INVESTIGATING MOTOR INHIBITION IN MOTOR IMAGERY

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Dedication:

This manuscript is dedicated to the memory of my late father. The value he placed on education and his inherent curiosity, led me to my introduction to the concept of scientific enquiry at a young age. He encouraged creativity and rational thinking in my early attempts to delve into research and his guidance inspired me to continue to develop the toolset for which he laid the foundation. Unfortunately, he is not here with me to read this document and provide me with his insight. I miss our incessant banter and, more importantly, I miss him dearly.

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Abstract:

Motor leaning can be achieved via motor execution and motor imagery (MI), where the difference is the lack of overt movement in MI. Many theories exist as to the timing and source of this motor inhibition, however no one theory has been proven. This project addressed this gap in the literature by examining the timing and source of the inhibition of overt movement in MI. We hypothesized that in MI, inhibition would be seen in the motor cortex, resulting from mechanisms involved in generating the simulation of movement mediated by the supplementary motor area (SMA). Through analysis of neuroimaging data, we confirmed the presence of motor inhibition in MI, and conclude that this inhibition likely occurred during the motor planning process. However, SMA was not the source of this inhibition, as decreased activity was also observed in premotor regions. These findings are discussed in the context of motor inhibition theories.

List of Abbreviations Used:

BOLD – blood oxygen level dependent

CB - cerebellum

CST – corticospinal tract

PFCDL – dorsolateral prefrontal cortex

PMCDL – dorsolateral premotor cortex

ERD – event related desynchrony

ERS – event related synchrony

fMRI – functional magnetic resonance imaging

IFG/PFCVL – inferior frontal gyrus

IPL/IPC – inferior parietal lobe

LJT – laterality judgement task

MEG – magnetoencephalography

M1 – motor cortex

MEP(s) – motor evoked potential(s)

ME – motor execution

MI – motor imagery

PostLobe – posterior lobe of the cerebellum

PPC – posterior parietal cortex

PMc – premotor cortex

ROI(s) – region(s) of interest

rTMS – repeated transcranial magnetic stimulation

S1 – somatosensory cortex

SPL/SPC – superior parietal lobe

SMA/PMCMed – supplementary motor area

TFR – time frequency response

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Chapter 1: Introduction

Motor learning is a fundamental human behaviour that refers to one's ability to acquire or improve a motor skill through repetition of the movement with the provision of feedback about performance [1]. Motor learning has importance in a wide variety of fields including occupational performance, athletics and rehabilitation. Motor learning can be realized using a variety of practice modalities, yet the two most well understood are actual and imagined practice, termed motor execution (ME) and motor imagery (MI) respectively. The primary behavioural difference between these two modalities is the lack of overt movement as an outcome of MI, whereas the opposite is true of ME.

While different in regard to their outcome, the neural representation of ME and MI are similar, in that the neural network underlying each have considerable overlap. However, differences do exist between these neural networks, most noticeably the involvement of the primary motor cortex (M1) in MI. While recent research has consistently identified M1 as part of the MI network, the magnitude of M1 activity in MI is greatly reduced in comparison to ME [2,3]. A plausible explanation for the reduction or absence of M1 activity in MI is motor inhibition. Throughout, we define motor inhibition as the suppression of a planned overt behaviour, in this case movement [4]. Three theories have been suggested to explain this phenomenon: 1) motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding ME; 2) cortical areas exert inhibitory influences to suppress the mobilization of the motor command

after the mental representation of the plan has been formed; and 3) sub-cortical structures (namely at the level of the spinal cord) may be responsible for motor inhibition after the signal is sent by M1 [5]. Recent work examining the contribution of spinal level structures to motor inhibition in MI has largely addressed the third theory, leaving the mechanism underlying motor inhibition in MI to cortical structures [6]. To further our understanding of the mechanisms underlying motor inhibition in MI, and to investigate the remaining theories, the purpose of this project was to identify the timing and source of the motor inhibition occurring in MI.

To address our two study objectives, which are 1) to verify if, and determine when, motor inhibition occurs during the performance of MI and 2) to determine which cortical areas are responsible for this inhibition, neuroimaging data of a two-target grasping task performed using MI or ME was obtained. Hypotheses related to our objectives were: 1) motor inhibition would occur in M1 contralateral to the movement being performed, and that this inhibition would occur as a result of mechanisms related to the process of generating a mental representation of a motor task; and 2) the brain region responsible for this inhibitory effect would be the supplementary motor area (SMA), which may be influenced by other areas of the brain within the MI network located in the frontal or premotor regions.

The hypotheses were addressed by investigating the neural networks underlying actual (ME) and imagined (MI) performance using magnetoencephalography (MEG), a neuroimaging modality with high temporal resolution that can highlight which brain regions are active during the planning and subsequent performance of a task.

Participants performed a unimanual grasping task using either MI or ME as directed while their brain activity was being recorded. Briefly, participants were asked to prepare to perform a grasping motion to one of two targets that was specified at the beginning of a performance phase. When the target was specified, the participant completed the task, either reaching out and grasping the target (ME), or imagining reaching out and grasping the target (MI). Sensor and source-level data (from 32 regions of interest, ROIs) were examined in regard to estimated source strength and oscillatory activity in the beta frequency band (15-30Hz).

Results showed reduced contralateral (left) M1 activity in MI for both estimated source strength and oscillatory activity, verifying that M1 inhibition occurs in MI. Reduced activity in MI relative to ME was also observed in ROIs comprising premotor regions, including the SMA. This finding suggests the SMA is not a source of inhibition to M1, providing support for the first theory of motor inhibition in MI, specifically, that M1 inhibition occurs as a result of mechanisms involved in generating the mental representation of a motor task. What this study adds is the notion that areas of the brain outside of the premotor region may be involved in the motor inhibition observed in MI, likely at an earlier stage of generating the mental representation of a motor task.

Chapter 2: Literature Review

2.1: Motor learning

Motor learning is a fundamental aspect of human behavior, referring to the ability to acquire or improve skills through the repetition of movement and the provision of feedback about performance [1]. A commonly referred to application of motor learning has been the achievement of excellence in athletics or music. Beyond athletics and music, motor learning is applicable to everyday life, as the ability to proficiently perform skilled movements in numerous domains is achieved through motor learning.

The correlation between time spent practicing a skill and increased level of performance has been reinforced in innumerable studies [1], including a series of experiments looking at musicians of varying skill level enrolled in musical academies in Germany. This series of experiments showed that regardless of the instrument being practiced, violin or piano, students deemed excellent by their professors reported to have performed a larger volume of practice in comparison to their peers who were not as proficient at playing the same instrument [7].

The process of motor learning is achieved through changes in communication between cells of the brain. The nervous system is composed of billions of distinct cells, called neurons, that mediate communication in the brain through chemical and electrical signalling between adjacent neurons [8]. This communication occurs at junctions, called synapses, between the axon terminal of the pre-synaptic neuron and

the dendrite of a post-synaptic neuron. All synapses have the ability to undergo activity-dependent changes in synaptic strength [9]. When this change results in a strengthening of communication at the synapse that lasts for more than an hour, long-term potentiation has been said to occur. It is this property, which in the most basic sense underlies synaptic plasticity within the brain. When neurons repeatedly fire together over a large number of iterations, the increase in synaptic strength will become substantial enough to create lasting physiological changes [10]. In the context of motor learning or motor skill acquisition, it has been demonstrated that repeated physical practice of a skill leads to changes in behaviour as well as functional and structural changes in the brain. For instance, learning a sequence of thumb finger oppositions over a period of 4 weeks leads to an increase in the number of sequenced repetitions in 30 seconds as well as enhanced M1 activity in response to performing the practiced sequence [11]. Despite a specific set of neural events that must occur in order to facilitate learning, the method in which one practices a motor skill in order to induce LTP can differ.

2.2: Introduction to motor imagery

Two established modalities for motor learning exist: ME and MI. Motor execution is the modality most commonly associated with motor learning, referring to the physical repetition of a specific task to improve performance. Motor imagery is traditionally defined as the mental representation of an action without any movement [5]. Motor imagery can be performed from two perspectives; first person (from behind the eyes of the imager) and third person (visualizing the movement from the perspective of

a spectator) [12]. Additionally, MI can be performed in two domains: kinaesthetic imagery, where the focus is on the mechanical and tactile sensations involved in performing a movement, and visual imagery, where the focus is on the visual representation of the performance of a movement. While visual imagery can be performed from either perspective (first or third person), kinaesthetic imagery is usually performed only from the first person prospective [12]. It has been demonstrated that kinaesthetic imagery, imagining the performance of a task "through your own eyes", is more effective than visual imagery for basic motor skill acquisition [13].

The theory underlying motor learning is that as the participant performs a motor skill, they first generate a plan (the efference copy) for the movement, execute the movement and subsequently use the information from the outcome of the movement (the reafference copy) to update their motor plan to improve their ability to perform the practiced skill. The reafference copy contains a variety of information including task outcome (often referred to as knowledge of results and knowledge of performance) as well as somatosensory feedback, including vision, touch and proprioception. Post movement, the reafference copy is compared with the efference copy established during the preparation for movement. The process of contrasting these two constructs defines a forward model where the discrepancy between the reafference copy and the efference copy is used to adjust or update the participant's motor plan such that their next attempt to perform the task can be more successful. Over a large number of repetitions, the error detection and correction that is facilitated by this forward model helps drive lasting changes in the areas of the brain associated with task performance

(i.e., plasticity), allowing learning to occur. At the cellular level, the repetition of the task reinforces the pattern of synaptic activity that defines task performance allowing LTP to occur at the synapses involved, while reducing the strength of the connection between synapses considered non-essential to task performance.

One notable difference between learning via MI and ME is that the participant receives feedback from their attempt to perform the task during ME, whereas in MI they do not. Given the concealed nature of MI, feedback about task performance is not available, as the task is not actually executed. As such, the comparison of the reafference copy to the efference copy cannot occur. Motor imagery is theoretically thought to parallel the internal movement planning portion of ME [14], indicating that MI practice does not improve the ME component of the task, and improvements in task performance are therefore only reflected by changes in the internal representation of a motor task [15]. This notion that MI-based practice improves the internal representation of the task is backed by a transfer study where participants learned a task using MI and then underwent either a perceptual transfer (a change from auditory to visual cues), or motor transfer (a change from the hand used during training to the untrained hand), between training and testing [16]. This study demonstrated impaired learning in conditions where participants underwent the perceptual transfer in comparison to a condition that did not undergo a transfer. This gives insight into the nature of MI-based learning by highlighting its reliance on the perceptual/cognitive components of learning a skill, such as encoding the spatial goal of a movement [16,17]. The most noticeable difference between the two modalities of motor skill acquisition is that ME has the

additional component of execution. This allows individuals to gain information about task performance to update their motor plan and improve their execution of the task [1].

Motor imagery has been applied in a number of fields including music, sports, strength training, surgical practice and neurorehabilitation to improve motor task execution in a variety of manners [18-22]. A study by Robin et al. 2007 tested the effects of MI as part of a training paradigm aimed at reducing the number of invalid serve returns in tennis. Participants were divided into 3 groups based on MI ability: good imagers, poor imagers and control (consisting of an equal number of good and poor imagers). The experimental groups underwent 8 weeks of MI training whereas the control group was given a reading task during the same time period. The researchers demonstrated that MI training decreased the number of service return errors in comparison to control and that the improvements in service return were better for those deemed 'good' at imagery relative to those deemed 'poor' at imagery. Thus, MI appears to be an effective form of training in tennis, dependant on an individual's ability to perform it [20]. Despite the large diversity of uses for MI, each application of this modality relies on the underlying principle of skill acquisition or improvement.

While ME has been demonstrated to be the most effective method of motor skill acquisition [23], there are situations in which MI can be used as an adjunct to ME or independent of ME to improve motor skill acquisition [24-26]. Recently, Kraeutner et al. 2016 developed a task to determine whether acquisition of a novel skill was possible using MI, without any contamination from a priori ME. This paradigm, based on implicit sequence learning, exposed participants to a sequence of cues embedded in training

blocks by mixing the sequence with a randomly generated series of cues. Participants were asked to either imagine or physically press a key corresponding to the cue based on their group designation. Participants were tested using a reaction time test to see if they were faster at responding to sequenced cues in comparison to random ones. Results of this study demonstrated that participants who trained using MI were faster at responding to sequenced cues (relative to random cues), and that the magnitude of this difference paralleled that of the physical practice group. These results indicated that those training via MI had learned the sequence embedded in the training blocks [26].

More recent work in our lab has built upon this finding and demonstrated that MI-based learning can be used to learn more complex skills. This specific study used a task that involved participants tracing complex shapes at varying speeds over the course of 5 training sessions[27]. Throughout the entire experiment there was a single shape that repeated, intermixed with randomly generated shapes. After training, the speed accuracy function was used to demonstrate learning of the repeated shape, evidenced by decreased error and a shift in the speed accuracy function. Participants in the physical practice group demonstrated a high degree of learning over the course of training that was not reflected in the physical practice group that didn't receive feedback (Figure 1; PP-FB vs. PP). Participants in the MI condition also demonstrated learning as their speed accuracy functions at day 5 indicated better performance than the day 1 scores from the physical practice group without feedback (Figure 1; MI vs. PP). The MI group also outperformed a perceptual control group that also only physically performed the task on day 5 (Figure 1; MI vs. P-Ctrl). However, the scores from the MI

condition are significantly lower than the scores from the day 1 scores of the physical practice with feedback condition (Figure 1; MI vs. PP-FB). This finding demonstrates the relative effectiveness of MI in comparison to physical practice. In order to better understand the differences between the outcomes from these learning modalities it is important to better understand the functional anatomy involved in each modality to evaluate how each modality affects the brain during learning.

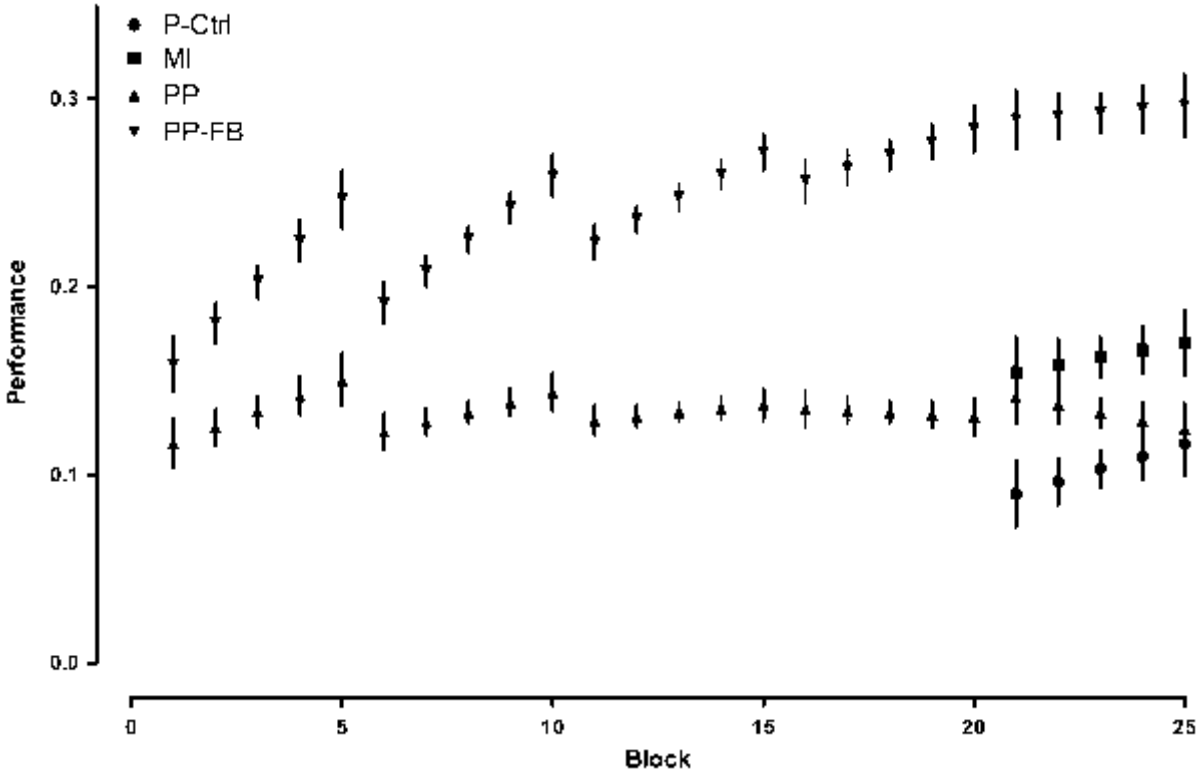

Figure 1: Learning of a complex upper limb multi-joint movement. Participants trained for 5 days (each day = 5 blocks) using a variety of modalities (motor imagery; MI, perceptual control; P-Ctrl, physical practice without feedback; PP & physical practice with feedback; PP-FB). The PP-FB group demonstrated superior learning relative to the other conditions, however the group that trained using MI also demonstrated learning superior to day 1 of the PP group. Note performance represents a shift in the speed accuracy function (from Ingram et. al., unpublished data).

2.3: Representing behaviours using neural networks

As alluded to previously, motor skills are often complex, requiring a population of neurons to function together in order to properly perform the skill. These neurons belong to a variety of spatially unique brain regions and it is the interaction or communications between these brain regions that underlie performance of the given motor task. These communications are facilitated by synchronous neural firing within similar ranges of frequency [28]. When the series of brain regions and the interactions involved in a task are considered as a whole, the ensemble can be referred to as a neural network.

While the brain is considered to be a single organ, it can be subdivided into a large number of spatially distinct areas, or cell assemblies, each with their own unique function [29]. When these areas communicate with each other, they form networks that represent human cognition and behaviours. Therefore, the brain can be conceptualized as a large network that is compartmentalized into smaller networks responsible for controlling individualized initiation, regulation and termination of unique functions. For the purpose of this project, the structural and functional organization of the brain created a platform in which to compare MI and ME. Since both unique, albeit related, behaviours rely heavily on the motor system, they are represented by similar neural networks involving areas of the brain associated with motor control [2,3,30]. However, the lack of a behavioural response, namely movement resulting from MI, suggests that there must be differences in the neural underpinnings of the behaviour that reflects the different outcomes resulting from MI and ME. These neural differences can be

addressed from two perspectives. Firstly, one could address differences between MI and ME by looking at which brain areas are associated with each network. Areas that are active in either MI or ME but are not common to both networks may help explain the behavioural differences between the two tasks. Secondly, the nature of the interaction between brain areas, even if the areas are common to both MI and ME networks, may differ. Analyzing and comparing patterns of activity between the MI and ME networks can provide a means to identify areas in the brain that underlie the key difference between these modalities, namely the lack of overt movement in MI.

2.3.1: Network underlying MI

Mapping the neural correlates of MI has primarily been accomplished using functional magnetic resonance imaging (fMRI) and positron emission tomography [30,31]. While several theories exist about the parallels between MI and ME [12,32], a common explanation used to compare the two behaviours is that MI is thought to be analogous to the preparation portion of ME without actual execution. As such, the two behaviours were thought to use similar brain areas and processes [31,33]. An extensive meta-analysis of the literature aiming at identifying the neural substrates of MI was first performed in 2001 [31]. Results of this meta-analysis suggested that M1, premotor cortex (PMc), SMA, inferior and superior parietal lobules (IPL/SPL), anterior cingulate cortex and the cerebellum (CB) were all involved in the MI network [31]. Recently, a second meta-analysis of the literature surrounding the neural substrates of MI was performed using more quantitative approaches that built on findings from Grézes & Decety, 2001. This meta-analysis incorporated methodological differences to quantitatively address the effects of

which body part was the target of MI, modality of MI and type of MI task being performed [30]. Additionally, the updated meta- analysis used a revised version of the activation likelihood estimate technique for coordinate-based analysis of the results of neuroimaging studies. The activation likelihood estimate technique identifies areas of the brain that show convergence of activation across studies and compares the convergence to a null distribution to test whether the area demonstrates a spatial association of activation across studies. Results of this meta-analysis on general MI demonstrate heavy involvement of the fronto-parietal network, including the PMc, SMA, IPL and SPL, and reliance on several subcortical structures, including the putamen and thalamus, as well as the CB [30] (see Figure 2).

When comparing the networks underlying MI and ME, there are many areas of the brain that contribute to both networks. While there is a large amount of variability between studies about which brain areas are considered to be involved in both MI and ME, areas including the posterior parietal cortex (PPC), CB, SMA and PMc are consistently found to be active in both networks [2,3,34]. However, the attributions of areas of the brain such as S1 and M1 to MI remain highly debated [2,3,30,35]. Another key difference between the two networks is that the ME network tends to be lateralized to the hemisphere contralateral to the task being performed, assuming that the task is unimanual, whereas the MI network is less lateralized and more widespread throughout the brain [2,3]. Motor imagery tends to recruit areas from the ipsilateral hemisphere whose functions mirror those required in order to perform MI such as visuospatial processing and inhibition of response [36,37]. Another factor contributing to the dispersal

of the MI network is the novelty of the task. A study comparing the brain activity of elite archers to non-archers as they perform MI of an archery task was performed to evaluate the effect of skill level on the neural network underlying MI. The authors determined that elite archers have a more efficiently organized MI network lateralized in one hemisphere in comparison to their non-archer counterparts who demonstrated a much more diffuse bilateral MI network [38]. The noted effect of expertise on MI networks parallels the effects of expertise on ME networks, whereby brain activity is decreased in cortical areas that were not associated with the demands of the task [39].

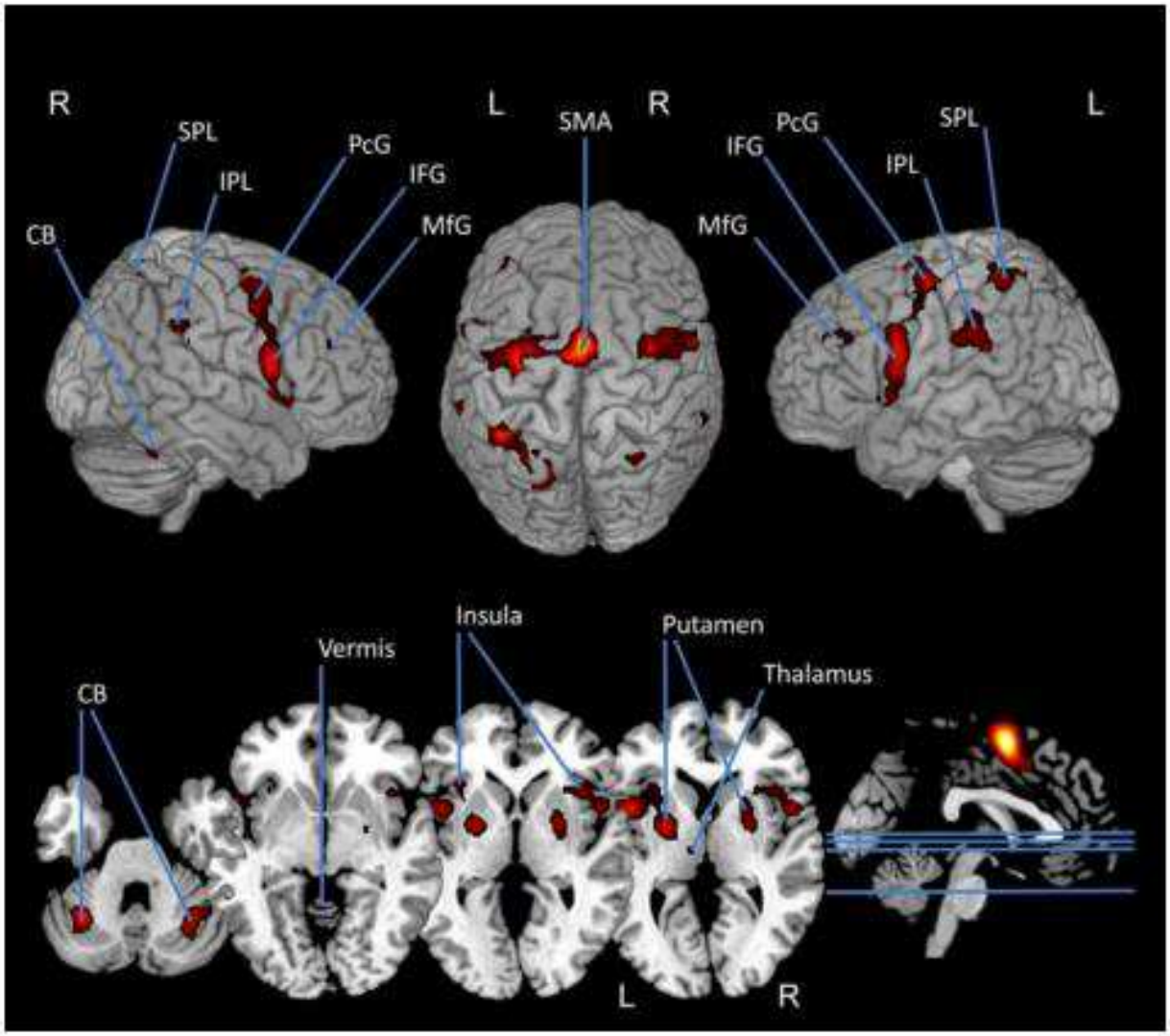

Figure 2: The neural network underlying general MI. Red areas indicate consistent activation during MI as indicated by an activation likelihood estimate meta-analysis (taken from Hétu at al. 2013).

2.3.2: Factors that bias the neural network underlying MI

One of the primary reasons Hétu et al., 2013 performed a second meta-analysis of the neural networks underlying MI is that the meta-analysis performed by Grézes & Decety, 2001 did not account for which body part was being imagined, the modality of MI or the type of MI task when forming their conclusions based on the existing

literature at the time. Each of these variables is known to affect the neural network underlying MI [30,40,41].

The neural map presented in Figure 2 is also representative of MI of the upper-limbs as 54 of 81 studies included in this meta-analysis performed MI of tasks involving the upper-limbs, limiting the effect of studies that performed MI of the lower-limbs on the general MI neural network [30]. Transitivity, whether an object is (transitive) or is not (intransitive) involved in the action being imagined, introduces bias into the MI network. Transitive MI appears to rely more heavily on the premotor portions of this network in comparison to intransitive actions [30]. This result is surprising given the parietal lobe's involvement in encoding the spatial and visual properties of items as well as visuomotor manipulations of the objects to transform them into orientations appropriate for grasping [42-44]. This bias towards the frontal part of the fronto-parietal network may therefore reflect an increased demand on the motor planning portion of MI [30].

The modality of MI, kinaesthetic and visual, also plays a role in determining the network underlying either task. As indicated previously, kinaesthetic imagery refers to one imaging themselves performing a movement, whereas visual imagery refers to an individual imagining someone perform a movement. These two forms of MI differ behaviourally as visual MI involves perceiving visual cues about how an action is performed whereas kinaesthetic imagery relies on the perception of the movement through the mental simulation of the appropriate proprioceptive information [45]. It would therefore be logical to hypothesize that kinaesthetic imagery would rely on a network that involves brain areas associated with motor planning. Results of

neuroimaging studies indicate that kinaesthetic MI has a larger, more expansive network than visual MI, which includes concentrations of consistent brain activity in the left PMc as well as the parietal cortex [30,40]. Visual imagery, on the other hand, seems to rely more heavily on the occipital lobe, which is involved in the encoding and processing of visual stimuli [40]. Despite the effects of a variety of factors on the MI network, it relies heavily on five spatially distinct areas of the brain (cerebellum, motor cortex, parietal lobes, premotor & frontal lobes). Understanding the respective contributions of these five areas to the MI network will help explain the differences in MI network activation as noted above, and will also provide insight about possible sources for the mechanism underlying the lack of overt movement in MI.

2.4 Cerebellum

The cerebellum is located in the posterior aspect of the brain on the ventral surface, separate from the cerebrum. The cerebellum can be divided into two hemispheres, each containing multiple lobules, connected by mid-line region called the vermis. The cerebellum is consistently active during MI [2,3,30]. This in unsurprising given the role of the CB in the motor network, and specifically its role in the modulation of motor commands, where the CB is the site of error detection and subsequent correction that is facilitated by comparison of the reafference and efference copy as part of a forward model [46]. This function of the CB is supported by the presence of structural connections with a variety of cortical areas involved in motor control and execution including M1, SMA, and PPC [47,48], which allows the CB to indirectly modulate motor commands. The consistent activity of CB during MI intimates that it is possible that

efference copies are similarly created in MI as they are in ME further supporting the parallels between both modalities and providing CB with a possible role in MI.

The importance of the CB in MI can be speculated by investigating the degree of impairment to MI caused by damage to the CB. A recent review of the impact of a variety of specific brain lesion on MI ability demonstrated that in stroke and Parkinson's patients with damage to the CB only 33% showed MI impairments [49]. Despite the lack of MI impairment due to CB lesions noted by McInnes et al., several earlier studies of patients with lesions to the CB did not corroborate this finding and indicated the damage to this area of the brain resulted in MI impairments [50,51].

In the review performed by McInnes, Friesen and Boe, it was noted that a patient performing MI of a previously learned task may not report an impairment in MI ability given the role of the CB in the initial stages of motor learning [52]. If the task to be performed was novel, the authors suggested that consistent impairment in MI ability due to CB damage would have been noted. Since the confound of task familiarity is not well controlled for in the clinical literature, it is difficult to make definite conclusions about the role of CB in MI based on examining MI impairments due to damage to the CB.

Another recent study attempted to ascertain the role of the CB in MI using anodal transcranial direct current stimulation on the CB during MI performance [53]. These authors postulated that the CB has a direct inhibitory effect on M1 via activation of Purkinje cells in the CB during MI and that excitatory anodal transcranial direct current stimulation would further increase the inhibitory effect of CB on M1. In this

study, participants were asked to perform MI of two unilateral tasks pre- and post-transcranial direct current stimulation that differed in complexity. The transcranial direct current stimulation was either excitatory in nature or sham. Motor evoked potentials (MEP) were obtained using transcranial magnetic stimulation from the contralateral M1 during MI of the tasks and compared to see if the additional CB activity reduced the amplitude of the MEPs obtained post- transcranial direct current stimulation. A suppression of the amplitude of MEPs obtained from M1 following transcranial direct current stimulation on the CB was only seen in the "simpler" of the two tasks in the group receiving excitatory stimulation. Sham transcranial direct current stimulation did not affect the M1-facilitated MEP while performing MI of either task. Thus, the authors concluded that activation of the CB during MI reflects an inhibitory mechanism acting on M1 [53]. This finding corroborates similar findings in ME when receiving electrical stimulation to the CB caused Purkinje cell activity that inhibited M1 via the inhibititory dentato-thalamo-cortical pathway [54].

2.5: Parietal lobes

The parietal lobes, one each in the left and right hemispheres, is situated superior to the lateral fissure and is clearly separated from the frontal lobe by the central sulcus. The lobes cover an area that stretch caudally towards the parieto-occipital fissure that serves as the posterior boundary between the parietal and occipital cortices. This lobe can be divided into two areas, S1 and the PPC.

Somatosensory cortex is not consistently active during MI [30,31,34]. Given that performing MI does not provide somatosensory input, this finding is consistent with the

classic role of S1 including the perception of sensory input including touch, proprioception and pain [55]. However, recent studies using effective connectivity have demonstrated that S1 is a causal target in the MI network receiving signals from a variety of sources, such as the SMA, indicating that these areas active in the MI network are providing input to S1 [35,56]. It was also noted that S1 shares a bi-directional connection with M1 only when performing ME, and therefore may be representative of an interaction between these two areas as a result of the overt motor output from each ME trial [35].

The PPC is divided along the superior/inferior axis by the intraparietal sulcus where the area superior to the sulcus forms the SPL and the area inferior to the intraparietal sulcus forms the IPL. The most generally accepted function of the PPC is to act as a sensory integration hub in the motor control pathway as its sub-regions, the SPL and IPL, project to various cortical areas involved in ME such as the PMc and M1 [57]. The PPC is integral for visuo-motor transformation processes and is important in visually guided motor tasks [58,59]. Given its importance in the integration of visual sensory input from the primary visual cortex in movement, this area, especially the left PPC, has been associated with processes related to motor attention and motor planning during MI [60,61]. Additionally, the PPC, specifically the IPL, is thought to be involved with higher order cognitive and motor functions [57].

In contrast to the results reported by Hétu et al. 2013 suggesting that the SPL is active during MI (Figure 2), more recent literature has failed to report similar findings [2,3]. To support these more recent findings, a study performed by Pelgrims, Andres & Olivier

used repetitive transcranial magnetic stimulation (rTMS) to create virtual lesions in the SPL and IPL to test their effects on kinaesthetic (referred to as 'motor' in this specific study) and visual imagery [62]. The kinaesthetic task consisted of a laterality judgment task (LJT), in which participants see a series of pictures of hands in different orientations and have to determine the handedness of the image, whereas the visual imagery task required participants to decide whether or not a rotated letter was in canonical form or not. Results of this study demonstrated that virtual lesions to the SPL only significantly interfered with the visual imagery but had no effect on the kinaesthetic imagery. In contrast, lesions to the IPL interfered with the participant's ability to perform MI yet did not impact the subject's ability to perform visual imagery [62]. A suggestion for why there has been some debate in the literature is that activation in the SPL is driven in part by muscle activity. The contamination of muscle activity in these recordings was theorized to have been caused by poor controls for movement during MI trials, and by processes involved in interpreting environmental cues and external spatial orientation [3,63]. Additionally, the task being imagined may also play a role in determining if activity occurs in the SPL during MI. Figure 2 represents results from all types of MI tasks included in the Hétu et al. meta-analysis, but when that group is divided based on task, either 'Pure MI', the mental simulation of an body movement, LJT and sequence-based MI tasks (Figure 3), it becomes apparent that 'Pure MI' does not activate SPL as significantly as the other two tasks [30]. One explanation for this result could be that a majority of LJTs and sequence-based tasks use visual cues, hence there is a need for interpretation of environmental cues, a known function of the SPL. On the other hand,

pure MI tasks do not necessitate integration of visual information hence there is less activation in the SPL. The lower activation in SPL likely reflects MI's reliance on motor planning and proprioception rather than processing one's environment and body position in space [64].

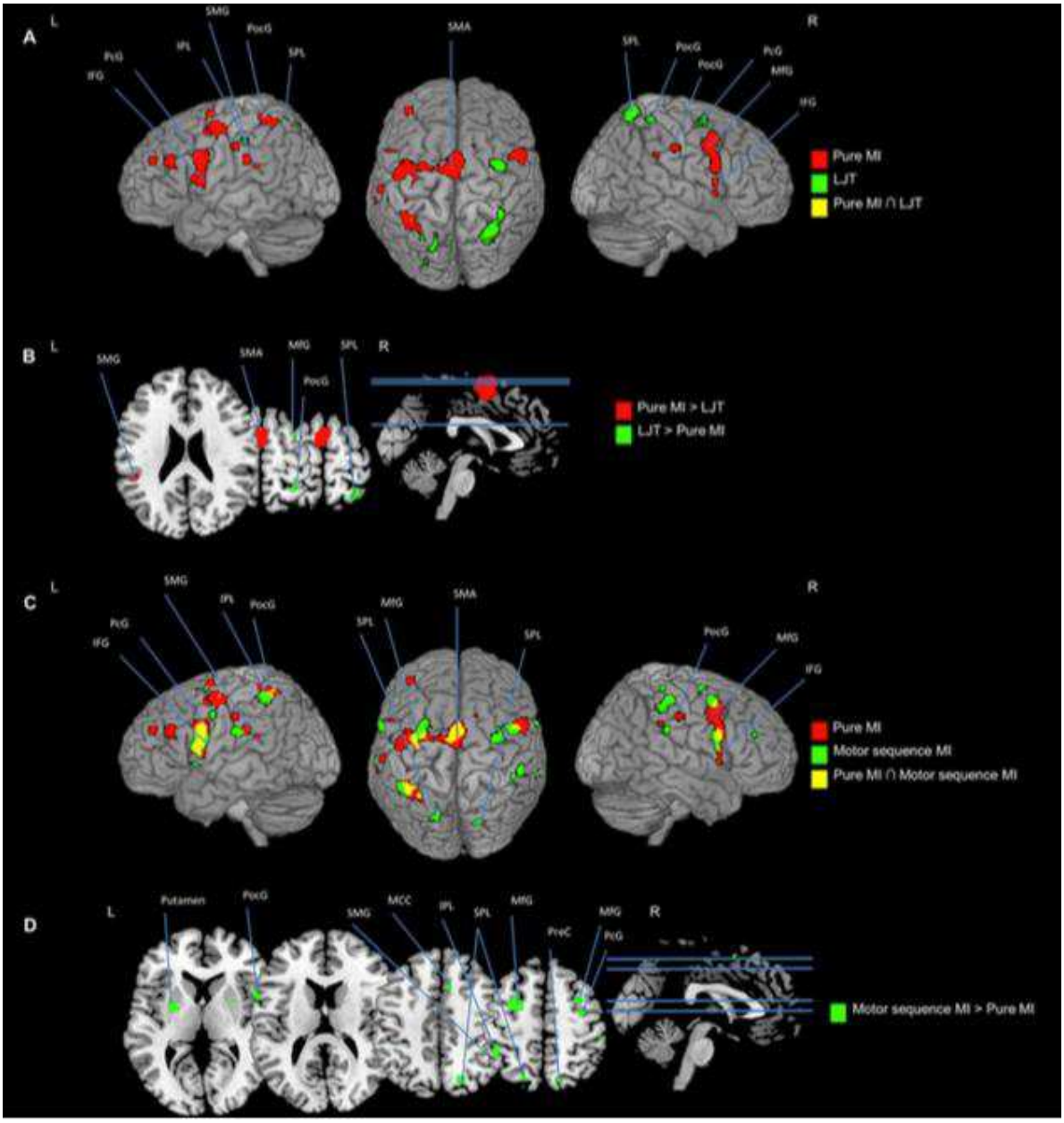

Figure 3: Differences in activation between different MI tasks. A: Pure MI versus late LJT, B: Difference network between Pure MI and LJT, C: Pure MI versus Motor Sequence MI,

D: Difference network between Pure MI and Motor Sequence MI. (Taken from Hétu et al. 2013)

The other portion of the PPC, the IPL, is consistently active during MI [2,3,30,31]. The IPL, especially the anterior intraparietal area, has been proposed to be the parietal node in the fronto-parietal mirror neuron system whose function is to dictate communication between observed and executed movements [37,65]. As part of the mirror neuron system, a system of neurons active when both viewing and performing a given task[66], the IPL is known to be responsible for storing motor representations which may be internally recalled when performing MI [30,67]. These representations are thought to be effector independent in nature, meaning that the information transfers between effectors (e.g., left to right hand), and are implicated in identifying the spatial goal of a movement [43,67]. Since MI lacks the feedback associated with ME, it is theorized that MI relies more on recruiting effector independent motor representations and mapping perceptual cues to the goal of the movement [16].

The importance of the IPL in MI has also been demonstrated by studies using rTMS to induce virtual lesions in the IPL. A recent study by Kraeutner et al. 2016 utilized this technique to determine if inhibiting IPL prior to an MI-based implicit sequence learning task would affect the participant's ability to learn the task. There were two groups in this study, a group that received the inhibitory rTMS to their IPL and a control group that received sham stimulation. The implicit sequence task, described above, was used to determine if participants demonstrated a difference in reaction times between random and repeated sequence elements, indicating that they learned the sequence via MI. Results indicated that participants who received inhibitory rTMS were unable to

learn the sequence while the sham group demonstrated a difference in reaction times between random and repeated sequence elements, indicating that they learned the sequence. Given the rTMS group's reduced ability to learn the task, it was suggested that the IPL is essential to MI performance and potentially to MI-based skill acquisition [68]. These findings are also reflected in a rTMS study using a LJT where rTMS inhibition of the IPL reduced the participant's ability to perform the task [62].

2.6: Premotor areas and frontal lobe

The "frontal" portion of the fronto-parietal network, located anteriorly to M1, consists of areas often involved in creating and preparing motor plans for execution [69]. These areas include the ventral and dorsal premotor cortices, SMA and inferior frontal gyrus (IFG) [2,3,30,34]. The time taken to perform MI or ME for a given movement suggests that the brain undergoes similar computational steps when performing either task [70]. Given that the premotor areas active in MI are also included in ME neural networks, it is likely that the planning phases of ME and MI are analogous [30]. Results of training studies looking at the neural correlates of MI highlight the importance of the PMc and IPL to MI as after a week of ME training, MI of the task demonstrated increased activity in PMc and IPL, while ME of the task activated M1 and S1 more heavily [52]. This finding is consistent with the theory that MI relies heavily on motor planning. Additionally, PMc has also been shown to form functional connections with both the SPL and IPL [35,56]. These connections exist in both ME and MI indicating that the areas interact to mediate sensorimotor transformations especially in visually cued tasks [71]. Interestingly, it was

found that PMc was active bilaterally in both tasks despite these results occurring as a product of a unimanual task [35].

The IFG is considered to be a critical portion of the mirror neuron system that is responsible for communication between observing and imitating a motor plan [37] . Specifically, the dorsal section of the pars opercularis was found to be significantly more active during kinaesthetic than visual imagery or ME [72,73]. The authors of this study identified the dorsal pars opercularis as the frontal node of the mirror neuron system responsible for action-observation matching, as it was active in both kinaesthetic and visual imagery but the blood oxygen level dependent (BOLD) signal was greater during kinaesthetic than visual imagery [73]. Given the consistent activation of the IFG in MI, it has been postulated that this area is involved in updating and recalling internally generated motor plan representations during MI [12]. The importance of the IFG in MI has been reinforced by a study of individuals with MI impairments on a LJT due to cardiovascular accidents. It has been demonstrated that if a lesion occurs in the left frontal lobe, MI performance on the task is significantly impaired [74]. Additionally, lesion and inhibitory rTMS studies of the right hemisphere IFG have shown this area to be essential for regulating inhibition of movement, the cognitive process required to cancel an intended movement [36,75].

The SMA can be divided into two spatially distinct areas, pre-SMA (anterior) and post-SMA (posterior), each with different functions. The pre-SMA is densely connected with the frontal lobe and is thought to play a role in cognitive evaluation of tasks such as movement selection and preparation while the post-SMA is more closely tied to M1 and

ME [12,72,76]. A study conducted by Zantgraf et al. 2005, demonstrated differences in levels of activation across the two SMA regions by performing an observational study. In this study, there were two groups of participants who watched a video of a male gymnast perform a sequence of movements. In one group, participants were asked to watch the video repeatedly with the goal of subsequent MI performance of the sequence of movements. In the other group, participants were asked to watch the video and score the accuracy of the sequence of movements. The authors of this study found that the MI group had less activity in the pre-SMA than the evaluation group but greater activity in the post-SMA in comparison to the evaluation group [76]. They concluded that while both parts of the SMA are active during imagery, the greater activation in post-SMA during MI reflects the greater parallels between MI and ME while action observation tends to more closely parallel mental stimulation [76].

Parallels between MI and ME in the SMA have also been investigated by capturing changes in the magnitude of oscillatory brain activity in response to a given task, otherwise known as event related synchronization or desynchronization (ERS/ERD) [77]. Given the oscillatory nature of brain activity, different types of brain activity are characterized by the frequency at which they oscillate. In the motor system, brain activity related to motor task performance has been associated with ERD in the range of 15-30Hz, called the beta band [78]. Given that beta band activity is inhibitory by nature, ERD in this frequency range is thought to be representative of increased brain activity associated with performance of a motor task [77]. In the SMA, analysis of ERD in two separate MEG studies showed no significant difference between MI and ME [2,3]. These

findings are also supported in multivariate analysis of BOLD signals during MI which also demonstrate similar activation between MI and ME [34].

Despite the commonalities shared between MI and ME, investigations of the SMA using functional and effective connectivity indicate that the SMA's role during MI is different than during ME [35,56,79,80]. During MI, the SMA has been shown to have functional connections with M1, PMc, PPC and S1 [35,56]. Functional connections are defined as connections indicating the transfer of information to and or from another area of the brain. The connections between SMA and PMc have been noted on several occasions [35,56,79,80] but the function of this connection demanded more attention [35]. The most remarkable connection between any area and the SMA is the connection between the SMA and M1. In contrast to the functions of the SMA in ME, it has been proposed that the SMA exerts inhibitory influences on M1 during MI rather than excitatory [35,56,79,81]. This effect was first demonstrated by Kasess and colleagues, who applied dynamic causal modelling to fMRI data obtained from participants performing a unimanual finger-tapping task [79]. In this study, the researchers were only concerned with two ROIs, M1 and SMA. Results from this study showed that the SMA was similarly active in both MI and ME conditions, however, the BOLD signal in M1 was significantly lower during MI in comparison to ME (Figure 4). Using effective connectivity and dynamic causal modelling, the authors identified a causal link between SMA and M1 where SMA excites M1 during preparatory phases of the movement but subsequently changes post-movement onset, inhibiting M1 [79]. Following this finding, an additional study by Gao et al. 2011 used Granger's Causality in a larger number of ROIs, detecting a

similar relationship between both contralateral and ipsilateral SMA and M1 contralateral to the performed task. Additionally, M1 also provided information back to the contralateral SMA but not the ipsilateral. This finding indicates that bilateral SMA may be involved in MI and that their roles may be different given their involvement with contralateral M1 [35].

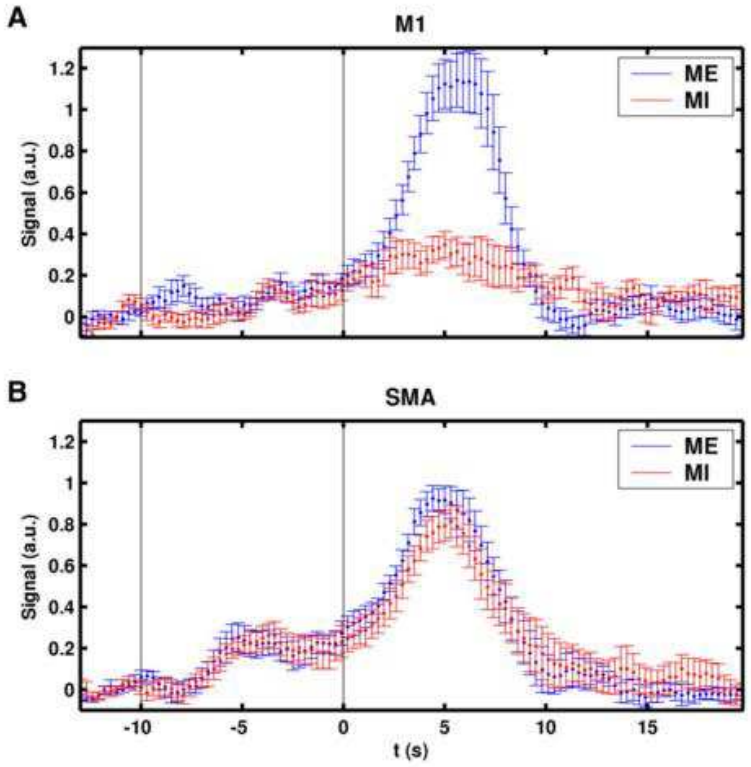

Figure 4: BOLD signal activation in the motor cortex (A) and the supplementary motor area (B) during MI (red) and ME (blue). (taken from Kasess et al. 2008)

2.7: Motor cortex

The classical definition of M1 function is to provide input to the corticospinal tract (CST) to execute voluntary movements. Historically, the involvement of M1 in MI has been debated and best presented by Hétu et al. 2013 who indicates that only 22 of 122 experiments included in their meta-analysis reported M1 activation as captured by

fMRI and positron emission tomography [30]. However, TMS studies of M1 have shown that MI increases CST excitability, which is thought to be a function of M1 and therefore requires some activation in that region of the brain, in comparison to rest and visual imagery [6,82,83]. This seemingly contradictory set of findings was attributed to a lack of temporal resolution in fMRI combined with a limited magnitude of the BOLD signal response in M1 as a result of MI [30]. Mapping the network using imaging techniques with higher temporal resolution such as MEG has demonstrated M1 activation during MI, however, the peak ERD in M1 during MI is smaller and occurs later in time across a smaller range of frequencies (Figures 5 and 6) [2,3].

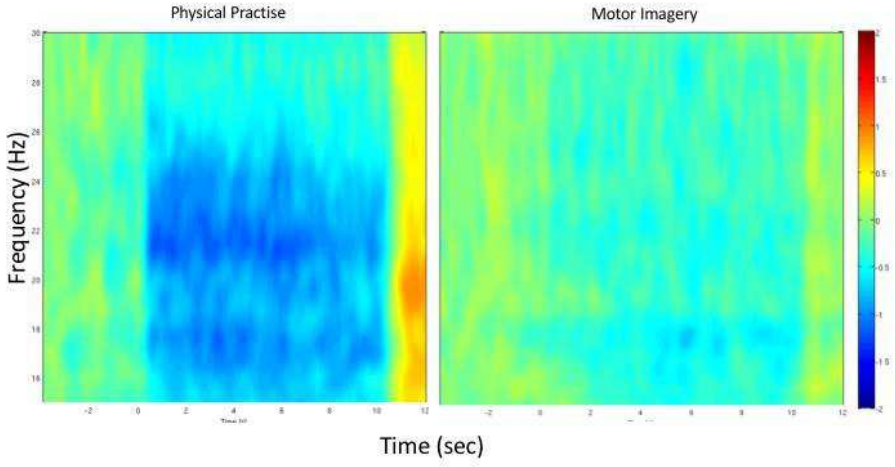

Figure 5: Group averaged time-frequency response plots showing beta band ERS/ERD of ME and MI in M1. (adapted from Kraeutner et al. 2014)

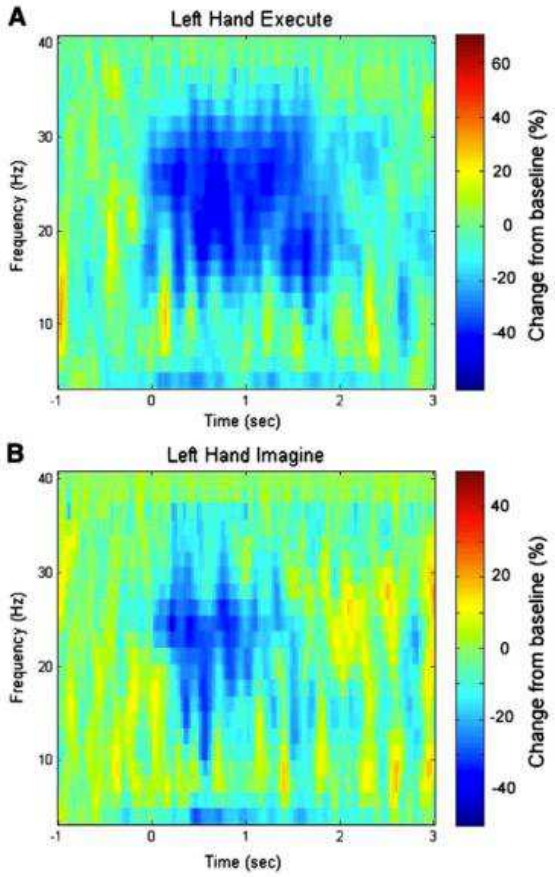

Figure 6: Group averaged time-frequency response plots showing beta band ERS/ERD of M1 during (A) ME and (B) MI. (taken from Burianová et al. 2013)

The activity generated in M1 has also been shown to be dependent on a variety of factors including MI modality and task [83,84]. A study performed by Stinear et al. 2006 used TMS to determine if MI modality, either kinaesthetic or visual, differentially impacted the excitability within the CST by measuring MEPs from the target muscles in response to the different types of imagery. The authors found that only kinaesthetic MI increased MEP amplitude of the target muscle while results from visual MI were not significantly different from their control condition. The increased amplitude of the MEPs during MI could be explained by modulation of the CST at either the supraspinal or spinal level. However, the authors noted that there was little evidence in support of CST

modulation at the spinal level and concluded that cortical activity in M1 was the source of corticospinal modulation [83]. More recently, Pilgrimm et al. 2016 conducted a study to see whether MI is associated with action dependent patterns of activation, which would allow researchers to decode the content of a participant's MI activity. Participants were instructed to perform 3 different MI tasks while having their brain activity recorded by fMRI. Results from this study showed activation in M1, PMc, PPC, visual cortex and SMA as a result of MI and patterns of activity in M1, PMc and PPC could be used to determine which MI task was performed at a rate significantly higher than chance. These results indicate that activity within parietal motor and frontal areas in the brain associated with MI are representative of the content of MI [84]. The implications of these findings in contralateral M1 are of interest as they indicate that M1 displays action-dependent activity. It is theorized that M1 may be holding premotor information for short periods of time [85] and, as such, represents higher order functions other than ME. This suggests that the role of M1 in MI might emerge from a functional network within MI rather than treating the cortical area as a single entity with a single discrete function [84].

In line with earlier finding of consistent M1 activation, TMS has been used to demonstrate that M1 modulates CST activity during MI [12,86]. This increase in CST excitability is thought to be initiated by increased neuron responsiveness to magnetic stimulation [86] and/or a decrease in intracortical inhibition [87] . The CST excitability contains many parallel characteristics to CST excitation during ME, including spatial, temporal and contextual aspects [82]. From the spatial and temporal perspectives, it has been noted that increased MEP amplitude during MI occurs in a similar temporal

pattern to ME and only occurs in the target muscle(s) rather than in muscles that are not utilized during execution of the imagined task [83]. These findings indicate that M1 is active in MI despite the absence of an executed movement.

2.8: Network summary

In summary, the neural network underlying MI can be subdivided into five broad groups of brain regions: cerebellar, motor, parietal, frontal and premotor. The CB is consistently active during MI; it is thought to be involved in the storage of efference copies. Within the parietal region, the PPC seems to play the most consistent role in MI [30] as it is thought to be involved in visuo-motor transformations during movement as well as motor attention and planning [57,58,60]. The PPC contains two sub-regions, the IPL and SPL. While the role of the SPL in MI is debated, the IPL is vital to MI [30,62,68]. The IPL is thought to be responsible for storing internal representations for movements that are recalled during MI performance [30,67]. The frontal region most active in MI is the IFG, which is responsible for neural communications between observing and imitating a movement [37]. Additionally, this area is responsible for recalling and updating motor representations used during MI [12]. Within the premotor areas, the PMc and SMA are considered to be the most involved in MI [30]. Given that the chronometry between MI and ME are similar [70], it has been concluded that the two tasks are likely to use similar processes. The activation in PMc has been shown to be greater in MI than in ME, supporting the theory of increased reliance on motor planning during MI in comparison to ME [52]. The posterior portion of the SMA has also been consistently associated with MI, however, unlike the PMc, the SMA is thought to play different roles in MI and ME [72,76,79]. In ME, the SMA has

been shown to supply M1 with excitatory activity that largely results in an overt movement, but in MI the nature of this activity is thought to be switched, providing inhibitory signals to M1 instead of excitatory [79] . The involvement of M1 in MI has been one of the most controversial subjects with respect to mapping the MI network with many neuroimaging papers supporting either side of the debate [2,3,30]. Given its function as the brain region responsible for sending motor commands to the CST, M1 involvement in MI was questioned, as MI does not result in any overt movement. However, more recent work using TMS has demonstrated the M1 modulates CST excitability during MI and, as such, is likely active to some degree during MI [83,84]. The question of how M1 activity can occur during MI without resulting in an overt movement has yet to be fully explained.

2.9: Theories behind the lack of overt movement as a result of MI

Three theories have been put forward to explain the phenomenon of the lack of overt movements as an outcome of MI (Figure 7); 1) motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding execution, 2) cortical areas exert inhibitory influences to suppress the mobilization of the motor command after the mental representation of the plan has been formed and 3) CST influences may be responsible for motor inhibition after the signal is sent by M1 [5].

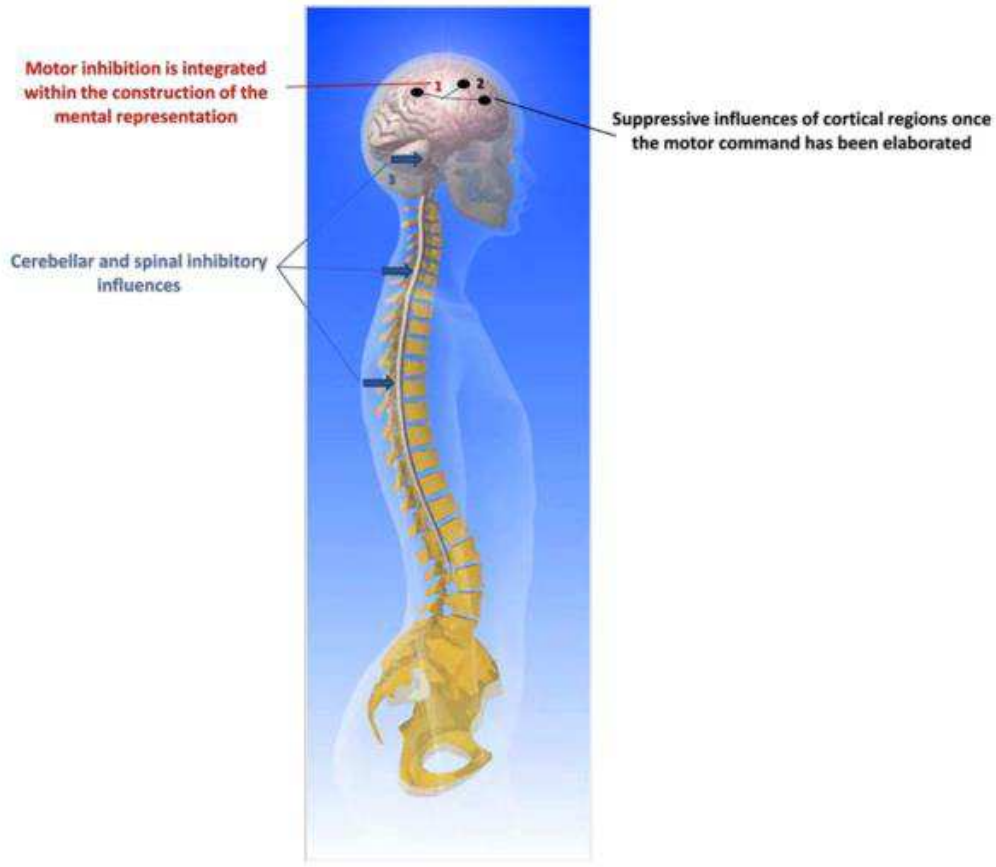

Figure 7: Possible sources of motor inhibition during MI. (taken from Guillot et al. 2012)

Since the publication of the review by Guillot and colleagues, the third theory (CST influences may be responsible for motor inhibition after the signal is sent by M1) was tested using TMS in an investigation of the effect of the descending signal from M1 on the CST during MI [6]. This study used a variety of complementary techniques, including MEPs, cervico-medullary-evoked potentials and Hoffmann-reflexes, to determine the effect of MI on CST structures and its interaction with the peripheral nervous system. The effect of MI on spinal excitability was assessed using cervico-medullary-evoked potentials and Hoffmann-reflexes, which are two independent measures of spinal excitability [88]. The peripheral level of the CST was evaluated using

MEPs. Additionally, novel techniques involving passive lengthening of the target muscle and a conditioning manoeuver during MI was used to address the role of MI on spinal structures with thresholds lower than the alpha-motor neurons. During the study, participants were asked to perform MI of a maximal voluntary contraction of the target muscle, the flexor carpi radialis. Results showed that during MI, MEP amplitude in the target muscle increased as did cervico-medullary-evoked potential amplitude, however Hoffmann-reflex amplitude remained unchanged. The novel techniques revealed that MI retained Hoffmann-reflex magnitude in comparison to rest where Hoffmann-reflex amplitude was diminished. These results indicate that MI causes subthresold activation of the CST that is insufficient to recruit alpha-motor neurons, yet these descending volleys during MI were able to activate low-threshold interneurons decreasing presynaptic inhibition (Figure 8) [6]. This finding means that MI could represent an intermediate cortical output somewhere between rest and voluntary contraction [89]. When taken in combination with the theories posed by Guillot et al. 2012, it suggests that motor inhibition likely occurs at the cortical level, indicating theory 1 or 2 are more likely to explain the absence of overt movement in MI.

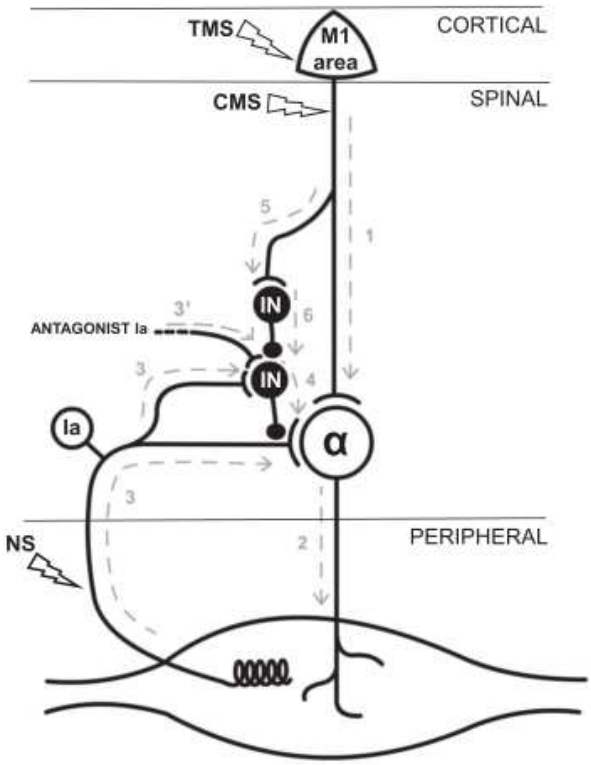

Figure 8: Simplified diagram of the hypothetical spinal neuron network involved in MI. It is suggested that MI generates a subthreshold impulse that is not strong enough to cause activation of alpha-motor neurons (2). Instead, MI might activate a system of interneurons (5 + 6) with lower thresholds of activation. The results of this interneuron activation is the reduction of presynaptic inhibition on the alpha-motor neuron (from Grosprêtre et al. 2016).

While the first two theories both indicate cortical inhibition of the motor command, they differ in regard to the timing of the inhibition. Theory 1 proposes that inhibition occurs as part of the process of generating a mental representation of a motor task, whereas theory 2 proposes that inhibition occurs after the mental representation has been generated. Given that ME is classically thought to be a function of M1, it is theorized that reducing activity in this part of the brain is a likely explanation for the lack of movement in MI. Suggestions have been made in support of both theories, indicating numerous cortical areas involved in MI that may be involved in the

inhibitory processes (Table 1). Additionally, the authors suggested that more research needs to be done to investigate the degree to which contralateral M1 is inhibited by other areas of the brain and whether or not neural impulses elicited by M1 during MI are inhibited by spinal mechanisms [5]. The results from Kasess et al. 2008 indicate that the SMA was responsible for the inhibition of M1 during MI (Figure 4 & Table 1) [79]. In this study, the task was broken into two phases; a countdown and execution phase. During the preparation phase for both conditions (MI and ME), activity at both ROIs were identical indicating similar processes underlie both tasks prior to execution. It was only during the execution phase that the lack of activity in M1 during MI became noticeable, suggesting that the inhibition of overt movement occurred after the mental representation of the task was generated during MI [79]. The timing of the inhibition in MI, as reported in this study, supports theory 2.

Table 1: A list of studies that have identified cortical areas possibly involved with inhibition of M1 during MI. (taken from Guillot et al. 2012)

Authors	Type of study	Method	Participants	Potential inhibitory regions
Alkadhi et al. (2005)	Motor imagery of foot movement	fMRI	Healthy ($n = 8$) Patients ($n = 8$)	Motor command suppression but no clear inhibitory regions
Bonnet et al. (1997)	Motor imagery of a foot pressure on a pedal	Reflex stimulation	Healthy ($n = 20$)	Inhibitory spinal influences
Di Rienzo et al. (submitted)	Case study with a C6–C7 quadriplegic patient	MEG	Patients ($n = 1$)	Primary sensory area and supplementary motor area
Jeannerod (2001, 2006)	Review papers	–	–	prefrontal cortical areas and/or brainstem and spinal influences
Kasess et al. (2008)	Motor imagery of finger movements	fMRI	Healthy ($n = 8$)	Supplementary motor area
Lotze et al. (1999b)	Motor imagery of hand movements	fMRI	Healthy ($n = 10$)	Posterior cerebellum
Schwoebel et al. (2002)	Case study with a patient suffering from bilateral parietal lesions	Psychophysic experiment	Patients ($n = 1$)	Fronto-parietal network
Solodkin et al. (2004)	Motor imagery of a finger-to-thumb opposition task	fMRI	Healthy ($n = 18$)	Superior parietal lobule and supplementary motor area
Deiber et al. (1998)	Motor imagery of finger movements	TEP	Healthy ($n = 10$)	Inferior frontal cortex
Lebon et al. (2012)	Motor imagery and mental rotation of a pinching movement	TMS	Healthy ($n = 11$)	Inferior parietal lobule

A more recently published article by Eagles et al., 2015 supports theory 1 [90]. In this study, the authors created a startle response paradigm where participants were

asked to drop a weight when they heard an auditory 'release' cue while their brain activity was being recorded via electroencephalography. The task was performed via ME and MI, such that the MI group imagined dropping the weight without physically doing so. Prior to the release cue, the participants were occasionally presented with a startle cue at different intervals from the release cue (e.g., 200ms or 500ms prior to the release cue), causing them to prematurely drop the weight. Results demonstrated that participants in the ME group were increasingly prone to dropping the weight when presented with a startle cue that was presented closer in time to the release cue whereas the MI group remained impervious to the startle cues regardless of their proximity to the release cue [90]. Additionally, the magnitude of the neural activity during MI at M1 at the time of the latest presented startle cue, 200ms prior to the release cue, was greater than the neural activity recorded in ME at a startle cue 1500ms prior to the release cue (Figure 9). In the ME condition, the startle cue 1500ms prior to the release cue caused the participant to prematurely drop the weight whereas in the MI group, even the startle cue closest to the release cue was unable to cause the participants to prematurely drop the weight [90]. Therefore, only interpreting the magnitude of activity in M1 does not explain the differences between ME and MI. If the processes were analogous, then sufficient M1 activity should have caused release of the weight regardless of condition. These results suggest that there are fundamental differences in preparation for ME and MI, a finding that supports theory 1.

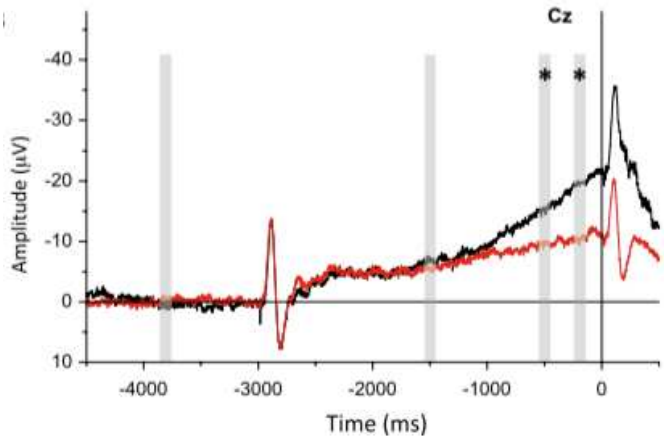

Figure 9: Grand average electroencephalography activity in response to the startle task designed by Eagles et al. 2015 from both the MI group (red) and the ME group (Black). (Eagles et al., 2015)

As outlined above, two possible theories are likely to explain the lack of overt movement in MI: 1) motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding execution, and 2) cortical areas exert inhibitory influences to supress the mobilization of the motor command after the mental representation of the plan has been formed [5]. While both theories are valid, it is unknown which is more representative of the neural mechanisms underlying the inhibition of overt movement during MI.

2.10: Research Objectives and Hypotheses

Given the limited understanding of the interactions between brain areas while performing MI, the purpose of this study was to determine the timing and source of the mechanism responsible for the absence of overt movements in MI, the primary behavioural difference between MI and ME. As such, the primary objective of the analysis was to determine the presence of motor inhibition occurring during MI. Once

the presence of the motor inhibition during MI was established, a secondary objective was to determine which brain region(s) may be responsible for this inhibition of the motor output. To address these research objectives, brain activity was obtained during the actual (ME) and imagined (MI) performance of a two-target grasping task to determine the networks underlying both. Brain activity in specific ROIs was examined in the spatial, temporal and spectral domains to investigate the timing and source of motor inhibition during MI. Given the scope of the project, the analysis performed was exploratory in nature and did not include formal statistical analysis; however, exploration of the data was aligned with the research objectives by focusing on the following hypotheses:

1. Motor inhibition will occur in MI, evidenced by decreased activity in contralateral (left) M1 post-task onset in MI in comparison to ME, and that this motor inhibition will result from mechanisms related to the process of generating a mental representation of a motor task.
2. The brain region responsible for this inhibitory effect will be the SMA, which may be influenced by other areas of the brain within the MI network located in the frontal or premotor regions. Regions possibly involved in motor inhibition during MI will be active immediately prior to M1.

Elucidating the mechanism by which movement is inhibited in MI is important to the MI field of research as the findings of this study will further the understanding of MI as a modality for motor learning, which has implications to fields such as sport, music, skilled vocations and rehabilitation.

Chapter 3: Methodology

3.1: Participants

Eighteen young, healthy, right-handed participants free of neurological impairments and who were MEG compatible, as determined by a pre-screening questionnaire, were recruited for this study. Handedness was confirmed by the Edinburgh handedness questionnaire [91]. Ethics approval was obtained from the research ethics board of the IWK Health Centre. Written informed consent was obtained from each participant prior to his or her involvement in the study.

3.2: MI Assessment

Prior to engaging in the experimental task, the MI ability of each participant was assessed using the Movement Imagery Questionnaire-Revised, Second Edition. This questionnaire is a highly reliable tool for assessing the ability to perform MI in individuals with and without brain injury [92,93].

3.3: Task Overview & Apparatus

Neuroimaging data was obtained using MEG (see below for details) during the performance of a unilateral power grasp task with the dominant hand. A unilateral, as opposed to a bilateral, power grasping task was selected in order to constrain task dependent brain activity to the hemisphere contralateral to the limb used for performance of the task [94,95]. Additionally, two targets were included in the task to introduce variability to ensure participants adhered to the timing of the task and were not pre-emptively preparing for task execution.

3.3.1: Apparatus

To address the study objectives, the experimental task required participants to prepare and execute a motor plan with the goal of imagining or executing a grasp to one of two targets in response to a series of cues while their brain activity was recorded. The apparatus used was built to function within the confines of the magnetically shielded room in which the MEG scanner is housed. Two handles protruding from the top of the apparatus served as the targets for the power grasp. The targets were presented 20.32 cm above the participant's leg when seated in the MEG and were oriented to the left and right of the participant's right arm such that the targets were equidistant from the participant's hand when at rest. To increase ambiguity between the two targets, the right and left targets were oriented differently, 45° and 135° above the horizontal respectively (Figure 10).

Figure 10: Experimental apparatus used. Two targets (rotated 45° and 135° above the horizontal) were presented to the participant on either side of their right leg.

Task performance was guided by a custom script generated using the Presentation software package (Neurobehavioural Systems, Albany, CA). Specifically, the custom script included a visual presentation of the apparatus and both auditory and visual cues to guide task performance. The presentation was projected on a screen one meter in front of the participant once they were comfortably seated in the MEG scanner. The presentation also appeared on the screen with consistent dimensions across participants to ensure that each participant had a similar viewing angle and that the entire presentation was viewable to the participant without having to move their eyes. Additionally, the apparatus was oriented such that both targets were in the participants' field of view when they were looking at the presentation. These

specifications helped minimize the effects of eye and head movement on the neuroimaging data.

3.3.2: Task Details

In general, the experiment was broken up into blocks of 15 trials (described below), with each trial lasting 6 seconds and sub-divided into two phases: preparation and execution (Figure 11). The beginning of each trial was indicated to the participant by a preparation cue. This cue consisted of an image of the two targets, from the participant's point of view (i.e., first person), with a superimposed fixation cross appearing in the middle of the screen. The preparation cue was present on the screen for 3s, the total duration of the preparation phase. While this cue was present on the screen, participants were instructed to prepare for task onset. Task onset was indicated by the appearance of a translucent green circle superimposed on the preparation cue over either the left or right target. Participants were told that the highlighted target indicated the goal for the current trial. During this performance phase, which lasted 3s, the participants respond by grasping the highlighted target using the appropriate modality (MI or ME) based on the block type. To introduce further ambiguity between the movements required for each target, participants were instructed to grasp the left handle with an overhand grip and the right handle with an underhand grip. Each trial was then separated by an inter-trial-interval where participants were presented with a fixation cross. The duration of inter-trial-intervals was randomized, being 3, 4 or 5s in length, such that the average inter-trial-interval was 4s.

Figure 11: Outline of a single experimental trial. Each trial lasts a total of 6 seconds beginning with a preparation phase and ending with a performance phase. Each phase is indicated by a cue (see text for details). Trial are separated by a 3-5 second inter-stimulus-interval.

3.4: MEG Acquisition

Neuroimaging data was obtained using a 306-channel whole head Elekta Neuromag MEG system (Elekta AB, Stockholm, SE) located within and operated by the IWK Health Centre. Data was sampled at 1000Hz and a bandwidth of 0.1-330Hz. Additionally, bi-polar electrodes used to obtain physiological signals (e.g., electromyography, EMG) were sampled at 1000Hz using the same electronics as the MEG, and head position continuously monitored via the head position indicator coils placed on each participant (described below).

3.5: Session Overview

3.5.1: Participant preparation

Upon arrival at the IWK MEG lab, participants were asked to remove metallic objects that may interfere with the MEG recordings. Participants were shown the MEG scanner and comfortably seated in it to perform an artefact check. The purpose of the artefact check was to ensure that the participants were not generating artefacts during subsequent recordings of neural activity in the MEG. Afterwards, participants were removed from the MEG scanner to complete the preparation for the MEG scan.

The remainder of the preparation process can be divided into two parts; attaching electrodes for electrophysiological recordings and acquiring the head position estimates (see '*Head position estimates*' section below). Four electrodes were placed around the eyes to record vertical (above and below the left eye) and horizontal (just lateral to each eye) electrooculograms to facilitate the removal of artefacts related to eye movements and blinks. Two electrodes were then placed on the inside of each bicep to obtain the electrocardiogram to facilitate removal of the electrocardiogram artefact in post-processing. Electrodes were also placed, according to SENIAM guidelines, one finger width anterior and distal to the acromion process on the dominant arm in a bipolar arrangement with a 2 cm inter-electrode distance to obtain the EMG of the anterior deltoid to facilitate monitoring of muscle activity during MI. An additional electrode was placed on the participants' left collarbone to serve as a ground. The sites for each electrode were prepared by lightly abrading the skin with NuPrep skin gel (Weaver and Company, Aurora, CO) and cleaned using an alcohol swab to ensure

minimal impedance of the recorded signal and improved adhesion of each electrode. A generous layer of Elefix, a conductive paste that serves as a medium for the desired signal to reach the electrode, was then applied to each electrode before they were affixed to the skin using Tagaderm transparent film dressings (3M, London, Ont).

3.5.2: Head position estimates

The location of the participant's head was tracked throughout the scan to ensure they remained still during data acquisition. Four head position indicator coils, affixed to the scalp (bilaterally on the forehead above each eyebrow just below the hairline and overlying the mastoid process bilaterally) were digitized to allow for monitoring of the participant's head position during data acquisition. In addition to the four head position indicator coils, three anatomical landmarks (nasion and bilateral pre-auricular points) were digitized using an electromagnetic stylus (Polhemus digitization device, Polhemus Incorporated, Vermont, USA) to facilitate co-registration with a template MRI brain for subsequent analysis. The electromagnetic stylus was then run across the participant's head to collect 200 evenly distributed points along the scalp to create a 3D representation of the participant's head. This 3D structure was then used to verify the accuracy of the co-registration process, to ensure the process will provide highly accurate MEG source localizations [96].

3.5.3: Experimental procedure

The study consisted of a single session (duration: 35:00 minutes) divided into 9 blocks: 4 blocks each of MI and ME, and 1 block of rest (Figure 12). The rest block was 5 minutes in duration during which participants were asked to relax in the scanner with

their hands resting in their lap while baseline (resting state) neural activity was recorded. Immediately following this rest block, participants listened to an audio script that explained the task and how to perform it in detail, including watching two videos illustrating task performance from the participant's (first person) point of view. Each video demonstrated ME task performance for trials where either the left or right target was highlighted. During the MI and ME blocks (duration 2:15min per block), participants completed 15 trials of the task during which brain activity was obtained throughout. The only difference between the MI and ME blocks related to task execution, such that participants actually execute the movement during ME, and imagine executing the movement during MI. The blocks were arranged such that the participants performed alternating two-block sequences of MI and ME (Figure 12). Participants were reminded via auditory instructions at the beginning of each segment of two blocks whether to physically execute the task (ME block) or imagine executing the task (MI block). The order in which the task modalities appeared was counterbalanced across participants to eliminate ordering effects. Throughout data acquisition, markers relating to the different components of the task (e.g., preparation cue, task onset and the inter-trial-interval) and block type (MI, ME, rest) were placed on the continuous data file to facilitate subsequent analysis.

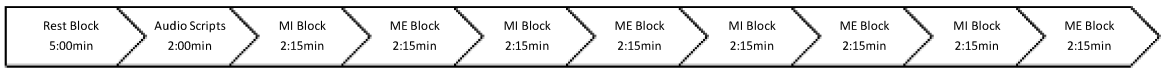

Figure 12: Outline of a single session. After participants were prepped for data collection and completed both a rest block (5:00min) and listened to the audio script (see text for details) they engaged in alternating sequences of MI and ME of the task.

3.6: Data Analysis

3.6.1: EMG analysis

The EMG data recorded during MI was used to reject segments of data that contained muscle activity relevant to the task being performed. The absence of activity during each MI trial was determined by comparing EMG activity during MI performance to the preceding rest block. All EMG data was rectified and then low-pass filtered at 10Hz. The EMG activity from the middle 4 minutes of the 5-minute rest block was used to calculate a mean and standard deviation that characterized EMG activity at rest (i.e., baseline). Similar to the analysis techniques of Kraeutner et al. [26], EMG activity in any given MI trial that exceeded the mean + 2 standard deviations of the EMG activity at rest constituted a trial with excessive muscle activity. If >15% of all comparisons for a

given participant demonstrated excessive movement while performing MI, the participant was removed from the analysis. In participants where excessive muscle activity was detected but did not exceed the >15% threshold, the individual trials exhibiting excessive muscle activity were removed. Trials remaining following the EMG analysis were carried forward for the remainder of study analysis.

3.6.2: MEG pre-processing

Pre-processing of the neuroimaging data proceeded according to standard laboratory procedures. First, data was reviewed to identify segments with excessive head movement (defined as > 5mm shift in any direction and/or > 3° rotation). Identification of excessive head movement was achieved via head position indicator coil data, tracking their coordinates in 3D space throughout the scan. Second, a visual inspection of all raw data was performed to identify and remove malfunctioning sensors to prevent them from affecting the data during the subsequent filtering techniques. Briefly, malfunctioning sensors typically either had large artefacts that greatly exceeded the average signal amplitude at neighbouring sensors or demonstrated changes in the recorded signal at a rate too fast to be naturally occurring. A Maxfilter process (Elekta AB, Stockholm, SE) was then applied, which uses a temporal signal spatial separation method to spatially filter the data to improve the signal to noise ratio [97]. During this step, the headspace for each MI and ME block were transformed to align with the rest block such that comparisons of brain activity during task and rest could be performed. Pre-processed data was notch filtered at 60Hz then bandpass filtered between 1-70Hz. Finally, cardiac artefacts and those artefacts resulting from eye movements were

identified and removed using a custom Python script employing independent component analysis to identify artefacts in the electrocardiogram and electrooculograms channels.

3.6.3: MEG-MRI co-registration

Following pre-processing, MEG data was co-registered to a template MRI (fsaverage; Freesurfer, Boston MA) to facilitate source localization. Co-registration was performed by aligning the anatomical landmarks digitized prior to data acquisition with the corresponding landmarks on the template MRI using the MRILab program (Elekta AB, Stockholm, SE), and adjusted so that the three digitized anatomical landmarks on the participant's head aligned with the fsaverage template brain. After co-registering the functional MEG data with the template MRI, a boundary element model was created for the template brain using a custom MATLAB script. The quality of the co-registration and subsequently created boundary element model file was visually inspected by overlaying the boundary element model onto the co-registered MRI using MRIview (Elekta AB, Stockholm, SE).

3.6.4: Evoked Potentials

Prior to beamforming (i.e., source-level analysis), a data check was performed to ensure that the appropriate physiological responses were appearing in the sensor level data for each modality. To achieve this, the evoked response was obtained by averaging the data for all sensors (magnetometers and gradiometers) across participants, trials, and blocks to obtain the average response for a trial of the paradigm. Each epoch was locked to task onset (i.e., the 3s point of each trial), and a 7s window was selected (-4 to

+3s from task onset). This window captures the entire trial and the 1s prior to the presentation cue. This grand averaged data was then inspected for the presence of expected responses including: 1) an early visual response to the presentation of the preparation cue; 2) a second visual response to the cue for task onset; and 3) a motor evoked field resulting from either the actual (ME) or imagined (MI) execution of the grasping task.

3.6.5: Source-level analysis (Beamforming)

Thirty-two anatomically pre-determined nodes selected based on their involvement in the ME and MI networks [94] defined by their Talarich-Tournoux coordinates were used as ROIs in the present study (Figure 13 & Table 2). In this study the PMCMed nodes (11 and 25) were interpreted as the corresponding left and right SMA. The nodes representing these ROIs were aligned with the 3D representation of the participant's brain in MEG space based on the transformations underlying the MEG-MRI co-registration.

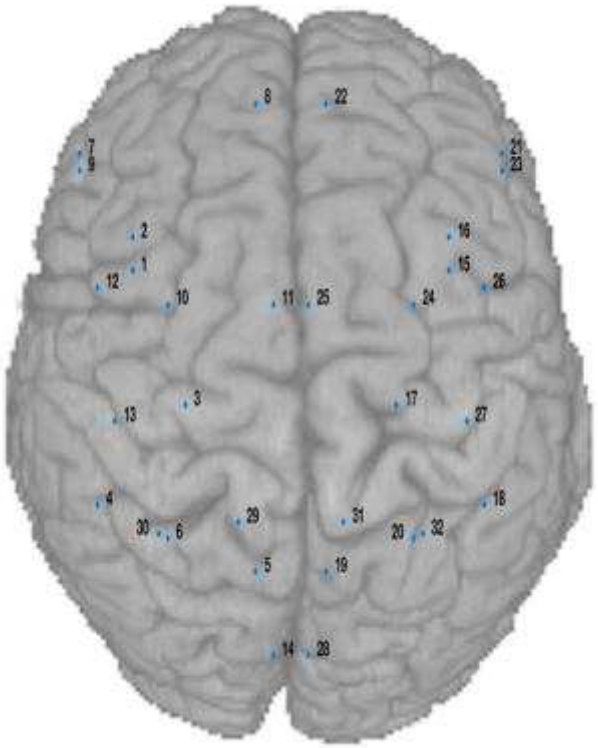

Figure 13: Location of the 32 predefined anatomical nodes used as ROIs (from Bardouille & Boe, 2012).

Table 2: Labels associated with the 32 predefined anatomical nodes used as ROIs (from Bardouille & Boe, 2012).

Region of Interest:	Label:		Region of Interest:	Label:	
	Left Hemisphere	Right Hemisphere		Left Hemisphere	Right Hemisphere
Frontal Eye Fields (FEF)	1	15	Ventrolateral Prefrontal Cortex (PFCVL)	9	23
Anterior Insula (AntI)	2	16	Dorsolateral Premotor Cortex (PMCDL)	10	24
Motor Cortex (M1)	3	17	Medial Premotor Cortex (PMCMed)	11	25
Inferior Parietal Cortex (IPC)	4	18	Ventrolateral Premotor Cortex (PMCVL)	12	26
Pre-Cuneus (PreCun)	5	19	Somatosensory Cortex (S1)	13	27
Superior Parietal Cortex (SPC)	6	20	Visual Cortex (V1)	14	28

Dorsolateral Prefrontal Cortex (PFCDL)	7	21	Dentate	29	31
Medial Prefrontal Cortex (PFCMed)	8	22	Posterior Lobe (PostLobe)	30	32

An estimate of the source level activity at each node across all trials within each block was obtained. Data from each block were trimmed to the length of the shortest block prior to beamforming. A whole head virtual electrode, event-related beamformer spatial filter was then applied to continuous data from each block to determine activity at each node [98]. The result of the beamformer analysis is 32 source-level virtual electrodes representing the estimated source strength during each block at each of the ROIs. Source level data was then plotted for each virtual electrode and imported into MATLAB for further analysis.

3.6.6: Time Frequency Response (TFR) analysis

The TFR analysis was performed as per Boe et al. 2014 [99] to obtain the average ERS/ERD in the beta frequency band (15-30Hz) associated with trials in ME and MI separately. For each virtual electrode, data was epoched such that each trial was a single 8-second segment (1s pre-preparation cue). A Morlet wavelet transformation (size= 512 samples) was then applied to each trial. The entire frequency range of the virtual electrodes (1-70Hz) was subjected to this transformation resulting in 70 frequency bins. The data from each trial was then trimmed by 0.5s on each side to remove the edge effect introduced by the transformation. The ERS/ERD was calculated from the remaining 7s of data using a base-2 logarithm of the ratio of beta band (15-30

Hz) ERS/ERD in a rest period to the period of time representing each trial. For this experiment, the rest period for each trial was the half-second prior to the preparation cue. The TFR was plotted using a pseudo-color plot across the entire range of frequencies (1-70Hz) over the course of a trial to optimize visualization the ERS/ERD response. For each virtual electrode, the TFR for each trial was averaged across trials and subjects within the beta band to obtain the power change in the beta band for each modality. These results were then plotted for each virtual electrode with the average power change in the beta frequency band from each trial for each modality superimposed on the same graph to make comparisons between conditions.

3.6.7: Source strength and oscillatory activity

The result of the above described analysis is activity from two 'levels', sensor and source, where the source level activity can be divided into two domains, time and frequency. Results that were interpreted from the sensor level were obtained from the evoked responses for each condition. This information relates to the strength of the magnetic field from each of the 306 sensors (i.e., measured in fT). At the source level, data from the time domain relates to the estimated source strength as a function of time at each of the desired 32 virtual electrodes (i.e., ROIs). Estimated source strength is measured in arbitrary units, interpreted as the estimate of neural activity at a given ROI in comparison to a non-active baseline segment. Data from the frequency domain was obtained as a result of applying the TFR analysis to the time domain data from each of the 32 ROIs. Such analysis provides information related to the oscillatory activity (ERS/ERD) in a given frequency band. Specifically, ERS is an indicator of an increase in

magnitude of localized rhythmic activity, whereas ERD refers to a decrease in magnitude [100]. Event related desynchronization in a specific ROI is indicative of a desynchronization of activity in the included neuronal assemblies representing a state of maximal readiness and information capacity. In contrast ERS is on the opposite end of the spectrum and is indicative of synchronized activity at in the brain region underlying the response representing a brain state with reduced information processing and lacking readiness [100]. For the purpose of this study, analysis will focus specifically on changes in the beta frequency band, as such changes have been linked with the performance of voluntary movement in the upper limbs [77].

3.6.8: Evaluation of ROIs

From the 32 nodes selected, the primary analysis was contained to areas thought to be involved in the inhibition of MI as outlined in previous literature (Table 1) [5]. These regions included S1, posterior CB, IPL & SPL, SMA, and the frontal cortex, specifically the inferior frontal cortex [5]. The analysis started with the evaluation of a subset of these areas thought to be involved in the preparation of motor commands to identify which, in any, were involved in motor inhibition. If the pattern of activation indicated possible inhibition of M1, the analysis was expanded to other ROIs within that area of the cortex. Finally, the analysis was expanded to the remaining areas in Table 1 to ascertain if they were contributing to motor inhibition in MI.

Chapter 4: Results

4.1: Participants

Of the 18 participants, 13 (4 females, aged 24.4 ± 2.5 years; range 18 to 32) were included in the final analysis. Participants displayed right hand dominance as defined by the Edinburgh Handedness Inventory (mean score of 86 ± 17.3). Importantly, all participants scored above the threshold for determining 'right handedness', which is >40 [91]. Of the 5 participants removed from analysis, 2 were removed during pre-processing for artefacts, 2 were removed for the presence of head motion that exceeded the threshold, and 1 was removed owing to the presence of artefacts after pre-processing was completed. Values reported throughout are mean ± standard deviation unless stated otherwise.

4.2: Movement Imagery Questionnaire

As indicated above, the ability to perform MI was determined using the Movement Imagery Questionnaire-Revised, Second Edition. All participants were deemed able to perform MI given scores in excess of the threshold of greater than 25 (mean score across all participants of 69 ± 24.8; range 52 - 86). Participants performed similarly on the visual and kinaesthetic domains of imagery (visual: 35.1 ± 5.1, kinaesthetic 33.9 ± 7.6). Participant scores were similar across all questions, evidenced by a low standard deviation associated with the mean (5.3 ± 0.3). The lowest scoring question had a mean score of 4.8 ± 1.4, while the highest scoring question had a mean score of 5.7 ± 0.8.

4.3: EMG

On average, participants demonstrated excessive EMG activity in 1.15% of MI trials, based on the criteria outlined in the methods sections. None of the participants were rejected based on the threshold of 15% of trials containing excessive movement. As indicated in the methods section, individual trials were removed from the analysis (i.e., removal was done on a trial by trial basis for each participant); the greatest number of trials removed from a single participant was 4 (out of a possible 60) or 6.7% of all possible trials in the MI blocks.

4.4: Magnetoencephalography Results

4.4.1: Sensor Level Activity

The grand average for an entire trial (preparation and performance phases) for both modalities (ME and MI) are shown in Figures 14 and 15. In each response, the preparation cue was presented to the participant at 0s and the green circle highlighting the target for the given trial was shown to the participant at the 3s mark. In both ME and MI, there were 3 visible peaks in activity (see Figures 14 and 15). The first peak occurring within the first 500ms corresponds to the participant's visual response to the presentation of the prep cue (Figures 14 and 15). The second and third responses occur shortly after task onset (time 3000ms). The first spike in activity occurring approximately 100-200ms after task onset is a second visual response which is followed shortly thereafter by the motor evoked response, approximately 200ms later (Figures 14 and 15). While the motor response is present in both modalities, it has a larger magnitude in ME (Figure 14) relative to MI (Figure 15) and lasts slightly longer. These responses

confirmed the expected physiological responses from a trial and validated further analysis of the data during each task at the source level.

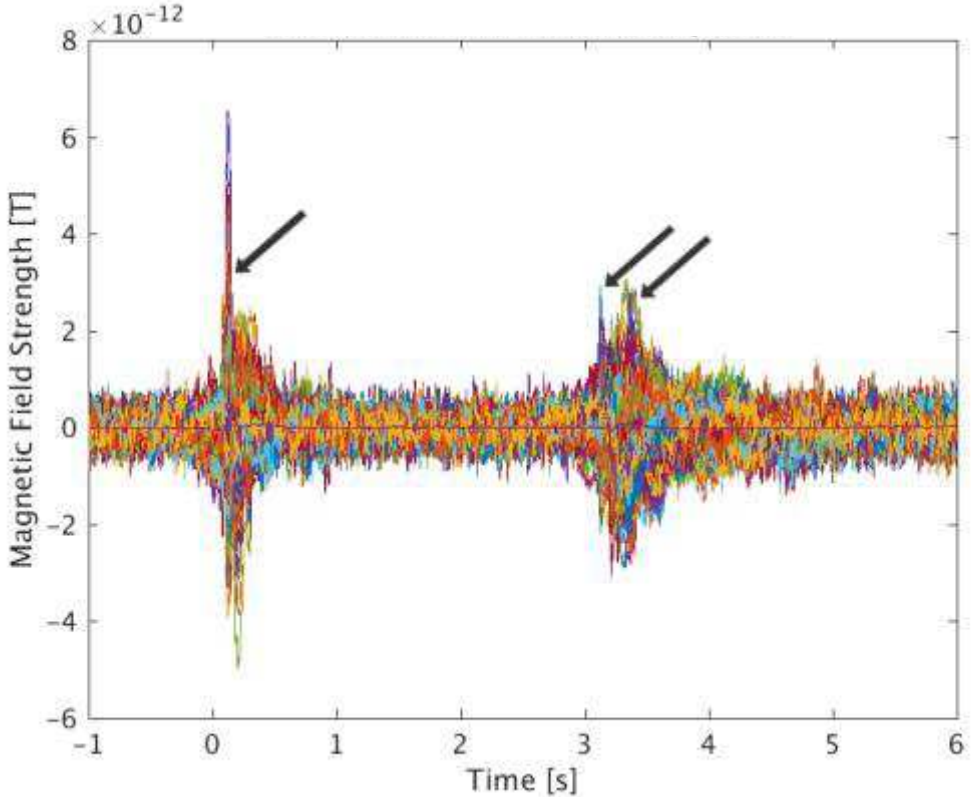

Figure 14: The ME evoked response represented in sensor space from all 305 magnetometers and gradiometers. The visual responses to the preparation and task onset cues are visible at 100-200ms and 3100-3200ms respectively. The motor evoked response is also visible at 3300-3700ms. These responses are indicated by the black arrows (left to right for the 3 responses noted above).

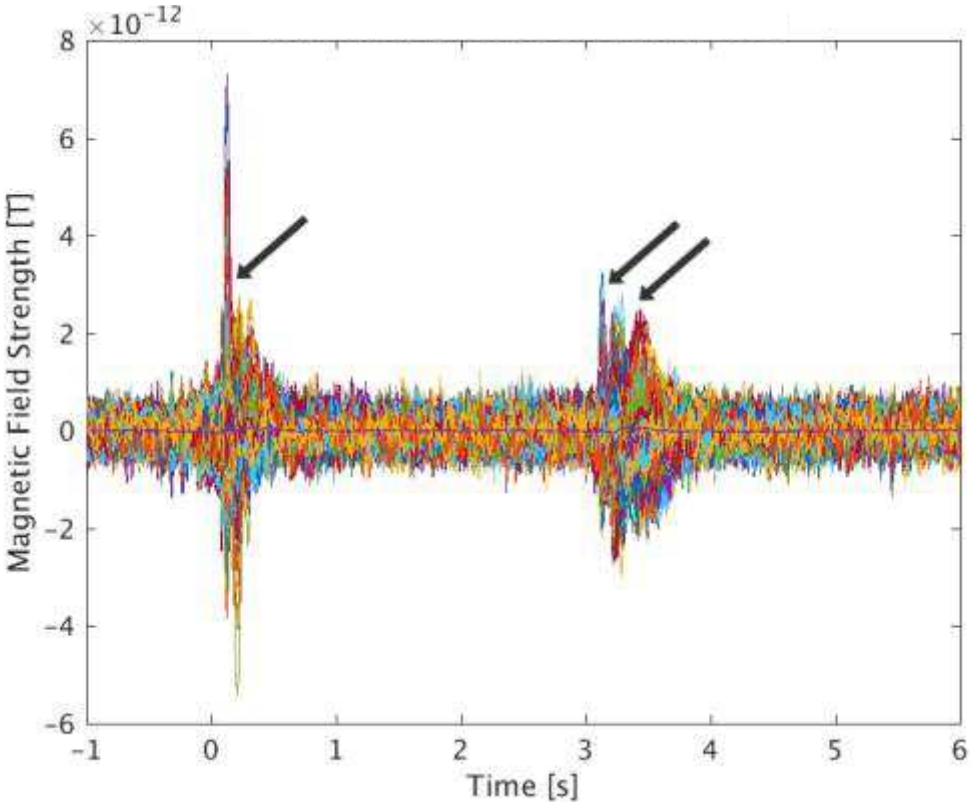

Figure 15: The MI evoked response represented in sensor space from all 305 magnetometers and gradiometers. The visual responses to the preparation cue and target highlight are visible at 100-200ms and 3100-3200ms respectively. The motor evoked response is also visible at 3300-3600ms. These responses are indicated by the black arrows (left to right for the 3 responses noted above).

4.4.2: Source level activity

Source level activity is shown for specific ROIs (i.e., virtual electrode) in Figures 16 through 19. This subset of the 32 ROIs (virtual electrodes) in the hemisphere contralateral to the hand executing the grasping task (left hemisphere) included M1 and areas of the brain identified as possible sources for M1 inhibition during MI, including the SMA, S1 and the posterior lobe of the CB (as per Table 1). Each figure comprises 4 plots: the upper plot (A) represents the estimated inter-trial average signal strength (source level) of the virtual electrode over the course of trial for ME (blue line) and MI (orange line). The two middle plots (B and C) are the grand average TFRs for both ME

(right plot) and MI (left plot), displaying ERS/ERD in the 1-70 Hz. The lower plot (D) shows power change in the beta frequency band for ME (blue line) and MI (orange line).

The results shown in Figures 16 (A-D) for M1 indicate that inhibition is present in MI, but not ME. The estimated source strength (Figure 16A) reveals a peak in activity shortly after task onset (~3.3s). This peak in activity is considerably larger in ME than in MI (Figure 16A). The TFR plot for contralateral M1 (Figure 16B and C) reveals ERD in the beta frequency band in both ME and MI; this ERD appears to be somewhat temporally aligned with the peak in activity observed for the estimated source strength (Figure 16A), however the ERD peak occurs a bit later than that observed for the estimated source strength (~3.8 vs. 3.3s respectively; Figure 16A vs. Figure 16D). In comparing ME and MI, it is clear that the magnitude of ERD in the beta frequency band is larger in ME than MI (Figures 16 B and C respectively), and in addition to a larger magnitude of ERD in ME, the shift towards ERD starts earlier for ME relative to MI, with the onset of ERD occurring at the beginning of the preparation phase (~0.5s), whereas in MI, the changes in ERD are more constrained in time beginning shortly before the onset of the execution phase (~2.8s) (Figure 16D).

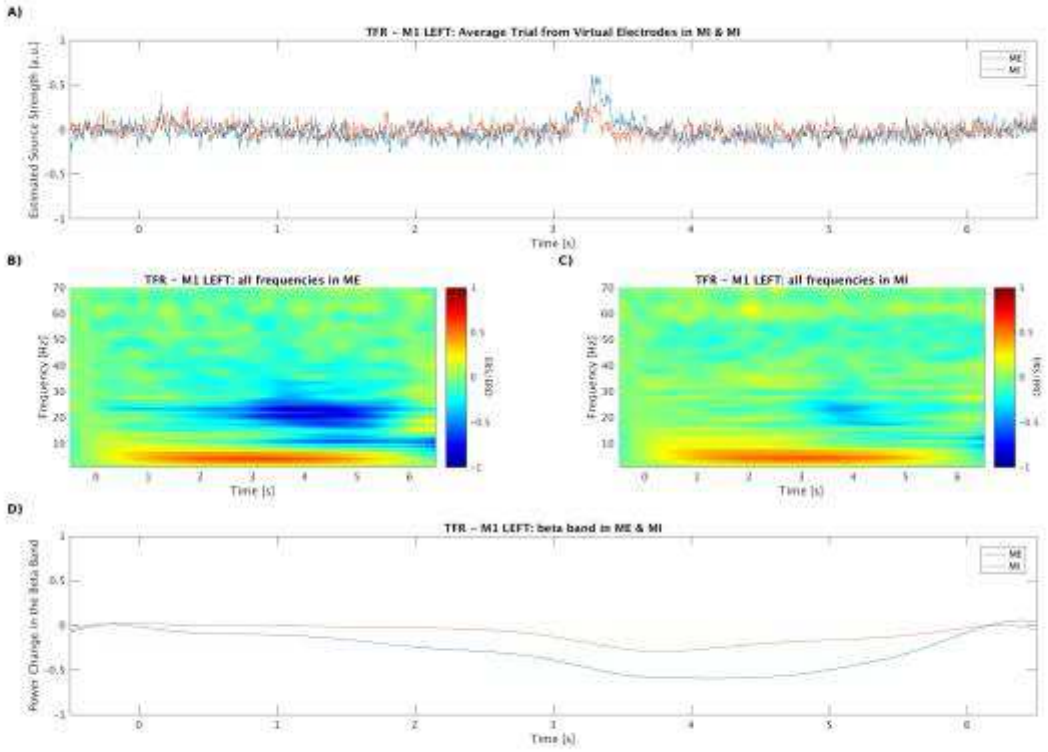

Figure 16: Virtual electrode data for left (contralateral) M1. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). M1 inhibition in MI is present based on the reduced magnitude in the estimated source strength immediately after task onset in comparison to ME. This difference is also reflected in the frequency domain, evidenced by a reduced magnitude of the ERD in MI relative to ME.

Given that inhibition of M1 occurred in MI (as shown in Figures 16A-D), we further investigated the activity at the previously identified ROIs that represented the possible source of this inhibition, including the SMA, S1 and the posterior lobe of the CB (referred to as 'PostLobe'). Results for the SMA are shown in Figures 17 (A-D). The estimated source strength (Figure 17A) reveals a peak in activity shortly after task onset (~3.3s) that is of similar magnitude in both conditions. Despite the similar estimated source strength between conditions, the TFR analysis of contralateral SMA revealed

stark differences between conditions. In ME (Figure 17B), there is a large peak in ERD in the beta frequency band starting early in the preparation phase (~1s; Figure 17D) that occurs later in time in comparison to the peak in estimated source strength (~3.3s; Figure 17A versus ~3.6s; Figure 17D). In contrast to ME, the magnitude of ERD in the beta frequency band is considerably less in MI (Figure 17C). There does appear to be a small ERD peak ~1s after task onset (i.e., the 4s mark; Figure 17D) but it is not well aligned with the peak in estimated source strength (Figure 17A).

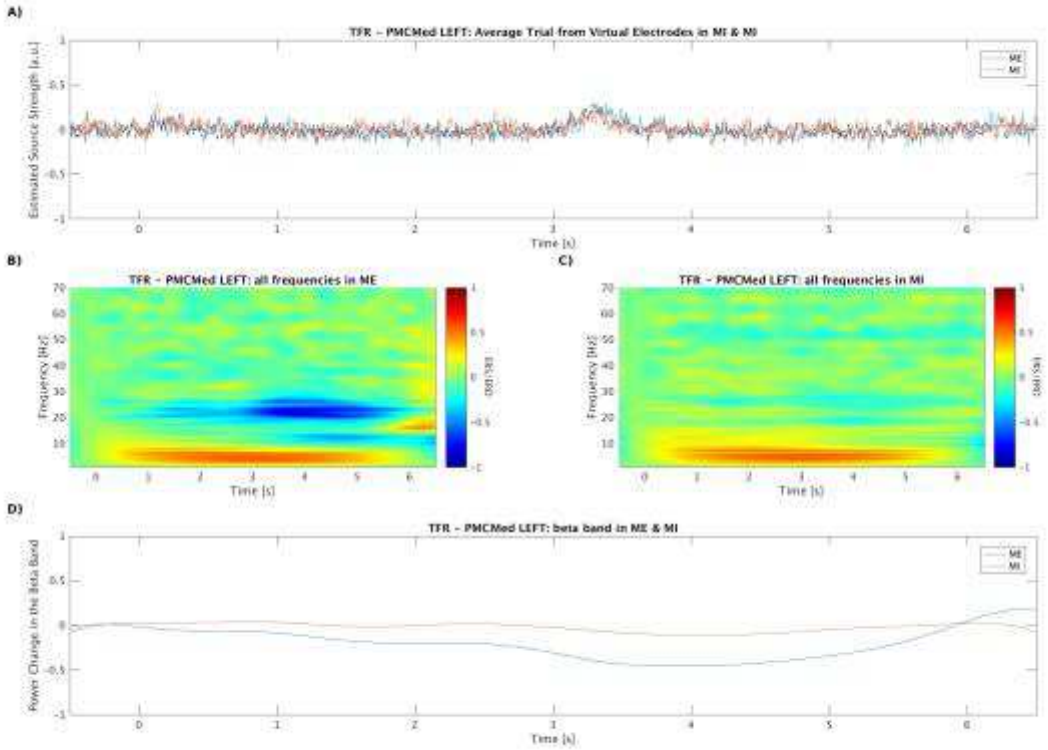

Figure 17: Virtual electrode data for left (contralateral) PMCMed (SMA). Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). While there is very little difference in the magnitude of the estimated source strength between conditions, there is a substantial ERD response in ME that is greatly reduced in MI.

In S1, the estimated source strength (Figure 18A) demonstrates a peak in activity shortly after task onset in both conditions, however, in ME the magnitude of the peak is larger and slightly delayed in comparison to MI (~3.4s in ME vs. ~3.3s in MI; Figure 18A). Additionally, the difference in the magnitude of activity between conditions is not as large as the reported difference in estimated source strength magnitude in M1 (Figure 18A). The TFR plot for contralateral S1 (Figure 18B and C) reveals ERD in the beta frequency band in both ME and MI; this ERD appears to be somewhat temporally aligned with the peak in activity observed for the estimated signal strength (Figure 18A), however the ERD peak occurs later than that observed for the estimated signal strength (~4.2 vs. ~3.4s in ME and ~3.8 vs. ~3.3s in MI) (Figure 18D vs. Figure 18A). There is also a considerably larger magnitude of ERD in the beta frequency band in ME in comparison to MI (Figures 18B and C respectively). Additionally, the onset of the ERD occurred earlier in ME (~0.5s) than in MI (~3s; Figure 18D).

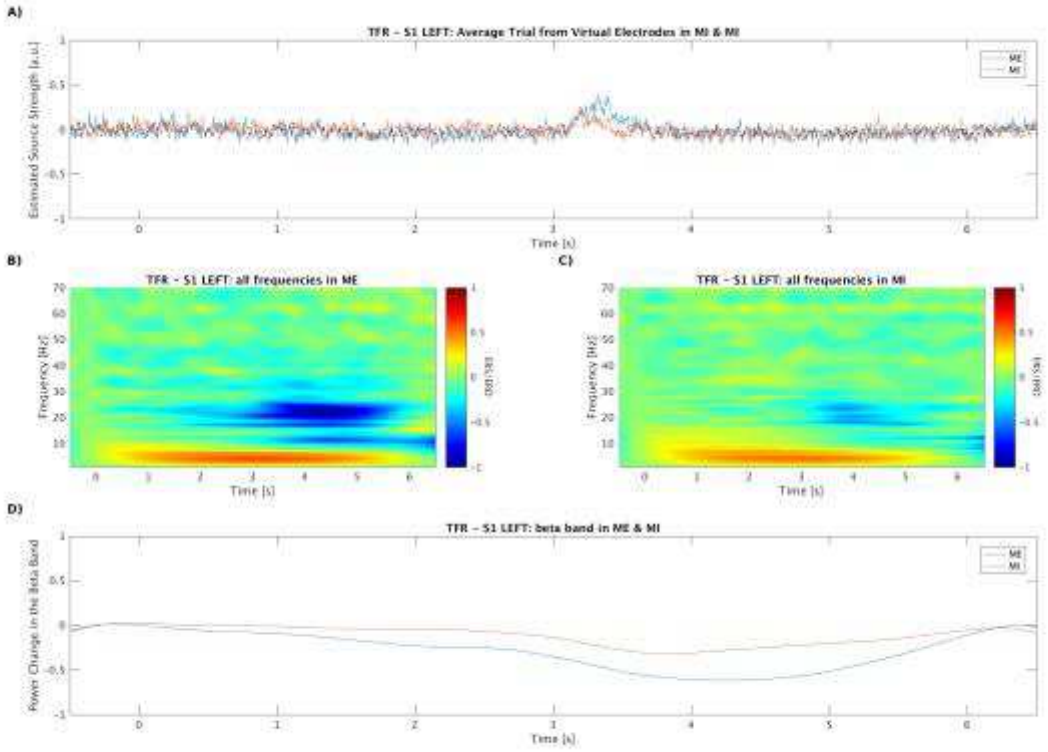

Figure 18: Virtual electrode data for left (contralateral) S1. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). There is a peak in magnitude of estimated source strength in both conditions shortly after task onset but the peak is larger and slightly delayed in ME. This difference is also reflected in the frequency domain as there is a larger ERD response in ME that peaks later than it does in MI.

Unlike M1, SMA or S1, the PostLobe was not very active during the task (Figures 19A-D). The largest response in estimated source strength obtained in the PostLobe occurred shortly after the preparation cue (~0.1s) in both conditions and otherwise remained fairly inactive during task performance (Figure 19A). The TFR analysis also demonstrated little change in ERS/ERD in the beta frequency band during the trials in either condition (Figures 19B and C respectively), with only a slight peak ERS occurring in ME very late during the course of the trial (~4.8s; Figure 19D).

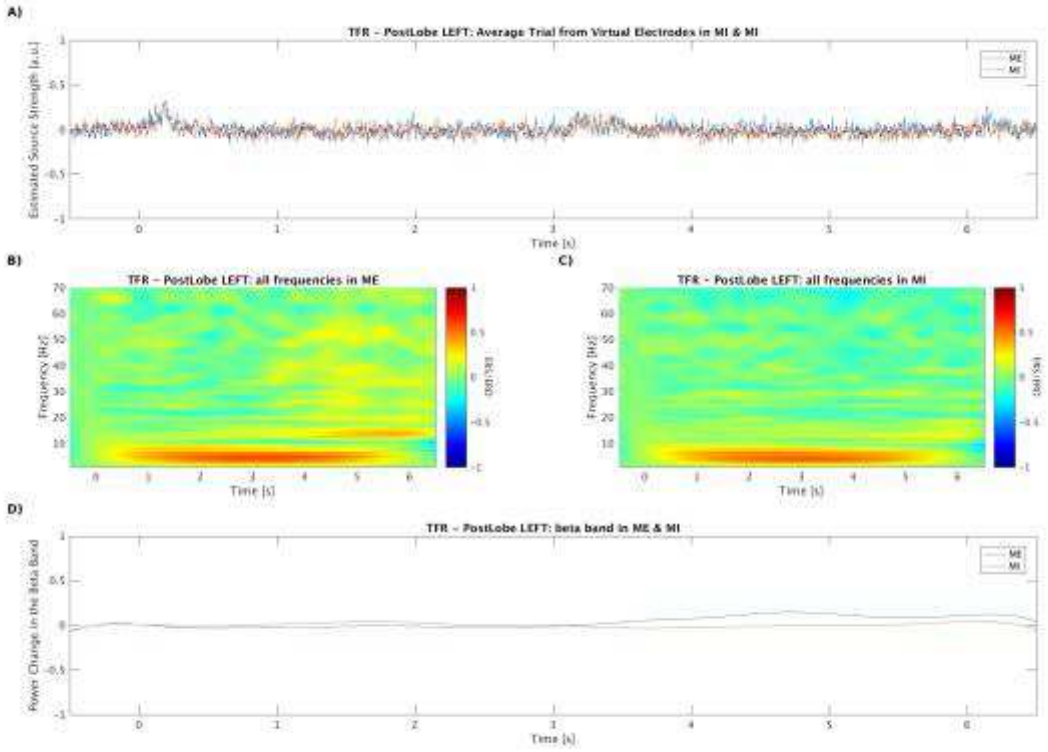

Figure 19: Virtual electrode data for left (contralateral) PostLobe. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). While there is not a noticeable peak in the magnitude of the estimated source strength in either condition after task onset, there is a small ERS response in ME late into the performance phase that is not present in MI.

To aid in the investigation of which brain areas (ROIs) were candidates for being the source of the inhibition of M1 during MI, the time-course of the average trial for each ROI was plotted separately for each condition (Figures 20 and 21). In ME, the timing of estimated source strength in M1 was similar to SMA and S1 with small differences, however, the peak activity in S1 occurred slightly after the peak in M1 whereas the opposite is true of the peak in SMA (Figure 20A). The most notable difference in the estimated source strength was that the magnitude of the peak in M1

was larger than in the other ROIs (Figure 20A). In the frequency domain, similar patterns of beta frequency ERS/ERD were demonstrated in each ROI with the exception of the posterior lobe (Figure 20B). The difference in magnitude of ERS/ERD between each ROI was small, with very similar peak magnitudes observed between M1, SMA and S1 (Figure 20B). The temporal dynamics in this domain replicated the patterns seen in the time domain with SMA reaching peak activity first, followed by M1 and S1 respectively (Figure 20B). The PostLobe did not show any change in estimated source strength or ERS/ERD (Figures 20 A and B).

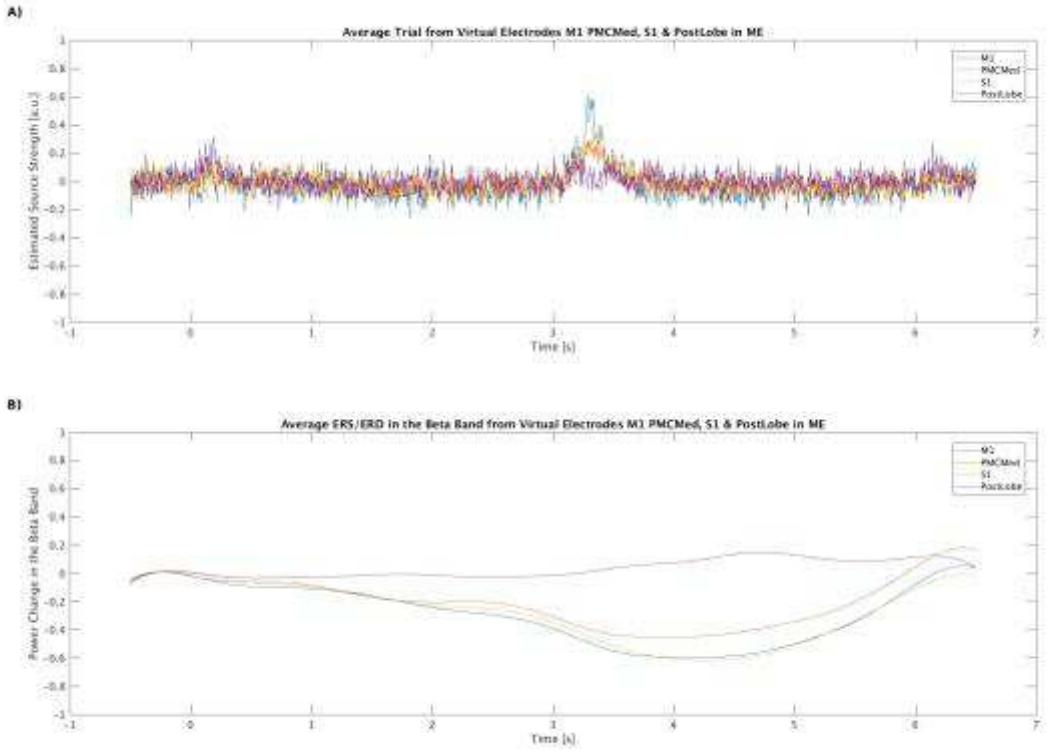

Figure 20: Virtual electrode data from M1, PMCMed, S1 and PostLobe represented by estimated source strength (A) and ERS/ERD change in the beta band in ME (B). The development of peak activity in M1 is mirrored by PMCMed and S1 in both the time and frequency domains. Notably, the peak estimated source strength for M1 is greater than peak activity from the other regions.

In MI, the timing of estimated source strength in M1, SMA, S1 and PostLobe replicated the patterns seen in ME except with smaller magnitude (Figure 21A). The estimated source strength of all ROIs during MI was greatly reduced in comparison to ME, yet the peaks of estimated source strength occurred at roughly the same time in both conditions (Figure 20A and Figure 21A). The beta frequency band ERS/ERD also showed reduction in magnitude in MI in comparison to ME (Figure 21B vs. Figure 20B respectively). The temporal dynamics of the ERS/ERD responses changed considerably as peak ERD in the SMA occurred well after M1 and S1 (Figure 21B). Similar to ME, the PostLobe in MI did not display any changes in estimated source strength or ERS/ERD in the beta frequency band (Figures 21A and B).

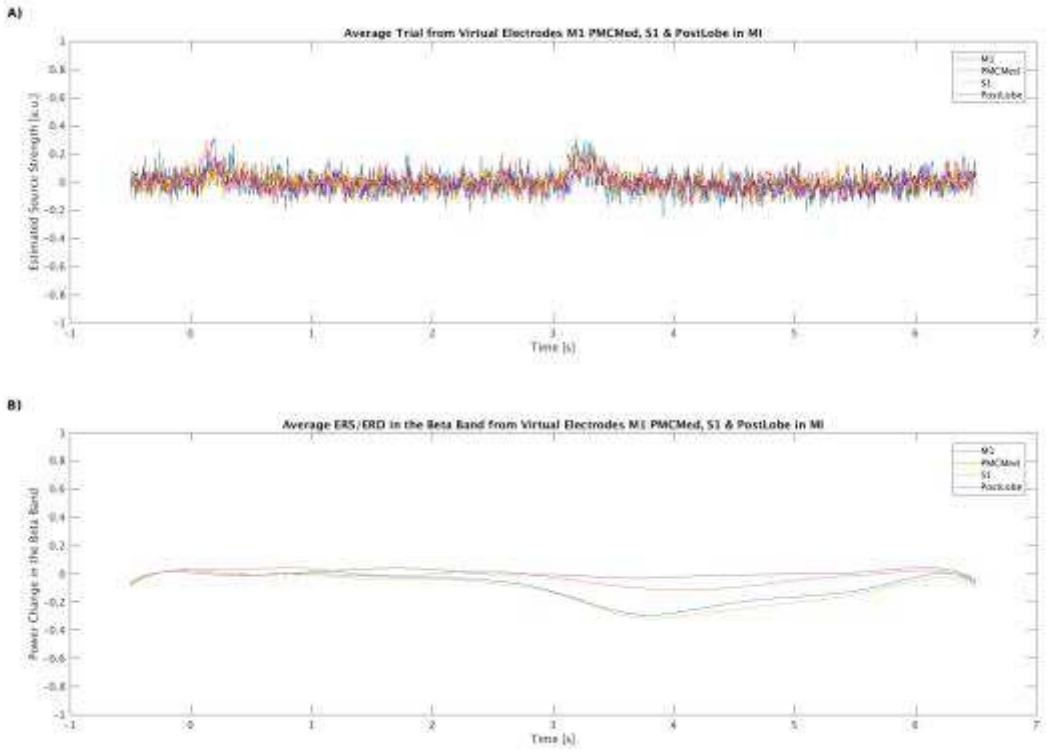

Figure 21: Virtual electrode data from M1, PMCMed, S1 and PostLobe represented by estimated source strength (A) and ERS/ERD change in the beta band in MI (B). The

development of peak activity in in MI replicated the results seen in ME except with smaller magnitude.

Given the lack of involvement in PostLobe and the peak beta band ERD in S1 and SMA occurring after M1 in MI, other candidates for M1 inhibition during MI were evaluated from ROIs in other premotor areas. The most notable activity of the remaining ROIs in the premotor regions was PMCDL. The estimated source strength in PMCDL (Figure 22A) demonstrated a peak in activity shortly after task onset in ME (~3.4s); however, the response is entirely absent in MI (Figure 22A). The TFR plot for contralateral M1 (Figures 22B and C) reveals ERD in the beta frequency band in both ME and MI. In ME, this ERD appears to be temporally aligned with the peak in activity observed for the estimated source strength (Figure 22A), however the ERD peak occurs later than that observed for the estimated source strength (~4.1s) (Figure 22D). There is also a considerably larger magnitude of ERD in the beta frequency band in ME in comparison to MI (Figures 22B and C respectively). Additionally, the onset of the ERD occurred earlier in ME (~0s) than in MI (~3s; Figure 22D).

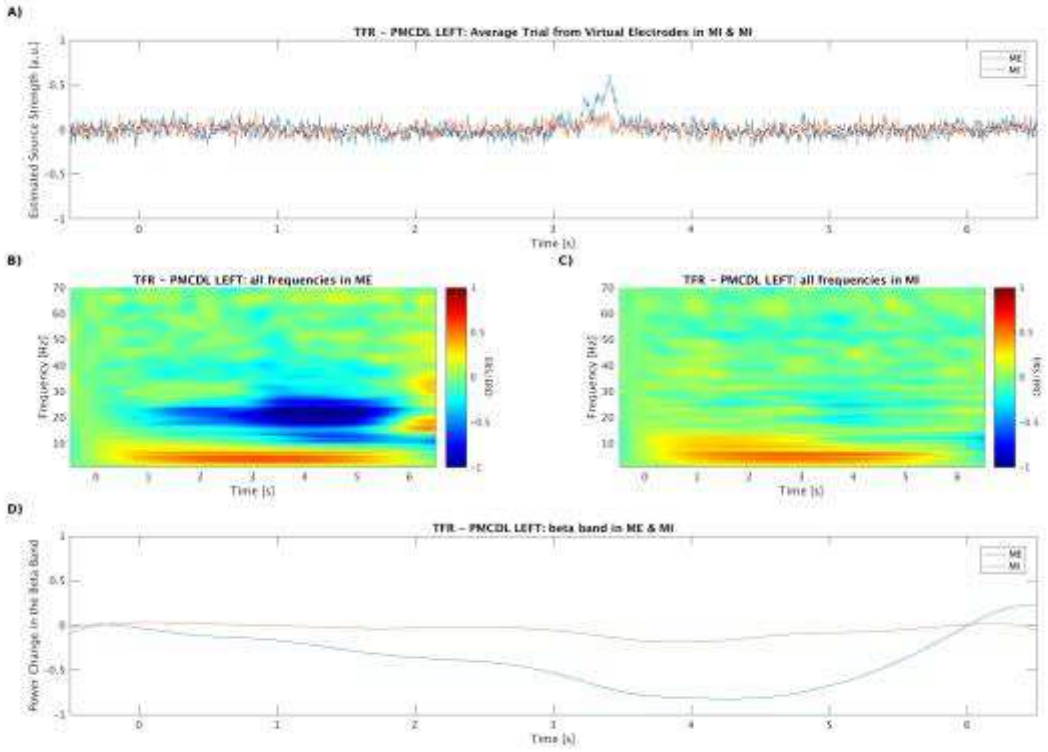

Figure 22: Virtual electrode data for left (contralateral) PMCDL. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). There is a peak in magnitude of estimated source strength in ME shortly after task onset but is absent in MI. This difference is also reflected in the frequency domain, as there is a substantially larger ERD response in ME that peaks later than it does in MI.

To determine if PMCDL is involved in the inhibition of M1, the time-course data of estimated source strength and beta frequency band ERS/ERD for both ROIs were compared between conditions (Figures 23A-D). In ME, the timing of estimated source strength in M1 (Figure 23A) was similar to PMCDL (Figure 23C), however, the peak activity in PMCDL occurred slightly after the peak in M1 (Figure 23C vs. Figure 23A respectively). The most notable difference between conditions is the reduced magnitude of estimated source strength in both ROIs in MI (Figures 23A and C). In ME in the frequency domain, similar temporal patterns of beta band ERS/ERD were

demonstrated in each ROI with both demonstrating a shift towards ERD shortly after the beginning of each trial and peaking around ~4s (Figures 23B and 23D). The difference in magnitude of ERD between each M1 and PMCDL was also small (Figures 23B and 23D). In comparison to ME, the magnitude of beta frequency ERD is greatly reduced in both M1 and PMCDL yet the temporal dynamics of the responses appeared similar to ME (Figures 23B and 23D).

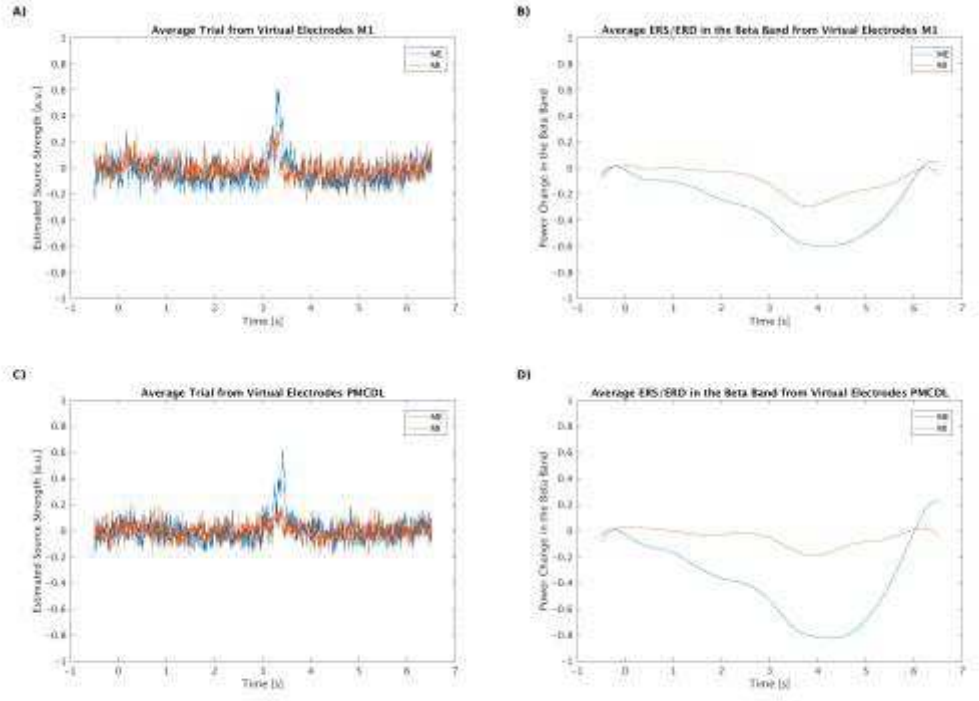

Figure 23: Virtual electrode data from M1 and PMCDL represented by estimated source strength (A: M1, C: PMCDL) and ERS/ERD change in the beta band (B: M1, D: PMCDL) in ME and MI. The time course data from ME was similar across both domains (time and frequency) but in PMCDL there was a large response in both domains in ME that was absent in MI.

Chapter 5: Discussion

5.1: Overview

Currently, there are three proposed theories describing how motor inhibition occurs in MI (Figure 7): 1) motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding execution; 2) cortical areas exert inhibitory influences to suppress the mobilization of the motor command after the mental representation of the plan has been formed; and 3) CST influences may be responsible for motor inhibition after the signal is sent by M1 [5]. Recent work utilizing TMS has since been used to investigate the effect of the descending signal from M1 on the CST during MI [6]. From this work, it was determined that MI causes subthreshold activation that is insufficient to recruit alpha-motor neurons and that the output from M1 to the CST during MI could represent an intermediate cortical output somewhere between rest and voluntary contraction [6,89]. When this result is taken in context of the 3 theories underlying motor inhibition in MI, it suggests that motor inhibition likely occurs at the cortical level, indicating theory 1 or 2 is a more likely explanation. Theories 1 and 2 have each received additional support in the literature leaving some ambiguity as to which theory is more likely to explain the lack of overt movement in MI [79,90]. The authors who proposed the 3 theories underlying motor inhibition in MI suggested that further research was needed that focused on the degree to which M1 (contralateral to the side

of task performance) is inhibited by other areas of the brain, especially by those indicated in the literature to have possible inhibitory effects on M1 during MI (Table 1) [5].

As such, the focus of this work was to first provide additional evidence that motor inhibition (i.e., inhibition of M1) occurs during MI by contrasting estimated source strength activity and beta frequency ERS/ERD in M1 as a result of performing MI or ME of a unimanual grasping task. If motor inhibition was present in MI, the secondary objective was to determine which areas of the brain are responsible or involved in the inhibition of M1 during MI by comparing source level activity in the time and frequency domains between ROIs in the ME and MI networks [2,3,30,94]. Source level activity in M1 revealed that there was decreased magnitude of estimated source strength immediately after task onset in MI, and that this reduction in activity was paired with a greatly reduced ERD in the beta frequency band in comparison to ME. These results indicate that there is indeed inhibition of M1 during MI, and that the timing of this inhibition aligns with the execution phase of the task in either modality. These results indicate that motor inhibition in MI is likely occurring at the cortical level providing additional support for theories 1 and 2 as likely explanations for motor inhibition in MI [5].

Since motor inhibition in MI was observed in this study, a select number of ROIs taken from Table 1, including the SMA, S1, CB and PMc, were investigated as regions possibly involved in this inhibitory mechanism. Given our results, we conclude that the posterior lobe of the CB (PostLobe) and S1 are not likely involved in the inhibition of M1 during MI. We arrive at this conclusion as the CB was not active during the task and both the peak magnitude in estimated source strength and in beta frequency band ERD

obtained for S1 occurred after the corresponding peaks in M1 (Figure 20). Activity in the SMA provided conflicting results as the peak of the estimated source strength was similar in magnitude and timing between ME and MI, but this activity was accompanied by a small beta frequency band ERD in MI that peaked well after the peak beta frequency band ERD in ME. When the estimated source strength results are taken in context with M1, they loosely replicate the results obtained by Kasess et al. 2008 supporting the 2nd theory of M1 inhibition in MI. However, when the results of power change in the beta frequency band in MI are considered, they highlight the possibility that SMA is subject to inhibition in MI rather than being responsible for inhibition of M1 in MI. Furthermore, if activity in the PMCDL is considered, there is even more evidence indicating inhibition of PMc in MI as there is a large peak in both estimated source strength and beta frequency ERD in ME that is greatly attenuated in MI. When the results from PMCDL are taken in combination with those from SMA, they support the 1st theory of motor inhibition in MI indicating that motor inhibition occurs during the process of generating a mental representation of a motor task as the PMc, a brain region involved in motor planning, is inhibited in MI [5]. The finding of pre-motor inhibition and the decreased magnitude of activity in M1 during MI provides support for the study's first hypothesis, however, the second hypothesis that SMA is providing the inhibitory input to M1 is appears unlikely, as pre-motor regions (including the SMA) appear to be subject to inhibition in MI. Given that the analysis performed was exploratory in nature, there is no statistical evidence to support or refute the null-hypothesis for either hypothesis. As such, results from this study need to be re-

evaluated using a statistical approach in order to make robust conclusions regarding the study's hypotheses.

Ultimately, these findings highlight inhibition of PMc as well as M1 in MI, supporting theory 1 related to motor inhibition. Specifically, our findings indicate that a brain region involved in an earlier stage of generating the mental representation of a motor plan is likely responsible for the lack of overt movement during MI. While these findings appear promising, further research needs to be conducted using methods such as effective connectivity to establish which connections are reliably expressed in MI and what the nature of these connections are (i.e., inhibitory or excitatory). These findings are discussed in greater detail below and the role of key ROIs are reviewed in light of this study's findings.

5.2: Motor Inhibition in Motor Imagery

As highlighted in the introduction, MI is the mental representation of an action without any movement [5]. In contrast to ME, the most noticeable difference between these two modalities is a lack of a behavioural outcome, physical movement, as a result of MI. Given that both modalities can drive motor learning [7,26], MI, at least in theory, is thought to parallel the movement planning portion of ME [14] and, therefore, can only drive changes in the internal representation of a motor task as learning occurs [16,17]. The similarity between the two modalities is further reinforced by the overlap in the neural networks involved in each [2,3,30]. However, as demonstrated in these studies, the ME and MI networks are not identical, and there are some key differences in the pattern of

brain activity that occur in key ROIs in each network that might explain how movement is inhibited in MI [2,3,30].

As indicated above, there are two viable theories related to the presence of motor inhibition in MI (Figure 7): 1) motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding motor execution and 2) cortical areas exert inhibitory influences to suppress the mobilization of the motor command after the mental representation of the plan has been formed [5]. Each of these theories has received support from independent neuroimaging studies [79,90] and, therefore, it is difficult to definitively conclude as to which theory is a more likely explanation of motor inhibition in MI. In order to discern which areas of the brain are responsible for motor inhibition in MI, this study aimed to address two hypotheses: 1) determining if motor inhibition occurs in MI and 2) identifying if the SMA, which may be influenced by other areas of the brain within the MI network located in the frontal or premotor regions, is responsible for motor inhibition in MI. These hypotheses were addressed by evaluating source level activity in two domains (time and frequency) in multiple ROIs between modalities (ME and MI).

5.2.1: Motor Cortex

To address the study's hypotheses and determine if motor inhibition was occurring in M1 during MI, the source level activity at this ROI was compared between ME and MI. Given that the primary function of M1 is to provide input to the CST to execute voluntary movements, it has been heavily debated whether or not M1 is active

during MI. A meta-analysis performed by Hétu et al. 2013 reported that only 22 of 122 experiments demonstrated M1 activity in MI, however, since that publication, there have been numerous papers demonstrating reduced M1 activity in MI [2,3] capable of modulating CST excitability during MI [83,84].

Results of this study align with the most recent findings regarding M1 activity in MI. Analysis of the estimated source strength of M1 during both conditions showed a greatly reduced magnitude in activity immediately after task onset in comparison to ME (Figure 16A). This reduction in estimated signal strength in MI also coincided with a greatly reduced magnitude of ERD (Figures 16C and D), replicating findings reported by Kraeutner et al. and Burianová et al. (see Figures 5 and 6). These results indicate that inhibition of M1 occurs in MI; however, the inhibition is not complete as M1 still shows some activity that is temporally related to task performance. This finding of some activity aligns with those obtained at the level of the CST indicating that M1 modulates CST excitability during MI [83,84], and represents an intermediary cortical output between rest and ME [89]. Furthermore, these findings also suggest that inhibition of overt movements occurs at the cortical level, reinforcing theory 1 or 2 as likely explanations for the lack of overt movement as a result of MI [5].

5.3: Brain Regions Thought to be involved in the Inhibition of the Motor Cortex:

There were a number of possible ROIs identified by Guillot et al. 2012 as possible sources for inhibition of M1 during MI (Table 1). Results of this study relating to the CB,

S1, SMA and PMc indicated that they were unlikely to be responsible for the inhibition of M1 during MI.

5.3.1: Cerebellum

Results obtained from the posterior lobe of the CB (Figure 19) showed that it was generally inactive throughout the course of ME and MI of the grasping task. The estimated source strength over the course of the trial showed a small peak estimated source strength in reaction to the onset of the preparation cue but otherwise, did not show any deviation from baseline in either ME or MI (Figure 19A). In the frequency domain, there were few changes in power in the beta frequency band with only a small ERS response occurring in ME late in the performance window (Figure 19B). While this result indicates that the CB is not involved in M1 inhibition, it is surprising that there is little to no CB activity throughout the course of the average trial.

The classically defined function of the CB has been to modulate motor commands and implement error detection / correction that is facilitated by comparison of the reafference and efference copy as part of a forward model [46]. In MI, the CB has also been demonstrated to be consistently active [2,3,30] and this activity had been tied to inhibitory mechanisms during MI [53]. Results of this study conflict with these findings given the lack of activity obtained in the CB. This incongruence between the findings of the current study and past literature can be explained by methodological limitations, as it is difficult to localize source level activity in deep brain structures and the CB [101]. This possible methodological limitation may explain why CB activity was not noted in either

condition as this methodological confound would have been common to both ME and MI trials.

5.3.2: Somatosensory Cortex

The somatosensory cortex also appears to be an unlikely candidate for M1 inhibition in MI. Results of this study demonstrate that both the peak estimated source strength and beta frequency band ERD in S1 are dramatically reduced in MI in comparison to ME (Figure 18). This finding aligns well with the generally accepted function of S1, as it is thought to be a region primarily responsible for the processing of conscious somatosensory input including pain, temperature, touch and proprioception [55]. Given that movement is not produced during MI, it is logical to conclude that the lack of activity in S1 is due to a lack of somatosensory input. Furthermore, the literature has not demonstrated consistent activation of S1 during MI (Figure 2) [30,31,34]. The minimal S1 peak in estimated source strength seen in MI could be explained by the task's transitive nature (movement directed towards an object) where somatosensory activity was noted specifically in the case of MI of upper limb transitive movements [30].

Additionally, the findings obtained from S1 align well with the pattern of source level activity in M1, where increased magnitude of the estimate peak source strength and beta frequency band ERD was seen in M1 in ME. Given the presence of bi-directional connections between M1 and S1 during ME [35], the presence of a large potential in both ROIs would support the concept of an excitatory relationship between both areas. Results from this study demonstrated that the timing of peak estimated source strength and beta frequency band ERD in both ROIs revealed that S1 lags slightly

behind M1 (Figure 20), further supporting the concept of an excitatory relationship between the regions. The pattern of peak estimated source strength and beta frequency band ERD is also replicated in MI where S1 activity again lags slightly behind M1, except with greatly reduced magnitude (Figure 21). This lag in activity precludes S1 as a candidate for M1 inhibition during MI as in order for one area of the brain to cause excitation or inhibition of another, the area providing the excitatory/inhibitory signal must be active prior to the reduced peak or lack of peak in activity in the area receiving the excitation/inhibition.

5.3.3: Supplementary Motor Area

According to the literature, the SMA appeared to be the region most likely responsible for M1 inhibition during MI. Consideration of SMA as a possible brain region responsible for M1 inhibition in MI stems largely from the work of Kasess and colleagues [79]. As summarized in the introduction, in this study, participants performed a unimanual finger-tapping task using ME and MI. A similar pattern of BOLD signal was found in SMA in both modalities followed by a peak in M1 activity unique to ME (Figure 4). Using effective connectivity, it was then concluded that SMA was inhibiting M1 in MI and exciting M1 in ME [79]. These results led to the hypothesis that if M1 inhibition occurred in MI, then the SMA would be responsible for this inhibition.

Results of the current study generally replicate the results obtained by Kasess and colleagues [79]. The present results demonstrate a peak in estimated source strength in SMA shortly after task onset in both ME and MI (Figure 17A) that appear to precede the peak in estimated source strength in M1 for both ME and MI (Figures 20A and 21A).

The peak magnitude of the estimated source strength was similar in the SMA between conditions and the subsequent peak in estimated source strength in M1 was substantially larger in ME than in MI (Figures 17A and 16A respectively) as seen in Kasess et al. 2008. The only difference between the results of the current study and Kasess et al. 2008 was that in Kasess et al. 2008, the magnitude of the peak of BOLD signal in SMA was more similar to the magnitude of the peak of estimated source strength in M1 in ME, whereas in the current study, the magnitude of the peak of estimated source strength in SMA was more similar to the magnitude of the peak of estimated source strength in M1 in MI rather than ME (Figures 17A and 16A).

When the changes in beta band frequency power are considered, the function of SMA in MI becomes more complex. In ME, there was substantial ERD in the beta frequency band, achieving peak magnitude shortly after task onset and immediately prior to the peak in beta frequency band ERD in M1 (Figure 20B). However, in MI, the peak in beta frequency band ERD in SMA was substantially smaller and occurred much later in time, well after the reduced peak magnitude beta frequency band ERD response seen in M1 in MI (Figure 21B). Given the reduced magnitude of the beta frequency ERD seen in the SMA in MI in comparison to ME as well as the delayed peak beta frequency ERD in MI, it appears as if the SMA itself is being inhibited in MI rather than being responsible for M1 inhibition in MI as proposed by Kasess et al. 2008. In light of the findings related to the lack of ERD in the SMA, and in particular its temporal relationship with those from M1 (during MI), the findings do not provide any evidence to indicate that the study's second hypothesis is likely to have occurred.

To optimize the temporal resolution (i.e., decrease the repetition time) of their data collection using fMRI, Kasess and colleagues were only able to image a small portion of the brain, including their specific ROIs (SMA and M1). As such, only SMA and M1 were included in their effective connectivity analysis. Had they included a greater number of ROIs in their effective connectivity analysis, one might hypothesize that a larger network of brain regions was indeed involved in the motor inhibition observed in MI, and specifically a more complex mechanism underlying M1 inhibition in MI.

The differing nature of the connections between SMA and M1 has also been investigated outside of the current context of ME and MI [102-104]. Past studies have investigated the relationship of SMA to M1 using a variety of TMS protocols and outcome measures [102]. One of these studies ultimately concluded that SMA activity moderately facilitates M1 activity as evidenced by enhanced short-interval intracortical facilitation induced by TMS activation of the SMA by activating excitatory neurons in M1, excluding corticospinal neurons, as SMA activity did not affect recorded MEPs [102]. Additionally, this study investigated the possibility that this facilitation arose from dis-inhibition of common inhibitory systems. The two systems investigated were short-interval intracortical inhibition and contralateral silent periods, which are mediated by gamma-aminobutyric acid A ($GABA_A$) and $GABA_B$ respectively [105,106]. It was noted that SMA activity did not modulate either system, excluding dis-inhibition of either of these systems as a mediator for the increased facilitation of M1 caused by SMA activity (Shirota 2012)[102]. However, studies of individuals with Tourette's syndrome (TS) have shown increased GABA in the SMA, a region thought to be involved in the generation of

tics in TS [103,107]. This increase in GABA in SMA in TS participants is negatively correlated with both a SMA BOLD response to a motor task and M1 excitability to the task, indicating that increased tonic inhibition in the area in TS patients may be responsible for suppression of tics [103]. This finding could also potentially be generalized to the control of behaviour in a healthy population, where increased GABA in the SMA may be indicative of reduced activity in the area and the subsequent lack of excitability in M1.

In contrast to the above findings, increases in SMA GABA have also been associated with response inhibition in a reverse mask priming task [104]. In this study participants performed a standard task where they were asked to respond to a target arrow pointing either left or right where each target is preceded by a backward-masked prime for a duration of time below threshold for conscious discrimination of the direction of the arrow in the prime. The two conditions of interest to this study were positive compatibility effect (PCE) and negative compatibility effect (NCE). The PCE occurs when the RT to the target is decreased due to the presentation of the prime arrow shortly before the target in the same direction. The NCE occurs when RTs are delayed in response to a target preceded by a prime in the same direction that is presented earlier in time. The NCE effect has been attributed to the SMA and the study demonstrated that GABA concentration in the areas inversely related to the size of the NCE response indicating that the SMA is part of the inhibitory response responsible for NCEs [104] . However, this result seems specific to the NCE as neither SMA GABA concentrations or the NCE correlate well with results of other tasks including the Simon task, the Eriksen flanker task and the STOP task [104].

5.3.4: Premotor Cortices

Given that the SMA appeared to be inhibited in MI, the analysis of ROIs was extended to other regions of the PMc to ascertain whether the inhibitory effect of MI on the SMA was replicated in other areas commonly associated with planning movements. Results from the PMCDL suggest this region was also inhibited during MI (Figure 22). The peak magnitude of estimated source strength of PMCDL in MI was substantially lower than in comparison to ME (Figure 22A) and there was also an associated reduction in beta band ERD in MI (Figures 22C and 22D) in comparison to ME (Figures 22B and 22D). In ME, the temporal relationship of PMCDL and MI activity suggest a causal relationship that is excitatory in nature (Figure 23). While the peak magnitude of estimated source strength and beta frequency ERD in PMCDL in ME occurs after the corresponding peaks in M1 in ME (Figures 23A-D), in the frequency domain, the development of the beta frequency band ERD response seems to lead the ERD response seen in M1 (Figures 23B and D). This finding supports the concept of an excitatory relationship between the two areas in ME. However, in MI, the reduction in peak magnitude of both the estimated source strength and beta frequency ERD and the delay in peak beta frequency ERD (Figures 23A-D) replicates the patterns seen in the SMA, indicating that the relationship between both areas in ME may be different in MI.

If the 2nd theory of motor inhibition in MI was to explain the lack of overt movement in MI, then it would be expected that areas involved in movement planning, such as the SMA or PMCDL, would show similar activity between ME and MI [5]. However, it appears that PMc is inhibited during MI, as there were reductions in beta frequency

ERD in both the PMCDL and SMA. Additionally, the large peak in estimated source strength in PMCDL seen only in ME indicates that this region is either receiving additional excitatory input from another brain region involved in an earlier stage of movement planning, or is receiving less inhibitory input (from other brain regions or interneuronal network) during ME. When the results of PMDCL and SMA are considered together, they seem to support the 1st theory of motor inhibition in MI, namely that motor inhibition occurs *during* the process of generating a mental representation of a motor task. The results of the present work lead to this conclusion, as there is evidence to indicate there is inhibition in both M1, the brain region responsible for the execution of movement, and the PMc, which contains a group of areas involved in movement planning [5]. It is therefore likely that the region responsible for the inhibition of overt movement in MI is involved in an earlier stage of generating the representation of a motor task in MI.

5.4: Candidates for the Inhibition of the Motor Cortex and Premotor Cortices

5.4.1: Right Frontal Cortex

One of the other regions postulated to be a candidate responsible for M1 inhibition in MI is the frontal cortex (Table 1) [5]. The area of the frontal cortex with the most pertinence to the motor task employed in this paradigm is the IFG (Table 1; reported as inferior frontal cortex) [5]. Results from the nearest node to IFG, contralateral (left) PFCVL, were not included in the results section of this study as the corresponding ROI showed virtually no change in estimated source strength or beta frequency band ERS/ERD. There was, however, a response in the ipsilateral (right) PFCVL (Figure 24). The

peak estimated source strength in both conditions immediately after task onset (Figure 24A) corresponds well with previous literature implicating right IFG with response inhibition [75]. Additionally, recent findings have also implemented the right IFG in motor inhibition during overt (ME) and covert (MI) actions [108]. In this study participants performed a Go/NoGo task where participants were provided with a cue and a subsequent target indicating that they must either perform or withhold a button press, depending on the target. This task was performed in two blocks: one using ME, and one using MI. This design resulted in four conditions (Go/NoGo & MI/NoGoMI). High density electroencephalography was used to capture the participants brain activity during the task. Sensor level data was evaluated using a microstate segmentation approach [109] that identified a series of time period called segmentation maps each defining temporally discrete computational steps during the response process. The segmentation maps between MI and ME were compared, and those that occurred during a time window that showed significant differences between conditions were subjected to source level analysis. The results of the source level analysis showed similarities between regions involved in inhibitory mechanisms of overt and covert actions revolving around increased activity in the right IFG during MI, NoGo and NoGoMI [108]. While these findings do not align entirely with those of the present study, the difference between these results could be explained by the fact that inhibition occurred during all trials of this study regardless of modality. Given that in the present study the participants only became aware of the goal for a given trial after the green circle was presented to them at task onset, they were unable to pre-emptively inhibit the 'incorrect' response prior to

the task onset (i.e., the 3s mark). Thus, inhibition of the 'incorrect' response occurred regardless of the modality used to perform the movement, hence the similar peak estimated source strength between conditions.

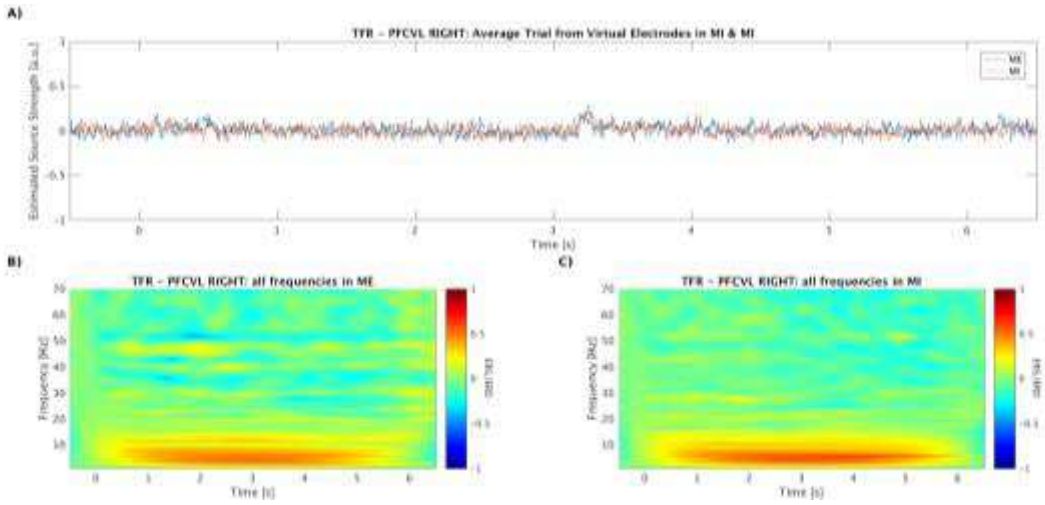

Figure 24: Virtual electrode data for right (ipsilateral) PFCVL. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). There is a peak in magnitude of estimated source strength in both modalities shortly after task onset but is absent in MI. There is however a difference in the frequency domain as there is a larger low frequency ERS response in MI than ME.

The magnitude of ERS in the alpha frequency band may also help explain the inhibition seen in the PMc and M1; a recent study exploring MEG signatures of right pre-frontal involvement in response inhibition used a go/no-go paradigm and determined that there was substantially more alpha synchronization in the right PFCDL in trials where participants withheld their responses (correctly or incorrectly) in comparison to trials where participants executed the task [110]. Results from the current study for the right PFCDL support these findings in both the time and frequency domains (Figures 25A-C). The right PFCDL demonstrates a peak in estimated source strength in both

conditions shortly after task onset, corresponding with inhibition of the motor plan for the target not selected as the goal of a given trial (Figure 25A). This peak in activity is accompanied with large alpha frequency band ERS peaking at ~3s in both conditions, however, the ERS response appears slightly larger in MI (Figures 25B and C). This pattern of activity in the right PFCDL is also replicated in the right PFCVL with alpha frequency ERS seen in both modalities and a slightly larger ERS response is again seen in MI (Figures 24B and C). It is therefore possible that the increased alpha frequency ERS seen in both the PFCVL and PFCDL represents a right prefrontal inhibitory mechanism unique to MI that could explain the inhibition of PMc and M1 in MI.

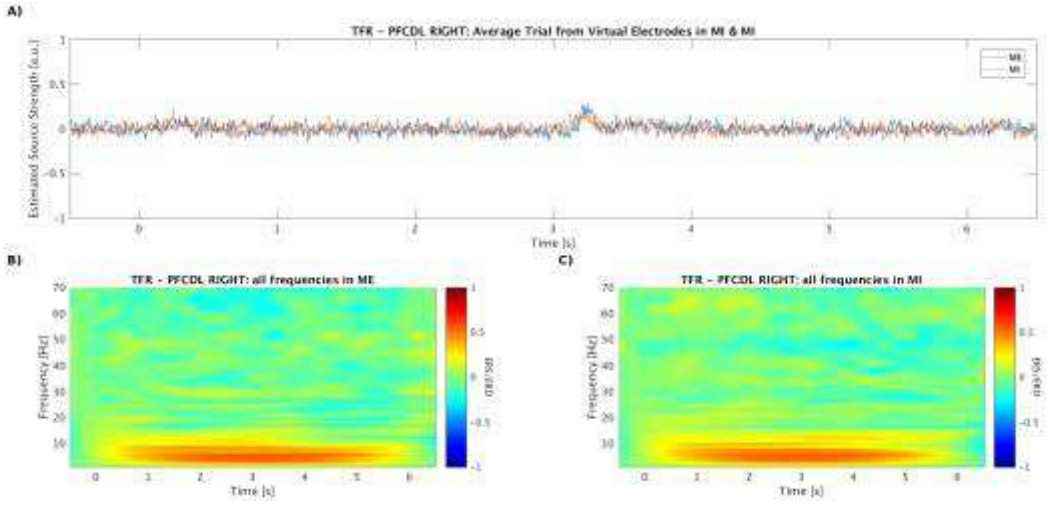

Figure 25: Virtual electrode data for right (ipsilateral) PFCDL. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). There is a peak in magnitude of estimated source strength in both modalities shortly after task onset but is absent in MI. There is however a difference in the frequency domain as there is a larger low frequency ERS response in MI than ME.

5.4.2: Parietal Cortices

The final region postulated to be a candidate responsible for M1 inhibition in MI is the parietal cortex (Table 1) [5]. The importance of the parietal cortex to the

performance of MI has been well documented in numerous studies[49,62,68,111]. The dependence of MI on parietal cortex function, particularly the PPC, is unsurprising, given its involvement with processes related to motor attention, planning, and visuo-spatial transformations [60,61].

Results of this study demonstrate that the left (contralateral) IPL, labeled as IPC, showed a small peak in estimated source strength shortly after task onset (Figure 26A). This peak in estimated source strength was accompanied by a minimal peak in beta frequency band ERD occurring at ~4s that is not present in MI (Figures 26B-C). The magnitude of this beta ERD peak is minimal however. Given the lack of response in left IPL, the left SPL was additionally investigated. Similar to the IPL, there was a peak in estimated source strength shortly after task onset that was present in both modalities. In the frequency domain both modalities showed ERD peaks occurring between ~4 and 4.5s into the trial. The additional activity seen in SPL in comparison to IPL could be due to the use of visual cues as one of the known functions of the SPL is interpretation of environmental cues [30]. With respect to M1 inhibition in MI, it doesn't appear as if the IPL or SPL is involved in the inhibitory mechanism as the peak in beta frequency band power change over the course of the average trial occurs well after the corresponding response in M1. However, given the size of the response in the SPL, coupled with findings from Solodkin et al., who used a connectivity analysis to conclude that SPL inhibits M1, future analyses should continue to include the SPL as a possible source of M1 inhibition in MI [80].

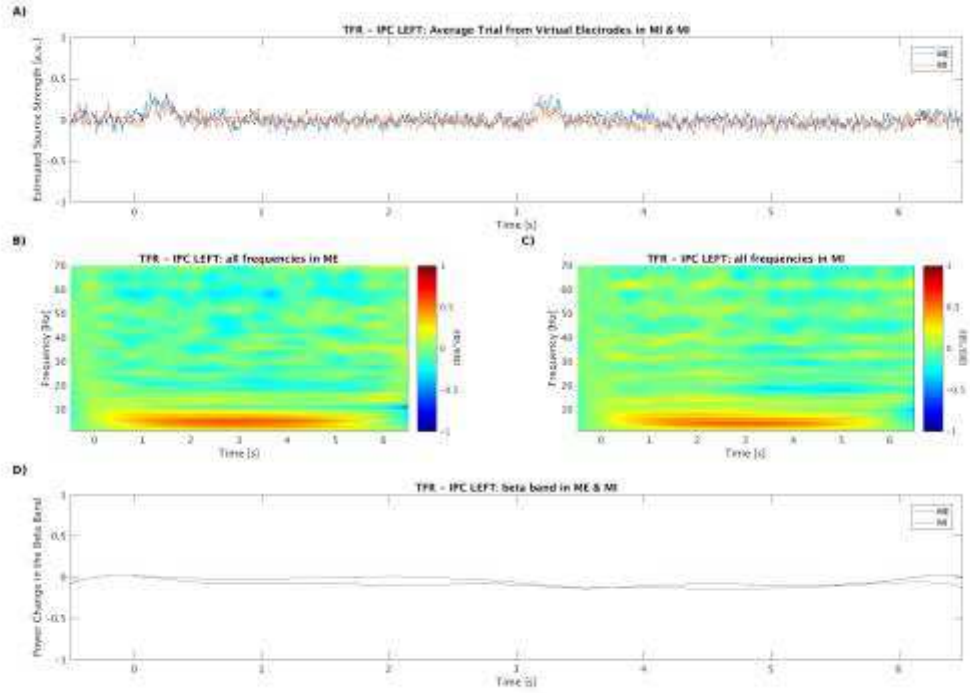

Figure 26: Virtual electrode data for left (contralateral) IPL. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). There is a peak in magnitude of estimated source strength in both modalities shortly after task onset in both conditions. There is a minimal ERD response in MI that is not present in ME.

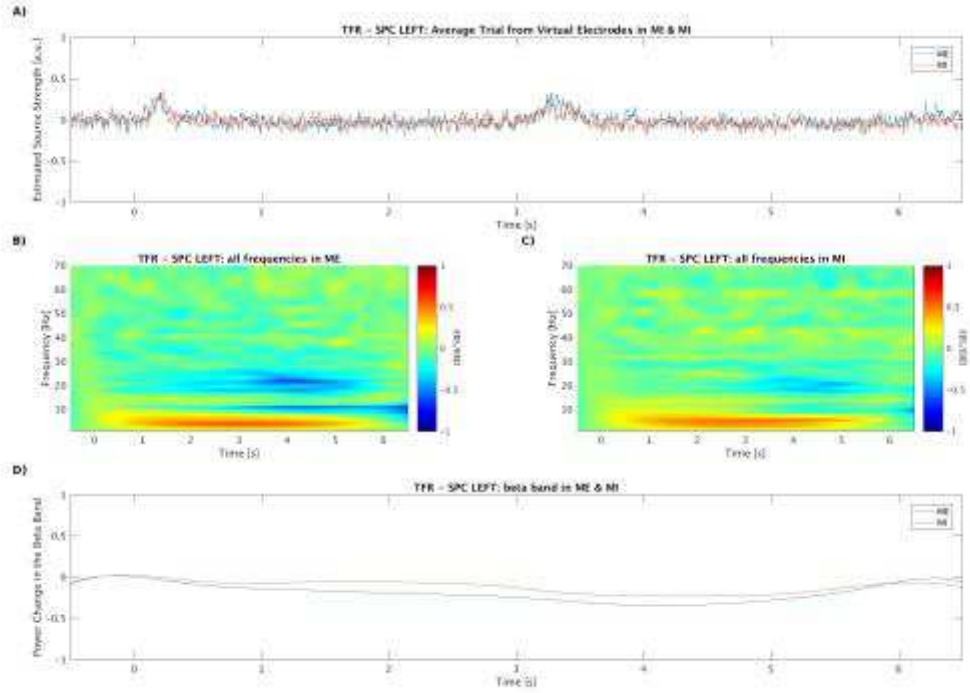

Figure 27: Virtual electrode data for left (contralateral) SPL. Panel A depicts the estimated signal strength over the course of an average trial in each condition. Panels B and C depict event related synchronization and de-synchronization (ERS/ERD) for ME (B) and MI (C). Panel D depicts the change in power for the beta frequency band (15-30 Hz). There is a peak in magnitude of estimated source strength in both modalities shortly after task onset in both conditions. There is a ERD responses in both conditions that is larger in magnitude in ME in comparison to MI.

5.5: Future Directions & Limitation

The primary limitation of the present work is that the conclusions made about communication between brain regions are based on qualitative comparisons of source level time and frequency results from spatially independent brain regions. Specifically, we did not undertake formal connectivity analyses that would provide greater support for the presence and nature of the relationship between brain areas. This type of analysis was not undertaken as it was beyond the scope of the thesis. In order to make more substantiated conclusions about the presence and nature of connections between

brain areas in either modality, a form of connectivity analysis would need to be applied to the source level data. Addressing this limitation by applying an effective connectivity analysis would address this limitation, and as such represents a logical future direction. Measures of effective connectivity allow one to determine and quantify the directionality of connections between two brain regions rather than simply determining that a connection exists (as in functional connectivity analysis). Adding directionality to a functional network allows effective connectivity measures to make inferences about causality within the network and, as such, effective connectivity measures can provide valuable information that can be used to identify the mechanism behind the lack of overt movement in MI. One technique used to extract effective connectivity measures is dynamic causal modeling, which estimates and makes inferences about directed influences of a variable on others within the network being modeled [112]. Dynamic causal modeling is historically thought of as a hypothesis driven approach to EC, where each hypothesis can be defined as a single version of a model, with set priors, for a given neural network. However, dynamic causal modeling has been adapted for network discovery and can effectively model large networks without any a priori knowledge of how the network will function [113,114]. If dynamic causal modeling was used to re-analyze the data from this study, the ROIs identified would be included as nodes in the network. Conclusions could then be drawn from a comparison of the selected models for each condition (MI and ME), identifying which node(s) or brain region(s) is responsible for the inhibition of the overt movement in MI.

An additional limitation that should be noted relates to source imaging using MEG. In addition to the difficulties of localizing source level activity in deep brain structures [101], the spatial resolution of MEG, while better than electroencephalography, [115] is inferior to fMRI [116]. When interpreting group level analysis after each participant is individually co-registered, the spatial resolution of MEG has been estimated to be approximately 1cm [117]. As a result, estimated activity from ROIs that are close together (e.g., right and left PMCMed) are likely to reflect the same activity. It is important to further note that the lack of activity in a given brain region (i.e., a 'negative finding') does not necessarily mean that the area was not active, but rather the chosen modality (MEG) was not sensitive to the activity occurring in the region. For example, we did not observe task-related activity in the posterior lobe of the CB (i.e., PostLobe). This lack of activity may in fact be due to the inability of the MEG to detect and subsequently capture activity from this region, as opposed to the region not having any task-related activity. These possible limitations notwithstanding, the advantage of MEG is its very high temporal resolution, which allows for analysis of estimated signals at the source level on the scale of milliseconds. We selected MEG for this particular study given the advantage of its high temporal resolution, in order to assess the timing of activity between various brain regions at a level not possible with fMRI. Given the limited spatial resolution of MEG, multimodal imaging methods have been implemented, such as combining MEG with fMRI [116] or TMS [118], in order to combine the strengths of each imaging modality to attain a more comprehensive understand the responses being imaged (i.e., high spatial and temporal resolution).

Chapter 6: Conclusions

Motor imagery, the simulation of movement without overt execution, is thought to parallel the motor planning portion of ME [14]. The most notable behavioural difference between ME and MI is the lack of overt movement as a result of MI. By demonstrating reduced M1 activity in MI in both the time and frequency domains, the current study verified that M1 inhibition occurs in MI. However, by additionally demonstrating inhibition of ROIs in the PMc, the current study objects to the theory that the SMA is responsible for M1 inhibition in MI [79] and supports the theory that motor inhibition occurs during the process of generating a mental representation of a motor task, such that only subthreshold motor commands are sent to the effectors, precluding execution [5]. These findings support the studies first hypothesis that contralateral M1 inhibition would occur as a result of mechanisms involved in creating the mental representation of a motor task. However, these results do not support the study's second hypothesis as SMA appears to be subject to inhibition in MI rather than inhibiting M1 during MI. The current study also indicates that another area involved in an earlier stage of generating the mental representation of a motor task in MI is likely responsible for inhibition of both PMc and M1. Two possible candidates for this inhibitory effect could be the right frontal cortex or left SPL, however, without quantification of the connectivity within the networks underlying each modality, it is difficult to make any definitive conclusions. Future work should apply an effective connectivity analysis in order to determine the dynamics and nature of information

transfer within each network and more decisively identify the brain region(s) involved in the inhibition of overt movement in MI.

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