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Interhemispheric modulation of corticomotor excitability following i-wave periodicity transcranial magnetic brain stimulation (iTMS)

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INTERHEMISPHERIC MODULATION OF CORTICOMOTOR EXCITABILITY FOLLOWING I-WAVE PERIODICITY TRANSCRANIAL MAGNETIC BRAIN STIMULATION (iTMS)

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USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

ABSTRACT

Aims

Transcranial Magnetic Stimulation (TMS) has been recently demonstrated to have potential therapeutic benefits by promoting cortical plasticity through modulation of corticospinal excitability. We have previously shown in healthy adult subjects that paired-pulse TMS (1.5ms ISI) applied over M1 at 0.2Hz for 15min (known as iTMS), can raise corticospinal excitability for a period (~10min) that outlasts the intervention. Since interhemispheric changes in corticomotor excitability are considered to have fundamental importance in the control of voluntary movement, and recovery of motor function following unilateral damage, importance is placed on understanding the mechanisms involved. The aims of the current study were therefore to investigate if the raised corticomotor excitability following iTMS intervention over M1 will be paralleled by an increase in contralateral M1, and whether this may be brought about by a reduced transcallosal inhibition.

Method

STUDY ONE: In eleven healthy adult volunteers (7 Male, 18-45yrs), the mean amplitude of the MEP was recorded (single pulse, 110% resting motor threshold, optimal site for first dorsal interosseous muscle on each hemisphere) pre and post 15 min of iTMS (left hemisphere, 100% of resting motor threshold).

STUDY TWO: In six healthy adult volunteers (2 Male, 19-36yrs), Transcallosal Inhibition was investigated pre and post 15 minute iTMS, by delivering a conditioning pulse over the comparable site on the contralateral cortex at 110% resting motor threshold (RMT) between 9-13ms ISI. The effect of the conditioning stimulus on the test stimulus MEP amplitude was compared pre and post iTMS and was expressed as an index of Interhemispheric Inhibition.

Results

iTMS applied over the left M1 (Primary motor cortex) during study one, produced a post intervention increase in MEP amplitude for RFDI in the first post collection of 227%±34% SEM ($p<0.000$) of baseline, with a corresponding increase of 123.8% ±12% ($p<0.001$) in the contralateral hemisphere. Similarly during study two, MEP amplitude for first collection following 15 minutes iTMS produced increases of

197%±26% ($p<0.001$) and 125%±14% SEM ($p<0.05$) for the intervened and non intervened hemispheres respectively. IHI following 15 minutes iTMS was significantly reduced by 20% (pre intervention: 57±12%, post intervention: 77±14%, $p<0.05$).

Conclusion

These studies have reliably produced raised corticomotor excitability following iTMS, and have demonstrated that this effect is not confined to the stimulated area. The findings support that the effects of repetitive TMS can be distributed across motor networks, with raised excitability being partially transferred to the contralateral cortex. Whilst facilitatory interhemispheric pathways may be involved in this phenomenon, it can be confirmed that the observation of bilateral increase in corticomotor excitability does involve transcallosal inhibitory pathways, and that the contralateral cortex is disinhibited. Such findings may be of importance for therapeutic TMS application where the aim is to enhance corticospinal output, and due to the nature of lesion, stimulation over the lesioned cortex is not possible or contraindicated.

DECLARATION

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ABBREVIATIONS AND DEFINITIONS

Abbreviations

AP:	anterior to posterior
CNS:	central nervous system
EMG:	electromyography
LFDI:	left first dorsal interosseous muscle
RFDI:	right first dorsal interosseous muscle
GABA:	gamma-aminobutyric acid
IHF:	interhemispheric facilitation
IHI:	interhemispheric inhibition
ISI:	inter-stimulus interval
LTP:	long-term potentiation
LTD:	long-term depression
MEP:	motor evoked potential
M1:	primary motor cortex
mV:	millivolts
rTMS:	repetitive transcranial magnetic stimulation
SEM:	standard error of the mean
TMS:	transcranial magnetic stimulation

Definitions

Baseline:	a set of motor evoked potentials recorded in the absence of the movement intervention
Contralateral:	pertaining to the other/opposite side
Corticomotor:	the pathway from the primary motor cortex to the muscle and represents the corticospinal tract and corresponding spinal motoneuron pool.
Homologous:	pertaining to the same area
Heteronymous:	pertaining to a separate structure
IHI Index:	the ratio of the conditioned to unconditioned MEP (Conditioned/unconditioned x 100)
Normalise:	a method of expressing data obtained during movement in relation to the baseline data (movement/baseline x 100)

CHAPTER 1
INTRODUCTION

There has been emerging interest in the use of transcranial magnetic stimulation (TMS) as an interventional tool used to modulate output from the primary motor cortex and thus having potential therapeutic application. Short-term local changes can be induced in motor cortex excitability, these changes are dependant on a number of factors such as stimulation site, intensity, and the interval between stimuli. Its effects can be either inhibitory (Chen et al., 1997; Huang and Rothwell., 2004; Wasserman, 1998; Maeda et al., 2000) or facilitatory (Pascual-Leone et al., 1994; Berardelli et al., 1999; Wu et al., 2000), and in some cases, these changes can affect distant cortical sites.

Through studies employing single, paired-pulse and repetitive TMS (rTMS), and additionally functional imaging, the existence of interhemispheric connections between the primary motor cortex within humans has been suggested. These connections, supported by primate studies are thought to be mediated transcallosally (Gould et al., 1986; Matsunami and Hamada, 1985), and are predominantly inhibitory in nature (Cook, 1986). Current available evidence suggests that interhemispheric inhibitory interactions are more apparent between homologous motor cortices, than facilitation (Ferbert et al., 1992). Although not exclusive, such inhibitory interactions are necessary for the control on unilateral limb movements and bilateral control (Schnitzler et al., 1996) in healthy adults. These interactions could be involved in the suppression of the homologous contralateral motor cortex during strictly unilateral movement, and non-symmetrical bimanual tasks, as well as providing focal motor cortex activation in symmetrical tasks by inhibiting extraneous movements. Its importance can be seen in the case of unilateral neurological lesion where interference to the normal interaction results in an imbalance and is associated with poor limb control (Murase et al., 2004).

At present, the majority of studies that have investigated such interactions using repetitive TMS (rTMS) have done so by applying inhibitory protocols. There is evidence that such protocols may reduce the excitability on the stimulated hemisphere by targeting intracortical interneurons, whilst raising the excitability on the contralateral hemisphere through disinhibition of transcallosal pathways (Gilio et al., 2003). There is only one study that has examined interhemispheric effects using an excitatory rTMS

protocol. This study has also documented a contralateral increase in excitability (Gorsler et al., 2003). Whilst it is unlikely that this is due to the same mechanisms acting with an inhibitory protocol, little has been proposed about the possible physiological mechanisms surrounding this increase.

It is presently unknown as to what effects iTMS, a novel technique which involves paired pulse stimuli at specific I-wave periodicity would have on other more distant sites. The rationale for iTMS was to increase cortex excitability by reinforcement of trans-synaptic facilitation (Thickbroom et al., 2006). This is contrasting to protocols previously used to explore effects that have examined the excitatory effects of higher frequency stimulation (Maeda et al., 2000; Pascual-Leone et al., 1994). iTMS protocol characteristics include a relatively low intensity and number of stimuli that has been reported to be comfortable throughout the procedure (Thickbroom et al, 2006). The magnitude of the observed increase in excitability can be compared to that seen in suprathreshold trains of high frequency rTMS. Furthermore, there is developing interest as to whether potential changes may be mediated by alterations in transcallosal inhibition.

There is a paucity of literature describing projections to the contralateral hemisphere of induced increases in corticomotor excitability, and the role of transcallosal inhibition. To date, only one study documents a direct contralateral increase in homologous primary motor cortex via excitatory rTMS (Gorsler et al., 2003), with another study reporting facilitatory effects in the primary motor cortex (M1) by applying excitatory rTMS over the premotor area (Bäumer et al., 2006). The mechanisms in both cases remain inconclusive and require further investigation.

The aims of the current study were therefore to investigate if the raised corticomotor excitability following iTMS intervention over M1 would be paralleled by an increase in contralateral M1, and whether this may be brought about by a reduced transcallosal inhibition. The first study involved applying iTMS on the left, dominant primary motor cortex, whilst observing its effects on the contralateral homologous cortex. The second study aimed to establish if iTMS, and its respective changes in excitability, involve a modulation of interhemispheric inhibition.

CHAPTER 2
LITERATURE REVIEW

The human brain provides an extensive arrangement of functions that are involved in the control of human voluntary movement. Their significance is even more prominent following neural injury. These responses do not solely occur in the brain, but in interaction with the lower level sensory and motor structures. The following review will highlight some of the key anatomical and physiological components of the motor pathways that are thought to be involved, along with their role in recovery. Another component that will be addressed is the use of magnetic brain stimulation (rTMS and iTMS), and its various application protocols as a way of exploring the physiological mechanisms and their influence on control of movement. By having an understanding of the contribution of these features in motor control, future research is able to be directed at increasing and improving functional recovery after damage.

2.1 Motor Pathways

In order to execute voluntary movement, it requires ongoing awareness of both internal and external environments. In order to activate appropriate movements, direct and indirect cortical motor pathways are facilitated (Kuypers, 1981). The indirect motor pathways generally arise in the motor cortex and terminate in subcortical targets, which then project to centres that form the origin of peripheral nerves that through an organised thalamic rely provide feedback to the motor cortex. These subcortical targets include the basal ganglia, motor thalamus, red nucleus, reticular formation and pontine gray. The tracts arising from the brainstem nuclei, the reticulospinal, rubrospinal, and to a lesser extent, the tectospinal tract, all play a role in the control of voluntary movement (Nolte, 1999).

The direct motor pathways arise from many motor cortical areas, and terminate on motor neurons whose axons leave the central nervous system (CNS) to form peripheral nerves. These pathways include the corticobulbar projections, that originate in the motor cortex and terminate directly on brainstem cranial nerves, and the corticospinal projections, whose synapses are on spinal motoneurons and interneurons (Morecroft and Van Hoesen, 1996). The corticospinal tract is considered to be the principle mediator of voluntary movement of the limbs and the trunk (Nolte, 1999).

Early beliefs suggested that corticospinal cell bodies were located in the primary motor cortex, it is now evident that possibly less that half originate from this location, with the remainder originating from the pre-motor areas (lateral pre-motor cortex, supplementary motor cortex, and cingulate motor cortex combined) (Dum and Strick, 1991; He et al., 1995) and the parietal lobe (more particularly the somatosensory cortex of the post central gyrus and the superior parietal lobule) (Kuyper, 1981). M1 has previously been viewed somatotopically, organised into separate groups of upper motor neurons, of which each controlling a pool of spinal motoneurons, therefore resulting in the movement of a particular body segment. More recent views (Schieber, 2001) suggest that motoneuron pools receive input from broad, overlapping cortical territories, with M1 neurons having projections that diverge to more than one motoneuron pool.

Through the use of electrical stimulation over the motor areas, movement can be elicited. Generation of movement in the supplementary and pre-motor areas have displayed a higher threshold that the primary motor cortex. Also noting that movements evoked through stimulation of the primary motor cortex usually isolate to small groups of muscles or a single muscle, compared to that of other motor areas where the resulting movement is of multiple muscle or the production of a more complex movement.

Most of the corticospinal axons course through the internal capsule, cross the mid-line at the level of the lower medulla and form the lateral corticospinal tract, then terminate in the gray matter of the spinal cord. The axons that do not cross the mid-line at the level of the medullar form the anterior corticospinal tract. There remain a small percentage of the anterior corticospinal tract fibres that remain ipsilateral throughout their course, whilst the larger percentage eventually cross the midline at or near the spinal segments that they innervate (Nathan et al., 1990; Ziemann et al., 1999).

Throughout their course from the cortex to the spinal cord, corticospinal fibres may give rise to collaterals that end in locations including the basal ganglia, reticular formation, and various sensory nuclei such as the posterior column nuclei. In the spinal cord, some fibres end in the posterior horn and others in the immediate gray, with some directly on alpha and gamma motor neurons. It is unlikely that the corticospinal tract has a single specified function knowing its numerous connections.

With the majority of corticospinal fibres crossing in the medullar, if there were a lesion in the primary motor cortex, generally a contralateral paresis is produced that is more evident in the distal musculature. Evidence would suggest that ipsilateral signs and symptoms may be present due to the small portion of anterior corticospinal fibres that do not cross (Jones et al., 1989)

After damage to the corticospinal tract, axons that are carried in the uncrossed ventral corticospinal tract from the opposite hemisphere may have the ability to exert compensatory control over muscles over the affected side (Fisher, 1992). These axons are more likely to exert more control over the proximal and axial musculature rather that distal segments (Kuypers, 1981). It is therefore believed that these spared ventromedial inputs to spinal motoneurons, controlling the affected side of the body may be the response for relative preservation after stroke of axial and proximal motor control (Colebatch and Gandevia, 1989).

2.2 Plasticity and motor learning

It was previously thought that the nervous system of adult mammals was not able to change. This was because it was assumed that the number of available synapses and organisation was in fact fixed. If true, this would limit the ability for the human brain to reorganise and recover function following a lesion. Advances have shed light over new mechanisms underlying the function and capabilities of both the central and peripheral central nervous systems. We now know that the CNS is able to adapt to changes in the environment (Kaas, 1991). It is this process of change that we term 'plasticity'. Modern concepts suggests that the nervous system is permanently changing, adapting to changes in the internal and external environments.

In physiological terms, synaptic plasticity refers to the ability of the synapse, between two neurons to change in strength. It is postulated that memories are stored within synapses of the brain. Therefore synaptic plasticity is the predominant neurochemical foundations of learning and memory. It was Hebb in 1949 who introduced a model for the encoding of information in the brain, which is now termed the Hebbian theory (Hebb, 1949) In this model he proposed that the repetitive activation of a presynaptic neuron together with simultaneous activation of its postsynaptic strength would lead to a change in one or both neurons, resulting in an increased synaptic strength between neurons. Two types of long lasting alterations in synaptic strength are long-term potentiation (LTP) and long-term depression (LTD). These

alterations have been demonstrated at glutamatergic synapses throughout the CNS, whilst also used as a model within biochemical and cellular process that may underlie learning and memory.

LTP refers to the sustained increase in synaptic strength elicited by brief, high frequency stimulation of excitatory afferents, however to avoid network saturation, decreases in strength must also take place. This decrease is referred to as LTD. It is these mechanisms that are thought to underlie induction of long-term plasticity (Bashir and Massey, ND).

Mechanisms underlying these plastic changes include modulation of neuronal excitability and synaptic efficacy, as well as inhibition. A generalised increase in postsynaptic excitability, not synaptic specificity of LTD or LTP, may underlie other forms of reorganisation. The generation of new dendritic connections, which result in an increase in the number of dendritic developments or increase axonal collaterals through horizontal pathways, for example sprouting, set up morphological changes that may have functional implications.

Cortical motor representations are no longer thought to be static, but fluid and regulated by use. A common method to investigate the potential for cortical plasticity has been to evaluate reorganization of motor maps following central lesions, and creating a comparison model to healthy animals. The synaptic mechanisms underlying plasticity have become an important focus within research in order to apply findings to rehabilitation of neurological conditions.

Some of the changes that have been documented include lesion-induced changes, experience-dependent changes, simple movement tasks and motor learning. Evidence provided by Nudo et al. (1996) states that motor cortical map plasticity is dependant on limb use, both after cortical ischemic damage and in the intact monkey, with representations increasing in size for specific muscle groups used in a skilled task. It should be noted that the repetitive motor movement alone, without the need for learning, does not change functional organization of cortical maps (Plautz et al., 2000). Experience has now been associated to motor cortical plasticity according to Ohlsson and Johansson (1995) who suggest that environment can alter cortical representation.

Reorganisation of cortical representations acting through the mechanisms that have been described previously may provide important directional assistance for

recovery of function after damage to the nervous system, with the possibility of changing this reorganisation to enhance functional recovery. Recent studies provide evidence that interventions can be designed to promote recovery of function through the utilisation of remaining neural circuitry (Ojakangas and Donoghue, 2005).

2.3 Transcranial Magnetic Stimulation (TMS)

TMS is a safe, non-invasive technique used extensively for examining human corticomotor properties in health and disease (Chen, 2000; Di Lazzaro et al., 2004; Maertens de Noordhout et al., 1989; Pascual-Leone et al., 1998; Rothwell, 1997;). The technique is based on electromagnetic induction, in which a rapid discharge of current through a coil held over the scalp induces an electrical field into neuronal tissue which as a result can bring neurons to firing threshold (Ruohonen and Ilmoniemi, 1999). Some factors that affect the depth of penetration include anatomical factors, coil size, coil geometry, and intensity of the applied stimulus.

In terms of activation, TMS preferentially activates, either directly or transynaptically (D- or I-waves) (Lemon, 2002), fast conducting cortico-spinal fibres of the pyramidal tract which project monosynaptically (Burke et al., 1993; Day et al., 1989; Rothwell et al., 1991), or via spinal interneurons (Burke et al., 1994; Pierrot-Deseilligny, 1996) to alpha motoneurons.

Motor Evoked Potential (MEP) is the biphasic electromyographic response to the target muscle, and is accepted as a measure of corticospinal excitability. It represents the firing of some fraction of the pool of spinal motoneurons projecting on the target muscle (Wasserman, 2002), resulting from the summation of nearly synchronous motor unit potentials (Devanne et al., 1997). The MEP amplitude reflects the balance of excitatory and inhibitory inputs to the cortico-motoneuronal pathway.

The motor threshold represents the stimulus intensity needed to activate the most excitable corticospinal elements and motoneurons (Devanne et al., 1997). MEPs are evoked only when the cortical stimulus produces a volley of impulses in the corticospinal tract that is a sufficient size to bring the spinal motoneurons to their firing threshold (Wasserman 2002). In order the produce a lower effect in the brain, the threshold required is much lower. The likelihood of evoking a response provides the most logical way in which motor threshold can be defined. With no agreed protocol for defining this probability, different laboratories often adopt many different protocols

(Reid et al., 2002). One of the most common methods is to increase the stimulus intensity in 5% increments until reaching a level that induces reliable responses in 50% of stimuli (Rossini et al., 1994). In order to detect small changes in the motor threshold that may not be otherwise detected in the above described method, threshold curves may be used (Devanne et al., 1997).

TMS as an interventional technique proved painless, non-invasive and relatively short in application time. Its use in clinical neurophysiology is continually increasing due to minimal contraindications, its positive characteristics, and possible therapeutic benefits.

2.4 Rationale for Repetitive Transcranial Magnetic Stimulation (rTMS)

Introduced in 1989, repetitive transcranial magnetic stimulation (rTMS) is a non invasive and painless technique used to stimulate the human brain in order to alter the excitability or function of the cortex or connections (Wassermann, 1998; Ziemann, 2004). The effect of rTMS is measured using the amplitude of the MEP.

During rTMS, MEP amplitude increases with TMS intensity in a curvilinear fashion. When above MEP threshold intensity, TMS elicits a complex corticospinal volley consisting of multiple waves, also referred to as I-waves (Di Lazzaro et al., 2004). These waves are likely to be mediated through cortical excitatory interneurons (Ziemann and Rothwell, 2000).

Studies involving rTMS have used variations in the train of pulses and stimulus intensities. Initial studies tested MEP amplitude using a rTMS train of 20 or less pulses, with no change in MEP amplitude being observed at a low frequency (1Hz) (Pascual-Leone et al., 1994). However, increases during higher frequency rTMS (>2Hz) were seen (Pascual-Leone et al., 1994; Berardelli et al., 1999). Advancements from this led to single short rTMS train of up to 30 pulses which were sufficient enough to change the MEP amplitude and outlast the rTMS train by up to a few seconds. Interesting to note that a short high-frequency rTMS train with a low stimulus intensity decreases MEP amplitude (Wassernman et al., 1996).

rTMS effects may not be limited to the stimulated cortex, but may have an effect on brain areas connected to the cortex. More specifically to the hand areas of the primary motor cortex, there is evidence that although sparse, the left and right hemispheres are interconnected through the corpus callosum (Rouiller et al., 1994). This

would support the variable changes in excitability of the opposite non-stimulated motor cortex seen in the majority of studies. Other reported features include a decrease in short interval intracortical inhibition (SICI), or increased intracortical facilitation (ICF) (Gilio et al., 2001; Schambra et al., 2003). These effects are explained through mutual inhibitory effects in which the motor cortices exert on one another (Netz et al.,1995). Therefore the depression of one hemisphere, should lead to disinhibition of the other as a resultant consequence (Ziemann, 2004). Although the exact mechanisms involved in the long-lasting changes in MEP amplitude with those seen in rTMS trains remain inconclusive, the authors propose that synaptic plasticity in the form of LTP and LTD are good representatives to help explain the changes in MEP amplitude.

With very few of the very large possible combinations of stimulation parameters having been tested experimentally, safety places strict boundaries on the parameters that can be used in human studies (Wasserman, 1998). This provides likely limitations of the efficacy of rTMS, directed more too increasing cortical excitability. Another limitation of such a technique may be related to the depth of penetration of the stimulating currents. There is the possibility that stimulation could be created to induce effective currents deeper into the human brain. With this comes the likelihood of an increased strength of current at the surface which could as a result be epileptogenic or harmful to tissue (Wassernmann and Lisanby, 2001).

After initial observations more than 90 years ago, rTMS experiments in humans have provided an opening into the nature of cortical plasticity and learning. It is these links between cortical plasticity and learning that improve possibilities for the development of future strategies to improve learning.

2.5 I-wave Periodicity Transcranial Magnetic Stimulation (iTMS)

A hypothesis was made by Thickbroom et al. (2006), that facilitatory I-wave interaction, set up by paired pulse TMS delivered at an I-wave periodicity (iTMS) may result in a reinforcement of transynaptic events and provide a means for directly targeting synaptic plasticity. Results supported with a steady increase in the level of corticospinal excitability (assessed by an increase in MEP amplitude) (Thickbroom et al., 2006). A five fold increase in MEP amplitude was recorded during the stimulation and by a mean of four fold increase for 10 minutes after stimulation. Findings suggest that iTMS is an effective method for manipulating synaptic plasticity in the primary motor cortex.

Thickbroom et al. (2006) have introduced a novel and effective technique that is non-invasive and results in an increased cortical excitability in M1. Its effectiveness is theorised to be through modulation of synaptic efficacy. This stimulation technique is relatively short in time application, with an intervention last no longer than 30 minutes, and having no adverse side effects during and after stimulation. Some caution may need to be implemented as although providing a low intensity stimulus, the excitability changes induced are of a larger magnitude.

It is believed that this technique may in fact have some parallels with the concept of Hebbian plasticity and learning (Hebb, 1949). Postulating that with the correlated firing of presynaptic and post synaptic neurons, modifications of synaptic efficacy could emanate. While other techniques like rTMS are thought to exert effects through LTP/LTD-like mechanisms, these methods target synaptic transmission indirectly. It is proposed that reinforcement of trans-synaptic events by iTMS, may provide a more direct way to not only investigate, but modulate synaptic plasticity (Thickbroom et al., 2005). In order to characterise the physiological effects of this intervention and allow for optimal stimulation parameters, further research was proposed. The current research will use the given parameters in order to achieve an increase in cortical excitability that outlasts the intervention.

2.6 Transcallosal pathways and interhemispheric inhibition

Using stroke as a model, it is suggested that the unaffected primary motor cortex ipsilateral to the paralytic limb may take over functions for the damaged M1 (Miller-Fisher, 1992). What remains unclear is to where along the neuraxis the descending modulatory influence of the motor cortex on muscles in the ipsilateral limb is relayed (Gerloff et al., 1998).

It has been proposed that this route is transcallosal (Ferbert et al., 1992; Borroojerdi et al., 1996) and responsible for transmission of inhibitory interactions between the bilateral M1 hand representations (Wassermann et al., 1991), however the connections between cortical hand motor representations remain sparse (Gould et al., 1986). It has not been established whether these few interhemispheric connections transmit predominantly inhibitory and facilitatory commands (Jones, 1993). Other pathways which could also mediate these ipsilateral effects due to there bilateral

organisation include the reticulospinal tract, or spinal interneuron circuits (Mazevet et al., 1996).

Through the use of TMS, three different cortico-cortical inhibitory processes have been demonstrated. These include: interhemispheric inhibition (IHI), SICI and Long Interval Intracortical Inhibition (LICI) (Daskalakis et al., 2002). IHI involves applying a Conditioning Stimulus (CS) to the motor cortex, which in turn inhibits the size of the (MEP) produced by the Test Stimulus (TS) of the opposite cortex (Ferbert et al., 1992). The Interstimulus Intervals (ISI) in which IHI can be observed is said to be between 6 and 50 ms (Ferbert et al., 1992; Gerloff et al., 1998). SICI and LICI involve pairing a subthreshold CS with a suprathreshold TS at a short ISIs (1-5ms) which inhibits the MEP produced by the given TS, and a suprathreshold CS paired with a suprathreshold TS at long ISIs (50-200 ms) which weakens the MEP, respectively (Wassermann et al., 1996).

Evidence would suggest that IHI is related to the activity of inhibitory interneurons and by transcallosal pathways, which can be supported by several findings. Ferbert et al. (1992) noted that test responses evoked by a small anodal electrical shock were not significantly inhibited by the contralateral magnetic conditioning stimuli, supporting Rothwell (1997) who stated that low intensity electrical stimuli excite descending pyramidal axons, found in the white matter, that are not sensitive to changes in cortical excitability.

Another key finding was that H-reflexes in a relaxed forearm flexor are unaffected by conditioning stimuli to the ipsilateral hemisphere, this indicates that the ipsilateral motor cortex stimulation does not change spinal excitability (Ferbert et al., 1992; Gerloff et al., 1998). The neurons responsible for mediating IHI must originate from contralateral sites and travel to the opposite hemisphere to apply their inhibitory effects. Since GABAergic synapses mainly serve local circuits, IHI is more than likely mediated through the excitatory axons present in the corpus callosum, that cross to act of local inhibitory neurons in the contralateral motor cortex. More research is required to determine the relationship in which IHI has with SICI and LICI, and whether they are mediated by similar or different GABAergic mechanisms (Daskalakis et al., 2002).

Results provided by Daskalakis et al. (2002), supported also by Ferbert et al. (1992) and Di Lazzaro et al. (1999) show that ipsilateral inhibition does occur at the cortical level, compared to that of Gerloff et al. (1998) who suggests that ipsilateral

inhibition does not necessarily occur through interhemispheric connections, rather at subcortical sites. Conclusions made by Daskalakis consider that since both LICI (Chen et al., 1999) and SICI (Di Lazzaro et al., 1998) are both phenomena that are mediated cortically, then the findings that the contralateral motor cortex stimulation influences LICI and SICI, would suggest the cortical level dominance of IHI.

Conclusions around research involving transcallosal pathways and the interhemispheric connections associated are still undefined. More research is required to determine the extent and effect of inhibition and facilitation between the hemispheres and their role when disruption (lesion) in one hemisphere occurs.

2.7 Interhemispheric interactions and motor control

With research suggesting that interhemispheric interactions being mediated transcallosally, then damage or agenesis to the corpus callosum would no doubted lead to changes in brain dynamics. In humans, the corpus callosum is one of the last fibre tracts to be myelinated (Rakic and Yakovlev 1968; Cowell et al., 1992), maturing at around 10 years of age. Evidently, this coincides with adult levels of bimanual co-ordination and control (Jeeves et al., 1998). Functional deficits have been observed in patients with corpus callosum agenesis or lesion suggesting that this structure plays an important part in the execution of fast and complex motor tasks involving the hands in humans (Jeeves et al., 1998; Meyer et al., 1998). The proposed idea is that interhemispheric facilitation or inhibition of the contralateral motor cortex, via the corpus callosum may assist in performance of symmetrical and asymmetrical bilateral movements involving the hands. Whilst also suppressing unwanted movements with the opposite hand during unilateral hand tasks (Schnitzler et al., 1996).

2.8 Brain Injury

Neuroscientists have attempted to understand the neurological bases for functional recovery after brain injury (Ogden and Franz, 1917), with part of functional recovery requiring an understanding of how the normal brain works and how the damaged brain reorganises itself (Lang et al., 2006). It was poorly understood processes like substitution that have remained a neural model. In knowing that, short term recovery from cortical injury probably involves the resolution of acute pathophysiologic processes in and around the site of injury. However, since improvements in motor abilities can continue for months, other mechanisms must play a role.

Studies over the past 15 years using neuroimaging and non-invasive stimulation have begun to shed light on the neurological bases of motor recovery in greater detail. The common theme within the findings suggests the cerebral cortex undergoes significant alterations in functional organisation after peripheral and central nervous system injury (Kaas et al., 1990; Donoghue and Sanes, 1987; Cohen et al., 1993).

After chronic stroke, activity in the unaffected hemisphere with movements of the paretic hand is more prominent in patients with poor motor function (Berg et al., 2002; Cramer et al., 1997; Netz et al., 1997,) which decreases over time with rehabilitation. It is this functional role which remains unclear. Some possible explanations may be that the increased activation in the intact hemisphere may reflect its direct contribution to motor performance or planning (Luft et al., 2004; Krakauer et al., 2004), or that the activity does not impact directly on motor function in the paretic hand, but actually reflects the increased influence that the intact hemisphere has on homonymous regions of the lesioned hemisphere through transcallosal inhibitory interactions (Ferbert et al., 1992).

These inhibitory interactions between the primary motor cortices contribute to general motor control in healthy subjects (Luft et al., 2004; Krakauer et al., 2004), and are generally disrupted in patients with brain lesions (Murase et al., 2004). Murase et al. (2004) demonstrated that interhemispheric inhibition influencing the motor cortex responsible for controlling the paretic hand in preparation for voluntary movement is more prominent than that identified in healthy individuals. These finding were further extended by Duque et al (2005), who also demonstrated differing magnitudes of IHI between the hemispheres following damage. It is these findings that assist the view that down-regulating inhibitory influences from the unaffected hemisphere to the lesioned hemisphere in these particular patients may help contribute to neuro-rehabilitation efforts (Ward and Cohen, 2004).

2.9 Changes in corticospinal excitability

When applied to the motor cortex, TMS using difference paradigms can study different components of cortical excitability, whilst also giving an insight to different neurotransmitter systems (Pascual-Leone et al., 1998). The motor threshold, which refers to the lowest TMS intensity that is required to evoke MEP in 50% of trials, is believed to represent a measure of membrane excitability in pyramidal neurons. This threshold can be determined using single-pulse TMS. When using a single-pulse

paradigm over the motor cortex with a progressive increase in intensity, the generation of an input-output curve results. This modulation of amplitude of MEP to increasing intensity of TMS stimuli would appear to give a measure of excitatory feedback to corticospinal efferent output (Valls-Sole et al., 1994). This feature is thought to be glutamatergically mediated.

If TMS were to be applied in trains of multiple stimuli, or at a specific I-wave periodicity, cortical excitability can apparently be enhanced or decreased in a sustained fashion, this is depending upon the stimulation frequency and intensity (Pascual-Leone et al., 1998; Thickbroom et al., 2006).

These mechanisms responsible for the longer lasting modulation of cortical excitability are relatively unclear, however are thought to have some relationship with LTP and LTD and in paired-pulse techniques due to intracortical shifts in inhibition and facilitation (Tergau et al., 1997). The possibility for such techniques to have clinical applicability is spreading. The therapeutic potential, which has previously been based on rTMS, but now more recently iTMS is based on the ability to modulate cortical excitability for longer periods than the duration of the intervention. In addition, due to the transynaptic effects, this modulation is not limited to the cortical area stimulated, but more widely distributed. (Pascual-Leone et al., 1998).

With the known effects of the previously mentioned techniques, it remains unclear about the mechanisms or extent of effect on the contralateral hemisphere during and following stimulation. This small amount of research provides little insight into the possible clinical application that may be present to assist in advanced motor recovery following brain injury.

This research, through the use of iTMS aimed to look at the effect of iTMS on the contralateral hemisphere and through this, giving us a better understanding of the transcallosal effects of inhibition and facilitation, and to hypothesise about any other possible pathways or mechanisms. In all, the theories derived from the research may provide assistance into clinical work in which interhemispheric pathways play a key role in recovery.

CHAPTER 3
METHOD

3.1 Participants

All participants had normal neuromuscular and orthopaedic function. Informed consent was obtained prior to the commencement of the study which had ethical approval from both the Human Ethics Committee at Edith Cowan University, and Ethics Board at Sir Charles Gairdner Hospital, Western Australia.

3.2 Positioning and Set up

Each participant remained seated for the duration of the experiment (approximately 90-min), with forearms gently rested on a pillow placed on their lap. Participants were instructed to remain relaxed and ensure quiescent muscle activity in the upper limbs, whilst looking forward. Real-time EMG was monitored to ensure muscle relaxation during the experiment.

3.3 Motor cortex stimulation

A flexible, translucent rubber cap with pre-marked stimulus sites at 1 cm spacings is secured and aligned with the vertex, determined by measuring the mid-point intersection of the nasion-inion and interaural. Transcranial magnetic stimulation is performed using a Magstim 200 stimulator, with a figure-eight coil to stimulate the hand area of the motor cortex. The coil is held tangential to the scalp, with the handle obliquely posterior (approximately 45 degrees), such that it is perpendicular to the line of the pre-central gyrus, or primary motor cortex (M1) (Figure 3.1)

3.4 Motor evoked potential recordings

MEPs are recorded from the first dorsal interosseous muscle (FDI) using surface electrodes attached to the muscle belly (Figure 3.2). Electromyographic (EMG) signals are amplified (x1000) and band-pass filtered between 20 and 2000 Hz, before being digitised at 2000 Hz for 500 ms following each stimulation. The site which elicited the largest amplitude MEP for a given stimulus intensity is maintained throughout the experiment. Cortical motor threshold of the M1 was determined by stimulation centrally

over the hand area of cortex, starting at 30% with the stimulus output increasing in 5% increments. Threshold was defined when 3 out of the 4 stimuli produced a clear MEP.

3.5 Study Design

The design of the experiments were within-subject, single factor repeated measures design [Portney, 1993 #499]. Comparisons were drawn between pre and post intervention recordings within subjects, and were performed within one experimental session for each subject. Measures are taken to address potential confounding factors including maintaining a consistent, quiet experimental laboratory with minimal verbal interaction after commencement of the experiment, consistent subject instructions, consistent visual outlook, minimising length of the experiment, and familiarisation with TMS (during attainment of optimal site and stimulus intensity). The facilitatory I-wave interaction at 1.5ms (Thickbroom et al., 2006) was confirmed for each participant, to ensure that facilitatory networks were being re-enforced during the intervention. The total experimental session and familiarisation had duration of approximately 2 hours.

3.6 Protocol

3.6.1 Study One

11 healthy right handed adult participants (7 male, 4 female, 18-45yrs) volunteered for the first study. Setup/ positioning, magnetic brain stimulation and muscle recordings were carried out as previously described. 12 baseline recordings, using a single pulse stimulus at 10% above resting motor threshold (RMT) were collected from the optimal site in both hemispheres. An interventional protocol was then applied using a paired-pulse stimulus (1.5ms ISI), over M1, at 0.2Hz and 100% of RMT for 15 minutes (iTMS). Post intervention recordings (12) were then taken from both hemispheres using the same single pulse stimulus, at 10% above motor threshold as initial baseline recordings. This process was achieved by alternating between the hemispheres after each 1 minute collection, with tracking lasting 10 minutes post intervention (Figure 3.3)

3.6.2 Study Two

Six healthy right handed adult participants (4 females, 2 males, 19-36yrs) volunteered for the second study. Setup/ positioning, magnetic brain stimulation and muscle recordings were carried out as previously described. Twelve baseline recordings, using a single pulse stimulus at 10% above RMT were collected from the optimal site in

both hemispheres. Followed then by a recording of interhemispheric inhibition, in which a conditioning stimulus (110% RMT, dominant hemisphere) was applied prior to the test stimulus (contralateral hemisphere). The optimal time for the preceding stimulus was chosen by delivering 4 stimuli at time intervals 9-13ms (Ferbert et al. 1992), and selecting the time in which the greatest level of inhibition was displayed. Twelve baseline recordings where then obtained using the optimum interstimulus interval.

The 15 minutes iTMS interventional protocol was then applied over the dominant (left) hemisphere. Post intervention recordings (12 single pulse stimuli) were then taken from each hemisphere using the same single pulse stimulus, at 10% above motor threshold as initial baseline recordings, followed then by 12 MEP recordings using the conditioning model. Shown in Figure 3.4, this process was achieved by alternating between the hemispheres after each 1 minute collection, and tracking for approximately 14 minutes post intervention. Recording minutes 0, 3, 6, 9 and 12 post intervention represent single pulse stimulation on the dominant hemisphere, whilst 1,4,7,10 and 13 minutes are on the non dominant or contralateral hemisphere. 2, 5,8,11, and 14 minutes are the times in which the conditioning protocol (i.e. preceding conditioning stimulus) was recorded.

3.8 Data Analysis

The digitised peak-to-peak MEP amplitude (units= mV) was measured by manual cursoring of each waveform using TMS acquisition and analysis specific software (previously developed in the laboratory). For both studies, the model of data analysis was the same. For each participant, the mean of twelve baseline MEPs was compared to the mean of five sets of MEPs, in the ten minute period following the intervention. Post intervention data was normalised to baseline. This data was tested for normality (Kolmogorov and Smirnov test), then tested for significance above baseline using a Z-test with an alpha level being set at 0.05. MEPs recorded during accidental muscle activity were regarded as outliers and removed prior to calculation of mean values.

3.8.1 iTMS intervention

Twelve paired stimuli with an ISI of 2.0ms were delivered prior to the iTMS intervention (100%RMT). This is to establish if 1.5ms sufficiently raises MEP amplitude when compared to 2.0ms, which has been shown to approximate the

amplitude of the test pulse alone (Ziemann et al., 2000). Data were analysed using a paired t-test and are reported as mean (+/-SEM).

Increase in iMEP (the MEP resulting from the conditioned test stimulus) over the intervention period, was analysed in both studies by averaging the MEP (12) amplitude for each minute of the intervention (15 minutes). The value for each successive minute was expressed as a percentage of the first minute. Group mean data was tested for progressive increase in amplitude over time using a linear regression.

3.8.2 Interhemispheric Inhibition (IHI)

To ensure the conditioning stimulus significantly reduced the amplitude of the test stimulus, each participant's unconditioned stimulus was compared to their conditioned stimulus prior to the intervention. This was then analysed for significance using a two-tailed paired t-test ($p<0.05$). To establish if the effect of conditioning stimulus changed as a result of the intervention (raised excitability), the ratio of conditioned to unconditioned MEP (conditioned/unconditioned x 100) was compared pre and post intervention, using a paired t-test ($p<0.05$). Ratio data, referred to as Interhemispheric Inhibition Index (IHI index) across the group are presented as mean +/- standard error of measurement (SEM). One-tailed t-tests were used to test the hypothesis that intervention raises both the excitability of the contralateral hemisphere, and correspondingly the IHI ratio.

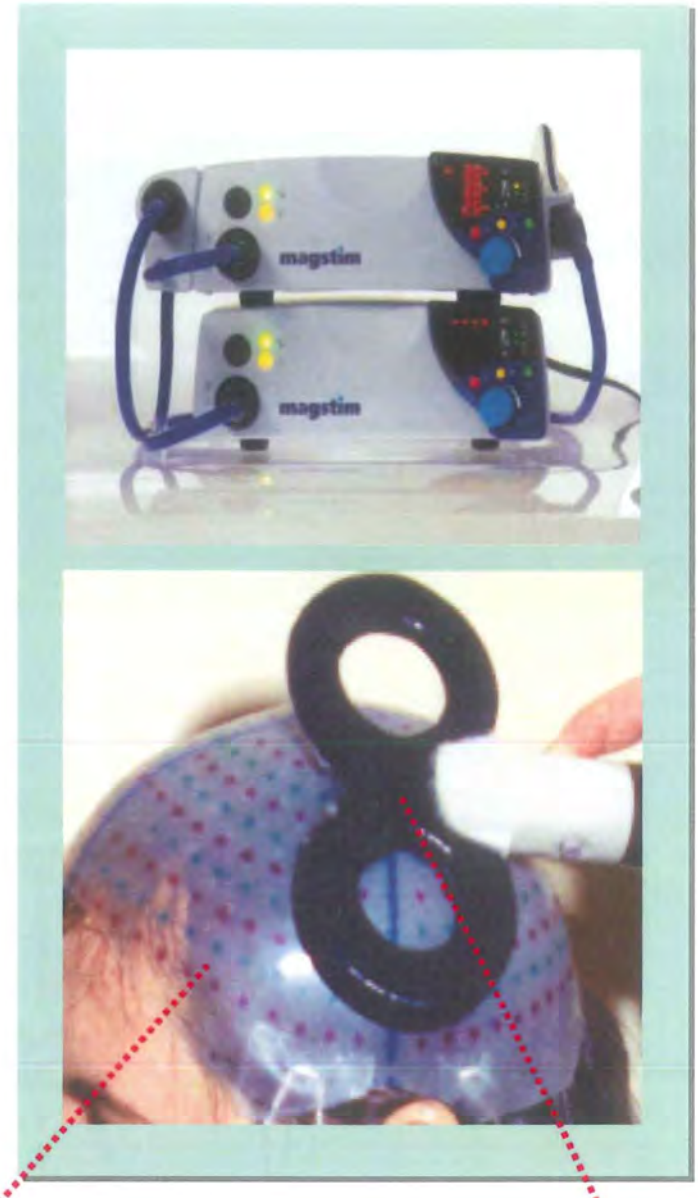
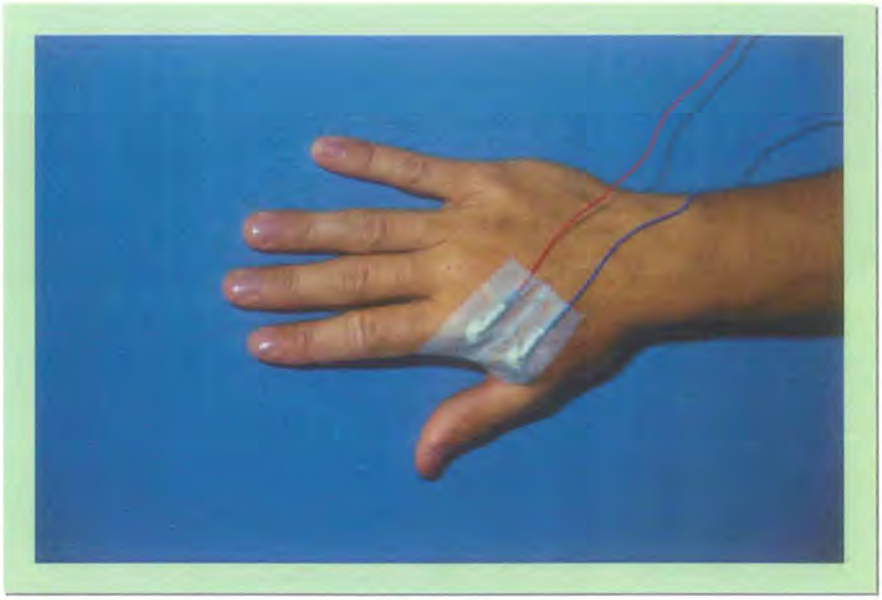


Figure 3.1. The magnetic stimulator, figure-eight coil, and latex reference cap used in the TMS experiments.

(a)



(b)

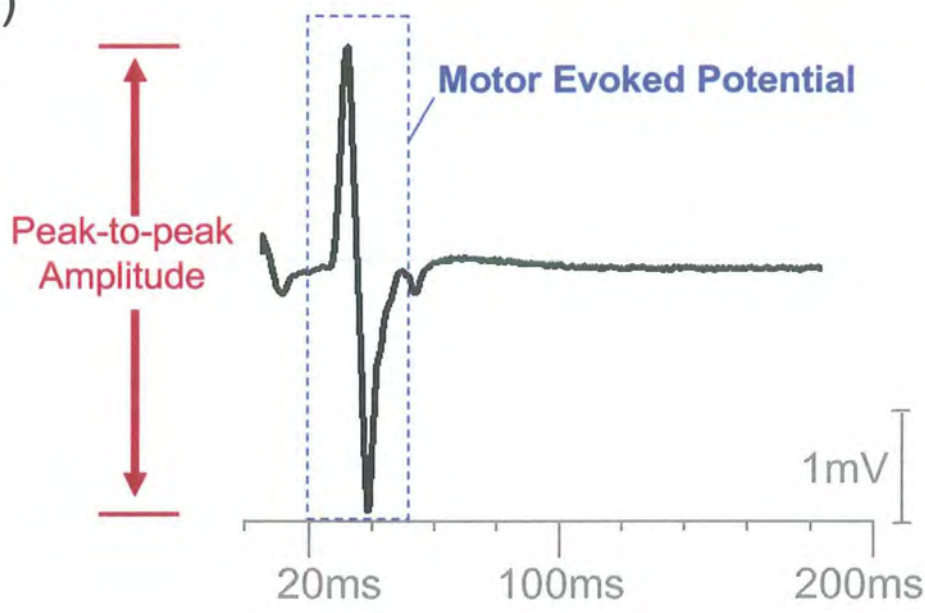


Figure 3.2 (a) Electrode placement over the first dorsal interosseous muscle with the proximal electrode over the muscle belly. The ground electrode was attached to the bony prominence of the lateral humeral epicondyle. (b) The biphasic EMG deflection referred to as a Motor Evoked Potential (MEP) occurring at approximately 26ms. The peak-to-peak amplitude of the potential was measured.

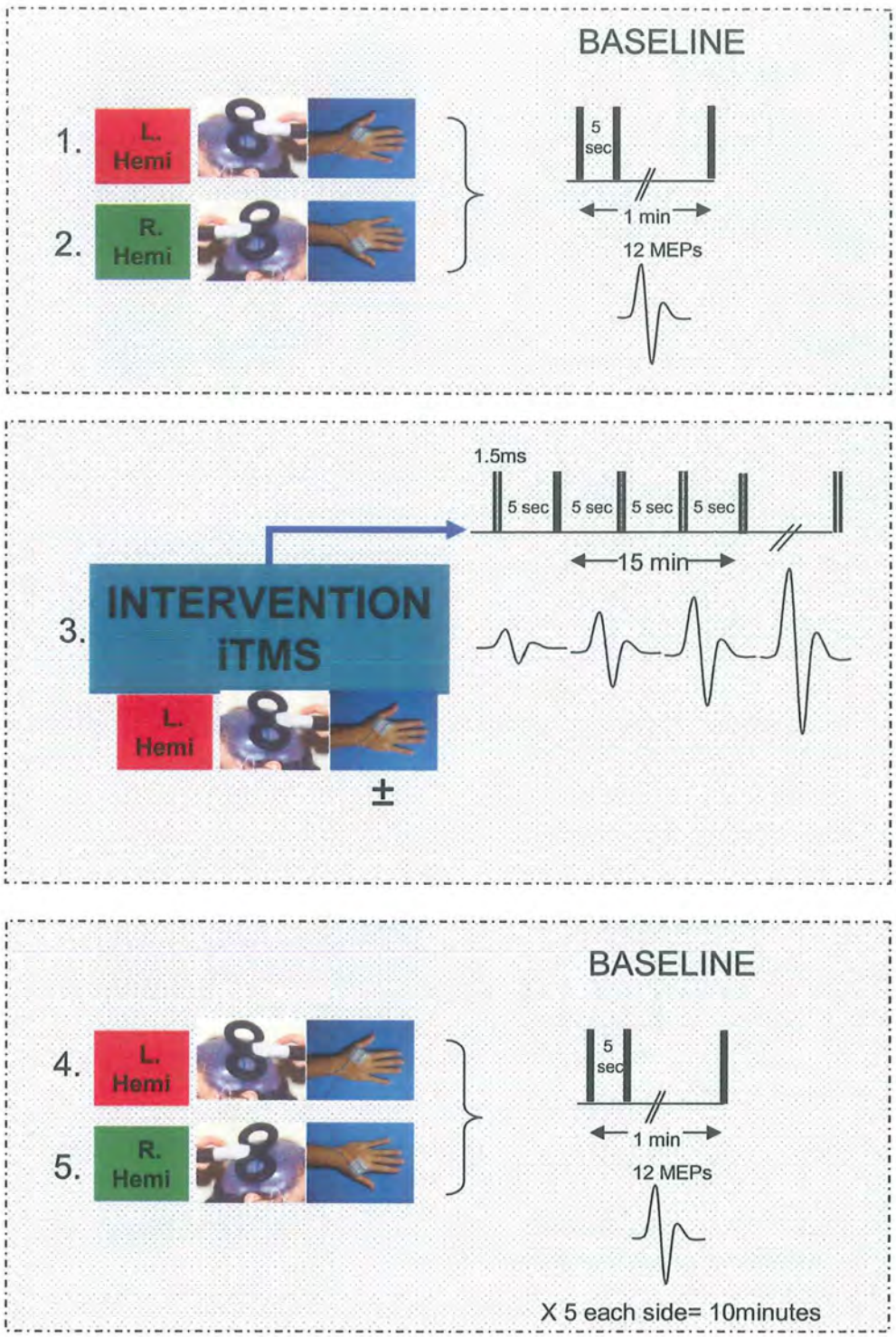


Figure 3.3. Experimental protocol for study one. Pre and post intervention recordings taken using single pulse suprathreshold stimuli (12). The intervention (iTMS) was applied for 15 minutes using paired pulse stimuli at resting motor threshold with 1.5 ms interstimulus interval. Post intervention collections were taken 5 times on each hemisphere

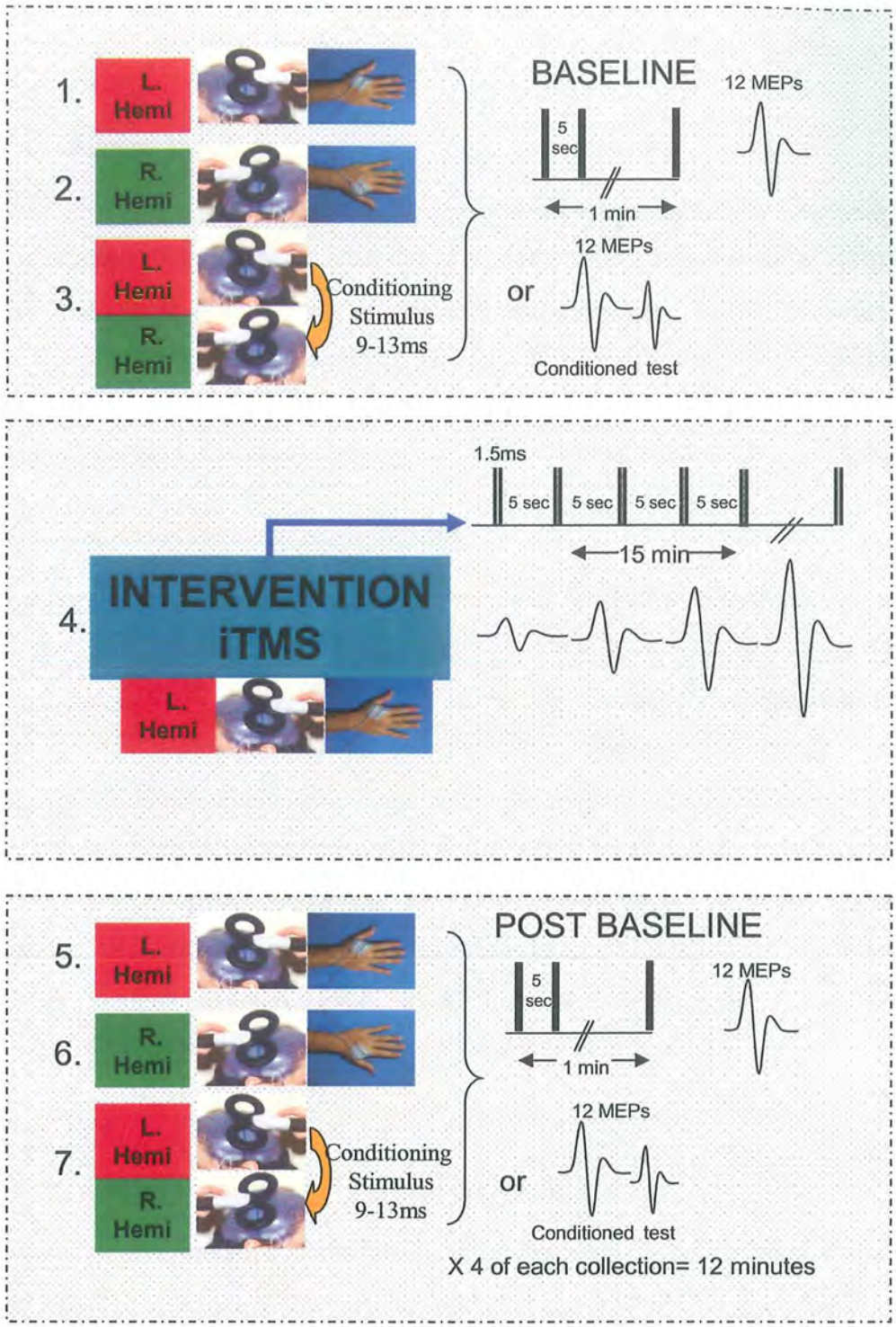


Figure 3.4. Experimental protocol for study two. Pre and post intervention recordings were taken from both hemispheres (12) with additional collection measuring interhemispheric inhibition by applying a conditioning stimulus (between 9-13 ms) on the dominant hemisphere. The intervention comprised 15 minutes iTMS at 1.5ms ISI.

CHAPTER 4

RESULTS

4.1 Study One

4.1.1 Paired-Pulse ISI

With 1.5 ms ISI for paired pulse stimuli at resting motor threshold, MEP amplitude increased by 100% (2.57±0.63mV SEM) from pre intervention baseline. This indicated that facilitatory I-wave interaction was occurring at this interval, when compared to a mean single pulse amplitude of 1.29±0.13mV with a 5% higher stimulus intensity. Such reinforcement was not evident at 2.0 ms where mean MEP amplitude reduced 32% (0.88±0.37 mV) (Fig 4.1).

4.1.2 Effect of iTMS on cortical excitability

Figure 4.2 shows a progressive increase in iMEP amplitude across the intervention period ($r=0.79$, $p=<0.001$) reaching 160±20.4% of baseline on the left, intervened hemisphere. Single-pulse MEP amplitude was significantly increased from baseline at each time point for 10 minutes after intervention, with no significant variation in MEP amplitude over time being noted (intervened hemisphere $r=0.04$, $p=0.87$; non-intervened hemisphere $r=0.1$, $p=0.87$) (Fig 4.3a). When pooled to represent a group mean, both the ipsilateral and contralateral sides to iTMS stimulation significantly increased to 227±34% ($p=0$) and 123.8±12.1% ($p=0.0001$) respectively (Fig 4.3b). Sample overlaid MEP waveforms from one participant, which are representative of the finding of the group, are presented in Figure 4.4.

4.2 Study Two

4.2.1 iTMS and post intervention cortical excitability

As shown in previous study, mean iMEP amplitude increased significantly from baseline over the 15 minutes intervention period (Fig 4.5; $p=0.001$ & $r= 0.40$), with an increase in iMEP amplitude reaching 155%±25%. Corresponding also to that seen in the first study is a sustained increase of 197.3±26.4% ($p=0.0001$) and 125.2±14.5 ($p=0.04$) for the intervened and non-intervened hemisphere respectively. Figure 4.6 displays the increased MEP amplitude in each hemisphere following iTMS in both study one and two, demonstrating a distinct pattern and supporting the reliability of this finding

4.2.2 Change in Interhemispheric Inhibition

Represented in figure 4.7 is the MEP amplitude taken from the sample of ISI collections in which interhemispheric inhibition was most pronounced. Shown here in one participant, this demonstrates the typical fluctuations seen between time-points (Appendix A). MEP amplitude for group data was significantly reduced by ~50% (unconditioned 1.57mV and conditioned 0.78mV; p=0.004) when preceded by an optimal ISI for interhemispheric inhibition (Figure 4.8). Using sample waveforms taken from one participant, the magnitude of the effect from the conditioning stimulus on interhemispheric inhibition can be seen. Using the optimal ISI (11ms), MEP amplitude is reduced following the conditioning stimulus. However following the intervention MEP amplitude visibly increased regardless of conditioning (Figure 4.9) which was a common trend throughout subjects.

IHI following the 15 minute intervention (iTMS) was significantly reduced as indicated by a ~20% increase in IHI ratio for mean data (Fig.4.10; Pre 57±12%, Post 77±14% p=0.047). Individual participant data is shown in Figure 4.11, comparing the mean conditioned MEP amplitude pre and post iTMS for each subject, with participant 4 being removed from mean data values due to large variance and extreme pre intervention inhibition. This highlights subject variability, but supportive of the common trend of reduced interhemispheric inhibition shown in previous pooled data.

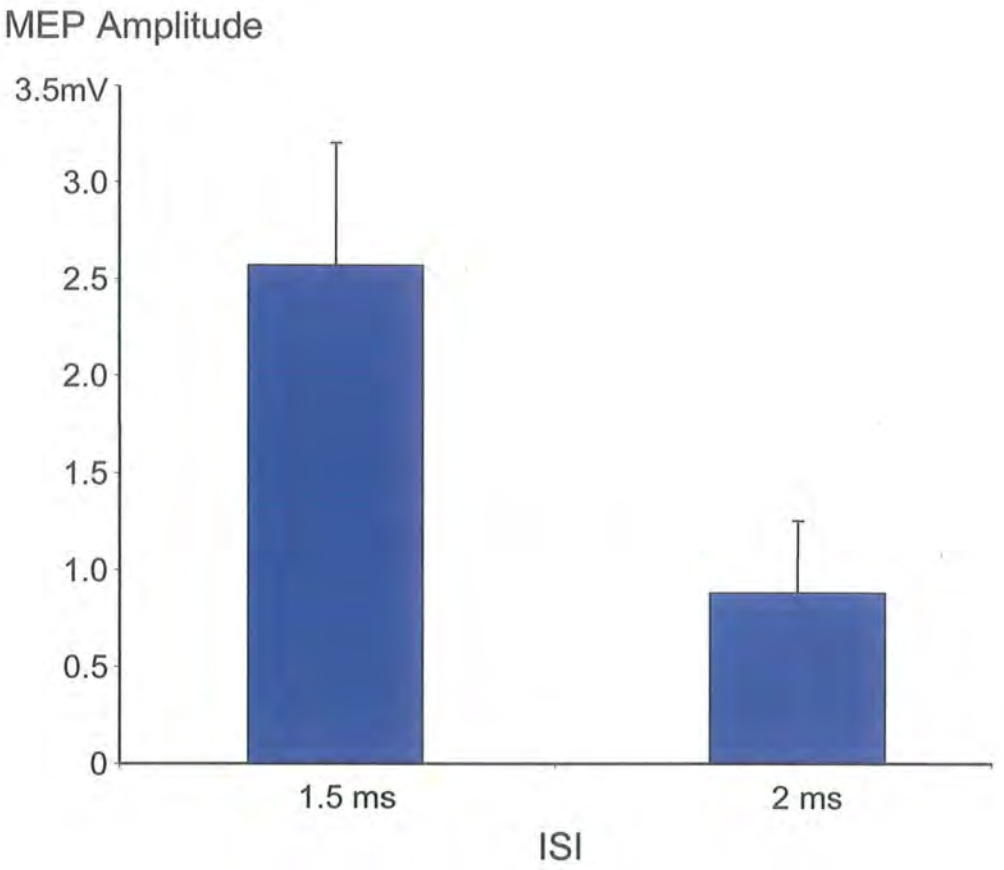


Figure 4.1. Mean (±SEM) conditioned MEP amplitude for iTMS across subjects for ISI of 1.5 and 2ms, showing facilitation at 1.5ms when compared to 2.0ms.

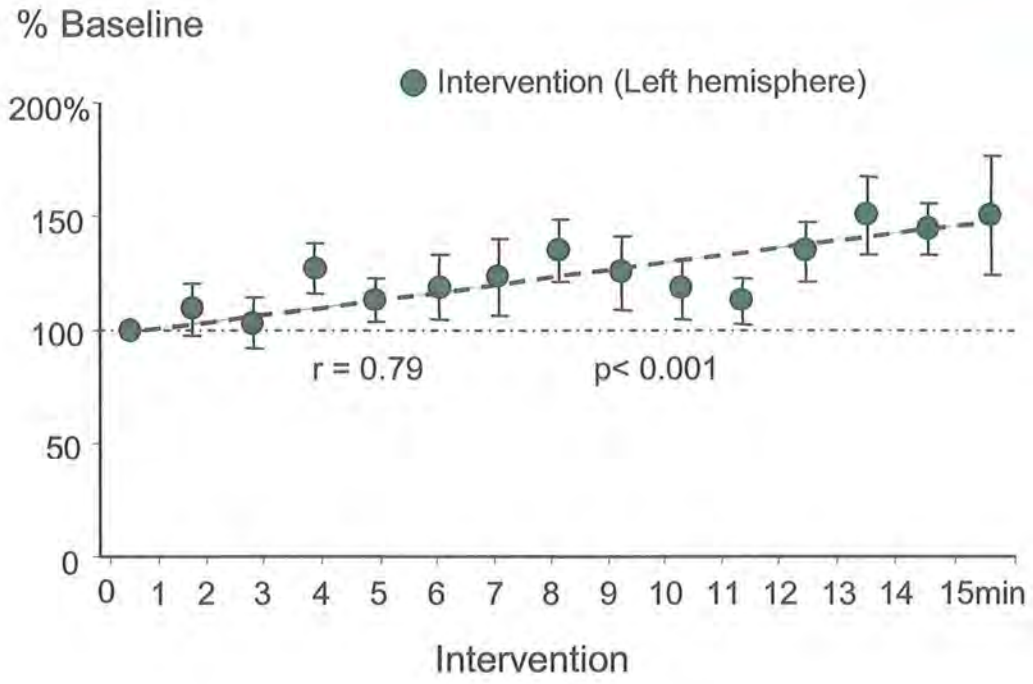


Figure 4.2. Normalised mean (±SEM) MEP amplitude across subjects during 15 minute period of iTMS. Demonstrating the significant increase in corticomotor excitability over the dominant, left hemisphere during the intervention.

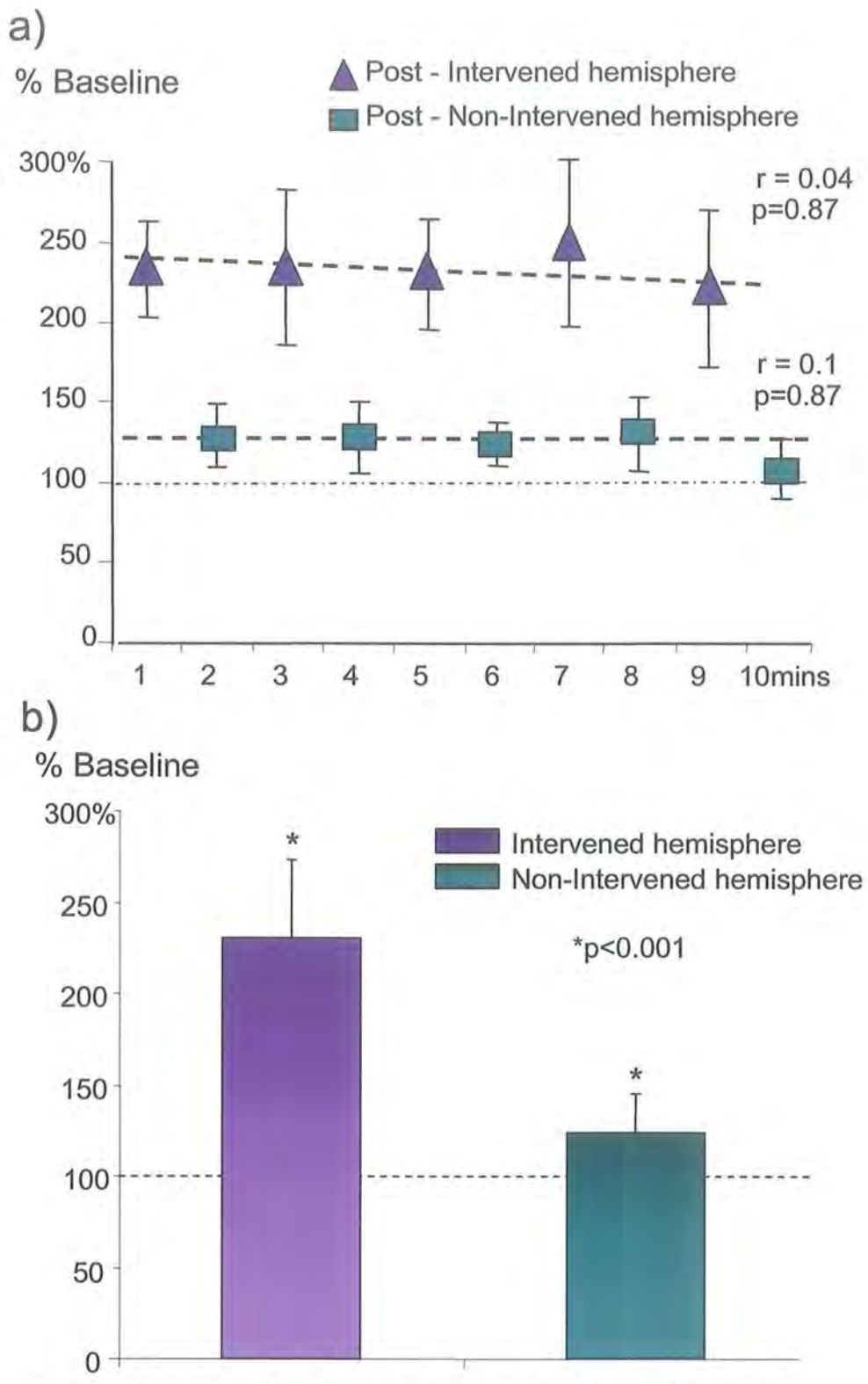


Figure 4.3 a) Normalised group mean data for the 10 minutes post intervention period indicating a steady elevation across the time period. b) pooled mean for 10 minutes post iTMS. Representing a significant increase in both the intervened and non intervened hemispheres after iTMS application.

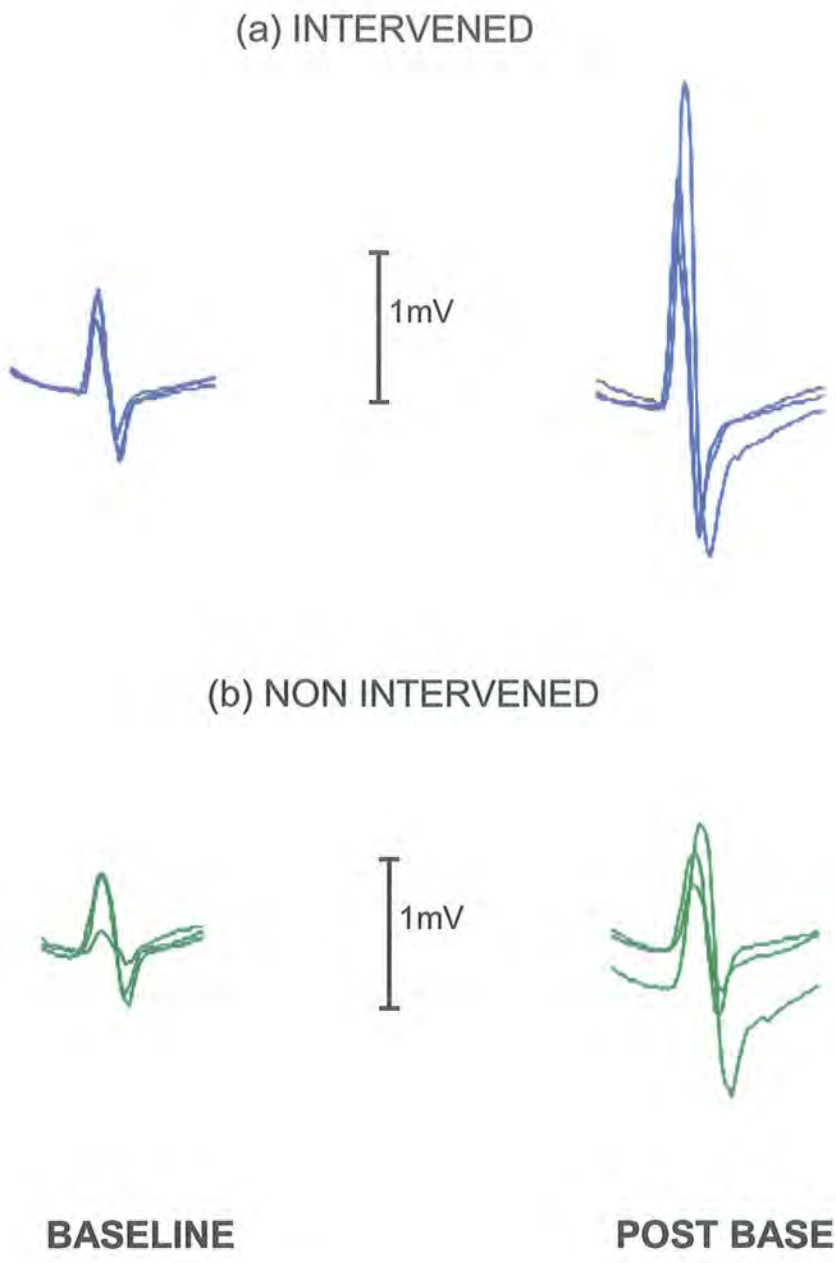


Figure 4.4. Sample overlaid MEPs from one subject. Pre and post intervention recordings from both a) intervened and b) non-intervened hemispheres, taken at corresponding time points. Illustrating an increase in MEP amplitude following 15 minutes iTMS.

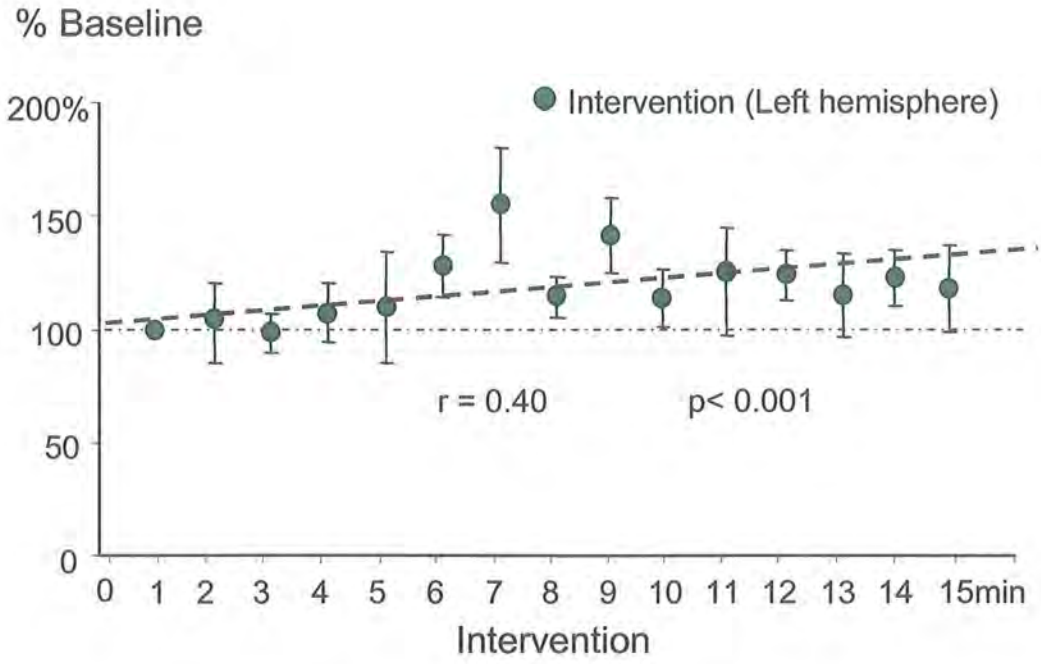


Figure 4.5. Normalised mean (±SEM) MEP amplitude across subjects during 15 minute period of iTMS in the second study. Demonstrating the progressive increase in corticomotor excitability over the dominant left hemisphere during the intervention.

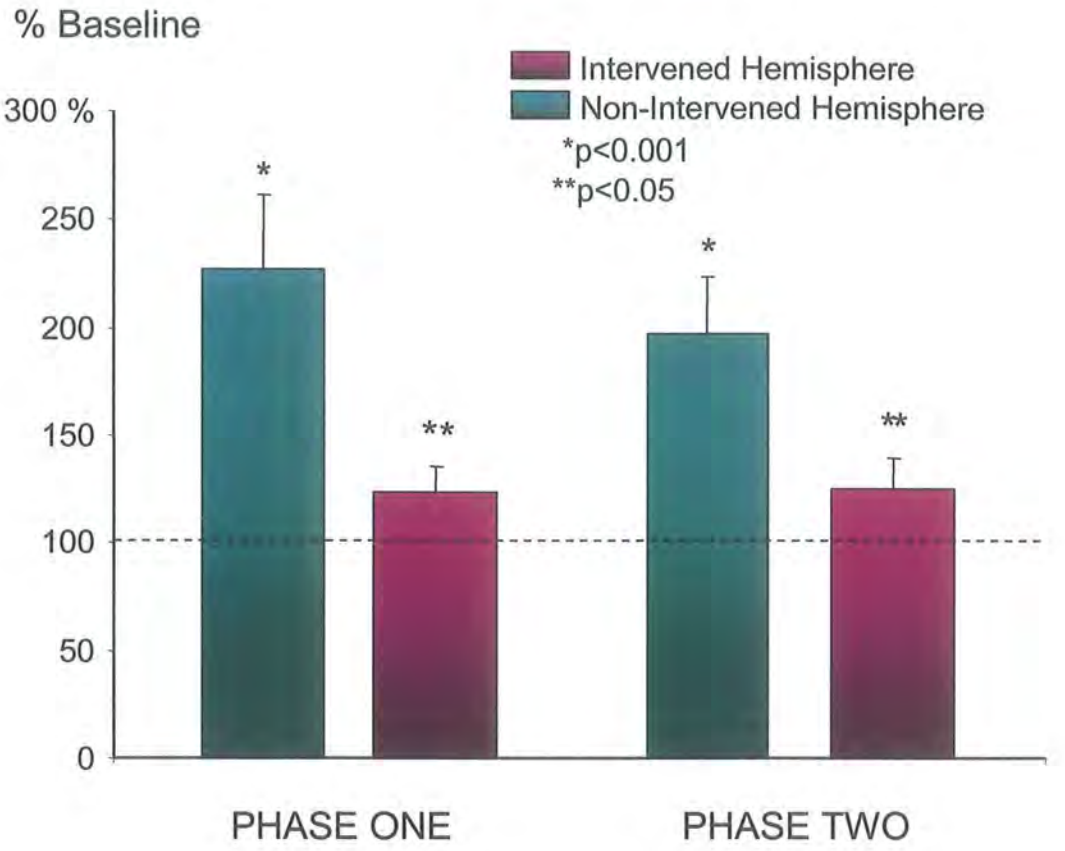


Figure 4.6. Normalised group mean (±SEM) MEP amplitude, of the 10 minute period following iTMS for intervened (left) and non-intervened (right) hemispheres during studies one and two. Representing a sustained significant increase in both the left and right hemispheres after iTMS application.

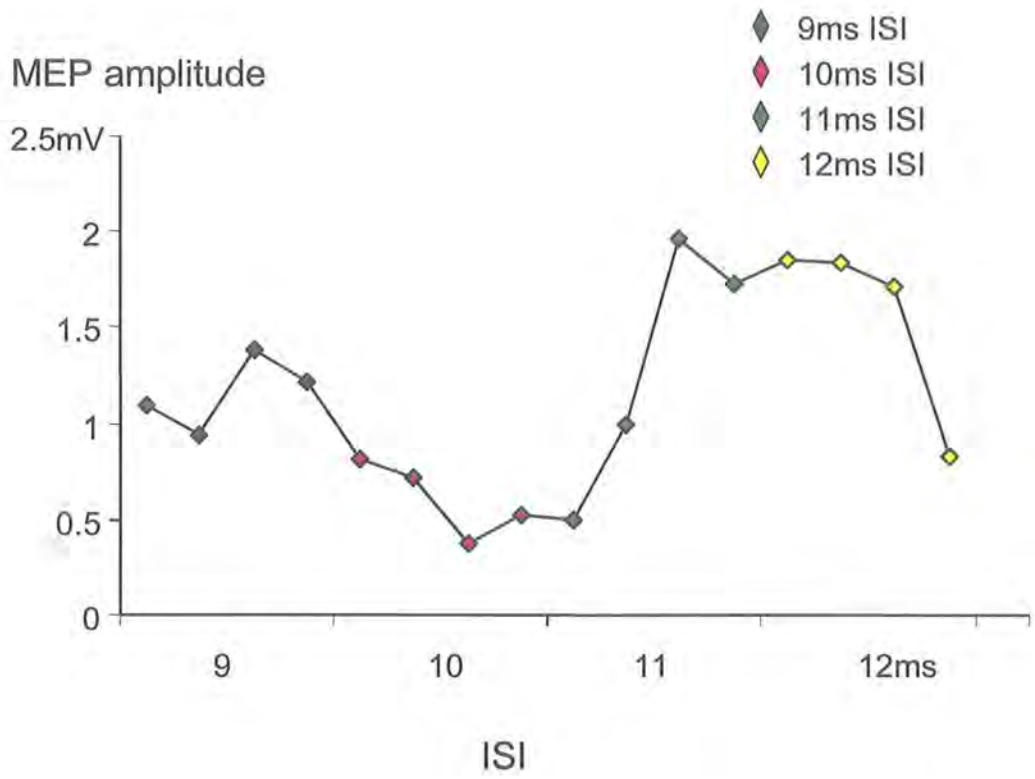


Figure 4.7. Interhemispheric Inhibition: Sample ISI collection taken from one subject represented by mean MEP amplitude at ISI's of 9-13ms. This demonstrates the optimal time for interhemispheric inhibition which could be seen across subjects.

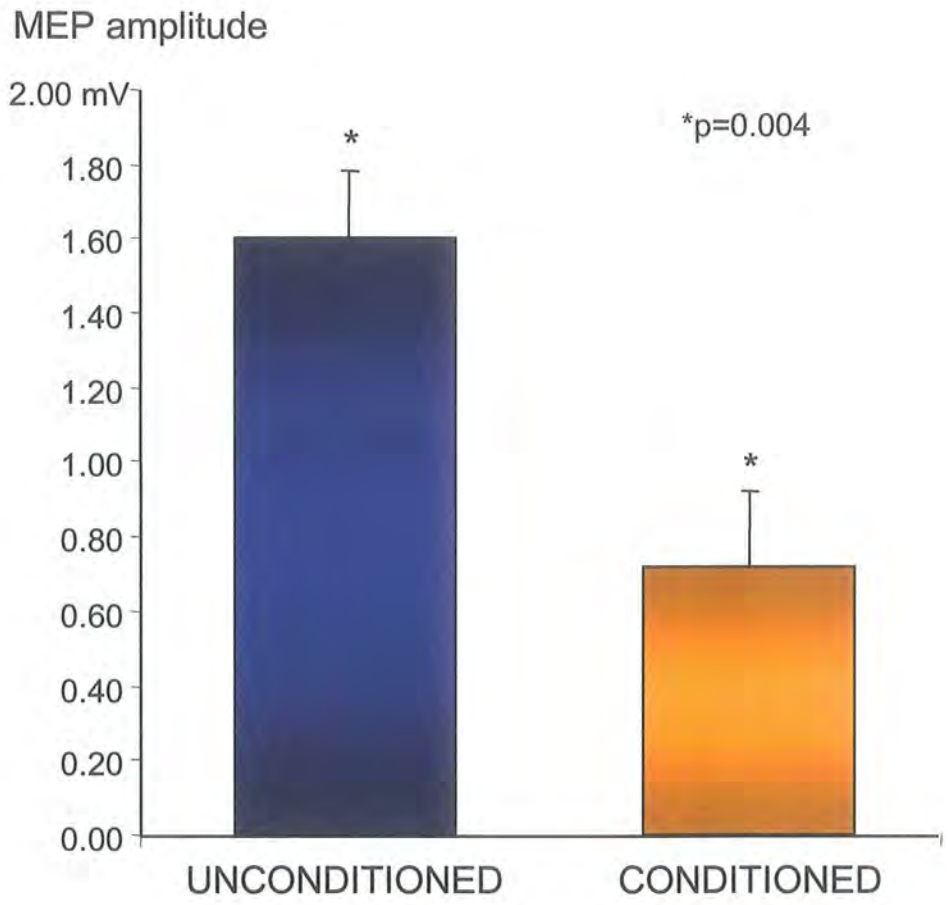


Figure 4.8. Normalised group mean (±SEM) MEP amplitude for conditioned and unconditioned stimulus representing the IHI effect of the conditioning stimulus on the test stimulus at baseline.

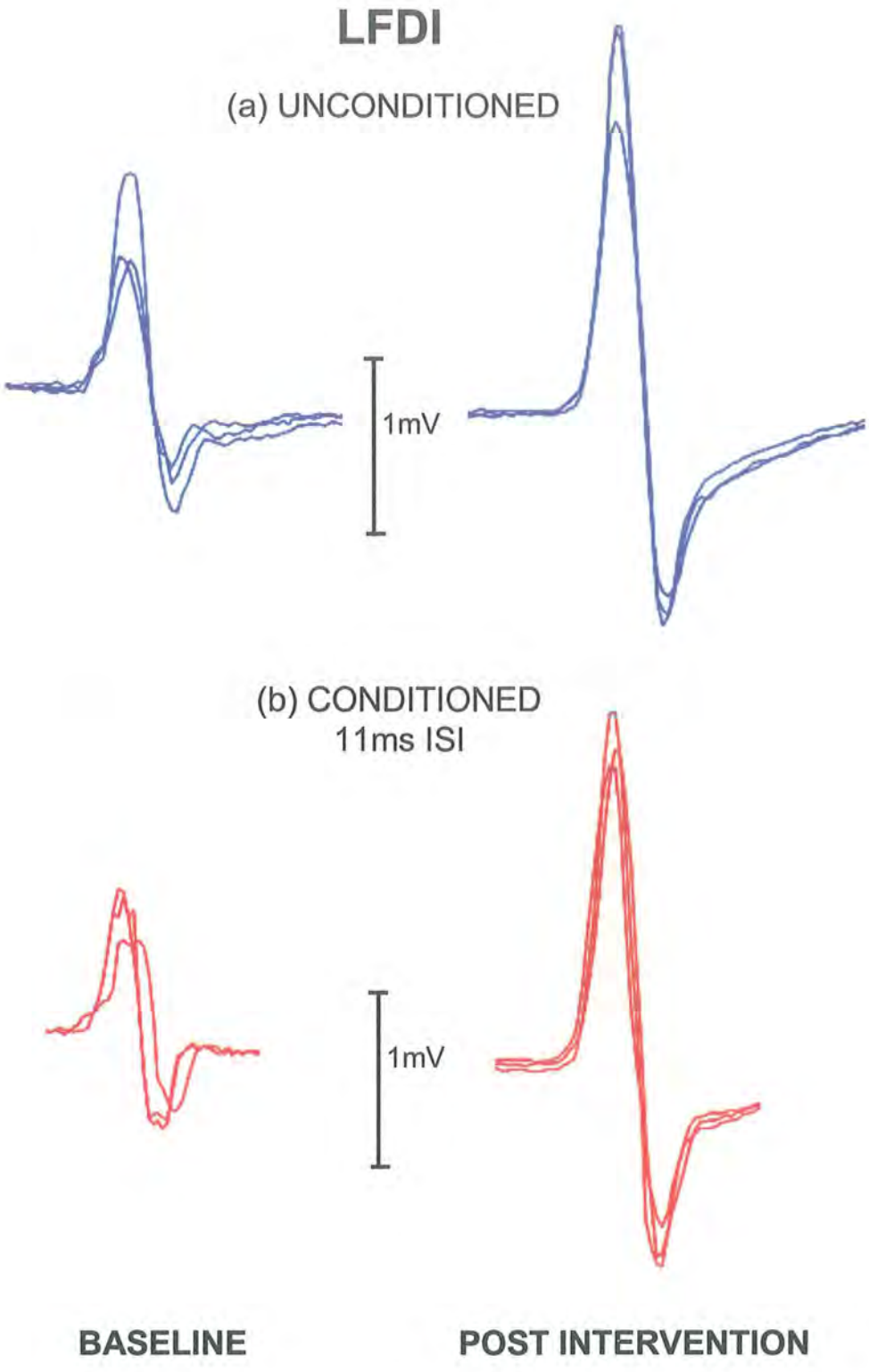


Figure 4.9. Sample MEP waveforms demonstrating IHI for one subject. Pre and post intervention recordings of a) unconditioned LFDI, and b) conditioned LFDI. Showing a significant increase in in both a) & b) following 15 minutes iTMS.

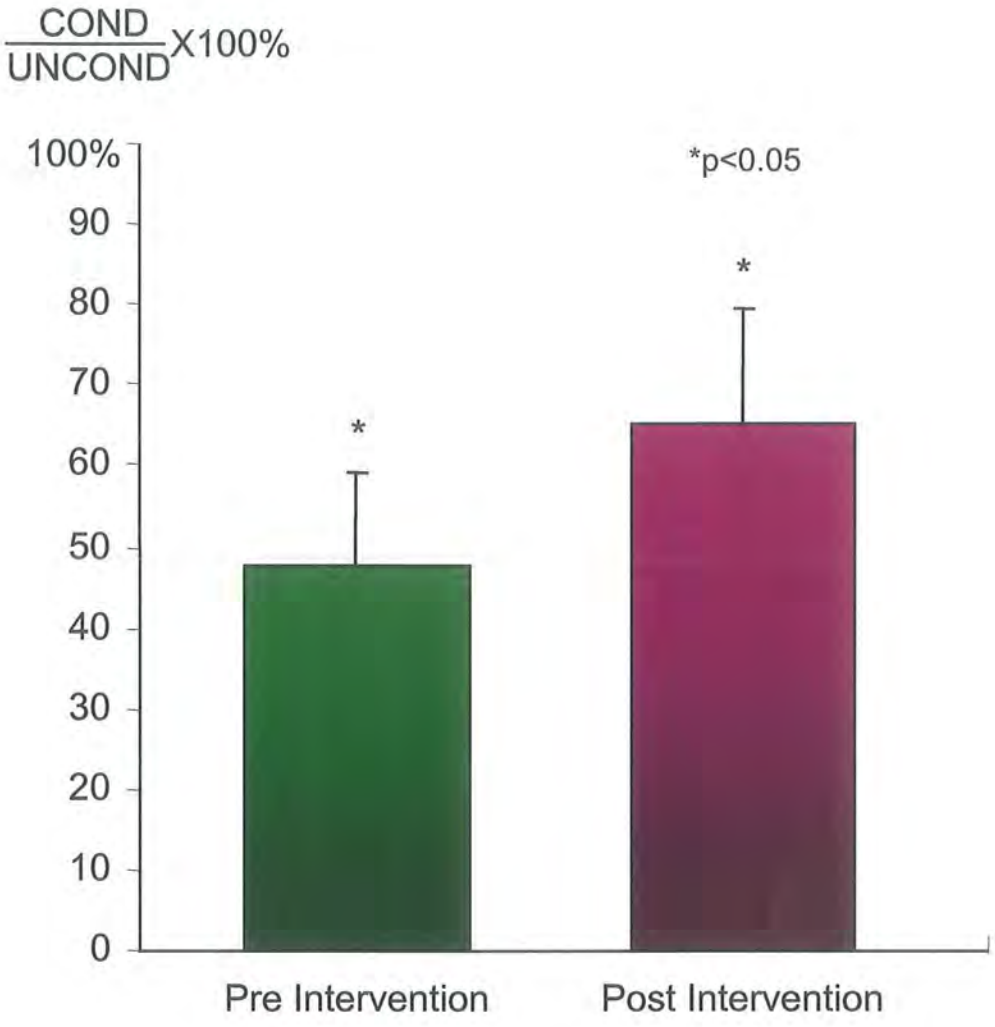


Figure 4.10. Percentage change of interhemispheric inhibition pre and post intervention (iTMS). Group data demonstrated an increase in the IHI index, which represents a reduced interhemispheric inhibition.

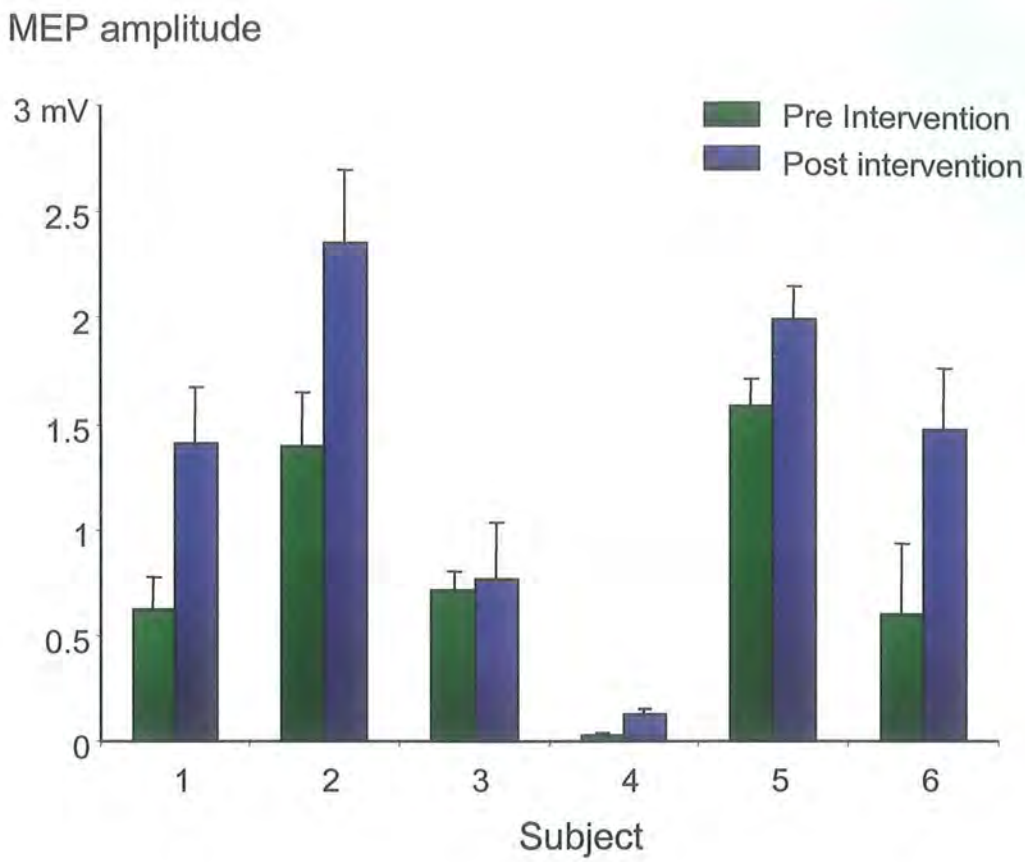


Figure 4.11. Mean (±SEM) *conditioned* MEP amplitude for each subject pre and post 15 minutes iTMS showing the relative increase observed post intervention, and supporting reduced interhemispheric inhibition.

CHAPTER 5
DISCUSSION

In the present study it has been shown that by raising primary motor cortex excitability using iTMS, contralateral homologous cortex excitability is modestly but significantly raised. This finding is in contrast to the view that raised excitability will lead to greater interhemispheric inhibition. Corresponding to the bilateral increase in corticomotor excitability following 15 minutes of iTMS, was a reduction in interhemispheric inhibition from the dominant (left) to non-dominant (right) hemisphere.

Taken with previous inhibitory protocols, this finding demonstrates another way in which the excitability to the contralateral cortex can be temporarily increased using TMS. Other methods have been documented and similar outcomes achieved. However these have all used rTMS protocols, being either inhibitory (1 Hz)(Chen et al., 1997) which act by increasing the excitability in the contralateral cortex through disinhibition, or excitatory (5 Hz), which is documented in one study (Gorsler et al., 2003). However the mechanism of action remains unclear. The present study differs from those aforementioned, with the use of a novel technique which differs in stimulus frequency, intensity and is used to target I-wave interactions specifically.

Possible mechanisms have been suggested for the increased contralateral cortex excitability following application of an inhibitory protocol (low frequency rTMS). These effects are thought to be attributed to a decrease in the efficacy of inhibitory synapses in the non-intervened hemisphere due to repeated activation. This may also be possible in the stimulated hemisphere, in which if interhemispheric fibres are normally activated trans-synaptically, then repeated activation could reduce the effectiveness, decreasing IHI (Gilio et al., 2003). Due to the nature of the interventional protocol, it is unlikely that the increases found in the present study are a resultant of such mechanisms.

Increases in motor cortex excitability observed in this study support those seen in the first iTMS study by Thickbroom et al. (2006), in which an increase in excitability is seen on the stimulated hemisphere both during the intervention, and sustained for a period of 10 minutes following application. iTMS design is based on the reinforcement

of transynaptic I-wave facilitation through using a repeated optimal interstimulus interval (Thickbroom et al., 2006; Ziemann et al., 1998). Interestingly, the previous study (Thickbroom et al., 2006) documented a sustained increase for 10 minutes following the 30 minutes intervention, seeing a four-fold increase, whilst the present study applied the intervention for only 15 minutes in which was sustained also for 10 minutes with a two/three fold increase. Noting that the application time for iTMS did not affect the duration it was sustained, but might be responsible for the difference in magnitude.

With interhemispheric effects proposed to be mediated transcallosally, it was thought that by increasing the excitability in one hemisphere, it is possible to increase excitability of the contralateral cortex via transcallosal connections. These transcallosal connections have been studied in humans for the last decade using transcranial stimulation. The effects of interhemispheric inhibition via transcallosal connections have been well documented (Ferbert et al., 1992; Meyer et al., 1995, 1998) with interhemispheric facilitation between hemispheres being described as capricious. Whilst there is empirical evidence that supports this view, the elements of these transcallosal facilitatory connections remain unclear and require further investigation.

Current ideas suggest that the predominant interhemispheric connection is inhibitory, with facilitation being relatively weak and focal (Boroojerdi et al., 1996; Ferbert et al., 1992; Meyer et al., 1995). This would likely suggest a hypothesis whereby increasing the excitability in one hemisphere, one would raise the level of transcallosal inhibition, presuming it is mediated transcallosally. To date, there appears to be only one other study that has looked at the effects of an excitatory protocol on the contralateral homologous cortex. Gorsler at al. (2003) documented an increase in MEP amplitude at the stimulation site and on the contralateral cortex after 5 Hz rTMS (Gorsler et al., 2003), which outlasted the stimulation period. If prior assumptions are true, then what has been demonstrated by Gorsler et al. (2003) and seen in the present study must depict an alternative phenomenon.

The increase in contralateral cortex excitability following iTMS, was significantly elevated when compared to baseline. However it remains unclear as to why the effect is not as extensive as on the iTMS stimulated hemisphere (left), and relatively capricious in appearance when subjects are individually analysed. With the design of iTMS to target trans-synaptic facilitation, one might speculate that the dominant

hemisphere is likely to have a larger increase. This may be due to the fact that direct application would cause an increase in focal synaptic activity in that region, which then branches to other more distant sites. If actually targeting interhemispheric circuits other than the aforementioned inhibitory pathways, it would be anticipated that interhemispheric inhibition would still be acting on the contralateral cortex, which as a result would suppress any further increase. It would be naïve to think that these processes, either inhibitory or facilitatory, act independently and are not affected by one another.

It remains unclear as to the exact mechanisms surrounding the effects seen in the first study, but it seems likely that the iTMS protocol is targeting different pathways to those seen in low frequency rTMS studies. It is proposed that facilitatory pathways are existent and it is not unlikely that the parameters used could be targeting these connections. From these findings, further research was carried out to explore the effects of iTMS on IHI, and what role possible role it may play in raising contralateral corticomotor excitability following iTMS.

The second study was a logical progression to examine the effect iTMS had on interhemispheric inhibition, and to determine whether this was the reason for a bilateral increase in excitability. With the idea that this protocol was affecting facilitatory networks rather than inhibitory, it remained unknown as to the changes that would occur in IHI, or more specifically transcallosal inhibition.

In a separate group of participants, a similar experimental protocol to the initial study was applied. A bilateral increase in corticomotor excitability was again observed and was of comparable magnitude. This provides evidence of experimental reliability for iTMS to increase contralateral cortex excitability. The second finding was a reduction in IHI following 15 minutes iTMS. This would suggest that a bilateral excitability increase may be a result of altered IHI through disinhibition of intracortical interneurons in the right hemisphere, via transcallosal inhibitory networks. This appears to be a different mechanism to those demonstrated using 1Hz TMS as the physiological processes and rationale behind the protocols vary.

iTMS (Thickbroom et al., 2006) as an intervention aims to modulate synaptic plasticity by targeting I-wave facilitation. It was found that stimulation at a specific periodicity resulted in volleys that arose from trans-synaptic activation of corticospinal neurons via excitatory cortical interneurones. Through the modulation of synaptic

efficacy, an increased cortical excitability has been documented, which outlast the period of stimulation. The present study supports the effectiveness of the protocol to increase excitability and in addition, project to other more distant sites.

Through the use of paired-pulse TMS, interhemispheric interaction in the human brain have been explored, thereby supporting increased evidence for the inhibitory interaction between the primary motor areas of both sides (Ferbert et al., 1992). RTMS induced inhibition has been proposed to occur at a cortical level from epidural studies in which was recorded from descending corticospinal volleys (Di Lazzaro et al., 2002). Furthermore, it was reported to be transcallosally mediated by studies carried out using patients with lesions to the corpus callosum (Boroojerdi et al., 1996; Meyer et al., 1995).

Changes in IHI could be influenced by modifying excitability on the side of stimulation, or the side of MEP test, or on both. This is similar to other studies that have documented a reduction in IHI following TMS. However, these studies have used inhibitory protocols (rTMS) (Gilio et al., 2003). Although changes in excitability in each hemisphere are likely to occur via different processes, this does not necessarily suggest that similar mechanisms are not responsible for the changes in IHI. Gilio et al., (2003) noted that repeated activation of pathways could have decreased the efficacy of inhibitory synapses in the right hemisphere, resulting with a conditioning pulse exerting less inhibition. Due to the present experiment design, this can not be concluded and would require further investigation.

An important factor that may play some role in the effects seen following iTMS, or TMS in general, is stimulus intensity. Varying intensities can have contrasting effects, for example, sub-threshold conditioning stimuli may have facilitatory effects, whilst suprathreshold may be inhibitory, depending also on the ISI and region of application (Kujirai et al., 1993; Ziemann et al., 1999). iTMS uses 100% resting motor threshold which is likely to be sufficient enough to activate interhemispheric fibres. It is not known if iTMS intensity were increased to suprathreshold paired stimuli, whether the effects would be more pronounced, more specifically on the contralateral hemisphere. It could be hypothesized that an increase in stimulus intensity is likely to activate a larger network of surrounding excitatory neurons and be more efficient in activating interhemispheric fibres. This could as a result alter the interhemispheric distribution, and could act on both inhibitory and facilitatory pathways.

Observations suggest that an increase in M1 excitability is partially transferred to the contralateral cortex following iTMS application. This is thought to be a result of interhemispheric pathways to the non-intervened hemisphere. What has not been explored in the present study is the effect that the increased MEP amplitude in the contralateral cortex would have on transcallosal inhibition back to the intervened hemisphere. However, there is no evidence of a decrease in MEP amplitude on the intervened hemisphere as a result of the acting inhibition from the contralateral cortex. Therefore the changing level of inhibition is not necessarily a direct result of an increase in MEP amplitude on the opposite hemisphere.

It has been put forward that the functional role of transcallosal connections is that they play an important part in bimanual coordination. Possible modes of callosal interaction outlined by Schnitzler et al. (1996) include the rapid transfer of motor commands for symmetrical bimanual movements; inhibition acting on the contralateral cortex could be important for unilateral movements; and finally, in non-symmetrical bimanual movements, transcallosal effects may modulate the onset of contralateral movements. In the case of inhibition, a reduced transcallosal inhibition is likely to result in ipsilateral activation (mirror movements) which supports the idea that inhibition is necessary for control of unilateral movements.

These present studies have reliably produced raised corticomotor excitability following iTMS, and have demonstrated that this effect is not confined to the stimulated area. It was shown that an increase in M1 cortex excitability following iTMS can be partially transferred to the contralateral cortex. Whilst facilitatory interhemispheric pathways may be involved in this phenomenon, it can be confirmed that the observation of bilateral increase in corticomotor excitability does involve transcallosal inhibitory pathways, and that the contralateral cortex is disinhibited. These findings support the idea that the effects of repetitive TMS can be distributed across motor networks. This may be important for therapeutic TMS application aiming to increase corticospinal output following neurological damage. This may be particularly useful where due to the nature of lesion, successful stimulation over the lesioned cortex is not possible or contraindicated.

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APPENDIX A

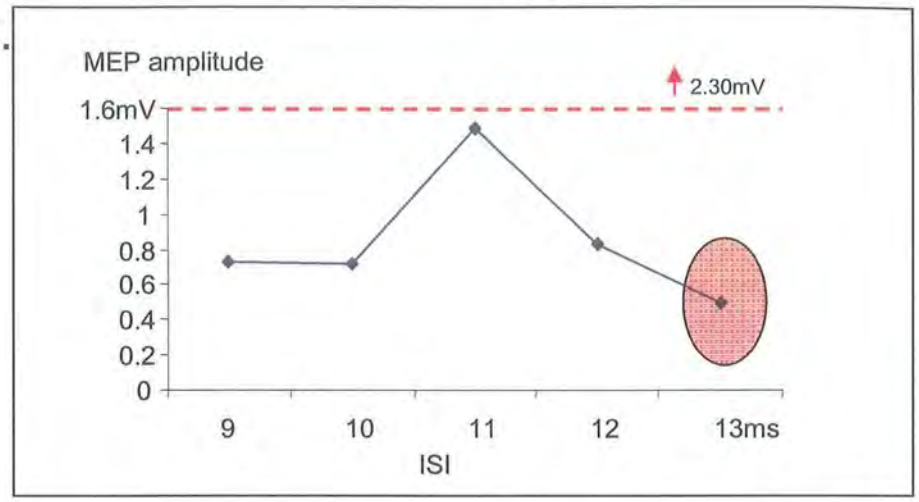
INDIVIDUAL SUBJECT

IHI DATA

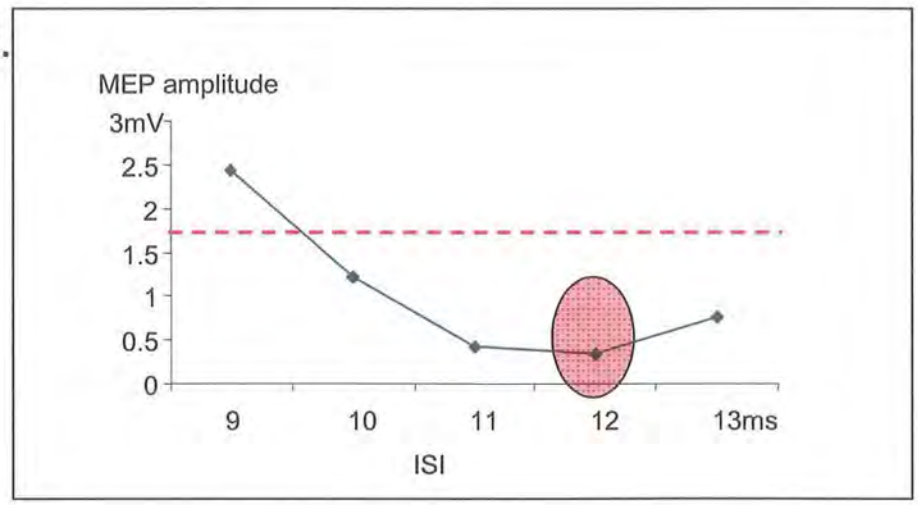
ISI used in each subject for study 2

Baseline

1.



2.



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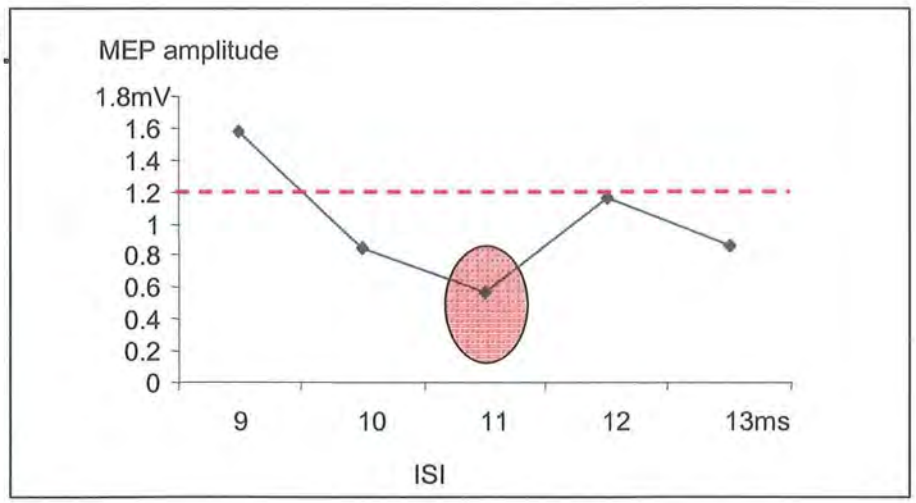

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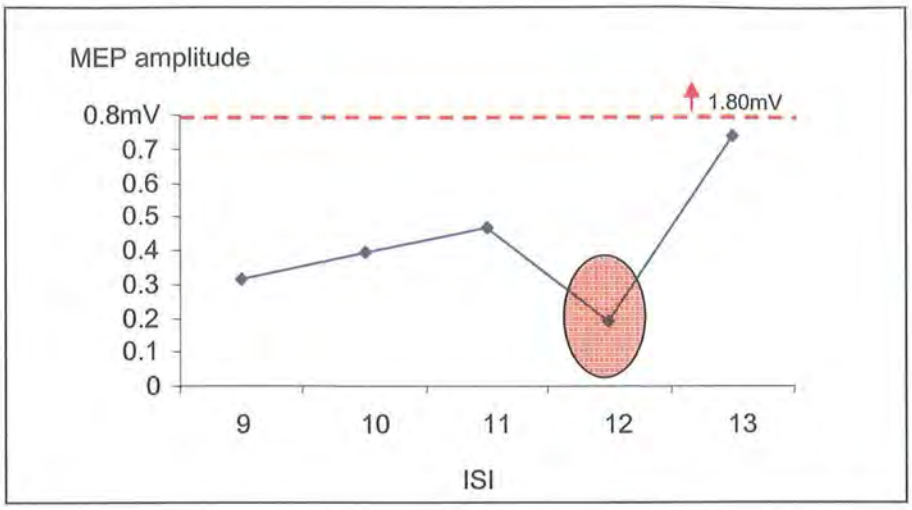
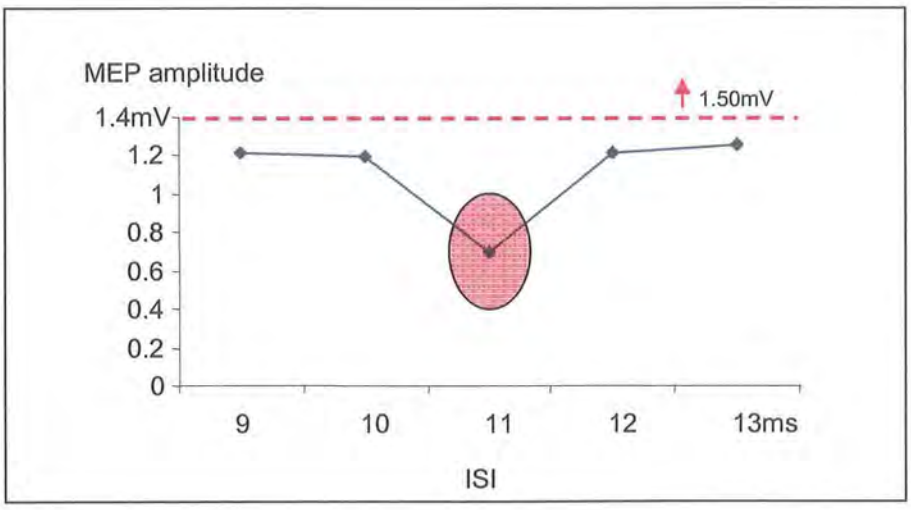
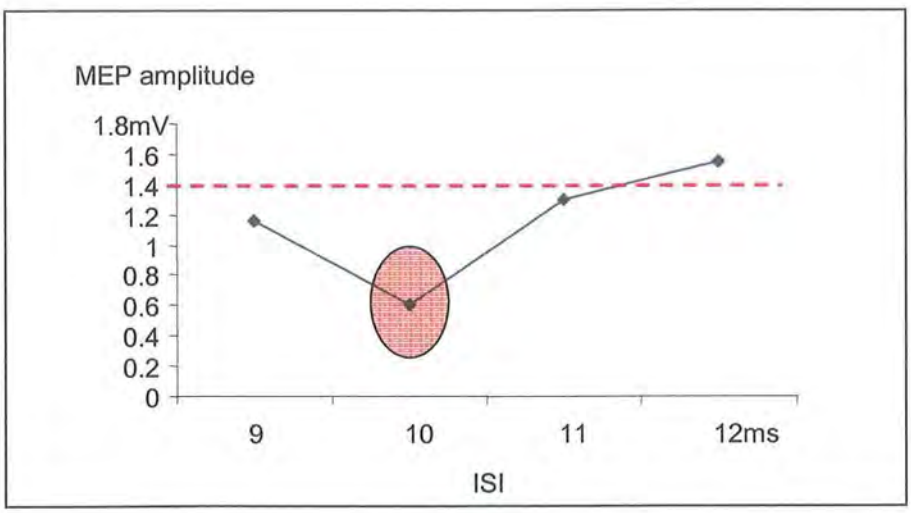

MEP amplitude
1.80mV
0.8mV
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
9
10
11
12
13
ISI

5


MEP amplitude
1.50mV
1.4mV
1.2
1
0.8
0.6
0.4
0.2
0
9
10
11
12
13ms
ISI

6


MEP amplitude
1.8mV
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
9
10
11
12ms
ISI

APPENDIX B

PRESENTATIONS ARISING

FROM THESIS

ORAL PRESENTATION
INTERNATIONAL SOCIETY OF ELECTROMYOGRAPHY
AND KINESIOLOGY CONGRESS, TORINO, ITALY, 2006

BILATERAL INCREASE IN CORTICOMOTOR EXCITABILITY FOLLOWING INTERVENTIONAL TRANSCRANIAL MAGNETIC STIMULATION (iTMS)

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AIMS:

Transcranial Magnetic Stimulation (TMS) has been recently demonstrated to have potential therapeutic benefits by promoting cortical plasticity through modulation of corticospinal excitability. We have previously shown in healthy adult subjects that paired-pulse TMS (1.5ms isi) applied over M1 at 0.2Hz for 15min (known as iTMS), can raise corticospinal excitability for a period (~10min) that outlasts the intervention. Since inter-hemispheric changes in corticomotor excitability are considered to have fundamental importance in the control of voluntary movement, and recovery of motor function following unilateral damage, the aim of the present study was to investigate if the unilateral facilitation produced by iTMS influenced contralateral corticomotor excitability.

METHODS:

In 11 healthy adult volunteers (7 Male, 18-45yrs), the mean amplitude of the Motor Evoked Potential (MEP) was recorded (single pulse, 110% resting motor threshold, optimal site for first dorsal interosseous muscle on each hemisphere) pre and post 15 min of iTMS (left hemisphere, 100% of resting motor threshold). The mean amplitude post iTMS was expressed as a percentage of the pre iTMS mean for each subject.

RESULTS:

iTMS applied over the left M1, produced a post intervention increase in MEP amplitude for the right FDI that peaked at 365% ± 44% SEM ($p<0.000$) of baseline, with a corresponding increase of 154% ±25% ($p<0.05$) in the contralateral hemisphere.

CONCLUSIONS:

The profound increase in corticomotor excitability following iTMS is partially transferred to the contralateral hemisphere. This finding strengthens the role of each hemisphere influencing the output of the contralateral hemisphere and is contrary to the current opinion that the net action of one hemisphere is to inhibit the output of the contralateral hemisphere. It remains to be determined whether this effect is acting by direct transcallosal pathways, or is a more elaborate and independent phenomenon.

APPENDIX C

INFORMATION SHEET

AND CONSENT FORM

Sir Charles Gairdner Hospital

PARTICIPANT INFORMATION

STUDY TITLE: Interventional Transcranial Magnetic Stimulation.

INVESTIGATORS: Lucy Millar / Dr Dylan Edwards / Assoc Prof Gary Thickbroom / Prof Frank Mastaglia.

AIM OF STUDY: To identify changes in the nervous system occurring as a result of repetitive magnetic stimulation.

PROCEDURE:
The procedure is non-invasive. Electrode discs will be taped onto the hand / forearm and the activity in muscles will be recorded via these electrodes and the information will be fed to a computer. Magnetic stimulation will be used. A snuggly fitting cap with pre-marked spacings will be placed on the head. A magnetic coil will be positioned on specific sites on the cap and that part of the brain will be stimulated. Each stimulation will be very short, much less than 1 second. This is not painful, but some small movements may be noticed. For example, when we stimulate the part of the brain responsible for small hand movements, the muscles in the hand will contract and a small movement of the hand will be felt. During the session, your hand will be resting relaxed on a pillow in your lap. There are very few possible discomforts associated with these procedures. On rare occasions magnetic stimulation may cause a headache. If this occurs and you wish to stop the session, we will stop the session. You may withdraw from the study at any time without prejudice.

Transcranial magnetic stimulation uses magnetism and as such, there are various factors which may exclude you from participating in this study. These include having a pacemaker or metal objects like cerebral aneurysm clips inside your body. You will be asked a series of questions to determine if there are any factors which may stop you from participating in this study.

All information obtained will remain confidential and no names will be used in any publications.

CONSENT:
The study will be carried out in a manner conforming to the principles set out by the National Health and Medical Research Council. You are free to withdraw your consent and discontinue with your participation at any time. Your participation in this study does not prejudice any right to compensation, which you may have under statute or common law.

FURTHER INFORMATION:
If you have any questions regarding this study you can contact Dr Dylan Edwards on 9346 7309 or 6304 5158.
You will be given a copy of this information sheet and consent form to read and keep prior to indicating your consent to participate by signing the consent form.

CONSENT FORM

STUDY TITLE: Interventional Transcranial Magnetic Stimulation.

INVESTIGATORS: Lucy Millar / Dr Dylan Edwards / Ass Prof Gary Thickbroom / Prof Frank Mastaglia.

I have been given clear information (verbal and written) about this study and have been given time to consider whether I want to take part.

I have been told about the possible risks of taking part in the study and I understand what I am being asked to do.

I have been able to ask questions and all questions have been answered satisfactorily.

I know that I do not have to take part in the study and that I can withdraw at any time during the study without affecting my future medical care. I understand that participation in this study does not affect any right to compensation, which I may have under statute or common law.

I agree to take part in this research study and for the data obtained to be published provided my name or other identifying information is not used.

.....	.....	.....
Name of Subject	*Signature of Subject*	*Date*

.....	.....	.....
Name of Investigator	*Signature of Investigator*	*Date*

All study participants may obtain a copy of the Information Sheet and Consent Form for their personal records upon request

APPENDIX D

MEDICAL HISTORY

QUESTIONNAIRE

MEDICAL HISTORY – Date:_____			
SURNAME:_____ GIVEN NAMES:_____ DOB:_____			
QUESTION	**YES**	**NO**	**COMMENTS**
Brain Surgery			
Shunt			
Craniotomy			
Cranioplasty / Metal Plates in Skull			
Aneurysm Clip			
Deep Brain Electrodes			
Other Devices			
Pacemaker			
Valve Replacement			
Hearing Aid			
Cochlear Implant			
Metal Foreign Bodies			
e.g. shrapnel			
Intracranial			
Orbit / Eyeball			
Other region			
Epilepsy			
Migraine			
Medication			
Braces			
Other			

APPENDIX E

SAMPLE DATA

RECORDING SHEET

IHI Study

SUBJECT:_____
DATE:_____
FILENAME:_____
STUDY:_____

CAP PLACEMENT
Nasion-inion:_____ Channel 1:_____
Inter-aural:_____ Channel 2:_____

SITES
Sites explored: _____;_____;_____;_____;_____;_____;_____;_____
Sites used:_____

THRESHOLD
TMS% intensities explored; Right_____
Left_____
Right hemisphere:
Left hemisphere:

BASELINE
Right hemisphere; 12 @_____
Conditioned (L&R); 12@ _____
Left hemisphere; 12 @_____

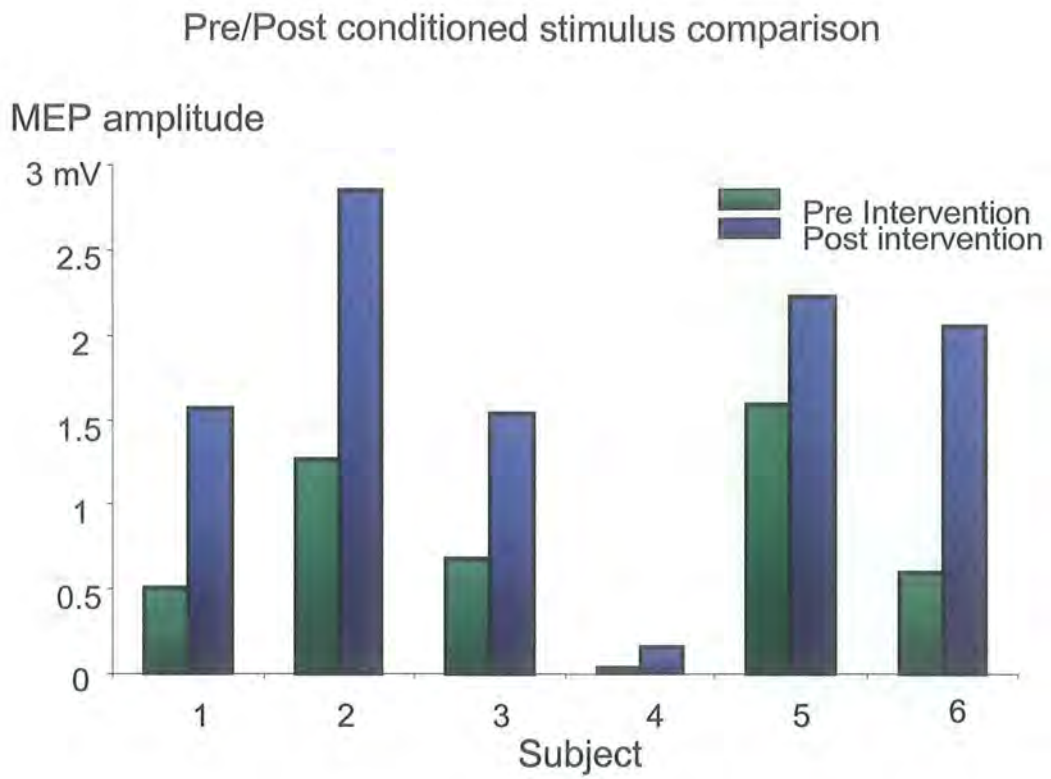
INTERVENTION
I1_____
I2_____
I3_____
I4_____

POST INTERVENTION (minutes post)
1. Conditioned 1_____
2. Unconditioned Lfdi 1_____
3. Conditioned 2_____
4. Unconditioned Lfdi 2_____
5. Conditioned 3_____
6. Unconditioned Lfdi 3_____
7. Conditioned 4_____
8. Unconditioned Lfdi 4_____
9. Conditioned 5_____
10. Unconditioned Lfdi 5_____
11. Conditioned 6_____
12. Unconditioned Lfdi 6_____
13. Conditioned 7_____
14. Unconditioned Lfdi 7_____

APPENDIX F

SUPPLEMENTARY IHI

DATA ANALYSIS



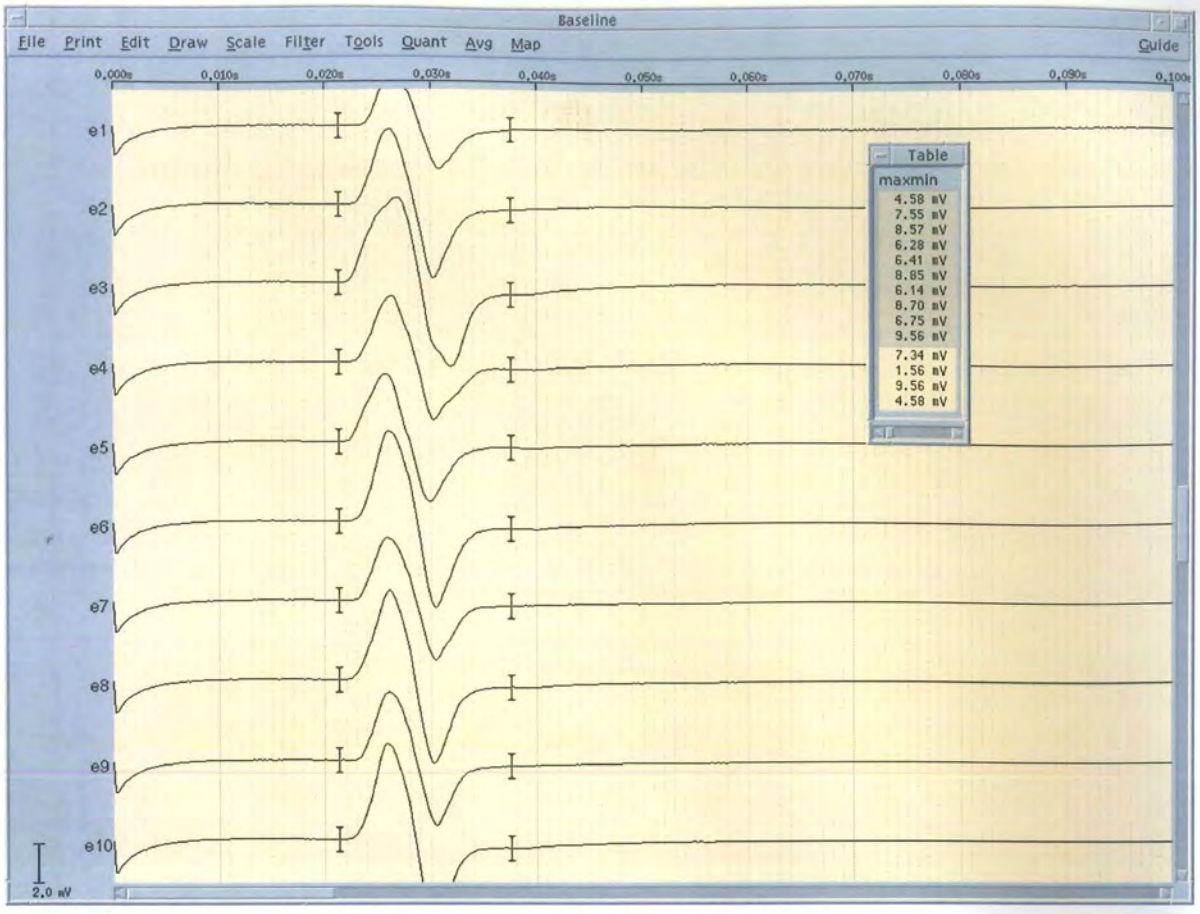
Due to individual variation over the experimental time-course. Another method of analysis used individual peak values. These were attained by taking the peak MEP amplitude and analysing using the corresponding time-point for the unconditioned stimulus. The above graph demonstrated similar results to those seen in Figure 4.11, however peak median MEP amplitude was used and compared to pre intervention baseline. Regardless of the method of analysis, a significant reduction in IHI can be noted. Analysis was also carried out for study one and two post intervention collections.

APPENDIX G

SCREEN SHOTS: MEP WAVEFORMS

Presented are sample stacked MEP waveforms with characteristic negative/positive deflection. Included is an illustration of manual cursoring used to establish peak to peak amplitude.

LFDI BASELINE



LFDI: CONDITIONED