). [A8]Further, neurons in area 5 are engaged by hand manipulation or reach to grasp tasks (Hyvarinen & Poranen, 1978;Mountcastle *et al.*, 1975). These latter findings, in addition to the greater representation of the hand in area 5 compared to the other body parts, lend support that area 5 is involved in tasks requiring the entire hand such as in power grip (Padberg *et al.*, 2007). In the present experiment, the influence from area 5 to M1 will be investigated during power grip.

Pinch grip, also called precision grip, involves grasping an object between the thumb and the index finger (Flament *et al.*, 1993). In humans, precision grip activates ipsilateral (Ehrsson *et al.*, 2000b), contralateral (Ehrsson *et al.*, 2001) and bilateral (Dettmers *et al.,* 1995) area 5. Electrophysiological studies in monkeys reveal that a disproportionately larger territory in area 5 is dedicated to the thumb and index finger compared to other digits and the hand (Padberg *et al.*, 2005). Further, area 5 is only found to be present in monkey species capable of executing opposable thumb movements (Padberg *et al.*, 2007). Taken together, the authors suggest that area 5 evolved with the ability to execute precision grip (Padberg *et al.*, 2007). Thus, the findings in humans and monkeys suggest that area 5 participates in pinch grip. To explore this further in humans, the present experiment will investigate the neural interaction between area 5 and M1 during pinch grip. Changes observed in the nature and timing of the area 5 to M1 interaction would support the involvement of area 5 in pinch grip.

Both pinch and power grip involve the recruitment of the thumb and index finger. However, it is possible that tasks involving only one of these digits and not both may act to influence the area 5 to M1 interaction. If true, the function of area 5 may be more general than the previous suggestion that area 5 evolved with the ability to manipulate tools (Hinkley *et al.*,

2009a). Electrophysiological studies in monkeys show that isometric contraction of the hand recruits neurons in area 5 (Kalaska *et al.*, 1983;Hamel-Paquet *et al.*, 2006). In humans, a PET study showed activation in left area 5 during simple isometric contraction of the index finger (Dettmers *et al.*, 1995). In summary, there is substantial evidence that area 5 neurons are driven by tasks that do not require both the muscles of the index and thumb to be active. To test the possible role of area 5 in motor tasks that do not require both the thumb and index finger, the area 5 to M1 interaction will be probed during isometric contraction of the index finger only.

To assess the timecourse of the area 5 to M1 interaction, the ISI between the CS and TS will be tested using a range of short and longer latencies that include 4, 6 and 15 ms. Previous research has shown facilitation from the PPC area to M1 during rest at 4, 6 and 15 ms (Koch *et al.*, 2007). Further, Experiment 1 in this thesis demonstrated facilitation of the M1 output at 6 ms.

4.2 Hypotheses

In a paired-pulse TMS study of the ventral premotor cortex (PMv), MEP amplitude was greatest during pinch compared to power grip and smallest during rest when the ISI was between 6 and 8 ms (Davare *et al.*, 2008). Similarly, isometric contraction increased MEPs compared to rest in a TMS study investigating the influence of dorsal premotor cortex (PMd) and supplementary motor area (SMA) on M1 output (Civardi *et al., 2001)*. It was therefore hypothesized that compared to rest, MEPs will be facilitated at all ISIs during all active tasks. Further, comparing the motor tasks, it is hypothesized that the facilitation seen at 6 ms will be greater during pinch versus power grip and least during isometric contraction.

4.3 Methods

4.3.1 Subjects

Twelve right-handed subjects (5 males, mean = 27 years, SD = 4.1 years) participated in the experiment. Right-handedness was determined using a subset of the Edinburgh Handedness Inventory (Oldfield, 1971). All subjects gave informed written consent. The study was approved by the Office of Research Ethics at the University of Waterloo and conformed to the Declaration of Helsinki.

4.3.2 Experimental approach

All subjects were tested during a single 2 hour session [A9]at the University of Waterloo.

EMG recording

Surface EMG was recorded from the first dorsal interosseous (FDI) and abductor pollicis brevis bilaterally (APB) with 9 mm diameter Ag-AgCl surface electrodes on both hands. The active electrode was placed over the muscle belly and the reference electrode was placed over the metacarpophalangeal joint of the index finger and thumb for FDI and APB, respectively. EMG was amplified 1000 x, band-pass filtered between 2 Hz to 2.5 kHz (Intronix Technologies Corporation Model 2024F, Canada), digitized at 5 kHz by an analog-to-digital interface (Micro1401, Cambridge Electronics Design, UK) and stored on a computer for off-line analysis. The EMG signal for right FDI was also passed through a leaky integrator and the level of EMG was displayed on an oscilloscope as a horizontal line. The position of the line was controlled by muscle contraction of right FDI. The EMG signal from right APB was also sent to a speaker and provided auditory feedback to the participant and experimenters that reflected the level of muscle activity.

Neuronavigation and Transcranial magnetic stimulation

TMS was performed using two customized 50 mm inner diameter figure-of-eight branding coils with Magstim 200^2 stimulator (Magstim, Whitland, UK). The motor hotspot was defined as the optimal location within left hemisphere precentral gyrus to elicit a MEP in the right FDI muscle with the coil oriented 45 degrees to the mid-sagittal line. From the motor hotspot, rest motor threshold (RMT) was determined as the lowest intensity that evoked a peak-to-peak response of at least 50 μV in a series of 10 consecutive stimuli in the relaxed right FDI muscle (Rothwell *et al.*, 1999). The active motor threshold (AMT) was defined as the lowest intensity that evoked a peak-to-peak response of at least 200 μV in the right FDI muscle during 10% MVC in a series of 10 consecutive stimuli (Orth & Rothwell, 2009). The test stimulus (TS) coil was positioned over the motor hotspot and the intensity was adjusted to evoke a MEP of ~1 mV in right RFDI. The acceptable range for this value was between 0.5-1.5 mV based on previous paired-pulse studies (Chen *et al.*, 1997;Koch *et al.*, 2007). The conditioning stimulus (CS) coil was positioned over putative area 5 within the medial superior parietal lobule and oriented to induce a current flowing posterior to anterior in the underlying tissue. The intensity of the CS stimulus was set at 90% of RMT (Koch *et al.*, 2007). The ISIs between the CS and TS was tested at 4, 6 and 15 ms. Twenty responses were collected for TS alone and each ISI for a total of 80 trials in each condition (below). A six second interval separated trials. In all subjects, MRI-guided Brainsight Software (Rogue Research, Canada) was used to verify and monitor the position of both coils with respect to the motor cortex and area 5 throughout the experiment. MRI was conducted on a 3T GE scanner (172 images) with 3DFSPGR-IR sequences using a 20 cm FOV (256 x 256).

Experimental tasks

There were four task conditions. In the 'rest' condition the muscles of the right hand were relaxed as determined by EMG feedback from right FDI and APB. For this condition, the CS and TS intensity was determined using RMT. The remaining three tasks involved active contraction

of the right index finger or index finger and thumb and TMS intensities were determined using AMT values. For these conditions the subject was always required to maintain 10% MVC of the right FDI as determined by visual feedback on the oscilloscope. MVC was first determined by a maximum isometric abduction against a stabilized post. The second condition 'isometric FDI' required 10% MVC abduction of right FDI against the stabilized post. The third condition 'pinch grip' required flexion of the thumb and index fingers to grasp a 140 g object using an aperture width of 4.5 cm. The object was equipped with a 20 lbs maximum force, 2-D load cell (Schaevitz Engineering, Virgina, USA). The load cell recorded the sum of forces exerted by the thumb and index finger and the output was amplified by a LVDI conditioner (Daytronic, model 3130, Ohio, USA) and acquired using Signal software. The fourth condition, a power grip was performed such that the palm of the hand grasped a tennis ball. For all active conditions, subjects were instructed to maintain 10% MVC of the right FDI using the visual feedback on the oscilloscope. For rest and isometric index conditions, subjects additionally used auditory feedback to minimize activity of right APB. Short breaks were given to subjects at 4 minute intervals during active conditions to minimize fatigue of FDI muscle. The left hand was relaxed during all task conditions. The posture of the right forearm was held constant throughout all task conditions. The order of task conditions was randomized across participants for each experiment.

4.3.3 Data analysis

TMS intensity values

To determine the average TMS stimulator output for Experiment 2, individual values for resting motor threshold (RMT), active motor threshold (AMT) and corresponding 1 mV intensities were averaged and reported with the standard deviation values.

Similarity of 4 and 6 ms

The ISIs of 4 and 6 ms were initially chosen to probe the possibility of a monosynaptic versus di-synaptic route from area 5 to M1, respectively. However, data suggests that area 5 projections to M1 may require a wide range of conduction times that incorporate the two ISIs (Zarzecki *et al.*, 1978a). Further, a study investigating the influence of IPL on M1 output demonstrated facilitation at both 4 and 6 ms. To investigate whether ISI latencies of 4 and 6 ms were different or could be grouped together as one ISI (i.e. the average of 4 and 6 ms), a preliminary analysis was performed. A two-way ANOVA with within subject factors *ISI* (2 levels; 4 ms, 6 ms) and *condition* (4 levels; rest, isometric contraction, pinch, power) was completed. The purpose was to expose whether there is a main effect of ISI, or an interaction between ISI and condition – if neither exists, the data from 4 and 6 ms will be averaged. This approach is used in other TMS studies whereby the CS and TS intervals are similar and thought to be mediated by the same neural mechanisms (Nelson *et al.*, 2009;Nelson *et al.*, 2010).

Background EMG

To obtain EMG measures for the ongoing muscle activity immediately prior to the CS-TS pair, the average EMG area was calculated over a 50 ms window for each trial of every condition. This analysis was performed for FDI and APB on the right and left hands. One-way ANOVAs were conducted for each muscle on each hand with within-subject factor *condition* (4 levels; rest, isometric contraction, pinch grip and power grip). For right FDI it was hypothesized that active tasks would demonstrate greater EMG activity than the rest condition. Three *a priori contrasts* were conducted and each compared rest versus one active condition and used a Bonferroni correction to accommodate comparisons. *Post-hoc Tukey's* test was used to identify any differences in background EMG between the active conditions. For right APB, it was hypothesized that active tasks of pinch and power grip would have greater EMG activity than rest and isometric contraction. Four *a priori contrasts* were used to compare the following; rest

versus pinch grip, rest versus power grips, isometric contraction versus pinch grip, and isometric contraction versus power grip. *A priori* comparisons for right APB were Bonferroni corrected for four comparisons. *Post-hoc Tukey's* test was used to identify any further differences among task conditions. EMG area was also calculated for the left FDI and APB muscles, the hand that was anticipated to be relaxed throughout testing. A one-way ANOVA was conducted with within-subject factor *condition* (4 levels; rest, isometric contraction, pinch grip and power grip). *Post-hoc Tukey's* test was conducted to reveal any differences amongst conditions.

Rest condition

The rest condition of Experiment 1 did not reveal a statistically significant influence of area 5 on M1 output. To test whether rest would lead to a facilitation or inhibition of the MEP in the present study, a one-way ANOVA comparing non-normalized TS values to the ISIs was conducted. For this analysis, 4 and 6 ms were averaged and included as a single level of *ISI*. Therefore the within subject factor *ISI* had three levels (TS alone, 4-6 ms averaged and 15 ms).

Comparison of conditions

To identify whether area 5 influences M1 at specific ISI and/or conditions, a two-way ANOVA was conducted with within-subject factors *ISI* (2 levels; 4-6 ms, 15 ms) and *condition* (4 levels; rest, isometric contraction, pinch grip, power grip). *A priori contrasts* compared rest versus each active task, isometric contraction versus pinch, isometric contraction versus power, and pinch versus power using a p-value Bonferroni correction for twelve comparisons. Statistical significance was set at $p < 0.05$ for all analyses.

4.4 Results

TMS Intensity values

All participants successfully completed the experiment. Data from one participant was excluded due to the very large MEP amplitudes obtained during TS alone that exceeded the criteria of 0.5-

1.5 mV. Average TMS stimulator output for RMT and AMT was 54% (SD = ± 8.9) and 39% (SD = ± 8.7), respectively. The average TMS stimulator output to achieve ~1 mV MEP output was 58% (SD = ± 8.6) for rest and 41% (SD = ± 8.7) for the active conditions.

Similarity of 4 and 6 ms

The two-way ANOVA revealed a statistically significant main effect of *condition* ($F_{(3, 30)}$ = 4.0; p = 0.0167) but not *ISI* ($F_{(1,10)}$ = 2.84 ; p = 0.123), and no interaction between *condition* and *ISI* ($F_{(3,30)}$ = 0.05; p = 0.986). These results suggested that 4 and 6 ms were not statistically different. Therefore, for the following analyses, the data obtained at 4 and 6 ms were averaged for each participant for each condition.

Background EMG

The ANOVA for right FDI revealed no effect of *ISI* ($F_{(1, 10)}$ = 1.78 ; p = 0.212), no interaction between *ISI* and *condition* ($F_{(3,30)}$ = 0.24 ; p = 0.867) and a statistically significant effect of *condition* ($F_{(3,30)}$ = 98.02 ; p < 0.0001). Figure 4.1 displays the group-averaged (with standard error) background EMG area (mV * ms) for right FDI. As hypothesized, *a priori contrasts* revealed greater activity during isometric contraction (p < 0.001), pinch grip (p < 0.001) and power grip (p < 0.001) compared to rest. *Post-hoc Tukey's* test revealed no difference among the remaining conditions (p > 0.05). For right APB (Figure 4.1B), the one-way ANOVA revealed a statistically significant effect of *condition* ($F_{(3,30)}$ = 10.82 ; p <0.0001). *A priori contrasts* revealed greater activity in pinch grip and power grip compared to rest (p = 0.0005, p < 0.0001, respectively), and greater activity in power grip compared to isometric contraction (p = 0.012). In contrast to the hypotheses, EMG activity in APB was similar during isometric contraction and pinch grip (p = 0.31) suggesting that the isometric contraction task did not isolate activity within FDI but also engaged APB. *Post-hoc Tukey's* test further confirmed that rest was significantly different than isometric contraction (p < 0.05). For the left hand, one-way ANOVA for FDI and

APB revealed no effect of *condition* (FDI, $F_{(3,30)} = 1.91$; $p = 0.1487$, APB, ($F_{(3, 30)} = 1.70$; $p = 0.1881$). Figure 4.2 displays the group-averaged data (with standard errors) for left FDI and APB respectively.

Rest condition

To test whether area 5 influences M1 output during rest, a one-way ANOVA was conducted on the non-normalized MEP data and revealed no effect of *ISI* ($F_{(3,30)} = 2.29$; $p = 0.096$). Similar to the results of experiment 1, these data indicate that during rest, area 5 does not statistically facilitate or inhibit M1 output for the ISIs tested.

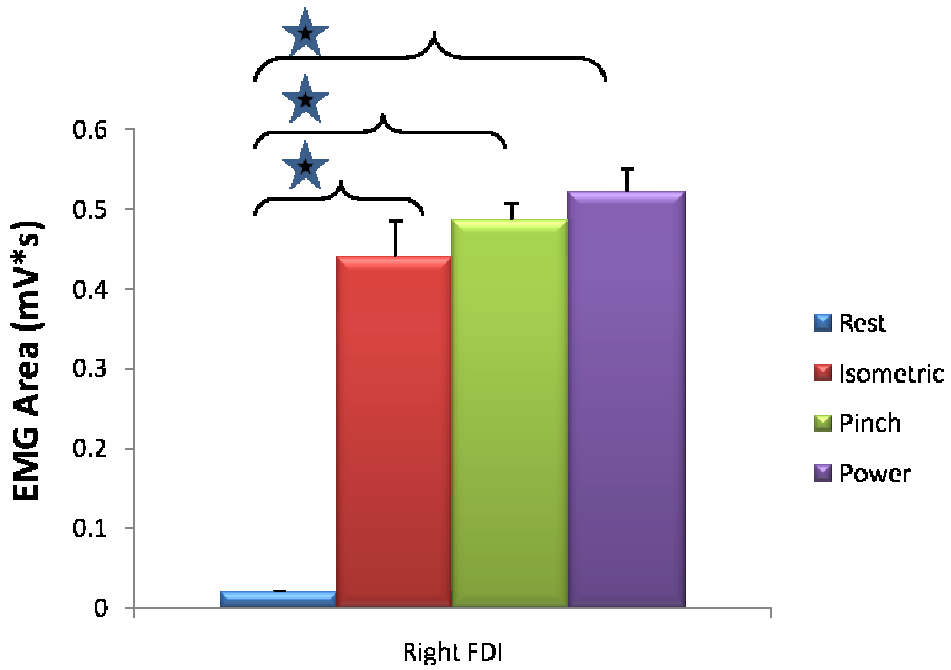
Comparison of conditions

To determine the conditions and ISIs whereby area 5 facilitates or inhibits M1 output, a two-way ANOVA was conducted with within-subject factors *ISI* (2 levels; average of 4 and 6 ms, 15 ms) and *condition* (4 levels; rest, isometric contraction, pinch grip and power grip). To conduct this analysis, the MEP amplitudes for each ISI were normalized to the values for TS alone for each condition. The ANOVA revealed no effect of either *condition* ($F_{(3,30)} = 1.75$; $p = 0.1776$) or *ISI* ($F_{(1,10)} = 2.90$; $p = 0.1196$) but a statistically significant interaction between *condition* and *ISI* ($F_{(3,30)} = 2.93$; $p = 0.0496$). Figure 4.3 displays the group-averaged data (n = 11) for all conditions normalized to TS alone. Values above the horizontal line indicate facilitation of MEP and values below the line reflect inhibition of the MEP. *Post-hoc Tukey's* test revealed that MEPs during rest were increased compared to isometric contraction ($p = < 0.0001$) and pinch grip ($p = 0.0006$) but not power grip ($p = 0.176$) at 4-6 ms ISI. Figure 4.4 plots the four task conditions for this ISI in each individual participant. Eight subjects show this effect for rest versus pinch (subjects 3, 4, 5, 7, 8, 9, 10 11) and nine subjects show the effect for rest versus isometric contraction (subjects 2, 3, 4, 5, 7, 8, 9, 10, 11). *Post-hoc Tukey's* test also revealed that isometric contraction was found to be inhibited compared to power grip ($p = 0.0025$) at 4-6 ms an observation that is seen in

eight participants (2, 3, 4, 6, 7, 8, 9, 10). The alpha values for the comparisons were Bonferroni corrected to account for twelve post-hoc comparisons.

EMG from Right FDI and APB

4.1A



4.1B

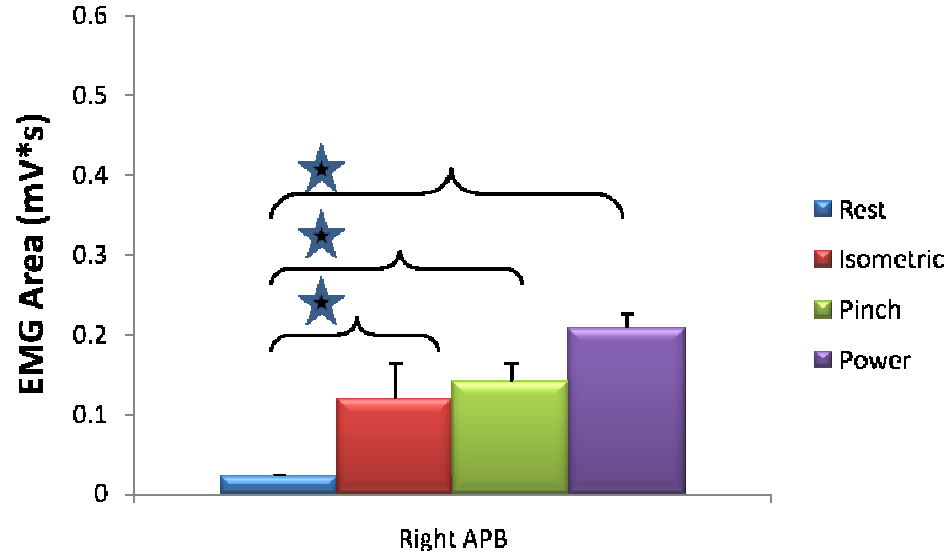


Figure 4.1 **EMG for right FDI and APB prior to CS-TS pairing for each condition. A)** EMG activity for right FDI was greater during the active conditions compared to rest. **B)** EMG for right APB was also greater during active conditions compared to rest. There was no difference between active tasks in right FDI or APB.[A10]

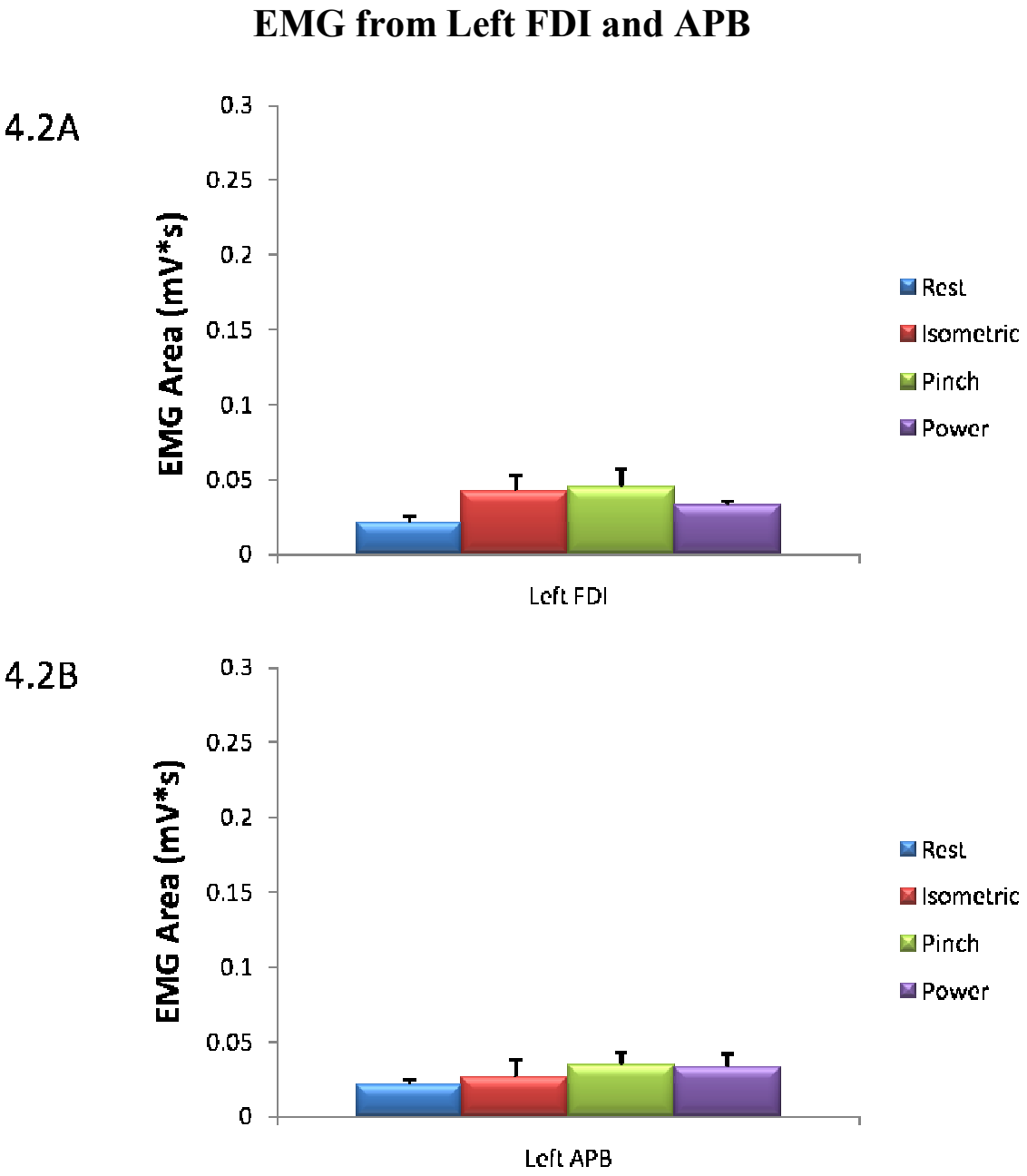


Figure 4.2 **EMG for left FDI and APB prior to CS-TS pairing for each condition**. **A)** EMG activity for left FDI. ANOVA revealed no effect of *condition* (*p*= 0.1487). **B)** EMG activity for left APB also showed no effect of *condition* (*p*= 0.1881).

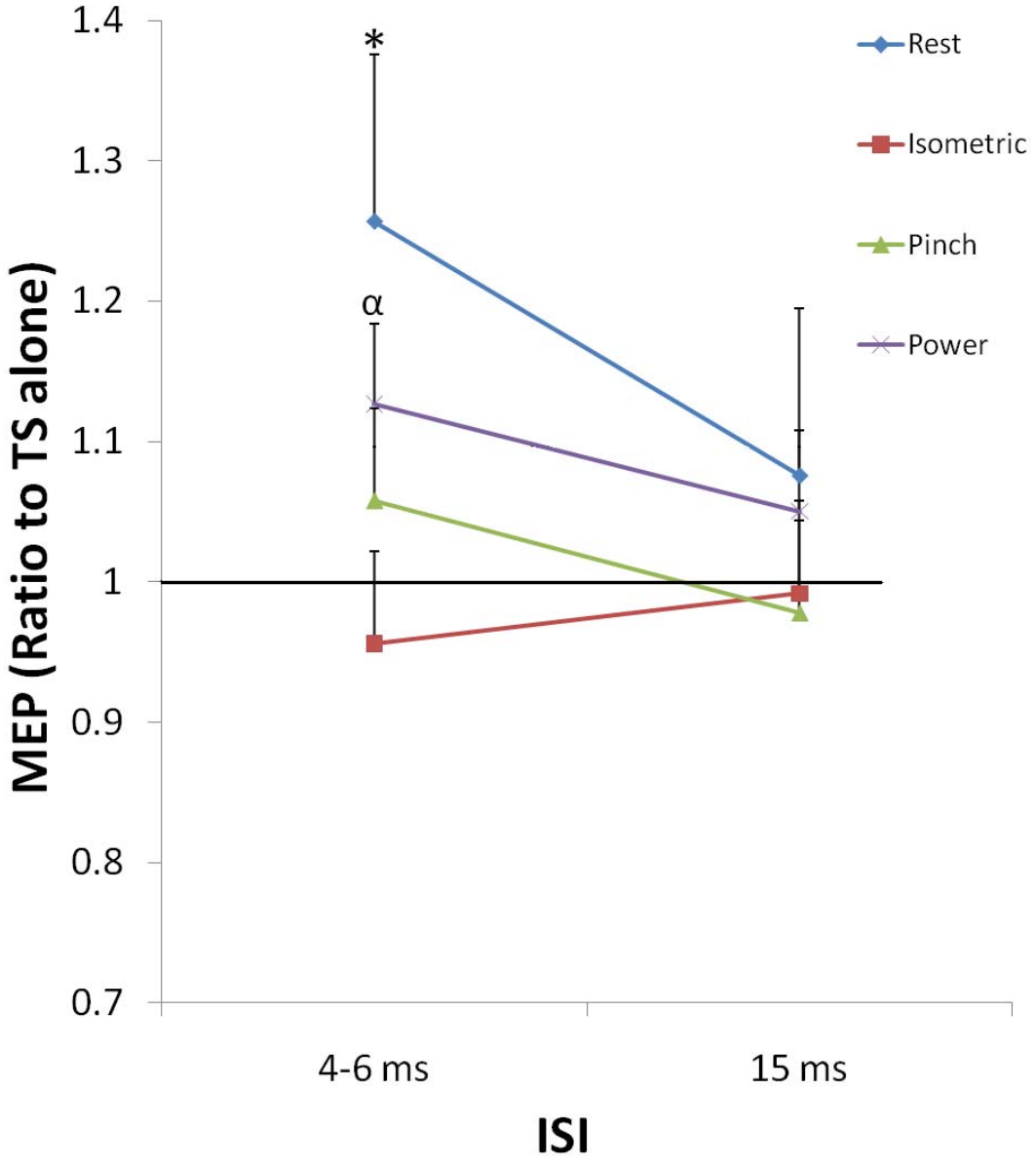


Figure 4.3 **MEP amplitude for each task condition.** Group averaged (n=11) MEP amplitude normalized to TS alone for each of the four conditions. Post-hoc Tukey's test revealed that MEPS are depressed during motor tasks compared to rest as denoted with the asterisk (*). Isometric contraction is also inhibited compared to power grip signified with the α symbol. Significance set at *p* value <0.004.

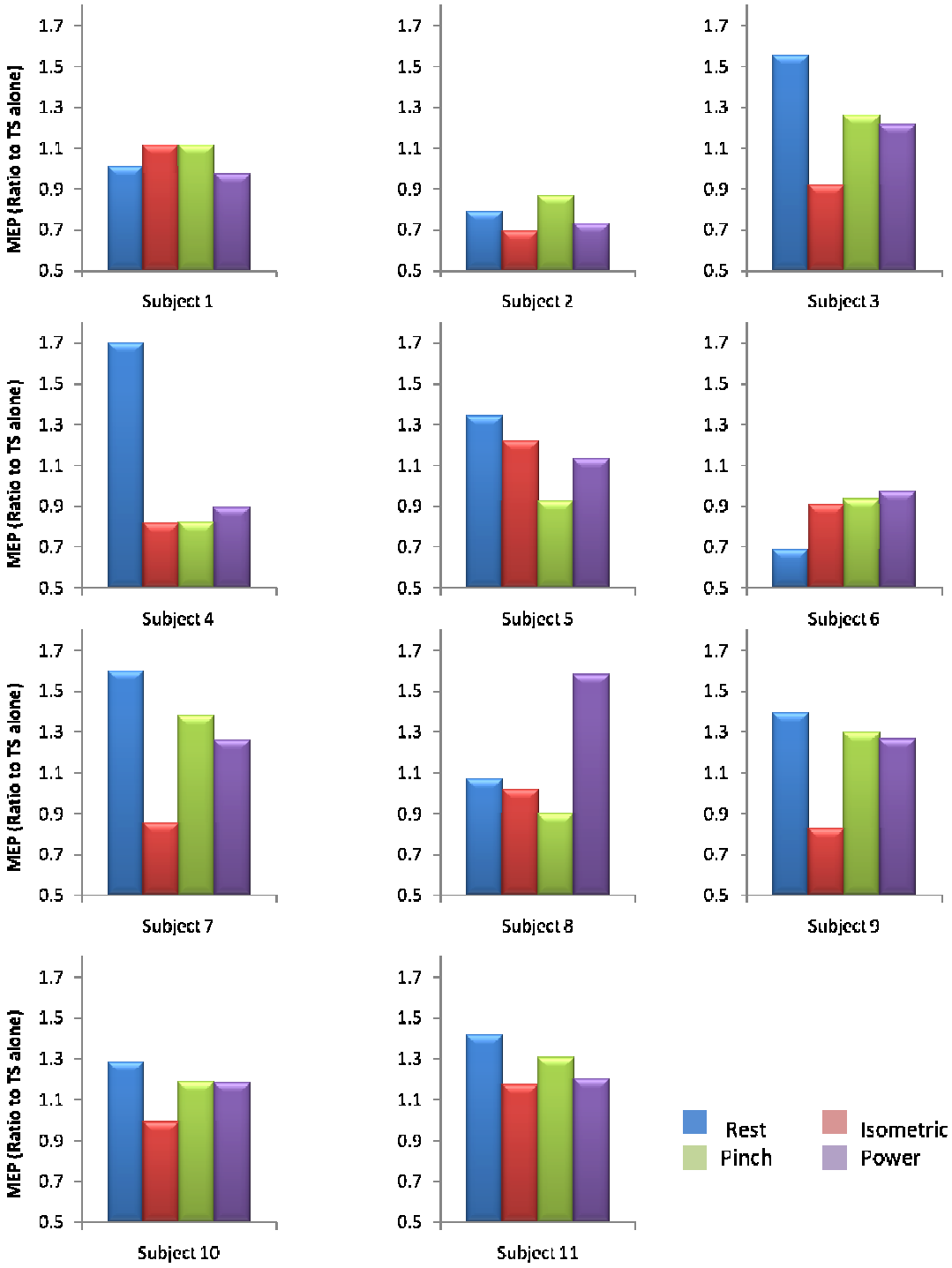


Figure 4.4 **Individual subject data for each task condition.** Subject data for 11 subjects. MEP amplitude normalized to TS alone for each of the four conditions.

4.5 Discussion

The present study investigated the functional connectivity between area 5 and ipsilateral M1, two areas important for the control of hand movement. Using paired pulse TMS whereby a CS was delivered to area 5 followed by a TS to M1, the influence of area 5 on M1 output was studied during motor tasks involving the hand. Several novel observations were found. First, the influence of area 5 on the amplitude of the MEP was dependent on the motor task. Compared to rest, MEPs during isometric contraction and pinch grip were reduced, suggesting that area 5 acts to inhibit M1 output during these tasks. Second, the effects were specific to short latencies and were not observed when the interval between the CS and TS was increased to 15 ms. In addition to these novel findings, the data from the rest condition replicated that from Experiment 1 which found that area 5 had no net influence on M1 output when the contralateral hand was relaxed.

In contrast to the hypotheses, the present study showed that MEPs are reduced, not increased during motor tasks compared to rest. This finding is incongruent to the observations made in studies investigating the influence of ventral premotor (Davare *et al.*, 2008) and dorsal premotor cortex (Civardi *et al., 2001)*. Davare and colleagues (2008) found suppression of the MEP at rest when the ventral premotor cortex was conditioned at 6 and 8 ms seconds prior to M1. This suppression was reduced during performance of a power grip task and facilitation occurred when the motor task required a pinch grip with the thumb and index finger. Based on these results it was argued that the inhibition during power grip is released during pinch grip since this area has been shown to play a role in pinch, not power grip. Similarly, Civardi (2001) found MEP suppression was greatest when the CS was delivered to frontal areas including ventral premotor and mesial supplementary motor cortex 6-8 ms prior the test pulse to M1. This suppression was released during an isometric contraction of the right FDI at 10% MVC. The authors argued that the reduction of inhibition during grasp may be necessary to permit voluntary hand movement. However, the discrepancy between the latter studies and the present work may

relate to differences between premotor and posterior parietal cortex. At rest, parietal cortex appears to facilitate and not inhibit M1 (Ziluk *et al.*, 2010;Koch *et al.*, 2007;Koch *et al.*, 2008)[A11]. This is in direct contrast to the inhibitory influence reported between premotor cortex and M1. The PPC-M1 TMS study by Koch and colleagues (2007) revealed facilitation of M1 at rest by conditioning the left PPC at 6 and 15 ms before delivery of the TS. Second, since the rest state is facilitation, tasks that facilitate M1 when probed from premotor areas are revealed as inhibition when probed from area 5.

The present study demonstrated that MEPs were reduced during isometric contraction and pinch grip but not power grip. The purpose for testing isometric contraction was to determine whether a task that did not engage the thumb was necessary in order for area 5 to modulate M1 output; we did not anticipate the thumb muscles to be active during isometric contraction of the index finger. However, our EMG data clearly shows that the thumb was indeed engaged to a similar extent during isometric contraction and pinch grip. Further the EMG data indicate that both the FDI and APB muscles were engaged similarly in the isometric and pinch grip tasks, the two tasks that led to significantly decreased MEPs. It is unfortunate that our study could not dissociate the thumb and index finger contributions. However, future studies may probe this hypothesis.

The reduction of MEPs during isometric contraction and pinch grip may have biological significance. Inhibition of the MEP may reflect cortical suppression for enhancing motor control by reducing the excitation of cortical spinal neurons within M1. This mechanism may prevent unwanted movements during precision grip tasks where fine motor skills are required. In contrast, strong muscle contractions or tasks that do not require fine motor control such as in power grip, may not have the need to prevent or inhibit specific muscles. Our data for power grip supports this argument since no statistical differences were found between this condition and rest. It is important to note however that power grip demonstrated a similar trend as isometric

contraction and pinch grip. This may suggest that all tasks increase the inhibition but tasks requiring fine more control do this to a greater extent.

Some limitations warrant discussion and could impact the present results. First, the CS may have spread to activate surrounding structures such as area 2 or area 7. Current spread for TMS is highly dependent on stimulus intensity and coil type. Figure-of-eight coils such as the one used in this study, activate approximately a 3 cm diameter distance from the coil hotspot so while it is unlikely that other structures besides area 5 were stimulated, this possibility cannot be ignored. In Davare's (2008) study, a control condition was introduced to test the possibility of current spread to M1[A12]. Conditioning and test pulses were delivered to M1 at the same intervals as the premotor to M1 condition. Results indicated that M1-M1 interaction was different from PMv-M1 suggesting that current spread was not a factor. [A13]This finding supports the claim that the CS spread to M1 is not occurring since the distance from area 5 to M1 is greater than the ~6 cm distance from PMv to motor cortex.

Another consideration is the disparity between area 5 locations in each participant. There have only been general inferences available that place area 5 in the medial SPL, therefore it is possible that areas outside of area 5 were targeted by the conditioning coil. Further, it is possible that muscle fatigue during completion of the motor tasks influenced the present results. The isometric contraction, pinch grip and power grip tasks required participants to perform a constant contraction for 2.5-3 consecutive minutes without a break. Although the intensity of contraction was low (10% MVC) fatigue of the muscles may have occurred. These tasks may have also not mimicked real life muscle activation when gripping an object. [A14]During normal pinch and power grip situations, individuals do not typically maintain the same force of contraction around an object for an extended length of time. For example, holding a pen or ball requires dynamic changes in grip forces applied to an object to ensure that the safety margin (determined by the skin-object friction) is appropriately met (Johansson & Westling, 1984). These necessary

adjustments to grip forces are automatic and change as the object is manipulated in the hand to avoid object slip (Flanagan & Wing, 1995).

Paired-pulse TMS allows for the investigation of corticocortical connections to primary motor cortex from other non motor areas. The present study demonstrates the existence of such connections between parietal area 5 and M1. Research into understanding the interactions between these cortical loci opens the possibility for future studies to document changes in connectivity during normal movement and in disease. Testing how this is impaired in different neurological disorders such as in stroke or focal hand dystonia could lead to novel insights into the pathophysiology of such disorders.

Chapter 5: General discussion

5.1 Summary of results

The goal of this thesis was to investigate the task-specific modulation of the influence of area 5 on ipsilateral M1 output. Two experiments were performed to examine the interaction between area 5 and M1 and its task-specific modulation. Experiment 1 investigated the influence of tactile processing while Experiment 2 explored the influence of motor tasks on the area 5 to M1 interaction. Both experiments were focused on the hand since evidence suggests that area 5 is dominated by inputs arising from this body part (Padberg *et al.*, 2007). Taken together, the findings presented in this thesis indicate that area 5 influences M1 output during specific tasks related to sensory input to the hand or motor tasks involving the hand.

Experiment 1 investigated the effect of passive tactile inputs delivered to the hand and its consequent influence on the area 5 to M1 connection. Participants were asked to relax their right hand while the thumb and index finger received passive stimulation in the form of a 23 Hz vibration applied to the volar surface of each digit tip. Paired-pulse TMS was applied whereby a subthreshold stimulus was first delivered to area 5 (CS) followed by a suprathreshold to ipsilateral M1 (TS) at short and long latencies. This study revealed that area 5 facilitates and inhibits M1 output at 6 and 40 ms, respectively, during the tactile stimulation of digits 1 and 2. These changes were not observed during the rest condition when no tactile stimulation was delivered. These results suggest that area 5 has a task dependent and temporally specific influence on M1 output.

To further investigate the task dependence of the area 5 to M1 interaction, Experiment 2 tested whether motor tasks of the right hand, involving the thumb and index finger, modulated the output of ipsilateral M1. To test for such task dependence, three motor tasks were performed with the right hand during an identical CS-TS paired- pulse paradigm. A selection of short and medium ISIs were tested based on the results of Experiment 1 and previous paired-pulse TMS research examining the interaction of the inferior parietal lobule and M1 (Koch *et al.*, 2007). The

tasks selected for the study included a simple isometric contraction of the index finger, a pinch grip around an object with the thumb and index finger and a power grip around a tennis ball involving the whole hand. Results of Experiment 2 revealed that pinch grip and isometric contraction reduced the MEP at 6 ms compared to rest. Similar to Experiment 1, there was no net facilitation or inhibition present at rest. These results suggest that the influence of area 5 on the amplitude of the MEP was dependent on the motor task. Specifically, tasks requiring a higher level of precision and low muscular activation of the index finger and thumb (such as a precision grip and isometric contraction) have an inhibitory effect on motor output compared to gross motor tasks involving multiple hand muscles at greater forces such as in power grip.

Experiments 1 and 2 present novel information on how peripheral inputs and motor tasks may affect output from M1 via area 5 conditioning. As suggested by the results of each experiment, the possible neural mechanisms and cortical pathways mediating these effects may depend on the processing requirements of area 5. Several possibilities exist as to the neural pathway by which area 5 influences M1 output. Although it is not possible to clearly identify which neural circuits are traversed based on the present experiments, the following section explores candidate mechanisms that may mediate the neural interaction.

5.2 Neural mechanisms mediating influence of area 5 on M1 output

5.2.1 Effects observed at 6 ms

Compared to rest, tactile processing facilitated the MEP at 6 ms. In direct contrast, motor tasks that involved the thumb and index finger reduced MEPs at this same latency. Figure 5.1 displays a schematic to explain the neural mechanisms that mediate both facilitation and inhibition of the MEP at 6 ms. As shown by the red arrow, facilitation may be mediated by an excitatory glutamatergic projection (Bakiri *et al.*, 2009;Dingledine *et al.*, 1999b) via division I of the SLF (Makris et al., 2005) that synapses directly on cortical spinal neurons (CSN) in M1 (Jones et al., 1978;Strick & Kim, 1978) that in turn project to alpha motor neurons in the spinal cord

(Grandjean et al., 2007). Evidence to support the existence of this pathway is derived from anterograde and retrograde transport studies in monkeys that reported reciprocal connections between area 5 and M1 (Jones et al., 1978;Strick & Kim, 1978;Strick & Kim, 1978). The giant Betz cells within lamina V of M1 form the majority of cell bodies that comprise the corticospinal tract (Rivara et al., 2003).

Figure 5.1 displays the path traversed during motor tasks such as pinch, isometric and power grip as shown by the blue arrows. Via this pathway, MEP amplitude is reduced.[A15] This mechanism relies on an inhibitory interneuron within M1 and previous data in mice indicates that the excitatory projection from area 5 terminates on inhibitory interneurons within lamina II/III (Weiler *et al.*, 2008b) of M1. The inhibitory interneuron subsequently synapses on corticospinal neurons followed by a final synapse on alpha motor neurons in the spinal cord. Such inhibitory interneurons are considered to be mediated by the neurotransmitter GABA. Specifically, it is believed that the ionotropic receptor type $GABA_A$ is responsible for mediating fast inhibitory processes including short interval intracortical inhibition (Kujirai *et al.*, 1993) and interhemispheric inhibition.

5.2.2 Effects observed at 40 ms

Compared to rest, tactile processing inhibited the MEP at 40 ms. Figure 5.2 displays a schematic to explain the neural mechanisms that underpin MEP suppression at this longer latency. As shown by the red arrow, the possible pathway is similar to the one at 6 ms, but rather uses another GABA receptor type known as $GABA_B$. $GABA_B$ is the slow-acting metabotropic receptor that functions using a second messenger. It has been shown to mediate the pathway involved in long interval intracortical inhibition (LICI) (Kujirai *et al.*, 1993). However, an alternative possibility, shown by the green arrow in Figure 5.2, is that area 5 influences M1 output via a polysynaptic route that involves remote cortical locations. Anatomical studies in monkeys have shown that

area 5 has direct connections to premotor cortex, supplementary motor area, S2, visual areas, 7b and the cingulate cortex (Pandya & Kuypers, 1969;Jones *et al.*, 1978).

5.3 Facilitation during tactile versus inhibition during motor tasks

Effect of area 5 conditioning at 6 ms was present in Experiment 1 during cutaneous stimulation and in Experiment 2 during the performance of specific motor tasks involving the thumb and index finger. The direction of these effects were found to be opposite to each other. Passive stimulation resulted in a facilitation of the MEP relative to rest while the motor tasks suppressed motor output of the hand. What is the biological significance of the task-specific modulation revealed in the present experiments? It appears as though mechanisms within M1 serve to modify output based on the relevance of the current motor demand. For instance, during cutaneous stimulation, motor cortex may up regulate output to enhance tactile processing of the vibration in the absence of movement. In contrast, during the movement conditions, motor control may take precedence over tactile processing resulting in a down regulation of M1 output. As mentioned in the previous section, inhibition of motor output during tasks requiring fine motor control of the hand serves to prevent unwanted movements. A similar phenomena known as sensory gating is found in sensory systems. A study by Chapman (1994) revealed that tactile perception is reduced if a simultaneous movement is performed by the same limb. Similarly, Brooke and colleagues (1997), reported that sensory evoked potentials are attenuated during active or passive movements of the leg. Sensory gating is a process by which the central nervous system increases the processing of relevant inputs while it minimizes or suppresses inputs not relevant or that require little attentional resources.

5.4 Scientific and clinical significance of the thesis

The present study demonstrated that the area 5 influence on M1 output is task-dependent. Specifically, it was found that area 5 reduces M1 output during the execution of motor tasks

involving the thumb and index finger, and conversely, facilitates M1 output when cutaneous inputs were applied to the tips of these digits. The above findings provide novel neuroscientific information for understanding area 5's role in the generation of hand movements. Further, the information discovered in this thesis provides a basic framework into the underpinnings of facilitatory and inhibitory pathways between area 5 and M1. Understanding the balance between these pathways in healthy adults may further our knowledge of similar pathways in clinical populations. [A16]This may be achieved by either increasing or decreasing motor output depending on the requirement of the patient. Neural injury as a result of a stroke may cause irreparable damage of the primary sensorimotor cortex. In fact, it has been shown that over 50% of stoke patients lose functional use of the affected upper limbs for the remainder of life (Duncan *et al.*, 1992). Therefore, maximizing on pathways to M1 that remain intact post stroke, may promote hand recovery. The present study has identified two ways in which M1 may be modulated via area 5. Increasing M1 excitability may be achieved through cutaneous stimulation to the hand while suppression of M1 output can occur with simple isometric contraction and pinch grip. By optimizing on this information, a balance between facilitatory and inhibitory circuits within M1 may be restored.

Mechanisms at 6 ms

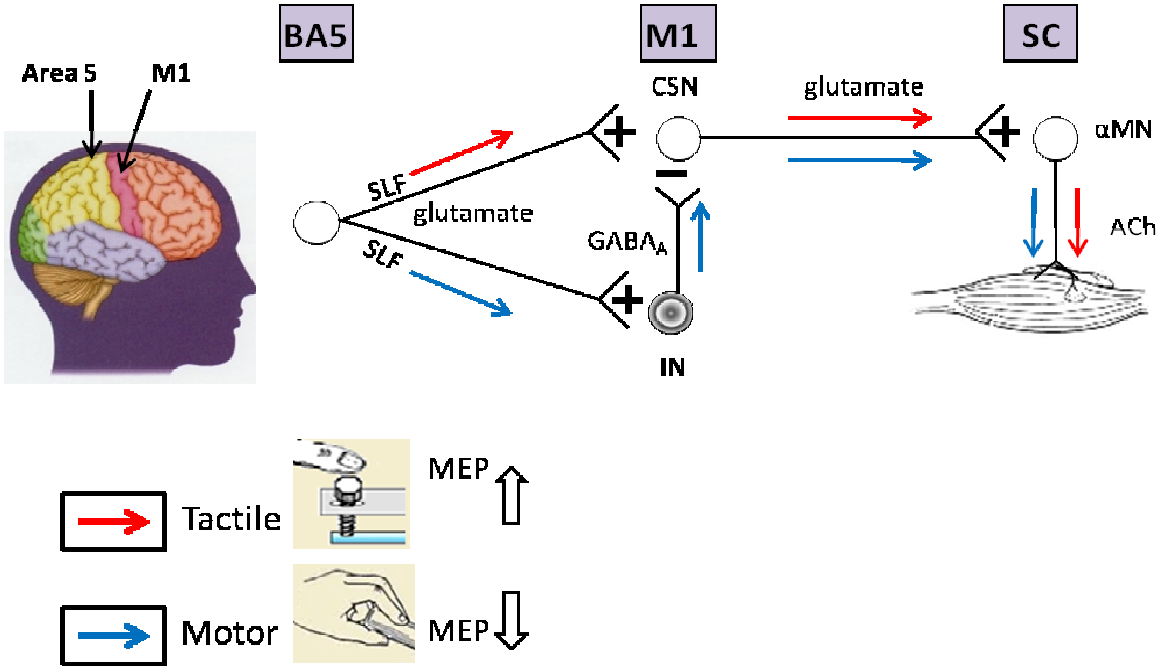


Figure 5.1 **Neural mechanism mediating effect at 6 ms.** Schematic depicting area 5 to M1 connection. Red arrows represent the possible pathway traversed during tactile stimulation. This pathway results in an increase in M1 output. Blue arrows show disynaptic path occurring during motor tasks. This pathway results in an inhibition of the MEP. **BA5-** area 5, **SLF-** superior longitudinal fasciculus, **IN-** inhibitory interneuron, **CSN-** cortical spinal neurons, **SC-** spinal cord, **αMN-** alpha motor neuron, **ACh-** acetylcholine. Legend displays task represented and direction of MEP output.

Mechanisms at 40 ms

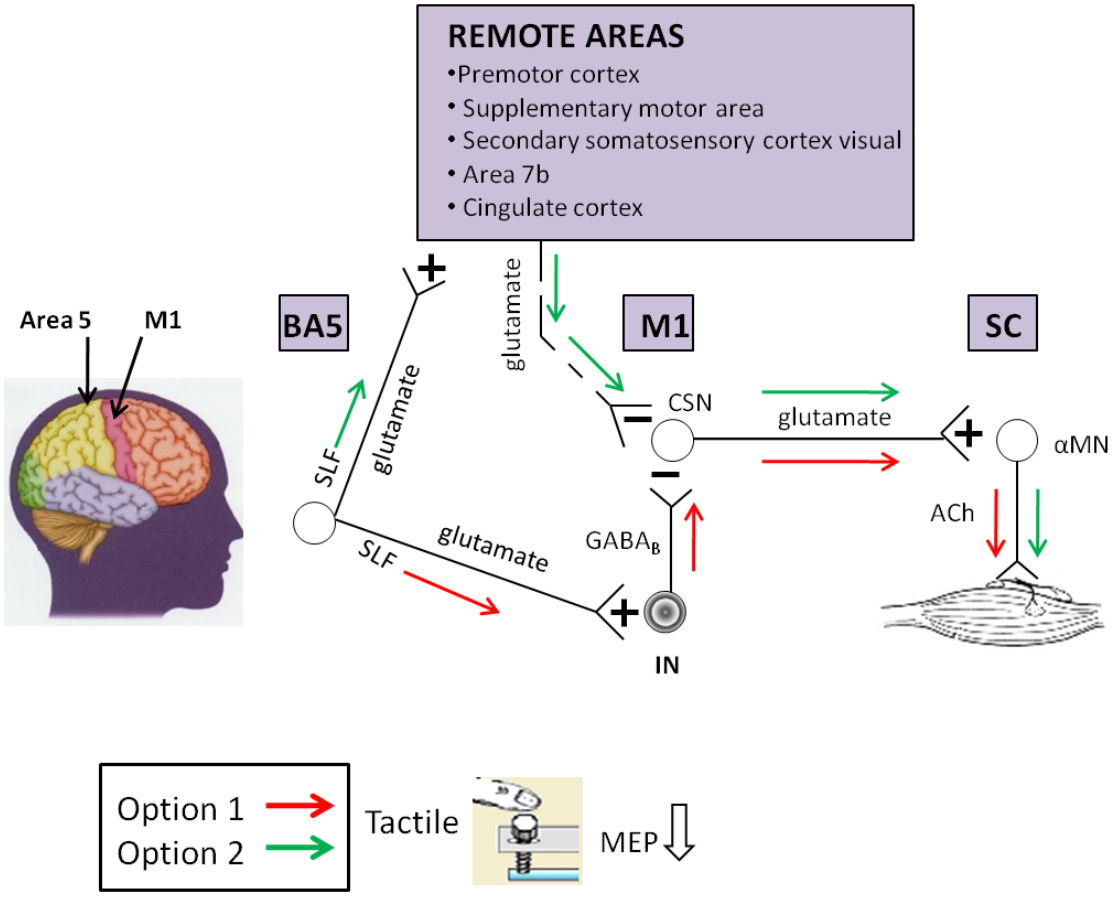


Figure 5.2 **Neural mechanisms mediating effect at 40 ms.** Schematic depicting area 5 to M1 connection. Red arrows represent the possible pathway traversed during tactile stimulation via remote brain areas that ultimately synapse in M1. This pathway results in a decrease in M1 output. Red arrows show a direct pathway between area 5 and M1. This pathway results in an inhibition of the MEP. **BA 5-** area 5, **SLF-** superior longitudinal fasciculus, **IN-** inhibitory interneuron, **CSN-** cortical spinal neuron, **SC-** spinal cord, **αMN-** alpha motor neuron, **ACh-** acetylcholine.

5.5 Limitations of current research

The physiological mechanisms underpinning TMS effects on the underlying cortex is not well understood in areas outside of M1 (O'Shea *et al.*, 2008). Previous paired-pulse studies have overlooked the possibility that the observed effects may not be related to functional connections between cortical areas, but rather to the effect of TMS within the target region. For instance, it is not clear whether the area directly beneath the CS is being activated or inhibited. In fact, it has not been proven (or disproven) that the conditioning pulse is generating excitatory projections to neurons within M1 via the SLF. It may be that the effects of the CS on area 5 acts to excite inhibitory interneurons within area 5 which in turn inhibits area 5 output to M1. This possible mechanism would result in the inhibition of the SLF rather than recruitment of this white matter tract. Disentangling this effect would assist in dissociating which neural models presented in this thesis actually account for the results shown.

Another limitation of this research is the questionable location of the CS coil over the area 5 target. As described in the literature review, the location of area 5 within the SPL is not differentiated from area 7 by any gross anatomical features. Only post-mortem brains can provide exact location of area 5 in the SPL. Therefore while anatomical studies have shown that area 5 is in the medial aspect of the lobule, it is possible that portions of the coil were placed over adjacent areas such as area 2 or area 7. To overcome this particular limitation, an fMRI activation map for each participant may be obtained. This would assist in determining the proper position for the TMS coil over the 'hand area' within the area 5 borders.

5.6 Future avenues

The goal of this thesis was to examine how area 5 modulates the output of M1 during rest, passively applied cutaneous inputs and during the performance of motor tasks involving the hand. [A17]While this investigation merited novel findings regarding area 5's role in modulating the motor output of M1, there is potential to expand this investigation to include other phases of

movement. For instance, single-unit studies in macaques demonstrated that area 5 is involved in motor preparation (Bioulac *et al.*, 1995) and in preshaping the hand prior to object grasp (Debowy *et al.*, 2001). Lesion studies in monkeys have also revealed that removal of area 5 creates deficits in the coordination of arm and hand velocity as well as the postural relationship between the hand and wrist (Rushworth *et al.*, 1997). Applying TMS during the phases of a reach-to-grasp movement may reveal an additional role for human area 5. It would also be of interest to investigate bilateral movements of the hands during object manipulation since area 5 has been shown to have bilateral receptive fields (Iwamura, 2000;Iwamura *et al.*, 2001;Taoka *et al.*, 2000).

5.7 Conclusion

This thesis is the first investigation into the functional connectivity between area 5 and M1 in humans. Paired-pulse TMS has allowed for this influence to be probed in awake and functioning participants. The results revealed that area 5 influence on M1 is task dependant. By using short and long latencies, this research explored the possible neural mechanisms and pathways that mediate this interaction. It has been shown that various neural circuits may be involved that include inhibitory, excitatory and possible connections in remote brain areas.

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