Movement-induced motor cortical excitability changes of upper limb representations during voluntary contraction of the contralateral limb:

A TMS investigation of interhemispheric interactions

by

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

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Abstract

Humans possess the ability to generate an incredible degree of complex, highly skilled, and coordinated movements. Although much is known about the anatomical and physiological components of upper limb movement, the exact means by which these different areas coordinate is still far from understood. The ability to perform symmetrical, bimanual tasks with ease suggest a default coupling between mirror motor regions – a default coupling that is perceptible in unilateral movements. During intended unimanual movement in the upper limbs, bilateral changes to motor cortex output occur. The purpose of this study was to investigate the neural underpinnings of these bilateral changes and investigate the involvement of intracortical inhibitory circuits. Previous studies have shown that transcallosal connections between cortical representations of the intrinsic muscles of the hands are relatively sparser than the more proximal muscles of the upper limbs. It was hypothesized that differential responses in overall motor output or intracortical inhibition to ipsilateral muscle activation between the FDI and ECR could infer the involvement of transcallosal pathways; although interhemispheric transfer was not directly investigated in this thesis. Two studies used focal transcranial magnetic stimulation (TMS), specifically paired-pulse protocols, to investigate changes in short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI) in response to contraction of contralateral homologous muscle groups to the inactive test muscle. Also, the response to activation of a non-homologous, but spatially close, muscle was investigated. Lastly, two muscle groups were investigated, a distal, intrinsic muscle of the hand (first dorsal interosseous) and a relatively more proximal muscle of the upper limb (extensor carpi radialis). These studies revealed that at low levels of force

generation, unilateral isometric contractions facilitate ipsilateral mirror motor representations and reduce local $GABA_A$ receptor mediated inhibition. Notably, while similar facilitation occurred in both the distal and proximal effectors, decreases in SICI were much more robust in the ECR. Findings from this thesis provides insight into the neural mechanisms governing bilateral changes with unilateral movement and is important in the guiding the focus of future research.

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List of Abbreviations

AMT	Active Motor Threshold
ANOVA	Analysis of Variance
AP	Anterior-Posterior
ATS	Active Test Stimulus
CIMT	Constraint Induced Movement Therapy
CS	Conditioning Stimulus
ECR	Extensor Carpi Radialis
EMG	Electromyography
FCR	Flexor Carpi Radialis
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resonance Image
GABA	Gamma (γ) Amino Butyric Acid
ICF	Intracortical Facilitation
ICI	Intracortical Inhibition
IHI	Interhemispheric Inhibition
IPSP	Inhibitory Post-Synaptic Potential
ISI	Interstimulus Interval
LICI	Long-Interval Intracortical Inhibition
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MRI	Magnetic Resonance Image
Ms	Milliseconds
MT	Motor Threshold
PA	Posterior-Anterior
RMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic Stimulation
SE	Standard Error
SICI	Short-Interval Intracortical Inhibition
SMA	Supplementary Motor Areas
SPSS	Statistical Package for Social Sciences
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Simulation
TS	Test Stimulus
μV	Microvolts

Chapter 1 - Introduction

A net balance between simultaneous excitatory and inhibitory interactions is necessary for normal human motor function. It has been shown that communication between primary motor cortices and GABAergic intracortical inhibitory circuits play important roles in the coordination of upper limb movements and disruptions to which are symptomatic of specific pathological disorders of the CNS. The goal of the following review is to discuss the interhemispheric interactions that contribute to normal motor control, methods for investigating cortical excitability, changes to cortical excitability that occur in stroke patients and the implications that these may have to stroke recovery and rehabilitation.

1.1 Stroke & Stroke Rehabilitation

A stroke impairs blood flow to the brain that leads to the rapid death of cells in the brain. Not only is stroke the fourth leading cause of death in Canada, but it is the foremost cause of adult neurological disability with greater than 50% of stroke survivors left with some form of chronic motor deficit (Calautti & Baron, 2003). And while most patients regain the ability to walk, up to 60% fail to regain functional use of their upper limbs (Kwakkel et al., 1999). A great deal is known about the process of neurological damage incurred by stroke, yet little is known about the mechanisms of stroke recovery (Calautti & Barron, 2003). Further, the formulation of effective rehabilitation strategies for motor control is not only dependent upon working knowledge of recovery, but on the underlying neurophysiologic mechanisms governing human movement.

After focal damage to the brain, many changes to neuronal organization can be observed in both the lesioned area as well as in distant neural networks (Ward & Cohen, 2004). Among the changes in the stroke affected area is an increase in motor threshold excitability, a decrease in corticospinal output, and shifts to the cortical motor map during recovery (Swayne et al., 2008; Byrnes et al., 1999). Additionally, in acute stroke patients, a significant disinhibition in the contralateral unaffected hemisphere has been documented (Liepert et al., 2000; Shimizu et al., 2002). The disinhibiting effect is thought to arise from a reduction in the inhibitory influence of the affected hemisphere and may contribute to the unintended movement of the opposite limb during deliberate unilateral movements (called mirror movements) sometimes experienced by recovering stroke patients (Farmer, 2005).

The purpose of the contralateral disinhibition and whether or not it provides a beneficial effect to the patient recovery is debatable. Strens and colleagues (2003) conducted a repetitive TMS study in a healthy population and found that changes in the ipsilateral primary motor cortex (M1) may in fact play a compensatory role to dysfunction in the lesioned hemisphere. Force production was recorded from seven neurologically normal participants during a right hand finger-tapping exercise after having undergone rTMS over the contralateral M1. A transitory change in ipsilateral hemisphere excitability was recorded, however no differences were observed in tapping force between the rTMS participants and controls. When simultaneous bilateral rTMS was applied to the participants, there was a marked decrease in finger tapping force. It is believed that the bilateral rTMS temporarily removed the compensatory changes in ipsilateral hemispheric excitability that preserved finger tapping force production in the

single rTMS group, thus demonstrating a potential role for contralesional excitability changes in stroke patients. While some have adopted the view that hyperexcitability of the unaffected hemisphere represents an acute motor response to cortical damage, others suggest that it is merely an unmasking of latent functional pathways. Regardless of its intended purpose, there exists a correlation between changes to excitability in the unaffected hemisphere and stroke recovery, where poor recovery is associated with persistent imbalances in interhemispheric inhibition (Manganotti et al., 2002). The potential therefore exists for contralesional hyperexcitability to not only be used as a predictor for good recovery, but to be exploited in newly developed stroke rehabilitation strategies.

Stroke survivors with resulting hemiparesis routinely undergo rehabilitation therapy to help improve motor function and increase independence post-stroke. Each rehabilitation program is customized to each patient's condition, however constraint-induced movement therapy (CIMT) and bilateral movement training are two of the more common and promising techniques administered by healthcare providers.

Constraint-induced therapy is used to overcome learned non-use in stroke patients who adapt to their hemiplegia by favouring their non-affected limb for tasks normally accomplished using their affected limb. CIMT discourages such behavior by constraining the undamaged limb (typically with a mitt) during functional activities, thus forcing the use of the paretic limb. Many studies have investigated the effectiveness of CIMT and results suggest that the intervention has long lasting benefits for patients with mild to moderate motor impairments of the upper limbs (Boake et al., 2007; Taub et al., 2006; Wolf et al., 2008). The effectiveness of CIMT on patients with severe hemiparesis

is less clear. There is limited evidence that suggests that CIMT can significantly improve functional ability in those with severe upper extremity impairment, however the resulting paretic limb remain largely impaired and is mostly useful as an assist to bimanual movements (Bonifer et al., 2005). Bonifer and Anderson (2003) monitored the functional recovery of a 53 year old stroke survivor with severe upper-extremity deficits as she completed a 3 week CIMT program. While all outcome measures initially increased post-treatment, Motor Activity Log scores returned to baseline after 6 months and the patient reported no lasting functional improvements in her paretic limb.

CIMT uses unilateral strengthening and training, focusing exclusively on the paretic limb, however many daily tasks require the coordination of both upper limbs. Further, while CIMT can improve motor recovery in a significant number of patients, there is a subset of stroke survivors who are not candidates for the intervention as they are unable to generate the unilateral motor activity necessary for CIMT. Consequently, there is a growing amount of research focusing on the efficacy of bimanual training for post-stroke upper limb rehabilitation, especially in the more severe cases, and several studies are reporting favorable results (Summers et al., 2007; Harris-Love, 2005).

Staines and colleagues (2001) in a longitudinal study of 2 acute stroke patients observed an enhancement in neural activation of the stroke-affected hemisphere during coordinated bimanual movement. This bilateral observation occurred in the early weeks following stroke and ceased as the recovery progressed. The implication is that pathways in the hemisphere ipsilateral to the paretic limb may play a role in functional recovery after stroke and that bilateral training may lead to favorable changes in excitability and cortical representations. A 2005 study by Harris-Love et al. investigated the efficacy of

bilateral training in a pathological population. Thirty-two participants with chronic unilateral stroke were recruited for a study comparing unilateral versus bilateral task training (Harris-Love et al., 2005). In subjects with moderate to severe hemiparesis, the paretic limb recorded higher on performance measures with bilateral training tasks compared to unilateral reaching. However, the beneficial effects of bilateral training were immediate and there was no measure of their durability over time. Further investigation into the long-term effects of bilateral training is warranted.

No single post-stroke rehabilitation strategy has been shown to improve motor outcomes in all stroke survivors. In fact, the majority of patients are still unable to restore full functional use in their paretic limb despite rehabilatory interventions (Rose & Winstein, 2004). Thus, a better understanding of the neural correlates that contribute to the bimanual effects of unilateral movements – in both the stroke and healthy population - will not only contribute to our knowledge of cortical function, but provide insight into the appropriate rehabilitation prescription for specific patient characteristics in the hopes of improving the quality of life for many people.

1.2 Motor Irradiation

Mirror movements occur when intended unilateral movements result in the involuntary co-contraction of the homologous muscles in the opposite limb. Considered normal in developing children, the occurrence of mirror movements typically disappears around the age of ten and is thought to coincide with the myelination of the corpus callosum. While they have been documented in normal individuals during prominent physical effort and severe fatigue, the presence of mirror movements in the mature motor

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system is typically associated with certain pathological conditions such as cerebral palsy, Parkinson's disease, epilepsy and stroke-induced cortical damage (Carson, 2005; Ueki et al., 2004).

Though overt mirror movements do not typically occur in neurologically healthy adults, there is growing evidence that there exists a tendency for simultaneous movements of upper limbs to be drawn to one another. Motor irradiation, defined as an increase in the excitability of the opposite homologous motor area during unilateral contractions, has been documented in a growing body of literature and demonstrates that there exists a continued communicative pathway between contralateral homologous motor regions in the adult brain (Ghacibeh et al., 2006; Kobayashi et al., 2003; Christova et al., 2006; Stinear et al., 2001). Although the many terms have been used to describe this unintended activity in the contralateral muscle – physiological mirroring, motor irradiation, associated activity, motor overflow, global synkinesis – in this thesis the term 'motor irradiation' will be used.

*1.3 **Pathways Mediating Motor Irradiation***

In the human cortex, the two hemispheres are continuously communicating through excitatory and inhibitory pathways and the maintenance of interhemispheric balance is important for normal brain function (Chen et al., 2004). It is evident that in the normal human brain there exists a strong interhemispheric interaction between the primary motor cortices, with inhibitory influences being more prominent than facilitatory pathways (Fecteau et al., 2006). The exact means by which the two M1s communicate is, however, under debate. Different theories attempt to explain the control of motor

irradiation and interhemispheric inhibition. There are a myriad of cortical and subcortical regions capable of exchanging information via commissural fiber systems, though recent research have narrowed them down to a handful of possible candidates; uncrossed corticofugal fibers, branched bilateral corticomotoneuronal projections, bilateral ineractions between primary motor cortices, bilateral interactions between supplementary motor areas, common inputs to both motor cortices such as cingulate cortex, and subcortical areas including the basal ganglia and cerebellum (Rose & Winstein, 2004; Carson, 2005).

Once thought to be controlled though uncrossed corticofugal fibres or segmental networks, recent studies indicate that the mediation of bilateral interactions of the upper limbs occurs at the cortical level. Hortobagyi and colleagues (2003) investigated the effects of voluntary contraction on the motor pathway of contralateral homologous muscles using TMS with and without direct stimulation (magnetic stimulation to the back of the head, at the level of the cervicomedullary junction). They observed a facilitation of TMS evoked potentials in the homologous muscle representation during voluntary contraction of the ipsilateral wrist flexors and no affect to the direct stimulation evoked potentials in descending tracts (cervicomedullary MEPs). These findings suggest a cortical level component to the interhemispheric interactions contributing to motor irradiation. Likewise, Carson et al. (2004) noted an interhemispheric interaction between muscle representations during rhythmic wrist flexion and extension. Their results indicated a patterned modulation where the greatest facilitation occurred during the phases of movement when homologous muscles where engaged simultaneously.

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Emerging evidence from recent research indicates that the pathways mediating

bilateral interactions may occur at least in part, trough transcallosal connections. Shimizu

et al. (2002) investigated 21 stroke patients; 12 with unilateral cortical stroke and 9 with

subcortical stroke caudal to the corpus callosum. Using paired-pulse TMS, they found an

ipsilateral motor cortical disinhibition during the acute phase of stroke recovery

accompanied by changes to transcallosal inhibition, but *only* in the cortical stroke

patients; the subcortical group showed normal excitability patterns. These findings have
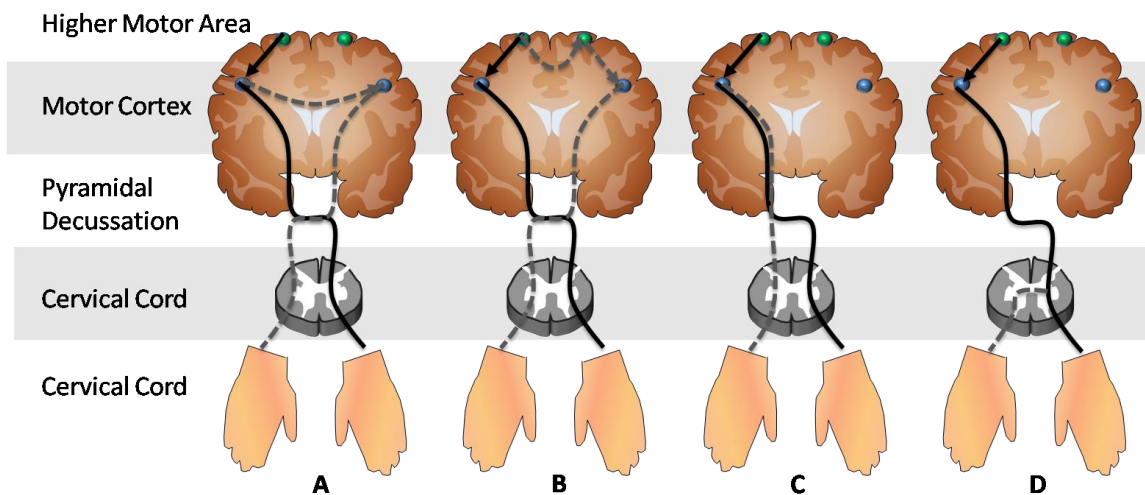
Figure 1.1 **Schematic diagram of possible motor irradiation pathways**
Unintended motor output, signified by the dashed lines, could occur via (A)
excitatory transcallosal pathways connecting the motor cortices (B) common
inputs to both motor cortices from higher order areas (C) uncrossed corticofugal
collaterals, or (D) branched bilateral corticospinal connections. Adapted from
Carson (2005)

been taken to suggest the involvement of transcallosal pathways in the control of

interhemispheric excitability. Earlier insights into the role of transcallosal connectivity in

bilateral interactions came from the study of callosectomy patients. People with partial or

complete agenesis of the corpus callosum often demonstrate an uncoupling of bimanual

movements (Diedrichsen et al., 2003). Meyer and colleagues (1995) demonstrated that

patients with agenesis of the anterior trunk of the corpus callosum showed impaired or absent transcallosal inhibition. What remains to be determined is whether the proposed interhemispheric communication occurs through direct transcallosal connectivity between the primary motor cortices or between regions upstream of M1 (Carson, 2005).

The standard view is that the transcallosal connectivity between distal arm muscle representations in the primary motor cortex is negligible or non-existent. Rouiller et al. (1994) used microstimulation and antero- and retrograde tracer substances to investigate the distribution and density of callosal projections in macaque monkey. Their results showed that hand representations of the supplementary motor areas (SMA) have dense and widespread callosal connectivity unlike those of the primary motor cortex representations, which were markedly less dense. One would expect then that if the primary means of communication arises from a direct linkage between motor cortical representations, then contraction of a distal effector of the upper limb would have diminutive effect on the ipsilateral homologous representation. Conversely, if unilateral activation of the intrinsic muscles of the hand does demonstrate bilateral effects, then conceivably higher order cortical areas (such as the SMA) are responsible.

Despite the focus on transcallosal connectivity with regards to motor irradiation, there still exists the possibility that sub-cortical structures such as the cerebellum and basal ganglia account for motor irradiation, however little evidence exists to support this supposition.

1.4 Transcranial Magnetic Stimulation (TMS)

The understanding of normal human neuromodulation has increased dramatically over the past few decades thanks to emerging neuroscience research methods such as electroencephalography (EEG), positron emission tomography (PET), computerized tomography (CT), functional magnetic resonance imaging (fMRI), transcranial electrical stimulation (TES), and transcranial magnetic stimulation (TMS). Each of these techniques can be used as complimentary methods to study normal or abnormal function of specific cortical regions. Functional MRI can be used to reveal regions of the brain activated during specific tasks by examining the haemodynamic response to a cognitive and/or motor behaviour. Though fMRI can identify regions associated with specific cognitive functions it, however, lacks temporal resolution and therefore cannot prove unequivocally which cortical regions are essential to the task. Conversely, TMS's ability to produce focal and transient virtual lesions presents a significant contribution to the determination of causality. When a cognitive function is suppressed by TMS stimulation, it provides evidence towards regions necessary for the task performance.

Since its inception in the mid 80's, TMS has been used extensively as a non-invasive method to investigate the excitability of neurons; indicated by the growing body of TMS research published over the years (Illes et al., 2006). TMS operates under the basis of Faraday's Law of Induction; an electromotive force can be created by a changing magnetic environment. A transcranial magnetic stimulator consists of a capacitor and an inducer. The capacitor is charged to 2-3 kV of electricity and can produce a brief pulse of up to 5000A when discharged. A transient magnetic field is induced by passing this brief pulse of electrical current through the inducer – a coil of copper wire called the magnetic

coil. As the high-current pulse travels through the coil of wire, a magnetic field is produced with a line of force running perpendicular to the surface of the coil. This in turn induces an electric current flowing perpendicular to the magnetic field. The end product results in a weaker electrical current that flows in loops parallel to the surface of the coil (Hallet, 2000).

The electrical field produced is contingent upon three factors; the shape of the magnetic coil, the orientation of the coil, and the electrical conductivity of the cortical tissues. Magnetic coils are manufactured in different shapes, most notably round and figure of eight. While a round coil produces a larger, more robust stimulation, a figure of eight coil provides a more focal stimulation with maximal current at the intersection of the two coils that define its shape. The optimal orientation of the coil changes depending on the target cortical structure. For the motor cortex, Ellaway et al. (1998) found that a coil handle directed 45° to the midline (perpendicular to the central sulcus) was most favorable for eliciting MEPs since the induced current runs perpendicular to corticospinal neurons at that orientation.

Although TMS is routinely used in the investigation of neural activity, the exact structures activated by the stimulation have been debated. It is thought that TMS normally activates corticospinal neurons indirectly through the stimulation of synaptic inputs; however as the stimulation intensity increases direct activation of the corticospinal track can occur, also indirect stimulation is still preferred (Hallet, 2000; Terao & Ugawa, 2002).

1.5 Safety of TMS

TMS has been accepted as a safe and acceptable method of investigating the human neural system (Hallet, 2000). For instance, the peak magnetic field strength of TMS, 1.5 – 2 T, is less than other methods used in neurophysiologic research such as MRI, which produces field strengths of 3 – 8 T (Hallet, 2000). Nonetheless, although the changes in neural activity induced by TMS are transient and without long-lasting effects, the possibility that TMS presents long term risks cannot be excluded.

Single-pulse TMS appears to pose no significant risk to healthy adult participants beyond mild discomforts. The most common reported side effects of TMS are headaches and discomfort at the site of stimulation, both of which are discontinued upon the cessation of TMS and can easily be treated with over the counter pain medication (Anand & Hotson, 2002). A study by Counter et al. (1990) on the effects of extracranial magnetic field stimulation acoustic artifacts on the unprotected ears of experimental animals found that the auditory clicks that accompany TMS may raise the hearing threshold in rabbits; however the findings have not been reproduced in the human population (Pascual-Leone et al., 1992). Both short and long-term studies addressing the safety of magnetic simulation of presumed healthy participants found no significant changes in neurological, neuropsychological, EEG, hormonal levels, and cardiovascular function (Chokroverty et al., 1995).

As the frequency of TMS stimulation increases, so does the risk of short-term adverse effects. With rTMS, a magnetic stimulation technique that utilizes high frequency stimulation to produce longer lasting effects, there are no known long-term consequences, but there are increased short-term risks above and beyond those observed

in single-pulse TMS protocol. Immediately following rTMS, motor reaction times may be decreased, possible changes in endocrine function and short-lived decline in short-term verbal memory (Anand & Hotson, 2002). The greatest concern in terms of rTMS is the risk of seizure in epileptic and healthy participants. In single-pulse TMS studies, seizures have been reported in only 7 adults, all of which had pre-existing abnormal brain function (Hallet, 2000). However, a small number of seemingly healthy participants have had a seizure while undergoing high frequency repetitive TMS (rTMS).

In response to the known risks associated with TMS, Keel et al. (2000) have proposed a Transcranial magnetic stimulation Adult Safety Screen (TASS), a self-administered questionnaire to screen potential participants for those who are at greater risk for adverse events. A copy of the modified TASS can be found in Appendix 1.

1.6 TMS and the Motor Cortex

TMS has proven to be an invaluable tool in the investigation of the excitability and connectivity of the human motor cortex. The ability of TMS to selectively activate a specific muscle or muscle group, in addition to its ability to stimulate the corticospinal tract both directly and indirectly, allows for great flexibility in this research technique.

Patton and Amassian (1954) observed multiple descending volleys in the pyramidal tract when an electrical stimulus was applied directly to the exposed motor cortex of monkeys. The first wave, later called the direct or 'D-wave', is considered to be the product of the direct excitation of the corticospinal axons. Later volleys are speculated to be a result of the indirect, transsynaptic activation of corticospinal cells and are thus called indirect or 'I-waves'. When the stimulus intensity is greater than

threshold, multiple I-waves can be observed and are named in order of their latency (I_1, I_2, I_3, etc…).

While direct stimulation of the exposed cortex has a long history, it was not until the work of Merton and Morton in 1980 did noninvasive brain stimulation techniques gain in popularity. Since then, several methods of transcranial electrical and magnetic stimulation have been developed. As the names imply, transcranial electrical stimulation (TES) uses a direct current to activate the motor cortex, while transcranial magnetic stimulation (TMS) induces a current through the use of magnetic fields. Several studies using direct recordings of descending spinal cord volleys in humans have shown that TES activates the corticospinal tract directly producing D-waves while TMS activates the pyramidal cells indirectly through excitatory interneurons, resulting in I-waves (Brocke et al., 2005). However, as the intensity of TMS is increased to higher levels and in specific coil orientations, D-wave activation is possible (Di Lazzaro et al., 1999).

The magnetic field induced by TMS creates a weak electric current that flows parallel to the surface of the cortex. Coil orientation, and the resulting direction of the induced current, is a significant factor in determining the mechanism of activation for TMS (Brocke et al., 2005). Sakai et al. (1997), using a figure-eight coil, investigated the effect of eight different coil orientations, each separated by 45°, on the latencies of responses to TMS in the intact human brain. When the TMS coil was medially and anteriorly directed, I_1 waves were preferentially elicited whereas when the TMS was laterally and posteriorly directed, the current readily evoked I_3 waves. D-waves can be predominantly recruited when the TMS coil is held in a lateral-medial direction (Di Lazzaro et al., 2001). fMRI studies have revealed that the differential effects are related

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to the direction of the induced current in relation to the central sulcus. Di Lazzaro et al. (2001) confirmed that posterior-anterior (PA) coil orientation generates I_1 waves and that when the current flow is reversed to an anterior-posterior (AP) direction, I_3 waves are produced at lower intensities. Their results suggest that different coil orientations preferentially activate different subpopulations of cortical neurons.

1.7 Paired-pulse TMS Protocol

Normal functioning of the human cortical activity is dependent upon a balance of excitatory and inhibitory systems (Chen, 2004). TMS is an effective method to non-invasively investigate cortical circuits mediating motor output, especially when a paired-pulse protocol is used. A paired-pulse paradigm involves stimulation with two distinct stimuli, a conditioning stimulus and a test stimulus, separated by varying interstimulus intervals (ISIs). The test stimulus is suprathreshold and large enough to produce a motor response in resting muscles. The effect that the preceding conditioning stimulus has on the test stimulus is dependent on its intensity and the interstimulus interval. If a single subthreshold conditioning stimulus occurs 1-5 ms prior, the resulting MEP elicited by the test stimulus will be inhibited (Di Lazzaro et al., 1998). Conversely, the same conditioning stimulus occurring 8-20 ms before the test stimulus results in a facilitated response. Lastly, if a suprathreshold conditioning stimulus precedes the test stimulus by 50-200 ms, the resulting MEP is decreased (Lee, Gunraj, & Chen, 2007). The different responses to changing paired-pulse parameters indicate the recruitment of separate inhibitory and facilitatory circuits.

1.8 Intracortical Inhibition

Intracortical inhibition of the human motor cortex – the process by which interneurons attenuate the activity of other cortical neurons – can be investigated through the use of paired-pulse TMS. Di Lazzaro and colleagues (1998) recorded descending volleys produced by paired-pulse stimulation in healthy adults using high cervical, epidural electrodes. They observed no descending activity caused by the conditioning stimulus and concluded that the inhibition caused by the conditioning stimulus was most likely due to an activation of local inhibitory mechanisms. Further research has established that the majority of inhibitory synaptic transmission is controlled more specifically by the neurotransmitter gamma-aminobutyric acid (McCormick, 1989).

GABA, or Gamma-Aminobutyric Acid, is an amino acid first discovered in Berlin in 1883. Originally known only as a product of plant and microbe metabolism, GABA was later discovered in the 1950s to serve an inhibitory function in vertebrates. Approximately 20% of neurons in the human cortex are GABAergic (Gottesmann et al., 2001).

It is generally accepted that GABA-mediated inhibition functions by generating hyperpolarizing inhibitory postsynaptic potentials (IPSP), changing the membrane voltage of the postsynaptic neuron and making it more difficult for a membrane potential to reach threshold for generating an action potential. How GABA neurotransmitters act on the human cortex specifically is dependent on the receptor subtype.   Based on their pharmacological profiles, three distinct GABA receptors have been identified: $GABA_A$, $GABA_B$, and $GABA_C$.

The GABA$_A$ receptor was the first of the three receptor types to be identified. GABA$_A$ is an ionotropic receptor that functions by regulating the release of negatively charged chloride ions. The receptor itself has several subunits – α, β, γ, ρ, δ, π, ε, and θ – and is the most widely expressed GABA receptor. Since GABA$_A$ is an ionotropic receptor, it is responsible for the faster inhibitory activity processes (called short interval intracortical inhibition) associated with GABA and is involved in the regulation of many processes including anxiety, muscle tone, memory functions, vigilance, epileptic seizures and sleep (Bateson, 2004). Short interval intracortical inhibition (SICI) can be demonstrated by delivering a subthreshold conditioning stimulus 1-6 ms prior to a suprathreshold test stimulus (Kujirai et al., 1993).

GABA$_B$ was the second GABA receptors to be identified. It differs from both GABA$_A$ and GABA$_C$ receptor types in that it is metabotropic, meaning that it functions through second messenger systems; specifically it is coupled to Ca2$^+$ and K$^+$ ion channels and acts in slow-acting or long interval intracortical inhibition (Gottesmann et al., 2001). Long interval intracortical inhibition (LICI) can be demonstrated by delivering a suprathreshold conditioning stimulus 50-200 ms prior to a suprathreshold test stimulus (Kujirai et al., 1993).

GABA$_C$ is the latest GABA receptor to be identified. First thought to be exclusively located in the retina, it is now known that GABA$_C$ receptors exist in select areas of the central nervous system (Gottesmann et al., 2001). Similar in structure to the GABA$_A$ receptor, GABA$_C$ is an ionotropic receptor and has several subunits: ρ1, ρ2, and ρ3. Though structurally similar to GABA$_A$ receptors, GABA$_A$ and GABA$_C$ are distinct

from one another. An important dissimilarity is that $GABA_C$ has a greater affinity for the neurotransmitter GABA than $GABA_A$; however its distribution is much sparser.

Intracortical inhibition subserves human motor function by maintaining a balance in the excitability of corticospinal neurons. Stinear and Byblow's (2003) study of intracortical inhibition during phasic index finger flexion demonstrated an increase in intracortical inhibition for abductor pollicis brevis muscles during selective activation of the ipsilateral flexor dorsal interosseous. Their findings suggest that intracortical inhibition may serve to prevent unwanted muscle activation during selective muscle contractions. A recent study by Schneider et al. (2002) investigated the processes involved in the coupling of motor cortical points using intracortical microstimulation and anaesthetized cats. The authors identified two cortical points on M1 that activated separate muscles at threshold. When they injected the cat with a $GABA_A$ receptor antagonist at the test point, stimulation of the other cortical point produced activation of both cortical points. Further, they found that simply increasing the intensity of the test stimulus did not result in co-activation of both cortical points and concluded that the muscle synergy was not simply a result of the stimulation spreading, but rather the release of inhibition of the test cortical point. This study lends further evidence to the role of intracortical inhibition in the control of co-activation of separate muscle motor representations.

Chapter 2 – Goal of Thesis

2.1 Overview

The overall objective of this thesis was to investigate the changes in motor cortical excitability that accompany movement of the upper limb ipsilateral to the M1 and the underlying mechanisms that may be contributing to these activity-dependant modulations.

Changes in ipsilateral M1 excitability have previously been documented; however the mechanisms controlling the ipsilateral influences remain relatively unknown. Both spinal and supraspinal elements have been implicated in the changes to homologous muscle motor output during unilateral movement, though recent research implicate cortical mechanisms. Transcranial magnetic stimulation, the research tool utilized in this thesis, not only can modify the excitability of the primary motor cortex, but is an effective instrument in the investigation of cortical level influences including interhemispheric and intracortical inhibitory circuitry. Understanding the neural underpinnings controlling upper limb movement may not only provide valuable insight into upper limb motor deficits observed with neuronal damage, but can be an invaluable tool in the development and prescription of rehabilitative strategies.

The specific aims of the thesis were to:

1) Compare excitability changes in distal and proximal arm representations in the primary motor cortex in response to ipsilateral homologous muscle activation.

2) Contrast changes in short and long interval intracortical inhibition associated with ipsilateral hand movements.

3) Compare excitability changes in the primary motor cortex during contraction of ipsilateral homologous and non-homologous muscles in order to investigate the specificity of interhemispheric cortical modulation in M1.

2.2 Hypothesis

Short and long interval intracortical inhibition can be demonstrated using a paired-pulse TMS protocol where a conditioning stimulus precedes a test stimulus by either 1 – 6 ms or 50 – 200 ms respectively. The conditioning stimulus is believed to activate inhibitory interneurons which suppress motor cortex output to the spinal cord. It has been suggested that with activation of the right primary motor cortex, glutamatergic pathways connecting the primary motor cortices via the corpus callosum activate local inhibitory interneurons in the homologous region of left motor cortex (called interhemispheric inhibition; Daskalakis et al., 2002). These inhibitory interneurons in turn inhibit the local inhibitory populations responsible for SICI. The resulting effect of the interhemispheric inhibition is a decrease in the short interval intracortical inhibition induced by the paired-pulse TMS and, subsequently, an increase in motor cortex output.

In order to address the first aim of the thesis, we hypothesized that discrete unilateral movements of the ECR would increase the excitability of the homologous muscle representation in the ipsilateral M1 compared to when the muscle was at rest. In contrast, contralateral movement of the FDI would have had little effect on the MEP amplitude of the test muscle due to the relatively sparse transcallosal connections between the homologous FDI cortical representations. The second aim was addressed by testing the hypothesis that any observed excitability increases in the M1 ipsilateral to the muscle contraction would be associated with a release of intracortical inhibition.

Further, with reference to the third aim of the thesis, we hypothesized that movement of the antagonist muscle group (flexor carpi radialis) would show no effect on the MEP amplitude of the contralateral test muscle (ECR).

2.2 Summary of Experiments

Single and paired pulse TMS protocols were used to evaluate changes in SICI, LICI, and motor cortex excitability in response to ipsilateral isometric contractions of isolated hand muscles. Specifically, an intrinsic muscle of the hand, the FDI, as well as a relatively more proximal muscle of the upper limb, the ECR, were targeted. The strength of the contraction was fixed to 10% of the subject's maximal voluntary contraction (MVC) for the non-dominant test effector. All participants were right hand dominant and TMS was delivered to the left primary motor cortex. Our research was separated into two studies described below:

1) 10 healthy adult participants were tested while at rest and during performance of a voluntary unimanual contraction. Single pulse TMS evaluated the changes to cortical excitability of the ipsilateral M1 during unilateral isometric contractions in the wrist and hand. Resting and active level SICI was evaluated by preceding a suprathreshold (120% RMT) test stimulus with a subthreshold (80% RMT) conditioning stimulus by 3ms.

2) Both SICI and LICI were evaluated in 5 healthy participants. SICI response to active conditions was reevaluated by adjusting TMS stimulator output to match the average MEP created by a single pulse during ipsilateral movement to that created by a single pulse at rest. The newfound TMS output was then

used as the active test pulse, and was preceded by an 80% subthreshold conditioning pulse for evaluating SICI during movement conditions. LICI was investigated by preceding a test pulse with an equal suprathreshold (120% MT) conditioning stimulus using an ISI of 100 ms.

Full descriptions of each study can be found in Chapters 3 and 4. Each study was prepared as a separate manuscript for submission to a scientific journal, as such some of the material presented in both chapters may be overlapping.

Chapter 3 – Study One

Changes in motor cortex excitability and short interval intracortical inhibition during ipsilateral performance of a proximal versus distal muscle of the upper limb

3.1 Overview

Changes in ipsilateral primary motor cortex excitability are observed during performance of unilateral movements of the upper limb. To help understand some of the neural mechanisms modifying the corticospinal output, we investigated whether local intracortical neural circuits are modulated by ipsilateral homologous and non-homologous motor activity. Additionally, we tested both distal and proximal arm musculature to see if they had comparable effects to contralateral homologous muscle movement. Previous studies have shown that transcallosal connections between cortical representations of the intrinsic muscles of the hands are relatively sparser than the more proximal muscles of the upper limbs. It was theorized that differential responses by the distal and proximal effectors could implicate involvement of callosal pathways connecting the primary motor cortices. Focal transcranial magnetic stimulation (TMS) was delivered to healthy, right-handed subjects. A paired-pulse protocol was applied to the primary cortex contralateral to the test hand using an interstimulus interval of 3 ms; stimuli specifically targeted either the extensor carpi radialis (ECR) or first dorsal interosseous (FDI) motor representations. The conditioning stimulus was set at 80% of resting motor threshold (RMT) and the test stimulus was suprathreshold at 120% RMT. Motor-evoked potentials (MEPs), recorded using surface electrodes, were measured while the homologous muscle groups contralateral to the test hand were both active and at rest. Peak-to-peak MEP amplitudes were averaged for each condition and the resulting data normalized as a percentage change from the average MEP produced by an

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unconditioned test pulse at rest. Results showed that discrete unilateral movements of both the FDI and ECR increased the excitability of their respective contralateral homologous muscle representations, compared to when the same muscle was at rest. Further, SICI was almost completely disinhibited during low-level ipsilateral ECR contraction. In contrast, while activity-dependent decreases in SICI did occur during ipsilateral movement of the FDI, the effect was to a great extent less than that observed with the ECR. Further, movement of the antagonist muscle group (flexor carpi radialis) showed no effect on the MEP amplitude of the contralateral test muscle (ECR). Our findings suggest that reductions in intracortical inhibition contribute to the overall facilitation observed in homologous motor representations during unilateral movement. Further, direct transcallosal connections between mirror movement representations in the primary motor cortex may figure in these activity-dependent changes.

3.2 Introduction

In normal humans, voluntary unimanual hand movements result in bilateral changes in corticomotor excitability. Facilitation of the primary motor cortex (M1) contralateral to contraction forces in the hand have been reported in several functional magnetic resonance imaging (fMRI) (Cramer et al., 1999; Verstynen et al., 2005) and transcranial magnetic stimulation (TMS) studies (Muellbacher et al., 2000; Liepart et al., 2001; Woldag et al., 2004; Renner et al., 2005). Similarly, activity dependant changes have also been demonstrated in the M1 ipsilateral to hand movements. At higher levels of force, unilateral hand performance results in facilitation of corticomotor excitability targeting the non-task hand (Hess et al., 1986; Meyer et al., 1995; Tinazzi & Zanette, 1998; Muellbacher et al., 2000; Woldag et al., 2004), while force levels of around 1-2% of maximal voluntary contraction (MVC) have been shown to have an inhibitory effect on the motor output of the contralateral hand (Liepart et al., 2000). These changes also appear to be task dependant, with stronger facilitation observed during more complex movement sequences (Ziemann & Hallet, 2001).

The mechanisms responsible for these ipsilateral effects are relatively unclear, however both spinal and cortical level mechanisms have been suggested. For instance, Meyer et al. (1995) argued that the modulations were occurring at the spinal level since patients with agenesis of the corpus callosum still presented with ipsilateral facilitation during voluntary unimanual hand contractions. Subsequent TMS studies, however, have implicated the involvement of supraspinal mechanisms, particularly transcallosal pathways (Stinear, Walker, & Byblow, 2001; Gilio et al., 2003; Carson et al., 2004). Tinazzi and Zanette (1998) assessed the role of the ipsilateral motor cortex in the

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production of unilateral movements using three complementary methods of investigation; median nerve stimulation, transcranial electrical stimulation (TES), and TMS. Consistent with previous research, TMS-invoked MEPs were facilitated by homologous hand performance, whereas both the H-reflex induced by median nerve stimulation and the MEPs from TES were relatively unchanged. As TES bypasses changes in cortical excitability, the authors proposed that interhemispheric mechanisms are involved in the ipsilateral corticospinal activation observed.

Transcranial magnetic stimulation (TMS) provides a non-invasive method to probe transient modulations in corticospinal excitability. Further, cortical inhibitory systems can be studied using paired pulse TMS paradigms; a sequence of two stimuli where the initial conditioning pulse activates local GABAergic interneurons which suppress the corticospinal output stimulated by the subsequent suprathreshold test pulse. The purpose of the present study was to use TMS to investigate possible mechanisms controlling modulation of ipsilateral M1 during unilateral hand movements, specifically with a focus on fast acting GABAergic inhibitory interneurons. Further, we were interested in the in the modulation of intracortical neural circuits in response to ipsilateral performance of an intrinsic muscle of the hand versus a more proximal effector. Callosal connectivity between homologous cortical representations of the intrinsic muscles of the hand are generally sparse compared to the more proximal musculature of the limb. It was hypothesized that marked differences in the modulation of TMS evoked MEPs and/or intracortical inhibition between distal versus proximal musculature could implicate transcallosal influences, although direct interhemispheric connectivity was not directly tested by the current protocol. Finally, the influence of voluntary activation of an

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ipsilateral non-homologue (yet spatially close muscle representation) to the modulation of corticospinal excitability and intracortical inhibition will be investigated.

3.3 Methods

3.3.1 Subjects

Ten young (23-38 years of age, mean 26.5 yrs, 4 males, 6 females) healthy adult volunteers participated in the study. Each participant gave their informed written consent to participate in the study. The experimental procedures conform to the guidelines set forth by the Human Research Ethics Committee and the protocol was approved by the Office of Research Ethics at the University of Waterloo.

Participants were asked to complete a modified version of the Transcranial Magnetic Stimulation Adult Safety Screen Questionnaire – a 23 point screening tool used to exclude those participants who may be predisposed to adverse events during TMS (Keel et al., 2000) (*see Appendix 1*). In addition, participants were screened for right-hand dominance according to a modified Waterloo Handedness Questionnaire (WHQ) (*see Appendix* 2). All ten participants were given specific instructions to follow prior to the TMS testing to help eliminate possible confounding variables.

3.3.2 Experimental Approach

Testing was completed during a single 2.5 hr session in the Neurophysiology Lab in the Lyle Hallman Institute at the University of Waterloo.

Setup

Participants were seated comfortably in a modified office chair with their right and left forearms supported by armrests in a pronated position and head placed in a firm chinrest for stability and support. A metal rod, connected to the TMS support frame, rested vertically and flush against the left armrest in an adjustable position and provided support for the required isometric contractions. The TMS coil was supported on the left side by a variable friction arm, also attached to the TMS support frame. A photograph of the manipulation used for the experiment as well as a graphical overview of the experimental setup is provided in Figure 3.1.

Electromyography

Bipolar surface electromyogram (EMG) activity was recorded from three test muscles of both the right and left upper limbs; the first dorsal interosseous (FDI), flexor carpi radialis (FCR), extensor carpi radialis (ECR). A pair of self-adhesive Ag-AgCl Meditrace surface electrodes (Tyco Healthcare Group LP, Mansfield, MA) were placed longitudinally over the FDI, FCR, and ECR muscle bellies with grounds placed over the ulnar styloid process, medial epicondyle, and lateral epicondyle respectively.

EMG signals were amplified (5000x) and bandpass filtered (3-1000 Hz) using a standard EMG amplifier (Grass Technologies, West Warwick, RI), sampled at 1000 Hz using an analog-to-digital converter (NI DAQCard 6024E, National Instruments, Austin, TX) and stored on a PC computer for off-line analysis.

A

B
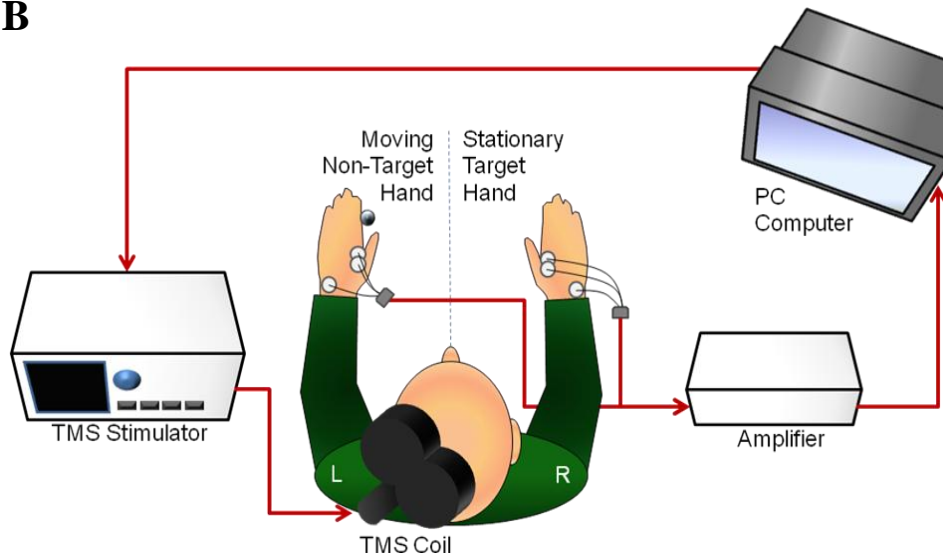
Figure 3.1 Manipulation used for the experiment
(A) Participants were seated comfortably in a modified office chair with arms
placed on wooden arm rests. A support frame was provided to support the
subject's head as well as the coil during the study. A vertical metal rod extended
downwards from the left side of the support frame, and sat flush at the end of the
left arm rest. (B) EMG activity recorded on a PC computer which was also used
to trigger TMS delivery.

Transcranial Magnetic Stimulation

TMS was delivered using a single MagPro (Medtronic-Dantec, Minneapolis, MN) stimulator and discharged through a figure-of-eight coil (outer diameter: 9 cm). Stimulus intensities were expressed as a percentage of maximal stimulator output. Prior to the experiment, an anatomical magnetic resonance image (MRI) was obtained for each participant using a 3 T MR system (GE HealthCare, Milwaukee, WI; TR = 12.4 ms, TE = 5.4 ms, FA = 35°, FOV = 20 x 16.5, 124 slices, 1.4 mm slice thickness). Coil placement and orientation was continuously monitored using BrainSight (Rogue Research Inc., Montreal, QC), a TMS neuronavigation system that displays real-time coil placement and target location on an anatomical magnetic resonance image (Figure 3.2). The coil was oriented tangentially to the surface of the skull, with the handle of the coil positioned dorsolaterally at an approximate 45° angle to the midline of the scalp. This particular orientation induces posterior-to-anterior directed current in the motor cortex and has been previously shown to be optimal for evoking MEPs (Ellaway et al., 1998).

For each participant, the stimulation occurred over the left hemisphere. Guided by the MRI image, the coil was placed over the section of the precentral gyrus known as the 'hand knob' and moved in small increments until the site that produced the largest MEP in the test muscle was identified (Yousry et al., 1997). The site location and coil trajectory was marked on BrainSight as a reference to reduce variability within and across conditions.
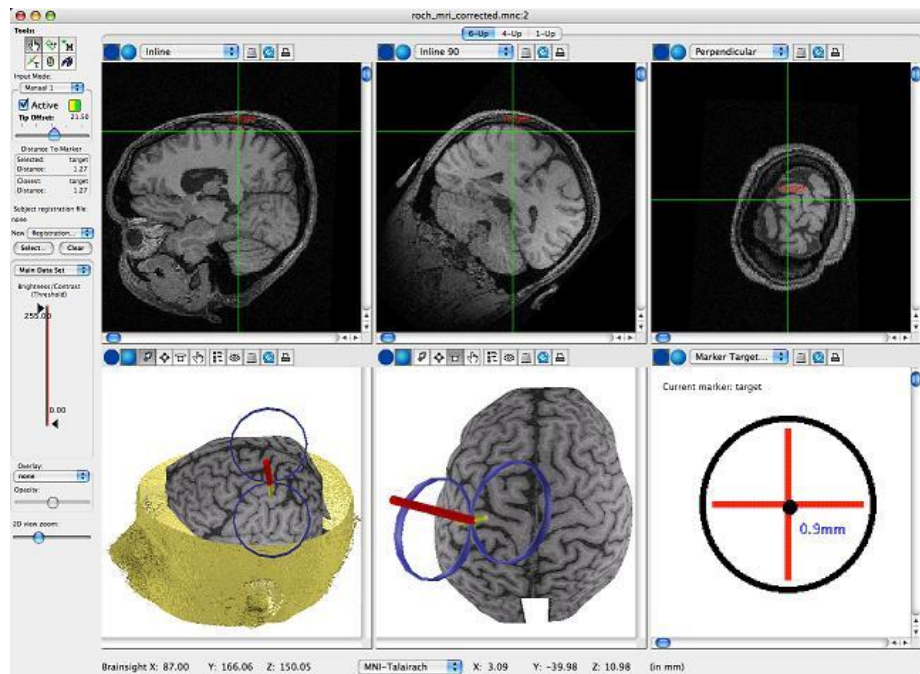
Figure 3.2 BrainSight main interface
Brainsight software allows for a curvilinear reconstruction of the brain from anatomical magnetic resonance images and aids in the stereotactic guiding of the TMS coil over specific anatomical locations. Trajectory and targeting views (red crosshairs) allow for repositioning of the TMS coil over target locations and aids in the reduction of stimulation site variability. 'Inline' and 'inline 90' views (top left images) help locate optimal coil trajectory for motor cortex stimulation.

Experimental Protocol

Participants were asked to perform three separate tasks with their non-dominant hand (hereto referred to as the task hand); a resisted isometric radial abduction of the index finger (FDI activation), a resisted isometric extension of the wrist (ECR activation), and a resisted isometric flexion of the wrist (FCR activation). A single experimental session consisted of 10 different conditions, each defined by the test muscle (in the resting right arm), number of pulses being delivered, the interstimulus interval, and whether the contralateral mirror agonist/antagonist muscle was active or at rest. Details and pictorial representations of experimental task conditions can be found in Table 3.1

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and Figure 3.3. Each run involved 20 trials per condition, with conditions being blocked according to distal (FDI) or proximal (ECR) effector and randomized within each block for a total of 200 trials. TMS was delivered as either a single pulse or as a short interstimulus interval paired-pulse (ISI 3 ms); each trial separated by a 3 to 5 second break. For all conditions, the test muscle was maintained in a state of rest and visual inspection excluded MEPs contaminated by preceding EMG activity. Prior to the onset of the TMS trials, the maximum EMG output was recorded for the left ECR, FCR, and FDI separately while the participant maintained a maximal voluntary contraction (MVC).

Rest conditions required participants to sit with both forearms at rest in a pronated position while the TMS was delivered, triggered externally using a customized LabView program (National Instruments, Austin, TX). The conditioning stimulus intensity for SICI was set to 80% resting motor threshold (RMT) and the subsequent test pulse adjusted to 120% RMT for all rest conditions. RMT was defined as the minimum stimulus intensity necessary to evoke a MEP with a peak-to-peak amplitude of 50 µV or greater in 5 out of 10 consecutive trials while the test muscle was at inactive (Rossini & Rossi, 2007).

During the movement task, participants were asked to perform a voluntary isometric contraction with either the contralateral homologous or mirror antagonist to the resting test muscle. This was accomplished by resting the inactive test hand against a metal rod attached to the TMS support frame. Trials in which the FDI was the test muscle, the index finger sat flush against the metal support at the proximal interphalangeal joint. Trials where the intended movement was flexion or extension of the wrist, the hand rested against the rod at the metacarpophalangeal joint. Subjects were instructed to keep their right limb in a state of rest between trials, and upon verbal cue,

initiate a resisted contraction of the test muscle. Participants were instructed to try to isolate activation to the test muscle only, and to maintain all other muscles in a relaxed state. A custom written LabView (National Instruments, Austin, TX) program triggered TMS delivery when the measured EMG level reached 10% of the activated muscle's maximal voluntary contraction.

Table 3.1 Experimental task conditions

Condition	Pulse Type	ISI (ms)	CS (% RMT)	TS (% RMT)	Task
FDI					
1	SP	-	-	120%	Rest
2	SP	-	-	120%	Movement
3	PP	3	80%	120%	Rest
4	PP	3	80%	120%	Movement
ECR					
5	SP	-	-	120%	Rest
6	SP	-	-	120%	Agonist Movement
7	SP	-	-	120%	Antagonist Movement
8	PP	3	80%	120%	Rest
9	PP	3	80%	120%	Agonist Movement
10	PP	3	80%	120%	Antagonist Movement

10 study conditions varying by test muscle (ECR, FDI), presence of conditioning stimulus, CS, (SP, PP), and whether the agonist (ECR/FDI) or antagonist (FCR) muscle is active or at rest. TS – test stimulus; rMT – resting motor threshold; SP – single pulse, PP – paired pulse; ISI – interstimulus interval.
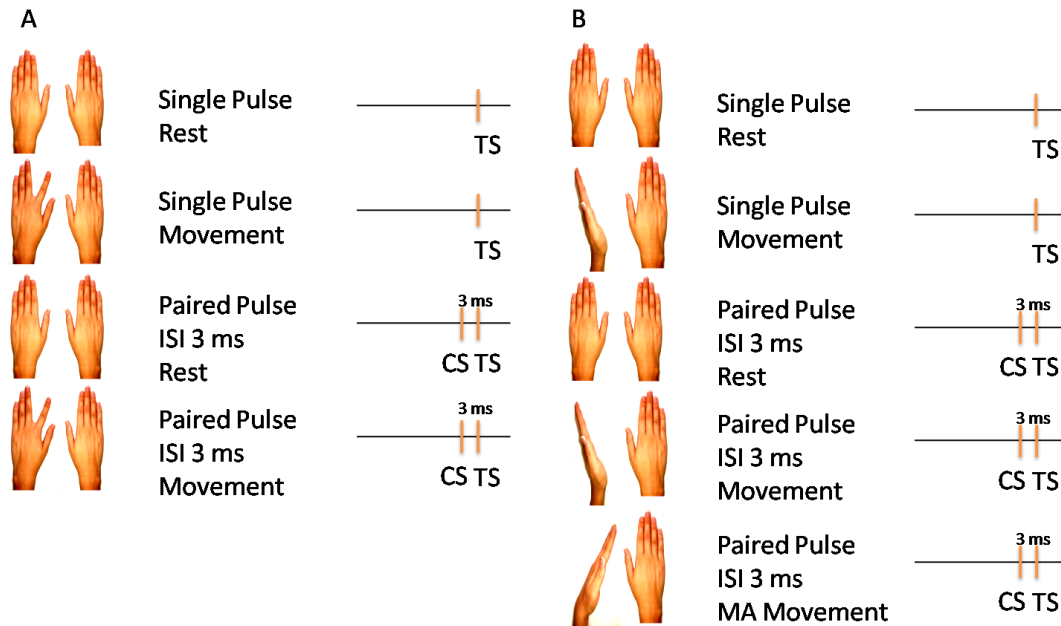
Figure 3.3 Diagram of experimental task conditions
Participants were asked to either maintain both arms and hands in a state of rest or to contract the left FDI (A), ECR or FCR (B). The above diagram shows overt movements however this is purely a pictorial representation of the actions requested of the participants. Isolated contraction forces were generated against a stationary bar, and were isometric as a result. MA = mirror antagonist (FCR).

3.3.3 Data Analysis

Mean peak-to-peak MEP amplitudes were calculated for each condition off-line using a customized program written in LabView (National Instruments, Austin, TX). The effect of voluntary unilateral movement on motor output and SICI of the ipsilateral mirror motor representation was assessed using a two-way repeated measures analysis of variance (ANOVA) to determine the effect of MOVEMENT (rest or 10% isometric contraction) and STIMULATION (single or paired-pulse) on right test muscle MEP amplitudes. To evaluate the hypothesis that any observed increases in excitability would be associated with modulation of intracortical inhibition, paired *t* tests were used to contrast the change in EMG activity between 'single pulse at rest' and 'paired pulse at

rest' conditions to the difference between 'single pulse during movement' and 'paired pulse during movement' conditions. Differences were considered significant at p<0.05 for all parameters. Paired *t* tests were also used to contrast changes induced by the contraction of the non-homologous FCR on the ipsilateral ECR M1 representation. Results are reported as mean ± standard error (SE) unless otherwise stated. All statistical analysis was completed using Statistical Analysis Software (SAS Institute, Cary NC, USA).

3.4 Results

3.4.1 Unconditioned Test-Pulse

Figure 3.4 illustrates the effects of single and paired-pulse TMS on the MEPs from both test muscles in a representative subject while the contralateral mirror muscle was at rest and during 10% MVC isometric contraction. For both the FDI and ECR, voluntary isometric contractions led to facilitation in the ipsilateral homologous M1 representation. Recorded from the right ECR muscle, when the test pulse was delivered during 10% MVC activation of the left ECR the average MEP increased by 35 ± 24%. Similarly, with the FDI, concurrent activation of the left FDI increased the MEP amplitude produced by the test stimulus 32 ± 19%.

3.4.2 Paired-pulse During Homologous Muscle Performance

Figure 3.5 illustrates changes in SICI in the left M1 during contraction of the left FDI an ECR. During resting conditions, when the test pulse was preceded by a subthreshold conditioning stimulus (80% RMT; ISI 3ms) the average MEP was suppressed in both test muscles.
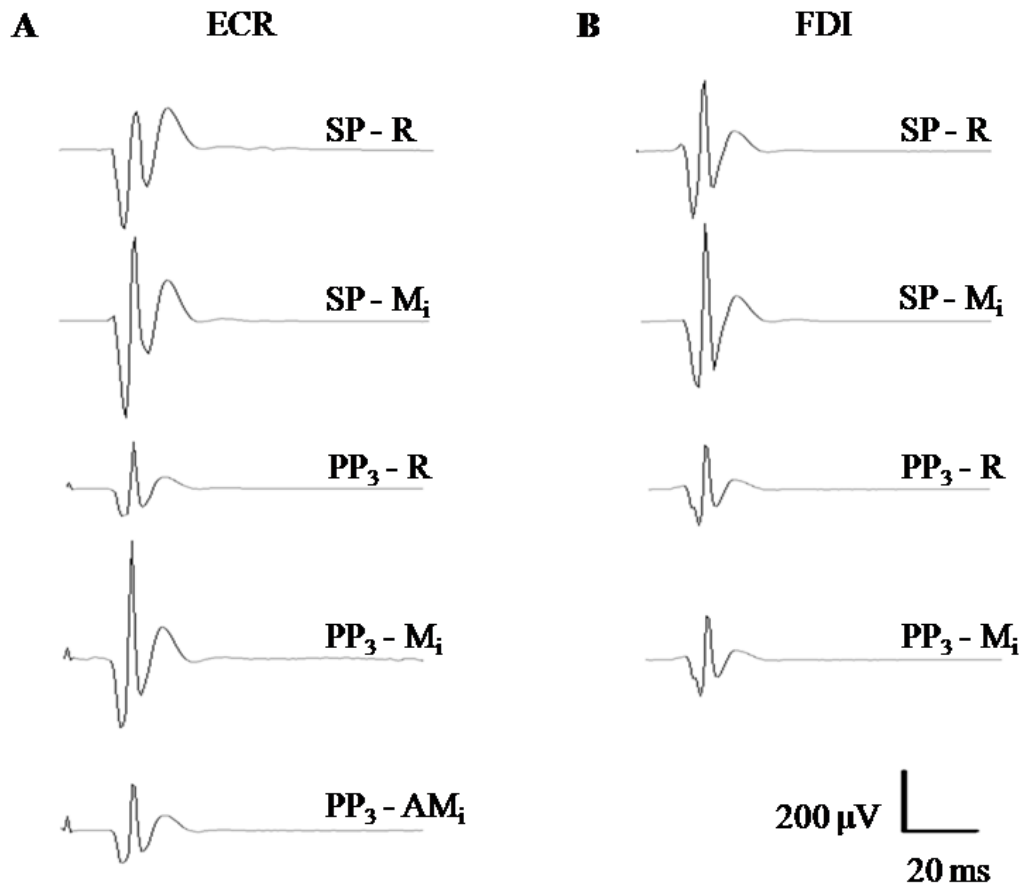
Figure 3.4 EMG responses to task conditions
Recordings from the right ECR (A) and FDI (B) of a representative subject during performance of each task condition. SP – single pulse; PP_3 – paired pulse with an interstimulus interval of 3 ms, R – rest, M_i – movement of ipsilateral mirror muscle, AM_i – movement of ipsilateral antagonist to the mirror muscle.

The difference in the mean results obtained for the conditions from 'single pulse at rest' to 'single pulse during movement' were compared to the change from 'paired-pulse at rest' to 'paired-pulse during movement' for both muscle effectors. A two-way repeated measures ANOVA of observed EMG activity in the right ECR revealed a main effect of MOVEMENT ($F(1,9)=6.40$, $p = 0.03$) with no significant effect of STIMULATION ($F(1,9)=3.21$, $p = 0.11$) or their interaction MOVEMENT X

STIMULATION ($F(1,9)$=0.76, $p = 0.41$),. Post-hoc tests revealed that the conditioning stimulus (ISI 3ms) significantly inhibited the size of the MEP compared to the unconditioned test stimulus ($F(1,9)$=3.75, $p = 0.06$). Simultaneous contraction of the contralateral ECR increased EMG activity in the right ECR to levels not significantly different from MEPs recorded during unconditioned test stimuli at rest ($F(1,9)$=0.36, $p = 0.55$).

Table 3.2 Normalized peak-to-peak MEP amplitudes from right FDI / ECR during task conditions

EMG %	Left FDI Rest	Left FDI Movement	Left ECR Rest	Left ECR Movement	Left FCR Movement
Single Pulse	100 ± 0 *	132 ± 19	100 ± 0 *	135 ± 24	
Paired Pulse	32 ± 16	49 ± 9	49 ± 8	98 ± 18	52 ± 10

* EMG levels expressed as % ratio ± SE of the average MEP measured during the single pulse at rest task condition, setting single pulse at rest conditions to 100%.

With the FDI as test muscle a two-way repeated measures ANOVA showed a main effect of MOVEMENT ($F(1,9)$=6.39, $p = 0.03$), STIMULATION ($F(1,9)$=22.06, $p = 0.001$), however no interaction MOVEMENT X STIMULATION (F(1,9)=0.01, $p = 0.94$). Post-hoc analysis showed that EMG activity in the right FDI was reduced by the short interval conditioning stimulus ($F(1,9) = 12.72$, $p = 0.001$). For the FDI, the conditioning TMS stimulus was still effective in suppressing the MEPs during movement of the contralateral FDI , however a small yet significant disinhibition occurred ($F(1,9) = 11.99$, $p = 0.002$).

3.4.3 Paired-pulse During Contralateral Antagonist Muscle Performance

Voluntary contraction of a non-homologue ipsilateral muscle, the FCR, did not significantly disinhibit SICI in the motor representation of the ECR muscle *(*Paired t-test; *p* = 0.6*)*. As Figure 3.6 depicts, during the resting condition, a pre-conditioned test stimuli produced on average an MEP 49 ± 8% of the mean MEP produced by an unconditioned test pulse at rest. During left wrist flexion (contraction of the left FCR), the average MEP changed to 52 ± 10% which did not prove statistically significant.
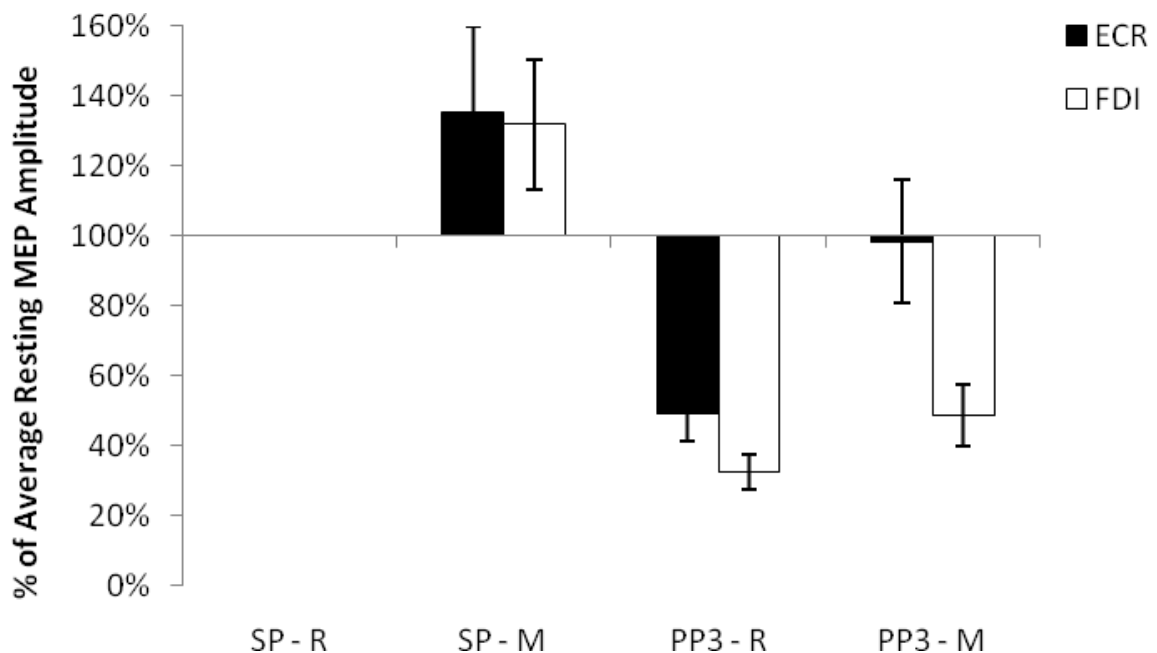
Figure 3.5 Group results illustrating changes in the relative amplitudes of MEPs produced by TMS for ECR and FDI effector groups.
Data from 10 subjects, grouped by TMS condition. PP_3 – paired pulse with 3 ms interstimulus interval; R – rest; M – movement. The main effect of movement was found for both effectors, FDI and ECR (p<0.05). Paired pulse TMS produced significant MEP inhibition at rest for both FDI and ECR (p<0.05), although only a main effect of stimulation was found in the FDI when changes SP-R to PP-R and SP-M to PP3-M were compared (p<0.05).
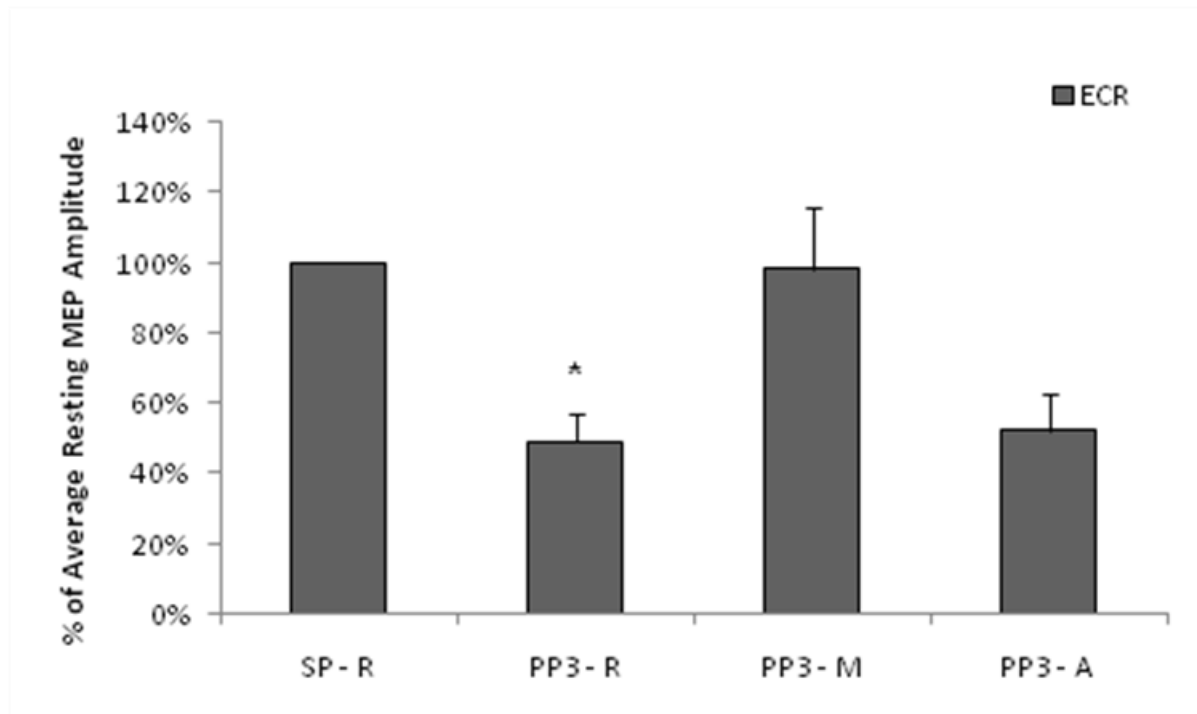
Figure 3.6 Group results illustrating changes in the relative amplitudes of MEPs produced by TMS during ipsilateral homologous or non-homologous muscle activation.
Data from 10 subjects, grouped by TMS condition. PP_3 – paired pulse with 3 ms interstimulus interval; R – rest; M – movement; A – antagonist movement. Voluntary isometric muscle contraction of the left ECR resulted in significant disinhibition of the mirror ipsilateral cortical representation *($p < 0.05$). Conversely, contraction of the antagonist (FCR) had no significant effect on MEPs recorded from the contralateral ECR test muscle.
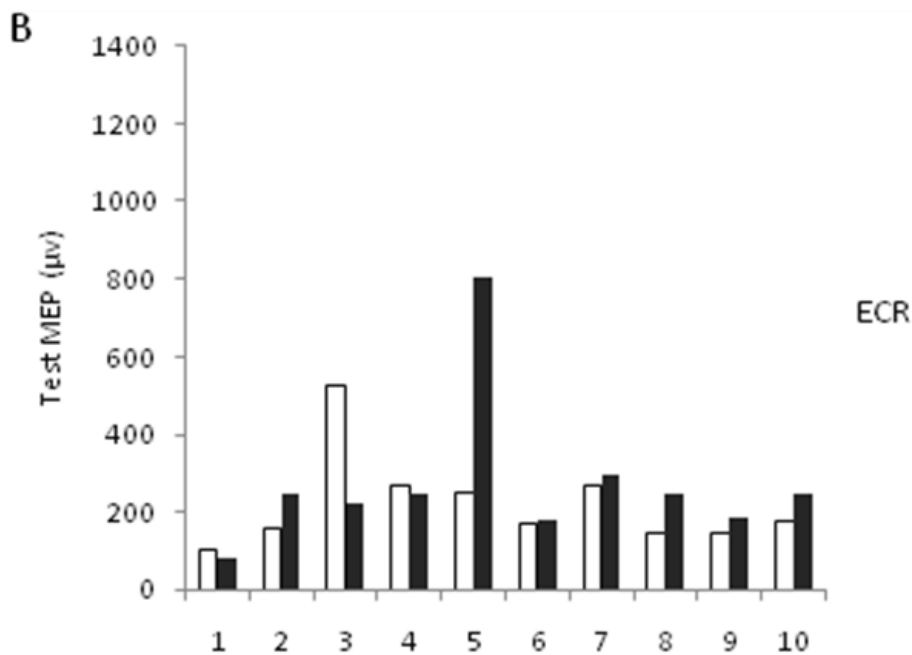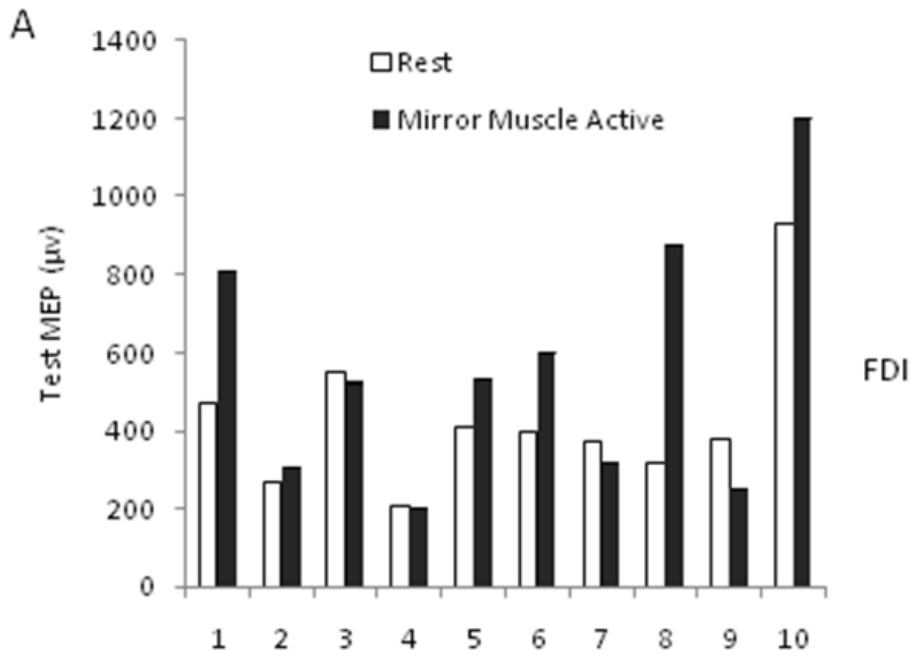
Figure 3.7 Mean size of unconditioned test MEP in both test muscles while the homologous mirror muscle is active or at rest.
Raw EMG recorded during delivery of un-conditioned test stimuli for the FDI (A) and ECR (B) muscle effectors. Data represents mean peak-to-peak MEP amplitudes for all 10 participants and expressed in microvolts. Overall, greater levels of EMG were found in the FDI compared to ECR. In the majority of participants, ipsilateral mirror contractions facilitated TMS-evoked potentials.

3.5 Discussion

The novel finding of the present study is that unimanual contraction influences SICI in the ipsilateral homologous motor representation and the degree of modulation is dependent on the location of the muscle on the proximal-distal axis.

3.5.1 MEP Facilitation in Distal vs. Relatively Proximal Musculature

Previous studies have shown that moderate to high levels of unilateral activity in muscles of the upper limb lead to an increased excitability in ipsilateral mirror motor representation (Hess et al., 1986; Meyer et al., 1995; Tinazzi & Zanette, 1998; Muellbacher et al., 2000; Hortobagayi et al., 2003). A study by Liepert et al. (2001) contrastingly reported an inhibition in the non-task hand during performance levels of 1-2% of MVC. However, in the same study tonic contractions at 20% and 40% MVC facilitated MEPs in the ipsilateral M1. Consistent with previous research, our results demonstrate a similar trend with contralateral performance increasing both ECR and FDI muscle MEPs by more than 30% of the average MEP obtained from a single pulse at rest. Presumably, the increase in MEP size could be due to changes at the cortical or subcortical level. While the present study was not designed to investigate elements of the corticospinal pathway other than the primary motor cortex, previous research suggests that the cross-facilitation is occurring primarily at a supraspinal level.

3.5.2 SICI at Rest

When a suprathreshold TMS stimuli, delivered to the motor cortex, is preceded by a below threshold conditioning stimulus at a short interval (≤ 5ms), EMG responses

evoked by the second test stimulus can be suppressed (Kujirai et al., 1993). The effect, known as SICI (short interval intracortical inhibition), is argued to be a result of supraspinal inhibitory mechanisms since the intensity of the conditioning stimulus is below the threshold required for active motor responses or the H reflex (Di Lazzaro et al., 1998). While the H-reflex is unaffected, data from both single motor unit recordings and recordings from epidural electrodes placed directly into human cervical spines have shown that conditioning stimulation reduces the amplitude of I-waves (with the exception of I_1) suggesting that the excitability of pyramidal cells are not directly influenced by the conditioning stimulus (Di Lazzario et al., 1998). Further, pharmacological studies strongly suggest that SICI is mediated by GABAergic inhibitory systems in the motor cortex (Florian et al., 2008)

Our results were consistent with previous literature with the conditioning stimulus suppressing the size of test pulse-produced MEPs delivered 3ms afterwards in both the ECR and FDI test muscles by approximately 50% of the average MEP produced during single pulse at rest.

3.5.3 SICI During Ipsilateral Performance

It was previously argued that unimanual movements were accomplished by suppressing or inhibiting activity in the contralateral homologous cortex, preventing bilateral contractions. As our findings suggest, however, unimanual movement at levels as low as 10% MVC are accompanied by a facilitation in the homologous motor areas and a decrease in intracortical inhibition. Results from the present study showed that paired-pulse TMS was less effective at suppressing the MEPs when contralateral muscles

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were activated. As shown in Figure 3.3, increases in FDI and ECR MEP amplitudes did occur when conditioned TMS pulses were introduced with simultaneous performance of the opposite hand. When each condition's MEP amplitudes are expressed as a percentage of the average MEP at rest, the same trend remains, however, the increase in FDI MEPs were markedly smaller than those observed in the ECR which almost returned to unconditioned test pulse MEP amplitudes. Due to overall increases in excitability observed during movement performance, a direct comparison between SICI at rest and during simultaneous ipsilateral movement could not be made. Instead we compared the overall change in MEP induced by an unconditioned test pulse between rest and movement conditions to the difference in MEPs evoked by paired-pulse TMS while the ipsilateral muscle was at rest or performing an isometric contraction. Subsequent analysis showed no significant effect of stimulation type in the ECR, meaning that increases observed between rest and movement conditions during single pulse stimuli were comparable to those recorded during paired-pulse stimuli. It is possible that the disinhibtion observed in the ECR merely reflected an overall modulation of corticospinal excitability and not decreases in inhibitory influences. Conversely, it could be argued that a reduction in SICI could be the reason for the increased corticospinal excitability observed. Changes to SICI in the FDI were significantly different from differences in single pulse rest and movement conditions and therefore cannot be explained by increases in facilitation of the motor representation, nor can attenuation of SICI fully explain the increases in corticospinal excitability recorded.

As mentioned earlier, changes to MEP amplitudes could reflect modifications at either a cortical or subcortical level. This specific study was designed using a TMS

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protocol and while an effective measure of cortical excitability, TMS alone cannot identify all mechanisms contributing to neuromuscular control. Nevertheless, it is agreed upon that SICI is a cortical phenomenon and therefore the observed reduction in intracortical inhibition offers strong evidence towards a supraspinal component to the MEP enhancements recorded during mirror muscle activation.

Several possible mechanisms for the modulation of SICI exist. It is feasible that unilateral movement increased the excitability of a separate facilitatory network that offset the effects of inhibitory influences. Sohn and colleagues (2003) investigated changes in facilitatory and inhibitory intracortical networks during voluntary hand movements using TMS and found a significant increase of intracortical facilitation (ICF) in ipsilateral homologous motor areas when a unimanual FDI contraction was performed. However, in contrast to our results, isometric contraction of the FDI muscle at lower levels of force suppressed the excitability in the ipsilateral MI and showed no affect on ipsilateral intracortical inhibition. Separate mechanisms control intracortical inhibition and facilitation though they are able to influence a common neuron (Ziemann et al., 1996). It is possible that two separate processes worked in conjunction to determine overall motor excitability in our study, however further investigation using paired-pulse parameters for the assessment of excitatory networks, such as ICF, is required (Floeter & Rothwell, 1999).

Another explanation for the reduction of SICI could be an increase in presynaptic inhibition of SICI circuits. Interhemispheric inhibition (IHI) is another inhibitory process thought to contribute to the control of motor functions and is most likely mediated by excitatory fibers crossing the corpus callosum and acting on local inhibitory interneurons

(Daskalakis et al., 2002). Daskalakis and colleagues (2002) studied the relationship between intracortical inhibition and ipsilateral cortico-cortical inhibition using TMS. They found that SICI was reduced in the presence of interhemispheric inhibition (IHI) and suggested that IHI was inhibiting SICI and not the other way around. It is possible that within the context of the present study, reductions in SICI were a result of increases in inhibitory drive from the contralateral M1. Ipsilateral IHI has been shown to remain constant through pre-movement periods and, at the onset of movement, shifts in the direction of the ipsilateral primary motor cortex (Duque et al., 2007).

Differing levels of inhibition between the FDI and ECR also support the role of IHI in task-dependant changes to the ipsilateral M1. Evidence exists for differences in IHI between varying upper limb muscle representations. Some have suggested that the degree of IHI follows a proximal-distal gradient (Ferbert et al., 1992; Sohn et al., 2003) while others propose that the degree of IHI is dependent on the muscle's functional role in everyday behaviour (Harris-Love et al., 2007). In general, direct callosal connectivity between M1 representations of distal arm musculature is sparse, however certain regions have been shown to have more dense connections than what was previously thought (Gould et al., 1986). If IHI differs on a proximal-distal gradient, since IHI is mediated through transcallosal connections between M1s, one would expect less interhemispheric inhibitory influence between FDI representations than the more proximal ECR motor areas. Likewise, if IHI is guided more by the behavioral context of the muscle, it would be expected that IHI would be less between FDI representation since the muscle contributes to more low force and precise unilateral tasks than the ECR which are typically used in more forceful, bilateral movements which would favour mirror

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activation; assuming that increases in IHI lead to a decrease in local inhibitory influence on corticospinal neurons.

While the process of IHI leading to the presynaptic inhibition of SICI neurons may explain the activity-dependent disinhibtion observed and explain the recorded differences between FDI and ECR, it cannot account for the comparable levels of overall facilitation of MEPs in both the distal and proximal effectors. One explanation is that other local inhibitory or excitatory mechanisms not investigated in this particular study (such as LICI or ICF) are differentially activated in the FDI and ECR resulting in the overall comparable MEP facilitation; further investigations into these networks is necessary. Our results support the view that interactions of two or more separate mechanisms are responsible for the facilitation observed in the ipsilateral mirror M1 representation. One theory that has been presented implies that motor commands are by default bilateral and that the ipsilateral motor cortex receives a copy of the motor command. The theory continues to state that performance of a unilateral movement requires inhibition of the non-test muscle. While our observed facilitation of homologous motor areas is explained under this hypothesis, our results are not consistent with this theory as we showed *decreases* in inhibition in the ipsilateral hemisphere. Yet another theory explains that during unimanual voluntary muscle activation, motor commands irradiate to contralateral muscles at either cortical (ex: SMA, M1) or spinal levels and that suppression or release of mirror movements is under the control of local inhibitory interneurons in the contralateral hemisphere. When a motor command is initiated, in addition to action on corticospinal output, transcallosal projections are activated (Avanzino et al., 2007). This excitatory transmission, which crosses the corpus callosum

and synapses on inhibitory interneurons, could in turn inhibit the interneurons

responsible for SICI, creating an overall disinhibition of pyramidal cells. While the exact
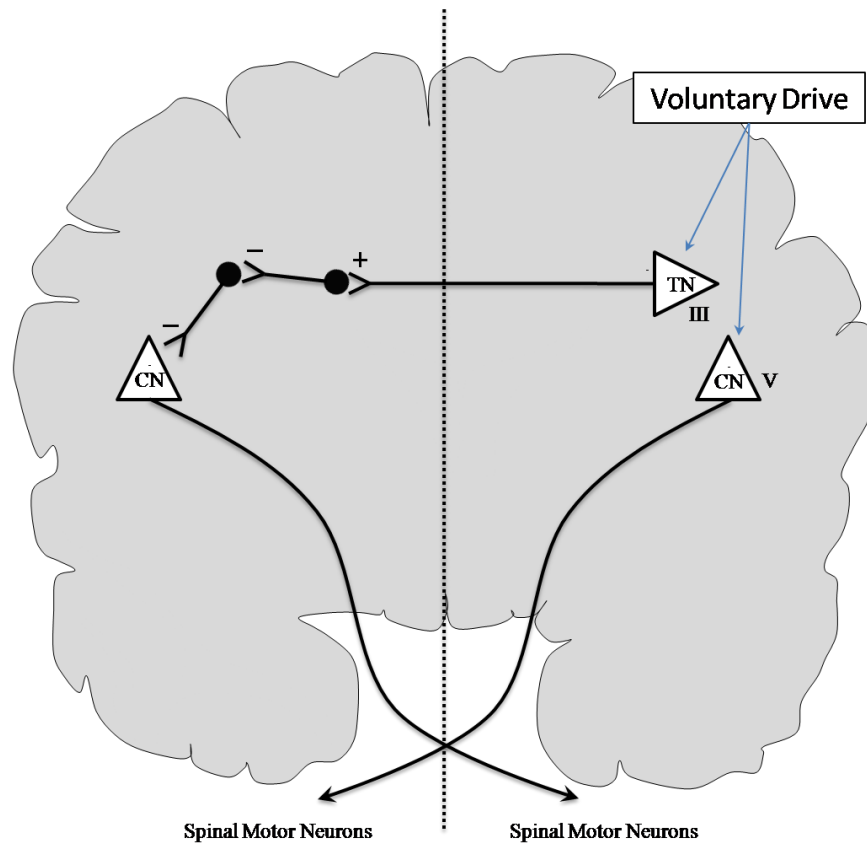
Figure 3.8 Schematic diagram of a possible mechanism for SICI disinhibition
Voluntary drive initiates action of both corticospinal output and transcallosal projections.
An excitatory drive is sent via callosal pathways to the contralateral hemisphere and
projects onto GABAergic interneurons (thought to underlie the effects of IHI) that inhibit
local inhibitory populations (responsible for SICI). The result is an overall facilitation of
corticospinal neurons. +/− represents excitatory/inhibitory synapses respectively.
Adapted from Avanzino, Teo, & Rothwell, (2007)

neurophysiological purpose of this motor irradiation is unknown, it could reflect the

combined efforts of parallel pathways to focus and promote the recruitment of

symmetrical bimanual activities when the task demands it.

Our study used a very specific set of parameters and had the participants used

stronger force levels during their isometric contraction (>10% MVC) perhaps different

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effects could be observed. Indeed, differences in our experimental design from others exploring similar motor system modulations limit the direct comparison in findings that can be made. Further, careful consideration should be taken when directly comparing effects of SICI during rest and movement conditions in our study given that ipsilateral movement increased the MEPs evoked from a single test pulse. The possibility exists that the modulation of MEP amplitudes signifies that different neural pools were activated and, if so, direct comparisons cannot be made. To account for the dissimilarity, we contrasted the changes between single and paired-pulse MEP amplitudes in rest conditions to those during movement conditions.

The task-dependant effects observed in ipsilateral SICI, and the theorized control from IHI, may shed light onto the neural underpinnings of both unilateral and bilateral limb control. Further, it may provide an explanation for unwanted mirror movements observed when patients with motor disorders perform unilateral tasks. It is clear the ipsilateral facilitation that accompanies unilateral movement involves a complex network of facilitatory and inhibitory influences, and further investigation into these issues, specifically with a focus on the interaction of IHI and ICI, is warranted.

Chapter 4 – Study Two

Interactions between inhibitory intracortical pathways and the modulation of ipsilateral M1 excitability in the human motor cortex during unimanual voluntary movement

4.1 Overview

Voluntary unilateral movements of the upper limb can increase the cortical excitability of the ipsilateral homologous motor representations. However, the mechanism underlying this modulation of excitability is not clear. The purpose of this study was to investigate the role of short and long latency intracortical inhibition in the previously observed interhemispheric modulation of motor cortical excitability. We delivered focal transcranial magnetic stimulation (TMS) to healthy, right-handed subjects targeting either the extensor carpi radialis (ECR) or first dorsal interosseous (FDI) motor representations. A paired-pulse protocol was applied to the left primary motor cortex using interstimulus intervals of 3 ms (short interval intracortical inhibition, SICI) and 100 ms (long interval intracortical inhibition, LICI). For rest conditions, the conditioning stimulus was set at 80% and 120% of resting motor threshold (MT) for the SICI and LICI trials respectively. In both cases the test stimulus was suprathreshold at 120% MT. Motor-evoked potentials (MEPs), recorded using surface electrodes, were measured while the homologous muscle groups contralateral to the right test hand were both active and at rest. In the active condition, EMG activity from the initiation of a dynamic contraction (10% of the maximal voluntary contraction) of either the ECR or FDI ipsilateral to the stimulated motor cortex was used to trigger the TMS. When test stimuli were facilitated by ipsilateral movement, the stimulus intensity was reduced to match MEP amplitudes produced during rest conditions and conditioning stimuli adjusted to

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80% of the active condition motor threshold.. For SICI and LICI conditions, the peak-to-peak MEP amplitude was averaged for each condition and the resulting data was normalized as a percentage change from the average MEP for single pulse stimulation. Results showed that discrete unilateral contractions of both ECR and FDI increased the excitability of the contralateral homologous muscle representation compared to when the muscle was at rest. Further, voluntary contraction of the contralateral ECR significantly reduced SICI whereas contralateral activation of the FDI had a marginal effect on SICI in the homologous motor areas of the opposite hemisphere. Active conditions for both ECR and FDI had little effect on LICI. Our preliminary findings suggest that the interhemispheric modulation of motor cortical excitability between homologous muscle representations is primarily mediated through pathways acting on $GABA_A$ mediated inhibitory intracortical interneurons.

4.2 Introduction

Transcranial magnetic stimulation (TMS) applied over the motor cortex provides a non-invasive method to study cortical excitability changes. A single suprathreshold stimulus applied over the primary motor cortex depolarizes neurons and creates a motor-evoked potential measurable in the contralateral limb. These motor evoked potentials (MEPs) can not only be modulated by contraction of the muscle from which they are recorded, but by activation of the homologous muscle ipsilateral to TMS stimulation (Hess et al, 1986; Meyer et al., 1995; Tinazzi & Zanette, 1998; Muelbacher et al., 2000). Studies using moderate to high levels of force have consistently reported facilitation of ipsilateral corticomotor excitability, while studies using low level contractions have produced conflicting results, with unilateral movement facilitating, inhibiting or not significantly affecting MEPs simultaneously recorded in the contralateral mirror muscle (Chiappa et al., 1991; Liepart et al., 2001).

The mechanisms responsible for the task-dependent changes to ipsilateral MEPs remain unclear, with both cortical and spinal level circuits being implicated. Meyer et al. (1995) compared effects of motor responses in on hand during forceful contractions of the opposite hand in both healthy subjects and patients with abnormalities of the corpus callosum. They found similar facilitation in both populations and concluded that mechanisms for the observed facilitation are most likely at a spinal level. However, however, recent TMS research has suggested otherwise and implicate the involvement of the ipsilateral M1 in unilateral movement. In more than one study, TMS-evoked potentials were increased in the non-task hand when the coil was placed over the ipsilateral M1, while MEPs due to direct stimulation of the spinal cord were unaffected

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by homologous muscle activation (Hortobagyi et al., 2003). Uncovering the mechanisms controlling this physiological mirroring will not only help in the understanding of unimanual and bimanual movements in the healthy population, but may help us gain insight into the neural correlates behind congenital and acquired mirror movements.

The primary focus in the present study is on the changes in the primary motor cortex ipsilateral muscle activity; specifically focusing on the contribution of intracortical inhibitory systems. In addition to facilitatory influences, cortical excitability is subject to a network of inhibitory influences. Intracortical inhibition (ICI) of the motor cortex can be studied using paired-pulse stimulation that involves preceding a test stimulus by a conditioning stimulus by specified interstimulus intervals (ISI). Short-interval intracortical inhibition (SICI), suggested to be mediated via $GABA_A$ receptors, is demonstrated by a delivering a subthreshold stimulus 1-6 ms prior to the test stimulus. A second inhibitory system, long-interval intracortical inhibition (LICI), can be investigated by separating two suprathreshold stimuli by 50 – 200 ms and is attributed to slower acting $GABA_B$ receptors. It is possible that changes to SICI and LICI, separately or in conjunction, contribute to the excitability changes in the M1 ipsilateral to unilateral muscle activation.

4.3 Methods

4.3.1 Subjects

Five young (23-38 years of age, mean 28 yrs, 2 males, 3 females) healthy adult volunteers were recruited. All participants gave their informed written consent to participate in the study. The experimental procedures conform to the guidelines set forth by the Human Research Ethics Committee and the protocol was approved by the Office of Research Ethics at the University of Waterloo. Participants completed a modified

version of the Transcranial Magnetic Stimulation Adult Safety Screen Questionnaire – a 23 point screening tool used to exclude those participants who may be predisposed to adverse events during TMS (Keel et al., 2000) (*see Appendix 1*). In addition, participants were screened for right-hand dominance according to a modified Waterloo Handedness Questionnaire (WHQ) (*see Appendix* 2).

4.3.2 Experimental Approach

Setup, Electromyography, & Transcranial Magnetic Stimulation

Experimental setup, EMG and TMS procedures were identical to those used in study one. Please refer to Chapter Three for detailed protocol.

Experimental Protocol

Participants were asked to perform three separate tasks with their non-dominant left hand (hereto referred as the task hand); a resisted isometric radial abduction of the index finger (FDI activation), a resisted isometric extension of the wrist (ECR activation), and a resisted isometric flexion of the wrist (FCR activation). A single experimental session consisted of 15 different conditions (*see Table 4.1 and Figure 4.1*), each defined by the test muscle (always in the right upper limb), number of pulses being delivered, the interstimulus interval, and whether the contralateral agonist/antagonist to the right test muscle was active or at rest. Each run involved 20 trials per condition, with conditions being blocked according to distal (FDI) or proximal (ECR) effector and randomized within each block (300 trials total). TMS was delivered either as a single pulse or as a paired pulse with short and long interstimulus intervals (ISI 3 ms, ISI 100 ms). The

intertrial interval was randomized between 3 to 5 seconds. For all conditions, the test

muscle was maintained in a state of rest and visual inspection excluded MEPs

contaminated by preceding EMG activity. Prior to the onset of the TMS trials, the

maximum EMG output was recorded for the left ECR, FCR, and FDI separately while the

participant maintained a maximal voluntary contraction (MVC).

Rest Task

Rest conditions required participants to sit with both forearms at rest in a pronated

position while the TMS was delivered, triggered externally using a customized LabView

(National Instruments, eeAustin, TX) program. The conditioning stimulus intensity for

SICI was set to 80% resting motor threshold (RMT) and the subsequent test pulse

adjusted to 120% RMT for all rest conditions. LICI was evaluated by preceding the test

stimulus by 100 ms and using a suprathreshold conditioning stimulus set at 120% RMT.

RMT was defined as the minimum stimulus intensity necessary to evoke a MEP with a

peak-to-peak amplitude of 50 µV or greater in 5 out of 10 consecutive trials while the test

muscle was at inactive (Rossini & Rossi, 2007).

Movement Task

Prior to the onset of the task conditions, the active test stimulus (ATS) was

determined. ATS was defined as the adjusted stimulator output that matched the peak-to-

peak MEP amplitude produced by an unconditioned test stimulus during the movement

task (10% MVC) with the average peak-to-peak MEP amplitude produced by an

unconditioned test stimulus (120% RMT) delivered at rest. For assessment of SICI and

LICI, conditioning stimuli were then adjusted to 80% active motor threshold (AMT) (ISI 3 ms) and 120% AMT (ISI 100 ms) correspondingly. During the movement task, participants were asked to perform a voluntary isometric contraction with either the contralateral homologous or mirror antagonist to the resting test muscle. This was accomplished by resting the inactive test hand against a metal rod attached to the TMS support frame. Trials in which the FDI was the test muscle, the index finger sat flush against the metal support at the proximal interphalangeal joint. Trials where the intended movement was flexion or extension of the wrist, the hand rested against the rod at the metacarpophalangeal joint. Subjects were instructed to keep their right limb in a state of rest between trials, and upon verbal cue, initiate a resisted contraction of the test muscle. Participants were instructed to try to isolate activation to the test muscle only, and to maintain all other muscles in a relaxed state. A custom written LabView (National Instruments, Austin, TX) program triggered TMS delivery when the measured EMG level reached 10% of the activated muscle's maximal voluntary contraction.

Table 4.1 Experimental task conditions

FDI					
Condition	Pulse Type	ISI (ms)	CS (% MT)	TS (% RMT/AMT)	Task
1	SP	-	-	120% RMT	Rest
2	SP	-	-	120% AMT	Movement
3	PP	3	80%	120% RMT	Rest
4	PP	3	80%	120% AMT	Movement
5	PP	100	80%	120% RMT	Rest
6	PP	100	80%	120% AMT	Movement
ECR					
7	SP	-	-	120% RMT	Rest
8	SP	-	-	120% AMT	Agonist Movement
9	SP	-	-	120% RMT	Antagonist Movement
10	PP	3	80%	120% RMT	Rest
11	PP	3	80%	120% AMT	Agonist Movement
12	PP	3	80%	120% RMT	Antagonist Movement
13	PP	100	120%	120% RMT	Rest
14	PP	100	120%	120% RMT	Agonist Movement
15	PP	100	120%	120% AMT	Antagonist Movement

15 study conditions varying by test muscle (ECR, FDI), presence of conditioning stimulus, CS, (SP, PP), interstimulus interval, ISI, (3 ms, 100 ms), and whether the agonist (ECR/FDI) or antagonist (FCR) muscle is active or at rest. TS – test stimulus; RMT – resting motor threshold; AMT – active motor threshold; SP – single pulse, PP – paired pulse.
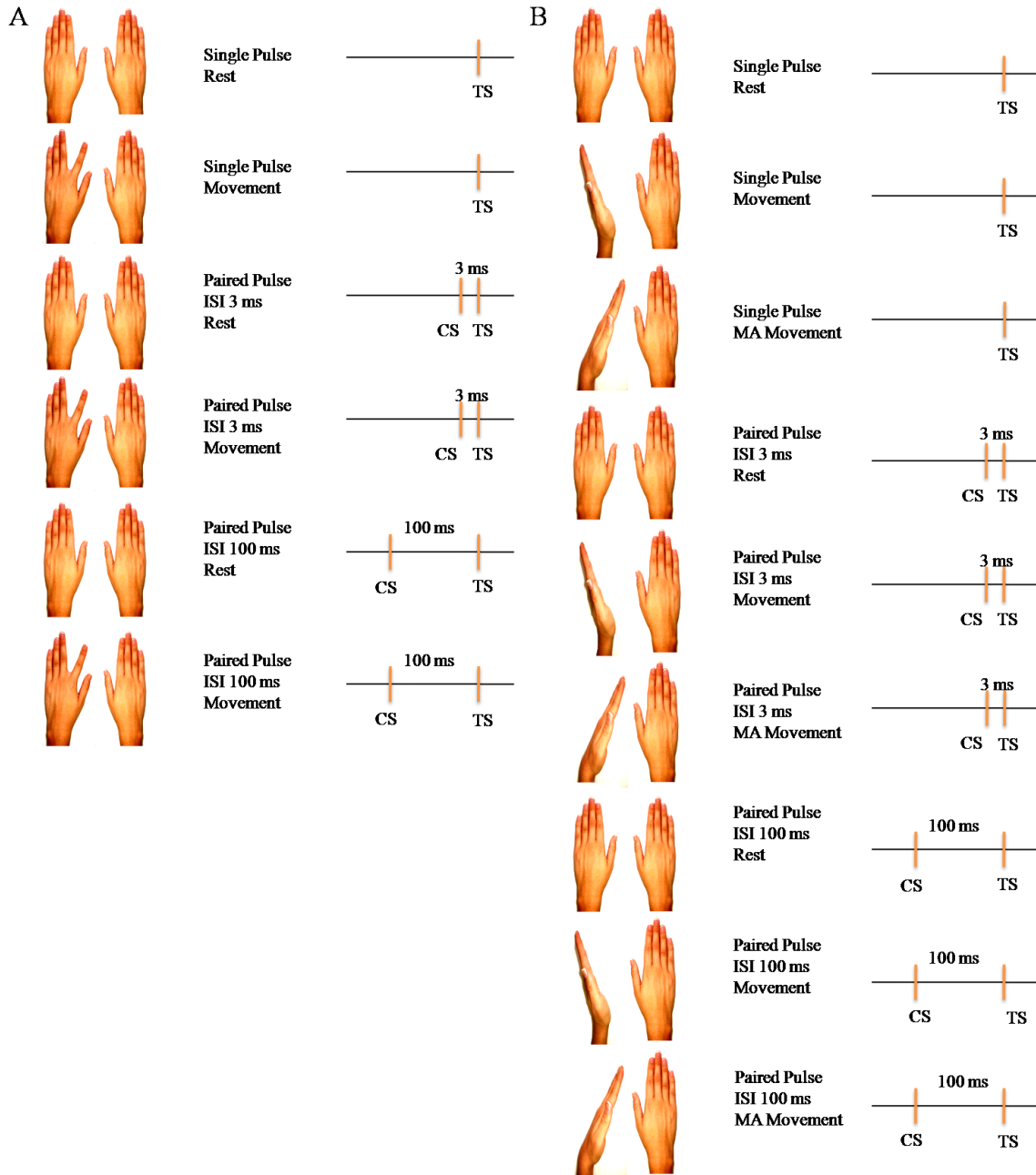
**Figure 4.1 Experimental task conditions**
Participants were asked to either maintain both arms and hands in a state of rest or to contract the left FDI (A), ECR or FCR (B). The above diagram shows overt movements however this is purely a pictorial representation of the actions requested of the participants. Isolated contraction forces were generated against a stationary bar, and were isometric as a result.

4.3.6 Data Analysis

Mean peak-to-peak MEP amplitudes were calculated for each condition off-line using a customized data acquisition program. The effect of voluntary unilateral movement on SICI, LICI, and motor output of the ipsilateral mirror motor representation was assessed using a repeated measures analysis of variance (ANOVA) to determine the effect of MOVEMENT (rest or 10% isometric contraction) and EFFECTOR (FDI or ECR) on right target muscle MEP amplitudes. Paired *t* tests were used to contrast the change in EMG activity between 'single pulse at rest' and 'paired pulse at rest' conditions to the difference between 'single pulse during movement' and 'paired pulse during movement' conditions. The size of the average MEP output for each condition was normalized as a percentage of the unconditioned MEP at rest to evaluate the effect of voluntary contralateral hand performance on SICI, LICI and corticospinal excitability. Differences were considered significant at p<0.05 for all parameters. Results are reported as mean ± standard error (SE) unless otherwise stated. All statistical analysis was completed using Statistical Analysis Software (SAS Institute, Cary NC, USA).

4.4 Results

4.4.1 Unconditioned Test Pulse

Figure 4.2 illustrates the effects of single and paired-pulse TMS on the MEPs from both test muscles in a representative subject while the contralateral mirror muscle was at rest and during 10% MVC isometric contraction. For both the FDI and ECR, voluntary isometric contractions led to facilitation in the ipsilateral homologous M1 representation. TMS output was reduced for all participants to match the unconditioned test pulse during homologous muscle activation to the average EMG output recorded for

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the test stimuli at rest. RMTs were lower in FDI ($46 \pm 2\%$ of maximum stimulator output) than in the ECR ($51 \pm 3\%$).
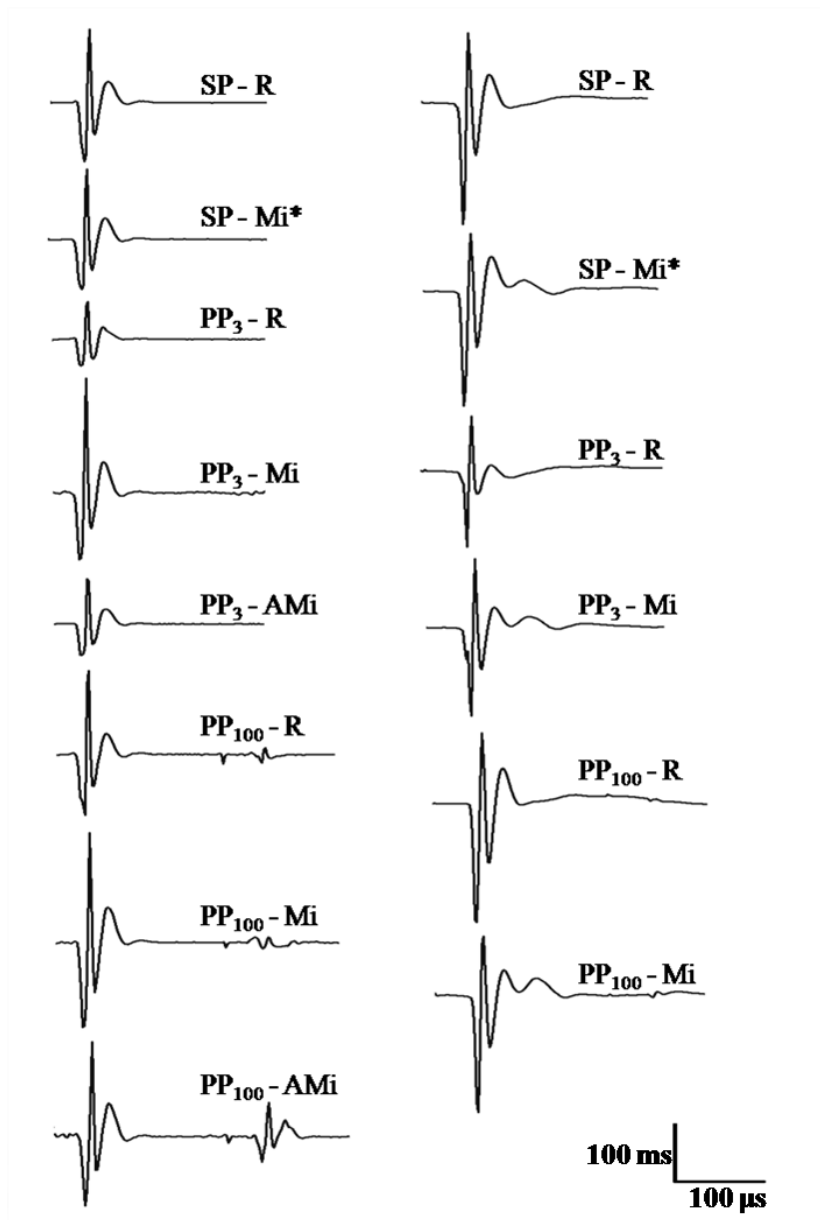
Figure 4.2 EMG recordings from the right arm of a representative subject during performance of each task condition
. *TMS output adjusted in single pulse movement condition to match MEP amplitude to that produced during single pulse at rest. SP – Single pulse; PP_3 – Paired pulse with isi of 3 ms; PP_{100} – Paired pulse with isi of 100 ms;,R – Rest, M_i – Movement of ipsilateral mirror muscle; AM – Movement of ipsilateral antagonist to mirror muscle

4.4.1 SICI During Homologous Muscle Performance

During rest conditions, preceding the test stimulus by a subthreshold CS (80% RMT; ISI 3ms) reduced the average MEP amplitude (expressed as a percentage of unconditioned test stimulus) in both the ECR and FDI. Refer to Figure 4.4 for results.

Table 4.2 Normalized peak-to-peak MEP amplitudes from right FDI / ECR during task conditions

EMG %	Left FDI Rest	Left FDI Movement	Left ECR Rest	Left ECR Movement	Left FCR Movement
SICI	43 ± 10	70 ± 15	63 ± 7	100 ± 18	51 ± 6
LICI	10 ± 3	17 ± 4	25 ± 3	37 ± 8	77 ± 16

*EMG levels expressed as % ratio of the average MEP measured during the single pulse at rest and movement task conditions

A repeated measures ANOVA of observed EMG activity during short interval paired pulse conditions revealed a strong trend towards an effect of MOVEMENT ($F(1,4) = 5.45$, $p = 0.08$), with no significant effect of EFFECTOR ($F(1,4) = 1.06$, $p = 0.4$) or their interaction MOVEMENT X EFFECTOR ($F(1,4) = 0.09$, $p = 0.8$). Due to inter-subject variability and a small sample population (n=5), post-hoc tests did not reveal significant differences between the mean MEP amplitudes produced during paired-pulse rest and homologous muscle activation for either the ECR or FDI ($p>0.05$). Although the difference did not reach statistical significance, the effectiveness of the conditioning stimuli was reduced to a greater extent in the ECR (100 ± 18 % of the average MEP measured during a single pulse during the rest condition) than in the FDI ($70 \pm 15\%$) (Figure 4.4).

In summary, concurrent isometric contraction of homologous muscles effectively disinhibited SICI regardless of effector; yet the movement-dependant facilitating effect may be greater for the more proximal muscle, the ECR, than the distal FDI.

4.4.2 LICI During Homologous Muscle Performance

Figure 4.4 illustrates that during resting conditions, preceding the test stimulus by a subthreshold CS (100% RMT; ISI 100ms) reduced the average MEP recorded for both the FDI and ECR test muscles.

A repeated measures ANOVA of observed EMG activity during long interval paired pulse conditions revealed a significant effect of MOVEMENT ($F(1,4) = 8.87$, $p \leq 0.05$), with no significant effect of EFFECTOR ($F(1,4) = 3.55$, $p = 0.3$) or their interaction MOVEMENT X EFFECTOR ($F(1,4) = 0.62$, $p = 0.5$). Post-hoc comparisons revealed no significant differences between mean MEPs during paired-pulse 100 ISI rest and movement in either the ECR or FDI (Paired t-test; *p=0.4, p=0.1* respectively).

4.4.2 SICI and LICI During Non-homologous Muscle Performance

Our results show that simultaneous performance of an ipsilateral non-homologous muscle, the FCR, does not alter the effectiveness of the conditioning stimuli at a short interstimulus interval (3ms) when delivered over an ECR motor representation ($p=0.3$). Conversely, as Figure 4.3 demonstrates LICI was decreased to a larger extent during FCR contraction (mean $77 \pm 16\%$) compared to ECR contraction and rest ($37 \pm 8\%$ and 25 ± 3), although not significantly ($p=0.1$). In summary, contraction of an antagonist to the

homologous to the test muscle results in an attenuation of LICI and does not appear to

affect SICI.
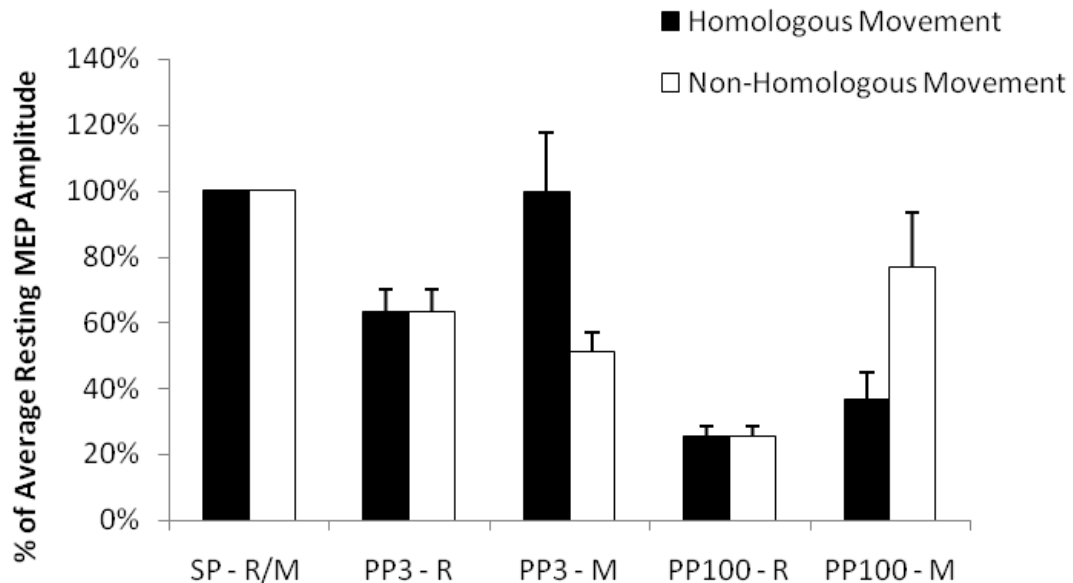
Figure 4.3 Group results illustrating changes in the relative amplitudes of MEPs produced by TMS during ipsilateral homologous or antagonist mirror muscle activation.
Data from 5 subjects, grouped by TMS condition. PP_3 – paired pulse with 3 ms interstimulus interval; PP_{100} – paired pulse with 100 ms interstimulus interval; R – rest; M – homologous movement; A – mirror antagonist movement. Voluntary isometric muscle contraction of the left ECR resulted in a disinhibition of SICI and LICI in the mirror ipsilateral cortical representation. Conversely, contraction of the antagonist (FCR) had no significant effect on SICI in the ipsilateral ECR motor representation, however, did attenuate LICI in the same representation.
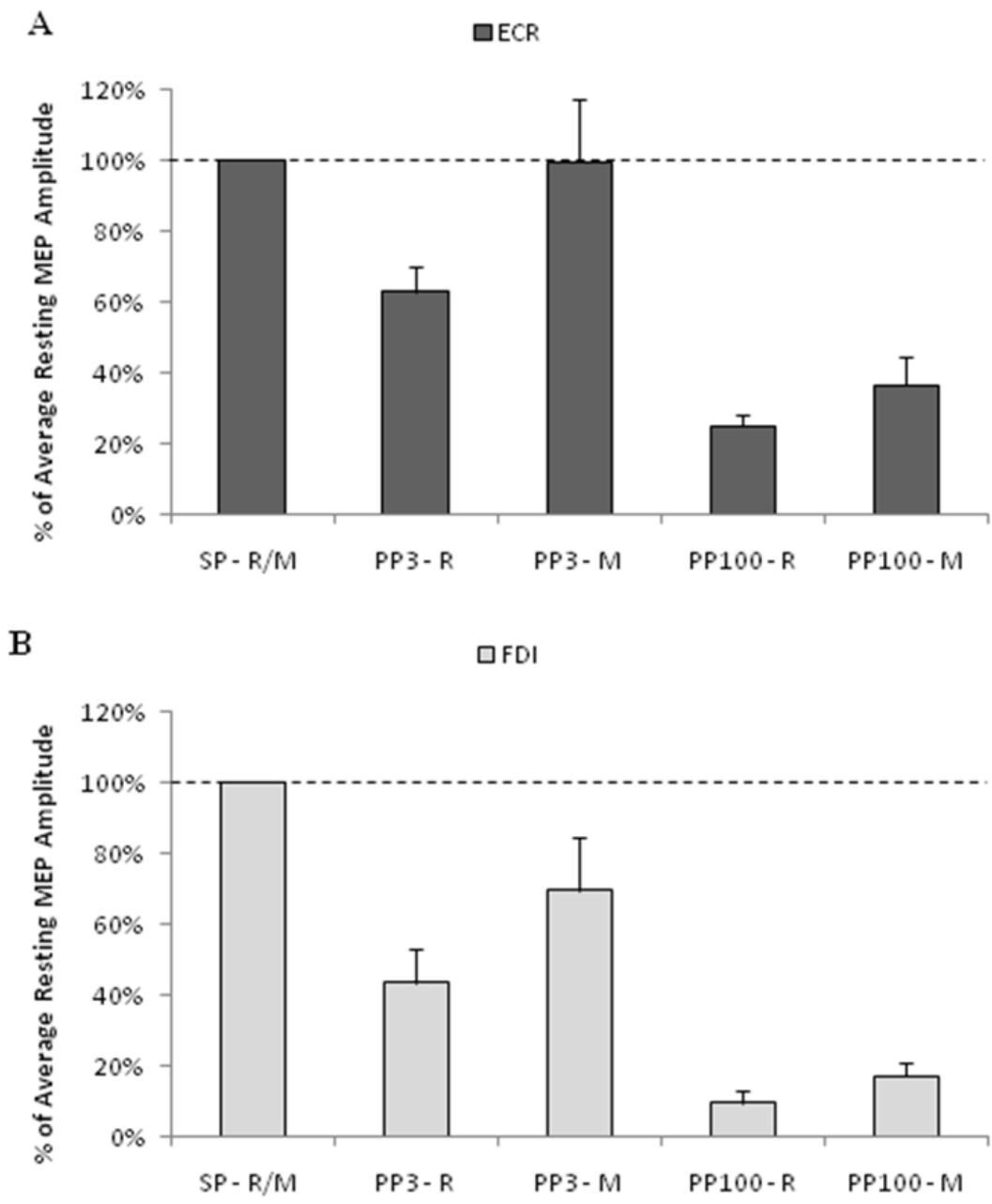
Figure 4.4 Group results illustrating changes in the relative amplitudes of MEPs produced by TMS for ECR and FDI effector groups.
Data from 5 subjects, grouped by TMS condition. PP_3 – paired pulse with 3 ms interstimulus interval; R – rest; M – movement. The main effect of movement was found for both effectors, FDI and ECR ($p<0.05$). Paired pulse TMS produced significant MEP inhibition at rest for both FDI and ECR ($p<0.05$).

4.5 Discussion

After controlling for the stimulus intensity and reducing the test stimuli intensity to match the size of the MEP in active conditions to those measured at rest, the results from the previous study stand. Again, our data demonstrate that voluntary unilateral contraction of the non-dominant hand and arm muscles results in facilitation of the ipsilateral motor cortex. Accompanying the excitatory effect is a decrease in SICI, which was found to be more profound in the relatively more proximal musculature. This disinhibition appears to be focused on homologous motor representations as SICI went unchanged during activation of the mirror antagonist. Further, a small modulation in LICI accompanied homologous muscle activation while contraction of the antagonist to the mirror muscle resulted in a larger release of LICI.

4.5.1 Excitability Changes in Ipsilateral M1

In the current study it was observed that TMS-evoked potentials measured from non-active FDI and ECR in the dominant arm were enhanced by concurrent isometric contraction of their homologues. These results support previous research, that likewise has reported facilitation in corticospinal excitability at higher force levels (Hess et al., 1986; Meyer et al., 1995; Tinazzi & Zanette, 1998; Muellbacher et al., 2000; Woldag et al., 2004). In contrast, one study reported inhibition in the non-task hand during unilateral performance at lower forces levels, 1-2% MVC, although their data also showed MVC facilitated MEPs at 20% and 40% MVC (Liepert et al., 2001). In our study we purposely chose 10% MVC since lower levels of force are hard to maintain and, in the context of real world application, would not be relevant for rehabilitative purposes. Further,

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resulting EMG activity from 10% MVC, used to trigger TMS stimulation, is easily distinguishable from background noise in EMG recordings. Although a single force level was used in our task protocol, emerging evidence suggests that the degree of activity-dependant changes we observed functions on a force level gradient, with higher levels of strength producing greater facilitation (Woldag, et al., 2004; Renner et al., 2005). The amount of facilitation also appears to be task relevant; one report citing differences between pinch grip and a power grip, with power grip resulting in a less MEP enhancement (Woldag et al., 2004).

There is still debate over where along the neuraxis that this ipsilateral M1 facilitation originates and both spinal and cortical level mechanisms have been implicated. Meyer and colleagues (1995) found no significant difference in ipsilateral facilitation during unilateral muscle activation when comparing healthy subjects and patients with anterior agenesis of the corpus callosum; the anterior portion of the corpus callosum having been previously shown to be integral for the interhemispheric integration during bimanual activities (Jeeves et al., 1988). However, Tanazzi and Zanetti (1998) probed the spinal excitability direct median nerve stimulation found no changes in the H reflex on the resting side during unilateral APB muscle activation. Further, the facilitation observed using TMS was absent when anodal TES was employed. While TMS mainly activates pyramidal cells indirectly via interneurons, TES likely activates corticospinal neurons directly implying that not only are the changes occurring at the level of the motor cortex, but require the modulation of facilitatory and/or inhibitory interneurons (Brocke et al., 2005). Stimulation directly at the level of cervicomedullary

junction is also unaffected by contralateral contraction, again implicating changes at the cortical level (Hortobagyi et al., 2003).

4.5. 2 Modulation of SICI

Our research investigated potential cortical level mechanisms that may contribute to these observed activity-dependent modulations. Contraction of both task muscles resulted in reduced SICI in their ipsilateral motor representation, while LICI showed a very slight decrease. Even when adjusted TMS output to obtain comparable test pulse MEP amplitudes (accounting for the facilitation observed during active conditions therefore reducing the possibility that different motoneuron pools are active) disinhibtion of SIC was still apparent. SICI and LICI, measured using paired-pulse TMS, presumably reflect two separate inhibitory circuits in the M1. While SICI appears to be $GABA_A$ receptor mediated, LICI appears to be controlled by $GABA_B$ (Kujirai et al., 1993). Our results further substantiate claims of a cortical component to movement induced ipsilateral facilitation and suggest that the enhancement of excitability may occur through a decrease in local inhibitory influences.

It has been suggested that motor commands are by default bilateral and that the ipsilateral motor cortex receives a copy of the motor command even though a unilateral movement is intended. Therefore, the successful performance of unilateral movements requires inhibition of the non-test muscle. While our observed facilitation of both homologous motor areas is explained under this hypothesis, our results are not consistent as we showed a significant *disinhibition* during unilateral contractions. Interhemispheric inhibition targeting the ipsilateral M1 is known to increase during unilateral contractions

(Ferbert et al., 1992; Duque et al., 2007; Lewis & Perreault, 2007). It is possible that the reductions of SICI could be a consequence of presynaptic inhibition by IHI. Interhemispheric inhibition (IHI) is another inhibitory process thought to contribute to the control of motor functions and is most likely mediated by excitatory fibers crossing the corpus callosum and acting on local inhibitory interneurons (Daskalakis et al., 2002). Daskalakis and colleagues (2002) studied the relationship between intracortical inhibition and ipsilateral cortico-cortical inhibition using TMS. They found that SICI was reduced in the presence of interhemispheric inhibition (IHI) and suggested that IHI was inhibiting SICI and not the other way around. It is possible that within the context of the present study, that reduction in SICI by IHI contributed to an overall facilitation of the ipsilateral corticospinal neurons.

Our results are in line with this viewpoint as we found differing levels of inhibition between the distal and more proximal test muscle. Evidence exists for differences in IHI between varying upper limb muscle representations. Some have suggested that the degree of IHI follows a proximal-distal gradient (Ferber et al., 1992; Sohn et al., 2003) while others propose that the degree of IHI is dependent on the muscle's functional role in everyday behaviour (Harris-Love et al., 2007). In general, direct callosal connectively between M1 representations of distal arm musculature is sparse, however certain regions have shown to have more dense connections than what was previously thought (Gould et al., 1986). If IHI differs on a proximal-distal gradient, since IHI is mediated through transcallosal connections between M1s, one would expect less interhemispheric inhibitory influence between FDI representations than the more proximal ECR motor areas. Likewise, if IHI is guided more by the behavioral context of

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the muscle, it would be expected that IHI would be less between FDI representation since the muscle contributes to more low force and precise unilateral tasks than the ECR which are typically used in more forceful, bilateral movements which would favour mirror activation; assuming that increases in IHI lead to a decrease in local inhibitory influence on corticospinal neurons. Further investigation of IHI and SICI-IHI interaction during ipsilateral unimanual movement is needed to substantiate these speculations.

4.5.3 Modulation of LICI

LICI, an intracortical inhibitory pathway mediated by $GABA_B$ receptors, were influenced by voluntary activation of homologous and non-homologous muscles ipsilateral to M1 stimulation. Due to inter-subject variability and the small sample population of the study, significance was not reached in many comparisons, however trends were revealed. Our data suggests that unilateral hand and wrist activation resulted in a small attenuation of LICI in the homotopic hand muscle representation and a much larger release of LICI when a non-homologous muscle was activated.

Both SICI and LICI are known to be involved in the modulation of motor output and are speculated to play an important role in the execution of voluntary movements (Hammond & Vallence, 2007). Further, LICI and SICI involve different neurons as demonstrated by the fact that increasing the intensity of the test pulse results in increases in SICI and decreases in LICI, with no correlation between the two (Sanger, Garg & Chen, 2001). Conflicting evidence exists, however, whether voluntary muscle activation significantly affects LICI and to what degree. A study by Wassermann and colleagues (1996) showed no effect of small amounts of voluntary contraction (increase from rest to

10% MVC) on LICI measured in the contralateral M1. In opposition Hammond and Vallence found that LICI decreases systematically with increasing levels of tonic voluntary contraction. Further, they observed an analogous release of SICI during task performance. These findings suggest that the two inhibitory processes work in parallel to control voluntary movement. Unlike the present study, Hammond and Vallence (2007) investigated changes in intracortical inhibition in the M1 contralateral to voluntary movement, however their results are similar to our study which investigated associated changes in the ipsilateral M1. It could be possible that the similar decreases in LICI subserve the same purpose, which is to promote and control voluntary movement.

It has been argued that LICI and IHI mediated through similar inhibitory neurons. Daskalakis et al. (2002) investigated the mechanisms of inhibition in the human motor cortex and their interactions. They found an interaction between LICI and IHI and argued that the reduction of one in the presence of the other may be explained by a resulting saturation effect of the overlapping inhibitory neurons. It has been previously demonstrated that unilateral activation results an increased IHI from the MI contralateral to the movement to the ipsilateral M1 (Ferbert et al., 1992). If SICI was reduced by an increase in IHI, as was argued earlier, it is conceivable that LICI would be concurrently reduced by a theorized saturation effect. Since LICI is reported to play a role in maintaining the resting state of the motor system, attenuating LICI in the homologous M1 representation ipsilateral to the unilateral contraction, would aid in the promotion of bimanual activation.

Interestingly, isometric contraction of the FCR resulted in a large attenuation in the ECR M1 representation in the ipsilateral hemisphere. The functional significance of

disinhibiting the antagonist to the mirror motor representation is unclear. It is possible that unlike the focal attenuation of SICI, release of LICI is more widespread. Further, changes recorded in the ECR during homologous muscle activation presumably occur in the FCR motor representation during activation of the ipsilateral FCR muscle. Modulation of intracortical inhibition induced by FCR activation may have induced changes to inhibitory circuits in the spatially close ECR muscle representation. Further investigation is required to confirm these suppositions and to evaluate the extent to which unilateral activity influences LICI in non-homologous muscle representations.

4.5.4 Homologous vs. Non-Homologous Task Conditions

It is known that voluntary movements of the upper limbs are drawn towards one another and that when performed in symmetry they are more stable and accurate than asymmetrical movements (Cohen, 1971; Riek et al., 1992; Carson, 1995; Carson et al., 2007). Neural pathways linking homologous regions are thought to play an integral role in this bimanual coordination and just as equally could contribute to bilateral changes observed during intended unilateral movements (Carson, 2005). In the present study, during unilateral isometric contraction of both the FDI and ECR, corticospinal excitability was facilitated and SICI decreased in their homologous motor regions. Conversely, contraction of an ipsilateral non-homologue and mirror antagonist, the FCR, had no effect on SICI. Evidence from the current study is insufficient to determine if the bilateral effects of unimanual movements extend exclusively to mirror musculature or if other motor representations can be influenced. We noticed no discernable differences in motor output and SICI associated with non-homologous movement relative to conditions

where both upper limbs are completely at rest. That being said, the representative muscle selected, the FCR, while a spatial close M1 representation is to the ECR, it is the test muscles mirror antagonist. If the purpose of the observed ipsilateral facilitations were to promote bimanual synchrony, then increasing the likelihood of FCR activation would be counterproductive to its goal and therefore it would be more efficient if inhibitory influences were predominant in the FCR, as reflected in our results (i.e. maintenance of resting state levels of SICI and LICI during ipsilateral contraction of the FCR).

Interestingly, the regions activated in the ipsilateral M1 are spatially distinct from those associated with contralateral movements (Cramer et al., 1999). Strick & Preston (1982) confirmed that primates have more than one spatially distinct M1 representation to produce the same movements. Further, Carson et al. (2007) demonstrated a postural context to movement-dependant ipsilateral facilitation. They found that when the postural context of the left and right forearm were symmetrical (pronated together or supinated together) the movements were more stable than when they were performed in a alternating fashion (one hand pronated while the other supinated). It could then be argued that the bilateral activations observed during unimanual actions are not linking muscles per se, but instead movements. We propose further investigation into the influence of multiple muscles in a synergy on the ipsilateral M1 representation of a muscle involved in the same pattern of movement.

Although the functional role of activity-facilitation in the ipsilateral M1 is unclear, focusing excitatory drive to the homologous motor representation could function to promote the simultaneous activation of bimanually coordinated movements. In summary, our data support the observation that voluntary unilateral contractions of upper

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limb musculature facilitates ipsilateral homologous motor representations and   that

decreases in intracortical inhibition are at least partially responsible. Further, modulation

of ipsilateral intracortical inhibition appears to involve transcallosal pathways.

Chapter 5 - Discussion

5.1 Summary of Results

The general purpose of this thesis was to investigate the impact of unilateral movement on the homologous motor representation in the ipsilateral M1 and the potential mechanisms that mediate the observed movement-induced excitability changes.

In the first of two studies, participants were asked to perform an isometric contraction of the ECR and FDI in the left arm while simultaneous single and paired-pulse TMS was delivered to a region in ipsilateral M1 that corresponded to the mirror muscle in the right hand. We demonstrated that lower level unilateral voluntary movements have facilitatory influences on the corticospinal neurons of the homologous motor representation. Further, these changes are observed in both distal and relatively more proximal musculature of the upper limb. When SICI was evaluated, again we observed movement-induced changes in both the distal and proximal effectors. Ipsilateral homologous movements of the ECR almost completely disinhibited SICI while the same concurrent contraction of the FDI produced a marginal yet significant reduction in SICI in the homologous motor region. In contrast, synchronized isometric contraction of a non-homologue, the FCR, had no significant effect on the MEPs recorded in the contralateral ECR.

In the second study, participants were re-tested for the effects of voluntary contractions of muscles ipsilateral to TMS stimulation on MEP amplitude and SICI. The previous study showed task-dependent facilitation in the homologous M1 representation. The larger MEP observed in active trials could be indicative that a different population of active motoneurons contributed to the MEP measured than what was recorded during rest

trials. If true, then a direct comparison could not be made between movement and rest

conditions since the effect by ICI would not be analogous. To ensure that this was not

confound, the test stimulus intensity was adjusted to match the average MEP amplitude

produced by a single 120% RMT pulse during rest, and the supposition made that the

matched MEP indicates the same population of motoneurons were active in both

conditions. The results supported the findings of the previous study, with significant task-

dependent disinhibition of SICI for both proximal and distal effectors. Again, unilateral

movement of the relatively more proximal ECR demonstrated greater effect on SICI,

though the second study's results demonstrated facilitation above the MEP produced by

an unconditioned test pulse. Similar to the preceding study, movement of the non-

homologue (FCR) had no influence on SICI. Study two also evaluated the effects of

ipsilateral mirror muscle movement on LICI and found no significant changes in either

the FDI or ECR motor representations.

There are two novel findings from the present research; voluntary low-level

contraction of upper limb musculature facilitates homotopic muscle representations in the

ipsilateral M1 and reduces the influence of surrounding GABAergic intracortical circuits

mediating SICI. It has been suggested that interhemispheric connections between

primary motor cortices is responsible for the modulation of the ipsilateral M1 during

voluntary unilateral movement. As previously highlighted, past studies have shown that

transcallosal connections between cortical representations of the intrinsic muscles of the

hands are relatively sparser than the more proximal muscles of the upper limbs. Based

upon the observations from studies one and two, it is hypothesized direct transcallosal

connectivity between homologous M1 motor representation is not the exclusive pathway

for the observed task-dependant facilitation, seeing as significant and comparable facilitation was recorded in both the intrinsic muscle of the hand and relatively more proximal effector. In contrast, movement of the FDI had appreciably less of an effect on SICI than did the ECR on its mirror motor representation. These notable differences could explained by the differences in behavioural context between a radial abduction of the index finger and the extension of the wrist, however it could also indicate that the observed modulation of SICI occurred via transcallosal pathways; though this interpretation is extremely limited as the present thesis did not directly investigate interhemispheric interactions. It is possible that voluntary unilateral movement of the upper limb activates two separate processes, both excitatory and inhibitory, that interact to determine the excitability of the ipsilateral corticospinal pathway.

5.2 Limitations

There are limitations in both the experimental manipulations used in the present thesis as well in the interpretation of the subsequent data produced.

TMS has been used for over 20 years in neurophysiology research to noninvasively investigate cortical excitability. Though TMS continuously proves to be an invaluable resource in drawing causal inferences of brain-function relationships, there is still an inherent variability in its measures within groups and within individual participants. There is an oft-reported interindividual variability in the MEP response to TMS stimulation, and for that reason we normalized each participant's data as a percentage of the average MEP produced by an unconditioned test pulse at rest to allow for comparison between subjects. In addition, variability can be introduced between

subject trials by a variety of changes in research conditions; however we employed a variety of measures to reduce the chances of such occurrences. Even small changes in coil placement and orientation can create significant changes in measured EMG activity. We tried to minimize such occurrences by placing the TMS coil in a stabilized mechanical arm and by using the neuronavigation system BrainSight to eliminate trials where the coil was displaced from its original position. Intra-subject variability can also be introduced through changes in subject fatigue, attention, and adherence to task instructions. We tried to minimize the influence of such occurrences by limiting the length of a single testing session, averaging across 20 trials for each condition, and randomizing the order of condition presentation. Post-study analysis of subject compliance was also done to eliminate trials where unwanted muscle activity was observed in either the test muscle or in the task muscle when resting conditions were tested. In addition, although instructions were given to the participant to perform an isometric contraction in a static position, the TMS was programmed to trigger when EMG activity registered 10% MVC which may have occurred during an early dynamic phase of the task performance. It is possible that the physiological responses measured in our study could respond differently to tonic contraction versus dynamic motion, however these cannot be distinguished by the present thesis.

There are also limitations to the interpretation of the data in the current thesis. The proposition made that changes in SICI were mediated through transcallosal connectivity is based on previous studies in healthy populations and persons with agenesis of the corpus callosum, and cannot be directly inferred by the present study. At no point were interhemispheric interactions directly studied and though the differences observed

between intrinsic hand muscles and relatively more proximal muscles of the upper limbs suggest the possible involvement of transcallosal pathways, this is but one interpretation; further studies investigating glutamatergic transcallosal connections as well as interhemispheric inhibitory influences are warranted. What's more, TMS stimulation involves the simultaneous activation of both inhibitory and excitatory neurons and one must be careful in the interpretation of disinhibtion which could result from a decrease in inhibitory influences but equally result from increases in excitatory circuits. Lastly, while reduced SICI may be found to be a main contributor to the facilitation observed in the ipsilateral M1, it is unknown whether concurrent changes occurred at the level of the spinal cord and further investigation is required to rule out sub-cortical involvement.

5.3 Future Directions

The goal of this thesis was to identify task-dependent changes occurring in the ipsilateral motor cortex during unilateral changes and hopefully provide direction for the focus of future research. Our findings suggest the involvement of the ipsilateral primary motor cortex in the generation of unilateral movement and, further, the involvement of ipsilateral local inhibitory neurons. It was previously suggested that the production of unilateral movement resulted in an increased inhibition in the mirror motor representation to prevent the occurrence of unwanted mirror activity. Conversely, our data not only shows an increased excitability in the ipsilateral M1 but a *release* of inhibition that may be a mechanism for the facilitation. However, in our experiments, we investigated but a subset of the potential mechanisms that can contribute to the control of ipsilateral motor output and we did so under a very specific set of conditions. Though we showed the involvement of GABAergic interneurons, we can only infer the methods by which the

disinhibtion occurred. Further research is needed to clarify the role of SICI in the motor output of the non-task hand.

To reiterate, it is important to keep in mind that our research was conducted under a very limited set of task conditions. We used a relatively low level of isometric contraction as our unimanual task. It would be advantageous to repeat our research under a multitude of force levels as well during isotonic contraction, passive stretch, and during the performance of real-world tasks such as object manipulation. The variety in research parameters could give further insight into the mechanisms of action and allow for a better understanding of how our observations may translate into the real world.

The task-dependent changes revealed during the two studies could not only contribute to the understanding of motor irradiation in the healthy population, but provide insight into movement deficits attributed to neurological damage and motor disorders; such as unwanted mirror movements. Further TMS studies investigating movement-induced changes to the ipsilateral M1 using a stroke population may help shed light into motor deficits that have reported in the intact hemisphere. The results could not only function to better our understanding of motor control in the healthy population, but could serve as a guide for the development and prescription of rehabilitative techniques.

Based on the results from our studies, in addition to the knowledge gained from previous research, the creation of a functional model for the bilateral effects of unilateral movement is needed to compartmentalize previous results, create hypotheses, and to provide guidance for future research questions. This model could begin by exploring mechanisms within and between primary motor cortices; including pyramidal neurons, inhibitory and excitatory intracortical interneurons in both the contralateral and ipsilateral

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M1s and interhemispheric interactions connecting both hemispheres. The working

strategy could then be modified through subsequent testing of each connection

independently and collectively under a variety of task conditions. Understanding of the

changes that occur within the primary motor cortices can allow the framework to evolve

to include other cortical areas and involvement of spinal level mechanisms.

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Appendix 1 - TMS Screening Form

University of
Waterloo

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TRANSCRANIAL MAGNETIC STIMULATION (TMS) SCREENING FORM

Below is a questionnaire used to exclude participants considered not suitable for transcranial magnetic stimulation (TMS). This information, as well as your identity, will be kept confidential in all future publications.

PLEASE COMPLETE FORM BELOW:

Participant Code: _____   **Age:** _____

Please **CIRCLE ONE**:

Neurological or Psychiatric Disorder	YES	NO	Multiple Sclerosis	YES	NO
Head Trauma	YES	NO	Depression	YES	NO
Stroke	YES	NO	treatment with amitryptiline and haloperidol	YES	NO
Brain surgery	YES	NO	Implanted medication pump	YES	NO
Metal in cranium	YES	NO	Intracranial Pathology	YES	NO
Brain Lesion	YES	NO	Albinism	YES	NO
Pacemaker	YES	NO	Intractable anxiety	YES	NO
History of seizure	YES	NO	Pregnant	YES	NO
Family history of epilepsy	YES	NO	Headaches or Hearing problems	YES	NO
History of epilepsy	YES	NO	Family History of Hearing Loss	YES	NO
Intracorporal electronic devices	YES	NO	Other medical conditions (please specify below)	YES	NO
Intracardic lines	YES	NO			

If you answered "yes" to any of the above questions, please provide details below.

_____

I hereby declare that all information given on this TMS screening form is true and complete in every respect.

_____   _____
Signature of Participant   Date

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Appendix 2 - Modified Waterloo Handedness Inventory

Name: _____   **Age**: _____   **Sex**: M / F

Instructions: Please indicate your hand preference for the following activities by circling the appropriate response. If you always (i.e. 95% a more of the time) use one hand to perform the described activity, circle **Ra** or **La** (for right always *or* left always). If you usually (i.e. about 75% of the time) use one hand circle **Ru** or **Lu** as appropriate. If you use both hands equally as often (i.e. you use *each* hand about 50% of the time), circle **Eq**.

1. Which hand would you use to spin a top?   **Ra Ru Eq Lu La**

2. With which hand would you hold a paintbrush to paint a wall?   **Ra Ru Eq Lu La**

3. Which hand would you use to pick up a book?   **Ra Ru Eq Lu La**

4. With which hand would you use a spoon to eat soup?   **Ra Ru Eq Lu La**

5. Which hand would you use to flip pancakes?   **Ra Ru Eq Lu La**

6. Which hand would you use to pick up a piece of paper?   **Ra Ru Eq Lu La**

7. Which hand would you use to draw a picture?   **Ra Ru Eq Lu La**

8. Which hand would you use to insert and turn a key in a lock?   **Ra Ru Eq Lu La**

9. Which hand would you use to insert a plug into an electrical outlet? **Ra Ru Eq Lu La**

10. Which hand would you use to throw a ball?   **Ra Ru Eq Lu La**

11. In which hand would you hold a needle while sewing?   **Ra Ru Eq Lu La**

12. Which hand would you use to turn on a light switch?   **Ra Ru Eq Lu La**

13. With which hand would you use the eraser at the end of a pencil?   **Ra Ru Eq Lu La**

14. Which hand would you use to saw a piece of wood with a hand saw? **Ra Ru Eq Lu La**

15. Which hand would you use to open a drawer?   **Ra Ru Eq Lu La**

16. Which hand would you turn a doorknob with?   **Ra Ru Eq Lu La**

17. Which hand would you use to hammer a nail?   **Ra Ru Eq Lu La**

18. With which hand would you use a pair of tweezers?   **Ra Ru Eq Lu La**

19. Which hand do you use for writing?   **Ra Ru Eq Lu La**

20. Which hand would you turn the dial of a combination lock with?   **Ra Ru Eq Lu La**

21. Is there any reason (e.g. injury) why you have changed your hand preference for any of the above
activities?   **YES / NO (circle one)**

Explain:

22. Have you ever been given special training or encouragement to use a particular hand for certain
activities?   **YES / NO (circle one)**

Explain: