INHIBITORY MOTOR PROCESSES DURING MOVEMENT PREPARATION

by

CHOTICA LAKSANAPHUK

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School of Sport, Exercise, and Rehabilitation Sciences
College of Life and Environmental Sciences
University of Birmingham
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ABSTRACT

The experiments presented in this thesis investigated the cortical inhibitory mechanisms mediated during the movement preparation. This was examined using response time tasks including the informative pre-cueing stimuli and conflict stimuli. The dual-coil paired-pulse transcranial magnetic stimulation (TMS) technique was used to measure the interhemispheric inhibition (IHI) occurred in both dominant and non-dominant hemispheres. The novel method of substitutional IHI measurement with the stimulus intensity to elicit the motor evoked potential of 1 mV in both hemispheres could elicit the comparable IHI in both hemispheres at rest as reported in other previous IHI studies. There was no lateralized effect of the IHI at rest. The novel method of IHI was further used to explore the cortical inhibition occurred in the motor cortex during movement preparation. It was found that the inhibition observed in the motor cortex contralateral and ipsilateral to the selected hand in order to prevent the premature response and inhibit the unwanted movement were not mediated by the opposite motor cortex.

The role of movement preparatory inhibition under the conflict condition was further investigated with the response time, EEG and single pulse TMS when the potential responses were manipulated into the homologous and non-homologous response-modes. The amount of preparatory inhibition to suppress the incorrect response induced by the conflict stimuli was influenced by the response-mode as observed in the response time, EEG, and TMS. The role of movement preparatory inhibition onto motor cortices was discussed in light of these results.

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Finally, I am proud of myself that I have the determination to make my dream come true.

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ABBREVIATIONS

APB	Abductor pollicis brevis
CC	Corpus callosum
CR	Competition resolution
CRP	Conditioned readiness potential
CS	Conditioning stimulus
CSE	Corticospinal excitability
cTS	Conditioned test-stimulus
dMFC	Dorsal medial frontal cortex
EEG	Electroencephalography
EMG	Electromyography
FDI	First dorsal interosseous
fMRI	Functional magnetic resonance imaging
IC	Impulse control
IHI	Interhemispheric inhibition
IHI_C	Interhemispheric inhibition from primary motor cortex ipsilateral to the selected effector onto primary motor cortex contralateral to the selected effector
IHI_I	Interhemispheric inhibition from primary motor cortex contralateral to the selected effector onto primary motor cortex ipsilateral to the selected effector
IHI_L	Interhemispheric inhibition from right primary motor cortex onto left primary motor cortex
IHI_R	Interhemispheric inhibition from left primary motor cortex onto right primary motor cortex

IO	Input-output
ISI	Inter-stimulus interval
LIHI	Long-latency interhemispheric inhibition
LPFC	Lateral prefrontal cortex
LRP	Lateralised readiness potential
LSP	Limb selection potential
MEP	Motor evoked potential
MPFC	Medial prefrontal cortex
MSO	Maximum stimulator output
PL	Peak latency
PMC	Primary motor cortex
PMC_C	Primary motor cortex contralateral to the selected effector
PMC_I	Primary motor cortex ipsilateral to the selected effector
PMC_L	Left primary motor cortex
PMC_R	Right primary motor cortex
PPC	Posterior parietal cortex
RMT	Resting motor threshold
rTMS	Repetitive transcranial magnetic stimulation
SI	Stimulus intensity
SIHI	Short-latency interhemispheric inhibition
TMS	Transcranial magnetic stimulation
TS	Test stimulus
uTS	Unconditioned test-stimulus

CHAPTER 1

General introduction to the movement preparatory inhibition

1.1 The control of movement

In everyday life, we interact with objects. Imagine that we see an apple on the table in front of us and we would like to reach and grasp it. All the goal directed movements require complex mechanisms of the sensory-motor coordination processed in the brain, which are classified into perception, cognition and movement execution. Perception is when we perceive the visual information from outside, in this example an apple. The visual information processing in the cerebral cortex provides us with identity, spatial information, colour, and shape of the object. Cognitive process is the internal process, which includes the stimuli analysis, manipulating the stimuli, making the decision (movement selection) and planning the associated movements. The processing of the sensory information begins in the primary sensory areas and sends the projections to prefrontal area in the frontal lobe and somatosensory association areas in the parietal cortex. The information from different sensory areas project to the premotor cortex. The movement execution is the control of the voluntary muscle contraction to produce the movement. Cortical areas that control voluntary movement are connected to basal ganglia and cerebellar circuits. Voluntary movement is mediated by the connections between the motor cortex and spinal cord, which sends the commands to the muscles (Cisek and Kalaska, 2010). The primary motor cortex (PMC) is located on the posterior-lateral side, just anterior to the central sulcus. Its fundamental function is to control voluntary movements on the contralateral side of the body. Each specific part of the body is represented in the motor homunculus that can control the movement of a specific area (Schieber, 2001). Neurons within the PMC transmit

neural impulses through the corticospinal tract, cross the body midline to terminate at the alpha motor neuron at the spinal level to activate muscles in the contralateral limbs.

In order to produce fast and precise movements, the motor cortex must receive sensory feedback from other brain areas to adjust the movement trajectory, this occurs frequently during hand movements (Bear et al., 2007, Georgopoulos, 1988). The brain areas connected to the motor cortex are the: somatosensory cortex, dorsal premotor cortex (Cincotta et al., 2004, Mochizuki et al., 2004), supplementary motor area (SMA) (Sadato et al., 1997), basal ganglia (Cincotta et al., 2006), posterior parietal cortex (Castiello, 2005, Rizzolatti and Luppino, 2001), and ipsilateral PMC (Duque et al., 2007, Hubers et al., 2008). These areas are involved in the sensory guidance, planning for the movement, and help control mirror movements when performing a unilateral movement or complex bimanual movement (Marteniuk et al., 1984, Debaere et al., 2004, Jancke et al., 2000).

Movement selection

Movement selection is one of the cognitive processes of choosing an action from among many possible alternatives (Cisek and Kalaska, 2010). A simple movement execution may be resulted from the competition between many potential actions. For example, to pick up an apple, we can use either left or right hand. It depends on the context such as the position of the object relative to the hands and the handedness (Romo and Salinas, 2003). The focus of the current thesis is how the brain achieves this so that one action is selected, whilst others are rejected. The brain area that is thought to play a role in the selection and preparation of the associated movement is the posterior parietal cortex (PPC) (Fagg and Arbib, 1998). The PPC provides the alternative options of potential movements as the brain synchronously processes the information of the

multiple potential movements (Welsh et al., 1999). It takes around 120-150 ms after the stimuli onset for the brain to provide the multiple potential movements that processes via the fronto-parietal sensorimotor control system. The multiple potential movements are presented within the different target neurons in the fronto-parietal region. The population of neurons that share similar representations can excite each other and lead to the movement activation, while the neurons that have different representations can suppress each other and lead to movement inhibition (Cisek, 2006). The decision process takes around 150 ms to integrate all the information to select the appropriate movement.

1.2 Assessment of movement preparation and selection processes using transcranial magnetic stimulation

1.2.1 Basic principles of transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS), as invented by Barker et al. (1985), is a non-invasive method of stimulating the human motor cortex in-vivo. The principle of TMS is based on electromagnetic induction as described by Faraday's law. In brief, a powerful and rapidly changing electric current is generated within the TMS coil, this produces an electromagnetic field, which induces a secondary electrical current in the underlying cortex. If the coil is held tangentially to the head, the current flow lies parallel to the surface of the skull. Pyramidal axons at a depth of 1.5-2 cm beneath the scalp will be stimulated as the electric current depolarises the transmembrane potential of the corticospinal axons (Mills et al., 1987, Barker et al., 1985, Kammer et al., 2001).

The application of supra-threshold TMS over the PMC elicits muscle contractions in the contralateral side of the body, which are recorded using electromyographic (EMG) recordings.

These motor evoked potentials (MEPs) provides a measure of corticospinal excitability (CSE) at the time of stimulation (Rothwell et al., 1987, Rossini et al., 1994) with an increase in the peak-to-peak MEP amplitude, or decrease in the onset latency indicating an increase in the excitability of the underlying neural circuitry. The resting motor threshold (RMT) is typically defined as the minimum intensity required to produce a 50 μV MEP peak-to-peak amplitude with 50% probability while target muscle is at rest (Rossini et al., 1994). RMT reflects excitability and local density of a central core of excitatory interneurons and corticospinal neurons (Mills and Nithi, 1997).

1.2.2 Physiology of the MEP

The physiological basis for the effects of cortical stimulation have been explored in detail. Initial recordings in macaque monkeys with implanted electrodes revealed that electrical stimulation applied to the exposed motor cortex produced a series of descending volleys from the cortex to the spinal cord (Amassian et al., 1987). The initial descending volley was termed a 'D-wave' as it resulted from the direct activation of corticospinal axons in the pyramidal tract. The later volleys were termed I-waves as they resulted from the indirect activation of corticospinal neurons via cortical interneurons. As reviewed by Di Lazzaro et al. (1998), who has performed extensive experiments utilising epidural recordings from the spinal cord of awake patients, transcranial magnetic stimulation produces similar effects on the human PMC. Briefly, low intensity TMS primarily recruits I-wave activity via the trans-synaptic activation of corticospinal neurons which last for approximately 3-5 ms. The corticospinal volleys travel down to the motoneurons in the spinal cord and project onto the motor units. This excitation can be recorded using EMG from the peak-to-peak MEP amplitude produced by the target muscle. As the intensity of the TMS pulse is increased, it will begin to also recruit D-wave

activity, which will proceed the initial I-wave because it activates at the proximal part of the pyramidal axon. This produces MEPs with shorter onset latencies (Day et al., 1989) the implication being that the MEP amplitude is less sensitive to fluctuations in cortical excitability.

The coil orientation can also influence the degree to which TMS recruits D- and I-wave activity. Figure-of-eight coils allow focal stimulation of the underlying cortical region. When held tangentially to the scalp, with the handle pointing 45 degrees postero-laterally to the midsagittal axis of the head, the current flows in the figure of eight coil are in an anterior-to-posterior direction, which is opposite to the current direction in the brain. Therefore, the direction of current flow in the brain is posterior-to-anterior that is perpendicular to the central sulcus (Mills et al., 1992, Brasil-Neto et al., 1992). This direction is best for producing trans-synaptic activation of the corticospinal neuron (i.e. later I-waves) when targeting over the hand motor area (Groppa et al., 2012).

1.2.3 Use of single pulse TMS to explore movement preparation and selection

Corticospinal excitability (CSE) can be evaluated in the context of simple reaction-time (RT) tasks. In these tasks, the participants are only required to make a single type of response, typically a button press when the imperative 'go' stimulus appears. Single pulse TMS has been used to measure changes in CSE at a number of time points after the imperative cue to assess the motor execution processes in the PMC. A consistent finding is that the CSE associated with the selected hand initially increases around 80-120 ms before the EMG onset (Chen et al., 1998, Leocani et al., 2000, Nikolova et al., 2006, Duque et al., 2007). However, the CSE in resting hand remains unchanged compared to the baseline (Duque et al., 2007, Leocani et al., 2000). Interestingly, Leocani et al. (2000) observed a bilateral increase in CSE in both selected and

resting hand around 180 ms before the EMG onset in the trials with slow response time. Therefore, the MEPs can be a marker to indicate when people have a delayed movement preparation process that has affected the movement execution. They suggested that the unchanged CSE in the resting hand may occur as a result of the inhibition from the PMC contralateral to the selected hand, that acts onto the PMC contralateral to the non-selected hand (Leocani et al., 2000).

Choice RT tasks contain at least two possible response choices to multiple imperative stimuli. A competitive process is generally involved in the decision making or selecting the response when there is a choice provided. The participants are required to choose one specific response that corresponds with the target stimulus indication and suppress those that do not. Therefore, in contrast to the simple RT task, the choice RT task requires both the on-line preparation and selection of the correct movement as well as the inhibition of others. Again, single-pulse TMS studies have examined CSE changes associated with these processes, including movement selection and inhibition of the non-selected movement. For example, Leocani et al. (2000), Duque et al. (2014), and Greenhouse et al. (2015b) observed the CSE suppression in the non-selected hand while the CSE in the selected hand increases before the EMG onset. This indicated facilitation of CSE in the selected hand in order to prepare for the response, while the suppression of CSE in the other hand prevents the non-required response. When the selected movement is activated in the cortical level prior to the movement onset, the homologous muscle, which is resting, undergoes inhibition (Leocani et al., 2000). The inhibitory effects that were found in the resting hand during the simple RT task and in the non-selected hand during choice RT task were thought to be produced at the cortical or spinal level (Leocani et al., 2000). This inhibition in the non-selected movement indicates the inhibitory mechanisms are involved

during movement selection to prevent the unwanted movement from the alternative competing movement responses.

Touge et al. (1998) included the warning stimulus prior to the imperative 'go' stimulus of the choice RT task in order to provide the information of the required response, which is thought to reduce the competitive process during movement selection and preparation. The participants were asked to make a speeded response when the imperative 'go' cue appeared. This cue was preceded by the informative preparatory cue which indicated the required response. Participants used the informative cue to prepare for the response in advance, but the selected action had to be withheld between the preparatory cue onset until the imperative cue onset (delay period). They observed the decrease of CSE in the selected hand during 500 ms interval between warning and imperative stimuli, indicating the inhibitory process to prevent the premature movement. This inhibition occurred prior to the changes in H-reflex, which indicated that this inhibitory mechanism processes in the cortical level.

1.2.4 Use of single-pulse TMS to explore inhibitory process during action selection

Followed on from Touge et al. (1998), Duque and Ivry (2009) used single pulse TMS to explore the inhibitory processes related to the selected and non-selected movements during an instructed-delay task, where the participants were given the advance information prior to the choice RT task. They observed a strong CSE suppression in the selected hand during the delay period, which was similar to that reported in the warning stimulus paradigm of Touge et al. (1998). It was proposed to reflect an inhibitory process, which they termed 'impulse control (IC)', that prevented premature movements during the response preparation phase. They also observed the inhibition of CSE in the non-selected hand during the delay period but the amount

of suppression was less than that observed in the selected hand. They tried to distinguish whether the IC was a global inhibition applied to both selected and non-selected hands. Alternatively they proposed that the 'inhibition for deselection' was applied to the non-selected hand while the IC was isolated to inhibit the selected hand.

The exact mechanism of IC was still unclear but it was the evident that the inhibition onto the selected response occurred at a spinal level. Duque et al. (2010) measured H-reflexes by applying electrical stimulator on the left median nerve and recording the EMG from wrist flexor muscle during the delay period when participants performing wrist movement in response to the delayed choice response time task to determine whether the IC extends to spinal circuits. The inhibition in selected response can be observed by the attenuation of H-reflex in the selected effector, indicating a parallel processing of cortical inhibition that acts onto spinal interneurons suppression (Duque et al., 2010) (see Figure 1-1). The inhibition in the selected hand or the PMC contralateral to the selected hand might occur from another brain regions, such as the frontal, cingular, and parietal areas, which affect the excitability of the corticospinal tract (Davare et al., 2008, Schmidlin et al., 2008). However, the prefrontal cortex was thought to be included in the CSE suppression in the selected hand to prevent the premature movement as it has its role to integrate the stimulus identification and select the response related to the stimulus instruction (Wallis et al., 2001, Koechlin and Summerfield, 2007). Moreover, there was evidence that the dorsal premotor cortex (PMd) and both ventrolateral medial aspects of the prefrontal cortex are involved during the early stages of response preparation and selection (Sawaguchi et al., 1996, Aron et al., 2007, Boulinguez et al., 2008, Kroeger et al., 2010).

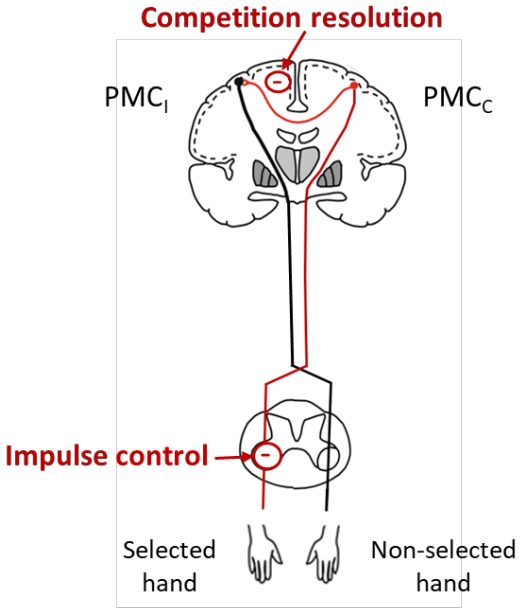

Figure 1-1. Schematic representation of two potential mechanisms of movement preparatory inhibition (indicated by red lines) as proposed by Duque and colleagues in 2009 and 2010. Impulse control (IC) is the inhibition of the selected movement to prevent a premature response, which is generated in the PMC contralateral to the selected hand (PMC_C) and acts at the spinal level. Competition resolution (CR) is the inhibition of the non-selected movement to suppress the unwanted movement, which was thought to be mediated through a transcallosal pathway.

Duque et al. (2010) also observed the CSE suppression in the non-selected hand during the instructed delay period. This was termed 'competition resolution (CR)' or the inhibition onto the non-selected candidate response. This type of inhibition was thought to be limited to the cortical level because it could not be observed by the H-reflex. They proposed that the inhibition occurred in the non-selected hand or the PMC ipsilateral to the selected hand possibly mediated from the PMC contralateral to the selected hand via a transcallosal pathway or the projection from premotor area to help sharpen the response selection in the competitive manner (Duque et al., 2010) (see Figure 1-1). Some evidence suggested that this inhibition originated from the prefrontal cortex and might include the lateral connection from the alternative response or the top-down control as the prefrontal cortex has its role in conflict monitoring and inhibit the competing response (MacDonald et al., 2000, Botvinick et al., 2001). This inhibition might help

control the mirror movement that possibly occurred when both hands were potentially selected or the response alternatives were similar (Labruna et al., 2014). Some evidences suggested that the CR occurred when there was a competition between several potential responses that were initially activated in parallel and act to inhibit each other because it helped to prevent the inappropriate response reaching the threshold, and therefore the response being executed (Coles et al., 1985, Duque and Ivry, 2009, Greenhouse et al., 2015b).

The previous sections have outlined how TMS, primarily single-pulse, has been used to establish the two main inhibitory mechanisms of impulse control and competition resolution during movement selection and preparation. Labruna and colleagues (2014) highlighted four possible models that could explain how the inhibitory mechanism of competition resolution operates to prevent the non-selected (unwanted) movements. The main difference between the models related to how the inhibitory processes were structured according to anatomical factors or the degree of similarity between potential responses (see Figure 1-2). The first two models were characterised as 'generic models' where all non-selected responses were globally inhibited to the same extent, therefore the level of inhibition would be independent from the task context. The first type of generic model is the 'self-contained' model. Here, when one PMC is preparing to execute the selected movement, a global inhibition originates within the opposite PMC that is ipsilateral to the selected movements (PMC_I) (see Figure 1-2A). In this 'dumb' situation each of the non-selected movements is inhibited in the same way. The second type of generic model is 'smart' model. Again, all non-selected movements receive the same level of inhibition but this time the inhibition originates from the PMC *contralateral* to the selected movement (PMC_C) (see Figure 1-2B).

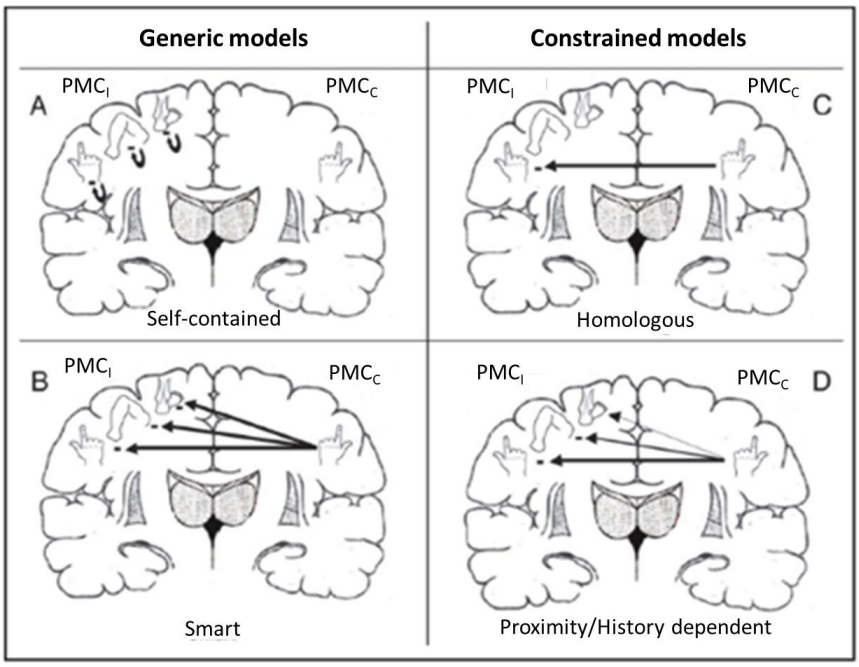

Figure 1-2. A model proposed by Labruna et al. (2014) to describe four possible architectures for the competition resolution. The generic model represents the effectors in the PMC_I were globally inhibited. The constraint model represents the non-selected task relevant effectors are selectively inhibited. (A) The non-selected effectors have the capability to inhibit themselves when they are not required to respond. (B) The selected effector has the capability to inhibit the non-selected effectors at the same amount for all of the task relevant effectors. (C) The selected effector has the capability to only inhibit the non-selected homologous effector. (D) The selected effector has the capability to inhibit the non-selected task relevant effecters with the graded amount depends on the proximity of the effectors and the experience of the effectors used.

In contrast to a generic inhibition, the inhibitory processes could also be represented as one of two type of 'constrained models' whereby the inhibition of the non-selected responses can operate in a more specific manner. The first of the constrained models is the 'homologous' model where the inhibition originates in the PMC_C and transfers to the opposite PMC but only acts on the homologous movement. The implication being that non-selected movements which involve non-homologous muscles will not receive inhibition (see Figure 1-2C). The final and second of the constrained models is the 'proximity/history-dependent' model (see Figure 1-2D). Again, the inhibition transfers from the PMC_C to the opposite hemisphere, but this time the strength of inhibition is graded by the response similarity (homologous) and/or the extent to which the possible, but non-selected, movements were previously paired with the selected

movement (i.e. history of competition between the movements). Labruna et al. (2014) observed that MEPs suppression in the PMC_I elicited from a single pulse TMS was graded depending on the proximity of the motor area in left and right PMCs. This potentially result from the PMC_C. However, they did not use an appropriate protocol to test the effect of interhemispheric inhibition and thus their hypothesis was unproven. Therefore, it was not totally be explained that the different amount of CR that occurred onto the PMC_I resulted from the excitation in the contralateral PMC. Moreover, Greenhouse et al. (2015b) reported the MEP suppression in PMC_I in other task irrelevant as observed in the decrease of MEPs measured from the other muscles even it was not the competitive alternating response. This suggested that the CR is a more general inhibitory process that acts on suppression of the other effectors' excitability to lower the background activity of other task irrelevant to allow the selected response to achieve the threshold easily in order to initiate the response.

1.2.5 Use of Dual coil TMS to explore connectivity in human cortex

The main supported model from Labruna et al. (2014), which is the proximity/history dependent model indicates that the inhibition transfers across hemispheres potentially from the interhemispheric inhibition through the corpus callosum pathway.

Anatomy and function of the corpus callosum

The corpus callosum (CC) is the largest neural pathway located beneath the longitudinal fissure of the cerebral cortex that connects the two cortical hemispheres (Banich, 1995). White matter bundle of fibres connecting two PMCs allow the coordination between left and right side of the body. Geometric and histological subdivision of the CC originally reported that the large, fast conducting and highly myelinated motor fibres connecting the PMCs pass through the anterior section of the CC mid-body (Witelson, 1989, Aboitiz et al., 1992, Gooijers and Swinnen, 2014).

Whereas, the sensory fibres from the primary sensory cortex and posterior parietal cortex pass through the posterior section of the mid-body of the CC (Gooijers and Swinnen, 2014). However, in-vivo imaging techniques in humans, primarily diffusion tensor imaging, have revealed that the white matter tracts of the primary motor fibres actually cross the CC more posteriorly (Hofer and Frahm, 2006, Wahl et al., 2007).

The CC allows cognitive and sensory information to be integrated between hemispheres and plays an important role in bimanual co-ordination (Gooijers and Swinnen, 2014). The signals transmitting information through the CC can have both excitatory and inhibitory influences. During normal unimanual movements the CC is required to mediate inhibitory processes to prevent mirror movements and suppress unwanted movements, which indicates an inhibitory influence. Ultimately, the balance of cortical excitability within and between the motor hemispheres will determine which movements are performed and coordinated (Aboitiz et al., 1992, Ferbert et al., 1992, Ugawa et al., 1993, Bloom and Hynd, 2005).

The findings that the disruption of inter-hemispheric information transfers through the corpus callosum agenesis or callosal lesions leads to a deficiency of bimanual movement and co-ordination, would suggest an excitatory influence (Lassonde et al., 1991, Paul et al., 2007). The dorsal premotor cortex has an important role during the performance of asymmetrical bimanual or unimanual movements. When the right dorsal premotor cortex was disrupted during left index finger abduction, the excitability in the PMC_I was increased, which reflects an increase of the mirror movement in the non-selected right-hand (Cincotta et al., 2004). The pathway between dorsal premotor cortex to the contralateral PMC was connected via transcallosal fibre (Mochizuki et al., 2004). A study in patients with impaired supplementary motor area (SMA)

showed that there was a mirror movement when writing and performing a co-ordination of bimanual movement (Chan and Ross, 1988, Laplane et al., 1977). The pathway from SMA projected bilaterally to PMC, premotor cortex, and contralateral SMA via the corpus callosum (Grefkes et al., 2008, Sadato et al., 1997). For the basal ganglia, it sends the indirect pathway to the SMA via thalamus. There was the evidence in patients with Parkinson's disease who had impaired basal ganglia that exhibited the mirror movement. This resulted from the increased of excitatory in the PMC_I due to loss of cortical inhibition when performing a voluntary movement (Cincotta et al., 2006).

Use of Dual coil TMS to explore cortico-cortical connection

Dual-coil TMS allows investigation of cortico-cortical connection, such as parieto-motor connections or interhemispheric connections within the human brain (Ferbert et al., 1992, Koch et al., 2009, Davare et al., 2008). Two coils are placed on the participants scalp. A 'test stimulus' (TS) is applied through the first coil which is placed over the PMC to elicit MEPs from the contralateral target muscle. The second coil is placed over a cortical region, such as the PMC, that is expectedly linked to the contralateral motor cortex. A 'conditioning stimulus' (CS) is applied through the second coil prior to the TS to determine what effect it has on the output of the PMC. If the MEP amplitude elicited by the TS decreases then the second cortical region is said to provide an inhibitory influence on the PMC. Whereas if the MEP amplitude increases, the cortical region is said to provide an excitatory influence.

Ferbert et al. (1992) first explored the interhemispheric interactions between the left and right PMC using a dual-coil TMS design. A TMS coil was positioned over the hand motor area of each PMC to determine the influence of inter-hemisphere connections on PMC excitability.

The main finding was that when the initial CS was applied 6 to 10 ms prior the TS over the contralateral PMC, it reduced the MEP amplitude by 50% as compared to the 'unconditioned' MEP. The inhibition produced with inter-stimulus intervals (ISI) of 6 to 12 ms was originally proposed to occur at the cortical level via a direct transcallosal pathway and was therefore termed short-latency IHI (SIHI) (Ferbert et al., 1992). The initial evidence was that the TS elicited by transcranial electrical stimulation or H-reflexes were unaffected by the CS with these ISI (Ferbert et al., 1992). Subsequent experiments using recordings of descending corticospinal volleys in humans confirmed this by showing that the CS inhibited the latter I-waves elicited by the TS (Di Lazzaro et al., 1999). The suppression of latter I-waves indicated that this inhibition occurred at the trans-synaptic level of the corticospinal neuron. The use of longer CS-TS intervals of 40 to 50 ms also produces a similar inhibition of MEP amplitudes at rest but it shows a minimal change of IHI when the target muscle is activated (Ridding et al., 2000, Chen et al., 2003). This is termed long-latency interhemispheric inhibition (LIHI) and is thought to occur via indirect pathways involving premotor regions (Ni et al., 2009).

Di Lazzaro et al. (1999) evaluated the IHI mechanism by recording the descending spinal volleys through high cervical epidural electrodes implanted in human after the TS was applied over one PMC and measured how it was influenced by the CS over the other hemisphere. They evaluated the effect of CS on the amplitude of the I-wave evoked by the TS and compared these changes to the EMG response changes measured from the left first dorsal interosseous (FDI) muscle. After receiving the CS prior to the TS at a variety of ISI, the first I-wave (I_1-wave) was unaffected at all ISI ranges, while the I_2-wave was less affected when using the ISI of 9-11 ms compared to the single-TS trials. There was a clear suppression of later I-wave (I_3-wave) when using the ISI of 6-10 ms. With the EMG response, there was a clear suppression at the ISI of 6-

11 ms. The suppression that was found in both the cortical level and the hand EMG activity simultaneously indicated that the EMG suppression in the hand muscle caused by the CS over the contralateral PMC, which affected the descending of corticospinal pathway that is directed to the hand muscle. Therefore, the EMG recording can be used as the indication of the inhibition of corticospinal excitability. In line with Ferbert et al. (1992) and Meyer et al. (1995), they concluded that when applying the supra-threshold TMS over one PMC, it suppressed the cortical activity in the contralateral PMC at 6-11 ms later via transcallosal connection. This was supported by the findings that the inhibition was generated via a transcallosal connection. The callosal conduction time had an onset latency of 8-9 ms and a duration of 7-15 ms obtained with electrical and magnetic stimulation corresponded with the ISI of 5-6 ms or longer that could generate a distinct inhibition onto the opposite hemisphere (Cracco et al., 1989, Saron and Davidson, 1989). However, the inhibition found in the early I-wave was generated by a different cortical neuron in a pyramidal cell compared to the inhibition found in the later I-waves (Di Lazzaro et al., 1998).

1.2.6 Roles of IHI in movement preparation

The appropriate amount of IHI between the PMC_C and PMC_I is required during unilateral movement preparation in order to control the mirror movement or unwanted movement in the non-selected hand. When the individual prepared to respond to a simple RT task with unilateral movement, the CSE (as measured by single pulse TMS) showed a reduction of MEPs in the PMC_I once after the 'go' signal, while the MEPs in the PMC_C progressively increased close to the movement onset. This inhibition of the MEPs in the PMC_I occurred to prevent the mirror movement while the increase of the MEPs in the PMC_C occurred to prepare for the response (Pascual-Leone et al., 1998, Chen et al., 1998, Leocani et al., 2000). These modulations of the

CSE in both PMCs was thought to be mediated by the interaction between PMC_C and PMC_I. Therefore, the IHI experiment had been conducted in a simple RT task to evaluate whether the CSE changes were mediated by the IHI. The IHI from PMC_I onto PMC_C (IHI_C) was increased after the 'go' signal and release when it was close to the movement onset, leading to increased CSE in the PMC_C as measured from the single pulse TMS. This indicated that the disinhibition of IHI_C helped generate the response in the selected hand. The IHI from PMC_C onto PMC_I (IHI_I) showed the inhibition after the 'go' signal and remained unchanged throughout the movement preparation period as the non-selected side was not required to respond to the task, which was corresponded to the MEP suppression in the PMC_I to inhibit the unwanted movement. This indicated that the IHI_I helped prevent the mirror movement in the non-selected hand (Murase et al., 2004, Duque et al., 2005a, Duque et al., 2007).

Kroeger et al. (2010) observed the mechanisms of the IC and CR as measured from the MEPs elicited from a single pulse TMS in the right PMC during the 2000 ms-delayed choice reaction time task. They also measured the IHI from the left PMC targeting onto the right PMC during the delay period when it was both contralateral and ipsilateral to the selected hand. The IHI_I was stronger than IHI_C during the delay period and these were stronger than the baseline. However, the IHI_C was initially increased and followed by a release of IHI when it was close to the movement onset to allow initiation of the movement. This was in line with the studies that explored the IHI during the simple RT (Murase et al., 2004, Duque et al., 2005a, Duque et al., 2007) as they found the IHI targeting on the PMC_I prevented the unwanted movement. These findings supported the 'proximity/history dependent' model from Labruna et al. (2014) that the PMC_C has the capability to inhibit the PMC_I through transcallosal pathway. However, Duque et al. (2007) reported the lateralised effect of IHI measured in the right-handed

participants. When responding with the dominant right-hand, the IHI_C was weaker than the IHI_I. While the IHI_C and IHI_I were comparable when participants responded with the non-dominant left-hand. Therefore, this suggested that the imbalance of IHI when responding with right-hand occurred to suppress the PMC_I as left-hand tended to display more mirror movement when the right-hand was selected.

1.3 Summary and aims of this thesis

I investigated whether the movement preparatory inhibition that occurred in the PMC ipsilateral to the selected effector inhibited the non-required movement is mediated by the PMC contralateral to the selected effector. These were based on a supporting models to explain that the competition resolution mechanism was mediated by many brain areas including the contralateral PMC to globally suppress the potential movements. From the literature review, I hypothesised that the inhibition occurred in the PMC contralateral to the selected hand to prevent the premature movement would not be mediated by the opposite PMC as it was self-inhibition within that PMC.

In order to explore the movement preparatory inhibition mechanisms in a comprehensive manner, I first developed a novel method for testing bilateral IHI in a quicker way in chapter 2. I focused on optimising the IHI measurement technique by controlling the factor that could interfere the amount of IHI. This was done by adjusting the stimulus intensity used to produce the IHI effect between two hemispheres by trying to eliminate the lateralized effect between dominant and non-dominant hemispheres. Specifically, the novel method of IHI measurement allowed me to measure the IHI in both hemispheres in the same experiment block because the total number of trials could be reduced by 50%. The aim was to compare if the novel IHI

technique could elicit a similar amount of IHI compared to the conventional method as it has been previously reported. Chapter 3 was then extended the findings from chapter 2 in terms of using the novel method of IHI measurement to evaluate whether the movement preparatory inhibition found in the PMC_C and PMC_I as measured by a single pulse TMS were mediated from the contralateral PMC.

The studies in chapter 4 and 5 focused on the movement preparatory inhibition under the conflict condition. These were included two limb systems as categorized into the homologous response-mode, where the potential responses were paired in homologous effectors, and non-homologous response-mode, where the potential responses were paired in different limb effectors. I used electroencephalography (EEG) to explore the correct response activation and incorrect response inhibition in a conflict condition when responding with two different response-modes. Based on the proximity/history dependent model, the inhibition onto the non-selected response was graded by the history of competition between the potential response alternatives. Therefore, I hypothesised that the amount of the incorrect response activation would be stronger when the response was made with the non-homologous response-mode as the amount of inhibition to suppress the incorrect response was thought to be lower when responding with the non-homologous response-mode. The EEG results would be correlated to the response time as the response time in the non-homologous response-mode would be slower as the incorrect response activation was stronger when compared to the homologous response-mode. Chapter 5 then applied the EEG findings from chapter 4 to determine the TMS timings to explore the CSE changes that reflects the amount of correct and incorrect response activations during a conflict task. I also expected that the MEPs would correspond with the response times and EEG findings.

CHAPTER 2

No effect of hemisphere dominance on the resting interhemispheric inhibition (IHI); a comparison of conventional versus substitutional approaches

2.1 Introduction

As outlined in the general introduction, the study reported in this chapter explored resting IHI in both left and right PMC using the dual coil TMS approach of Ferbert et al. (1992). These effects likely represent transcallosal inhibition mediated via the corpus callosum (Ferbert et al., 1992, Wahl et al., 2007, Ni et al., 2009). Left IHI (IHI_L) refers to the measurement of transcallosal inhibition from the right PMC onto left PMC. This is achieved by applying the conditioning TMS pulse over the right PMC and the test TMS pulse over the left PMC 10 ms later. Right IHI (IHI_R) refers to the same method but with the conditioning TMS pulse over the left PMC and the test TMS pulse over the right PMC. A TS intensity that elicits MEPs of 1 mV (termed the stimulus intensity (SI)-1mV) is typically used with the CS at supra-threshold intensity to produce SIHI. On average, this provides inhibitory effects of approximately 50%, which reduce as the TS intensity increases (Ferbert et al., 1992, Daskalakis et al., 2002b). The magnitude of SIHI is also determined by the CS intensity. Subthreshold CS intensities do not effectively produce SIHI (De Gennaro et al., 2004a), but a CS intensity of SI-1mV typically produces a SIHI of 50% onto the opposite PMC (Chen et al., 2003), and this effect increases as the CS intensity is increased (Chen et al., 2003, Uehara et al., 2013).

Novel approach used in current study

Several studies published in the past decade have a limitation when using single pulse TMS over one PMC to measure the MEP changes in a single hand during a uni-manual hand movement because the MEPs were measured when the hand was selected and non-selected in a different trials (Verleger et al., 2009, Klein et al., 2012, Labruna et al., 2014, Duque et al., 2014, Greenhouse et al., 2015b). The same issue applies to experimental designs for studies wanting to explore IHI effects in these tasks as adding double TMS trials (CS and TS pulses) will double the length of the experiment unless either the number of MEPs averaged in each condition or number of variables tested reduce accordingly. The former approach may negatively impact on the validity of the MEP measures (Cuypers et al., 2014) and the latter approach may reduce the insights gained from the study, for example, in previous studies, IHI was only measured from one hemisphere targeting a single hand at a time (Baumer et al., 2006, Chiou et al., 2013, Uehara et al., 2013). This approach was unable to observe the IHI in both left and right PMCs at a time. The results found in one PMC would possibly be applied onto the other PMC without direct measurement. Therefore, if there was the dominant effect on the IHI, it could not be detected with this approach.

For the reasons outlined above, the current study was designed to collect resting IHI-10ms measurements from both left and right hand muscles using a faster method. This was achieved by setting both the CS and TS to an intensity of SI-1 mV and running the typical IHI protocol with a mixture of single-TMS trials and the double-TMS trials in both PMCs (see Figure 2-1). A primary aim was to determine whether it was possible to obtain the same resting IHI effect in left and right PMCs when calculated using the conventional ratio approach or the novel substitutional ratio approach (see Equation 2-1A and B). The conventional IHI ratio is the mean

conditioned-test MEP amplitude divided by the mean unconditioned-test MEP amplitude (see Equation 2-1A). Our substitutional approach utilised the fact that we recorded the MEP amplitudes in the contralateral hand muscles resulting from *both* the CS and the conditioned TS in the double-TMS trials. The novel insight is that the MEPs elicited by the conditioning-stimulus in the double-TMS trials could provide the same measure of corticospinal excitability as the unconditioned-test MEPs in the single-TMS trials. To test this hypothesis, the mean unconditioned-test MEP was substituted for the conditioning-stimulus MEP. The substituted IHI ratio was then calculated as the conditioned-test MEP divided by the conditioning-stimulus MEP (see Equation 2-1B); enabling a direct comparison against the results of the conventional approach. The full methods used in the two approaches are also outlined in Figure 2-1 (page 28).

A) $$Conventional\ approach\ of\ IHI\ ratio = \left(\frac{mean\ conditioned\ TS\ MEP}{mean\ unconditioned\ TS\ MEP}\right)$$

B) $$Substitutional\ approach\ of\ IHI\ ratio = \left(\frac{mean\ conditioned\ TS\ MEP}{mean\ CS\ MEP}\right)$$

Equation 2-1. (A) Conventional approach for calculating the IHI ratio in terms of the mean of cTS MEP elicited from the double-TMS trials relatives to the mean of uTS MEP elicited from the single-TMS trials within the same PMC (Ferbert et al., 1992). (B) Substitutional approach for calculating the IHI ratio when substituting the mean CS MEP for the mean unconditioned TS MEP. The IHI ratio is the value of mean cTS MEP relatives to mean CS MEP when there were elicited within the same PMC.

The substitutional approach relies on two main assumptions. The first is that during the double-TMS trials, the MEP amplitude elicited by the CS will be unaffected by the later TS applied over the opposite PMC. To our knowledge this has not yet been directly explored, however, Duque and colleagues recently reported a new method to obtain bilateral MEPs from both hands simultaneously during tasks exploring movement preparation processes (Wilhelm et al., 2016, Grandjean et al., 2018, Vassiliadis et al., 2018). In all three studies, TMS was applied to both

PMCs with only 1 ms interval between the first and second pulses. The results consistently demonstrated that there was no interference between the first and second TMS stimuli on the bilateral MEP amplitudes for stimulus intensities ranging from 100 to 160% RMT. Our view is that if the initial MEP is unaffected by a later TMS pulse to the opposite PMC only 1 ms later then it will also be unaffected by one that is 10 ms later (as will be the case in our double-TMS trials).

An additional aim of the current study was to determine if there was a hemisphere dominance effect on resting SIHI when both the conditioning and test TMS pulses were set to the same relative stimulation intensity (i.e. SI-1mV). This is the basis for testing the second assumption behind our substitutional approach. If there is no hemisphere dominance effect on resting SIHI with SI-1mV, with both the conventional and substitutional approaches then we could apply the same intensity to both PMC for all conditioning and test stimuli. This would then allow us to remove the need for the single-TMS trials as the MEPs elicited by unconditioned-test stimulus would be replaced by the MEPs elicited by the conditioning stimulus. Although this may only have a minor impact on short experiments such as measuring resting SIHI, it would significantly reduce the time required to explore changes in movement related changes in corticospinal excitability and IHI during longer tasks such as the instructed-delay. This would avoid the need to only investigate one hemisphere per experimental session (Duque and Ivry, 2009).

In terms of dominance effects, IHI is thought to be more pronounced from the dominant onto non-dominant PMC (Duque et al., 2007, Netz et al., 1995). Functional magnetic resonance imaging (fMRI) and diffusion tensor imaging studies suggest this is possibly due to the

asymmetrical connections from PMC to other areas of the brain being broader in the dominant hemisphere (Guye et al., 2003). However, this remains controversial as Duque et al. (2007) reported no dominance effect of IHI at rest in right handed participants. This had been evaluated in both left and right handed participants. At rest, the IHI effects were similar across the two hemispheres (De Gennaro et al., 2004a).

The use of different stimulation intensities may provide a possible explanation for the inconsistent findings. It is possible that the hand dominance effects on SIHI caused by the dominant hemisphere received a relatively higher level of stimulation. For example, some studies demonstrate a comparable RMT for dominant and non-dominant hemispheres (Civardi et al., 2000, Rossini et al., 1992), while others have reported it to be lower when measured over the dominant hemisphere (De Gennaro et al., 2004a, Baumer et al., 2007). If this isn't taken into account and a fixed percentage of maximum stimulator output is applied to the PMC (Chen et al., 2003) then it would be expected that the hemisphere with lower RMT would receive a relatively higher level of stimulation. Baseline differences in RMT are commonly adjusted for by scaling the stimulus intensity relative to the % RMT. However, the use of input-output (IO) curves, first described by Devanne et al. (1997), which display the relationship between the TMS intensity and the resulting MEP amplitude from threshold to maximum responses. The correlation between MEP amplitude and TMS intensity used is a sigmoidal graph where the slope starts increasing at the intensity of motor threshold and a plateau phase is in the upper end of the curve which used high intensities evoked MEP (Vallence et al., 2015). This may be problematic. Daligadu et al. (2013) observed that in right handed participants, although both PMC showed comparable RMTs, the right 'non-dominant' PMC showed a steeper IO curve. The asymmetrical change in corticospinal excitability indicates that at 120% RMT, the non-

dominant PMC could be responding in a physiologically different way to the stimulation, which in turn could affect the manner in which IHI is elicited. As outlined in the general introduction, current evidence indicates that increasing the CS intensity enhances the inhibitory effect (Ugawa et al., 1993, Chen et al., 2003, Gennaro et al., 2004), whilst increasing the TS intensity reduces the inhibitory effect (Ferbert et al., 1992, Daskalakis et al., 2002b).

It is difficult to directly assess whether differences in stimulation intensity is a key factor in producing lateralised IHI effects due to the variety of different ways that the CS and TS intensities have been reported. For example, in %MSO (Chen et al., 2003), % RMT (Netz et al., 1995, Hinder et al., 2018, De Gennaro et al., 2004b), a fixed level of MEP amplitude such as SI-1mV (Baumer et al., 2007, Perez and Cohen, 2008, Uehara et al., 2013), or the CS intensity to elicit SIHI of 40-50% when using a TS of SI-1mV (Duque et al., 2007, Morishita et al., 2014). The IHI laterality study from Baumer et al. (2007) reported that the RMT in left- and right-handed participants were lower in left PMC compared to right PMC. They set the CS related to 120% RMT and TS at SI-1mV for both left- and right-handed participants. It was found that right-handed participants had a stronger IHI drive from their dominant PMC, while IHI drive from the non-dominant PMC was stronger in left-handed participants. Therefore, in both cases, the IHI effect from left onto right PMC was stronger. The study from Netz et al. (1995) who report the lateralised IHI in left and right handed participants, used both the TS and CS intensities of 105% RMT. The TS of 105% RMT evoked the TS MEPs of 0.2-0.8 mV, while the CS of 105% RMT evoked the CS MEP of 0.5-1.5 mV. However, the IHI study from De Gennaro et al. (2004b) who reported no dominant hemispheric effect on the IHI in left and right handed participants, used both the CS and TS intensities of 120% RMT. However, they observed a lower RMT in dominant PMC in both left and right handed participants. Duque et

al. (2007) who also reported no dominant hemispheric effect on the IHI only tested in right handed participants used the CS intensity to elicit the IHI effect of 50% and the TS of SI-1mV. These studies used different protocol of the stimulus intensities. The approaches that used different intensities between two PMCs would possibly have stimulated one PMC more than the other hence why the lateralised effects may have occurred.

The hypothesis of the current study is that by setting the stimulation intensity of both the conditioning and test TMS pulses to SI-1mV, the motor output is fixed to the stimulation. This means that the physiological response (ie. the pattern of D- and I-wave elicited which depend on the level of TMS intensity) to the stimulation should be consistent in both PMC and will provide a firm basis for exploring whether lateralised IHI effects exist. In summary, this study will explore the IHI effect in left and right hemispheres at rest and compare the conventional protocol to the novel approach of substitutional technique. If the CS MEP can be used to calculate the IHI ratio then this would allow us to assess the IHI in a much shorter experiment as we could eliminate the single-TMS trials. This would open up the opportunity to test for the effects of movement preparation and selection on bilateral IHI measures during the instructed delay task within a single session.

2.2 Methods

Participants

Based on sample sizes used in previous research, twenty-seven healthy volunteers (mean age 23.3 ± 5.7 years, seventeen males) participated in this study. All participants were right-handed as determined by the Edinburgh Handedness inventory (Oldfield, 1971). All participants gave written informed consent prior to starting the experiment. The protocol was approved by the

Science, Technology, Engineering and Mathematics (STEM) ethical review committee of the University of Birmingham, UK. The TMS safety screening questionnaire (Keel et al., 2001, Rossi et al., 2009) was administered before the experiment to ensure that participants were suitable to receive the TMS.

Electromyography (EMG)

EMG recordings were obtained with a Bagnoli 2-Channel EMG system (Delsys) placed on the belly of the left and right FDI muscles. The reference electrode was placed on the olecranon process of right elbow. The EMG signal was bandpass filtered (20-500 Hz) and digitized with a sampling rate of 2000 Hz using a Micro 1401 analogue to digital converter (CED) and transferred for offline analysis on a pc with Signal software Version 6.01 (CED).

Transcranial magnetic stimulation (TMS)

TMS was performed using two monophasic MagStim 200^2 stimulators (The Magstim Company, Whitland, UK). Each stimulator was connected to an identical 50 mm figure-of-eight coil, the size of which allowed them to be placed concurrently over the motor hotspots of the left and right PMC with the optimal orientations to produce low-threshold MEPs in contralateral FDI muscles. The coils were placed tangentially over each PMC with the coil handles pointing backwards about 45 degrees away from the midsagittal line and perpendicular to the central sulcus, which induces an induced brain current that flows in the postero-anterior direction (Mills et al., 1992, Chen et al., 2003) to activate the motor cortex trans-synaptically (Werhahn et al., 1994). The use of two small coils avoided the problem of some previous studies who found it impossible to keep both coils similarly oriented in a 45 degree fashion when using standard 70 mm coils (Duque et al., 2007, Harris-Love et al., 2007, Ni et al., 2009). The location and trajectory of each motor hotspot was marked using Brainsight™ version 2.2 (Rogue

Research Inc), which allowed simultaneous tracking of both coils throughout the duration of the study.

RMT was defined as the minimum stimulation intensity that produced MEP amplitudes of at least 50 μV on 5 out of 10 consecutive trials in resting FDI muscles (Rossini et al., 1999). A 6 second ISI was used and the RMT of left and right FDI muscles were determined in independent blocks. The RMT of each hemisphere was measured and expressed as a percentage of maximum stimulator output (% MSO).

Procedure

Determining stimulus intensity

For the main experimental session, both the CS and TS intensities were set to produce mean peak-to-peak MEP amplitudes of 1 mV in their respective resting FDI muscles (Ferbert et al., 1992, Boroojerdi et al., 1996). By setting the CS and TS to the same intensity, the IHI effect in both hemispheres could be measured with identical parameters, therefore the MEP from the conditioning-stimulus potentially could be used as a substitute for the unconditioned-test MEPs (single-TMS trials) to calculate the IHI effect. That is the IHI ratio calculated in Equation 2 1A could be swapped for Equation 2 1B. In order to find an accurate SI-1mv, we started with a stimulus intensity of 120% of an individual's RMT and readjusted until it produced a MEP of approximately 0.8-1.2 mV. Cuypers et al. (2014) and Biabani et al. (2018) report that the highest reliable mean MEP amplitudes require mid 20s trials within a session when using a stimulus intensities of 110 and 120% RMT and SI-1mV to reach a probability of 0.90 for hitting the 95% confidence interval, therefore 30 TMS pulses with an inter-trial interval of 6 secs were applied over the left PMC (PMCL) and right PMC (PMCR) separately. Trials with high background

EMG or coil position errors were removed prior to the calculation of the mean MEP amplitude. If the mean MEP amplitude was less than 0.8 or greater than 1.2 mV, the intensity was adjusted prior to delivering another 30 TMS pulses until the SI-1mV was found. This procedure was then repeated for the opposite PMC.

Determining the IHI effect

After establishing the SI-mV for each hemisphere, we determined the IHI-10ms effect for both IHI_R and IHI_L. Note that the subscript letter refers to the hemisphere receiving the test stimulus. Therefore, for the IHI_R, the conditioning stimulus was applied over PMC_L to elicit MEPs in the right FDI and the test stimulus was applied over PMC_R to elicit the MEPs in the left FDI (see Figure 2-1B). This means that IHI_R was testing the inhibitory effect from PMC_L onto PMC_R. This order was reversed when measuring IHI_L, which tested the inhibitory effect from PMC_R onto PMC_L (see Figure 2-1D). In addition to these two double-TMS trials, we also included two types of single-TMS trials, where one pulse of TMS was applied to either the PMC_R to elicit unconditioned MEPs in left FDI (see Figure 2-1A), or PMC_L to elicit unconditioned MEPs in the right FDI (see Figure 2-1C). We collected 30 trials of each condition (120 total) across two blocks with a five minutes rest in-between. Trial types were equally split across the blocks and presented in a random order with an inter-trial interval of 6 seconds. The experimental lasted 1 hour in total.

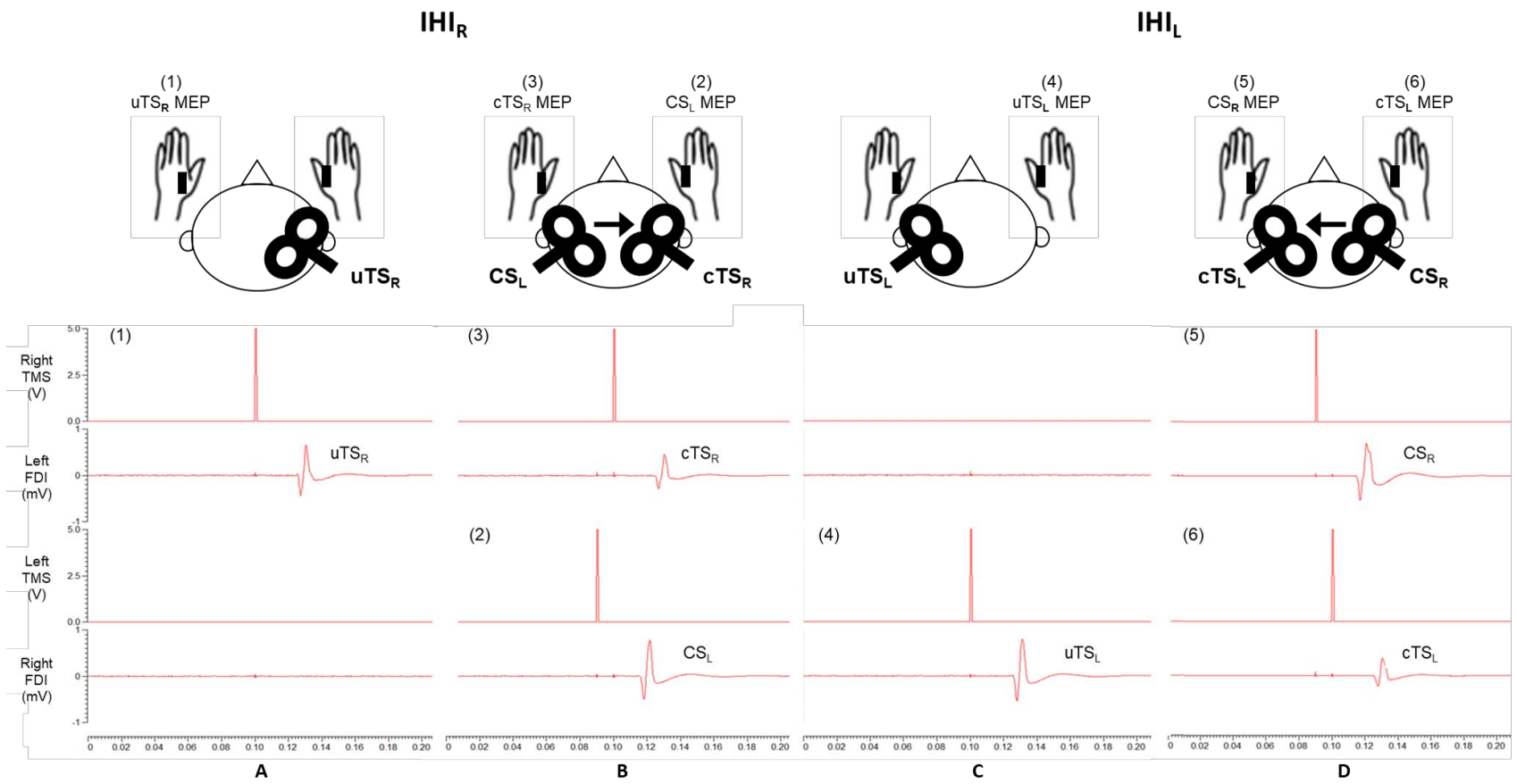

Figure 2-1. A schematic representation of the coil position and MEP recording from each hand when measured the IHI_R (IHI from PMC_L onto PMC_R) and IHI_L (IHI from PMC_R onto PMC_L). The measurement of IHI_R was first applied a single-TS stimulus over PMC_R (uTS_R) to elicit the unconditioned TS_R MEP (uTS_R MEP) in left FDI (1). Then, delivered the conditioning stimulus over PMC_L (CS_L) 10 ms prior to the cTS_R. This double-TMS trial would elicit the CS_L MEP in the right FDI (2) and the conditioned TS MEP (cTS_R MEP) in the left FDI (3). The conventional approach measuring IHI_R was to calculate the ratio of cTS_R MEP/uTS_R MEP or (3)/(1) to express the amount of MEP amplitude difference resulting from the cTS relative to the uTS. The measurement of IHI_L was first applied uTS_L to elicit the uTS_L MEP in right FDI (4). Then, delivered the CS_R 10 ms prior to the cTS_L. This CS-TS trial would elicit the CS_R MEP in the left FDI (5) and the cTS_L MEP in the right FDI (6). The conventional approach measuring IHI_L was to calculate the ratio of cTS_L MEP/uTS_L MEP or (6)/(4). The substitutional approach of measuring IHI was substituted the CS MEP for uTS MEP. Therefore, the measurement of IHI_R was calculated from the ratio of cTS_R MEP/CS_R MEP or (3)/(5), and the IHI_L was to calculate the ratio of cTS_L MEP/CS_L MEP or (6)/(2).

Data analysis

The background EMG activity was measured from both FDI muscles in the 11 to 61 ms before the CS and TS onsets. This time window avoided the stimulation artefact produced by the CS and TS impacting the background EMG measure. The magnitude of resting IHI increases during small contractions (5 to 10% of maximum) of the hand contralateral to the CS (Ferbert et al., 1992, Uehara et al., 2013). This may be a result of increasing descending cortical output and can remove any lateralised effect of IHI (Uehara et al. 2013). To ensure that both FDI muscles were at rest, trials were discarded from further analysis when the peak-to-peak background EMG activity of either FDI muscle exceeded 20 μV (McAllister et al., 2013). Maximal IHI occurs when the conditioning coil is placed over the motor hand area, but decreases with both 2 cm shifts either medially or laterally from that spot (Ferbert et al., 1992). Therefore, trials were also excluded if the location of either coil moved away more than 3 mm or 5 degrees from the original motor hotspot (Schmidt et al., 2015). Overall, a mean 9.2% of trials were excluded across each participant.

In both single-TMS and double-TMS trials, the peak-to-peak MEP amplitude of the contralateral FDI muscle was measured in the 10-50 ms after the CS and TS onset. The IHI effect was first expressed as the MEP amplitude resulting from the conditioned-test MEP in the double-TMS trials relative to that of the unconditioned-test MEP in the single-TMS trials (cTS MEP/uTS MEP) as displays in Equation 2-1A (see Figure 2-1). However, in double-TMS trials, the FDI MEP amplitude was also measured in the hand contralateral to the conditioning stimulus. As outlined within the introduction, we aimed to determine whether we would obtain the same IHI ratio when substituting the MEP elicited from the conditioning stimulus for the

unconditioned-test MEP. Figure 2-1B provides an example of a double-TMS trial when IHI_R was measured with the conditioning stimulus applied over the PMC_L and the test stimulus over PMC_R. The MEPs elicited from the conditioning stimulus over PMC_L as measured from right FDI in these trials (see label (2) in Figure 2-1B) were substituted for the unconditioned-test MEPs obtained from PMC_L as measured from right FDI during the single-TMS trials (see label (4) in Figure 2-1C). Figure 2-1D is an example of a double-TMS trial when IHI_L was measured with a conditioning stimulus applied over the PMC_R and the test stimulus over PMC_L. The MEPs elicited from the conditioning stimulus over PMC_R as measured from left FDI in these trials (see label (5) in Figure 2-1D) were substituted for the unconditioned-test MEPs obtained from PMC_R as measured in the left FDI during the single-TMS trials (see label (1) in Figure 2-1A).

Statistical analysis

The first aim of the current study was to determine whether it was possible to obtain a resting IHI effect when both the CS and the TS intensities were set to SI-1 mV. The second aim was to determine whether there was a hemisphere dominance effect on IHI such that it is greater when the CS is applied over dominant hemisphere and TS over non-dominant hemisphere (IHI_R). As mentioned in the introduction, the CS and TS intensity may influence the IHI ratio. Therefore, the stimulus intensities used to evoke a MEP of 1 mV and the relative RMTs that these produced within each participant were first compared between left and right PMCs using paired-samples t-tests to confirm whether they were consistent. Then a repeated measures ANOVA was run with factors of 2 PMC-SIDE (PMC_L, PMC_R) x 2 TEST-stimulus (unconditioned, conditioned) on the mean MEP amplitudes recorded in the contralateral FDI. The IHI_R and IHI_L were subsequently calculated and the results were compared using a paired-

sample t-test. The third and main aim of the current study was to determine whether the MEPs elicited from the conditioning stimulus obtained in the double-TMS trials could substitute for unconditioned-test MEP in the single-TMS trials. For this, another repeated measure ANOVA with factors of 2 PMC-SIDE (PMC_L, PMC_R) x 2 STIMULUS (conditioning, conditioned-test) was performed on the mean MEP amplitudes in the contralateral FDI. A paired-samples t-test was performed on the mean resting IHI to compare the difference between the substitutional approach and the conventional approach in PMC_L and PMC_R separately. Statistical testing was conducted with IBM SPSS Statistics 24 software package. Alpha level for statistical significance was set at 0.05. Post-hoc comparisons were conducted using the Sidak procedure.

2.3 Results

Table 1 shows the stimulation parameters used on the left and right PMCs. A paired-samples t-test revealed that the RMT conducted by the monophasic TMS pulse current showed no significant different between PMC_L (43.4 ± 1.6% MSO) and PMC_R (43.1 ± 1.7% MSO), t(df) = 26, P = 0.74. Moreover, the intensity used related to the % RMT and % MSO to evoke 1mV of MEP peak-to-peak amplitude of PMC_L (136.3 ± 3.4% RMT and 59.0 ± 2.4 % MSO) and PMC_R (137. 3 ± 3.5% RMT and 59.1 ± 2.7% MSO) were comparable (t(df) = 26, P = 0.67 and t(df) = 26, P = 0.96, respectively). Paired-samples t-tests also confirmed that the MEPs elicited from the conditioning stimulus over PMC_L (969.0 ± 69.5 μV) were not significantly different from the MEPs elicited from the conditioning stimulus over PMC_R (887.7 ± 51.8 μV, t(df) = 26, P = 0.29). The same was true for the unconditioned-test MEPs elicited from PMC_L (937. 0 ± 54.7 μV) vs. the unconditioned-test MEPs elicited from PMC_R (919.2 ± 45.7 μV, t(df) = 26, P = 0.76). The lack of a between-hemisphere difference in RMT or MEP amplitudes confirmed that both PMCs were stimulated in a comparable way, therefore any

lateralisation of the SIHI effect would likely relate to a dominance effect in the way that the inhibitory process was generated or acted on the opposite hemisphere.

Table 1. Comparative means for parameters between left and right hemispheres (n=27). Significant differences between each condition were examined for using paired t-tests.

IHI and parameters	Left PMC (mean ± SE)	Right PMC (mean ± SE)	t(df)	P value
RMT (% MSO)	43.4 ± 1.6	43.1 ± 1.7	26	.74
Stimulus intensity of 1mV				
• % RMT	136.3 ± 3.4	137. 3 ± 3.5	26	.67
• % MSO	59.0 ± 2.4	59.1 ± 2.7	26	.96
Conditioning stimulus MEP (μV)	969.0 ± 69.5	887.7 ± 51.8	26	.29
Test stimulus MEP (μV)	937. 0 ± 54.7	919.2 ± 45.7	26	.76
IHI	0.58 ± 0.04	0.65 ± 0.04	26	.23

Figure 2-2 shows the MEP amplitudes evoked by the conditioning stimulus, the unconditioned-test, and conditioned-test in both left and right PMCs. We first examined whether the current protocol produced a resting IHI effect when calculated using the conventional method. A repeated measures ANOVA applied on the unconditioned- and conditioned-test MEPs revealed a significant main effect of TEST-stimulus ($F_{1,26} = 85.0$, $P < 0.001$) but there was no main effect of PMC-SIDE ($F_{1,26} = 0.4$, $P = 0.53$) nor an interaction of these factors ($F_{1,26} = 2.2$, $P = 0.15$). This analysis indicates that the conditioning stimulus decreased the MEP amplitude (i.e. produced an IHI effect in both PMC_L (drop of 416.0 ± 61.8 μV) and PMC_R (drop of 319.1 ± 38.2 μV). A paired t-test (t(df) = 26, P = 0.23) conducted on the mean resting IHI_L and IHI_R

ratios (0.58 ± 0.04 vs. 0.65 ± 0.04, see Table 1) indicated that there was no detectable effect of hand dominance on resting IHI as measured with the conventional method.

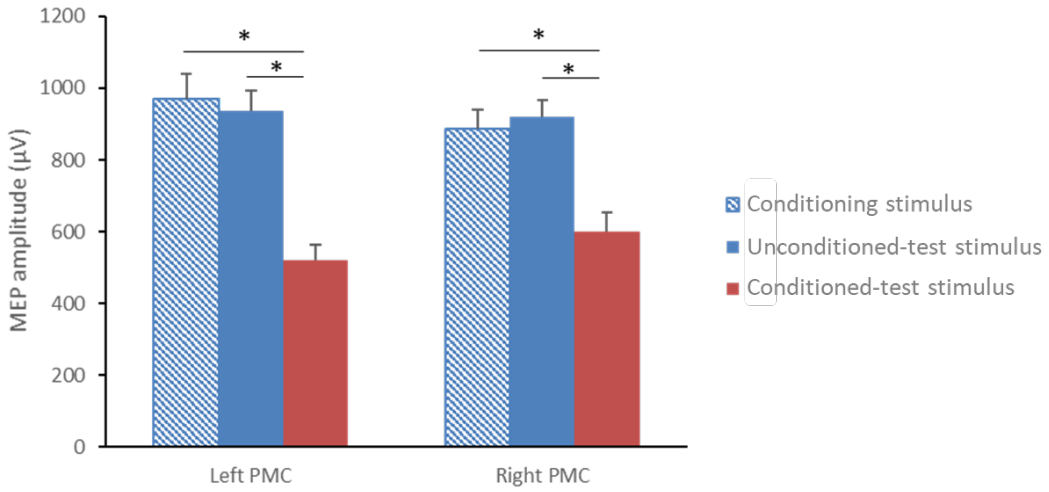

Figure 2-2. Interhemispheric inhibition (IHI) tested over left and right PMCs. To test the effect of IHI from right to left PMCs, TS was delivered to left PMC and CS was delivered to right PMC. Then the effect of conditioning stimulus onto the test stimulus was recorded as the conditioned-test MEP in left PMC in the double-TMS trials in relative to the unconditioned-test MEP in left PMC in the single-TMS trial and vice versa for the IHI from left to right PMC. When substituting the unconditioned-test MEP with conditioning stimulus MEP, the effect of the conditioning stimulus that reflect the IHI was similarly observed. * Indicates statistically significant differences ($P < 0.001$).

In the next analysis, the IHI ratio was calculated with the substitutional approach. Here the conditioning-stimulus MEPs in the double-TMS trials replaced the unconditioned-test MEPs from the single-TMS trials (see Figure 2-1 page 28). A repeated measure ANOVA revealed a significant main effect of STIMULUS ($F_{1,26}$ = 84.4, $P < 0.001$). Post-hoc pairwise comparisons revealed that the mean conditioned-test MEP was 367.9 ± 40.1 μV lower than the conditioning-stimulus MEP ($P < 0.001$). This indicated the presence of an IHI effect with the substitutional approach. There was no main effect of PMC-SIDE ($F_{1,26}$ = 0.0, P = 0.98) nor an interaction of PMC-SIDE and STIMULUS ($F_{1,26}$ = 3.7, P = 0.065). Post-hoc pairwise comparisons revealed that there was a comparable drop in MEP amplitude between the

conditioning-stimulus MEP and conditioned-test MEP in both PMC_L (drop of 448.0 ± 68.7 μV, $P < 0.001$) and PMC_R (drop of 287.8 ± 44.0 μV, $P < 0.001$). The paired t-test ($t(df) = 26$, $P = 0.104$) conducted on the mean resting IHI_L and IHI_R ratios (0.57 ± 0.05 vs. 0.68 ± 0.04) indicated that there was no detectable effect of hand dominance on resting IHI as measured with the substitutional method. In the final analysis, another set of paired t-tests confirmed that there were no significant differences in IHI_L (0.58 ± 0.04 conventional vs. 0.57 ± 0.05 substitutional, $t(df) = 26$, $P = 0.60$) or IHI_R (0.65 ± 0.04 conventional vs. 0.68 ± 0.04 substitutional, $t(df) = 26$, $P = 0.11$) between the two methods.

2.4 Discussion

This experiment demonstrated that it is possible to achieve a reliable level of resting IHI-10ms when using CS and TS intensities that, when given in isolation, elicited MEP amplitudes of 1 mV in the contralateral FDI muscles. Overall, we obtained resting IHI effects of 0.58 to 0.65 in the left and right PMCs, and this 35-42% reduction in MEP amplitude is comparable to that reported in previous studies (Ferbert et al., 1992, Duque et al., 2005b, Duque et al., 2007, Morishita et al., 2014). The novel approach of this study was that by setting both the CS and TS to SI-1mV, it was possible to substitute the MEPs obtained with the unconditioned TS in the single-TMS trials for those obtained with the CS in the double-TMS trials. These results demonstrated that the substitution approach reliably produced a similar level of IHI as the conventional approach, which were 0.57 to 0.68 in the left and right PMCs (equivalent to 32-43% reduction).

Mechanism of interhemispheric inhibition

The sites of corticospinal activation depends on the stimulator and stimulation technique. When using a transcranial electrical stimulation or a TMS with latero-medial coil direction, it produces the direct activation at the corticospinal axons resulting in D-wave. When using a TMS with anterior-posterior coil current direction, it produces an indirect activation of the corticospinal neurons at the axon of excitatory interneurons resulting in I-wave. Low intensity TMS is thought to activate corticospinal neurons trans-synaptically (Di Lazzaro et al., 1998). The resulting I-waves travel down the corticospinal tract to depolarise the spinal motoneurons and initiate MEPs in the contralateral hand muscles. With the SI-1mv intensity applied in the current study, the TMS may elicit a mixture of D- and I-waves, but predominately the latter, which means that the test-stimulus MEPs are sensitive to fluctuations in cortical excitability. The conditioning stimulus applied over the opposite PMC will activate inhibitory interneurons in the test PMC via the transcallosal pathway. This can take approximately 13 ms (Amassian et al., 1987), hence why the conditioning stimulus needs to be applied prior to the test stimulus with ISI of between 6-13 ms (Ferbert et al., 1992). In the current experiment, an ISI of 10 ms was used and by doing so it is likely that the excitability of the test PMC will be reduced during the initiation of the later I-wave volleys.

The effect of stimulus intensity on interhemispheric inhibition

The original IHI study of Ferbert et al. (1992) described inhibitory effects of around 0.4-0.5 or 50-60%, therefore, a popular approach for exploring IHI effects is to set the intensity of test stimulus to elicit mean MEP amplitude in the 0.5 to 1.5 mV range and the conditioning stimulus such that it produces an IHI ratio of 0.5 to 0.7 (Duque et al. 2005, Duque et al. 2007, Morishita et al. 2014). This would generally necessitate setting the conditioning and test stimuli to

different intensities during the double TMS trials and therefore it would not be possible to apply the substitutional analysis. Alternatively, we could have, however, set the intensity of both conditioning and test stimuli as a % of the RMT of each PMC. We choose not to do this as it has been reported that the input/output curves of each PMC may differ (Daligadu et al., 2013). If so this may have led to us stimulating one hemisphere to a greater extent and it is known that the inhibitory effect increases as the CS intensity increases (Ugawa et al., 1993, Chen et al., 2003, De Gennaro et al., 2004a). Instead, the physiological response was fixed to the stimulation (i.e. the MEP amplitude) by setting both the conditioning and test TMS pulse to SI-1mV. The rationale was that this should elicit similar patterns of D- and I-wave activity in both PMC and therefore any lateralised effects are not confounded by differences in stimulation intensity.

The SI-1mV values used in the main IHI experiment were determined in initial sets of single-TMS trials. It would not be possible to obtain a mean MEP amplitude of exactly 1 mV in each participant therefore we set a range of 0.8 to 1.2 mV. As seen in Table 1, when looking at the overall group averages this produced mean MEP amplitudes ranging from 0.89-0.97 mV. One limitation of the TMS approach is the inherent variability of MEP amplitudes. This is evidenced in that even although we used identical stimulation parameters in the main experiment, the mean MEP amplitudes of individual participants could now range from approximately 0.6 to 1.6 mV instead of the original 0.8 to 1.2 mV. It should be noted, however, that as shown in Figure 2-2, the overall group mean MEP amplitudes obtained from both the conditioning stimulus and the unconditioned-test stimulus were very similar to that of the baseline measures. Furthermore, they produced resting IHIs of 0.58 and 0.65, which

is in line with other studies where they used the supra-threshold stimulus intensities for CS and TS (Duque et al., 2005a, Duque et al., 2007, Baumer et al., 2007, Hinder et al., 2018).

In the current study, we attempted to reduce the mean MEP amplitude variability by collecting 30 trials in each condition; both when determining the initial SI-1mV in each PMC and also for each type of single-TMS and double-TMS trial in the main IHI part of the study. This approach was primarily based on Cuypers et al. (2014) who demonstrated that mean MEP amplitudes recorded using TMS intensities of 110 and 120% RMT showed the same results in terms of the 0.99 probability with 26 consecutive stimuli. The probability reduced to 0.86 and 0.71 with only 20 and 15 repetitions, respectively. On average 9.2 ± 1.8% of the total trials were rejected in each condition, either due to high baseline EMG activity or sub-optimal coil positions. This left a minimum of 25 trials remained in each of the TMS measurement conditions in every participant. Therefore, this could be confident that the mean MEP amplitudes had good reliability. Studies exploring IHI effects with low number of trials may have had less reliable responses which can lead to a higher incidence of false positive effects. For example, Baumer et al. (2007) used 10 trials of the double-TMS condition and found IHI lateralisation effects.

No interhemispheric dominance effect

Similar levels of IHI were found in both dominant and non-dominant PMCs in this group of right-handed participants. This suggests that there was no hand dominance effect on resting IHI-10ms, which fits with the results of De Gennaro et al. (2004b) who also examined the IHI with a variety of ISI in left- and right-handed participants and used a lower 120% RMT intensity for both CS and TS. In contrast to our results, Netz et al. (1995) and Baumer et al. (2007), who examined both left- and right-handed participants, reported a stronger IHI-10ms effect from

PMC_L to PMC_R in right-handed, but not left-handed participants. However, the experimental set up was different to our study as Netz et al. (1995) measured the IHI_L and IHI_R whilst the participants performed a tonic contraction of the FDI contralateral to the PMC where the CS was applied to maximize the interhemispheric effect, while the present experiment measured the IHI when both hands were relaxed. Many previous studies reported that the right PMC has a higher RMT than the left PMC regardless of the handedness of the participant (Baumer et al., 2007, Macdonell et al., 1991, Netz et al., 1995, Helmich et al., 2005). Baumer et al. (2007) suggested that the hemispheric difference affected the laterality of IHI. They also proposed that the dominance effects were rather weak because it could only be established in 66% of the right-handed and 57.5% in left-handed participants. In this study, RMTs were very similar in both left and right PMCs, which indicates that it was not influenced by handedness (all right-handed) in our group of participants.

The method used in this current study of setting the stimulus intensity of SI-1mV could be a solution for the neural drive asymmetry between dominant and non-dominant hemispheres that was found when using the stimulus intensity in relative value to the % RMT. Daligadu et al. (2013) investigated the asymmetry in neural drive between dominant and non-dominant hemispheres by generating the stimulus-response curve in left and right hands in right handed participants. When using the similar stimulus intensity relative to the % RMT, it evoked a higher MEP amplitudes in non-dominant PMC_R than dominant PMC_L. This suggests that the non-dominant PMC had an increased activation when compared to dominant PMC. The different MEPs between dominant and non-dominant PMCs when using the TMS intensity related to the % RMT to investigate the preparatory inhibition in both PMCs was also reported by Klein et al. (2016). However, it was in contrast to Daligadu et al. (2013) as they found that

the dominant PMC had lower RMT than non-dominant PMC and it resulted in a different intensity in terms of %MSO used to get 115 %RMT, which caused higher MEPs in dominant PMC than non-dominant PMC elicited at baseline measurement. Therefore, they changed the protocol to set the TMS intensity to SI-1mV. Interestingly, the TMS intensity in terms of %MSO was not different between dominant and non-dominant PMCs and the MEPs were comparable between both PMCs.

In this experiment, the advantage of no hemispheric dominance effect in right-handed participants allowed us to stimulate both PMCs with the intensity of SI-1mV. If there was a hemispheric dominant effect as presented by a different RMT between left and right PMCs, there would have shown a high IHI in one PMC and low IHI in the other PMC when the CS and TS intensities were set to SI-1mV for both PMCs because the dominant PMC would have a stronger drive onto non-dominant PMC. In this study, there was no dominant hemispheric effect on resting IHI. We ensured that it was not confounded by the stimulus parameters or other factors that affected the amount of IHI because similar RMT, SI-1mV, CS MEPs, unconditioned-test MEP, and conditioned-test MEP were observed in both dominant and non-dominant PMCs. One limitation is that the IHI was only tested with an ISI of 10 ms, therefore we are unable to say whether the substitutional approach is valid to examine the other form of IHI. For example, LIHI with the ISI of 40 ms, which is normally used to measure the inhibition from the other cortical areas that project onto PMC (Ni and Chen, 2011).

Corticospinal excitability is influenced by the participant's level of wakefulness or alertness (Gerloff et al., 1998, Ziemann et al., 1996). This might affect the amount of MEPs elicited with the SI-1mV and the amount of IHI. During the current experiment, participants were told to

keep their head still, and were allowed to blink but not close their eyes. There was no eye fixation point to keep the participant focused. These factors potentially made the level of alertness fluctuate in between participants. It is therefore suggested that future experiments should have the eye fixated point to reduce the variability of participants' alertness affected the corticospinal excitability.

Conclusion

The present study investigated the level of IHI-10ms, produced when setting the intensity of both the test and conditioning TMS pulses to SI-1 mV. Using the conventional approach could evoke a resting IHI of around 35-42% without hemispheric dominant effect. The substitution of the MEP elicited by the conditioning stimulus for the unconditioned-test MEP produced a similar IHI reduction of 32-43% without hemispheric dominant effect. Although the current experiments were fairly short (within 1 hour), the same approach could be used when studying IHI during the performance of unimanual or coordinated bimanual movements, which typically require much longer experiments. Indeed, the second study of this thesis explored the inhibitory mechanisms during movement preparation using the instructed-delay task and the role of interhemispheric interactions. Here, IHI-10ms was measured from bilateral PMCs with all stimulation intensities set at SI-1mV. As will be shown within the next chapter, the use of the substitutional approach allowed us to considerably shorten the length of the experiments as the need for single-TMS trials could be eliminated.

CHAPTER 3

Inhibitory motor processes during unilateral movement preparation in the instructed-delay task

3.1 Introduction

In everyday life, making a decision of hand response selection and preparation are required to interact with the various environments. The PMC is involved in motor program for a movement execution. Premotor area and supplementary motor areas also play an important role in movement preparation (Rouiller et al., 1994, Alexander and Crutcher, 1990). The dynamic interaction between left and right hemispheres is thought to enhance skilled and coordinated movements. The homologous areas of left and right PMCs are connected via the corpus callosum. When one PMC is activated, it can send excitatory impulses to the inhibitory interneurons in the contralateral PMC in order to inhibit its output excitability (Daskalakis et al., 2002a).

As outlined in the general introduction, an instructed-delay task has been used to study the mechanisms of IC and CR during movement selection and preparation (Touge et al., 1998, Duque and Ivry, 2009). Hinder et al. (2018) used dual-coil TMS to observe changes of IC and CR mechanisms by measuring CSE and IHI-10ms prior to the response onset in a 500 ms-delayed period of a choice reaction time task. Corticospinal excitability and IHI were measured at the preparatory cue onset, the imperative cue onset, and at three time-point prior to the response onset. MEPs elicited from the PMC contralateral to the selected hand (PMC_C) were inhibited at the imperative cue and turned to facilitation afterwards, while the MEPs elicited

from the PMC ipsilateral to the selected hand (PMC_I) were constantly inhibited at every time-point. When using IHI-10ms, there was a release of IHI_C (inhibition from the PMC_I to the PMC_C) at every time-point compared to baseline IHI, indicating a movement preparation in the selected hand. However, this pattern did not correspond to the changes of MEPs. This indicated that the IHI_C was not associated with the MEP suppression in the PMC_C. While the IHI_I (inhibition from the PMC_C to the PMC_I) remained unchanged at every time-points compared to the baseline, which corresponded to the MEPs suppression measured from the PMC_I. However, the study from Hinder et al. (2018) focused on the movement preparatory inhibition during movement execution period as they measured the CSE after the imperative cue until prior to the EMG onset. Therefore, the current study would focus on the movement preparatory inhibition during movement selection and preparation as observed with the modulation of CSE and IHI-10ms during the delay period of a choice reaction time task.

Labruna et al. (2014) suggested that the MEPs inhibition in PMC_I, resulting from CR, *was* affected by the response-mode; CSE of the non-selected hand reduced when the selected response was the hand and arm, but did not change when it was the foot. Therefore, the proximity/history dependent model was appropriate to explain the possible mechanism of CR (see Figure 1-2 page 11). The MEPs suppression in the PMC_C was stronger than the PMC_I in each condition, which suggested that IC was stronger than the CR and mediated from separate mechanisms. However, this has a limitation since they used a single pulse TMS to explore whether the MEPs reduction in the PMC_I was influenced by interhemispheric interaction from the PMC_C during the delay period of a choice RT task. Therefore, they could only confirm that the inhibition actually occurred but they could not identify whether it came from the opposite hemisphere. However, the inhibitory preparatory processes were influenced by the delay period

(Lebon et al., 2016). When participants could anticipate the onset of imperative cue, the inhibitory process occurs during a response preparation as the MEPs suppression were found only when it was close to the movement onset in the long delay period (≥ 500 ms). This indicated that the magnitude of MEPs suppression corresponded with the state of motor planning and the amount of time provided for the response preparation.

This current study evaluated the related mechanisms of the inhibitory process during 900 ms-delayed response task. I expected to observe a progress of MEP suppression from early, middle, and late delay period, which would show a clear inhibition at the late delay period (Lebon et al., 2016). If the variable delay periods were used, the participants could not anticipate the response. This would not allow us to get a reliable MEP suppression in both PMC_C and PMC_I across the delay period. Based on previous chapter, the similar IHI protocol would be used to investigate whether the impulse control mechanism that occurs on the PMC_C involves IHI_C (inhibition from the PMC_I to the PMC_C), and/or whether the competition resolution mechanism that occurs on the PMC_I involves IHI_I (inhibition from the PMC_C to the PMC_I). Stronger MEPs suppression or decrease in corticospinal excitability would be associated with an increase of IHI. If impulse control originated from the PMC_I acting upon the PMC_C, the IHI would be significantly increased. If the competition resolution found in the PMC_I originated from the PMC_C, less amount of IHI_I would be observed as compared to the IHI_C because there was the evidence that the IC was stronger than the CR (Labruna et al., 2014, Klein et al., 2016). By using the IHI protocol, the hypothesized result of the competition resolution could provide some supporting evidence for using the proximity/history dependent model to describe the CR mechanism that the PMC_C had the capability to specifically inhibit the alternative homologous response effector in the PMC_I.

3.2 Methods

Participants

Based on sample sizes used in previous research, twenty-one healthy subjects participated in this study (10 women; 24 $\pm$ 0.8 years old). All participants were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). All participants completed a TMS safety screening questionnaire before testing to ensure that they were no contraindications to the TMS and provided written informed consent. The protocol was approved by the STEM ethics committee of the University of Birmingham.

Instructed-delay task

Participants sat comfortably 70 cm in front of the computer screen with both hands resting on a wooden board placed over participants' lap, palms down with the elbows slightly flexed. The pegs on the board were designed to separate and restrain index from thumb and middle fingers (see Figure 3-1). During the experiment, participants were required to abduct their left or right index finger until it touched the wooden square block 'target', which was located in the middle of the peg board.

The instructed-delay task of Duque and Ivry (2009), which was used in this study, was implemented in E-prime 2.0 software (Psychology Software Tools, Pennsylvania, USA). The task protocol was described as a virtual soccer game (see Figure 3-2). Each trial began with the presentation of a central fixation cross for 100 ms, which was followed by a blank screen for 900 ms. After that, a central preparatory cue was presented at the centre of the screen and remained for 900 ms, which consisted of either an ')' or '('. The preparatory cue was always informative and indicated whether the participant should prepare a left [')'] or right ['('] index

finger response. The imperative cue appeared after the fixed delay period of 900 ms and consisted of a black circle next to the opening of the bracket (i.e. the ball was presented on the left side of the bracket when the bracket was opened to the left and vice versa). The position of the circle indicated whether the participant should abduct either their left or right index finger as if to push the 'ball' into the 'goal'. The cues remained for 300 ms and the screen went blank for 3000 ms before the next trial began.

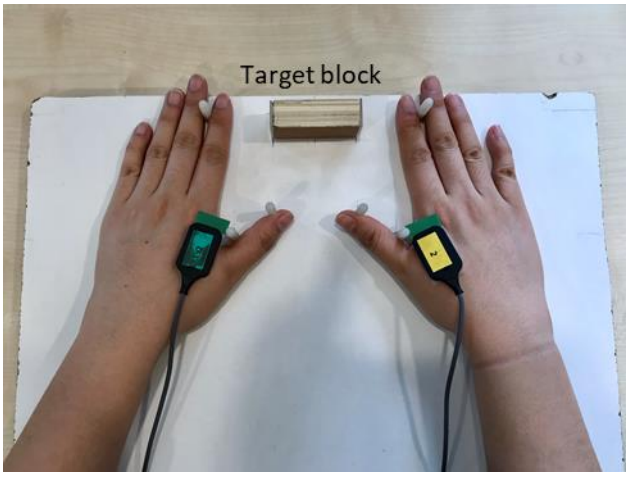

Figure 3-1. Hand positioned on the wooden peg board created to restrain both thumbs and middle fingers from left and right index fingers abduction. They were instructed to perform left or right index finger abduction movement according to the target stimuli on the computer screen until they touched the wooden square block target at the middle of the peg board as fast as possible.

The task was displayed on a 19 inch LCD monitor with a 60 Hz refresh rate. All stimuli were presented in black Courier New font size 150 on a light grey background. The onset of the fixation cross, preparatory cue and imperative cue were accompanied by a colour change within a square box presented in the bottom right corner of the monitor. A photodiode covered this area and generated precise markers of the cue onsets in Signal as its voltage changed according to screen luminescence (see Figure 3-3A and B; lower trace).

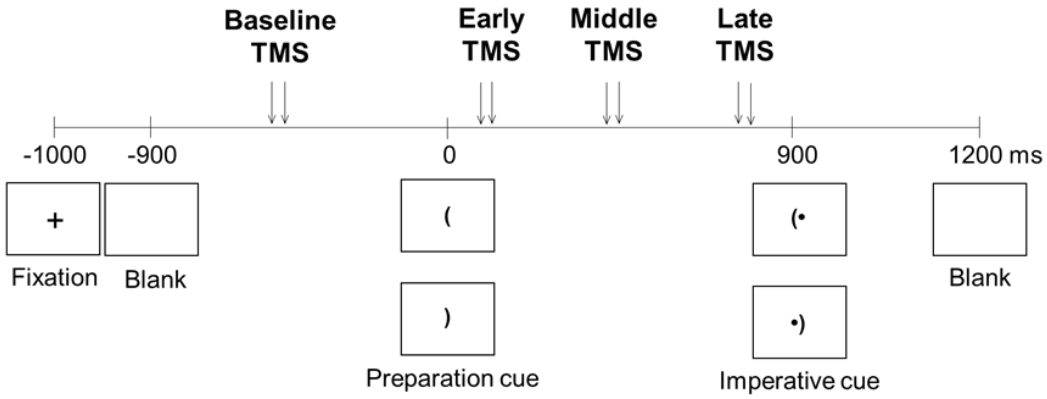

Figure 3-2. Instructed-delayed task protocol and four TMS timings. Participants prepared to move their right index following a preparation cue of the bracket opens to the right or left index finger following a preparation cue of the bracket opens to the left. However, they have to hold it until the imperative cue appeared at 900 ms later. The baseline TMS was delivered at the middle of fixation period or during the delay period of 100, 450, and 800 ms after the preparation cue in a separate trial.

Electromyography (EMG)

EMG recordings were obtained with a Bagnoli 2-Channel EMG system (Delsys) placed on the belly of the left and right first dorsal interosseous (FDI) muscles. The reference electrode was placed on the olecranon process of right elbow. The EMG signal was bandpass filtered (20-500 Hz) and digitized with a sampling rate of 2000 Hz using a Micro 1401 analogue to digital converter (CED) and transferred for offline analysis on a pc with Signal software Version 6.01 (CED).

Transcranial magnetic stimulation (TMS)

TMS was delivered over PMC_L and PMC_R using two Magstim 200^2 monophasic stimulators connected to 50 mm diameter figure-of-eight coils (Magstim Company Ltd). The size of the coils allowed them to be positioned on the participant's head targeting at the optimal stimulation sites of the FDI muscle in both primary motor cortices. The coils were placed tangentially over

the PMC with the coil handles pointing backwards about 45 degrees away from the midsagittal line and perpendicular to the central sulcus, which induced a brain current that flowed in the postero-anterior direction (Mills et al., 1992). Motor hotspot and RMT were first identified for the left and right FDI muscles using the same procedures as the previous study. We then calculated the SI-1 mV for both muscles using blocks of 30 trials (Cuypers et al., 2014). As per the previous study, the location and trajectory of each motor hotspot was marked using Brainsight^{TM} version 2.2 (Rogue Research Inc), which allowed simultaneous tracking of both coils throughout the duration of the study.

IHI measurement

A dual-coil TMS paradigm was used to explore the changes in corticospinal excitability and IHI during the delay period of the instructed-delay task. In the previous study, setting the CS and TS to SI-1mV produced similar levels of resting IHI-10ms in each PMC with both the conventional approach (IHI_L 0.58 ± 0.04; IHI_R 0.65 ± 0.04) and the novel substitution approach (IHI_L 0.57 ± 0.05; IHI_R 0.68 ± 0.04). We therefore continued with the substitution method in the current study, which allowed us to remove the single-TMS trials from the protocol and reduce the total length of the experiments by 50%.

The imperative cue instructed participants to abduct either their left or right index finger. Left and right hands could therefore be labelled as to whether they were selected or non-selected in each trial. However, it became apparent that this would prove problematic when labelling and reporting the IHI conditions. So when labelling the trials/conditions, we decided to take the perspective of the PMC in relation to the selected hand. The PMC contralateral to the selected hand was labelled as PMC_C and the PMC ipsilateral to the selected hand (i.e. contralateral to

the non-selected hand) was labelled as PMC_I. Therefore, in a trial in which the participants responded with their left hand, the right PMC, which was contralateral to the left selected hand, was defined as the 'PMC_{RC}', and the left PMC, which was ipsilateral to the left selected hand, was defined as the 'PMC_{LI}'. These roles were reversed in trials when the right hand was selected to provide PMC_{RI} and PMC_{LC}.

The IHI-10ms conditions were labelled using IHI_C and IHI_I with the subscript letter denoting which PMC which received the TS. So for IHI_C, the CS was delivered to the PMC_I 10 ms before the TS over the PMC_C (see Figure 3-3A). The addition of a subscript R or L further indicated whether the PMC that received the TS was in the left or right hemisphere. For example, IHI_{RC} had the CS delivered to the left PMC_I 10 ms before the TS over the right PMC_C. Alternatively, IHI_I was the measurement of IHI from the PMC_C to the PMC_I. Here the CS was delivered to the PMC_C 10 ms before the TS over the PMC_I (see Figure 3-3B). The example of IHI_{LI} trial in Figure 3-3B was when the CS delivered to the right PMC_C 10 ms before the TS over the left PMC_I.

The experiment began with ten practice trials to familiarise the participants with the instructed delay task. During the main experiment, dual-coil TMS (CS-TS with 10 ms delay) was applied at four time points. The first was during the middle of the baseline period (500 ms after fixation onset). The other three TMS measurements were obtained during the delay period: 100 ms after the preparatory cue, 450 ms after the preparatory cue, and 800 ms after the preparatory cue (100 ms before the imperative cue). These time points were labelled as early, middle and late TMS respectively (see Figure 3-2). The IHI_{RC}, IHI_{RI}, IHI_{LC}, and IHI_{LI} at each of these four time points were measured to obtain a total of 16 conditions. We performed 15 trials in each baseline

condition and 30 trials in each delay condition. 50% of the trials in the baseline condition were only required because the IHI measures were obtained before the participant saw the preparation cue, we could collapse the results of the selected and non-selected trials to 30 trials of IHI_L and 30 trials of IHI_R. In total, 420 trials were performed split into five blocks of 84 trials (approximately 7 minutes per block). Participants were given a five minutes rest between each block to cool the temperature of the TMS coils. Total length of experiment was 3-3.5 hours.

Data analysis

Screening of MEP data

Peak-to-peak MEP amplitudes were calculated in the 10-50 ms after the TMS onset. MEPs were discarded from further analysis when the background EMG activity of the target muscle exceeded 100 μV during the 200 ms prior to the TMS onset (Duque et al., 2014). This criterion was less conservative than the resting IHI study because the requirement for the participants to place their hands on the wooden peg board made it more difficult to keep the background EMG very low. Trials with response times faster than 80 ms or slower than 500 ms, incorrect or missing responses, or coil locations greater than 3 mm or 5 degrees from the original motor hotspot were also removed from further analysis.

Measurement of corticospinal excitability

Once all the basic MEP screening was complete, corticospinal excitability relating to the PMC_C and PMC_I were calculated. As outlined in the methods, the current protocol only included double-TMS trials, therefore all MEPs elicited by the test-stimuli were conditioned and therefore unsuitable for using as our basic measure of corticospinal excitability. Due to this, we instead used the MEPs elicited by the conditioning stimuli as our measure of corticospinal

excitability. The MEP resulting from the conditioning stimulus over PMC_C (contralateral to selected hand) provided a measurement of impulse control and the MEP elicited from the conditioning stimulus over PMC_I (ipsilateral to selected hand /contralateral to non-selected hand) provided a measurement of competition resolution. The MEPs elicited from the PMC_C and PMC_I were first split into separate sub-conditions according to whether they were also from the left or right side (i.e. PMC_{LC}, PMC_{RC}, PMC_{LI} and PMC_{RI}). The CS MEPs measured during the baseline were not different between left and right PMCs, therefore these values were pooled to obtain one baseline MEP measurement. The next step of measuring the inhibitory changes associated with movement preparation in the delay period was to pool the CS MEPs values recorded at each delay period across both left and right PMCs when that PMC acted as the PMC_C and normalised to a percentage change of the CS MEPs baseline. These MEPs elicited from the PMC_C would demonstrate the impulse control. This step was performed separately for the CS MEPs values elicited from the PMC_I to demonstrate the changes of the corticospinal excitability in the competition resolution.

Measurement of IHI

As per the previous study, IHI_R refers to when the test stimulus was applied on the right PMC and the conditioning stimulus was applied to the left PMC (see Figure 3-3A). The opposite is true for IHI_L (see Figure 3-3B). Each IHI ratio was calculated as the MEP elicited by conditioned-test stimulus (cTS) divided by the MEP amplitude elicited by the conditioning stimulus (CS) in the same FDI muscle (see Equation 2-1B and Figure 2-1). This meant that the conditioned-test MEPs were paired with the conditioning-stimulus MEPs from the alternative set of trials. The calculation of IHI_R used the MEP elicited by the conditioned-test stimulus over right PMC (label 2 in Figure 3-3A) and the MEP elicited by the conditioning stimulus over

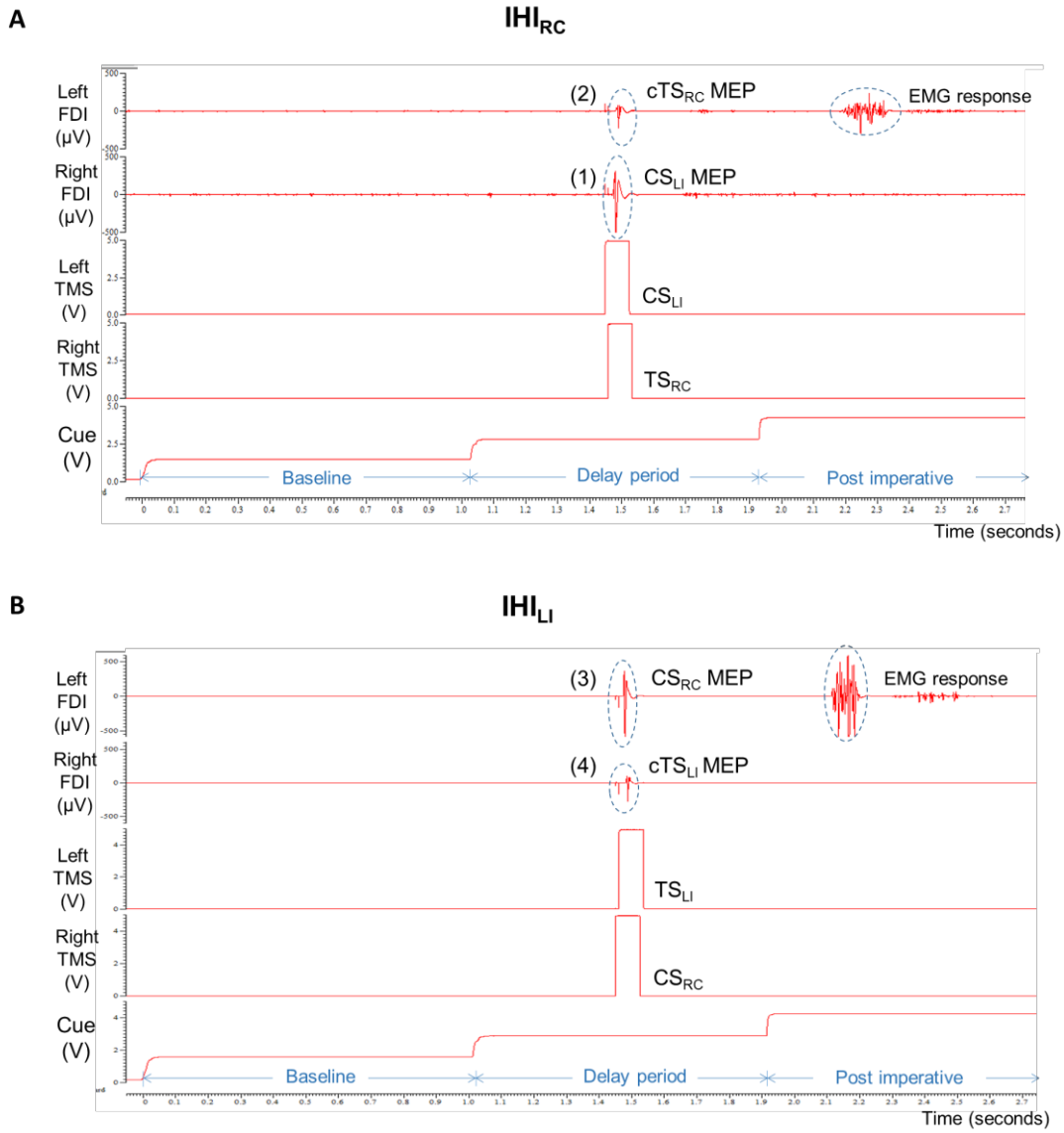

Figure 3-3. Signal program recording of the MEP amplitudes, EMG response, TMS timings, and timing of the task screen changes. **(A)** Example of a double-TMS trial when left–hand was selected as demonstrated by the EMG response presented in left FDI, therefore PMC_R was defined as a PMC_{RC} when it was the PMC contralateral to the selected hand. The IHI_{RC} was the measurement of the inhibition from PMC_{LI} onto PMC_{RC}. CS_{LI} was applied at the middle of delay period to the PMC_{LI} 10 ms prior to the TS_{RC} over the PMC_{RC}. The CS_{LI} MEP (1) was elicited in the right FDI and cTS_{RC} MEP (2) in the left FDI. The CS_{LI} MEP (1) resulting from the CS over PMC_{LI} was used in the substitutional approach to replace the uTS_{LI} MEP in order to calculate the IHI_L. In addition, the CS_{LI} MEP (1) resulting from the CS over PMC_{LI} also provided a measurement of a single pulse TMS, which illustrated the competition resolution in the PMC_{LI}. **(B)** Example of a double-TMS trial when left-hand was selected. IHI_{LI} was the measurement of the inhibition from PMC_{RC} onto PMC_{LI}. CS_{RC} was applied at the middle of delay period to the PMC_{RC} 10 ms prior to the TS over the PMC_{LI}. The CS_{RC} MEP (3) was elicited in the left FDI and cTS_{LI} MEP (4) in the right FDI. The CS_{RC} MEP (3) resulting from the CS over PMC_R was also used in the substitutional approach to replace the uTS_{RC} MEP in order to calculate the IHI_R. In addition, the CS_{RC} MEP resulting from the CS over PMC_{RC} also provided a measurement of a single pulse TMS, which illustrated the impulse control in the PMC_{RC}.

right PMC (label 3 in Figure 3-3B). The calculation of IHI_L used the MEP elicited by the conditioned-test stimulus over left PMC (label 4 in Figure 3-3B) and the MEP elicited by the conditioning stimulus over left PMC (label 1 in Figure 3-3A).

The IHI_L and IHI_R during the baseline and delay period were presented separately either when it was elicited from the PMC_C and PMC_I. There was no significant difference in IHI_L and IHI_R at baseline therefore these values were pooled to obtain one baseline IHI measurement. When evaluating the modulation of IHI during the delay period, the values recorded at each delay period were pooled across both IHI_L and IHI_R and normalized to a percentage change of the IHI baseline. The normalization of was performed separately for the IHI_C and IHI_I.

Statistical analysis

MEPs

As discussed in the previous chapter, the magnitude of IHI is influenced by the CS and TS intensities (Ferbert et al., 1992, Daskalakis et al., 2002a, Chen et al., 2003, De Gennaro et al., 2004a). Therefore, we wanted to determine that the resting motor thresholds and the intensities used to obtain SI-1 mV were consistent between the two hemispheres. This was tested using paired-samples t-tests to compare % RMT and % MSO between PMC_L and PMC_R. Following this, we determined whether selection and/or side of hemisphere affected corticospinal excitability during the delay period. A repeated measures ANOVA with factors 2 PMC-SIDE (PMC_L, PMC_R) x 3 SELECTION (baseline, contralateral, ipsilateral) was run on the MEP amplitudes. Note here that contralateral and ipsilateral refer to the mean MEPs pooled across all three delay periods. As previous studies have reported that the inhibition resulting from IC is stronger than CR (Labruna et al., 2014, Greenhouse et al., 2015b, Lebon et al., 2016), it was

hypothesized that the MEPs obtained from the PMC_C would be smaller than those obtained from the PMC_I during the delay period. It was further hypothesized that both effects would increase as the delay period increased. This was tested with a two-way repeated measures ANOVA with factors of 2 SELECTION (PMC_C, PMC_I) x 3 DELAY-PERIOD (early, middle, late) performed on the normalised CS MEP amplitudes.

IHI

For the IHI results, it was first determined whether the basic IHI-10ms effect was comparable in both PMCs during the baseline period. A repeated measures ANOVA of 2 PMC-SIDE (PMC_L, PMC_R) x 2 STIMULUS (CS, cTS) was conducted on the MEP amplitudes recorded during the baseline period.

Following this, the main hypothesis was tested: if competition resolution (inhibition of the non-selected hand) was mediated through IHI, then a decrease in the IHI_I ratio (reflected increased IHI) would be observed during the delay period. Alternatively, if impulse control (inhibition of the selected hand) was mediated through IHI then a decrease in the IHI_C ratio would be observed during the delay period. These hypotheses were tested using a repeated measures ANOVA with factors of 2 SIDE (IHI_L, IHI_R) x 3 SELECTION (baseline, contralateral, ipsilateral) on the IHI ratio values. A second repeated measures ANOVA including factors of 2 SELECTION (IHI_C, IHI_I) x 3 DELAY-PERIOD (early, middle, late) on IHI values after normalization to the baseline period. Statistical testing was conducted with IBM SPSS Statistics 24 software package. The significance level was set at 0.05. Post-hoc comparisons were conducted using the Sidak procedure.

3.3 Results

Baseline measurement

The current experiment used the same stimulation protocol as the previous resting IHI-10ms study. Both the conditioning and the test stimuli set to SI-1 mV and, as validated during the resting IHI results, we substituted the MEP elicited by the conditioning stimulus for the MEP elicited by the unconditioned-test stimulus. Table 2 shows that, similar to the resting IHI results of the previous chapter, we did not detect any between-hemisphere differences in RMT (t(df) = 20, P = 0.09) or the stimulus intensity when presented as % MSO (P = 0.44) or normalized to % RMT (t(df) = 20, P = 0.93). During the baseline period, the SI-1mV protocol also produced similar mean MEP amplitudes elicited from the left conditioning stimulus (877.5 ± 89.8 μV) and the right condition stimulus (927.2 ± 81.7 μV, t(df) = 20, P = 0.63).

Table 2. Comparative means for parameters across left and right hemispheres (n=21). Significant differences between each condition were examined for using paired t-tests.

IHI and parameters	PMC_L (mean ± SE)	PMC_R (mean ± SE)	t(df)	P value
RMT (% MSO)	45.4 ± 1.6	44.0 ± 1.4	20	.09
Stimulus intensity (% MSO)	64.4 ± 2.0	62.8 ± 2.5	20	.44
Stimulus intensity (% RMT)	143.8 ± 5.1	143.3 ± 5.1	20	.93
Baseline MEPs (μV)	877.5 ± 89.8	927.2 ± 81.7	20	.63
Baseline IHI	0.67 ± 0.05	0.72 ± 0.05	20	.39

Corticospinal excitability results

Since the substitutional approach measurement of IHI eliminated the single-TMS trials, we used the MEPs elicited by the conditioning stimulus as the measure of corticospinal excitability during the task. Figure 3-4A depicts the effects of selection on the MEP amplitudes obtained from PMC_L and PMC_R with the conditioning stimuli during the baseline and delay periods. A repeated measures ANOVA with factors 2 PMC-SIDE (PMC_L, PMC_R) and 3 SELECTION (baseline, contralateral, ipsilateral) on the MEP amplitudes revealed a main effect of SELECTION ($F_{2,40} = 15.7$, $P < 0.001$). Post-hoc analyses indicated that during the delay period, MEPs from both PMC_C (784 ± 55 μV, $P < 0.001$) and PMC_I (819 ± 59 μV, $P = 0.012$) were significantly lower than baseline (902 ± 69 μV), which established the presence of impulse control in the PMC_C and competition resolution in the PMC_I. There was no main effect of the PMC-SIDE ($F_{1,20} = 0.5$, $P = 0.51$) or an interaction between PMC-SIDE and SELECTION on the MEP amplitude ($F_{2,40} = 0.2$, $P = 0.77$).

Since both hemispheres showed a similar reduction in corticospinal excitability during the delay period, we decided to pool the MEPs across both sides for the remaining analysis. Instead MEPs were grouped into PMC_C (contralateral to the selected hand) and PMC_I (ipsilateral to the selected hand). The MEPs in each delay period were then normalized as a percentage of the baseline values. Figure 3-4B depicts the normalised MEPs amplitudes elicited from PMC_C and PMC_I during the delay period. A repeated measures ANOVA of 2 SELECTION (PMC_C, PMC_I) x 3 DELAY-PERIOD (early, middle, late) revealed a significant main effect of DELAY-PERIOD ($F_{2,40} = 14.1$, $P < 0.001$), but no main effect of SELECTION ($F_{1,20} = 0.1$, $P = 0.73$) and interaction ($F_{2,40} = 0.7$, $P = 0.50$) on the normalised MEPs amplitudes. Overall, the MEPs in both PMC_C and PMC_I showed a significant suppression as the delay period progressed. Post-

hoc analyses indicated that MEPs reduced from 97.1 ± 1.6% in the early to 88.1 ± 2.4% in middle delay period (P = 0.002), but the further small reduction to 85.8 ± 3.0% in the late delay period was not significantly different from the middle delay (P = 0.23). No significant difference between the PMC_C and PMC_I was found at any time point during the delay period (all, P > 0.05). The bilateral reduction in corticospinal excitability associated with both PMC_C and PMC_I during the instructed-delay period indicates that the strength of both impulse control and competition resolution processes increased by a similar amount during movement preparation and selection.

Interhemispheric inhibition results

The main aim of the current study was to explore whether the impulse control mechanism that occurs in the PMC_C involves IHI_C (inhibition from PMC_I to PMC_C), and/or whether the competition resolution mechanism that occurs on the PMC_I involves IHI_I (inhibition from the PMC_C to the PMC_I). If competition resolution was mediated by IHI, then the IHI_I ratio should decrease during the delay period and if impulse control was mediated by IHI then IHI_C ratio should decrease during the delay period.

Figure 3-4C displays the effect of selection on the IHI ratio as measured in PMC_L and PMC_R during the instructed delay task. It was first determined if there was a lateralization of the IHI effect during the *baseline* period only. Repeated measures ANOVA of 2 PMC-SIDE (PMC_L, PMC_R) x 2 STIMULUS (CS, cTS) revealed a significant main effect of STIMULUS ($F_{1,20}$ = 25.9, P < 0.001). The baseline MEP amplitudes with the conditioned-test MEP of 604 ± 60 μV being significantly lower than the conditioning-stimulus MEPs of 902 ± 69 μV. However, there was no main effect of the PMC-SIDE ($F_{1,20}$ = 1.2, P = 0.29), nor an interaction between PMC-

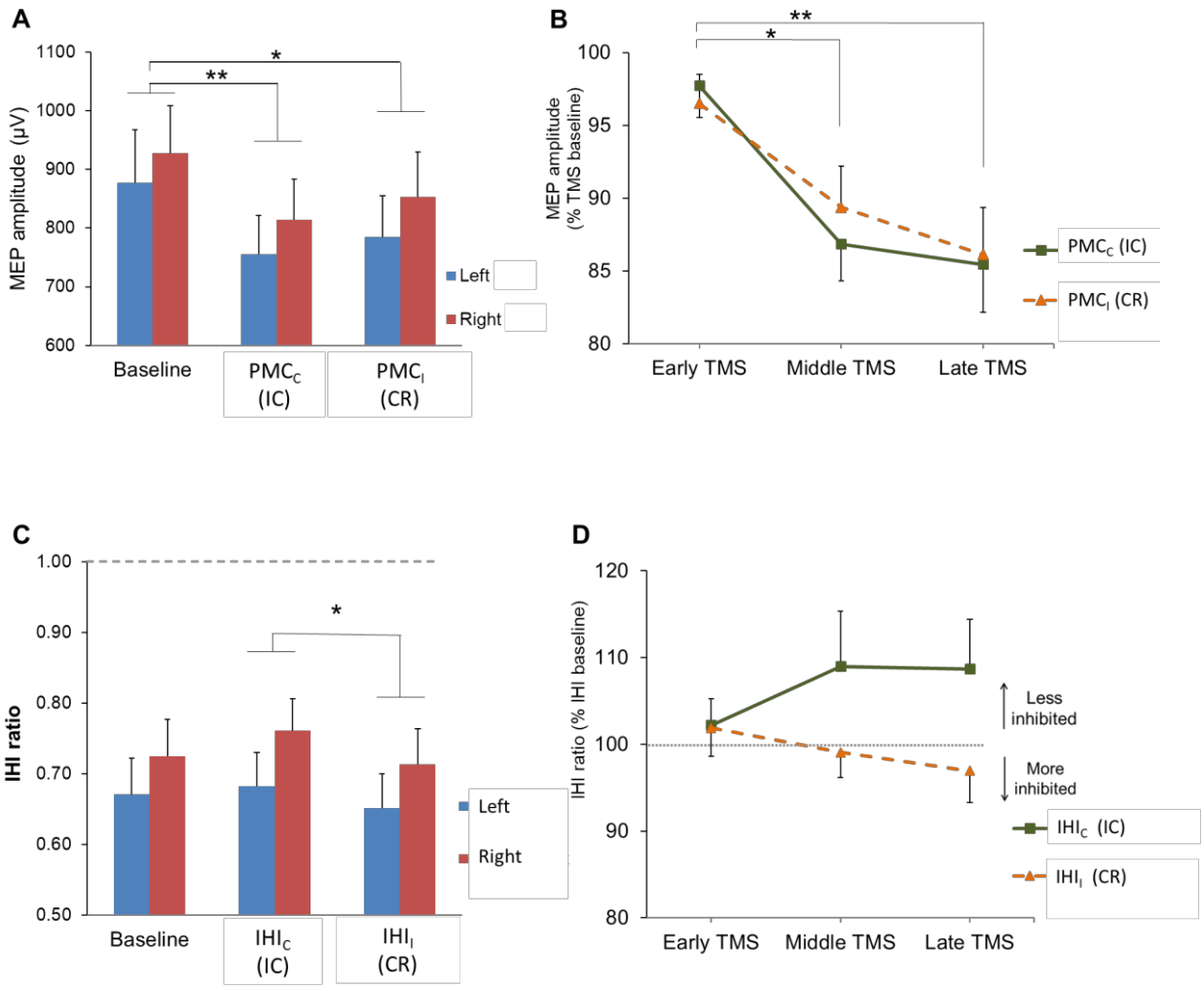

Figure 3-4. MEPs and IHI results during baseline and delay periods **(A)** MEP amplitudes elicited from the CS over left and right PMCs at the baseline and three TMS timings during the delay period when both PMC were the PMC_C and PMC_I. The MEPs measured from PMC_C and PMC_I during the delay period were significantly lower than the baseline; showing a presence of IC and CR during the delay period. **(B)** The MEPs during each time point of the delay period were averaged between left and right PMCs and normalized to a percentage of the MEPs at baseline measurement. It showed more pronounced suppression in the PMC_C and PMC_I as the delay period progressed. **(C)** IHI onto left PMC and IHI onto right PMC were measured at the baseline period and three timings during the delay period when both were IHI_C and IHI_I. The IHI ratio lower than 1.00 indicates the IHI. The IHI_I was significantly stronger than IHI_C during the delay period. **(D)** The IHIs measured at each time point of the delay period were averaged between IHI_L and IHI_R and normalized to the percentage of the averaged IHI between IHI_L and IHI_R measured at the baseline period. IHI_C and IHI_I showed no significant difference as the delay period progressed (*$p < 0.05$, ** $p < 0.01$).

SIDE and STIMULUS ($F_{1,20}$ = 0.3, P = 0.60) on the baseline MEP amplitudes. Post-hoc analyses indicated that the conditioned-test MEPs obtained from left PMC were 327 ± 88 μV lower than the conditioning-stimulus MEP (P = 0.001). In right PMC, the conditioned-test MEPs were 270 ± 70 μV lower than the conditioning-stimulus MEPs (P = 0.001). These translated into similar baseline IHI-10ms ratios of 0.67 ± 0.05 in IHI_L and 0.72 ± 0.05 in IHI_R (paired t-test, P = 0.39, see Table 2).

The next step was to investigate the effect of selection on IHI-10ms during the delay period. A repeated measures ANOVA of 2 PMC-SIDE (PMC_L, PMC_R) x 3 SELECTION (baseline, contralateral, ipsilateral) revealed a main effect of the SELECTION ($F_{2,40}$ = 3.7, P = 0.042) on the IHI ratios. Post-hoc analyses indicated that this was due to the IHI_I ratio of 0.68 ± 0.04 being significantly lower than the IHI_C ratio of 0.72 ± 0.04 (P = 0.004), which indicates that a stronger IHI effect onto the PMC associated with competition resolution as compared to the PMC associated with impulse control. There was no main effect of the PMC-SIDE ($F_{1,20}$ = 1.2, P = 0.28) or an interaction between PMC-SIDE and SELECTION ($F_{2,40}$ = 0.4, P = 0.70) on the IHI ratios, which indicates that the IHI_C and IHI_I effects were not lateralized according to either the left or right hemisphere.

Since there was no lateralised effect of PMC side on the IHI ratio, we explored the specific IHI-10ms changes within the delay period by pooling the results across both PMCs. The IHI effect in each (early, middle, late) delay period was first normalised as a percentage of the baseline IHI ratio. Figure 3-4D displays the normalised IHI_C and IHI_I values during the delay period. Values greater than 100% indicate that IHI decreased during the delay period and values less than 100% represent an increase in IHI. A repeated measures ANOVA of 2

SELECTION (PMC_C, PMC_I) x 3 DELAY-PERIOD (early, middle, delay) showed that the interaction between SELECTION and DELAY-PERIOD tended towards significance ($F_{2,40}$ = 2.7, P = 0.08). This was due to the small decrease in IHI_C from the early (102.2 ± 3.0%) to late (108.7 ± 5.7%) delay period and the small increase in the IHI_I from early (101.9 ± 3.3%) to late (97.0 ± 3.7%).

Comparison of corticospinal excitability and IHI results

Overall, the bilateral reduction of corticospinal excitability during the delay period indicated the presence of impulse control and competition resolution processes during response preparation and selection. However, the IHI_C and IHI_I results did not show the same pattern as the changes in corticospinal excitability during the delay period. If the impulse control resulted from IHI, we expected to observe greater IHI_C measured during the delay period compared to the IHI measured during baseline. If the competition resolution phenomenon resulted from IHI, we would have expected to observe greater IHI_I measured during the delay period compared to the baseline. The IHI_C and IHI_I results revealed no significant difference when it was measured during the delay period as compared to the baseline. There was only the small increase in IHI_C and the small decrease in the IHI_I during the delay period.

3.4 Discussion

Summary of impulse control and competition resolution results

The current study applied TMS over bilateral PMC to assess the mechanism of inhibitory control during an instructed delay task. In line with previous studies, it was found that MEPs obtained in both the selected and non-selected hands decreased during the delay period (Labruna et al., 2014, Greenhouse et al., 2015a, Lebon et al., 2016, Quoilin et al., 2016,

Vassiliadis et al., 2018). The MEP decrease indicates that the primary motor cortex situated both contralateral (PMC_C) and ipsilateral (PMC_I) to the selected hand are transiently inhibited during movement preparation and selection in this instructed delay task. Duque and colleagues first proposed that the inhibition, acting onto the selected hand, known as impulse control, prevents the premature release of the prepared action, while the inhibition acting onto the non-selected hand, known as competition resolution, prevents alternative but unrequired actions (Duque and Ivry, 2009, Duque et al., 2010).

Duque et al. (2010) first proposed that impulse control and competition resolution represent separate inhibitory processes because the strength of the MEP suppression associated with the selected action was stronger than the non-selected action. The inhibition of the competition resolution may involve in all the potential responses including the selected hand. The selected hand also has the additional inhibition of the impulse control by itself, therefore the inhibition in the selected hand as termed the impulse control was stronger than the inhibition in the non-selected hand or the competition resolution (Duque et al., 2010). Although some studies have reported similar results when they measured the MEPs only in right PMC (Labruna et al., 2014, Greenhouse et al., 2015b, Lebon et al., 2016), it isn't always a consistent finding (Klein et al., 2016, Vassiliadis et al., 2018). Similar to the latter studies, we measured the MEP from both PMCs, but we did not observe a significant stronger inhibition in IC (13.1%) than the CR (9.2%) and we did not find a dominant effect of IC and CR. We did, however, find that the inhibition of corticospinal excitability in both selected and non-selected hands increased as the delay period progressed.

Dominance effects for impulse control and competition resolution

Competition resolution and impulse control were limited to only measure the CSE or IHI in the right PMC when it was the PMC_C and PMC_I (Duque et al., 2014, Greenhouse et al., 2015a, Greenhouse et al., 2015b, Lebon et al., 2016). The decision to stimulate only in right PMC was based on the studies from Leocani et al. (2000) and Duque et al. (2007) who reported a stronger suppression in the right PMC. A dominance hemispheric effect possibly occurred from the lateralisation between both PMCs. In right-handed participants, the non-dominant left-hand tends to show more mirror movement (Armatas et al., 1994, Liepert et al., 2001). Another reason was that the right-hand dominant had more superior movement skill as it had higher performance in a task required rapid and precision of finger movement compared to left-hand non-dominant (Triggs et al., 1997, Roy et al., 2003). Moreover, the resting motor threshold was lower in the dominant PMC_L, leads to larger MEPs elicited from the dominant PMC relative to the non-dominant PMC. The dominant PMC was more excitable, especially just prior to the movement onset, therefore the effect of hemisphere dominance might influence the inhibition onto PMC_I when it was not required to move (Macdonell et al., 1991, Quoilin et al., 2016, Klein et al., 2016). If this suggestion was true, the IHI should have reflected the dominance hemispheric effect when a stronger suppression onto non-dominant PMC_I resulted from the higher excitability of the dominant PMC_C.

However, we did not detect any evidence for the dominance hemispheric effect on the movement preparatory inhibition as we found similar changes of corticospinal excitability associated with both left and right PMCs. These results are also in line with Klein et al. (2016), Quoilin et al. (2016), and Vassiliadis et al. (2018), who observed the MEPs in both PMCs during the delayed choice reaction time task. In addition, the present study did not find a lateralisation

in the RMT and MEPs measured at baseline period. Therefore the MEPs and IHI changes during the delay period would not influence from the natural dominance hemispheric effect in this group of participants.

Does IHI mediate competition resolution and/or impulse control?

In contrast to the clear attenuation of corticospinal excitability, the current IHI results only showed minimal changes during the delay period. There was a trend for a release of IHI_C, which could represent a release of inhibition onto the PMC contralateral to the selected hand just prior to the anticipated imperative cue. IHI_I showed very little change during the delay period, which indicates that it had a negligible role in the competition resolution process onto the non-selected response.

Based on the proximity/history dependent model from Labruna et al. (2014), the competition resolution should be observed with the IHI technique as it could provide the evidence that the inhibition generated in one PMC and affected on to the opposite PMC (Ferbert et al. 1992). However, this current experiment could observe the CR as showed in the decrease of MEPs in the PMC_I during the delay period but we could not observe any changes of the IHI_I during the delayed response task. Therefore, these findings indicate that the CR found in the PMC_I was not mediated by the PMC_C during the movement preparation period. It could possibly have some contribution from the prefrontal cortex as it had been previously observed by Duque et al. (2012) that the lateral prefrontal cortex had a generic effect on inhibiting both selected and non-selected responses during movement preparation and might occur via a basal ganglia pathway (Coulthard et al., 2008).

The exact mechanism of IC is also still unclear but it was the evidence that the inhibition onto the selected response occurred at the PMC contralateral to the selected hand originated from other regions such as dorsal premotor cortex and lateral prefrontal cortex in the opposite hemisphere (Duque et al., 2012). While Labruna et al. (2014) suggested that the IC was automatically originated when the activation in the PMC contralateral to the selected hand increased. This kind of automatic suppression onto the on-going preparation in the selected hand was thought to decrease the background activity during the delay period to help it generate a faster response once the imperative cue appeared. However, in this present study, we only evaluated whether the impulse control mechanism acting on the PMC_C mediated from the PMC_I. There was the presence of IC as observed by the decrease MEPs in both left and right PMCs, but we did not observe the increase of IHI_C measured during the delay period. This suggested that the impulse control which occurred in the PMC_C was not mediated by the opposite PMC via the transcallosal pathway. It possibly occurred from other brain areas such as dorsal premotor cortex, ventrolateral prefrontal cortex, and medial aspect of the prefrontal cortex that sent the inhibitory projecting onto the PMC_C (Sawaguchi et al., 1996, Kroeger et al., 2010, Aron et al., 2007, Boulinguez et al., 2008).

Kroeger et al. (2010) observed the presence of IC and CR as measured from the MEPs and IHI during the 2000 ms-delayed choice reaction time task. They reported a stronger IHI_I than the IHI_C during the delay period and there was a release of IHI_C when it was close to the movement onset, while the IHI_I remained inhibited. The limitation of this findings was that they reported the IHI value during the delay period without comparing to the IHI value measured at the baseline. Moreover, the distinct suppression found in Kroeger et al. (2010) is hard to interpret because the task protocol included the large amount of no-go trials for 25% of total trials at the

imperative cue and the MEPs and IHI were only measured from the go trial. Therefore, the participants had to expect to abort the prepared response for some of the catch trials, this might increase the inhibition from the top-down control of the inappropriate response (Klein et al., 2014, Quoilin and Derosiere, 2015). The amount of catch trials that were typically included to prevent the participants initiating the response prior to the imperative cue was around 5-8% of total trials as this would not affect the preparatory inhibition (Duque and Ivry, 2009, Duque et al., 2012). In the current experiment, catch trials were not included and the delay period was not varied. The protocol used in the current study was created with the awareness that participants may anticipate the imperative cue and so respond unintentionally and produce a number of premature responses. However, the aim of this study was to observe the inhibition when the participant already knew the required response, and they had to hold the response until the imperative cue appeared. Therefore, the fixed delay period and lack of catch trials allowed the participant to anticipate the imperative cue and so a consistent pattern of movement preparation and selection during the delay period could be expected. This was supported by Lebon et al. (2016) that the duration of the delay period could influence the preparatory inhibition.

Hinder et al. (2018) found the relationship of MEPs and IHI effect in movement execution period when measured after the imperative cue to the EMG onset. This suggested that the IHI is involved in the movement preparatory inhibition. However, we did not observe the progressed of IHI effect during movement selection and preparation period as we focused on the delay period which was different from Hinder et al. (2018) and they only measured the MEPs and IHI from one PMC. The current study had an advantage as the novel method of IHI was used with substitutional protocol that allowed us to measure the MEPs and IHI from both

left and right PMCs at three time-points after the preparatory cue until prior to the imperative cue.

Novel use of substitutional method

The novel substitution IHI approach used in the current study eliminated the need for the single-TMS trials and therefore the experimental trials were reduced by 50% and were able to obtain more data than other studies within the same session. This would help decrease the natural variability of the MEPs when increased the number of the MEPs as suggested in Cuypers et al. (2014). In the remaining double-TMS trials, the TMS pulses applied over both PMCs were always set to SI-1mV. This approach worked well for the resting IHI measurement as we obtained a clear IHI effect in both hemispheres prior to starting the main instructed delay experiment. The strength of this effect was similar to that reported in other studies. In addition, there were few studies that explored the IHI effects underlying IC and CR (Duque et al., 2007, Kroeger et al., 2010, Morishita et al., 2014), which examined only in right PMC or examined both PMCs in a separate experimental blocks. Duque et al. (2007) explored the IHI_C and IHI_I effect when the participants responded with left- and right-hands. In order to measure the IHI_C and IHI_I during the left-hand response trials, the CS was applied over left PMC and TS over right PMC to convey the IHI_C. While the CS was applied over right PMC and TS over left PMC to convey the IHI_I. But for the right-hand response trials, the CS was applied over right PMC and TS over left PMC to convey the IHI_C. While the CS was applied over left PMC and TS over right PMC to convey the IHI_I. The limitation from Duque et al. (2007) was that the four different measurements were performed in four separate sessions on different days. Moreover, the MEPs in each measurement condition were repeated for 10 trials, which possibly had less reliability with the lower trial numbers (Cuypers et al., 2014). This indicated that the

substitutional approach has allowed us to evaluate the IC and CR mechanisms obtained from the modulation of CS MEPs together with the IHI in both PMCs either when it was selected and non-selected within the same experimental block.

The preparatory inhibition was influenced by the task complexity (Greenhouse et al., 2015a, Quoilin et al., 2016). They suggested that greater control might be required in a complex task to prevent response errors. The PMC_I was also activated when the participants performed a complex task as observed in neuroimaging studies (Hackley and Miller, 1995, van den Berg et al., 2011). The increased activation in the PMC_I might contribute to a mirror movement, therefore the suppression onto the PMC_I was needed to sharpen and facilitate the correct response (Greenhouse et al., 2015b). They suggested that the competition resolution mechanism was not only restricted to the non-selected homologous response. This was also in line with the proximity/history dependent model from Labruna et al. (2014) that the CR could inhibit onto the non-homologous non-selected response. Therefore, in the next chapter, we would conduct the experiment including a homologous and non-homologous response-mode and observe the preparatory inhibition during movement preparation. The limitation of the instructed delayed response task was that the participants would always select and prepare the response after the preparatory cue and wait for the imperative cue. Therefore, it would demonstrate a greater extent of preparatory inhibition if the task included a conflict that would allow us to see how the brain cancelled the incorrect response activation induced by the conflict and reactivate the correct response activation.

Conclusion

During movement preparation, the motor system is transiently inhibited by the operation of two underlying mechanisms, impulse control (IC) and competition resolution (CR). As expected, IC and CR were both successfully elicited as observed with the reduce MEPs in selected and non-selected hands during the delay period of choice reaction time task. However, IC and CR were not associated with the IHI, this suggested that the transcallosal inhibition is not the main mechanism behind the suppression to prevent the premature response and to inhibit the unwanted movement during this task.

CHAPTER 4

EEG study of movement selection during a response conflict task involving homologous and non-homologous response-modes

4.1 Introduction

In everyday life, many situations require the selection of the either the left or right hand to perform an action, such as picking up a cup or pressing the button in a lift. Therefore, the hands are in regularly in the competition with each other. However, it is rare to find situations in which the response choices are between a hand and a foot so these effectors are not regularly in competition with each other. But for an important everyday task such as driving, it requires both hands and feet responses. Or some kind of sports, such as a football goalkeeper, they are allowed to use either hands or feet to catch the ball. Therefore, action selection in responding to the relevant stimuli requires the decision making process to make a correct choice between potential actions with either limb systems. From previous chapters, the inhibitory processes involved in the movement selection and preparation have been investigated during a delayed choice response time task. In the next two chapters, I will continue to investigate the inhibitory processes involved in the movement selection and preparation under the conflict, but shift away from the IHI approach onto the response-mode approach as outlined in the proximity/history dependent model of Labruna et al. (2014).

Labruna et al. (2014) hypothesized that the inhibition of the selected response (impulse control) was not influenced by the response-mode, but the selection process involved the inhibition of other non-selected responses (competition resolution) was influenced by the response-mode

where the non-selected response was similar to the selected response (homologous) or different from the selected response (non-homologous). They also raised a series of models to explain the possible underlying mechanisms of competition resolution. Thus they investigated the constraints on the operation of impulse control and competition resolution by manipulating the response-mode used for the task responding in a delayed response task. Overall, the MEP results suggested that the inhibitory mechanisms of competition resolution resulted from the motor areas contralateral to the selected response and the amount of inhibition was graded according to proximity or history of response-mode that are normally in competition (see Figure 1-2 D). The MEPs measured in the non-selected hand, which were elicited from the PMC_I, were more strongly inhibited when the participants responded with the hand vs hand (homologous response-mode) compared to the hand vs foot (non-homologous response-mode). Using the delayed response task to investigate movement preparation is limited to the extent of the participants already prepared to execute a movement that they knew in advance which hand or foot to use in responded to the imperative stimuli. In particular, it would be interesting to explore how the conflict information affects response preparation and whether it was influenced by the response-modes by using the flanker task.

4.1.1 Movement selection during response conflict

The Eriksen flanker task (Eriksen, 1995) is commonly used to study the cognitive processes involved in response conflict. The original flanker paradigm presented a row of letter stimuli and assigned the required response to the middle target letter by pressing left button in response to the letter ‘H’ and right button to the letter ‘S’. However the visual information from the letter stimuli does not correlate to the movement execution generated by left or right side of the body. Kopp et al. (1996) modified the flanker and target stimuli to the arrowheads pointing to the left

or right. A target stimulus is presented that require rapid choices movement, for instance, arrows pointing to the left or right instructed left or right hand responses, respectively. This type of stimuli reduces the complexity of the stimulus encoding and response identification processing (Kornblum et al., 1990). The target stimulus is typically flanked by two arrows on each side. Although participants should not respond to the flankers, these 'task-irrelevant' stimuli influence response selection. Congruent trials (flanker direction = target direction) are associated with faster response times than neutral trials (non-directional flanker), whereas incongruent trials (flanker direction ≠ target direction) have slower response times. The difference in response time between incongruent and congruent trials is the so-called 'congruency effect'. The arrow flanker can also produce a larger congruency effect than the letter flanker type because the arrow stimuli are the directional information that is more automatically conveyed by the cognitive process and trigger the side of response that related to the arrow direction (Peschke et al., 2013).

The processes underlying the congruency effect can be explained using the 'activation-suppression' model of Ridderinkhof et al. (2005) (see Figure 4-1). Once the stimuli have been perceived, the decision leading to the correct response activation occurs via two separate but parallel routes of processing. After initial stimuli processing, the task-irrelevant flanker stimulus activates an early, automatic response via the direct response activation route. While the task-relevant target stimuli activates response selection via the deliberate response route. Here a stimulus-response mapping is applied that is based on the task instruction, therefore processing in the 'conscious' deliberate route is generally slower than the automatic direct route. If the responses signalled from the two routes correspond, as will happen in the congruent flanker trials, then the correct response will be activated quickly. However, if the response

activation from the direct response route and deliberate route mismatch, as will happen during incongruent trials, then the response conflict must be resolved before a final motor response can be executed. This takes time, therefore, response times in incongruent trials are slower.

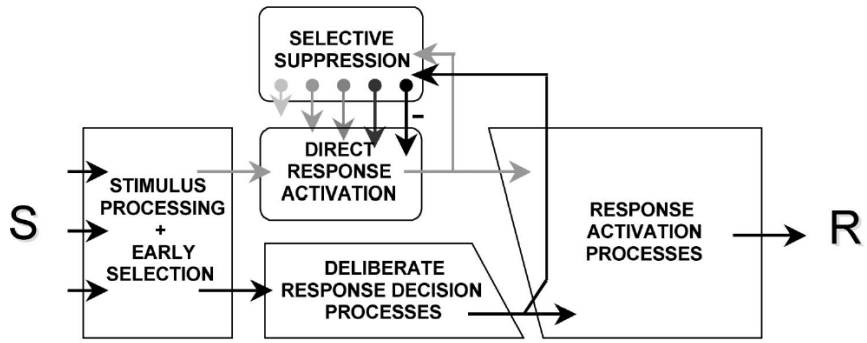

Figure 4-1. Dual-route model of response activation (Ridderinkhof et al. 2005). In a response conflict task, the irrelevant stimuli activate the response via the direct response activation route, which is more reflex-like route. While the relevant stimuli, which indicates that the response is needed to be executed, activate the response via the deliberate response route. The selective suppression is selectively reduce the activation of specific response induced by the irrelevant stimuli to control the inappropriate response activations. This suppression takes some time to build up, therefore it's only effective after the amount of time is provided.

A key feature of the activation-suppression model is the selective suppression that is applied onto the direct response activation pathway. This form of inhibitory control, which may originate in the prefrontal cortex and basal ganglia plays an important role in reducing response conflict (Iannaccone et al., 2015, Aron et al., 2007). It selectively suppresses the processing of the flanker stimuli and therefore reduces its influence on the response activation processes. The magnitude of selective suppression at the onset of each trial is therefore a key determinant of response time. When the 'baseline' level of selective suppression onto the direct response activation route is high, the flanker will have a relatively low influence on response selection. Thus, the response times will be less impaired by the incorrect response activation during incongruent trials and less facilitated by the correct direct response activation during congruent trials.

If the proximity/history dependent model of Labruna et al. (2014) was applied to test whether the selective suppression mechanism is similar to the competition resolution processes, this could be done by manipulating the potential responses into homologous and non-homologous response-modes. The prediction of competition resolution processes from Labruna and colleagues was that the amount of inhibition would be influenced by the response-modes. They reported a stronger inhibition of competition resolution onto the non-selected hand during a hand-hand (homologous) as compared to a hand-foot (non-homologous) response-mode in a delayed choice RT task (Labruna et al., 2019, Labruna et al., 2014). If the history dependent model is true, the selective suppression onto the direct response route will be lower with a non-homologous response-mode. The main implications of the change in selective suppression will now be covered in detail. First for the congruent condition and then for the incongruent condition.

In the congruent condition, the congruent flanker stimuli activates the correct response via the direct route. If the amount of selective suppression onto the direct response activation is lower in the non-homologous response-mode, then the correct response activation will receive more benefit from the congruent flanker stimuli. It will therefore be quicker to reach the decision threshold and response time will be faster with the non-homologous response-mode. For instance, when the irrelevant-flanker stimuli pointed to the right to automatically activate the right-hand, there would be less selective suppression onto the right-hand in the non-homologous (left-foot vs right-hand) than the homologous (left-hand vs right-hand) response-modes. When the target stimuli indicated that the right-hand is required to respond, right-hand in the non-homologous response-mode would be activated faster to the threshold level.

When experiencing the response conflict, the incongruent flanker activates the incorrect response via the direct response route. This needs to be selectively suppressed to allow the correct response activation to build up via the deliberate route. If the amount of selective suppression onto the direct route to inhibit the incorrect response is lower in the non-homologous response-mode, there will be stronger influence from the incongruent flanker stimuli in this condition. Therefore, the response time when responding with the non-homologous response-mode will be slower than the homologous response-mode. For instance, when the flanker stimuli pointed to the left to automatically activate left-hand (homologous response-mode) or left-foot (non-homologous response-mode) via the direct response route, the selective suppression would be lower onto the non-homologous left-foot. Therefore, the correct right-hand response could be activated via the deliberate route more slowly in the non-homologous response-mode.

4.1.2 Assessment of preparatory inhibition mechanism under conflict condition using electroencephalography (EEG)

Electroencephalography (EEG) recordings can measure the cortical activity associated with response preparation and selection (Eimer, 1999, Carrillo-de-la-Pena et al., 2006) and therefore provide an excellent method for testing the above predictions. Event-related potentials (ERPs) are average waveform voltages time-locked to specific events such as action-related stimuli or the responses they elicit (Coles, 1989). The specific cognitive processes that can be employed by using the ERP components are stimulus discrimination/ classification/ identification, memory operations or response selection and activation. For the movement related ERP that indicates the preparation of the voluntary movement, negative brain potentials of 10-15 μV can be observed around 100 ms prior to the movement onset. This negative potential was termed as

the readiness potential (Kornhuber and Deecke 1965). It typically shows a greater negativity when recorded from the electrode sites over the motor cortex contralateral to the moving hand, which corresponds to more activation of that brain motor region (Vaughan et al. 1968). This negative potential can be recorded bilaterally as it spreads larger in the frontal brain region (Kornhuber and Deecke 1965). When the left-hand prepares to move, the negative potential is larger over the right motor cortex at C4 electrode site of the International 10-20 system (Jasper, 1958), whereas when the right-hand prepares to move, the negative potential is larger over the left motor cortex at C3 electrode site. This negative component is considered to reflect response preparation processes. Figure 4-2A displays the EEG recordings during response preparation and selection in a warned reaction time task. Both left and right PMCs (C3 and C4 respectively) show negative potentials but there was a greater negative amplitude in the electrode contralateral to the responding hand. Therefore, when the participant is instructed to respond with left-hand, the negative potential in C4 is larger than C3, but the opposite is true when the right-hand prepares to respond (Coles 1989).

The lateralisation of the movement related ERP can provide an important electrophysiological indicator of the imbalance between left and right PMC (Gratton et al. 1988, Coles 1989, Eimer 1998). The averaging method of Coles (1989) first subtracts the potential recorded at the electrode ipsilateral to the responding hand from the electrode contralateral to the responding hand (see Figure 4-2B). This is performed separately for left and right hand responses. The resulting intermediate potential has a negative value when the electrode contralateral to the responding hand is more negative than the ipsilateral electrode (i.e. more active). Whereas, it will be positive when the ERP in the electrode contralateral to the non-responding hand is more negative (i.e. more active) than the electrode contralateral to the responding hand. The

intermediate potentials from left and right hand responses are then averaged to yield the lateralised readiness potential (LRP) by dividing the intermediate ERPs from left and right hand responses by two (see Equation 4-1 and Figure 4-2C). The advantage of this method is that brain potentials unrelated to the movement will average to zero and be eliminated.

$$LRP = [\text{mean } (C4 - C3)_{\text{left-hand movement}} + \text{mean } (C3 - C4)_{\text{right-hand movement}}] / 2$$

Equation 4-1. The formula for calculating the lateralised readiness potential at the C3 and C4 electrodes according to the averaging method introduced by Coles 1989

An alternative way of calculating the LRP is through the double subtraction method (Eimer 1998). The main difference between the double subtraction and the averaging method is the double subtraction method is always subtracting the ERP recorded at C4 site from C3 site regardless of left or right hand is selected. This subtraction is done separately for left-hand and right-hand response trials. The next step is subtracting the difference ERP between C3 and C4 in left-hand response from right-hand response to yield the LRP $[(C3 - C4)_{\text{right-hand movement}}] - [(C3 - C4)_{\text{left-hand movement}}]$. Therefore, when using the double subtraction method, the correct response activation is reflected by the positive LRP while the incorrect response activation is reflected by the negative LRP. Although the resulting LRPs can be twice the size of those produced by the averaging method, the double subtraction method can also exaggerate non movement-related potentials (Coles 1989, Eimer 1998). For this reason we decided to use the averaging method in this chapter.

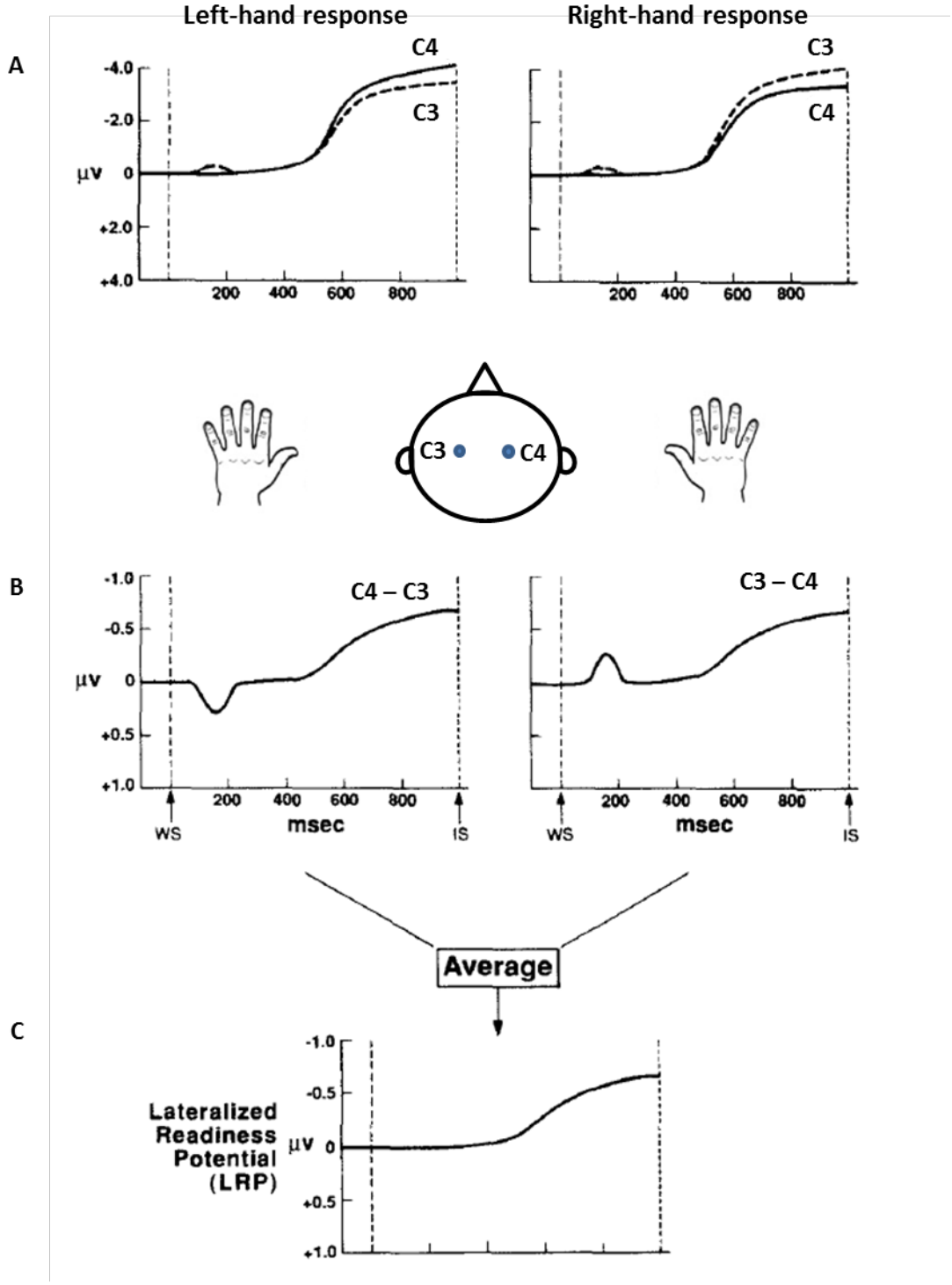

Figure 4-2. Averaging method in derivation of the lateralised readiness potential (LRP) (modified from Coles, 1989) (WS indicates warning stimulus onset, IS indicates imperative stimulus onset) (A) Brain potential recorded from the EEG electrode at the C3 and C4 sites during left and right hand responses to the warned reaction time stimulus. Greater negative potential elicits in the electrode contralateral to the left and right responding hands. (B) Asymmetry of the potential in the electrode between contralateral and ipsilateral sites to the responding hand is yielded by subtracting the potential recorded ipsilateral from the contralateral site to the responding hand. (C) The different potentials in left and right hand responses are averaged to get the LRP. The negative LRP component reflects the correct response activation.

When the arrow flankers were presented prior to the target stimulus, this priming effect of the flankers can automatically activate spatially compatible motor responses (Eimer 1995, Eimer and Schlaghecken 1998). Even when the participants are told not to respond to the task-irrelevant stimuli, they still perceive the flankers and the pre-activation of incorrect response resulted from the priming incongruent stimuli could be observed at a cortical level by the LRP measurement (Carrillo-de-la-Pena et al., 2006, Verleger et al., 2009) and at peripheral level measured by EMG response (Eriksen et al., 1985, Gratton et al., 1988, Smid et al., 1990).

The LRP results of Verleger et al. (2009), which are typically obtained during a flanker task with a homologous response-mode (hand-hand), are shown in Figure 4-3. In the congruent and neutral flanker conditions, the PMC contralateral to the responding hand was more active, the resulting LRPs show only negative polarities following the baseline period. This reflects that only correct responses were activated because the motor area contralateral to the instructed hand shows more negative potential (higher activation) than the opposite motor area (Coles, 1989, Kornhuber and Deecke, 2016). When the target stimulus and the flanker stimuli indicate opposite responses (incongruent trials), the LRP first shows a positive deflection (downward) before returning to negative component. The positive LRP values represent the incorrect response activation because the motor area contralateral to the non-instructed hand shows more negative potential (higher activation) than the contralateral to the instructed hand. The imbalance of activity in favour of the incorrect response activation reaches its peak around 300 ms after the onset of incongruent flanker and gradually declines and shifts towards the correct response activation.

As will be covered in more detail, Verleger and colleagues also measured corticospinal excitability associated with the selected and non-selected hands in their EEG study. They demonstrated that the LRP markers of correct and incorrect response activation reflected the MEP changes elicited by single-pulse TMS. In brief the key findings were that a) during congruent trials, the MEPs in the selected-hand increased with a similar time-course to the correct response activation reflected by the LRP; b) During the incongruent trials, the decrease of incorrect response activation indicated by the LRP occurred in parallel with a decrease in MEP amplitudes in the non-selected hand. Meanwhile the MEP amplitudes in the selected hand increased. The use of EEG and TMS indicated that the LRP changes did reflect the response activation and movement preparatory inhibition processes under the conflict condition.

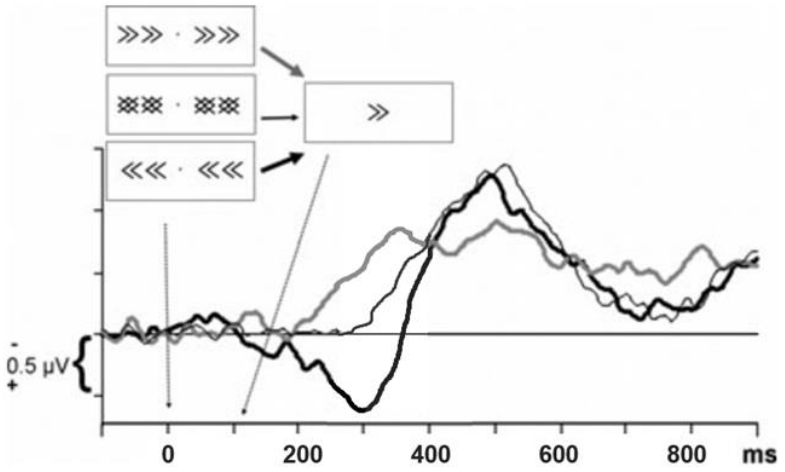

Figure 4-3. Adapted from Verleger et al. (2009). Grand mean LRPs recorded from left and right hand motor cortices when responding with right hand to a priming flanker task. The flanker stimuli appears 100 ms before the target stimulus onset. The congruent condition is when the flankers point in the same direction as the target. The neutral condition is when flankers provide no direction. The incongruent condition is when flanker stimuli point in the opposite direction to the target stimulus. The congruent LRP turned to a negative deflection earlier than the neutral LRP. This represents the correct response activation occurs earlier in the congruent as a result of the congruent flanker stimuli that prime the hand to respond. The incongruent LRP shows a positive deflection before returning to a negative deflection. The positive deflection that is only found in the incongruent flanker condition indicates that the incorrect response activation is prepared as a result of the incongruent flanker stimuli. After the incorrect response activation is suppressed and the correct response starts to build up after the target stimulus that indicate the opposite hand is required to respond, the LRP turns to a negative deflection that represents the preparation of the correct response.

In the current chapter, EEG was used to further explore how the response-mode influenced the amount of corticospinal inhibition in competition resolution based on the proximity/history dependent model raised by Labruna et al. (2014). We decided to approach this using a flanker task to test whether the mechanism of selective suppression to inhibit the unwanted movement under the conflict was a similar to the competition resolution. Because in general, we routinely encounter situations where multiple stimuli, which prime movements activate conflicting responses, selecting movement in responding to the relevant stimuli and attenuation of the irrelevant stimuli require the important aspect of the motor control to resolve conflict.

Many studies have explored the differences in movement preparation between hand and foot responses using ERP and LRP analysis techniques (Carrillo-de-la-Pena et al., 2006, Miller and Buchlak, 2012). The response times typically show that hand responses are faster than feet (Chan and Chan, 2010, Miller, 2012); however, dominant limb effects are generally inconsistent. LRPs can be obtained from C3 and C4 sites with hand responses, but these are not the optimal site for the foot responses as it shows responses of opposite polarity. For hand and foot responses, the LRP latency was longer in the incongruent than the congruent condition, reflecting a slower response time in the incongruent condition. Foot response trials had a longer negative LRP latency in the incongruent condition indicates a greater initial incorrect response activation and slower correct response time in the foot (Carrillo-de-la-Pena et al., 2006). The EEG studies that investigated the response selection focused on a single limb such as a task requiring hand response only or requiring foot response only in each experimental block, which was not designed to directly compare the response competition between hand and foot. The ERPs and LRPs were normally measured from left and right motor cortices. Foot response showed a reverse polarity and smaller LRP amplitudes compared to the hand response.

Therefore, the difference of the polarity between hand and foot was the limitation when using the ERPs or LRPs to directly compare the task requiring both hand and foot responses. A recent study from Miller (2012) explored the response competition involving hand and foot in a combined task. The EEG activities were recorded from the electrodes site at Cz, left (C3) and right (C4) motor hemispheres. The potentials at Cz were more positive when the participants responded with the hands than the feet. This measurement could produce a reliable LRPs as it was observed in the studies using separate tasks for the hand and foot responses. Therefore, this study adapted the Miller's (2012) approach by recording the ERPs from one electrode site, which allowed us to compare the potential differences between homologous and non-homologous response mode.

4.1.3 Hypotheses of the current study

If the inhibitory processes of competition resolution do act as the history dependent model proposes then we would expect less inhibition onto the non-selected actions with a non-homologous response mode task. Subsequently, competition resolution processes act like the selective suppression onto the direct response activation as outlined in the activation-suppression model (Ridderinkhof et al. 2005) then response preparation and selection processes will be more influenced by the irrelevant flanker stimuli during the non-homologous response-mode task. This would lead to the following specific hypotheses:

First, in terms of the behavioural effects, it was hypothesised that the non-homologous response-mode will show a larger congruency effect due to increased facilitation in congruent flanker condition and more slowing in the incongruent flanker condition based on lower selective suppression onto the direct response activation route. Second, in terms of the EEG, it

was expected that the congruent condition would show a larger negative component in the non-homologous response-mode, which should correlate with a higher benefit of the congruent flanker stimuli processed via the direct response route. For the incongruent flanker condition, I expected to observe a higher positive component and lower negative component in the non-homologous response-mode. This will reflect that the incorrect response is stronger activated as it is influenced by the incongruent flanker stimuli that has more impact on the non-homologous response-mode.

4.2 Methods

Participants

Based on sample sizes used in previous research, twenty one healthy volunteers (mean age 28 ± 5.7 years, twelve males) gave their written informed consent to participate in this study. Participants had normal or corrected-to-normal vision and were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). The protocol was approved by the STEM ethic committee of the University of Birmingham.

Flanker protocol

Participants sat comfortably on a height adjustable chair approximately 60 cm in front of a computer monitor with both arms resting on the desk, palms down with elbows slightly flexed. A Chronos device (Psychology Software Tools, Inc., Pennsylvania, USA) was used to record the response times; the participants' left and right thumbs rested on the leftmost and rightmost switches of the Chronos response box. Left and right feet were placed on the left and right foot pedals.

Participants performed a modified version of Eriksen flanker task (Eriksen, 1995) implemented in E-prime version 2.0 (Psychology Software Tools) using horizontally aligned arrows for both the flanker and target stimuli. Each trial started with a fixation cross positioned at the centre of the screen for 500 ms. Horizontal arrow flanker stimuli (horizontal arrow pointing either left or right) then appeared for 96 ms, prior to the presentation of a brief blank screen (16 ms) and then the central target stimulus for 112 ms. The screen then remained blank for 4200-5500 ms until the next trial began. Each trial took a maximum duration of 6224 ms. Participant responses were captured within the first 1500 ms (see Figure 4-4). Digital markers assigning stimuli onset, flanker conditions, response effectors, and response onset were added from E-prime to the EEG recording via the parallel port. Participants responded with either a left finger press or left foot press when the target arrow stimulus pointed to the left (<<), or responded with either right finger press or right foot press when the target arrow stimulus pointed to the right (>>). Both target and flanker stimuli were in a black Courier New 60 point size on a light grey background presented on the 19 inch LCD monitor screen (60 Hz refresh rate).

The experiment consisted of four tasks each with different response-modes (see Figure 4-5). Task 1: Participants responded with left-hand and right-hand (homologous effectors); Task 2: left-foot and right-hand (non-homologous effectors); Task 3: left-hand and right-foot (non-homologous effectors); Task 4: left-foot and right-foot (homologous effectors). Left and right target responses and one of three flanker stimuli (congruent, incongruent, and neutral), were presented with equal probability within each task. Each of the six combinations was performed 40 times within 4 blocks. Each task was presented in a separate block of 252 trials (including 12 practice trials at the start of each block). The block and the trial orders were presented in a

random fashion for each participant. A 5 minutes rest period was provided between each block and the full experiment took around 90 minutes to run.

The primary reason for including task 4 (homologous foot effectors) was so that the overall experimental design contained equal numbers of hand and foot responses. The data from this task was used for the response time analysis. However, this was not used in the EEG analyses as it did not include any hand responses. Moreover, left and right foot motor area is in between the longitudinal fissure which correlates to the Cz electrode location over midsagittal line (Penfield and Rasmussen 1950, Pfurtscheller et al. 1997), therefore we couldn't differentiate foot activity into left and right sides to get the lateralization between left and right motor cortices.

EEG recording

EEG data was recorded using a 64-channel silver/silver chloride electrodes embedded in an elastic cap (BrainCap MR model, BrainProducts GmbH, Munich, Germany) and two 32-channel BrainAmp MR amplifier systems (BrainProducts GmbH, Munich, Germany). In accordance with the International 10-20 system (Jasper, 1958), the reference electrode was positioned on FCz and the ground electrode was positioned on AFz. An additional electrode was placed below the left clavicle for electrocardiogram acquisition. Abralyte gel was used to keep the electrode impedance below 5 kΩ for the electrodes at C3, C4, FC3, and FC4. The cap connectors were linked to the amplifiers via two bundled cables. The EEG signal was sampled at 5 kHz rate with a bandpass filter of 0.016-250 Hz and stored on a pc using BrainVision Recorder (BrainProducts GmbH, Munich, Germany).

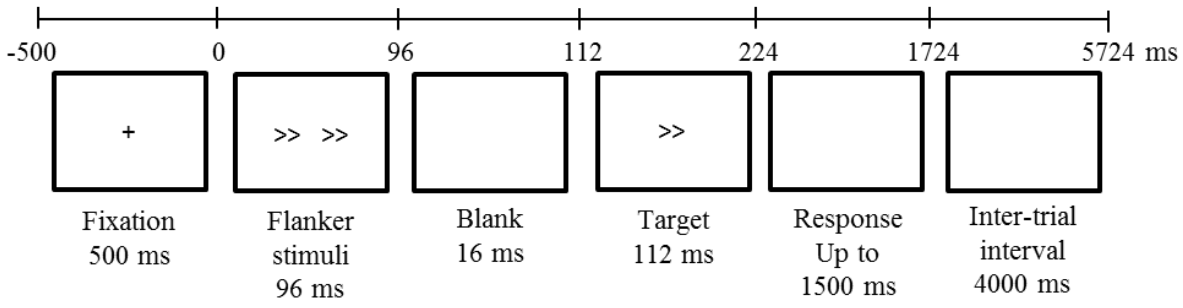

Figure 4-4. Example trial from the flanker task used in the current study. Sequence of the stimuli. Each trial started with a fixation cross for 500 ms. The flankers stimuli appeared for 96 ms followed by a blank screen for 16 ms. Then the target stimuli presented for 112 ms followed by a blank screen, when the participants provided a response within 1500 ms. The interval between each trial presented as a blank screen appeared for 4000 ms.

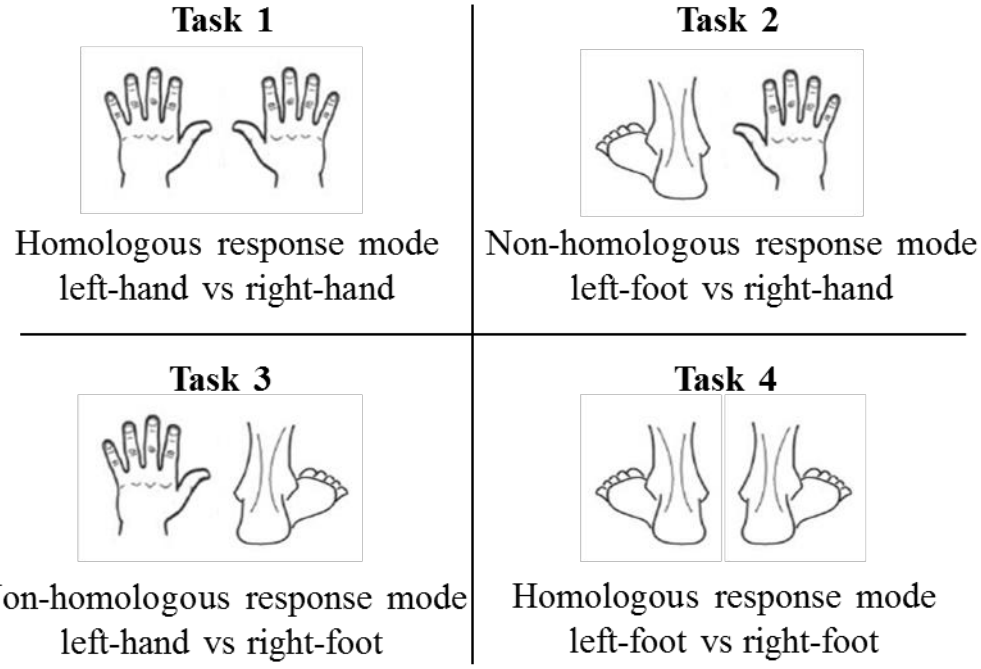

Figure 4-5. Experimental conditions of four tasks: homologous hand response-mode where participants responded with either their left or right hand (task1); non-homologous response-mode where participants responded with either their left-foot or right-hand (task 2) and their left-hand or right-foot (task 3); homologous foot response-mode task where participants responded with either their left or right foot (task 4).

Data analysis

Behavioural data

Response times were recorded on individual trials as the onset of the target stimulus to the onset of the button press recorded in E-prime. The response time markers in the EEG recording showed some inconsistencies with those in E-prime. As the Chronos device provided a direct timestamp, the latter were the most accurate measurement and so the E-prime RT onsets were subsequently transferred into the EEG data to override the original markers and used during analyses. Trials with response times faster than 112 ms, slower than 1112 ms, or with incorrect or missing responses were removed from the reaction time analysis. We excluded two participants who had > 25% errors in any single task condition.

To determine the behavioural congruency effect and to normalise across participants, the mean response time in the neutral flanker condition was subtracted from the mean response time of both the congruent and incongruent conditions. This was performed separately on each participant and task.

EEG data

The raw EEG data were processed offline and analysed using MATLAB (R2017b; Math Works, Massachusetts, USA) and EEGLAB (MATLAB toolbox version 12.0.1). The signal was first down-sampled to 600 Hz and the band-pass filter adjusted to 0.1 - 45 Hz. After filtering, each trial was segmented into 1000 ms epochs beginning from the 100 ms before the flanker stimulus onset. This 100 ms time window was used as a baseline. After epoching the data, any trials that contained an artifact or showed a noisy waveform in C3, C4 were identified by visual inspection (exceeding ± 100 μV) and rejected from the analysis. Before running the LRP calculation,

ocular artifacts were removed using independent component analysis (ICA) implemented in EEGLAB toolbox. Eimer (1998) suggested that at least 40 trials should be included into the averaged ERP for both side of responding effectors to compute a reliable LRP waveforms, however, they acknowledged this was a guideline and a lower number of trials could also provide suitable ERPs. Moreover, because both sides contributed to the LRP waveforms, an equal number of left- and right-sided responses is optimal. We were able to obtain a minimum of 30 trials in the LRP average of each task condition.

The LRP analysis on left and right motor cortices activity in the homologous hand response-mode (task 1) was straightforward, but proved more difficult in the non-homologous response-modes (tasks 2 and 3). The electrical dipoles from the foot area travel to the ipsilateral scalp site electrodes due to the location of the motor foot area on the medial surface of the PMC, which means that foot response ERPs show the opposite polarity to hand responses (Bocker et al., 1994). As described in the introduction, Miller (2012) first used ERP from Cz to examine the movement preparation in the task required both limb systems (hand and foot) (see Equation 4-2). The Cz ERP was more positive when responding with the hands than the feet. When the potentials at Cz were treated with the averaging method to yield the LRP, the hand response produced a positive LRP while the foot response produced a negative LRP. This procedure that represents the potential difference between the hand and foot responses recorded from Cz electrode site was found sensitive to use as an indicative of movement preparation in the task using hand and foot responses. This was termed as a limb selection potential (LSP) to use as an index of the limb system used in the task. However, the LSP method is only computed from the ERP activity at Cz electrode because it can display the movement related potentials for both

hand and foot movements. The limitation of this method is that it only provides the limb system difference, which cannot differentiate the side.

$$LSP = Cz_{(hand)} - Cz_{(foot)}$$

Equation 4-2. The formula for calculating the limb selection potential between hand and foot response derived from the potential difference recorded at the Cz electrode site when responds with hand and foot.

In the current experiment, we attempted to obtain a stimulus-locked LRP from C3 and C4 electrodes when responding with the hands and the Cz electrode site that resulted from the foot responses in the non-homologous response-modes in tasks 2 and 3. However, we were unable to obtain a reliable LRP from electrodes C3, C4 and Cz in the task conditions involving foot responses. This all relates to the issue of location of the foot region in the motor cortex, it makes it harder to measure, as there were a large difference in activity recorded in the C3/C4 compared to Cz. Activity occurred in the Cz can be contaminated by the hand response involved in the experiment. Therefore, any comparison between foot and hand regions become difficult.

To overcome this problem, we decided to compare the activity of the electrode over the PMC contralateral to the *selected hand* when the non-selected response was either the opposite hand (task 1) or the opposite foot (tasks 2 and 3). It was adapted from the LSP method as we were interested in the difference of ERPs measured from the same electrode site between responding with two conditions. However, the difference from the LSP methods was that it was always recorded the potential from the Cz electrode site contralateral to the responding hand when the hand was selected. We compared how the non-selected homologous response-mode affects the potential measured over the PMC contralateral to the *selected hand* and how the non-selected non-homologous response-mode affects the potential measured over the PMC contralateral to

the *selected hand*. The C3 and C4 electrodes were selected because it showed largest potential when responding with the hands. While the LSP was always recorded the potential only from Cz when the hand and the foot were selected and compare the potential difference between the two limb responses. The method used in this study will be illustrated using selected right-hand responses as an example (see right panel of Figure 4-12). ERP from the hand motor area (C3) contralateral to the selected right-hand was first calculated in task 1 (left-hand non-selected; see Figure 4-5). The corresponding ERP from C3 in task 2 was then calculated when the right-hand was selected (left-foot non-selected). We then subtracted the ERP recorded from C3 during task 2 from that recorded during task 1 to produce an intermediate ERP for the right-hand responses. For the left-hand responses (see left panel of Figure 4-12), the same steps were repeated but used the ERP from C4 contralateral to the selected left-hand in task 1 (right-hand non-selected) and task 3 (right-foot non-selected). We then averaged the intermediate ERP when right-hand and left-hand selected were combined that was termed the selected condition readiness potential (CRP) (see Equation 4-3). The CRPs were derived separately for congruent, neutral and incongruent flanker conditions.

$$CRP_{selected} = [\text{mean } (C3_{homologous} - C3_{non\text{-}homologous})_{\text{ right-hand selected}} + \text{mean } (C4_{homologous} - C4_{non\text{-}homologous})_{\text{ left-hand selected}}] / 2$$

Equation 4-3. The formula for calculating the conditioned readiness potential when the hands were selected in competition with homologous and non-homologous effector tasks

The same CRP method was also performed on the electrode over the hand motor area contralateral to the *non-selected* hand to compare how the activity was affected by whether the selected response was the opposite hand (task 1) versus the opposite foot (tasks 2 and 3). Using the non-selected right-hand as the example, ERP from the hand motor area (C3) contralateral

to the non-selected right-hand in task 1 (left-hand selected) was first calculated (see Figure 4-5). We then calculated the corresponding ERP from C3 in task 2 when the right-hand was non-selected (left-foot selected). The ERP recorded from C3 during task 2 was then subtracted from that recorded during task 1 to produce an intermediate ERP for the right-hand non-selected conditions. For the left-hand non-selected condition, we repeated similar steps but calculated the ERP from C4 contralateral to the non-selected left-hand in task 1 (right-hand selected) and task 3 (right-foot selected). We then averaged both non-selected intermediate ERPs to obtain the 'non-selected CRP' (see Equation 4-4). This method was performed separately for the congruent, incongruent, and neutral flanker conditions.

$$CRP_{\text{non-selected}} = [\text{mean } (C3_{\text{homologous}} - C3_{\text{non-homologous}})_{\text{ right-hand non-selected}} + \text{mean } (C4_{\text{homologous}} - C4_{\text{non-homologous}})_{\text{ left-hand non-selected}}] / 2$$

Equation 4-4. The formula for calculating the conditioned readiness potential when the hands were not selected in competition with homologous and non-homologous effector tasks

Statistical analysis

EEG data

The onset latency of the mean LRP was calculated for each of the congruent, incongruent, and neutral conditions using an automated script in MATLAB. This determined the first time point that the LRP signal changed by more than ± 3 standard deviations from the mean baseline period in the 100 ms prior the flanker stimuli onset. The sampling rate of the LRP data was 600 Hz. Therefore, the sample points were in 1.67 ms steps. The peak latency of the incongruent LRP set as the most positive deflection in the 0 to 400 ms after the flanker onset. Paired-samples t-

tests were performed on the mean amplitudes of the congruent and incongruent LRPs in the 400 ms after flanker onset to determine the time-course of the response activation processes. Statistical testing was conducted with automated scripts in MATLAB. The significance level was set to $P < 0.05$, but was adjusted for multiple comparisons using the Bonferroni correction (adjusted $P < 0.0002$).

The onset latencies of the mean CRP associated with the selected-hand was determined separately for each of the congruent, incongruent, and neutral conditions using an automated script. This determined the first time point that the CRP changed by more than ± 3 standard deviations from the mean baseline period in the 100 ms prior to the flanker stimuli onset. To determine the latency when the CRP showed a significant difference from zero, a paired-samples t-test was performed on the mean amplitudes of the neutral, congruent, and incongruent CRPs separately in the 0 to 400 ms after the flanker onset to determine the time-course of the different in response activation between homologous and non-homologous response-modes. The significance level was set to $P < 0.05$, but was adjusted for multiple comparisons using the Bonferroni correction (adjusted $P < 0.0002$). This method was repeated for the CRP associated with the non-selected hand to determine whether the response-mode affected the pattern of 'incorrect response activation' obtained in the incongruent condition.

4.3 Results

The following results include sixteen participants as five participants were excluded from the analysis because they had less than 30 trials remaining after removing errors or rejecting artefacts.

Behavioural data

Neutral flanker condition

Mean response times were first explored to determine whether the hand responses were faster than foot responses and whether there was a dominance effect. It was also then tested whether the homologous response-mode was faster than the non-homologous response-mode. A 3-way repeated measures ANOVA with factors of 2 RESPONSE-MODE (homologous, non-homologous) x 2 LIMB (hand, foot) x 2 SIDE (left, right) was conducted on the response times obtained in the neutral flanker condition only. This condition was not influenced by the flanker stimuli, therefore it would allow us to directly see the effect of the above factors on the response time.

Figure 4-6A shows the effect of the flanker stimuli on the mean response times in each of the four tasks. Figure 4-6B displays the same data but reallocated according to the perspective of the responding effector (left-hand, right-hand, left-foot and right-foot). This allowed direct comparisons of the effect of response-mode on the response times from each effector. In each responding effector, the congruent flanker trials were faster than the neutral and the incongruent flanker trials were slower than neutral. A repeated measures ANOVA on the response times of the *neutral* condition only revealed that there were main effects of RESPONSE-MODE ($F_{1, 15}$ = 37.9, P < 0.001) with a 21.6 ± 3.5 ms faster response time in the homologous response-mode, and LIMB ($F_{1, 15}$ = 29.8, P < 0.001) with hand responses being 83.3 ± 15.3 ms faster than foot responses. A main effect of SIDE ($F_{1, 15}$ = 6.0, P = 0.03) revealed that right-side responses (dominant) were 16.0 ± 6.5 ms faster than left-side responses. There was no interaction between these factors.

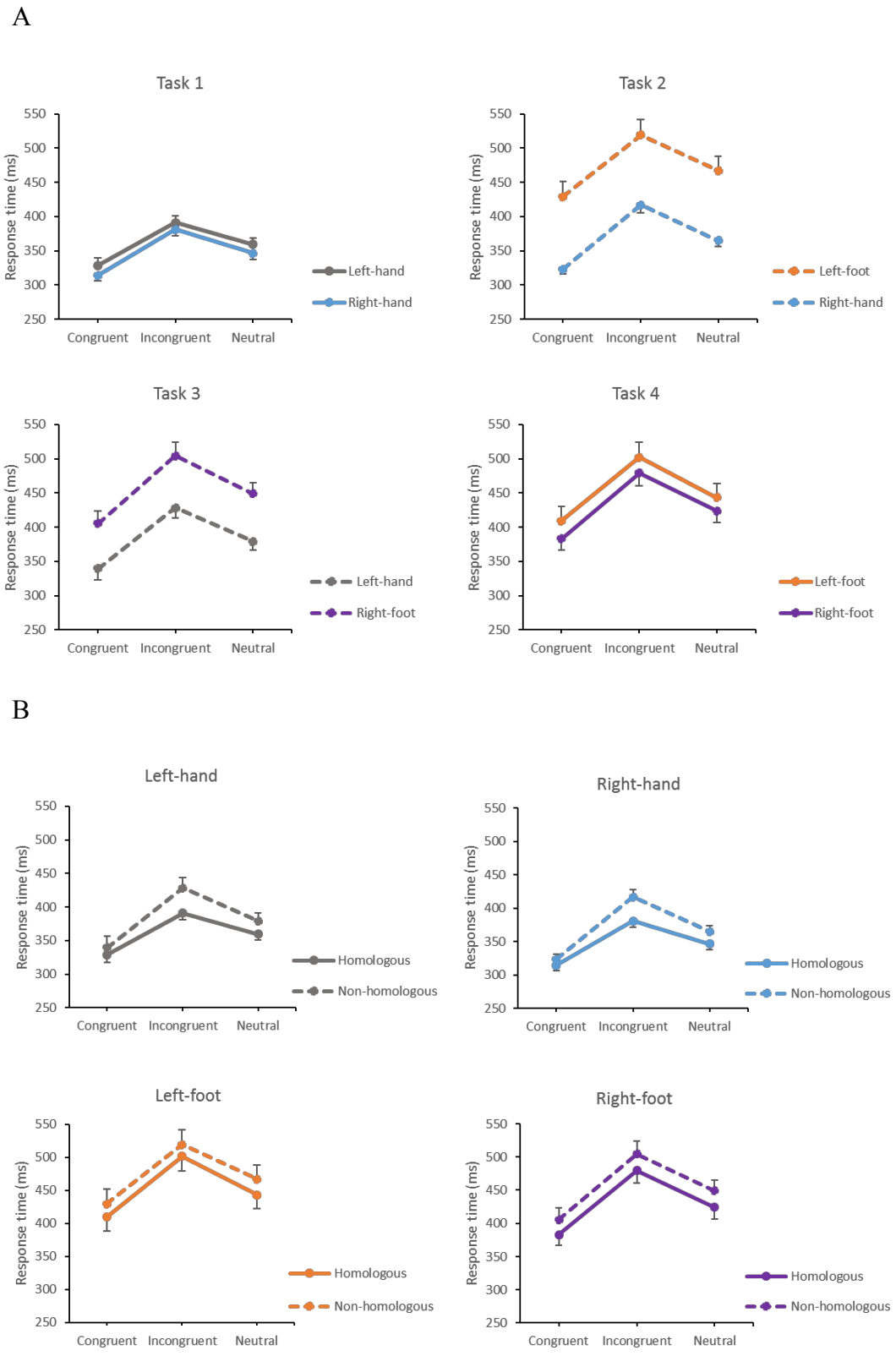

Figure 4-6. Effect of response-mode and congruency on the mean response times. Results displayed as; (A) separate tasks (B) separate effectors to compare between homologous and non-homologous response-modes. The ANOVA results presented in the main text are based on the conditions being allocated into separate effectors.

Effect of the response-mode on the congruency effect

In terms of behaviour, the main hypothesis was that the flanker stimuli would produce a greater congruency effect with the non-homologous response-mode. This is because the history-dependent model (Labruna et al. 2014) proposes that the non-selected effectors receive less inhibition during competition resolution with a non-homologous response-mode. As a consequence, the direct response activation route should receive less selective suppression and so the flanker stimuli should have more influence on the decision making process (Ridderinkhof et al., 2005). Therefore, it was expected to see a larger congruency effect in the non-homologous response-mode as the congruent flanker will speed up the response times and the incongruent flanker will slow down the response times at a greater extent.

Figure 4-7 depicts the data presented in Figure 4-6 but after the response times have been converted into the congruency effect. Panel A presents the results in terms of the four tasks. Congruency effects > 60 ms were observed in both left and right hand and foot responses. Panel B displays the same data but reallocated according to the perspective of the responding effector (left-hand, right-hand, left-foot and right-foot). The latter allows direct comparisons of the effects of response-mode within each effector. A repeated measures ANOVA of 2 RESPONSE-MODE (homologous, non-homologous) x 2 LIMB (hand, foot) x 2 SIDE (left, right) revealed a significant main effect of RESPONSE-MODE ($F_{1, 15}$ = 30.8, P < 0.001). The congruency effect was 13.3 ± 2.4 ms larger with the non-homologous response-mode. The main effect of LIMB ($F_{1, 15}$ = 10.5, P = 0.005) showed that there was a 16.7 ± 5.1 ms larger congruency effect with foot responses. There was also an interaction of RESPONSE-MODE * LIMB ($F_{1, 15}$ = 12.4, P = 0.003). The non-homologous response-mode was 26.7 ± 3.8 ms larger with hand responses (P < 0.001), but no different with foot responses (0.1 ± 5.1 ms, P = 0.99). This result

confirmed that the effect of response-mode was influenced by the limb in which the response occurred. In contrast, the congruency effect was not influenced by the response side as there was no main effect of SIDE ($F_{1, 15}$ = 1.6, P = 0.22) or significant interactions including SIDE (all P > 0.05).

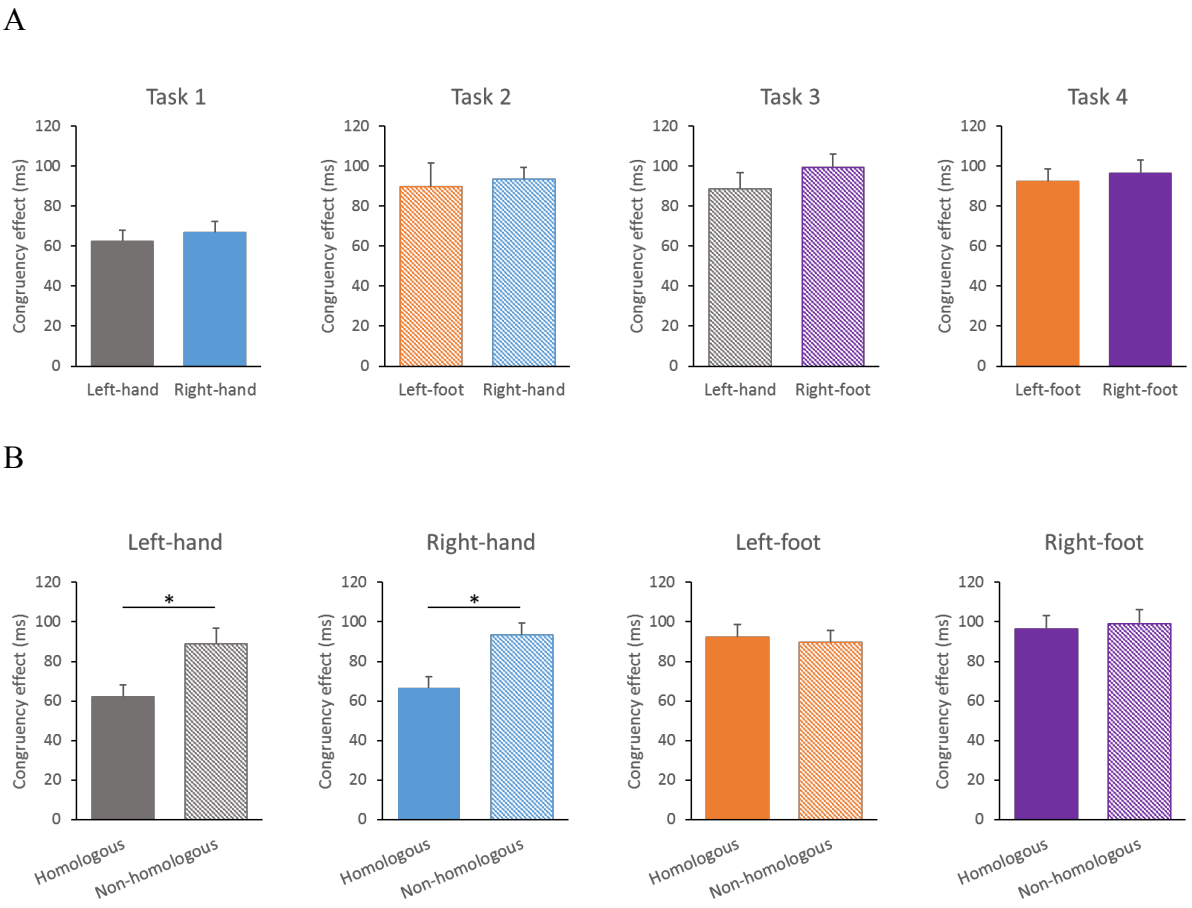

Figure 4-7. Effect of the response-mode on the congruency effect (response time difference between congruent and incongruent flanker conditions). Results displayed as; (A) separate tasks (B) separate effectors to compare between homologous (solid bars) and non-homologous (patterned bars) response-modes. The ANOVA results presented in the main text are based on the conditions being allocated into separate effectors. (*P < 0.01)

Effect of the response-mode on the normalised response time

The underlying reason for an effect of response-mode on the congruency effect was further explored (i.e. was it due to changes in the congruent or incongruent response times?) by examining the normalised response times. These are displayed according to the effector used in Figure 4-8. A 4-way repeated measures ANOVA with factors of 2 RESPONSE-MODE (homologous, non-homologous) x 2 LIMB (hand, foot) x 2 SIDE (left, right) x 2 FLANKER-CONGRUENCY (congruent, incongruent) was performed on the normalised response times. This revealed an interaction of RESPONSE-MODE * LIMB * FLANKER-CONGRUENCY ($F_{1, 15}$ = 12.4, P = 0.003), which demonstrated that for the congruent flanker conditions, although hand responses were significantly faster in the non-homologous response-mode (9.0 ± 3.0 ms, P = 0.008), foot responses were not (3.2 ± 5.1 ms, P = 0.54). For the incongruent flanker condition, hand responses were 17.6 ± 4.3 ms slower with the non-homologous response-mode (P = 0.001), but there was only a 3.1 ± 5.2 ms difference in the foot responses (P = 0.56).

In summary, the non-homologous response-mode produced a larger congruency effect for hand responses. This was due to a greater slowing of the response time in the incongruent flanker condition and a faster response time in the congruent flanker condition. No such effects of response-mode were detected for the foot responses.

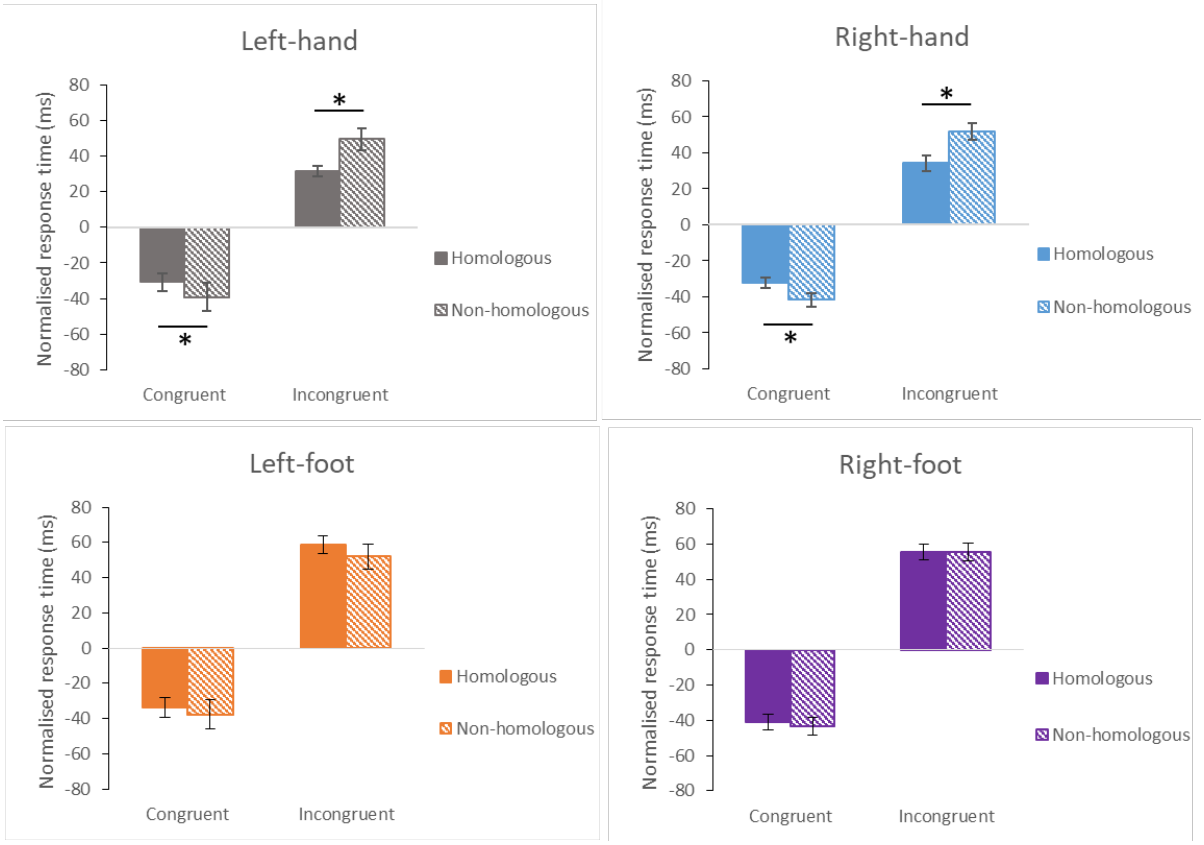

Figure 4-8. Normalised response times: mean response time in the neutral flanker condition was subtracted from the mean response time of both the congruent and incongruent conditions in both hands and both feet when it was in the homologous and non-homologous response-mode. (*P < 0.05)

EEG data

LRP data

The first part of the EEG analysis focused on the LRPs obtained during task 1 - hand responses with a homologous response-mode. Figure 4-9 displays the potentials recorded over electrodes positioned over left PMC (C3) and right PMC (C4) from an individual participant during task 1. For the congruent and neutral flanker conditions (see Figure 4-9 panel A and C), the potential recorded from the electrode contralateral to the responding hands generally showed a greater negative potential (more activated) than the ipsilateral side. This indicates the correct hand response activation. For the incongruent condition (see Figure 4-9B), a negative potential was

initially seen in the electrode ipsilateral to the responding hand prior to the negative potential in the electrode site contralateral to the responding hand. This indicated that the incorrect response activation occurred prior to the correct response activation. The intermediate ERPs were plotted as a waveform represented the potential differences after subtracting the potential in the electrode site ipsilateral to the responding hand from the contralateral to the responding hand. Therefore, a negative intermediate ERPs reflects the correct response activation as it showed in the congruent and neutral flanker condition, while a positive intermediate ERPs reflects the incorrect response activation as it can be found in the incongruent flanker condition. The intermediate ERPs in left and right-hand responses were averaged to provide the LRP waveform (see Figure 4-9D). This step was performed separately in each flanker condition.

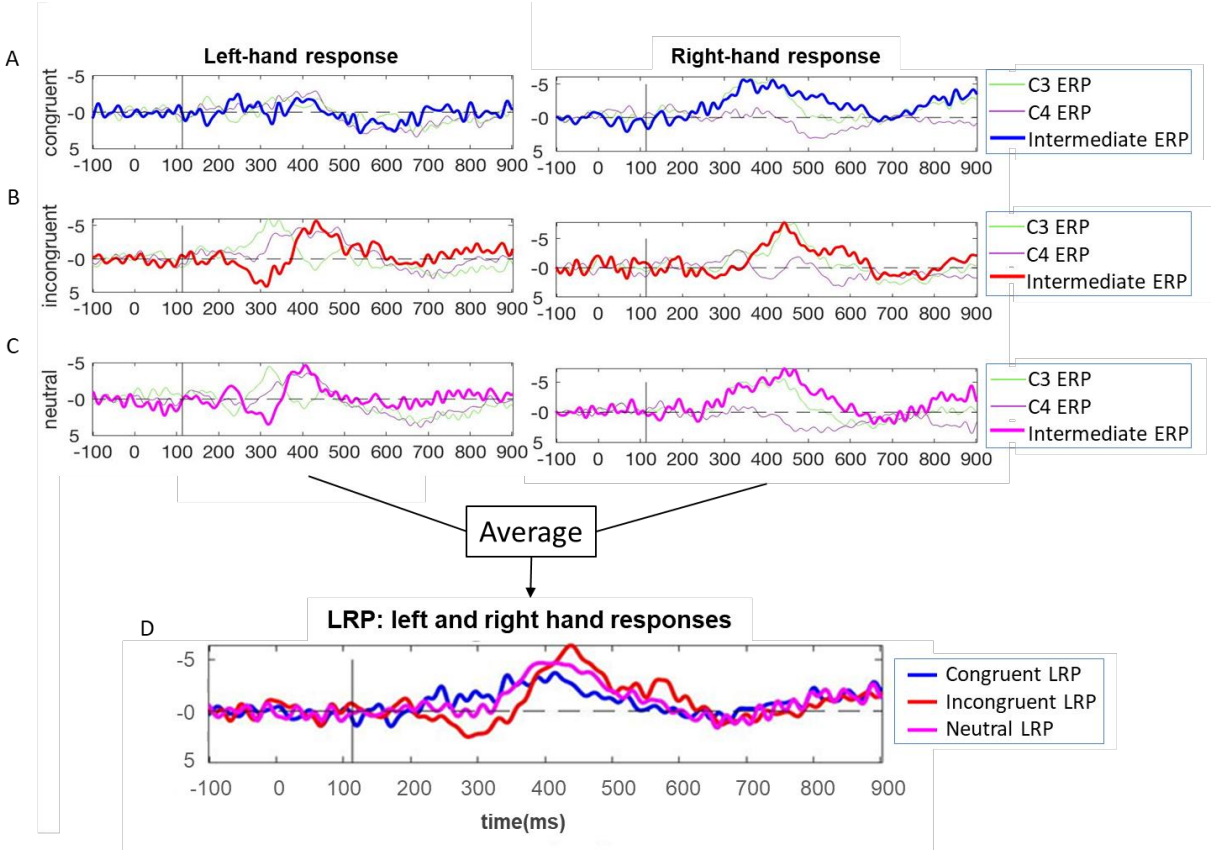

Figure 4-9. Example of the ERPs recorded from the C3 (green waveform) and C4 (purple waveform) electrodes in left and right hand response trials within an individual participant. Time zero denotes the onset of flanker stimuli and the vertical black line indicates the onset of target stimuli at 112 ms after the flanker onset. The intermediate ERPs after subtracting the potential in the electrode site ipsilateral to the responding hand from the contralateral to the responding hand are presented in the congruent (blue waveform in panel A), incongruent (red waveform in panel B), and neutral (pink waveform in panel C) condition separately. In left-hand response trials, the potential difference is calculated from C4-C3 site. In right-hand response trials, the potential difference is calculated from C3-C4 site. The average LRPs are averaged between left-hand and right-hand response trials.

Figure 4-10A depicts the grand average LRP across all 16 participants as recorded from both left and right hand responses. In the neutral condition the onset latency of the LRP was 215 ms after the target onset, which was 109 ms later than the LRP onset in the congruent condition and 92 ms later than in the incongruent condition and therefore close to the 112ms delay between the flanker and target onsets. This LRP data showed that the congruent flanker produced a negative LRP, which was earlier than the neutral LRP. For the incongruent trials, the flanker first evoked a strong positive deflection, which indicates the response activation of the incorrect hand (Coles et al. 1989). The incongruent LRP peak latency from the grand average waveform was at 292 ms after the flanker onset. This was obtained when the mean LRP was plotted across all participants and then the incongruent peak latency was determined. This peak latency was slightly earlier than the mean of incongruent LRP peak latency from each individual of 296.2 ± 29.4 ms after the flanker onset. The latency when the incongruent LRP was significantly different from the congruent LRP was between 258 and 353 ms after the flanker onset with the Bonferroni correction for a latency window of 0 – 400 ms (see Figure 4-10A; presents in a grey bar). After the peak of positive deflection, the LRP decreased from a positive component to a negative component. The lateralisation towards the correct response activation (i.e. when the LRP first became negative) was 368 ms after the flanker onset (256 ms after the target stimulus onset).

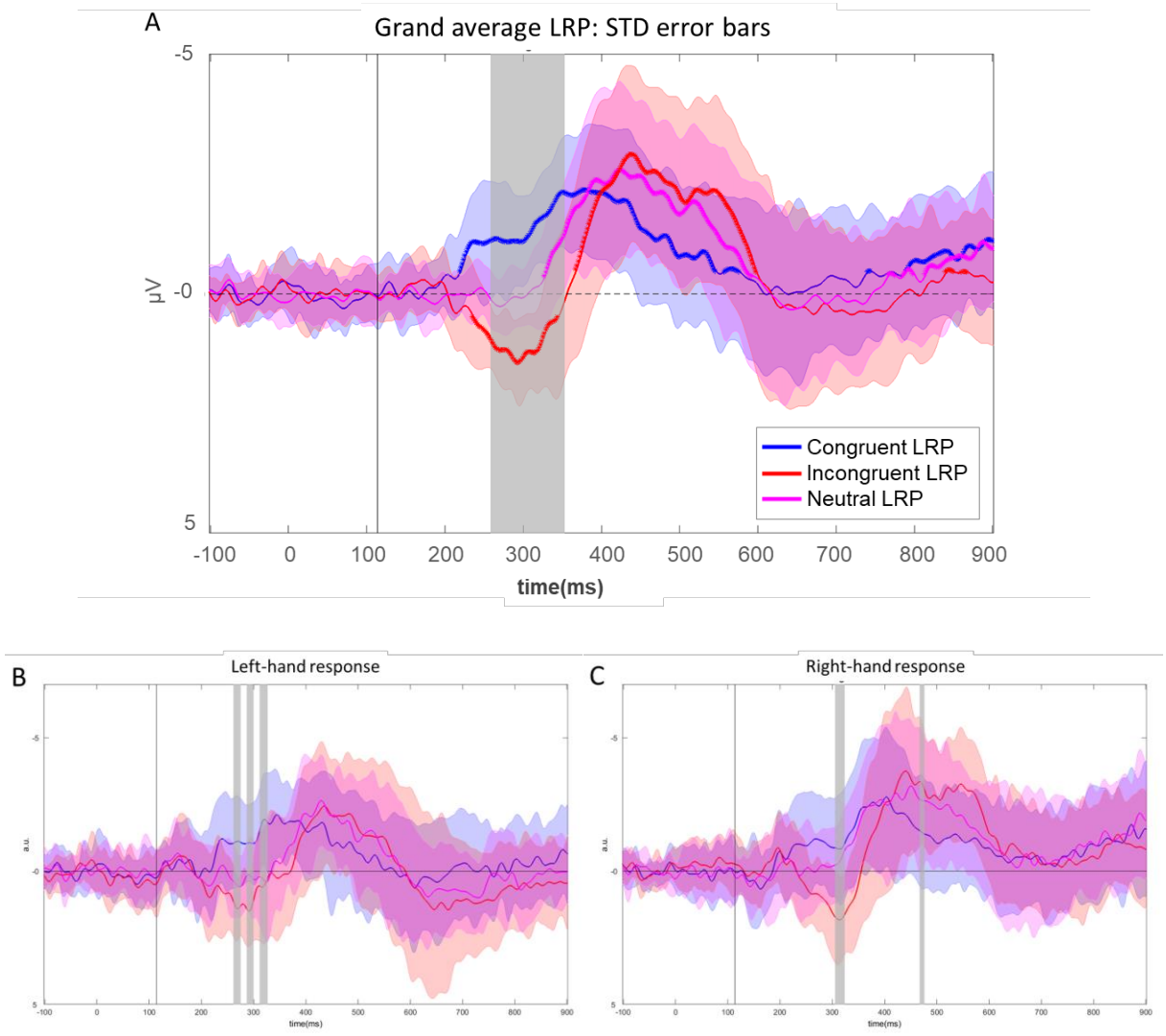

Figure 4-10. (A) Grand averaged LRPs average from left and right hand responses are displayed separately for the congruent (blue waveform), incongruent (red waveform), and neutral (pink waveform) flanker conditions. Time point zero denotes the onset of the flanker stimuli. The thin black vertical line indicates the onset of the target stimuli of 112 ms after the flanker onset. (B) The intermediate ERPs represent the different of voltage potentials in the left-hand response trials after subtracting the potential recorded at the C3 (ipsilateral to the responding hand) from the C4 (contralateral to the responding hand). (C) The intermediate ERPs represent the different of voltage potentials in the right-hand response trials after subtracting the potential recorded at the C4 (ipsilateral to the responding hand) from the C3 (contralateral to the responding hand). Stars represents the data point that exceed 3 SD from the baseline period. Grey box shows the latency when LRP from congruent trial was significant different from incongruent trial.

The aim was to explore whether the grand mean LRPs in three flanker conditions present in Figure 4-10A were strongly influenced from either left or right hand response. The grand mean LRPs were calculated from averaging the mean intermediate ERPs from left and right hand response trials together as shows in Figure 4-10B and C. Similar overall patterns can be seen but there are some small changes in the onset latencies. The onset latency was earlier in the right-hand as compared to the left-hand response for 41 ms in neutral, 33 ms in congruent and 18 ms in incongruent flanker conditions.

Selected Conditioned Readiness Potential results

The second part of the EEG analysis focused on the CRPs obtained during trials in which the hands were selected. The selected CRP analysis calculated the activity of PMC contralateral to the selected hand with homologous (task 1 – opposite hand) or non-homologous (tasks 2 and 3 – opposite foot) response-modes. The onset latency of the incongruent LRP occurred 235 ms after the flanker onset and its amplitude was significantly different from the congruent LRPs from 258 to 353 ms. Therefore the selected CRP analysis mainly focused on the 200 to 350 ms after the flanker onset. It was expected that the congruent flanker to produce a greater negative potential with the non-homologous response-mode, which would reflect more activation of the correct response. In contrast, for the incongruent flanker, a smaller negative potential in the non-homologous response-mode was expected to be observed. This would reflect less activation of the correct response as the incongruent flanker would initially produce a stronger activation of the incorrect response.

Figure 4-11A; pink waveform demonstrates the selected CRP in neutral flanker condition resulting from averaging the intermediate ERPs between left and right hand responses. The onset latency was at 228 ms after the flanker onset. No significant difference of the response activation between homologous and non-homologous effectors was found in neutral flanker condition. As outlined in the method, the first step of calculating the selected CRP was to record the ERPs from the electrode contralateral to the responding hand with the homologous and non-homologous response-mode (see Figure 4-12A). The ERPs data before subtraction demonstrates that when responding with left-hand and right-hand in the neutral flanker condition, the homologous response-mode had a greater negative potential during 200 to 350 ms after the flanker onset compared to the non-homologous response-mode suggested that the correct hand responses were slightly more activated in the homologous response-mode. Therefore, after subtracting the ERP recorded in the non-homologous from the ERP recorded in the homologous response-mode, the intermediate ERP showed a negative value.

For the congruent flanker condition (see Figure 4-11A; blue waveform), the selected CRP had an onset latency of 192 ms after the flanker onset in the congruent condition, which was 36 ms earlier than the neutral flanker condition. The intermediate ERPs (see Figure 4-12B) demonstrate that when responding with left-hand, the non-homologous response-mode had lower negative potential indicating less correct response activation in the non-homologous response-mode. However, when responding with right-hand, the intermediate ERP was close to zero indicating no ERP different between homologous and non-homologous response-modes. When averaging the intermediate ERPs between the left-hand and right-hand response trials, the selected CRP did not show any significant difference from zero during 200 to 350 ms

after the flanker onset. Therefore, the correct response activation in the non-homologous response-mode was comparable to the homologous response-mode in the congruent condition.

The behavioural results showed that there was a larger congruency effect in the non-homologous response-mode, which primarily resulted from slower response times in the incongruent flanker condition. If the incongruent flanker leads to a greater incorrect response activation, it will shows a higher negative potential in the PMC contralateral to the non-selected hand. Then the opposite hemisphere, which is contralateral to the selected hand would therefore show less negative potential that reflects less correct response activation in the non-homologous response-mode. Figure 4-11A; red waveform shows the mean selected CRPs in the incongruent condition averaged from both hand responses. The grey box on the graph represents the latency when the incongruent CRP was significant different from zero during 233 to 250 ms after the flanker onset (Bonferroni correction for a time window of 0 – 400 ms). The beginning of this time window was coincident similar to the LRPs onset latency of 235 ms after the flanker onset in the incongruent flanker condition when left and right hand responded in the homologous response-mode (see Figure 4-11; panel A and B). This suggests that in the incongruent condition, the non-homologous response-mode had significant lower correct response activation in the PMC contralateral to the responding hand (see Figure 4-12C). The motor cortex contralateral to the selected hand was less activated in the non-homologous response-mode when responded with left and right hands at the duration of 200 to 350 ms after the flanker onset.

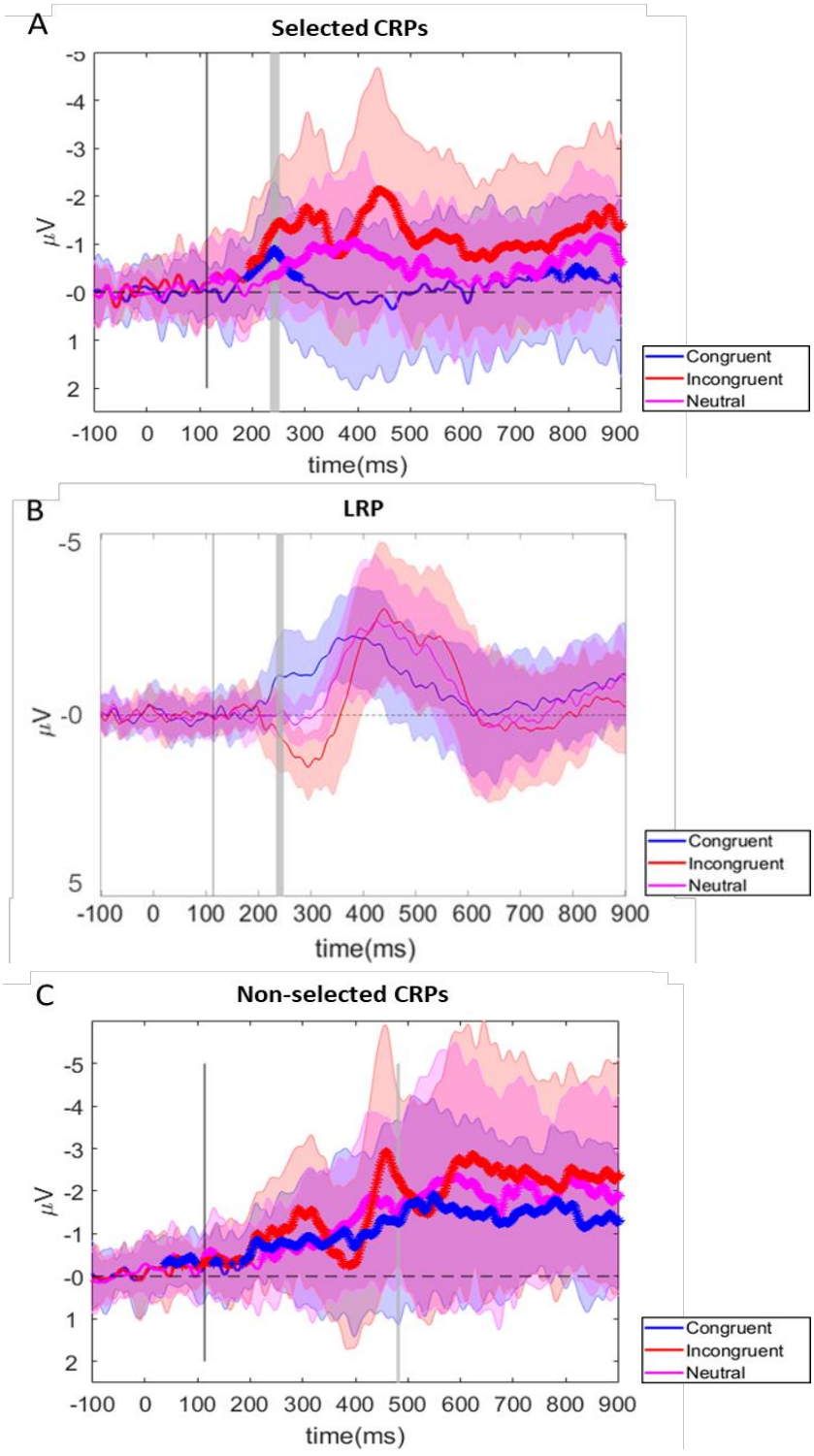

Figure 4-11. (A); CRPs when left and right hands were selected with the homologous and non-homologous effectors in congruent, incongruent and neutral flanker congruency conditions. Time zero denotes the onset of flanker stimuli and the vertical black line indicates the onset of target stimuli at 112 ms after the flanker onset. Grey bar represents the latency when the incongruent selected CRP shows a significant different from zero. (B); average LRPs from left and right hand responses with the homologous effector in congruent, incongruent and neutral flanker congruency conditions. Grey bar represents the onset latency of the incongruent LRP. (C); CRPs when left and right hands were not selected. Grey bar represents the latency when the data point in the incongruent condition shows a significant different from zero.

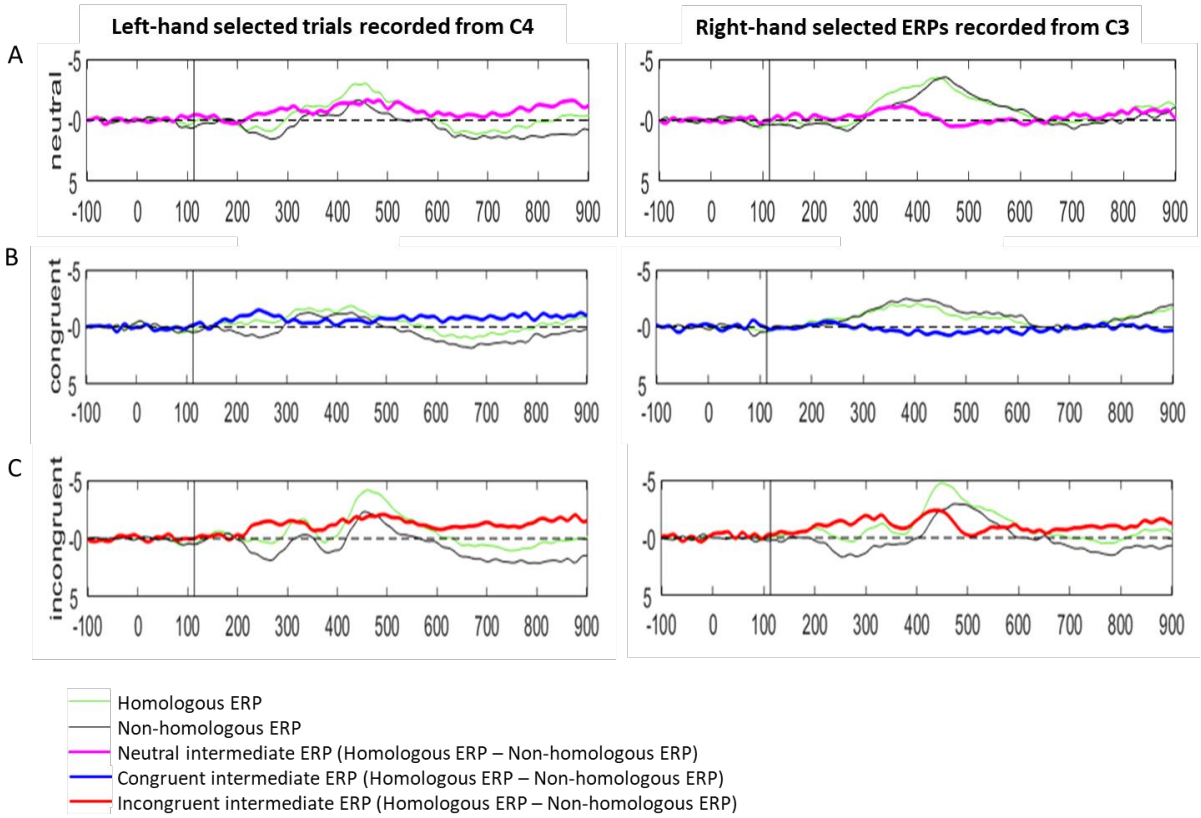

Figure 4-12. The mean ERPs from 16 participants recorded from the electrode site contralateral to the responding hand in the homologous (green waveform) and non-homologous response-modes (grey waveform). Time zero denotes the onset of flanker stimuli and the vertical black line indicates the onset of target stimuli at 112 ms after the flanker onset. Left panel display the ERP recorded from C4 electrode site in the left-hand response trials in the homologous response-mode (task 1; green waveform) and non-homologous response-mode (task 3; grey waveform). Right panel display the ERP recorded from C3 electrode site in the right-hand response trials in the homologous response-mode (task 1; green waveform) and non-homologous response-mode (task 2; grey waveform). The intermediate ERPs represents the voltage potential difference after subtracting the potential recorded in the non-homologous from the homologous response-modes are display separately in the neutral (panel A), congruent (panel B), and incongruent (panel C) flanker conditions. The intermediate ERPs in left and right hand response will be averaged to yield a selected CRPs.

Non-selected Conditioned Readiness Potential results

The final part of the analysis was to determine the effects of response-mode on the non-selected CRP. This was measured from the PMC contralateral to the non-selected hand and indicated the amount of incorrect response activation produced by the incongruent flanker (see Figure 4-11C). As the selected CRP showed that incongruent flanker was associated with reduced correct response activation with the non-homologous response-mode (see Figure 4-11A), it was expected that the non-selected CRP would show that the incongruent flanker produced higher incorrect response activation in the opposite PMC (contralateral to the non-selected hand). Each of non-selected CRP amplitude in three flanker conditions was tested against zero during the time window of 200 to 350 ms after the flanker onset. However, the non-selected CRP did not show a statistically significant change in any of the three flanker conditions (all $P > 0.05$). This indicated that response-mode had no effect on the incorrect response activation.

Using the LRP and CRP analyses, this indicated that a larger congruency effect that was found when responding with hands in the non-homologous response-mode primarily resulted from the incongruent flanker condition. The potential difference between homologous and non-homologous response-modes was only observed in the incongruent flanker condition as there was less correct response activation in left and right hands in the non-homologous response-mode. This corresponded to the slower response time in the non-homologous response-mode when responding with the hands to the incongruent flanker condition. However, the amount of incorrect response activation difference between homologous and non-homologous response-mode was unable to be observed.

4.4 Discussion

This study explored the influence of response-mode on the inhibitory control of response selection during a flanker task. The behavioural data demonstrated that the non-homologous response-mode showed a larger congruency effect for hand responses. This was primarily due to the slower response time in the incongruent flanker condition, but also a faster response time in the congruent flanker condition as compared to the homologous response-mode. This is consistent with our main hypothesis that that the direct response activation produced by the task-irrelevant flanker stimuli receives a lower level of selective suppression with the non-homologous response-mode. This main hypothesis was further tested using EEG recordings obtained over left and right PMC sites. These allowed us to explore the effects of response-mode on the correct and incorrect response activation processes at the cortical level. The LRP data provided the time window when the incorrect response activation was exerted during responded with the hands homologous response-mode in the incongruent condition. The incongruent flanker first evoked a positive deflection the incongruent LRP, indicated the activation of the premature preparation of the incorrect hand response. After the peak of incorrect response activation, the LRP turned to a negative deflection, which demonstrated the correct response activation. There was a limitation to observe the brain potential difference between homologous and non-homologous response-modes using the LRP approach. Therefore, the selected CRP approach was then performed by recording the brain potentials the electrode contralateral to the selected hand when responding with the homologous and non-homologous response-mode. This reflected the influence of response-mode onto the amount of correct response activation in three flanker conditions. The selected CRP data indicated that the non-homologous response-mode had a greater influence from the incongruent flanker stimuli

than the homologous response-mode. This was corresponded to slower response time in the non-homologous response-mode with the incongruent condition.

The processes underlying the congruency effect can be explained using the 'activation-suppression' model (Ridderinkhof et al., 2005). In the incongruent flanker condition, the response activation from direct response route is opposite to that of the deliberate response route, which leads to response conflict. The strength of the direct activation resulting from the task-irrelevant flanker stimuli can be reduced via the selective suppression pathway mediated by structures in prefrontal cortex and basal ganglia (Iannaccone et al., 2015, Garavan, 2002). Once the selective suppression is applied, the incorrect response will be inhibited before it reaches the threshold and allow the correct response activation elicited by the task-relevant target stimulus processed via the deliberate response route to be initiated. The processing time via the deliberate route builds up relatively slowly until it reaches the decision threshold, therefore, the response time in the incongruent flanker condition is slower than both congruent and neutral flanker conditions. If the competition resolution processes to inhibit the non-responding hand was influenced by the response-mode as described by proximity/history dependent model in Labruna et al. (2014), consequently the amount of selective suppression onto the direct response activation route would be greater when responding with the homologous response-mode.

Behavioural findings

The behavioural data in this study supported the activation-suppression model because the response time in the incongruent flanker condition was slower than the congruent flanker condition. The incorrect activation induced by the incongruent flanker stimuli via the direct

response route was required to be inhibited before the correct response activation reached the threshold via the deliberate response route. When the response-modes were modified into homologous and non-homologous response-modes, the behavioural data showed a larger congruency effect in the non-homologous response-mode. This suggested that the history-dependent model was supported by the response-mode effects (Labruna et al., 2014) that the suppression onto the non-selected homologous response-mode was greater than non-homologous response-mode. The non-homologous response-mode had a stronger influence from the incongruent flanker stimuli, which the incorrect response activation was then required a longer time to be inhibited. Therefore, the response time was slower than the homologous response-mode.

The present study manipulated the potential response effectors into left-hand, right-hand, left-foot, and right-foot. A larger congruency effect could be observed in the non-homologous response-mode task, but only for hand responses. A possible reason is that our foot responses were 80 ms slower than the hand responses. Miller (2012) also reported slower response time in the foot than the hand resulted from the decision making stage prior to the motor process, and prolonged conduction time of the peripheral motor response process. Ridderinkhof et al. (2005) highlighted that the speed of response can affect the interaction of the response activation processes within the activation-suppression model. If responses are slow enough to allow the selective suppression to build up, the response activation via the direct route would be suppressed. There will be less influence from the congruent and incongruent flanker stimuli onto the response activation processes. Therefore, we could not observe the different of congruency effect between homologous and non-homologous response-modes when responding with feet.

Moreover, Ridderinkhof et al. (2005) reported that the magnitude of the direct response activation route depends on the previous trial. For instance if the congruent trial is preceded by the incongruent trial, the activation via the direct route would be decreased. However, in this current experiment, the sequence of the trials was randomised and the flanker conditions were balanced, therefore it was expected the effect of conflict anticipation and trial sequence would be even out. This suggested that the modulation of the selective suppression in activation-suppression model as described by the proximity/history dependent could be only applied when the response was correct.

LRP findings

The LRP analysis performed in the current study enabled us to dissociate the activity between left and right PMCs when the tasks required the selection between left and right hand responses. A negative LRP indicates that the correct response activation is greater than the incorrect response activation, whereas a positive LRP means that the activation of the incorrect response activation is greater. The neutral and congruent conditions showed only negative deflections, indicating the correct response activation. The onset of neutral condition provides the indication that the correct response activation can be detected at cortical level approximately 215 ms after the target stimuli. The LRP onset latency in the congruent condition was 109 ms earlier than the neutral condition. This was because the congruent flanker stimuli facilitated the correct response activation via the direct route. It was only in the incongruent flanker condition that a positive LRP was observed. Once the positive LRP deflection reached its peak, it turned to the negative deflection. This indicated that the suppression onto the incorrect response occurred simultaneously as the correct response is activated. The peak of incorrect response activation

ranges between 258-353 ms after flanker onset, which was similar to Velerger et al. (2009) with the mean of individual's incongruent peak of 238-318 ms after flanker onset. However, the incongruent peak in this study was thought to be slightly later than Verlerger et al. (2009) because the flanker and target stimuli in this study displayed on the screen longer than Verleger's study of 11 ms.

The LRP recorded from C3 and C4 electrodes has previously been used to evaluate the effect of response conflict information in the flanker task on response preparation between hand and foot responses (Carrillo-de-la-Pena et al. 2005). This experiment tested hand and foot responses in separate experimental blocks. In the hand responses, LRP in the congruent condition demonstrated less correct response activation compared to the incongruent condition. However, foot responses showed less correct response activation in the incongruent condition. In general, the LRPs showed smaller correct and incorrect response activation with the foot responses in both congruent and incongruent condition. They suggested to record the foot activity from the ipsilateral PMC because the polarity from the foot response was opposite to the hand. In the present study, LRP method recorded from C3 and C4 electrode sites could only be used for the hand homologous response-mode. We wanted to differentiate the activation between left and right side responses as we would like to test the assumption of the competition resolution processes that the inhibition onto the PMC_I was originated from the opposite PMC. However, when the response is involved two limb systems including hand and foot in the same trial, more appropriate method was needed to use to index the different of movement preparation and inhibition between responding with homologous and non-homologous response-mode.

Consideration of the EEG method

Miller (2012) first used ERP from Cz to examine the movement preparation in the pre-cueing choice response time task, which required both limb systems (left-hand, right-hand, left-foot, right-foot). The activity from Cz was always used as an index of the foot response preparation because it is located at the mid-sagittal line closed to the foot motor area (Brunia and Vingerhoets, 1980, Bocker et al., 1994). Cz activity is more positive for the hand preparation than the foot because of the dipole projecting from the foot area to the scalp (Bocker et al. 1994). Therefore the LRP is more positive in the hand response, while it is negative in the foot response. Miller (2012) could not compare the onset latency between hand and foot response because the LRPs were different in amplitude and shape. Therefore, they compared the ERP recorded from Cz directly across two limb systems (LSP technique) but it is not possible to specify the movement side (Miller 2012). The LSP technique could not allow us to see the lateralisation between left and right side of movement because the ERP was measured from the Cz electrode located at the midline. Moreover, they did not include the conflict stimuli in the task, therefore these technique could not allow us to observe the correct and incorrect response activation made by left and right movement side.

Another possible approach to evaluate the different of ERP between left and right PMCs when the task required left and right foot responses was to record the activity from C1/C2 which were more lateral to left and right side of the Cz (Hari et al., 1983). However, we could not compare foot and hand directly in the same trial as the foot activity was much smaller when it was measured further away from Cz. Also the foot activity has the opposite dipole projection when compared with the hand activity when recorded over the hand motor area (C3/C4). I expected to observe a larger positive LRP in the incongruent trials with the non-homologous response-

mode. This is because the non-homologous response-mode had less selective suppression to inhibit the incorrect response activation in the incongruent flanker condition. This would lead to a greater and earlier activation of the incorrect response that required longer time to be inhibited before the correct response was generated. I expected to observe early onset latency of the positive deflection and later onset latency of the negative deflection in the incongruent LRP when responding with non-homologous response-mode as the incongruent flanker had more influence in this condition, but it was not possible to detect these changes using the LRP technique. Therefore, the CRP was performed as an alternative approach to compare the activation of hand and foot responses with homologous and non-homologous response-modes.

Selected CRP

The selected CRP data demonstrates the different amount of correct response activation between homologous and non-homologous response-modes. The flanker stimuli had a stronger influence onto the non-homologous response mode. Therefore, in the congruent condition, I expected to observe a larger correct response activation in the non-homologous response-mode. For the incongruent condition, the flanker stimuli would strongly activate the incorrect response activation in the non-homologous than the homologous response-modes, therefore I expected to observe less correct response activation in the non-homologous response-mode. Selected CRP data revealed that the non-homologous response-mode had less correct response activation than the homologous response-mode in all three flanker conditions. However, only the incongruent condition showed a significant effect of the response-mode. This suggests that the non-homologous response-mode didn't get more benefit from the congruent flanker stimuli, which was not in line with the faster normalised response time data. While the non-homologous response-mode got more interference from the incongruent flanker stimuli, which was in line

with the slower normalised response time data. In terms of the activation-suppression model, the stronger selective suppression onto the direct response route could only be applied onto the incongruent condition in this study. When the amount of suppression is graded as described by the history-dependent model, the incorrect non-homologous response-mode was less inhibited resulted in a greater activation of incorrect response, therefore the correct non-homologous response-mode was less activated when compared to the homologous response-mode. This could be confirmed by the intermediate ERP data in both left and right hand responses before taking the average. Both hand responses showed less correct cortex activation in the non-homologous response-mode.

The effect of response-mode on the correct response activation elicited in the congruent condition could not be observed with the CRP method. Greenhouse et al. (2015) demonstrated the inhibition associated with competition resolution increased as the task complexity increased. Therefore, we may not have found an effect as the task only required participants to perform simple finger and foot movements. The second reason was possibly from the averaging method used in the CRP. If the effect of response-mode is only found in one hand, it could be diminished after averaged the intermediate ERP between both left and right hands (see Figure 4-12B). Therefore, in case of the activations were not obviously showed the potential difference between two response-modes in both hand responses, the difference of potentials between homologous and non-homologous might be diminished after averaged.

Non-selected CRP

The non-selected CRP was measured over the PMC contralateral to the non-selected hand. This analysis attempted to measure the effect of response-mode on the incorrect response activation.

It was chose to focus on the 200 to 350 ms after the incongruent flanker onset as this corresponded to the positive deflection of the incongruent LRP, indicating that the incorrect response activation was greater than the correct activation. Based on the activation-suppression model and the history-dependent model, the flanker stimuli would activate the incorrect response activation greater in the non-homologous response-mode; therefore I expected to observe greater incorrect response activation. However, it was unable to detect an effect of response-mode on the non-selected CRP. The main limitation of measuring the activity from the hand motor area when the hand movement was not executed is that the activity would be very low and could be interfered from the volume conduction from the EEG measurement (Nunez and Westdorp, 1994, Tenke and Kayser, 2012, Burle et al., 2015). The activity recorded in the non-selected CRP method would pick up the mixture of the potentials form the underlying cortical activities around that area, which could distort the data. The intermediate ERP from non-selected left and right hands showed a non-consistent pattern that corresponded to the flanker stimuli during the latency of 200 to 350 ms after the flanker onset. Therefore, it was difficult to observe the incorrect response activation from the non-selected CRP method.

The mechanisms to resolve conflict

The competition resolution is thought to operate in the inhibition of incorrect response induced by a flanker stimuli to help decrease the threshold and sharpen the appropriate response selection by inhibiting the other candidate responses (Klein et al. 2014). Medial and lateral prefrontal area has its role in the competition resolution mechanism that acts onto the primary motor cortex (Burle et al., 2002, Duque et al., 2013) through the basal ganglia (Herz et al., 2014). The exertion of EEG activity from lateral and medial prefrontal area prior to the reduction of the incorrect activation measured from the PMC was observed when responding

to a Simon task (Burle et al., 2016). When the participants could not anticipate the response conflict, the level of inhibition could not be adjusted prior to the task. Therefore, the modulation of selective inhibition would occur after the target onset and only online adjustments could meet the response demands to prevent the response errors (Klein et al. 2014). They also suggested that using single pulse TMS to observe the MEPs changes in the PMC can reflect the modulation of the inhibitory of incorrect response produced by the conflict stimuli explained by the competition resolution.

Conclusion and focus of next study

The behavioural data indicated that the competition resolution was influenced by the response-mode. In the incongruent flanker condition, the non-homologous response-mode had slower response time than the homologous response-mode. This corresponded to the main EEG finding that the non-homologous response-mode has less correct response activation in the incongruent condition. These were supported by the activation-suppression model as the non-homologous response-mode had a lower level of selective suppression to inhibit the unwanted/incorrect response.

The main limitation is the assumption that the changes in LRP and selected CRP found in this study actually reflect response activation processes at the cortical level. Therefore, in the next study, the TMS was used to explore whether the effect of response-mode on the competition resolution involves in the corticospinal excitability during the flanker task. In particular, I expected to observe lower MEPs measured from the selected hand with non-homologous response-mode in the incongruent flanker condition as this would match with the selected CRP finding in this study. A TMS measure would provide more confidence about the CRP method

if it actually reflects the different between homologous and non-homologous response-modes. Moreover, the TMS would also allow me to further evaluate the effect of response-mode on the incorrect response activation, which I could not observe with the EEG in this study. The LRP and selected CRP data from this study when it showed a significant difference between the homologous and non-homologous response-mode will be used to provide a specific timings of the TMS to evaluate the corticospinal excitability changes in the homologous and non-homologous response-modes.

CHAPTER 5

Using transcranial magnetic stimulation to investigate the corticospinal excitability changes during movement selection in response to conflict stimuli

5.1 Introduction

In the flanker-EEG study of the previous chapter, the participants responded to the target stimuli either using both hands (homologous response-mode), or with a combination of hand and foot (non-homologous response-mode). The main behavioural finding was a larger congruency effect with the non-homologous response-mode, which was mainly due to an increased interference effect from the incongruent flanker. This effect was interpreted in the context of the activation-suppression model (Ridderinkhof et al., 2005) and proposed that this could be a result of a lower level of selective suppression with the non-homologous response-mode. Weaker selective suppression would lead to a greater direct activation of the incorrect response by the incongruent flanker. Consequently, more time is required to cancel the incorrect response and initiate the correct one with the non-homologous response-mode.

The limitation of using the EEG in the previous chapter was that it was only be able to perform the LRP analysis on the homologous response-mode data. This LRP analysis revealed that with the neutral trials, the activation in the PMC contralateral to the selected hand (correct response activation) increased following the target stimuli. With the congruent trials, the correct response activation occurred around 110 ms earlier than the neutral trials. This indicated that the flanker stimuli initiated the correct response activation around 220 ms after the flanker onset as

observed in the LRP onset latency. For the incongruent trials, the incorrect response activation elicited by the incongruent flanker began approximately 235 ms after the flanker onset and reached its peak around 270-325 ms after the flanker onset. This peak latency was slightly delayed compared to Verleger et al. (2009) as they observed the mean of individual's peak latency in the range of 238-318 ms after the flanker onset. The conditioned readiness potential (CRP) analysis was performed to assess the differences in cortical activation between homologous and non-homologous response-modes because we were unable to obtain LRPs with the non-homologous response mode. The 'selected' CRP obtained from the PMC contralateral to the selected hand reflected the difference in correct response activation between homologous and non-homologous response-modes. It revealed that the incongruent flanker had less influence in the non-homologous response-mode task. The timing of this effect, approximately 230-250 ms after the incongruent flanker onset, was consistent with the onset of the incorrect motor activation in the LRP analysis. The interpretation of the LRP and CRP results assumes that these signals represent the automatic preparatory response activation at the cortical level, but we don't yet have direct evidence of this.

When using single pulse TMS to evaluate the movement selection and preparation processes, it can reflect the amount of correct and incorrect motor outputs (Verleger et al., 2009, Michelet et al., 2010, Klein et al., 2014). Many previous studies have used TMS to assess the CSE changes associated with correct and incorrect response activation during a flanker task (Verleger et al., 2009, Michelet et al., 2010, Klein et al., 2014, Duque et al., 2016). They all found similar results that the congruent flankers facilitate the MEP amplitudes recorded from the selected hand, which reflects increased preparation of the correct response. In contrast, the incongruent flankers initially increase the MEPs in the non-selected hand, whilst MEPs in the

selected-hand are low. This effect then reverses as the trial progresses. The MEPs in non-selected hand indicated that the initial effects of the incongruent flanker was inhibited to prevent the unwanted/incorrect movement in the non-selected hand (Verleger et al., 2009, Michelet et al., 2010). For this reason all authors have proposed that the modulation of the CSE reflects the competition process in PMC when experiencing response conflict. All these studies used homologous response-mode, so the effect of non-homologous response-mode is still unknown.

Moreover, Klein et al. (2014) investigated how the top-down control of selective suppression can help resolve response conflict in the flanker task. They manipulated the percentage of incongruent trials presented in each block. In the 'mostly-incongruent' blocks (80% incongruent), participants would anticipate response conflict, therefore, the baseline level of selective suppression of the direct response would be high. The opposite was true for the 'mostly-congruent' blocks (80% congruent). The response times of the incongruent trials were faster and had lower error rates when response conflict was anticipated in the 'mostly-incongruent' condition. Klein et al. (2014) also measured the changes in corticospinal excitability associated with the selected and non-selected hands during the same conditions. They proposed that if the movement selection process during the conflict is operated by the top-down control, this could be probed via the corticospinal excitability. In agreement with the RT results, the MEPs measured in the non-selected hand during the incongruent trials in the 'mostly-incongruent' blocks (when conflict was anticipated) were smaller than during the 'mostly–congruent' blocks (conflict less anticipated). These results supported their hypothesis that the top-down inhibitory control onto the incorrect response activation increased when the conflict was expected. This occurred to reduce the incorrect response activation and could correspond to the selective suppression acting onto the direct response activation (from the task-

irrelevant stimuli) that was described in the activation-suppression model earlier (Ridderinkhof et al. 2005). Klein et al. (2014) hypothesised that the increased inhibition associated with the expected response conflict reflected stronger competition resolution.

The current study used single-pulse TMS to explore whether the response-mode could influence the amount of the selective suppression onto the direct response activation route during the flanker task. The 'history-dependence' model of Labruna et al. (2014) proposes that the level of inhibition onto the non-selected responses is graded according to the past history of competition between the potential response alternatives (see Figure 1-2D). If the alternative response competition is high such as left-hand vs right-hand, the inhibition onto the non-selected response will be stronger than the low competition such as left-foot vs right hand. The main hypothesis is to evaluate whether the inhibition onto the non-selected response is graded by the history dependence model. If this inhibition relates to the selective suppression onto the direct response route, then the flanker stimuli will elicit stronger changes in the corticospinal excitability with the non-homologous response-mode.

The results of the previous EEG study showed that the congruency effects were larger in the non-homologous response-mode. We therefore expected this to transfer into stronger changes in CSE in the non-homologous response-mode; in the congruent condition, the flanker would produce a greater increase of MEPs in PMC_C in the non-homologous response-mode. In contrast, during the incongruent condition, the flanker would initially produce a greater increase of MEPs in the PMC_I and/or greater MEPs suppression in the PMC_C in the non-homologous response-mode and the subsequent crossover of the MEPs between PMC_C and PMC_I at the later measurement would be less pronounced as the flanker would have a more persistent influence

on the incorrect motor activation (see Figure 5-1). The findings in the selected CRP from previous chapter also indicated that the difference of the correct response activation between homologous and non-homologous response-modes were prominent only in the incongruent condition. We therefore expected that the incongruent flanker condition would reveal a greater MEPs difference in the PMC_C between homologous and non-homologous response-modes when compared to the congruent and neutral flanker conditions.

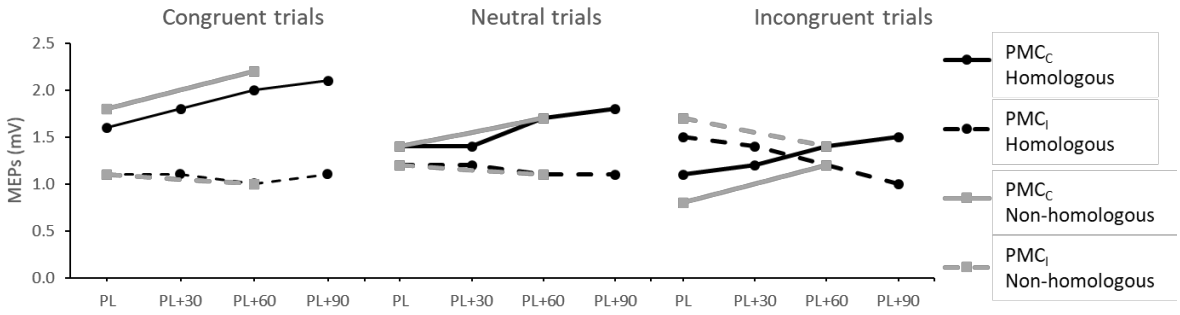

Figure 5-1. The MEPs recording from selected (PMC_C) and non-selected hands (PMC_I) in the congruent, neutral, and incongruent flanker conditions. Black solid and dash lines represents the MEPs recording in the selected and non-selected hands in the homologous response-mode adapted from Verleger et al. (2009) that were measured at 4 timings started from the peak latency of individuals' incorrect cortex activation (PL). For the congruent and neutral conditions, the MEPs elicited from PMC_C increases after the PL while the MEPs elicited from the PMC_I remain unchanged. This indicates a correct response activation in the selected hand and no incorrect response activation in the non-selected hand. However, the MEPs elicited from the PMC_C at PL in the congruent condition are higher than the neutral. This indicates the effect of the priming congruent flanker stimuli in the congruent condition. For the incongruent condition, the MEP elicited from PMC_I is higher than PMC_C at PL. This indicates the effect of the incongruent flanker that initially activates the non-selected hand. The MEPs in PMC_C increases from the PL afterwards, while the MEPs in PMC_I decreases. The MEPs cross-over effect represents the cancellation of the incorrect response activation and the activation of the correct response. The Grey solid and dash lines represents the expected MEPs in this experiment when responding with the non-homologous response-mode. In the congruent flanker condition, we expected to observe a higher MEPs elicited from PMC_C in the non-homologous response-mode because it has a stronger influence from the flanker. For the neutral condition, we expected no MEPs difference between homologous and non-homologous response-modes in both PMC_C and PMC_I as there is no effect of the flanker. For the incongruent flanker condition, we expected a greater MEPs in the PMC_I and lower MEPs in the PMC_C in the non-homologous response-mode as it has a stronger influence from the incongruent flanker stimuli that activate the incorrect response activation, therefore the correct response activation will take longer time to build up.

5.2 Methods

Participants

Based on sample sizes used in previous research, thirty-nine healthy subjects participated in this study (12 women; 24.1 $\pm$ 0.8 years old). All participants were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). The protocol was approved by the STEM ethics committee of the University of Birmingham. All participants provided written informed consent and completed a TMS safety screening questionnaire (Keel et al. 2001, Rossi et al. 2009) prior to the experiment to ensure that they had no contraindications to the TMS.

Electromyography (EMG)

Surface EMG electrodes (Bagnoli Electrodes DE-2.1, Delsys Inc, USA) were placed on the belly of right abductor pollicis brevis (APB) muscle. The reference electrode was on the olecranon process of right elbow. The EMG signals were amplified, band-pass filtered (20-500 Hz) and digitized at a sampling rate of 2000 Hz. The signals were acquired with a CED micro 1401 analogue to digital converter (Cambridge Electronic Design, Cambridge, UK) and transferred for offline analysis on a pc with Signal software Version 6.04 (Cambridge Electronic Design, Cambridge, UK).

Transcranial magnetic stimulation (TMS)

Single pulse TMS was performed using a monophasic Magstim 200^2 stimulator connected to a 50 mm alpha branding iron figure of eight coil (both, Magstim Company, Carmarthenshire, UK). The coil was placed tangentially over left PMC with the coil orientated in a posterior-lateral direction about 45 degrees from the midline and perpendicular to the central sulcus. The motor hotspot for the right abductor pollicis brevis was first identified and marked using

Brainsight ™ version 2.2 (Rogue Research Inc., Montreal, Canada). The resting motor threshold (RMT) was defined as a minimal intensity to evoke MEPs of 50 μV peak-peak amplitude in the targeted muscle on 5 out of 10 consecutive trials (Rossini et al. 1994). For the experimental session, the intensity of TMS was set to 115-120% of RMT.

Flanker protocol

Participants performed the same modified version of the Eriksen flanker task (Eriksen, 1995) that was used in the EEG experiment as programmed in E-prime version 2.0 (Psychology Software Tools, Inc., Pennsylvania, USA). Participants sat comfortably in front of the monitor with both arms resting on a height adjustable wooden table, palms down with the elbows slightly flexed. Participants responded with either, left and right thumb presses (task 1), or right thumb and left foot presses (task 2). Therefore, left and right thumbs were rested on the left-most and right-most switches of the Chronos response box and the left foot was on the left foot pedal (Psychology Software Tools, Inc., Pennsylvania, USA).

Both target and flankers stimuli were presented as same as in the EEG experiment (see Figure 5-2). The only difference of the flanker protocol between this experiment and the previous EEG experiment was that the inter-trial interval was shortened by 1000 ms because the TMS experiment required more time to complete than the EEG experiment. This duration was sufficient for the TMS to recharge and to limit the possibility of a repetitive TMS effect building up from multiple stimuli. The TMS was triggered from E-prime program via the parallel port on the pc.

In the previous EEG study, we obtained very similar behavioural congruency effects in both the left and right hands, therefore, as the TMS experiment required more time to complete than the EEG experiment, it was decided to only assess changes in corticospinal excitability associated with the right-hand. As the aim was to compare the effect of the response-modes between homologous and non-homologous, the experiment consisted of two tasks (see Figure 5-3). Task 1 (homologous response-mode), where the target instructed participants to respond with either their left-hand or right-hand. While task 2, the non-homologous response-mode, was the task when right-hand was in the competition with left-foot.

The conditions were separated according to whether the right-hand was selected or non-selected (left-hand or left-foot responses). Three possible flanker stimuli were presented prior to the target (congruent, neutral or incongruent), which provided six main conditions:
1) homologous right-hand selected congruent; 2) homologous right-hand selected incongruent; 3) homologous right-hand selected neutral; 4) homologous right-hand non-selected congruent; 5) homologous right-hand non-selected incongruent; 6) homologous right-hand non-selected neutral.

The TMS timing was determined by the group LRP and CRP data from the EEG experiment. However, Verleger et al. (2009) used the individual peak latency (PL) of the LRP in the incongruent flanker condition as a guideline to determine the timing of TMS. They recorded the MEPs at four time-points from the peak latency of the incongruent LRP until 90 ms afterwards. The results revealed that the cancelation of the incorrect cortex activation occurred simultaneously with the correct cortex activation during incongruent flanker trials as there was a cross-over of CSE from the non-selected to the selected hand (see Figure 5-1). However, as

the present study included both homologous and non-homologous response-mode tasks, it was not possible to obtain as many measurements. I therefore primarily based my TMS timings on the latency of LRP and CRP effects obtained in the previous EEG study to determine the modulation of correct and incorrect response activation in the conflict task. From the selected CRP data, the non-homologous response-mode showed lower correct response activation than the homologous response-modes in the incongruent flanker condition between 230 to 250 ms after the flanker onset. Therefore, in addition to a baseline measure of excitability (fixation period), the MEP was measured at two time-points after the flanker onset; FLK240 (240 ms after the flanker onset) and FLK300 (300 ms after the flanker onset) during both homologous and non-homologous response-modes (see Figure 5-2). As it was expected to observe the time course changes after the brain processed the target stimuli, the MEPs measured at 300 ms after the flanker onset would allow to detect the CSE changes in both selected and non-selected hands. No-TMS trials were also included as a control to determine if response times were directly influenced by the TMS.

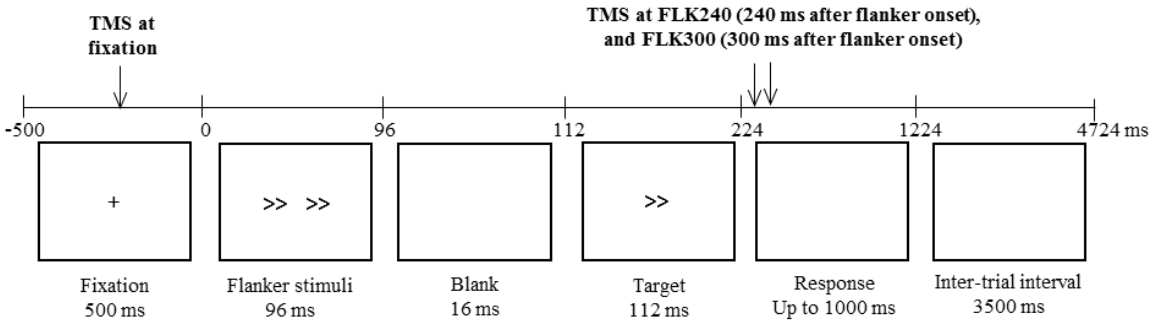

Figure 5-2. Sequence of the stimuli and timing of the TMS measurements. Each trial started with a fixation cross for 500 ms. The flankers stimuli appeared for 96 ms followed by a blank screen for 16 ms. Then the target stimuli presented for 112 ms followed by a blank screen, when the participants provided a respond within 1000 ms. The interval between each trial presented as a blank screen appeared for 3500 ms.

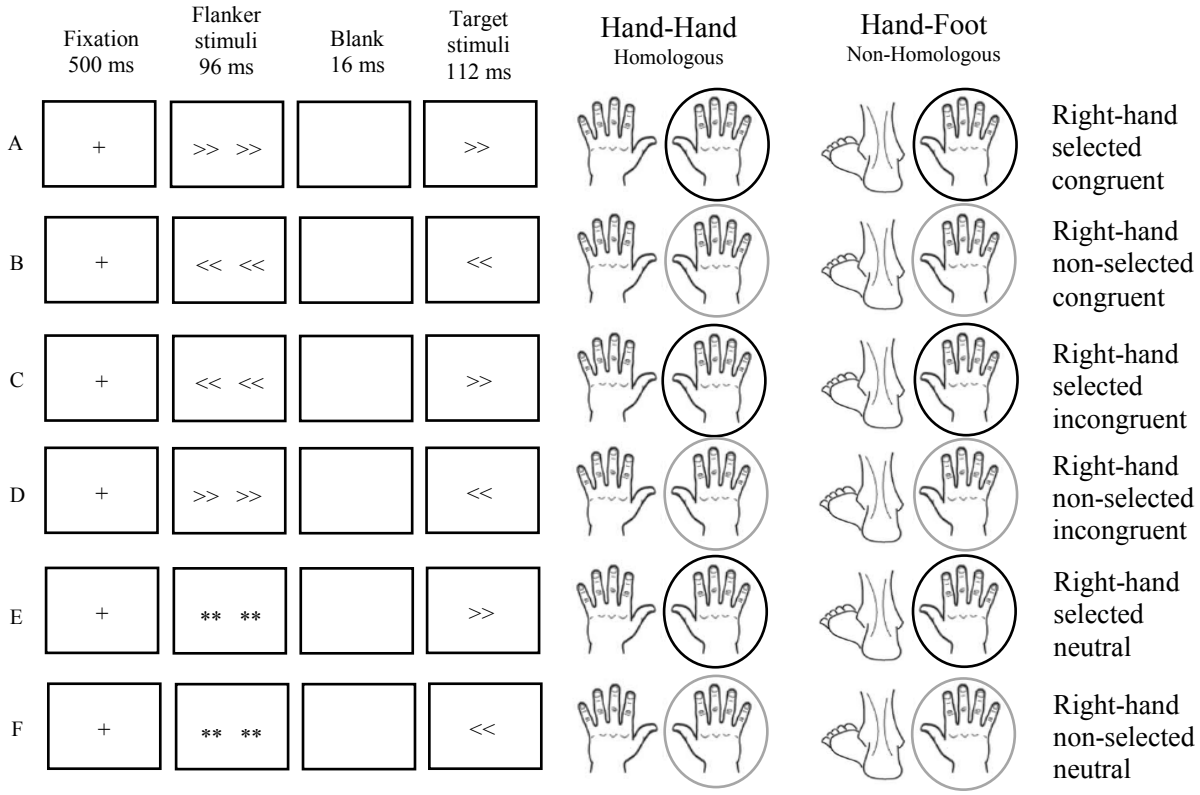

Figure 5-3. Experimental conditions of two tasks: homologous response-mode task where participants responded with either their left or right hand; non-homologous response-mode task where participants responded with either their left-foot or right-hand. Participants completed both tasks in a counter-balanced order. (A) and (B) demonstrate the congruent condition, when the flanker and target stimuli point in the same direction. (C) and (D) demonstrate the incongruent condition, when the flanker and target stimuli point in opposing directions. (E) and (F) demonstrate the neutral condition, when the flanker stimuli were not assign to a response. When the target stimuli point to the right, the participants were assigned to respond with their right-hand, and vice versa. The MEPs were always measured in the right abductor pollicis brevis muscle, regardless whether the participants responded with their left/right hand (homologous response-mode) or left-foot/right-hand (non-homologous response-mode). Therefore, the dark circle demonstrates the condition where the MEPs were measured when the right-hand was selected to respond. Whereas, the grey circle demonstrates the condition where the MEPs were measured from the right-hand when it was not selected to respond.

For the homologous response-mode, there were six blocks of 102 trials (total of 612 trials). Six MEPs were obtained in each condition at FIX, 36 MEPs were obtained in each condition at both FLK240 and FLK300 (see Table 3). There were also 24 trials in each condition without TMS in order to calculate the normal response times. MEPs measured at FIX were unaffected by the flanker and target stimuli, therefore the 6 FIX MEPs of each condition were combined to form a total of 36 MEPs. For the non-homologous response-mode (see Figure 5-3), again, task 2 was performed over six blocks of 102 trials (see Table 4). Task 1 and Task 2 were performed in the same sessions as alternate sequence of blocks (i.e Task 1 – Task 2 – Task 1- Task 2 etc.) (see Figure 5-4).

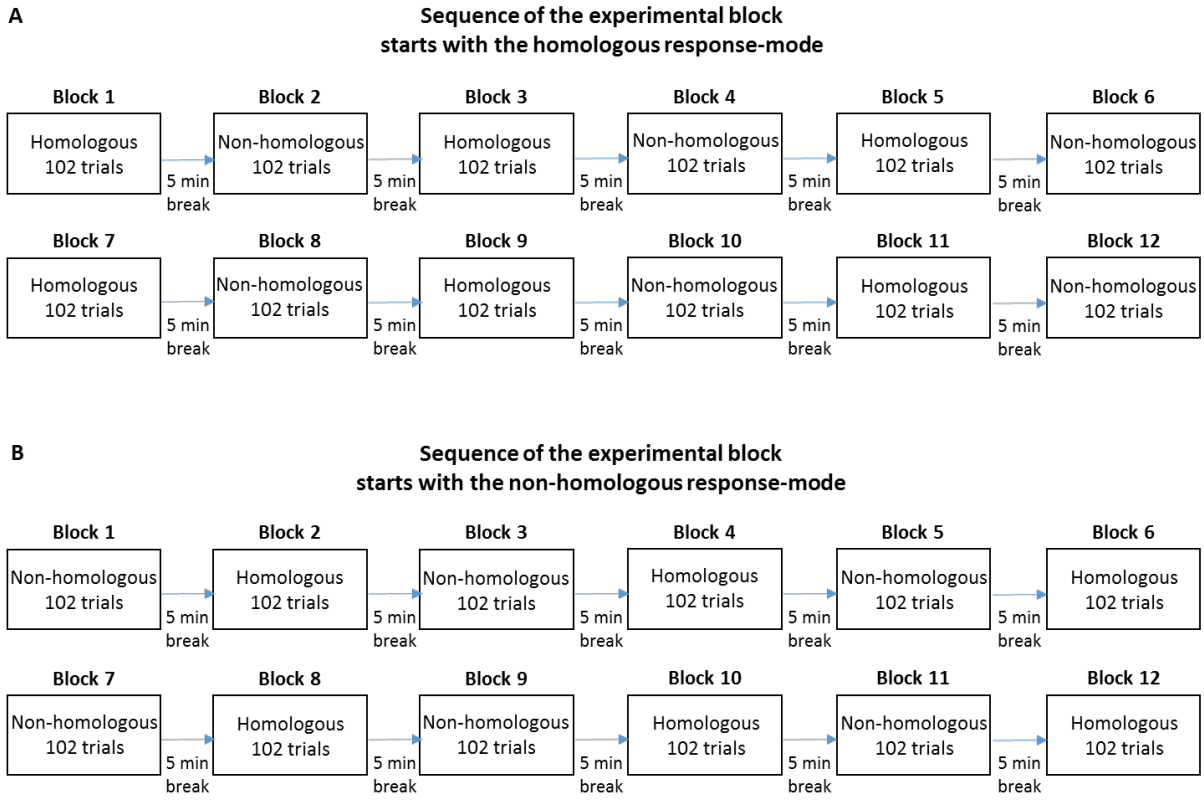

Figure 5-4. Alternate sequences of the experimental block. (A) Half of the participants started the experimental block with the homologous response-mode. These were performed for 6 blocks of 102 trials alternated with the non-homologous response-mode for 6 blocks of 102 trials. (B) Half of the participants started the experimental block with the non-homologous response-mode. These were performed for 6 blocks of 102 trials alternated with the homologous response-mode for 6 blocks of 102 trials. There was 5 minutes break between each block.

Table 3. Homologous response-mode (task1; left hand-right hand); number of the trials in each of the flanker condition at 4 different TMS timings

	No-TMS	FIX-TMS	FLK240-TMS	FLK300-TMS
Congruent	RH-LH	RH-LH	RH-LH	RH-LH
	LH-RH	LH-RH	LH-RH	LH-RH
Incongruent	RH-LH	RH-LH	RH-LH	RH-LH
	LH-RH	LH-RH	LH-RH	LH-RH
Neutral	RH-LH	RH-LH	RH-LH	RH-LH
	LH-RH	LH-RH	LH-RH	LH-RH
Number of trials in each condition (1 block)	4	1	6	6
Number of trials in each condition (total of 6 blocks)	24	6	36	36

Table 4. Non-homologous response-mode (task 2; left foot-right hand); number of the trials in each of the flanker condition at 4 different TMS timings

	No-TMS	FIX-TMS	FLK240-TMS	FLK300-TMS
Congruent	RH-LF	RH-LF	RH-LF	RH-LF
	LF-RH	LF-RH	LF-RH	LF-RH
Incongruent	RH-LF	RH-LF	RH-LF	RH-LF
	LF-RH	LF-RH	LF-RH	LF-RH
Neutral	RH-LF	RH-LF	RH-LF	RH-LF
	LF-RH	LF-RH	LF-RH	LF-RH
Number of trials in each condition (1 block)	4	1	6	6
Number of trials in each condition (total of 6 blocks)	24	6	36	36

Data analysis

Behavioural data

Response times were recorded on individual trials as the onset time of target stimuli to the onset of the button press recorded in E-prime. Trials with response times faster than 112 ms or slower than 1112 ms, or trials with incorrect or missing responses, were removed from further analysis. Baseline response times were influenced by the task, this variability was removed by normalising the response time in each condition to that of the related neutral condition. By subtracting the response time in neutral condition from the response time from congruent and incongruent conditions we could see the behavioural congruency effects.

MEP data

Peak-to-peak MEP amplitude of the right APB muscle was calculated in the 10-50 ms after the TMS onset. The MEP data was discarded from further analysis when the target muscle was not relaxed as measured by the peak-to-peak EMG activity exceeded 50 μV during the 50 ms prior to the TMS onset. Trials with response time faster than 112 ms or slower than 1112 ms, incorrect or missing responses, or coil locations greater than 3 mm or 5 degrees from the original motor hotspot were removed from further analysis (Schmidt et al., 2015). Participants who had less than 16 correct responses or less than 16 MEPs in every experimental condition were excluded from the analysis.

To reduce the variability from each participant, the raw mean MEP amplitude of each FLK240 and FLK300 condition were normalised by subtracting the mean MEP amplitude in FIX condition.

Statistical analysis

Behavioural data

We first wanted to test if the behavioural congruency effect was similar on left and right sides, as it was then more likely that the MEP data would also generalise to the other side. A 2-way repeated measures ANOVA was run on the normalised response times in the no-TMS condition of the homologous response-mode. This had factors of 2 SIDE (right-hand response, left-hand response) x 2 FLANKER-CONGRUENCY (congruent, incongruent).

The normalised response times in the no-TMS condition were determined whether the influence of response-mode on the behavioural congruency effect was comparable to that observed in the EEG experiment. This was tested by running a 2 way repeated measures ANOVA on the normalised right-hand response times in the no-TMS condition with factors of 2 RESPONSE-MODE (homologous, non-homologous) x 2 FLANKER-CONGRUENCY (congruent, incongruent).

In this experiment, TMS was used to measure CSE changes during movement preparation; however, the application of TMS over PMC may actually directly influence response times (Day et al., 1989, Pascual-Leone et al., 1998, Sawaki et al., 1999). Therefore, the influence of the stimulus site and timing on the behavioural congruency effect were determined. As highlighted previously, TMS was always applied over left PMC to elicit MEPs in the right-hand, therefore for right-hand responses, TMS was applied to the PMC_C, but for left-hand responses, TMS was applied to the PMC_I. Therefore a 3-way ANOVA was run on the congruency effect normalised to the no-TMS condition with a factor of 2 RESPONSE-MODE

(homologous, non-homologous) x 2 TMS-SIDE (PMC_C, PMC_I) x 3 TMS-TIMING (no-TMS, FIX-TMS, FLK240, FLK300). If the congruency effect was confounded by the TMS timing, there should have had more influence on the contralateral than ipsilateral side because TMS might disrupt the response activation by producing a twitch in the hand contralateral to the TMS (Walsh and Rushworth, 1999). Statistical testing was conducted with IBM SPSS Statistics 24 software package. The significance level was set at 0.05. Post-hoc comparisons were conducted using the Sidak procedure.

5.3 Results

After excluding trials with incorrect or missing responses, high background EMG activity, and response-time outliers, thirty-two of the thirty-nine participants had a minimum of 16 MEPs in every condition. The behavioural and CSE results will now be described for these participants.

Behavioural data

Figure 5-5A depicts the normalised response times measured from right and left hands in the no-TMS condition of the homologous response-mode. A repeated measures ANOVA of 2 SIDE (left-hand response, right-hand response) x 2 FLANKER-CONGRUENCY (congruent, incongruent) revealed a significant main effect of FLANKER-CONGRUENCY ($F_{1,31} = 358.2$, $P < 0.001$), with the congruent condition being 82.6 ± 4.4 ms faster than the incongruent condition. This confirmed that the current flanker protocol induced response conflict. There was no main effect of SIDE ($F_{1,31} = 0.4$, $P = 0.54$) or an interaction of those factors ($F_{1,31} = 0.6$, $P = 0.45$). This revealed that the congruency effect was comparable in both left and right hands. The implication being that we might expect the CSE profiles obtained from the right-hand MEPs to also apply to left-hand MEPs.

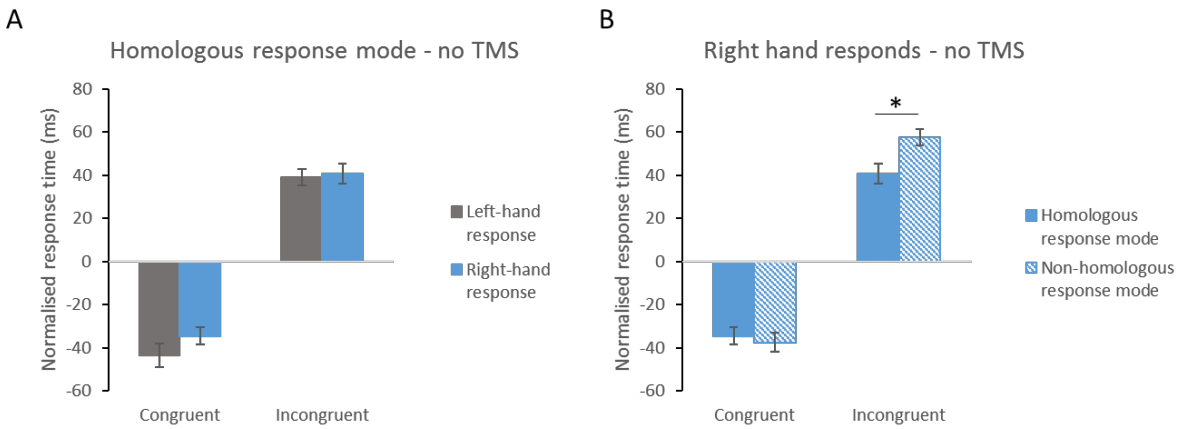

Figure 5-5. (A) Normalised response time recorded from left and right hand responses against the homologous response-mode for the congruent and incongruent trials at no TMS condition. (B) Normalised response time recorded from right-hand response against the homologous and non-homologous response-modes for the congruent and incongruent trials at no TMS condition. *P < 0.001

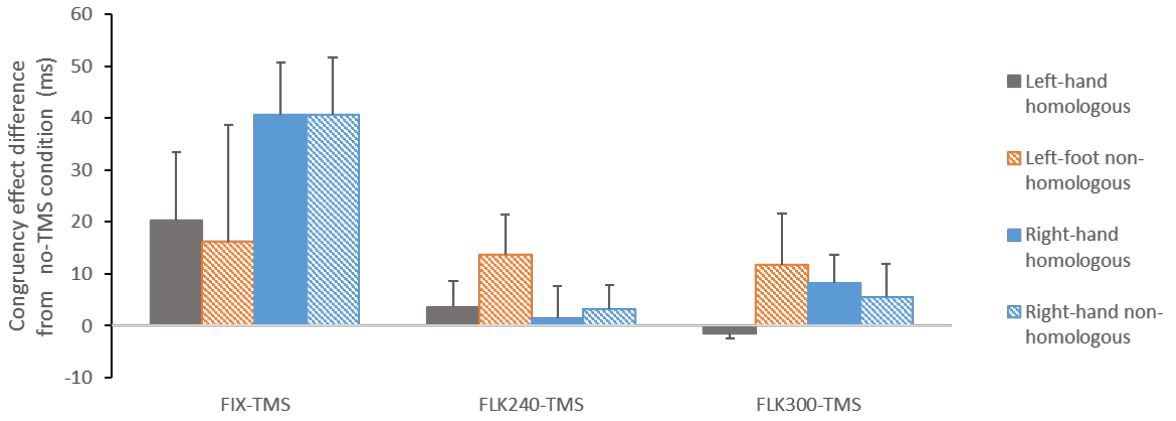

Figure 5-6. Congruency effect (response times difference between incongruent and congruent condition) when left and right hands and left foot responded against homologous and non-homologous response-modes in three different TMS timings.

This experiment only focused on measuring the MEP changes in the PMC contralateral to the right-hand. The normalised response times in right-hand response trials during no-TMS condition were also used to determine whether the influence of response-mode on the behavioural congruency effect was comparable to that observed in the EEG experiment. Figure 5-5B shows the normalised response times from the right-hand split according to the homologous and non-homologous response-modes. A repeated measures ANOVA of 2 RESPONSE-MODE (homologous, non-homologous) x 2 FLANKER-CONGRUENCY (congruent, incongruent) revealed a significant effect of FLANKER-CONGRUENCY ($F_{1,31}$ = 341.8, P < 0.001) and an interaction of RESPONSE-MODE * FLANKER-CONGRUENCY ($F_{1,31}$ = 31.2, P < 0.001), but no main effect of the RESPONSE-MODE was found ($F_{1,31}$ = 2.2, P = 0.15). The interaction reflects that the congruency effect of 100.8 ± 5.5 ms in the non-homologous response-mode was greater than the congruency effect of 81.1 ± 4.9 ms in the homologous response-mode. The increased congruency effect was primarily due to an enhanced effect of the incongruent flanker during the non-homologous response-mode. Post-hoc analyses indicated that incongruent response times were 13.5 ± 2.9 ms slower than in the homologous response-mode (P < 0.001). Overall, the congruency effects measured in the no-TMS condition were comparable to those reported in the previous EEG experiment.

Figure 5-6 demonstrates the normalised congruency effect across the three TMS conditions to no-TMS condition for both the homologous and non-homologous response-modes. We evaluated whether the congruency effect was influenced by the response-mode, hand-selection and TMS timing with a repeated measures ANOVA on the congruency effect normalised to the no-TMS condition including a factors of RESPONSE-MODE (homologous, non-homologous), TMS-SIDE (PMC_C, PMC_I) and three different TMS-TIMING (FIX-TMS, FLK240, FLK300).

A repeated measures ANOVAs revealed a significant main effect of TMS-TIMING ($F_{2,62}$ = 9.1, P < 0.001). Post-hoc analyses indicated that a significant larger congruency effect was found when the TMS was provided during the fixation period when compared with FLK240 (24.0 ± 7.3 ms, P = 0.008), and FLK300 (23.4 ± 7.8 ms, P = 0.015). The lack of a main effect of TMS-SIDE ($F_{1,31}$ = 0.5, P = 0.47) indicated a non-specific effect of TMS. The effect of TMS on the congruency effect was not influenced by the RESPONSE-MODE ($F_{1,31}$ = 0.2, P = 0.64) and there was no significant interactions between any of the factors.

MEP data

Figure 5-7 shows the changes in CSE in the right-hand for each of the flanker conditions (top row is the raw data and the bottom row is the normalised data). The results are separated according to hand selection and the homologous or non-homologous response-modes.

Neutral flanker condition

The neutral flanker condition was considered as a control measurement because there was no effect of the priming flanker to interfere the response time and allowed us to see the time course changes of the MEPs in the PMC_C and PMC_I in the absence of flanker effects. It was hypothesised that the CSE in the PMC_C would increase from FLK240 to FLK300 as it prepared to respond to the target stimuli. No such change would occur in the PMC_I as it was not required to respond. In addition, at FLK240, the MEPs in the PMC_C and PMC_I would be similar as there was no priming effect of the flanker stimuli onto the PMC_C. This hypothesis is based on the previous EEG data, as the onset latency of the neutral LRP was 215 ms after the target stimuli onset, therefore the MEPs measured at 240 ms after the flanker onset would be able to detect no effect of the neutral flanker stimuli. It was also expected to observe no MEP difference

between homologous and non-homologous response-modes as there was no interference from the irrelevant-flanker stimuli in this condition.

Figure 5-7 bottom row; middle panel displays the MEPs measured form the PMC_C and PMC_I in the homologous and non-homologous response-modes. A repeated measures ANOVA of 2 RESPONSE-MODE (homologous, non-homologous) x 2 SELECTION (contralateral, ipsilateral) x 2 TMS-TIMING (FLK240, FLK300) on the normalised MEPs revealed a significant main effect of SELECTION ($F_{1, 31}$ = 15.8, P < 0.001) and TMS-TIMING ($F_{1,31}$ = 18.6, P < 0.001) as well as the interaction of SELECTION * TMS-TIMING ($F_{1,31}$ = 10.6, P = 0.003). Post-hoc analyses indicated that when the right-hand was selected, the MEPs elicited from PMC_C showed a significant increase of 236 ± 52 μV from the FLK240 to the FLK300 period (P < 0.001) as the hand prepared to move, but it showed a slight change of 10 ± 38 μV when it was non-selected (P = 0.80). In addition, when the TMS was applied at FLK240, selection did not significantly increase the MEPs in PMC_C (P = 0.99), but at FLK300, the MEPs in the PMC_C were 246 ± 68 μV higher than the PMC_I (P = 0.001). This indicates that the target stimulus did not influence CSE in the PMC_C until 300 ms after the flanker onset (FLK300) or 188 ms after the target onset. There was no main effect of the RESPONSE-MODE ($F_{1, 31}$ = 0.7, P = 0.42) or interactions of RESPONSE-MODE * SELECTION ($F_{1, 31}$ = 2.4, P = 0.13); RESPONSE-MODE * TMS-TIMING ($F_{1, 31}$ = 0.1, P = 0.71), and RESPONSE-MODE * SELECTION * TMS-TIMING ($F_{1, 31}$ = 1.8, P = 0.18) were found. The difference of MEPs between PMC_C and PMC_I were comparable between homologous and non-homologous response-modes. Post-hoc pairwise comparisons revealed that at FLK240, there was no significant difference of the MEPs between PMC_C and PMC_I in both homologous (P = 0.76) and non-homologous response-mode (P = 0.76). At FLK300, there was a significant difference

of the MEPs between PMC_C and PMC_I in both homologous (P = 0.002) and non-homologous response-mode (P = 0.001). The MEPs in the homologous and non-homologous response-modes increased at the similar extent in the PMC_C and remained unchanged in the PMC_I.

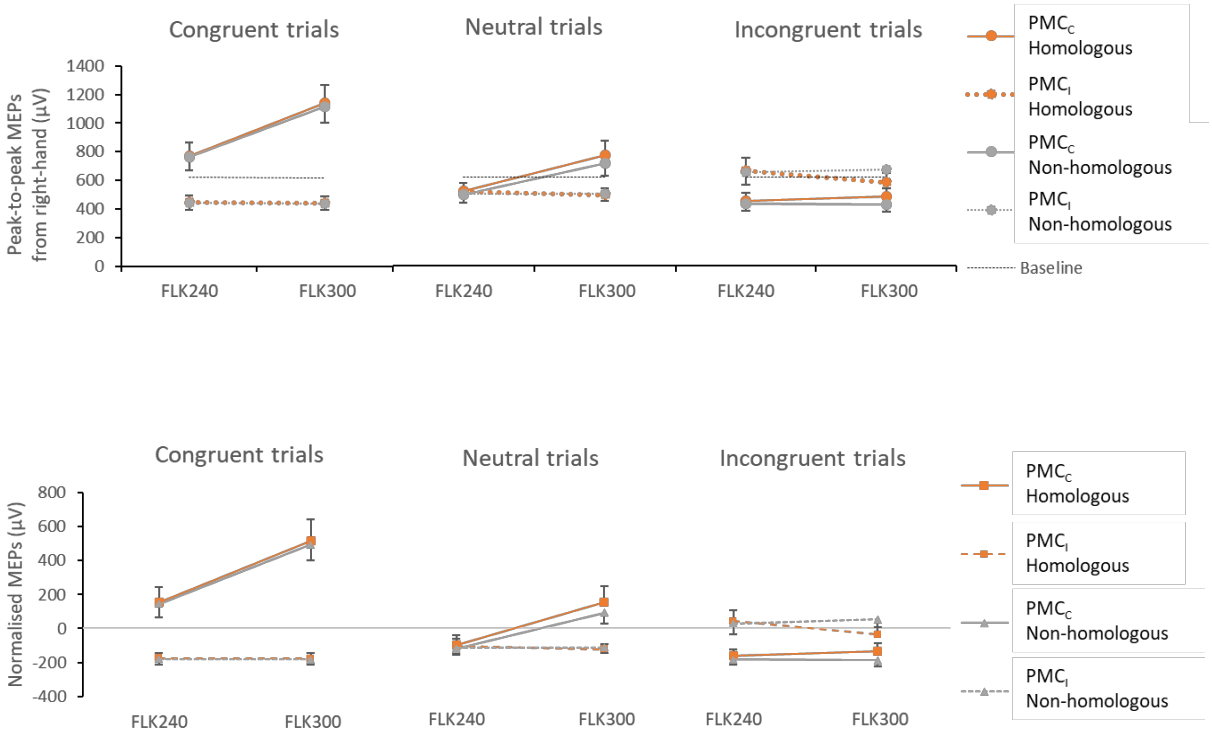

Figure 5-7. MEP amplitudes recorded from right APB muscle at 240 (FLK240) and 300 ms (FLK300) after the flanker onset when it was selected (solid line) and non-selected (dash line) and when responding with the homologous (orange colour) and non-homologous (grey colour) response-modes. Top row; the MEPs are presented in mean of peak-to-peak amplitude. Bottom row; the MEPs are presented in a normalised MEPs (raw MEPs – baseline MEPs). In the congruent and neutral conditions, the MEPs elicited from PMC_C increase from FLK240 to FLK300 while the MEPs elicited from PMC_I remain unchanged. However, at FLK240, the MEPs in PMC_C are higher than the PMC_I and also higher than the baseline measurement only in the congruent condition indicating a congruent flanker priming effect that activates the selected hand. While there is no flanker effect in the neutral condition, therefore the MEPs in PMC_C and PMC_I are comparable at FLK240. There was no significant difference between homologous and non-homologous response-mode in the congruent and neutral conditions. For the incongruent condition, the MEP elicited from PMC_I is higher than PMC_C at FLK240, indicating the effect of the incongruent flanker stimuli that initially activates the non-selected hand. The MEPs in PMC_C tend to increase only in the homologous response-mode, while the MEPs in PMC_I decrease. This represents the start of cross-over effect between correct and incorrect response activation in the homologous response-mode. However, for the non-homologous response-mode, the MEPs in elicited from PMC_I are constantly higher than the PMC_C during FLK240 and FLK300. There is no cross-over effect when responding with the non-homologous response-mode.

Congruent flanker condition

In order to see the effect of the flanker stimuli on the CSE changes, we begin with the congruent flanker condition (see left panel of Figure 5-7), which facilitated response times in the selected hand. It was expected to observe an increase MEPs in the PMC_C within 300 ms after the flanker onset as it prepared to respond to the target stimulus, but there would be no such change in the PMC_I. In contrast to the neutral condition, the flanker stimulus was informative and would also prime the selected hand. It is therefore expected that the MEPs in the PMC_C would be greater than the PMC_I at FLK240. In addition, the behavioural data from the previous EEG experiment showed that the non-homologous response-mode had a faster response time than the homologous response-mode. Therefore, it was hypothesised that the non-homologous response-modes would show a greater increase in MEPs in the PMC_C at both FLK240 and FLK300 when compared to the homologous response-mode. However, the selected CRP data revealed no significant different between homologous and non-homologous response-modes.

A repeated measures ANOVA of 2 RESPONSE-MODE (homologous, non-homologous) x 2 SELECTION (contralateral, ipsilateral) x 2 TMS-TIMING (FLK240, FLK300) revealed a significant main effect of SELECTION ($F_{1, 31}$ = 41.0, P < 0.001) and TMS-TIMING ($F_{1,31}$ = 46.0, P < 0.001), as well as the interaction of SELECTION * TMS-TIMING ($F_{1,31}$ = 48.9, P < 0.001). From FLK240 to FLK300, post-hoc pairwise comparisons revealed that the MEPs significantly increased by 357 ± 49 μV (P < 0.001) in the PMC_C but remained unchanged of 4 ± 17 μV in the PMC_I (P = 0.83). There were no main effects of the RESPONSE-MODE ($F_{1, 31}$ = 0.2, P = 0.66) or interactions of RESPONSE-MODE * SELECTION ($F_{1, 31}$ = 0.04, P = 0.84), RESPONSE-MODE * TMS-TIMING ($F_{1, 31}$ = 0.1, P = 0.82), and RESPONSE-MODE * SELECTION * TMS-TIMING ($F_{1, 31}$ = 0.1, P = 0.75).

In contrast to the neutral trials, the homologous response-mode revealed that the MEPs at FLK240 were 326 ± 73 μV higher in PMC_C than the PMC_I ($P < 0.001$). This indicates that the congruent flanker stimuli initiated a change in CSE within 240 ms. This effect was magnified to 695 ± 109 μV ($P < 0.001$) when the MEPs were measured at 300 ms after the flanker onset and the target stimulus instructing the selection of the right-hand had 188 ms to act. This effect was similar in the non-homologous response-mode. The MEPs at FLK240 were 325 ± 70 μV higher in PMC_C than PMC_I ($P < 0.001$). This effect was magnified to 678 ± 92 μV ($P < 0.001$) when the MEPs were measured at 300 ms after the flanker onset.

Incongruent flanker condition

The flanker stimuli firstly primed the incorrect response and followed by an opposite direction of a target stimuli that activate the correct response. So the MEPs in the PMC_C were expected to be increased within the interval between FLK240 and FLK300, started with a lower MEP when measured at FLK240 as there was a flanker stimuli that activated the non-selected hand. At FLK300, the MEPs in the PMC_C would be increased after the target stimuli appeared to activate the selected hand to prepare for the response. In the non-selected hand, the MEPs elicited from the PMC_I would decrease once the target stimuli indicated that it was not required to move. From the behavioural data in previous and current chapters, a larger congruency effect in the non-homologous was caused by the slower normalised response time in the non-homologous than the homologous response-mode. In addition, the selected CRPs data showed a lower correct response activation in the non-homologous response-mode as it received a greater influence from the incongruent flanker stimuli than the homologous response-mode. Therefore, it was hypothesised that the MEPs in the PMC_C in non-homologous response-mode would be lower at both FLK240 and FLK300. For the incorrect response activation, the MEP

elicited from the PMC_I in non-homologous response-mode would be higher at FLK 240 and FLK300 as it got more influence from the flanker stimuli and this would take longer time to be inhibited.

The right-hand panel of Figure 5-7 displays the CSE changes during the response conflict in the incongruent flanker condition. A repeated measures ANOVA on the normalised MEPs from the incongruent flanker condition revealed a significant main effect of SELECTION ($F_{1, 31}$ = 27.4, P < 0.001) but not RESPONSE-MODE ($F_{1, 31}$ = 0.0, P = 0.99) or TMS-TIMING ($F_{1, 31}$ = 0.14, P = 0.71). In contrast to the neutral and congruent conditions, post-hoc pairwise comparisons revealed that the MEPs measured from right-hand were 194 ± 37 μV lower when it was elicited from the PMC_C (P < 0.001). This indicates that the incongruent flanker initially primed the incorrect responding hand.

Interestingly, the response-mode appeared to be an important influence on CSE during the incongruent condition as there was a three-way interaction of RESPONSE-MODE * SELECTION * TMS-TIMING ($F_{1, 31}$ = 9.7, P = 0.004). This interaction was further examined by running two-way repeated measures ANOVA with factors of 2 RESPONSE-MODE (homologous, non-homologous) x 2 SELECTION (contralateral, ipsilateral) on MEPs of FLK240 and FLK300 separately. When examining the MEPs at FLK240, there was a main effect of selection ($F_{1, 31}$ = 16.0, P < 0.001), but no interaction between RESPONSE-MODE * SELECTION ($F_{1, 31}$ = 0.0, P = 0.85). Post-hoc analyses revealed that, at FLK240, the MEPs elicited from the PMC_I were significantly higher than the PMC_C in both homologous (210 ± 53 μV, P < 0.001) and non-homologous (218 ± 62 μV, P = 0.001) response-modes. Interestingly, at FLK300, there was also a main effect of SELECTION ($F_{1, 31}$ = 17.0, P < 0.001), however, at

this later time-point, the interaction between RESPONSE-MODE * SELECTION ($F_{1, 31}$ = 7.5, P = 0.01) *was* significant. Although, post-hoc analyses revealed that MEPs in the non-homologous response-mode were significantly higher in the PMC_I (246 ± 46 μV, P <0.001) at FLK300, with the homologous response-mode the MEP difference between PMC_I and PMC_C had dropped to 100 ± 53 μV and was no longer statistically significant (P = 0.07). This indicated the beginning of cross-over in CSE between PMC_C and PMC_I for the homologous response-mode, which was not present in the non-homologous response-mode.

5.4 Discussion

The corticospinal excitability findings revealed that the amount of selective suppression was influenced by the response-mode. The effects of response-mode on corticospinal excitability were a good fit with the behavioural results. Again the key difference was found in the incongruent flanker condition. With the homologous response-mode, the beginning of a 'cross-over' in corticospinal excitability was detected from the non-selected to the selected hand at the later time-point. In contrast, there was no sign of a cross-over with the non-homologous response-mode. Viewed through the lens of Ridderinkhof's activation-suppression model, this indicates a lower level of selective suppression onto the direct response activation induced by the incongruent flanker with the non-homologous response-mode and therefore the effects persisted longer. These findings were interpreted as supporting the inhibitory control of competition resolution via the history dependent model (Labruna et al., 2014). The reasons for which will be discussed in detail below.

Modulation of corticospinal excitability in the PMC_C and PMC_I

Neutral flanker condition

MEPs elicited from both PMC_C and PMC_I at FLK240 were suppressed compared to MEP at baseline. The TMS timing of 240 ms after the flanker onset was determined from the incongruent LRP onset latency and incongruent selected CRP, when it showed a significant different of correct response activation between homologous and non-homologous response-mode. The MEP results suggested that this TMS timing, which was averaged from every participant, was not influenced from the target stimuli as there were no increased MEPs observed in the PMC_C. The MEPs findings in the homologous response-mode in this study were comparable to the findings from Verleger et al. (2009), who used the priming arrow flanker protocol, as the MEPs elicited in the PMC_C and PMC_I were not different when measured at the early stage but the MEPs in the PMC_C was increased as the time progressed while it remained unchanged in the PMC_I. This suggested that the increase of MEPs elicited in the PMC_C reflected the correct response preparation while the suppression of the MEPs elicited in the PMC_I reflected the inhibition of the unwanted movement.

The TMS findings in this study matched with the LRP and selected CRP results from previous chapter. When delivering the TMS over the PMC to evaluate the CSE changes, the increase of MEPs in the PMC_C was observed, which corresponded to the LRP findings that there was the correct response activation in the neutral condition. The MEPs elicited from the PMC_C were not different between homologous and non-homologous response-modes, which was in line with the selected CRP data. When the TMS was applied over the PMC_I, the MEPs was suppressed throughout the period of measurement time. There was no difference of the MEPs

between homologous and non-homologous response-modes. These were in line with the LRP findings that there was no positive deflection (incorrect response activation) during the neutral condition and we could not observe any significant differences between homologous and non-homologous response-modes in the non-selected CRP.

Congruent flanker condition

The modulation of the MEPs during congruent flanker condition represented the effect of priming flanker stimuli when the conflict was not involved. The MEPs elicited from PMC_C increased from 240 to 300 ms after the flanker onset, while the MEPs elicited from the PMC_I were suppressed and remained unchanged throughout that period of time. This suggested that the increase of MEPs in the PMC_C reflected the correct response preparation while the suppression of the MEPs elicited in the PMC_I reflected the inhibition of the unwanted movement. When compared the MEPs elicited in PMC_C at 240 ms after the flanker onset between congruent and neutral condition, the MEP in the congruent was higher than the neutral conditions. This suggested that the TMS timing of 240 ms after the flanker onset, which was determined from the mean of LRP onset latency, was enabled us to detect the effect of priming congruent flanker stimuli on the response preparation.

The MEP findings in the homologous response-mode in this study were comparable to the findings from Verleger et al. (2009), who used the priming arrow flanker protocol. The MEPs elicited in the PMC_C were higher than PMC_I when measured at the early stage but the MEPs in the PMC_C was increased as the time progressed while it remained unchanged in the PMC_I. The MEP results in this study matched with the LRP, selected CRP, and non-selected CRP results from previous chapter. The increase of MEPs was observed only in the PMC_C, which

corresponded to the LRP findings that only showed a negative deflection (correct response activation) in the congruent condition. There was no positive deflection (incorrect response activation) observed in the LRPs, which was corresponded to the suppression of the MEPs in the PMC_I. In terms of the response-modes, the MEPs elicited from the PMC_C and PMC_I did not show any differences between homologous and non-homologous response-modes. This was in line with the selected CRP and non-selected CRP that we did not observe a significant difference between two response-modes.

Response interference from the priming congruent flanker stimuli can be observed in the normalised response time to the neutral condition. The response time in the congruent flanker condition was faster than neutral condition, suggesting that the congruent flanker stimuli automatically activated the associated response. There was no difference between homologous and non-homologous response-modes in the normalised response time, which was in line with the CRPs and MEPs findings in the previous EEG chapter.

However, these results were not supported by the activation-suppression model and the proximity/history dependent model. If the non-homologous response-mode had less selective suppression than the homologous response-mode to inhibit the effect of flanker stimuli processed via the direct response activation route, we should have observed faster response time and higher correct response activation in the non-homologous response-mode. This suggested that the amount of selective suppression onto the congruent flanker stimuli was not strongly induced by the different of response-mode. This would be further discussed in the other topics.

Incongruent flanker condition

When applying the single pulse TMS over one PMC, it can reveal the relationship between correct and incorrect response activation (Verleger et al., 2009, Soto et al., 2009, Klein et al., 2014). The competition in response selection processes can be observed in the motor output level, therefore, we could access the CSE changes during response execution and movement preparatory inhibition for the conflict resolution.

The modulation of the MEPs during incongruent flanker condition represented the effect of priming flanker stimuli when the conflict was involved. The difference in MEPs between homologous and non-homologous could be observed in this condition. In the homologous response-mode, the MEPs elicited from PMC_C remained suppressed from 240 to 300 ms after the flanker onset but it tended to increase at 300 ms after the flanker onset. While the MEPs elicited from PMC_I were higher than the PMC_C during this interval, the MEPs elicited from the PMC_I reduced from 240 to 300 ms after the flanker onset. This was in line with Soto et al. (2009) who reported higher MEPs elicited in PMC_I compared to PMC_C at 200 ms after the incongruent letter flanker stimuli onset. The MEPs elicited in the PMC_I further reduced from 200 ms to 305 ms after the flanker onset (Soto et al., 2009). This was related to the previous evidence from Gratton et al. (1988) that the incorrect response activation was optimally increased at 200 ms after the flanker onset. This indicated that the competition process between correct and incorrect response activation occurred at the early stages of response processing in the PMC (Soto et al., 2009). However, the MEP results in this study contrasted to Soto et al. (2009) as they found that the MEPs in the PMC_C were higher than the PMC_I when it was measured at 305 ms after the stimuli onset. This indicated the MEPs suppression in the PMC_I and MEPs excitation in the PMC_C occurred as the competition between correct and incorrect

response activations processes had finished. In this study, the MEPs in PMC_I were higher than PMC_C throughout this period of time. Higher MEPs in the PMC_I reflected the priming incongruent flanker stimuli could initially activate the spatial incorrect response. The decrease of MEPs in the PMC_I reflected that the incorrect response activation was inhibited. However, we could not observe the significant increase of MEPs in the PMC_C as the activation of the correct response should have been observed in this condition. The possible reason was Soto et al. (2009) used the five-letter array of the simultaneous target and flanker stimuli, while this study used a priming flanker stimuli with the interval between flanker and target of 112 ms, therefore this required longer time to process the target stimuli which presented later than the flanker stimuli. Michelet et al. (2010) measured the MEPs in right index flexor and extensor when the participants responded to the five-arrow array of the simultaneous target and flanker stimuli. In the incongruent trial, the MEP elicited in the incorrect response was initially increased at 160 to 320 ms after the stimuli onset and replaced by the increase of MEP elicited in the correct response at 400 ms after the stimuli onset. We could have possibly observed an increased MEP elicited from the PMC_C if we had measured the MEPs around 412 ms after the flanker onset (112 ms later than that of 300 ms after the flanker stimuli, which Soto and colleagues found significantly increased of MEPs in PMC_C) to allow enough time for the target stimuli processing. However, this would increase the chance to get high background EMG and it would be required to exclude from the analysis as the high background EMG would interfere with the level of MEPs.

The MEPs in the homologous response-mode reported by Verleger et al. (2009) reflected to the neuronal processes of the response selection observed in the LRPs. The MEPs elicited in the PMC_C showed a linear increase while the MEPs elicited in the PMC_I showed a linear decreased

when measured during a 90 ms period from the individual peak of incorrect response activation of the LRPs. It can reflect the inhibition of the incorrect response activation in the PMC_I and increase of correct response activation in the PMC_C as the MEPs elicited in both PMC_C and PMC_I showed an x-shaped pattern. The cross-over of MEPs between PMC_C and PMC_I occurred between 311 and 341 ms after the flanker onset, which was later than the measurement time of 300 ms after the flanker onset in our study. This could be the reason that we could only observe a start of cross-over pattern before the intercept. However, we could not delay the TMS timing to later than that of 300 ms after the flanker onset as we did not use individual LRPs to determine the TMS timings. Moreover, when delivering the TMS closer to the response time, the increase of background EMG activity resulted from the response onset would interfere with the MEPs. Therefore, any trials with exceed background EMG activity will be excluded from the MEP analysis.

When the participants responded with the non-homologous response-mode, the MEPs elicited in the PMC_I were constantly higher than the PMC_C throughout the period of time. However, the increase of MEPs elicited in the PMC_C and the decrease of MEPs elicited in the PMC_I could not be observed. This can be inferred that the incorrect response activation required longer time to be inhibited while the correct response activation required longer time to be initiated. The TMS timing of 300 ms after the flanker onset was too early to detect the modulation of CSE during the conflict task in the non-homologous response-mode. However, there was the limitation in this study as we could not extend the TMS timing because the fixed TMS-timing related to the mean of LRP and selected CRP result was used in this study.

When compared the MEPs between homologous and non-homologous response-modes, it was expected to observe higher MEPs elicited in PMC_I and lower MEPs in the PMC_C in the non-homologous response-mode. This was hypothesised based on the activation-suppression and proximity/history dependent models, therefore the incongruent flanker stimuli had a greater influence onto the non-homologous response-mode. Therefore, the incorrect response activation should be larger and slower inhibited when compared to the homologous response-mode. The correct response activation should be lower and slower increased when compared to the homologous response-mode. When comparing the MEP elicited at 300 ms after the flanker onset between homologous and non-homologous response-modes, the MEPs elicited from PMC_I in the non-homologous response-mode were higher than in the homologous response-mode. This supported the activation-suppression and proximity/history dependent models that the non-homologous response-mode had a less selective suppression onto the direct response activation route. Therefore, the effect of priming incongruent flanker has a stronger impact onto the non-homologous response-mode. It can be implied that these findings were comparable to the selected CRP in the previous chapter as we observed less correct response activation in the non-homologous response-mode. However, we could not observe the different of the incorrect response activation between homologous and non-homologous response-modes with the non-selected CRP method, but the finding in this study suggested that the incorrect response activation in the non-homologous response-mode was higher than the homologous response-mode as observed in the MEPs elicited from the PMC_I at 300 ms after the flanker onset.

Response interference from the priming incongruent flanker stimuli could be observed in the normalised response time. The response time in the non-homologous response-mode was slower than the homologous response-mode. This corresponded to the MEP findings that the

incongruent flanker stimuli had a stronger influence on the non-homologous response-mode. The incorrect response activation required longer time to be inhibited and the correct response activation required longer time to be initiated, therefore, the response time in the non-homologous response-mode was slower than the homologous response-mode.

The competition resolution mechanism is associated with the suppression of the incorrect response activation as it inhibits the unwanted movement. It was thought to help sharpen the correct response selection when there was a choice between two different candidate responses (Klein et al. 2014). The present study suggested that the inhibitory mechanism occurred to inhibit the incorrect response induced by the conflict stimuli was associated with the competition resolution. When the level of inhibition was modified by manipulating the response-mode, we could observe the changes of the CSE suppression that were corresponded to proximity/history dependent model from Labruna et al. (2014). However, we could not detect where the inhibition in the brain originated from as this study only focused on the PMC. Future experiments are required to understand the mechanisms at the cortical and subcortical levels that have the projection to inhibit the PMC in order to suppress the incorrect response induced by the conflict.

Task protocol

The present experiment used an arrow stimuli, which appropriates to observe the response activation between left and right body sides. The priming conflict stimuli automatically helped evoke the incorrect response activation, which related to the response side that was required to be inhibited. However, in the non-homologous response-mode trials, the position of hand and

foot are in the vertical alignment, therefore the flanker and target stimuli should have been presented in a vertical alignment to correspond with the response effectors.

It was expected to observe larger MEPs elicited from the PMC_C during congruent condition with the non-homologous response-mode (see Figure 5-1). However, we did not observe a distinct different between the two response-modes. We do not know whether it caused by the lower suppression in the homologous response-mode or stronger suppression in the non-homologous response-mode. The possible reason was the homologous and non-homologous response-mode were run in a separate block. When the participants were instructed whether homologous response-mode or non-homologous response-mode was required to respond at the beginning of the experimental block, we believe that the top-down control mechanism would influence the amount of baseline selective suppression. Therefore, the evaluation of the effect of response-mode onto the movement preparatory inhibition should have done when the potential responses including the homologous and non-homologous response-modes are involved in the same block.

This experiment included 48 task conditions that contained two response-modes, two selections, three flanker conditions and four TMS timings. The number of conditions was minimised by recording the MEPs elicited only from left PMC. Then the MEPs were compared when the other potential responses were homologous or non-homologous response-mode, and when the left PMC were contralateral to the selected hand or contralateral to the non-selected hand. We also compared the MEPs when the flanker conditions were congruent, incongruent, or neutral, and when the TMS was measured at the fixation, FLK240, FLK300, or no TMS. In each of these conditions, where the MEPs were measured at FLK240 and FLK300, a maximum

of 36 TMS trials were elicited. This study included extra trials in case that it were needed to be rejected when the response was incorrect, or baseline EMG was high. After rejecting the invalid trials, at least 16 trials of MEP remained for the analysis. The probability to reach 95% CI was 0.78 (Cuypers et al. 2014). However, we couldn't have the experiment longer as this experiment took 3.5 hours.

Top-down control during response selection under conflict

The mechanism of conflict resolution that occurs to suppress the incorrect response activation can be observed by the decrease of MEP amplitudes elicited in the PMC_I in the incongruent condition. Klein et al. (2014) and Duque et al. (2016) reported that the conflict resolution mechanism is a top-down control as the MEPs elicited in the PMC_I during the incongruent condition were more strongly suppressed when the participants anticipated for the conflict when the task included a greater proportion of the incongruent than the congruent trials compared to when the participants had less anticipated for the conflict in the task with mostly congruent trials.

Other cortical networks also contribute to the conflict resolution mechanism. Control of perception and attention also plays a role in the conflict paradigm. Participants were told to ignore the flanker stimuli and only pay attention onto the target stimuli. There is evidence that the flankers are not fully ignored as observed with the congruency effect, LRPs, and TMS. The posterior parietal cortex (PPC) involves at an early stage of conflict stimuli perception and encoding. When rTMS is applied to disrupt the PPC, the flanker effect was decreased as the response time in the incongruent trials was reduced and the accuracy was increased (Jin et al., 2010). Dorsal medial frontal cortex (dMFC) resolves the conflict by interacting with the PMC

as a part of the top-down control mechanism (Taylor et al., 2007). This was confirmed when rTMS is applied to disrupt the left dMFC, right hand produced higher error rate in the incongruent trials and the LRP displayed a higher positive deflection, which indicated a higher incorrect response activation. There is evidence from EEG, fMRI, and TMS studies showing that lateral prefrontal cortex (LPFC) and medial prefrontal cortex (MPFC) are associated with the online inhibitory control over incorrect response possibly through the basal ganglia (Burle et al., 2004, Aron et al., 2007, Duque et al., 2012, Burle et al., 2016).

Soto et al. (2009) suggested that during the response execution under the conflict condition, the competition between correct and incorrect response activation occurs at the level of motor output. When single pulse TMS is applied over the PMC at 200 ms after the incongruent stimuli onset, the MEP elicited from the PMC_I was higher than the PMC_C. This reflected the effect of the flanker interference onto the PMC. But when the TMS was applied at 300 ms after the stimuli onset, the MEP elicited in the PMC_I was lower than the PMC_C. This was in line with the present study that the different of MEP amplitudes elicited in the PMC_I were reduced at the later stage while the MEPs in the PMC_C was increased at the later stage as observed in the homologous response-mode. However, Soto et al. (2009) used the letter flanker stimuli that the target and flanker stimuli simultaneously presented in an array, but we could observe the effect of the flanker in the PMC when the TMS was delivered at 240 and 300 ms after the flanker onset. Michalet et al. (2010) used the arrow flanker stimuli that the target and flanker stimuli simultaneously presented in an array also found the MEPs elicited in the PMC_I was initially increased and was later replaced by the increasing of the MEPs elicited in the PMC_C in the incongruent flanker condition. These findings supported that the dynamic of CSE modulation reflects the competition process resulted from the selection of the correct response and

inhibition of the incorrect response, which distributed from the cortical and sub-cortical network that extend to the primary motor cortex.

This TMS experiment together with the behavioural study could only explore the indirect dynamic changes of the conflict stimuli processing and competition during response selection and execution when responding with the homologous and non-homologous response-modes. Therefore, it could not determine where the mechanisms originate from. This could possibly be further explored with the fMRI technique, which allow us to explore the exact brain areas that are involved in the conflict resolution when it is influenced with homologous and non-homologous response-modes.

Conclusion

The present study used the single pulse TMS to investigate the modulation of CSE during the conflict task and the influence of homologous and non-homologous response-modes on the amount of movement preparatory inhibition under the conflict. The selective suppression to inhibit the incorrect response activation was similar to the competition resolution processes as the amount of inhibitions were influenced by the response-modes. When experiencing the conflict, the incorrect response was initially activated prior to the cancellation of the incorrect response. While the correct response activation increased at the same time until it reached the threshold to execute the correct response. It was found that the amount of selective suppression to inhibit the incorrect response activation induced by the conflict stimuli was higher when the participant selected the appropriate response between left or right hands, which are normally in competition in daily life. When the movement selection was made when the potential responses

were hand and foot, which are not normally compete in the daily life, the amount of selective suppression to inhibit the incorrect response was lower than the hand-hand condition.

CHAPTER 6

General discussion

6.1 Discussion of experimental chapters

In this thesis, I have presented four studies, which have investigated the mechanisms of movement preparatory inhibition. In chapters 2 and 3, a novel method of conducting the IHI measurement was developed in a quicker way and applied to explore the inhibition mechanisms during movement preparation and selection. In theoretical basis of the inhibitory processes, in chapter 4 and 5, it was further investigated whether the competition resolution processes operate in a history dependent like fashion when selecting the movement during response conflict.

6.1.1 Inhibitory mechanisms during movement preparation

The measure of interhemispheric inhibition during movement preparation in this thesis was performed with a novel substitutional approach by setting the conditioning and test stimulus intensities to elicit the MEPs of 1 mV in both hemispheres. The MEP elicited from the conditioning stimulus can be replaced with the unconditioning test MEPs. Therefore, this can reduce the experiment for half of the total trials. The consistent amount of the resting IHI was observed with the novel method compared to previous studies (Ferbert et al., 1992, Duque et al., 2005, Duque et al., 2007, Morishita et al., 2014). Moreover, there was no asymmetry of resting IHI between left and right PMCs at rest when using either the conventional IHI method or the novel substitutional approach. By setting the stimulus intensity to elicit the similar level of MEPs of 1 mV in both PMCs, this decreases the effect of lateralisation between left and right hemispheres as the motor output from both hemispheres are similar.

This approach allowed me to explore the IHI effect in both PMCs during movement selection and preparation within an instructed delay task. When the participants prepare for the unilateral movement, a decision process is required to select the appropriate response. The several potential movement options are initially activated simultaneously and then gradually inhibited to allow the appropriate response execution (Coles, 1985, Cisek and Kalaska, 2010, Greenhouse et al., 2015). Two inhibitory mechanisms operate; these are 'impulse control', which prevents the premature release of the selected movement and 'competition resolution', which stops the competing but non-selected movement (Duque and Ivry, 2009, Duque et al., 2010). The evidence of both IC and CR was found when exploring changes in corticospinal excitability in the selected and non-selected hands.

Impulse control is thought to occur within the hemisphere contralateral to the responding effector as it has been suggested that the dorsal premotor cortex (Cisek and Kalaska, 2005, Duque et al., 2012) and lateral prefrontal cortex (Wallis et al., 2001, Koechlin and Summerfield, 2007) are involved in a process producing inhibition on the selected movement. The evidence in support of this idea was provided by demonstrating that the inhibition that was found in the PMC contralateral to the selected hand as observed in the MEP elicited from single pulse TMS. This inhibition was not mediated by the contralateral PMC as we could not observe the IHI effect in this condition. This suggested that the inhibitory mechanism to prevent the premature movement possibly occurs parallel to the activation and early processing of critical motor control strategies at spinal level.

The competition resolution mechanism has been thought to operate at the cortical level that inhibits the task-relevant response where the movement is involved in the response task (i.e.

the preparatory cue or imperative cue may signal this movement) and task-irrelevant responses where the movement is not involved in the response task (i.e. in the simple RT task when the participant knows which response is required and which responses are not required) (Duque et al., 2012). The underlying mechanisms of the competition resolution involved the interhemispheric inhibition from other brain areas such as lateral prefrontal cortex (MacDonald et al., 2000, Botvinick et al., 2001) and contralateral PMC (Labruna et al., 2014) that acts toward the responsible mirror movements or acts more globally to other motor representations (Duque et al., 2005, Greenhouse et al., 2015). This also demonstrated that the competition resolution as measured from the MEPs elicited from single pulse TMS but this inhibition is not mediated by the contralateral PMC as the progression of IHI could not be observed during the movement preparation period. This finding does not support the smart, homologous, and proximity/history dependent models proposed by Labruna et al. (2014). The inhibition found in the PMC_I that was thought to inhibit the unwanted movement is not mediated from the PMC contralateral to the selected hand via the transcallosal pathway. This possibly occurred from the lateral prefrontal cortex (MacDonald et al., 2000, Botvinick et al., 2001) or pre-supplementary motor area (Duque et al., 2013).

The limitation of this experiment was that it only evaluated the interhemispheric inhibition between left and right PMCs, therefore it was not possible to specify where other cortical and subcortical networks generate the preparatory inhibition. The task involved in this experiment might be an issue that the distinct IHI effect on the competition resolution could not be observed as the amount of inhibition was influenced by the task complexity (Greenhouse et al., 2015, Quoilin et al., 2016). To further understand the mechanisms of the movement preparatory inhibition, other brain areas such as the lateral prefrontal cortex and pre-supplementary motor

area that are thought to be involved in the competition resolution should be observed using the fMRI or dual-coil TMS. The response task is also needed to be more complex than index finger movement in one direction. The measure of inhibition action on other potential movements could provide the extensive of the inhibition onto task-relevant and task-irrelevant during movement selection and preparation.

6.1.2 Inhibitory mechanisms of movement preparation and selection during response conflict

Klein et al. (2014) proposed that the competition resolution is related to the selective suppression described in the activation-suppression model of Ridderinkhof et al. (2005). The common finding is the competition resolution and selective suppression both inhibit the competitive response or the unwanted response induced by conflict stimuli (Duque et al., 2012). In addition, it has been suggested that the inhibition of the competing response and conflict monitoring originated from prefrontal cortex and might include the contralateral PMC (MacDonald et al., 2000, Botvinick et al., 2001). Labruna et al. (2014) suggested that the amount of the inhibition to suppress the unwanted response is graded by the history or the similarity between the potential responses. Therefore, the focus of chapter 5 and 6 were to explore the effect of response-mode on the conflict resolution. If the selective suppression mechanism that is thought to inhibit the incorrect response induced by the conflict stimuli shared a common mechanism with the competition resolution, then the amount of inhibition in the homologous response-mode would be stronger than the non-homologous response-mode. The experiment was created to enhance the effect of conflict stimuli on the automatic activation of the incorrect response activation by using the priming arrow flanker task. The congruency effect was larger when responding with the non-homologous response-mode, which was mainly

contributed from the incongruent condition where the conflict was involved. The participant responded slower in the non-homologous response-mode when experiencing the conflict condition.

The response time in chapter 4 did indeed show a larger congruency effect for the non-homologous response-mode. The CRPs further provided the explanation that the non-homologous response-mode had less correct response activation than the homologous response-mode. This suggested that the selective suppression to inhibit the incorrect response activation was lower in the non-homologous response-mode. Therefore, it required longer time to resolve the conflict and initiate the correct response.

When using the LRP latency where the incorrect response activation was differ from the correct response activation and the latency where the selected CRP showed less correct response activation in the non-homologous response-mode to determine the TMS timings, the EEG findings were supported by the results in chapter 6. The response time showed a larger congruency effect with non-homologous response-mode. The MEPs provided the evidence of cross-over effect beginning to happen in the incongruent condition of the homologous response-mode, but this was not seen with non-homologous response-mode. This indicates that the effects of the flanker were stronger and more persistent with the non-homologous response-mode. It demonstrated that there was a stronger selective suppression to inhibit the incorrect response activation when responding with the homologous response-mode.

Thus, the findings in chapter 5 and 6 supported that the selective suppression, which plays important role in the conflict resolution was mediated by a similar mechanism to the

competition resolution in order to inhibit the unwanted movement in other potential responses. Moreover, these findings also suggested that the amount of competition resolution and selective suppression were influenced by the response-mode as proposed in proximity/history dependent model from Labruna et al. (2014). In chapter 5 and 6, the experiment protocol was more complex than that was performed in chapter 3. However, it was not observed whether the competition resolution is mediated from contralateral PMC or any other brain areas. Therefore, to further explore the competition resolution mechanism involving the conflict resolution, the IHI between left and right PMCs should be evaluated.

6.1.3 Contribution to knowledge and application of the thesis

This study is the first to establish the substitutional approach of IHI technique in a group of young healthy individuals. By setting the stimulus intensities to elicit the MEP output at a similar level between two hemispheres, this can eliminate the asymmetry of IHI between dominant and non-dominant hemispheres. Further research should apply this IHI protocol to clinical populations who have the impairments affecting the transcallosal pathway or have asymmetrical level of corticospinal excitability between lesioned and non-lesioned hemispheres (ie. individuals with stroke, multiple sclerosis). These impairments may influence the level of IHI during resting and performing a unimanual or bimanual movement. Moreover, the use of a stimulus intensity that elicits an MEP of 1 mV may be a problematic in patients with neurological impairments because it probably requires much higher intensity in terms of the percentage of the maximum stimulator output. On this basis, it would seem appropriate to use the stimulus intensity to produce the MEP amplitude of less than 1 mV equally in both hemispheres.

The CRP method in the EEG chapter that was used to evaluate the difference of correct and incorrect response activation between homologous and non-homologous response-modes should be explored further to determine the reliability of this method. Furthermore, as the CRP is novel, it is crucial that a different technique of analysis is carried out to assess the amount of incorrect response activation which could not be observed in this study.

6.2 General conclusions

The main aims of this thesis were to better understand the inhibitory motor processes during movement preparation. I sought to understand the inhibitory mechanisms that prevent the premature response of the planned movement and the inhibition that prevent the unwanted movement when there was a decision making to select the appropriate movement. Lastly, I was interested in whether the response-mode affected the preparatory motor processes. This thesis was able to demonstrate that the mechanism of the impulse control that prevents premature movement, as well as the competition resolution, are unlikely to directly involve inhibition from the contralateral PMC. It was also found that the inhibition of competition resolution decreased when the potential responses were not in the homologous response-mode. These findings support a proximity and history dependent model of competition resolution, but it is unlikely that the graded inhibition to the non-selected response operates directly from the opposite PMC through transcallosal pathway.

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