THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

INTERHEMISPHERIC INTERACTIONS ASSOCIATED WITH UNILATERAL BALLISTIC MOTOR TASKS

Lilian Min Yen CHYE
BSc (Sports Science) First Class Honours

A thesis submitted for the degree of Doctor of Philosophy at
The University of Queensland in 2015
School of Human Movement and Nutrition Sciences

Abstract

The aim of this research was to enhance our understanding of the spatial reference frames in which movement is represented in the primary motor cortices of both hemispheres during unilateral ballistic actions. The broader goal was to reveal how the coordinate systems of movement representation influence the interactions between the two cerebral hemispheres which presumably underlie cross limb transfer of motor skill. The issue of reference frames may be critical for cross-limb interactions because definitions of space and movement often conflict for opposite limbs in intrinsically-referenced coordinate systems (e.g. muscle or joint-based coordinates) due to their mirror symmetry. This thesis describes a series of experiments to investigate the reference frames in which movement representations are shared bilaterally, and whether alignment of reference frames influences the transfer of performance to the untrained limb after unilateral ballistic training.

There is increasing evidence that the primary motor cortex ipsilateral to the active limb is active during unilateral movement. Yet, the extent to which such activity represents functional details of movement with the ipsilateral limb, and the coordinates of any such representation, is unclear. Hence, Chapter 2 reports work aimed at understanding the timing and coordinates of interactions between the two motor cortices when movement with one hand is being prepared. Studying the time course of changes in twitch directions evoked by transcranial magnetic stimulation (TMS) enabled us to examine whether activity in the “resting” motor cortex functionally represents the impending movement, and the reference frame of any such representation. Our results showed that twitch directions in the resting limb shifted toward the impending direction of the active hand in a muscle-based reference frame. Evident changes in TMS-evoked twitch parameters right before movement onset might be associated with decreases in interhemispheric inhibition and intracortical inhibition, brought about by the release of motor commands to the active limb.

Use-dependent learning was previously reported to generalise according to extrinsic coordinates when posture changes were used to dissociate reference frames within the active limb, however it is unclear whether this form of learning generalises to the untrained limb. Chapter 3 assessed whether use-dependent bias in aiming performance occurred in the opposite untrained limb under postural manipulations that varied the extent to which spatial coordinates were aligned for the two limbs according to extrinsic, muscle-based and midline reference frames. The results revealed that systematic bias occurred only when both limbs were oriented such that training and aiming targets were aligned according to all reference frames. The data suggest that aiming biases in the untrained limb after contralateral ballistic training are represented according to a combination of coordinate systems.

In order to understand the role of reference frame conflicts in transfer of use-dependent learning, we examined the representation of learned movements within the trained hemisphere with TMS. When resting TMS-evoked twitches were observed in different postures (Chapter 4), twitch directions followed with the wrist according to joint- and muscle-based reference frames. When posture was manipulated after ballistic training (Chapter 5), there were systematic shifts in evoked twitch directions toward the training direction, but adaptation was represented either in extrinsic or muscle-based reference frames for different subjects.

In conclusion, the research described in this thesis increases understanding of the reference frames in which unilateral ballistic movements and learning are represented in the primary motor cortices. The findings showed that movement is represented in muscle-based reference frame during motor preparation, and that cross limb transfer improves when multiple reference frames of movement representation are congruent between limbs. We conclude that spatial reference frames play an important role in determining the functional effects of interhemispheric interaction during unilateral movement.

Declaration by Author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Conference abstracts:

Chye, L., Riek, S., de Rugy, A., & Carroll, T. (2015). TMS-evoked twitches in the passive limb change direction according to muscle activation of the active limb. Paper presented at the 1st International Brain Stimulation Conference, Singapore.

Chye, L., Riek, S., de Rugy, A., & Carroll, T. (2014). The direction of force twitches evoked by TMS in a passive limb shift according to the direction of impending contralateral muscle activation. Paper presented at the XII International Conference on Cognitive Neuroscience, Brisbane, Queensland, Australia.

Chye, L., Riek, S., de Rugy, A., & Carroll, T. (2013). Do TMS-evoked twitches shift towards a ballistic training direction in extrinsic or muscle-based coordinates? Paper presented at the Neuroscience Conference, San Diego, California.

Chye, L., Riek, S., de Rugy, A., & Carroll, T. (2012). Do TMS-evoked twitches shift towards the direction of high-force ballistic training according to extrinsic or muscle-based coordinates? Paper presented at the International Society of Electrophysiology & Kinesiology Conference, Brisbane, Queensland, Australia.

Publications included in this thesis

No publications included

Contributions by others to the thesis

Dr Welber Marinovic – statistical assistance for the studies in Chapter 2 and 3.

Statement of parts of the thesis submitted to qualify for the award of another degree

None

Acknowledgements

No words could express how grateful I am to the people who have helped me one way or another during my journey to complete my PhD.

My heartfelt gratitude to all of you.
Wishing you good health and success in life.

Keywords

Interhemispheric interaction, Primary motor cortex, Reference frame, Transcranial magnetic stimulation, Twitch direction, Ballistic movements, Use-dependent learning, Cross limb transfer, Ipsilateral motor cortex, Unilateral movements

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 110603, Motor Control, 80 %

ANZSRC code: 110602, Exercise Physiology, 10 %

ANZSRC code: 110903, Central Nervous System, 10 %

Fields of Research (FoR) Classification

FoR code: 1106, Human Movement and Sports Science, 90 %

FoR code: 1109, Neurosciences, 10 %

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List of Abbreviations

CCW - counter clockwise
CNS - central nervous system
CW - clockwise
ECR_b - extensor carpi radialis brevis
ECU - extensor carpi ulnaris
EMG - electromyography
FCR - flexor carpi ulnaris
FCU - flexor carpi ulnaris
FDI - first dorsal interosseous
GABA - ϒ-aminobutyric acid
LTP - long term potentiation
M1 - primary motor cortex
M1_contra - contralateral primary motor cortex
M1_ipsi - ipsilateral primary motor cortex
MEP - motor evoked potential
MVC - maximum voluntary contraction
NMDA - N-methyl-D-aspartate
NE - neutral extension
NF - neutral flexion
PE - pronated extension
PF - pronated flexion
RT - reaction time
TD - training direction
TES - transcranial electrical stimulation
TMS - transcranial magnetic stimulation

CHAPTER ONE

Introduction

A key feature of the human central nervous system (CNS) is its lateralisation. The brain and spinal cord are uniquely organized into two halves that are almost identical in structure. Voluntary control of movement of one side of the body is predominantly brought about by one cerebral hemisphere (contralateral to the moving side) via crossed corticospinal fibres at the pyramidal decussation and/or one spinal hemicord (ipsilateral to the moving side) (Kertesz & Geschwind, 1971; Levy, 2013; Siegel & Sapru, 2011). However, there is increasing evidence from neuroimaging studies showing substantial interaction between the two hemispheres, even during strictly unilateral movements (Bütefisch et al., 2014; Chiou et al., 2014). Transcranial magnetic stimulation (TMS) studies also revealed that corticospinal excitability, intracortical, and interhemispheric inhibition are all modulated in both hemispheres during unilateral movements (Howatson et al., 2011; Leocani et al., 2000; Perez & Cohen, 2008; Reid & Serrein, 2014; Teo et al., 2012). Although the gross structure of the hemispheres may be almost identical, many of their functions are asymmetrical. For instance in motor control, the dominant hemisphere is specialised for controlling limb trajectory (Flindall et al., 2014; Reid & Serrein, 2014; Sainburg, 2005; Wang & Sainburg, 2006) and response execution (Nisiyama & Ribeiro-do-Valle, 2014), while the non-dominant hemisphere is more efficient in position control (Sainburg, 2005; Wang & Sainburg, 2006). The mechanism of these functional asymmetries of the hemispheres remains unclear. There remains much to learn about the purpose and consequences of lateral interactions in the CNS.

One particularly interesting aspect of lateral interaction within the CNS with potential practical benefits is the capacity of training with one limb to improve performance of the same task with the opposite untrained limb (Adamson et al., 2008; Carroll et al., 2008; Munn et al., 2005; Perez et al., 2007; Wang & Sainburg, 2009). This phenomenon is widely known and has been termed cross education, cross limb transfer, inter-limb transfer, inter-lateral transfer or intermanual transfer. For the purpose of this thesis, the terms “cross education” and “cross limb transfer” are used interchangeably to describe the phenomenon. Cross education has been demonstrated in a number of tasks such as strength training (Adamson, et al., 2008; Farthing et al., 2009; Pearce et al., 2013), fast speed (ballistic) contractions (Carroll, et al., 2008; Hinder et al., 2013b; Lee et al., 2010), pegboard dexterity tasks (Schulze et al., 2002), sequential finger movements (Shea et al., 2011; Wiestler et al., 2014), force field perturbations (Criscimagna-Hemminger et al., 2003; Galea et al., 2007) and visuomotor distortions (Carroll et al., 2014; Sainburg & Wang, 2002; Taylor et al., 2011; Wang & Sainburg, 2009). The exact mechanisms for cross education are still not well understood, but presumably reflect the sharing between both limbs of specific information acquired by the trained limb. The form of information sharing has been described by two classes of model, termed “bilateral access” and “cross activation” (Lee & Carroll, 2007; Ruddy & Carson, 2013). Bilateral access models suggest that neural adaptation resulting from training resides either in cortical motor areas that project to both motor cortices, or in the trained motor cortex which is accessible to the untrained motor cortex via the corpus callosum (Anguera et al., 2007; Farthing et al., 2007; Lee & Carroll, 2007). In contrast, cross activation models suggest that bilateral activity occurs during unilateral training, which leads to neural adaptation in both trained and untrained hemispheres. The information can then be directly accessed by the untrained limb during task execution (Carroll et al., 2006; Koeneke et al., 2006). The “bilateral access” and “cross-activation” models are, however, not mutually exclusive and it is conceivable that both could occur concurrently during unilateral training. Although transfer of performance to the untrained limb is rarely complete, and frequently less than 75 % of what the trained limb has achieved after the training (Carroll, et al., 2006; Hinder et al., 2011; Taylor, et al., 2011), cross limb transfer has important implications for treatment of unilateral brain injury or movement disorders.

The effect of cross limb transfer has been reported to be asymmetrical in some contexts. For example, some studies suggest that cross education is stronger when the dominant arm is trained (Farthing, 2009; Farthing et al., 2005), while others reported that training the non-dominant arm is equally effective (Adamson, et al., 2008; Hinder et al., 2013a; Schulze, et al., 2002). Contradictory asymmetry results could imply cross limb transfer is influenced by multiple factors, such as age (Graziadio et al., 2015; Hinder, et al., 2013a), task complexity (Schulze, et al., 2002) and handedness (Sainburg, 2005; Wang & Sainburg, 2006). Apart from asymmetry of transfer, another important factor that has received little attention in the interpretation of results from these studies is the importance of reference frames. A reference frame can be referred to as a coordinate system or set of axes used to describe the location of an object. The location of an object in space can be represented in many different reference frames (Andersen et al., 1993; Cohen & Andersen, 2002). For example, consider a cup on a table. The position of the cup can be defined according to the visual field of the eyes, or the joint rotations needed to touch it with a hand, or a Cartesian coordinate system centred on the head, hand, or body. The cup’s location can also be defined with respect to the external world, such as its position relative to the table. Subsequently, if a decision is made to reach for the cup, the cup location must ultimately be transformed into the coordinates of muscle activation. This requires integration of positional information about the cup and the body from multiple sensory systems, each represented according to different coordinate systems. For instance, a visual target is initially represented in eye-based coordinates, and the reaching characteristics depend on the initial location of limb (which usually originates in joint angle coordinates). The information derived from these various reference frames must be combined and transformed to generate motor commands in muscle based coordinates at the limb. We are particularly interested in the question of whether neural adaptation is modulated by the reference frames in which a task is performed, and how it generalises to the untrained limb during transfer. Few studies have directly investigated cross limb transfer from the perspective of reference frames, but if reference frames are an integral aspect for transfer, there might be a greater generalisation of the learned skill to the untrained limb following unilateral training when reference frames of movement representation between limbs are congruent.

Previous reports on cross limb transfer of visuomotor adaptation illustrate the possibility that reference frames might be an important factor that determines transfer of motor skill between limbs. During this type of visuomotor adaptation paradigm, one arm typically performs visually guided aiming toward a target under altered visual feedback. The motor system adapts such that the aiming errors induced by the altered visual feedback are gradually corrected with practice. After the practiced arm has adapted to the error, transfer is tested with the unpractised arm. Very often during cross limb transfer studies, reference frames in which the movements defined are not aligned for the two limbs. For example, if you flex both wrists with your palms facing each other, the fingers of your left hand are moving to the right in external space, whereas the fingers of your right hand move toward the left. Therefore, while the flexion movement activates homologous muscles in each limb and is congruent in muscle-based coordinates, there is a conflict between the directions of limb motion in the extrinsic reference frame (one hand moving to the left, the other to the right). Although transfer is known to be asymmetrical in visuomotor adaptation studies for right-handed participants, it is reported that transfer is observed when the visual feedback is defined in identical extrinsic coordinates for trained and untrained arms. For example, after right-handers trained their right arms with a leftward shift in the visual feedback, the left arm showed rapid learning for leftward shifts by a reduction of direction error from the second trial (Sainburg & Wang, 2002; Taylor, et al., 2011). The same pattern of results occurred when the left (non-dominant) arm was trained, such that it can be concluded that training of the left (non-dominant) arm facilitates performance of the right (dominant) arm when the altered visual feedback is in the same direction defined in the extrinsic reference frame (Wang & Sainburg, 2004). However, these visuomotor studies were performed in horizontal plane reaching contexts, and therefore for any given target location, there was a conflict between the required visuomotor recalibration defined according to intrinsic (joint- and muscle-based) versus extrinsic coordinates for the two different limbs. However, Carroll et al. (2014) recently reported a symmetrical and immediate transfer of visuomotor adaptation, regardless of the hand trained, when the altered visual feedback had identical effects in the eye- (extrinsic) and joint-based (intrinsic) coordinates for both hands in the sagittal plane. It is thus suggested that the degree of alignment of the reference frames affects the behavioural responses during transfer. A more complete transfer occurs when movement for both limbs is defined in congruent extrinsic and intrinsic reference frames. Congruent reference frames could allow the untrained hemisphere to access the same motor plan adopted by the trained arm without the need to transform or recompute new motor commands, which might in turn lead to more rapid and complete transfer. Taken together, the issue of reference frames likely plays a vital role in influencing the interaction of both hemispheres during cross limb transfer. There are still many questions pertaining to the issue of the reference frames in which adaptation occurs during unilateral training, and how the information is made available to the opposite hemisphere. In this thesis, I will address three important research questions that are relevant to our understanding of cross education from the perspective of reference frames where adaptation occurs during unilateral training.

Interaction between hemispheres appears necessary for cross limb transfer, but at which stages of motor planning or execution do the critical interactions between the hemispheres occur? When the brain plans to move one hand in a certain direction, it is uncertain whether the other hemisphere prepares in the same manner and if so, in which reference frame any such movement plan is represented. In Chapter 2, we aimed at understanding the timing and coordinates of interactions between the two motor cortices when movement with one hand is being prepared. We used TMS to evoke twitches from the resting limb, during and just before movement production with the opposite limb. The direction of TMS-evoked twitches reflects the resultant force of the muscles activated by TMS. Studying the time course of changes in the twitch directions enabled us to examine whether the “resting” motor cortex represents the impending movement, and the reference frame of any such representation. In particular, if interaction between the hemispheres occurs during movement preparation, the twitch directions of the resting limb should shift either toward the target direction in extrinsic space or toward the homologous muscle responsible for the direction of action (i.e. toward the impending movement in muscle space). If we understand which reference frame the hemisphere ipsilateral to the moving limb encodes information about movement, training tasks could be manipulated to maximise reference frame alignments between limbs to enhance interactions between the hemispheres, which could potentially benefit performance transfer.

Learning to make a specific movement repeatedly reduces the error made toward the repeated target, and at the same time biases movements directed toward other locations toward the direction of the repeated actions (Diedrichsen et al., 2010; Verstynen & Sabes, 2011). Selvanayagam et al. (2012a) demonstrated that a single session of 40 ballistic isometric contractions toward a specific direction can bias subsequent aiming with the same limb toward the training direction defined according to extrinsic rather than intrinsic coordinates. This use-dependent learning that arises from repeated ballistic contractions biased aiming toward the training direction for target locations as far as 90° away from the training direction. Whether such aiming biases resulting from use-dependent learning generalise to the opposite limb is unknown. However, we speculated that cross education of use-dependent aiming bias might occur with ballistic training because ballistic training induces cross limb transfer of performance (Carroll, et al., 2008; Lee et al., 2009). Therefore in Chapter 3, we examined the aiming biases with the untrained limb after unilateral ballistic contractions. We manipulated the reference frames of movement between both limbs in congruent and conflicting manners. If reference frames are an important factor influencing interaction between hemispheres, then aiming bias should be more robust when the reference frames for the movement representation between both limbs are congruent (Carroll, et al., 2014).

Another consequence of use-dependent learning is demonstrated by a sustained, post-training shift of involuntary (TMS-evoked) twitch direction in the active limb toward the practiced direction (Classen et al., 1998; Giacobbe et al., 2011; van Elswijk et al., 2008). Such results are taken as evidence that use-dependent learning increases the corticospinal excitability of projections to the muscles activated for the training direction. However, these studies have not used methods that allow the dissociation of reference frames. The first step required to understand the role of reference frame conflicts in transfer of use-dependent learning as measured by TMS-evoked twitches is to identify the coordinates of evoked twitch representations within the trained hemisphere. It is still unclear how competing reference frames in movement representation between limbs influence the extent of central adaptation. Kakei et al. (1999) elegantly showed how reference frames can be easily dissociated into extrinsic and intrinsic reference frames by rotating the wrist joint between pronation and supination in primates. For example, an upward movement is produced by the wrist extensor muscles when the forearm is in pronation, but by the wrist flexor muscles when the forearm is in supination. In Chapter 4, we studied whether resting TMS-evoked twitch directions rotated with forearm between pronated and neutral (midway between pronation and supination) positions. de Rugy et al. (2012) recorded electromyography (EMG) signals via intramuscular electrodes and showed that the preferred muscle pulling directions of the human wrist muscles rotate with the wrist joint. Hence, if the TMS-evoked twitches follow changes in wrist joint orientation at rest, we could examine whether training causes an adaptation in joint- and/or muscle-based versus extrinsic reference frame by observing training-induced shifts of TMS-evoked twitch directions. In Chapter 5, we oriented the wrist into training and testing wrist postures and examined the resting twitch directions in these postures before and after a session of ballistic training. We sought to discover if ballistic contractions induce an adaptation toward the training direction in extrinsic or muscle-based reference frames, using the paradigm described by Selvanayagam et al. (2011). We chose two wrist postures in our study so that a shift of twitch direction in one direction would imply extrinsic representation of use-dependent learning, whereas a shift in the other direction would imply a muscle-based representation. By understanding how training could induce adaptation in a different reference frame, this knowledge could enable us to improve the design of subsequent experiments which study cross limb transfer.

This thesis aimed to examine the reference frames in which ballistic motor adaptation is represented and how the issue of reference frames influence the interaction between hemispheres during unilateral training. Specially, we investigated:

1) whether preparation of movement toward horizontal targets changes TMS-evoked twitch directions in opposite inactive limb;

2) whether transfer of aiming bias would be greater when the training direction defined according to extrinsic, muscle-based, midline, and/or training axis reference frames in both limbs are congruent in the opposite untrained limb;

3) whether the muscles activated by stimulation of the same part of motor cortex changes as a function of posture, and whether the neurons activated by TMS represent extrinsic or intrinsic movement direction within the same limb;

4) whether use-dependent learning was represented according to extrinsic or muscle-based coordinates within the same limb.

The advancement in the knowledge of cross limb transfer from the perspective of reference frames enables us to better understand why some cross limb studies reported incomplete or no transfer after unilateral training. Therefore, by understanding the importance of reference frame issues in interaction between hemispheres, the effect of cross education can be better harnessed to benefit rehabilitation programmes for unilateral movement disorders, and aid in the designing of human-machine interfaces for optimal motor control.

CHAPTER TWO

TMS-evoked twitches in the passive limb change direction according to the intrinsic coordinates of movement in the active limb

2.1 Introduction

Executing an accurate movement with our limbs, such as picking up an object, can seem effortless. Nonetheless, complicated sensorimotor integration is required in the brain even for apparently simple movements. Prior to a goal-directed action, the attributes of the movement are prepared well in advance for efficient execution (Cohen et al., 2010). Our brain processes information from our sensory systems, which originates in multiple reference frames, such as eye-based (visual), head-based (vestibular), joint-based (skin and joint afferent) and muscle-based (muscle afferent) coordinates, and ultimately transforms this information into a set of motor commands (Sabes, 2011). During unilateral movements involving the upper limbs, the primary motor cortex contralateral to the active limb (subsequently referred to as $M1_{contra}$) exerts control over voluntary movements via the ~80 % corticospinal fibres that cross over to the contralateral hemicord at the pyramidal decussation (Kertesz & Geschwind, 1971; Levy, 2013; Siegel & Sapru, 2011). However, there is increasing evidence, gathered from neuroimaging and transcranial magnetic stimulation (TMS) studies that the primary motor cortex ipsilateral to the active limb (subsequently referred to as $M1_{ipsi}$) is also involved during unilateral movement (Bütefisch, et al., 2014; Chiou, et al., 2014; Hinder et al., 2010; Howatson, et al., 2011; Kim et al., 1993; Lee, et al., 2010; McMillan et al., 2006; Verstynen & Ivry, 2011). Yet, it is still unclear whether, and if so in what coordinate frame, the activity in $M1_{ipsi}$ during unilateral movement represents functionally relevant characteristics of the movement executed with the ipsilateral limb, such as the movement direction.

In this study, we examined the representation of movement using non-invasive transcranial magnetic stimulation (TMS) over the left motor cortex during preparation of left hand movements to visual targets. TMS is widely used to assess the neurophysiological changes in M1 excitability. A suprathreshold TMS applied over the M1 representation of hand muscles elicits muscle responses in the contralateral muscles, which can be recorded as motor evoked potentials (MEP) using surface electromyography (EMG). TMS studies of unilateral movement have reliably found that corticospinal excitability in $M1_{contra}$ increases approximately 100 ms preceding voluntary movement (Chen et al., 1998; Hoshiyama et al., 1996; Leocani, et al., 2000; McMillan, et al., 2006; Nikolova et al., 2006;

Sommer et al., 2001; Soto et al., 2010; van Elswijk, et al., 2008). In contrast, the activity in $M1_{ipsi}$ during unilateral movement has received less attention and can either increase or decrease depending on the reaction time paradigm, such as simple vs choice reaction time tasks and pre-cued vs no pre-cued reaction time tasks (Duque et al., 2005; Leocani, et al., 2000; McMillan, et al., 2006).

Leocani et al. (2000) reported that the activity of $M1_{ipsi}$ during unilateral movement is inhibited before movement onset of the active limb when examined with a circular TMS coil over the vertex of the head. TMS responses were evoked simultaneously from $M1_{contra}$ and $M1_{ipsi}$ between 20 ms and 400 ms after presentation of acoustic stimuli during a two-choice thumb reaction time task. Subjects reacted by initiating thumb extension with their left or right thumb depending on the acoustic frequency of the imperative stimulus. There was a tendency for corticospinal excitability for the resting thumb to be inhibited within the final 100 ms prior the upcoming movement of active thumb, although the corticospinal inhibition did not reach a statistically significant difference from baseline. Assuming the reported 'inhibition' in $M1_{ipsi}$ is genuine, it could be the causal effect of stimulating both M1s simultaneously. Inhibitory circuits, such as interhemispheric inhibition and short-interval intracortical inhibition can be activated when both M1s are stimulated simultaneously. Hence the MEP data during unilateral movement for their study must be interpreted prudently as evidence for an inhibition or a reduction of excitability in $M1_{ipsi}$.

During a pre-cued two-choice wrist reaction time task, $M1_{ipsi}$ excitability is influenced by the activation of homologous muscle and the upcoming movement direction in extrinsic space of the active limb (McMillan, et al., 2006). Subjects were pre-cued with one of three target locations for 400 ms before an imperative signal indicating a response which required a flexion or an extension of their left or right wrists. The targets were located in a way that one of the targets required flexion of either wrist, whereas the other two targets required either a flexion in one wrist or extension in another wrist to acquire the targets in different trials. The corticospinal excitability of TMS-evoked wrist movements were measured and pooled into four arbitrary time bins in relation to the timing of movement onset, i.e. early (about 250 ms before EMG onset), middle (about 170 ms before EMG onset), late (about 85 ms before EMG onset) motor preparation stages and post movement (where TMS was presented following EMG onset). The authors reported that the $M1_{ipsi}$ excitability of projections to the passive flexor muscle increased during motor preparation when the pre-cued target was located between the two hands, and therefore would require a wrist flexion irrespective of which wrist was used to acquire it. When the pre-cued target required either a flexion with one hand or an extension with the other hand, the M1$_{ipsi}$ excitability of flexor and extensor muscles increased based on the direction response of the opposite active wrist. For example, the pre-cued target that was located to the right of the wrists would require an extension with the right wrist and a flexion with the left wrist to acquire it. However, because the target location was cued before the imperative signal that specified whether the left or right limb should be used, both M1s had reason to prepare a movement before the imperative signal. Therefore the M1$_{ipsi}$ excitability probed after imperative signal could possibly reflect the residual excitability from the motor preparation rather than the effects of unilateral movement on M1$_{ipsi}$ based on activation of homologous muscle or movement direction of the opposite active limb defined in space.

Another possible factor that might affect M1$_{ipsi}$ excitability during unilateral movement is a midline effect. Duque et al. (2005) measured MEPs from the resting left first dorsal interosseous (FDI) muscle in a pronated hand position while the right hand performed index finger adduction and abduction in three different hand positions (pronated, semi-pronated and supinated). The movement directions of finger adduction and abduction in these three hand positions were either mirrored (in pronated position) or non-mirrored (in semi-pronated and supinated positions). The M1$_{ipsi}$ excitability of projections to the left FDI recorded at about 70 ms before right finger movement onset decreased significantly from baseline when the movement direction of the active (right) index finger mirrored the muscle pulling direction of left FDI, irrespective which muscle was the prime mover (i.e. in the different postures). That is, movements directed towards the midline, in which direction the probed muscle in the passive limb pulled, resulted in reduced excitability of projections to the midline-corresponding passive muscle, regardless of the muscles engaged in the task with the active limb. However, these results also imply that the M1$_{ipsi}$ excitability of the left FDI increased from baseline when the movement direction of the active (right) index finger mimicked the same muscle pulling direction of left FDI defined in extrinsic space, i.e. away from the midline, irrespective of the right hand postures. Therefore it is not clear based on the inhibitory interactions in midline coordinates and excitatory interactions in extrinsic coordinates, whether the results represent midline or extrinsic-based movement representations in the M1$_{ipsi}$.

Taken together, M1$_{ipsi}$ excitability appears to be influenced by movement kinematics of the active limb, possibly through interhemispheric interactions between the two M1s via transcallosal pathways (Bütefisch, et al., 2014; Chiou et al., 2013; Chiou, et al., 2014). Although MEPs are the standard measurement of the corticospinal excitability used in the field, they may not provide comprehensive information to draw conclusions about the representation of movement direction in $M1_{ipsi}$ during unilateral movement. This is because measurement from a single muscle might not reflect the net muscular effect of TMS during unilateral movement. Information about the activity of a single muscle is not sufficient to identify the direction of evoked limb motion, because movement is generally brought about by coordination between multiple muscles. Furthermore, suprathreshold TMS typically activates several muscles in the upper limb, not only the ones for which MEPs are measured. One approach might be to record from all muscles acting at a relevant joint; however, simultaneous recording of MEPs from all targeted muscles is difficult due to the complex anatomical dispositions of muscles. Therefore, an alternative approach is to record the muscle forces evoked by TMS because directional information is contained within the pattern of excitability changes between muscles. TMS-evoked twitch direction at a joint reflects the vectorial summation of force vectors from all the muscles recruited by TMS. Using this method, we are able to assess the modulation of TMS-evoked force outputs (magnitude and direction) of $M1_{ipsi}$ during motor preparation of unilateral movement.

TMS-evoked twitch directions in the active limb have been reported to transiently shift away from the baseline twitch direction preceding the onset of voluntary thumb movements during reaction time tasks (Sommer, et al., 2001; van Elswijk, et al., 2008). There was an increase in the proportion of TMS-evoked twitches shifting away from the baseline twitch direction at about 100ms before movement onset. These studies suggested that TMS-evoked twitch directions reflect the functionally specific changes in corticospinal excitability of projections to the active limb during movement preparation. Therefore TMS-evoked twitch directions could be used to examine the directional-specific $M1_{ipsi}$ excitability changes which have not been characterised when movement direction with the opposite limb is uncertain prior to an imperative stimulus, i.e. target direction is not specified prior to an imperative stimulus.

The aim of the current study was to investigate whether preparation of movement toward horizontal targets changes TMS-evoked twitch directions in opposite inactive limb. Due to the mismatch of reference frames between limbs (refer to Section 2.2.5 and Figure 2.2 for more details), we examined whether a change of the twitch directions in the inactive limb was reflected in extrinsic or muscle-based directions. As these horizontal movements were systematically related to the body midline, putative muscle-based effects could not be dissociated from potential midline effects. In our experimental paradigm, participants could not predict the direction of movement to make until the onset of a visual cue.

Therefore, TMS delivered during motor preparation enabled us to study the movement representation in $M1_{ipsi}$ at different processing stages of movement generation.

2.2 Methods

Twelve right-handed participants (11 males and 1 female; aged between 20 and 37 years old) with no recent wrist, elbow or shoulder injuries volunteered for the study. Right-handedness was confirmed with Edinburgh Handedness Inventory (Oldfield, 1971). A medical questionnaire was used to screen the participants for neurological disorders and contraindications in relation to the application of TMS. The study was approved by the Medical Research Ethics Committee of The University of Queensland. All participants were briefed on the experimental procedures and gave written informed consent prior to the experiment which conformed to the Declaration of Helsinki.

2.2.1 Experimental protocol

In this cross-sectional study, the participants were required to attend a single 2 hours experimental session (including preparation time) in the laboratory. We aimed to assess whether preparation of left wrist forces toward horizontal targets would shift the twitch directions of the right wrist toward the direction of action of the homologous muscles (and/or body midline), or toward the direction of opposite limb movement defined in extrinsic space (Figure 2.1). Having an initial twitch direction which is evoked in a near vertical direction from the right wrist enables us to subsequently dissociate extrinsic and muscle reference frames based on the direction of twitches that has shifted during motor preparation toward horizontal targets. For example, when a left wrist movement is planned toward the right horizontal target, a leftward shift of twitch directions from the right wrist would imply $M1_{ipsi}$ activities represent movement direction in muscle reference frame and toward midline. However, a rightward shift of twitches from right wrist would imply that $M1_{ipsi}$ activities represent movement direction in extrinsic space.

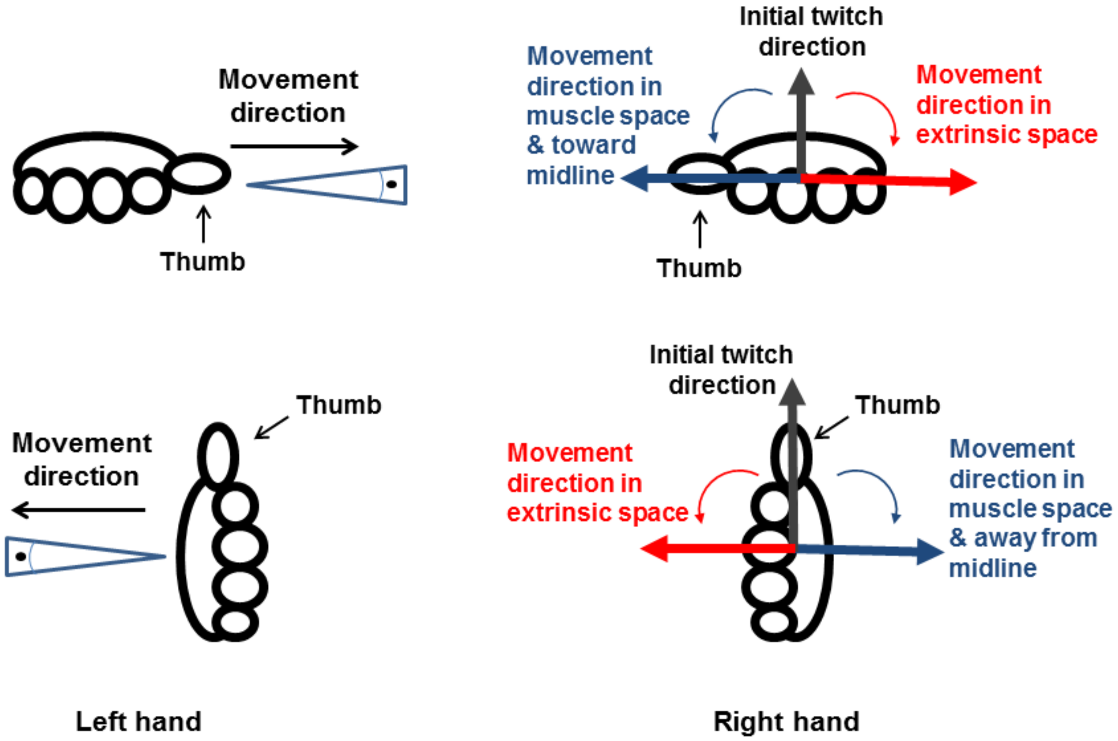


Figure 2.1. Schematic representation of the TMS-evoked twitch directions from right resting wrist during motor preparation of the left wrist toward horizontal targets in pronated and neutral hand positions. The figures on the left illustrate pronated and neutral hand positions with movement direction toward a horizontal target, i.e. 0° and 180° targets respectively. The right figures illustrate the possible reference frames where the twitch directions would shift during movement preparation of the left wrist.

Prior to the beginning of experiment, the participants completed two blocks (48 trials) of the choice reaction time task as familiarisation. In one of the familiarisation blocks, TMS was delivered at target appearance to one of the horizontal targets (0° or 180°, Figure 2.2a) to check if twitch directions varied from the directions previously assessed. Each individual’s average reaction time was estimated from the familiarisation trials to define the TMS time points to be delivered in the experiment.

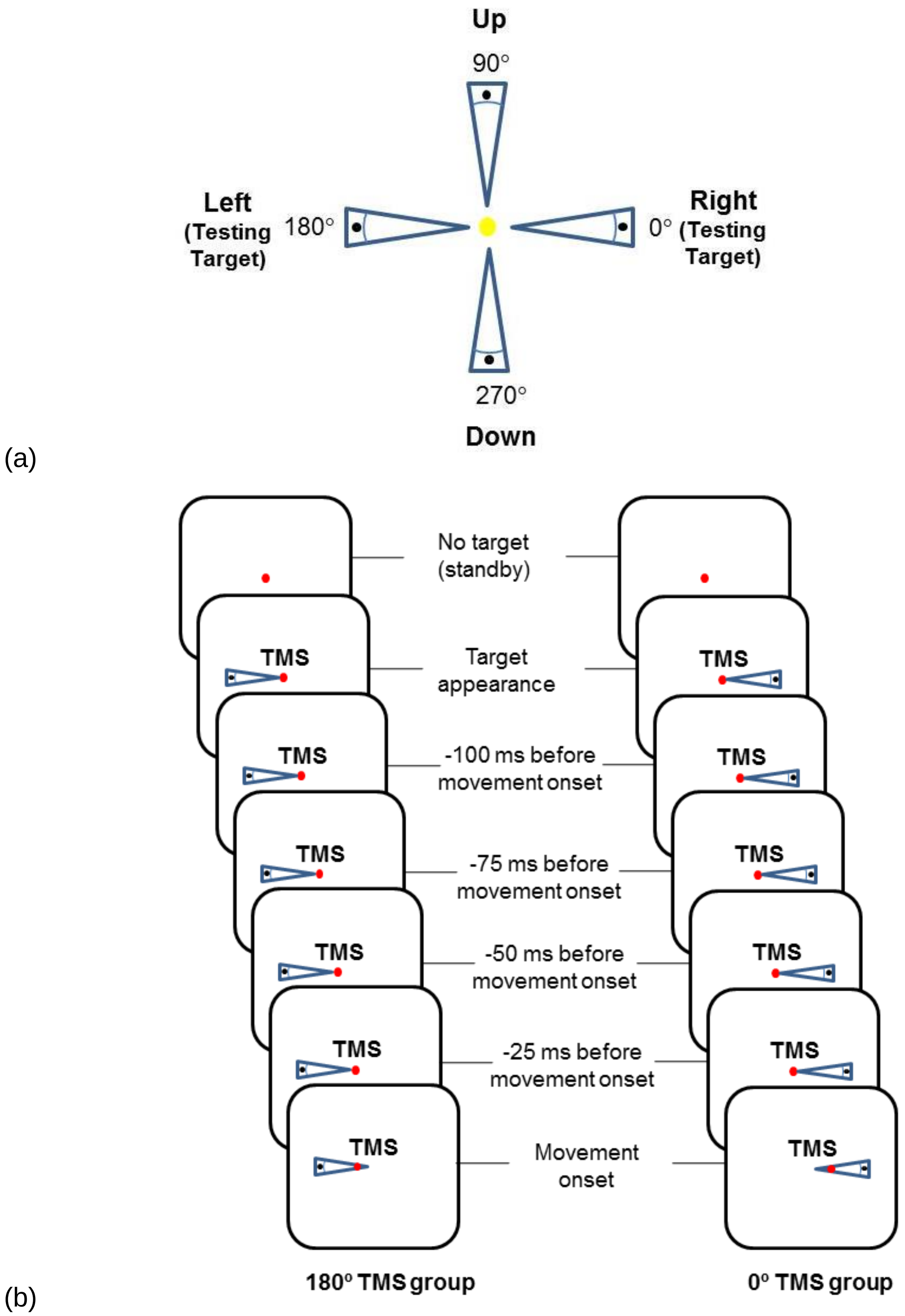


Figure 2.2. Experimental protocol. (a) Four alternatives choice reaction time task. The targets appeared in a randomised order and participants made quick isometric movements upon target appearance with their left hand. (b) An example illustrating the six time points where TMS was delivered during the trials. The time points before movement onset were estimated time points calculated based on each individual’s reaction time during familiarisation trials and adjusted according during the main experiment.

Each trial began with a circular warning sign displayed at the centre of the computer screen for between 1 s and 2 s before target appearance. Upon target appearance, participants were required to respond as fast as they could to acquire the target and relax in time for the next target with an inter-trial interval of 2 s. Each target appeared in a random order in every cycle of four trials to avoid anticipation. Each participant completed 8 blocks of 72 trials (4 targets x 18 trials each target) of the choice reaction task for the entire experiment. TMS was delivered for the 0° target presentation in four blocks of trials and for the 180° target in another four blocks of trials, i.e. 144 TMS trials for the experiment. TMS was delivered to the left motor cortex at target appearance, before predicted movement onset time (-100 ms, -75 ms, -50 ms and -25 ms from the estimated reaction time) and at movement onset (Figure 2.2b). The time points before movement onset were estimated from each individual's average reaction time from the familiarisation trials. Stimuli at movement onset were triggered by the onset of EMG activity in the relevant prime movers toward the horizontal targets. The average reaction time was monitored after each block of trials and adjusted for the subsequent block of trials to ensure that sufficient TMS trials were recorded throughout the desired movement preparation period prior to movement onset.

2.2.2 Experimental setup

Participants sat in front of a computer screen located approximately 1.2 m away at eye level (Figure 2.3). Left and right forearms were secured into a custom-made hand manipulandum, described previously (de Rugy, et al., 2012), which allowed passive rotation of wrist between neutral (midway between pronation and supination). Both elbows were kept at 110° with the forearm parallel to the table and supported by the manipulandum. The wrists were fixed by a series of twelve adjustable metal clamps contoured around the metacarpal-phalangeal joints and around the wrist proximal to the radial head. Wrist forces in radial-ulnar deviation and flexion-extension directions were recorded via a six degree-of-freedom force transducer (JR3 45E15A-163-A400N60S, Woodland, CA) attached to each manipulandum. Force data were sampled at a rate of 2 kHz via two 16-bit National Instruments A/D boards (NI BNC2090A, NI USB6221, National Instruments Corporation, USA). The online forces exerted in flexion-extension and abduction-adduction directions were displayed as a cursor in two dimensional space (x = flexion-extension, y = abduction-adduction) on the computer screen via a custom written Labview program (LabView2009, National Instrument, USA). The timing of the TMS pulses were also synchronised by the program.

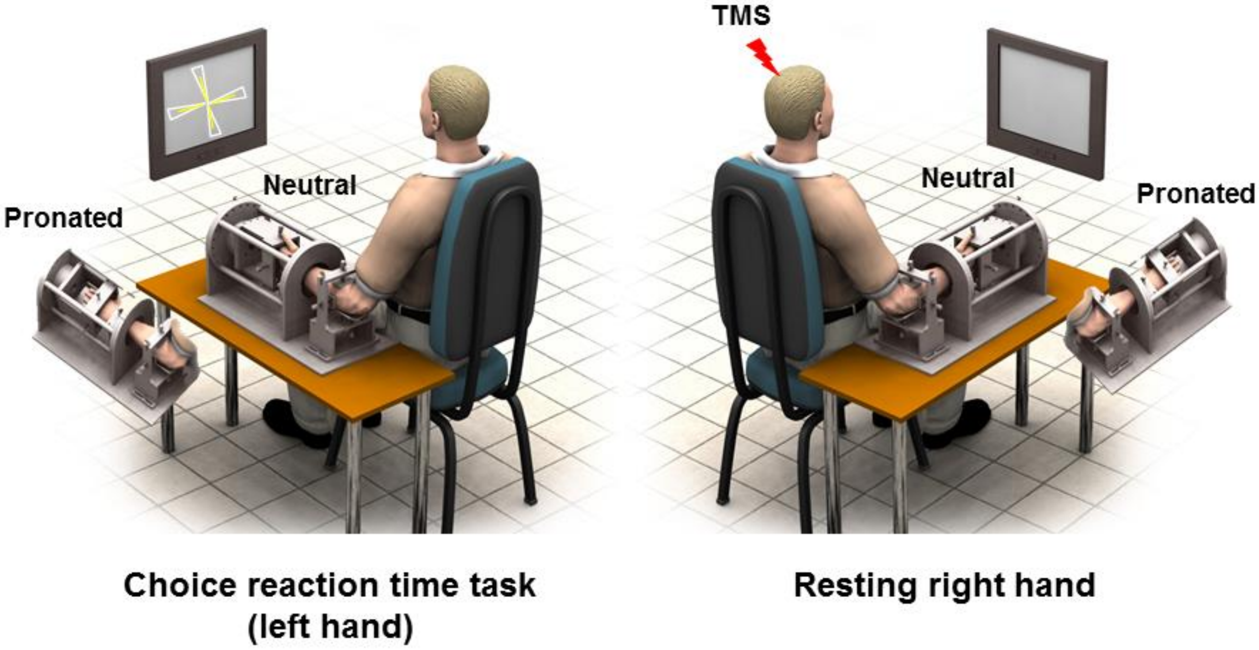


Figure 2.3. Experimental setup. Left figure shows participants making movements with their left wrist toward one of four targets in a choice reaction time task. Right figure shows TMS is applied the left motor cortex area to evoke twitches from the right wrist at rest, during movement preparation and movement onset of the left wrist.

2.2.3 Transcranial magnetic stimulation

Single-pulse TMS was delivered via a 70 mm diameter figure-of-eight magnetic coil (Magstim 200, Magstim, UK) over the forearm area of the left motor cortex. The magnetic coil was held tangentially on the scalp with the handle pointing backwards and 45° away from mid-sagittal axis. The coil was moved to locate a hotspot whereby the strongest and most consistent twitches elicited by TMS were identified online for each participant. The hotspot location for each participant was marked on the scalp to ensure the consistent coil placement throughout the experiment. The testing intensity was selected to elicit a resting muscle twitch of resultant magnitude between 0.5 N to 1 N. A twitch magnitude of a minimum 0.5 N is required to differentiate any noise from the actual twitch elicited by TMS. Participant’s hand position was readjusted to pronated or neutral posture, if necessary, to obtain twitch directions in a near vertical orientation. In our unpublished data (refer to Chapter 4), we have shown that the direction of TMS-evoked twitches follows the muscles when the wrist is rotated between pronated and neutral positions. For example, if the twitches evoked from the right wrist in the neutral position are oriented horizontally toward the right, then the direction of twitches will point upwards when the wrist is rotated to a pronated position. Therefore in this study, we could ensure individual participant’s twitch directions at baseline were toward a near vertical orientation by simply repositioning their hand in pronated or neutral position prior to the start of the experiment.

2.2.4 Surface electromyography recordings

Electromyography (EMG) signals were recorded from flexor carpi radialis (FCR), flexor carpi ulnaris (FCU), extensor carpi radialis brevis (ECR_b) and extensor carpi ulnaris (ECU) muscles of the both arms. Standard skin preparation was performed after the muscles were located and marked. Bipolar Ag/AgCl surface electrodes placed on the belly of the forearm muscles with an inter-electrode distance of 2 cm (centre to centre). The EMG signals were amplified with a gain of 500 ~ 1000 with Grass P511 amplifiers (Grass Instruments, AstroMed, West Warwick, RI) and band-pass filtered (10 Hz - 1 kHz).

2.2.5 Choice reaction time task

Participants were instructed to react as fast as possible in a four alternative choice reaction time task with their left hand. They were required to make a quick isometric contraction toward one of four targets which appeared in random order along the cardinal axes, i.e. 0°, 90°, 180°, 270° (Figure 2.2a). The targets appeared as a 10° wide wedge-shaped stimulus that extended to 75 % of the distance from the origin to the edge of the computer screen (10 cm). The cursor gain was set such that 20 N was required to reach the edge of the screen. Participants had to move the cursor from the origin toward the target within a movement time of 150 ms to 250 ms, with movement time defined as the time taken for the cursor to move from 10 % to 90 % of the target distance. A successful acquisition of target was cued with two high-pitched tones (500 ms, 800 Hz sinusoid) after the cursor remained within 10 % target radius from the centre of target for 10 ms.

2.2.6 Data analysis

Previous literature shows that corticospinal excitability in the $M1_{contra}$ gradually increases from approximately 100 ms before the onset of movement (Chen, et al., 1998; Leocani, et al., 2000). Therefore, the data in this chapter were analysed surrounding this time frame.

TMS-evoked twitch angles, twitch magnitudes, motor evoked potential (MEP) amplitudes and reaction times were recorded on disk and further analysed offline via a custom-written Matlab program (Mathworks, Natick, USA). Individual force traces were inspected visually and those with possible postural movements were removed manually. For example, the onset of a twitch following an MEP onset is between 20 ms and 40 ms. Therefore, any waveforms occurring before or after this time range were removed from subsequent analysis (13 % of TMS trials). All data were presented in mean ± SEM, unless stated otherwise. Statistical significance was set at the 0.05 level.

Twitch responses

Twitch angles and twitch magnitudes for each participant were defined as the resultant vectors calculated from the peak value of individual x and y force traces. Twitch angles and twitch magnitudes from each participant were normalised to the values obtained following stimulation at the time of target appearance (referred to as baseline) by subtracting the mean values from each trial. The differences in twitch angles from baseline determined the reference frame in which the twitches shifted during the trials. Positive deviations of twitch angle were defined as shifts toward the movement direction defined in muscle space whereas negative angle deviations were defined as shifts toward the movement direction defined in extrinsic space. The midline effects happened to be also consistent with the muscle-based effects in this case (refer to Figure 2.2), the direction of the twitch angle for each target condition were illustrated in Figure 2.4. The twitch data for both target conditions in each subject were pooled and grouped into bins of 20 ms width in relation to the movement onset for further analysis. One-way repeated measures ANOVAs were used to determine the effect of time on the twitch angles and twitch magnitudes for the pooled data. For each ANOVA, post hoc t-tests versus 0 for each time bin were performed to determine whether there were significant differences for the twitch angles and twitch magnitudes leading to post movement onset.

MEP responses

The peak-to-peak MEP amplitudes were calculated for each muscle in both target conditions and normalised to baseline by subtracting the mean baseline value from each trial. Subsequently, the normalised MEP data were pooled according to pronated (n = 9) and neutral (n = 3) hand positions by participants. The MEP data were grouped into bins of 40 ms width in relation to movement onset to ensure sufficient trials were obtained for each muscle and hand posture in each participant. The rationale behind analysing MEP amplitudes according to wrist postures was due to the prime muscles that elicit vertical twitch direction were different for the pronated and neutral hand positions. The onset of movement was defined as the EMG activity exceeds triple standard deviation range of EMG activity during rest (Konrad, 2005).

One-way repeated measures ANOVAs were used to determine the effect of time on the MEP amplitudes for each muscle in pronated hand position. Fisher’s least significant difference post hoc tests were performed for each ANOVA to determine significant changes in MEP amplitudes with the earliest motor preparation time point, i.e. ‘> -120 ms’ time bin. Statistical analysis was not performed for the MEP data in neutral hand position due to the violation of assumptions for the ANOVA with n = 3. However, the MEP amplitudes were plotted with mean ± 95 % confident intervals for references (refer to Figure 2.8).

Reaction time

Reaction time was defined as the interval between target appearance and the EMG onset of the agonist muscles of the responding hand (left wrist). It has been reported that TMS shortens reaction time when delivered during early motor preparation and delays reaction time when delivered during late motor preparation (Ziemann et al., 1997). Thus, reaction times with TMS from both target conditions were compared between the six time points, i.e. based on the time from stimulus presentation to the estimated reaction time, at target appearance, before movement onset (-100 ms, -75 ms, -50 ms and -25 ms) and at movement onset using 2 x 6 ANOVA (target conditions x time) to determine the effect of the TMS on reaction time. If reaction time was not significant across time points, the mean reaction time were compared between trials with and without TMS for the 0° and 180° target conditions. A 2 x 2 ANOVA (TMS trial x target conditions) was used to determine the main effect of TMS on reaction time between target conditions. Fisher’s least significant difference post hoc test was applied to determine the effect of TMS on reaction time between stimulation and non-stimulation trials for each target.

2.3 Results

2.3.1 Twitch angles of resting right wrist

Twitch angles for 0° and 180° target conditions

The twitch angles fluctuated from baseline for all stimuli delivered earlier than 20 ms before movement onset for the 0° and 180° target conditions. During late motor preparation, the twitch angles in both target conditions showed similar trend of twitch angles shifted toward the movement direction defined in muscle and midline space, i.e. stimuli delivered at 20 ms before movement onset (Figure 2.4).

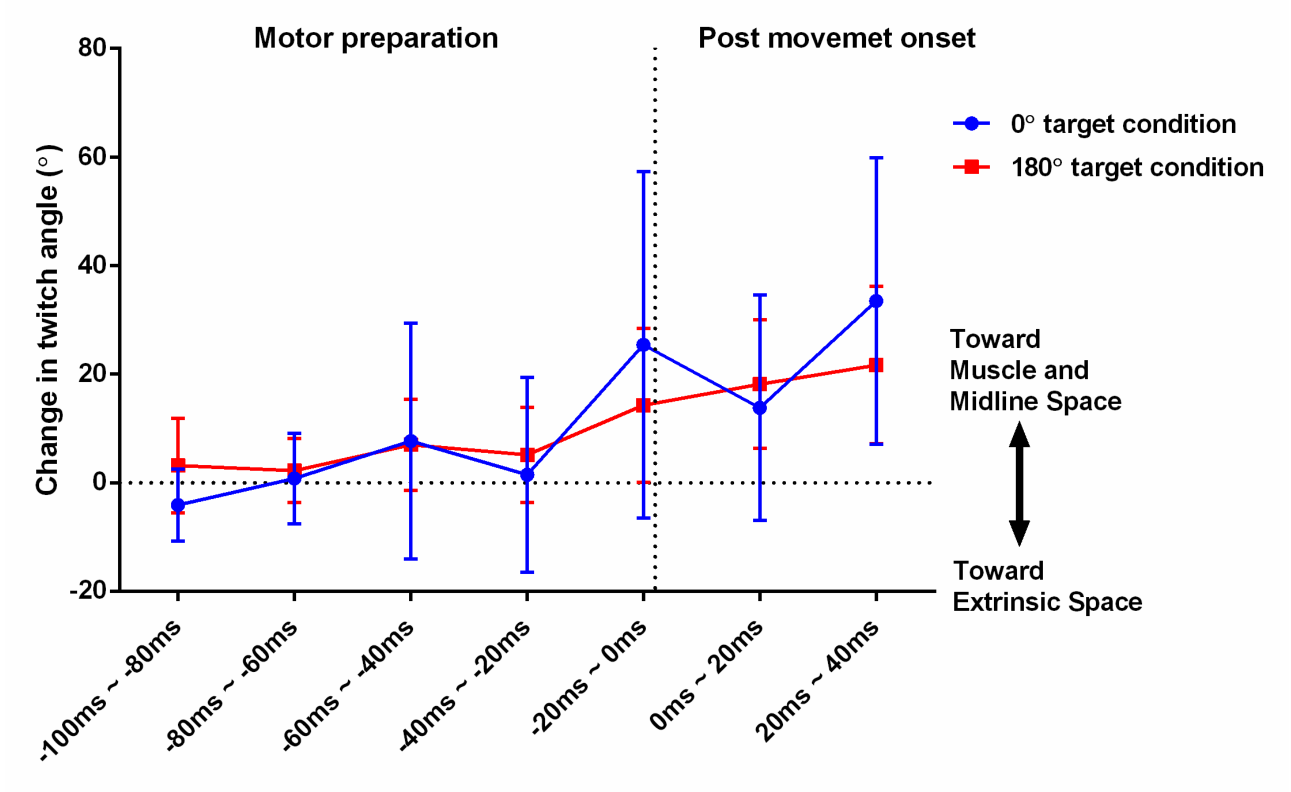


Figure 2.4. Change in twitch angles for the 0° and 180° target conditions. Blue line depicts change in twitch angles for the 0° target condition. Red line depicts change in twitch angles for the 180° target condition. Both target conditions showed similar trend of twitch angles shifted toward the movement direction defined in muscle and midline space during late motor preparation, i.e. from -20ms prior movement onset. Vertical dotted line depicts the movement onset of the active left wrist. Horizontal dotted line denotes the baseline. Data was presented in mean ± 95 % confident interval.

Pooled twitch angle data

The timing of TMS delivery had a significant effect on the pooled twitch angles during motor preparation and after movement onset ($F_{(6,66)} = 5.46$, $p = 0.0001$, Figure 2.5). Twitch angles remained fluctuated from baseline for all stimuli delivered earlier than 20 ms before movement onset, but were not statistically significant from baseline ($p > 0.05$). During late motor preparation, the twitch angles shifted 17 ± 7.5° from baseline toward the pulling direction of the homologous muscles responsible for force generation in the active left wrist, and the movement direction relative to the midline, i.e. stimuli delivered at 20 ms before movement onset ($p = 0.045$). The direction of twitch angles remained significantly toward muscle space and the midline direction after movement onset up to 40 ms post movement ($p < 0.05$ for all the time points after movement onset).

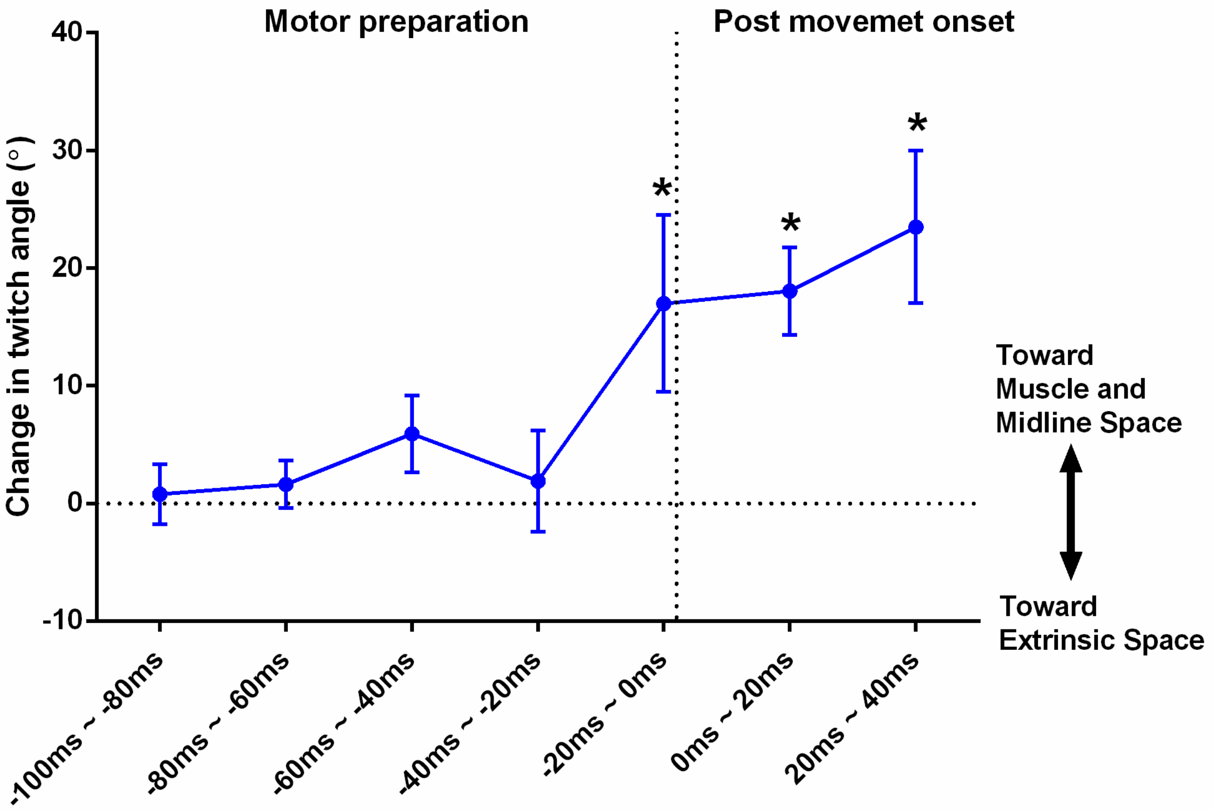


Figure 2.5. Change in pooled twitch angles of the resting right wrist during motor preparation and post movement onset. Positive twitch angle changes depict that twitch direction shifted toward the opposite limb movement direction defined in muscle and midline space. Vertical dotted line depicts the movement onset of the active left wrist. Horizontal dotted line denotes the baseline. Symbol '*' depicts significant difference from baseline ($p < 0.05$).

2.3.2 Twitch magnitudes of resting right wrist

Twitch magnitudes fluctuated from baseline during motor preparation, but were not statistically significant from baseline ($p > 0.05$, Figure 2.6). One-way repeated measures ANOVA showed a significant effect of time on twitch magnitudes ($F_{(6,66)} = 6.41$, $p < 0.001$). Twitch magnitudes significantly increased 20 ms after movement onset of the left active wrist (0.27 ± 0.08 N increase from baseline, $p = 0.004$), and remained elevated until 40 ms post movement onset.

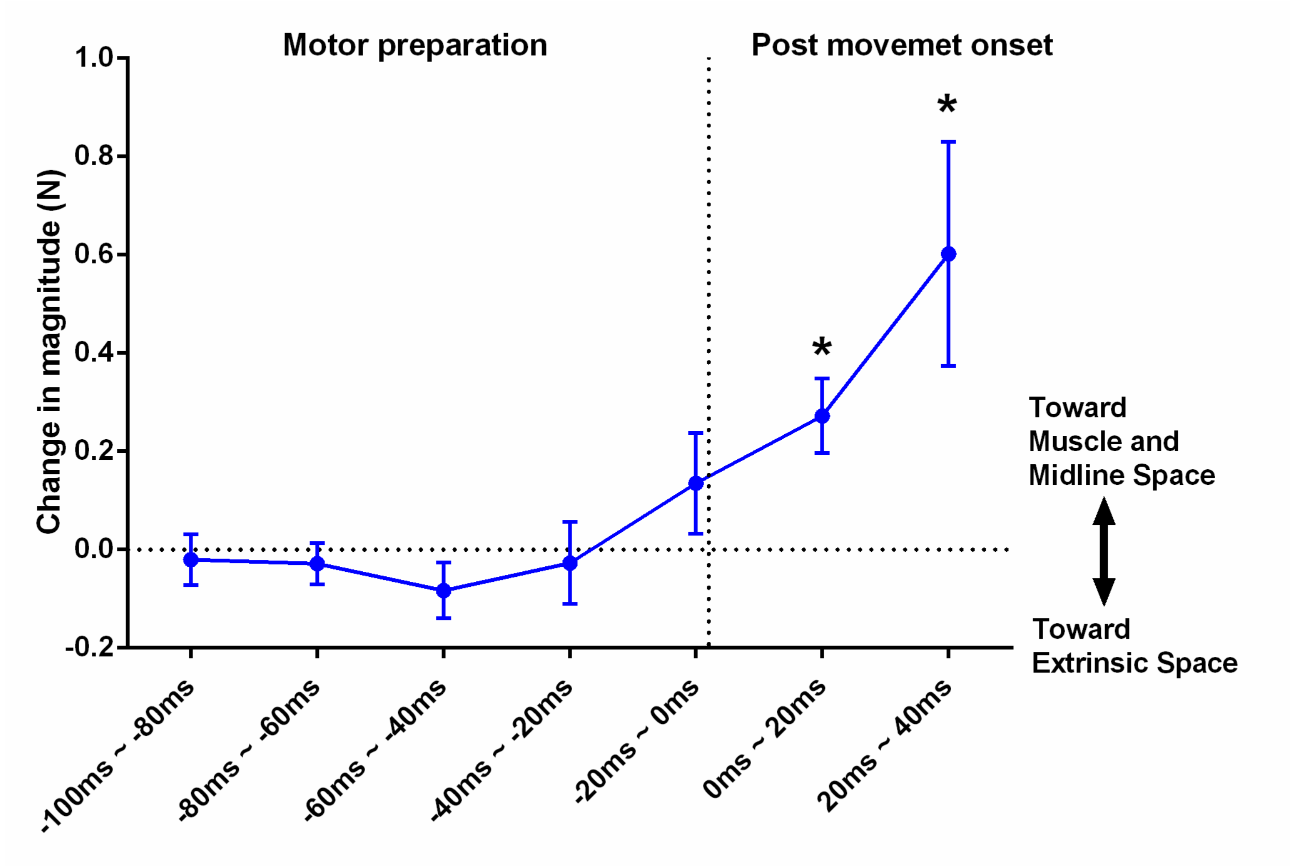


Figure 2.6. Change in twitch magnitudes of the resting right wrist during motor preparation and post movement onset. The twitch magnitude increased during late motor preparation. Vertical dotted line depicts the movement onset of the active left wrist. Horizontal dotted line denotes the baseline. Symbol '*' depicts significant difference from baseline ($p < 0.05$).

2.3.3 MEP amplitudes of resting right wrist

Pronated hand posture

The MEP amplitudes for all muscles in both target conditions remained closed to baseline during early motor preparation, i.e. stimuli delivered > -120ms, and gradually increased from about 100ms before the movement onset. There was a greater increase in the MEP amplitude for the homologous agonist muscles to the active limb for each target condition (Figure 2.7).

Two-way repeated measures ANOVAs revealed a significant main effect of time on the MEP amplitudes for all the muscles in both target conditions, i.e. ECU ($F_{(4,28)} = 22.59$, $p < 0.001$), ECR ($F_{(4,28)} = 16.97$, $p < 0.001$), FCU ($F_{(4,28)} = 20.05$, $p < 0.001$) and FCR ($F_{(4,28)} = 8.19$, $p < 0.001$). The changes in MEP amplitudes were significantly different from baseline from 80ms before movement onset (refer to Table 2.1 for all significant time points).

Table 2.1. Time points where MEP amplitude changes were significantly greater than the mean value at '> -120 ms' in pronated hand position. Homologous agonist muscles to the active limb for the 0° and 180° target conditions are highlighted with pink and blue shading respectively.

Pronated Hand Position (inactive limb)								
Time points	**ECU**		**ECR**		**FCU**		**FCR**	
	0° TMS	**180° TMS**	**0° TMS**	**180° TMS**	**0° TMS**	**180° TMS**	**0° TMS**	**180° TMS**
> -120ms	-	-	-	-	-	-	-	-
-120ms ~ -80ms	-	-	-	-	-	-	-	-
-80ms ~ -40ms	-	p < 0.001	p = 0.04	-	-	p < 0.001	p = 0.005	-
-40ms ~ 0ms	-	p < 0.001	p < 0.001	p = 0.04	-	p < 0.001	p < 0.001	p = 0.001
> 0ms	p = 0.01	p < 0.001	p < 0.001	p = 0.03	-	p < 0.001	p < 0.001	p < 0.001

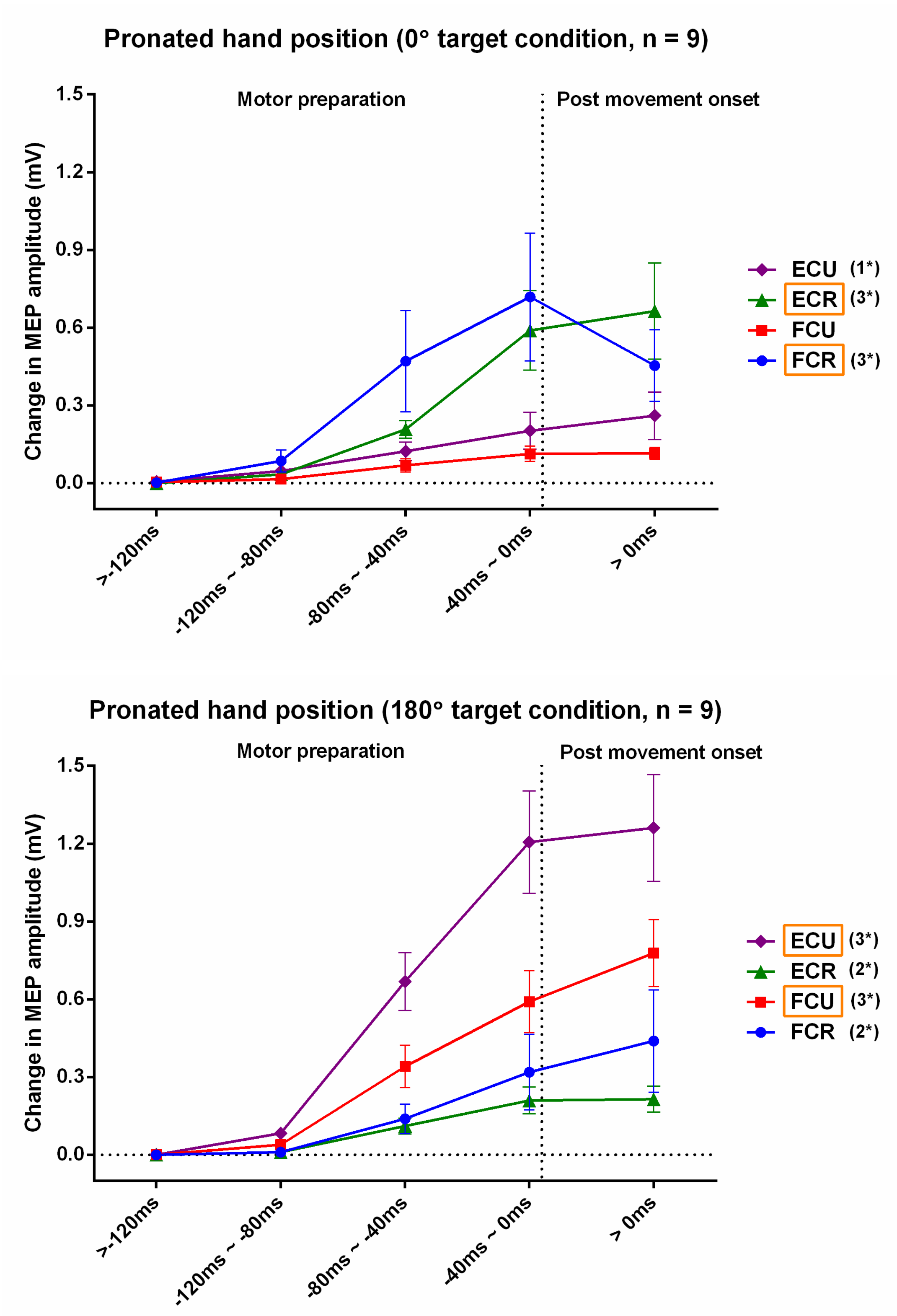
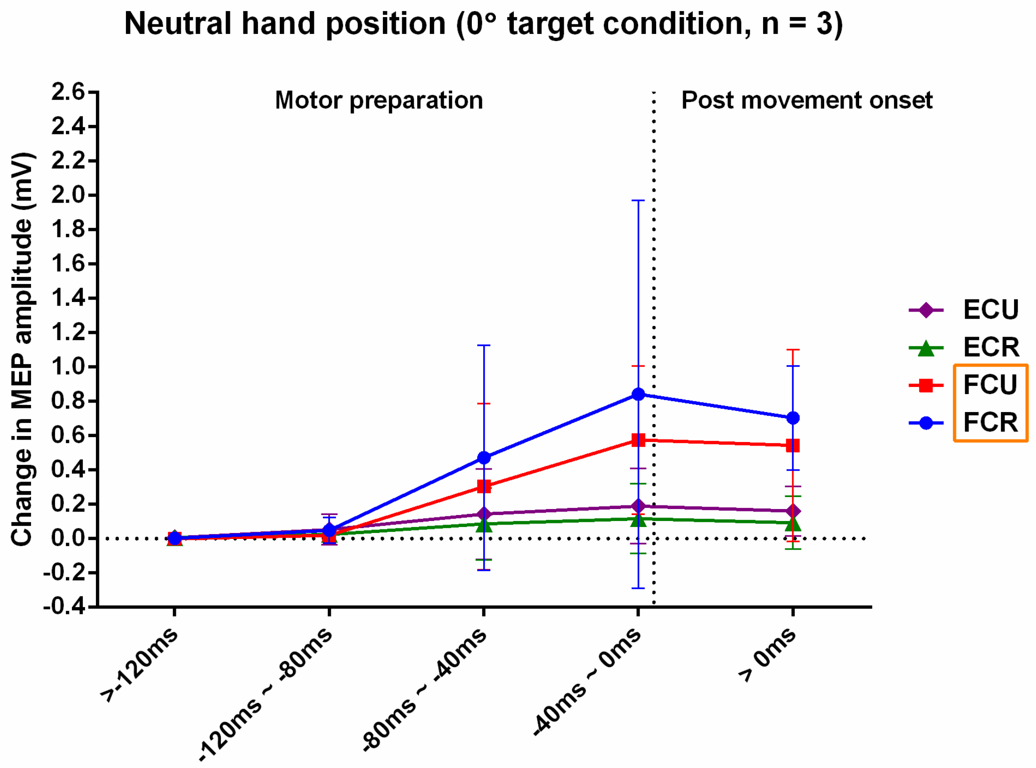


Figure 2.7. Change in MEP amplitudes of the resting right wrist during motor preparation and post movement onset in each muscle for the 0° and 180° target conditions in pronated hand position. Vertical dotted line depicts the movement onset of the active left wrist. Horizontal dotted line denotes the baseline. Homologous agonist muscles to the active left wrist toward the movement direction were highlighted with orange boxes in the legend. The number of ‘*’ besides each muscle depicts the number of time points significantly different from the mean value at ‘> -120 ms’ ($p < 0.05$).

Neutral hand posture

Three of twelve participants completed the study with their wrists in neutral position, thus fewer TMS observations were collected as compared to those in pronated hand position (about 200 observations for each target condition in neutral hand position vs about 600 observations for each target condition in pronated position). Hence, the changes in MEP amplitudes observed for neutral hand position should be interpreted cautiously. Statistical analysis was not performed for the MEP data in neutral hand position due to the violation of assumptions for the ANOVA with $n = 3$. However, the MEP amplitudes were plotted with mean ± 95 % confident intervals for references (Figure 2.8).

Similarly, the MEP amplitudes for the homologous agonist muscles to the active limb for both 0° and 180° target conditions increased from around 80ms prior to movement onset and remained elevated after movement onset. The MEP amplitudes for the homologous antagonist muscles remained closed to baseline.



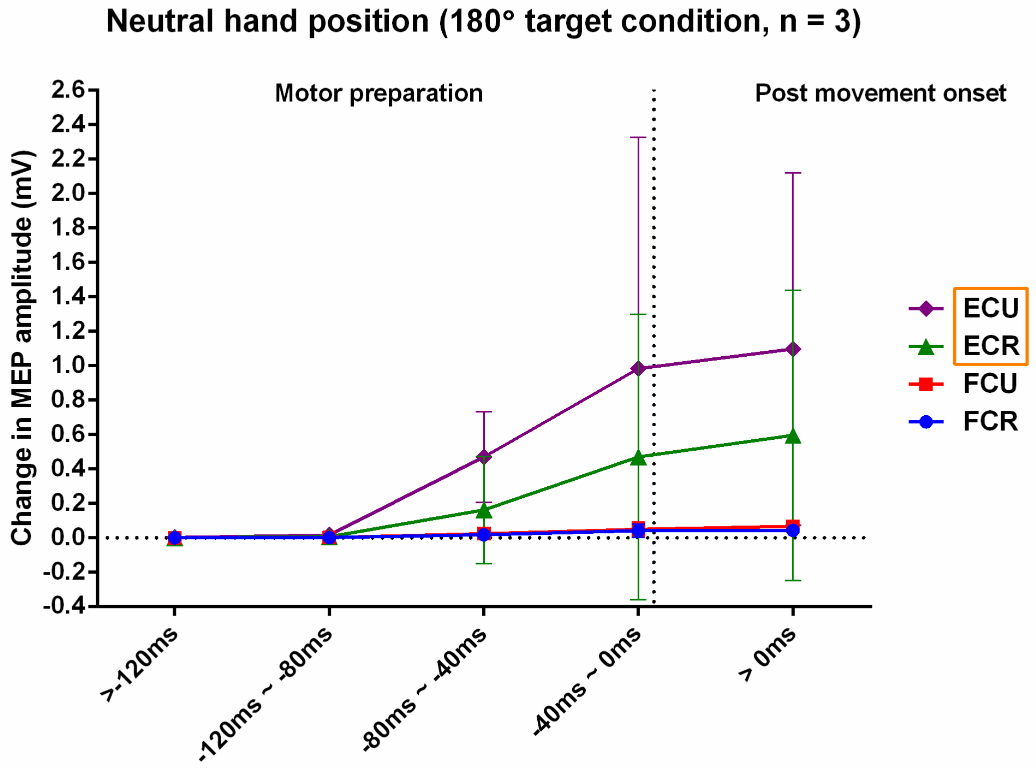


Figure 2.8. Change in MEP amplitudes of the resting right wrist during motor preparation and after movement onset for each muscle of the 0° and 180° target conditions in neutral hand position. Vertical dotted line depicts the movement onset of the active left wrist. Homologous agonist muscles to the active left wrist toward the movement direction were highlighted with orange boxes in the legend. No statistics performed, data presented in mean ± 95 % confident interval.

2.3.4 Reaction time of active left wrist

Reaction time was similar when TMS was delivered at various time points from target appearance to movement onset. TMS had no significant effects on the reaction time for both target conditions (Figure 2.9, $F_{(5,55)} = 1.72$, $p = 0.15$).

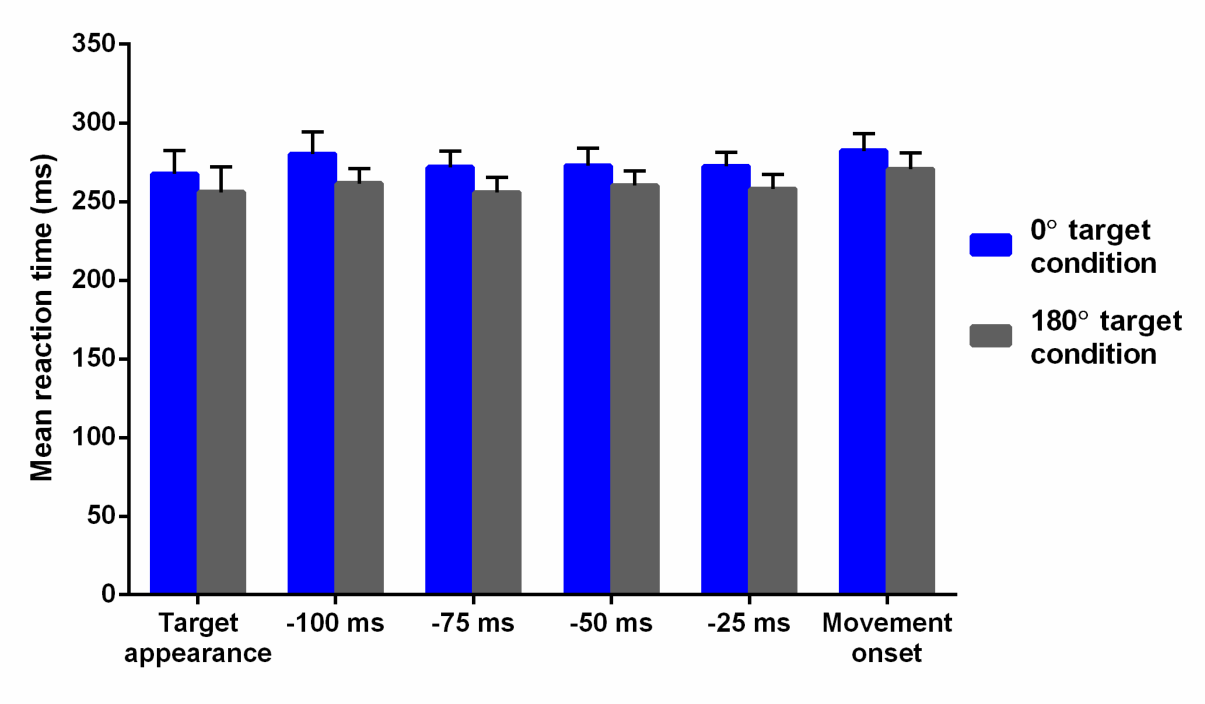


Figure 2.9. Mean reaction time for trials with TMS at target appearance, before movement onset (-100 ms, -75 ms, -50 ms and -25 ms) and at movement onset for the 0° and 180° target conditions.

The mean reaction time was significantly shorter on the TMS trials than non-TMS trials in both target conditions (Figure 2.10, $F_{(1,11)} = 8.1$, $p = 0.02$). For both movement directions targeted by TMS, reaction time was approximately 10ms shorter in stimulation trials than non-stimulation trials.

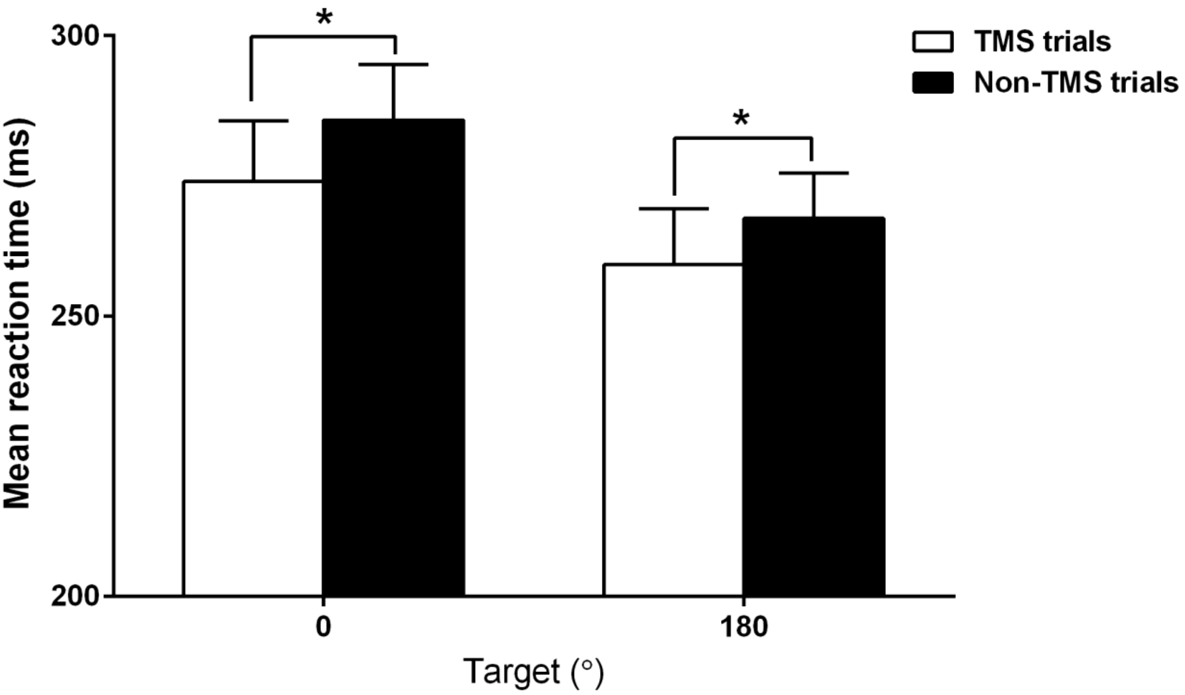


Figure 2.10. Mean reaction time for individual targets in the 0° and 180° target conditions. Symbol '*' depicts significant difference in reaction time between TMS (unfilled) and non-TMS (filled) trials ($p < 0.05$).

2.4 Discussion

The present study demonstrated that the corticospinal excitability of the resting right hand was modulated during motor preparation toward horizontal targets with the left hand in a non-precue choice reaction time task. Twitch directions, twitch magnitudes and MEP amplitudes remained unchanged during early motor preparation. The MEP amplitudes gradually increased from about 80 ms before EMG onset. Directionally specific information was only available during late motor preparation, i.e. from approximately from 20 ms before EMG onset and was represented according to muscle and/or midline reference frames.

During early preparation, little modulation of corticospinal excitability was shown for TMS delivered between target appearance and 100 ms preceding movement onset in the resting right hand. A large body of literature shows that corticospinal excitability for the agonist muscles of the responding hand increases only at approximately 100 ms prior to movement (Chen, et al., 1998; Evarts, 2011; Leocani, et al., 2000; Nikolova, et al., 2006).

Reaction time studies with TMS delivered between 400 ms and 100 ms prior to movement onset reported that twitch directions and MEP amplitudes remained close to baseline for both active and resting hands (Leocani, et al., 2000; McMillan, et al., 2006; Sommer, et al., 2001; van Elswijk, et al., 2008). Therefore, our results during early preparation of movement are consistent with the previous studies, whereby corticospinal excitability in the resting hand is modulated in the similar way with the active hand during early preparation, i.e 100 ms prior movement onset.

There was a general increase in MEP amplitudes for all of the muscles of the resting right hand during motor preparation, i.e. from 80 ms before movement onset, in both target conditions. Preliminary data analysis revealed that the direction of twitches shifted toward muscle space for both 0° and 180° target conditions, with a greater shift of twitch direction for the 0° target condition; suggesting corticospinal excitability might be influenced by the midline effects. A sharp deviation of the pooled twitch direction toward the impending contralateral movement defined according to muscle and/or midline reference frames at about 20 ms before movement onset in the right resting hand, suggesting that the MEP changes reflect directionally specific processing. Note that the latency difference between force and MEP changes is broadly consistent with the electromechanical delay in our setup (~30 ms). Thus, it appears that directional information only becomes available to the motor cortex ipsilateral to the impending movement at a very late stage of motor preparation under our choice reaction time conditions. While TMS elicits MEPs in both agonists and antagonists of the forearm simultaneously, the twitch direction provides a measure of the net excitability for the entire forearm. The shift of twitch direction thus reflects the selection of a particular set of muscles to prepare to move the arm in a given direction. However, the modulation in MEP amplitudes and twitch direction occurred prior to the muscle activation in the active left hand, i.e. before afferent feedback could possibly be involved. This timing of change may also coincide with the release of motor commands to the active limb.

Neuroimaging studies show that interhemispheric interactions occur between the two M1s via the transcallosal pathway during unilateral movement (Bütefisch, et al., 2014; Chiou, et al., 2013; Chiou, et al., 2014). Interhemispheric interactions were also reported by TMS studies of unilateral movement, whereby interhemispheric inhibition and short-interval intracortical inhibition decrease during unilateral movement (Howatson, et al., 2011; Muellbacher et al., 2000; Reissig et al., 2014; Soto, et al., 2010; Uehara et al., 2013). Therefore, we could speculate that the increase in MEP amplitudes and changes in twitch direction during late motor preparation and after movement onset in our results could be due to a decrease in interhemispheric inhibition and short-interval intracortical inhibition. Another factor to consider is that MEP data can be affected by cross-talk between wrist muscles with surface EMG techniques (Selvanayagam, Riek, & Carroll, 2012). As such, we acknowledge that part of the MEP increase observed in the current study could be also partly due to cross-talk effects.

Reaction time

Previous studies reported that reaction time was modulated by the time of TMS applied at the motor cortex contralateral to the active limb. For example, reaction time was shortened when stimuli were delivered to the $M1_{contra}$ at early stage of movement preparation (Leocani, et al., 2000; McMillan, et al., 2006; Michelet et al., 2010; Nikolova, et al., 2006; Soto, et al., 2010; van Elswijk, et al., 2008). However, when TMS was applied close to the expected movement onset to the $M1_{contra}$, reaction time was delayed due to disruption of movement production (Leocani, et al., 2000; McMillan, et al., 2006; Michelet, et al., 2010; Nikolova, et al., 2006; Soto, et al., 2010; Ziemann, et al., 1997). In contrast to these studies, the reaction time recorded with TMS delivered to $M1_{ipsi}$ at target appearance (early motor preparation) and late motor preparation was comparable. The $M1_{ipsi}$ does not directly contribute to movement production; therefore it is not clear whether we should expect reaction time to be affected when TMS was delivered to $M1_{ipsi}$ during motor preparation. However, our results show that reaction time was shorter with TMS trials as compared to non-stimulation trials in both target conditions. One possible explanation for this is that the reaction time might be affected by the acoustic noise produced by the TMS coil, because there is evidence showing that acoustic startle results in the early release of movement plans which causes a shortening of reaction time (Carlsen et al., 2011; Marinovic et al., 2014). Therefore, we could speculate that a shortening of reaction time in our results could be due to the side effect of the sound and tactile sensation produced by the TMS, and the activation of the inhibitory circuits, such as interhemispheric inhibition and short-interval intracortical inhibition, during $M1_{ipsi}$ stimulation.

2.5 Conclusion

The present study provides insights into the directional information of twitch directions in resting limb during motor preparation in a non-precued choice reaction time task. An increase of MEP amplitudes and a shift in twitch direction in the passive limb toward the impending direction of the active limb according to muscle and/or midline reference frames was shown during late motor preparation, i.e. about 20 ms before movement onset in the active limb, when the reference frames were mismatched. Our results suggest that the reference frame in which movement is represented could possibly influence the interactions between hemispheres.

CHAPTER THREE

Ballistic training induces systematic aiming bias in the opposite, untrained limb that is defined according to multiple spatial frames of reference

3.1 Introduction

Cross limb transfer is a well-known phenomenon whereby training with one limb leads to improved performance of the same task with the opposite untrained limb (Adamson, et al., 2008; Carroll, et al., 2008; Munn, et al., 2005; Perez, et al., 2007; Wang & Sainburg, 2009). The exact mechanisms for cross limb transfer are still not well understood, but two models, termed “bilateral access” and “cross activation” have been widely proposed (Lee & Carroll, 2007; Ruddy & Carson, 2013). Both models highlighted the sharing of specific information acquired by the trained limb with the untrained limb. Numerous studies have shown successful transfer of learning, in tasks including strength training (Adamson, et al., 2008; Farthing, et al., 2009; Pearce, et al., 2013), maximal acceleration (Carroll, et al., 2008; Hinder, et al., 2013b; Lee, et al., 2010), dexterity skills (Schulze, et al., 2002), sequential learning (Shea, et al., 2011; Wiestler, et al., 2014), and error-based learning (Carroll, et al., 2014; Criscimagna-Hemminger, et al., 2003; Galea, et al., 2007; Sainburg & Wang, 2002; Taylor, et al., 2011; Wang & Sainburg, 2009). However, the transfer of use-dependent learning has yet been reported.

Use-dependent learning can be induced by repeatedly executing movements toward a single target, and the learning outcome is illustrated by the reduction of error made toward the practiced direction. Repetitive movements not only enhance learning experiences and alter the neural networks associated with producing that particular movement, but also bias movements directed toward other locations toward the direction of the repeated actions (Diedrichsen, et al., 2010; Verstynen & Sabes, 2011). A recent study suggested that use-dependent learning strengthened the synapses in the network of neurons contributing to the movement, and is generalised to an extrinsic reference frame within the same limb (Selvanayagam, Riek, et al., 2012b). In that study, the authors demonstrated that a single session of 40 ballistic isometric contractions repeated in one direction biased movements aimed toward target locations that were up to 90° away from the training direction in extrinsic space. However, whether a similar use-dependent aiming bias would also generalise toward extrinsic space for the *opposite limb* after ballistic training is unknown.

In order to examine whether use-dependent aiming bias is transferable to the opposite limb, it is necessary to consider the reference frame in which use-dependent learning might be represented. The issue of reference frames is likely an important factor that influences the transfer of use-dependent aiming bias. We know that limb movement in space can be represented by multiple coordinate frames, such as extrinsic, muscle-based, and/or midline reference frames, and in many cases, movement representations between two limbs are mismatched. For example, an extension movement with the left wrist when in a neutral position would result in a conflict between reference frames when the same movement is transferred to the right wrist. In an extrinsic reference frame, extension of the left wrist would be represented as a leftward movement of the right wrist, but in a muscle-based reference frame extension of the left wrist would be represented as extension (rightward movement) of the right wrist. Furthermore, in a body centred reference frame, extension of the left wrist would be represented as a movement away from the midline, which in the right wrist would be also represented as a movement away from the midline (extension). In this example, there is a clear conflict between how the movement would be represented for the right wrist between an extrinsic reference frame vs muscle and body centred reference frames (Figure 3.1a). However, the reference frames between two limbs can be matched via postural manipulation (Figure 3.1b). For example, an extension movement with the left wrist in pronated posture matches the same upward extension movement in the right wrist defined according to the extrinsic, muscle and midline reference frames.

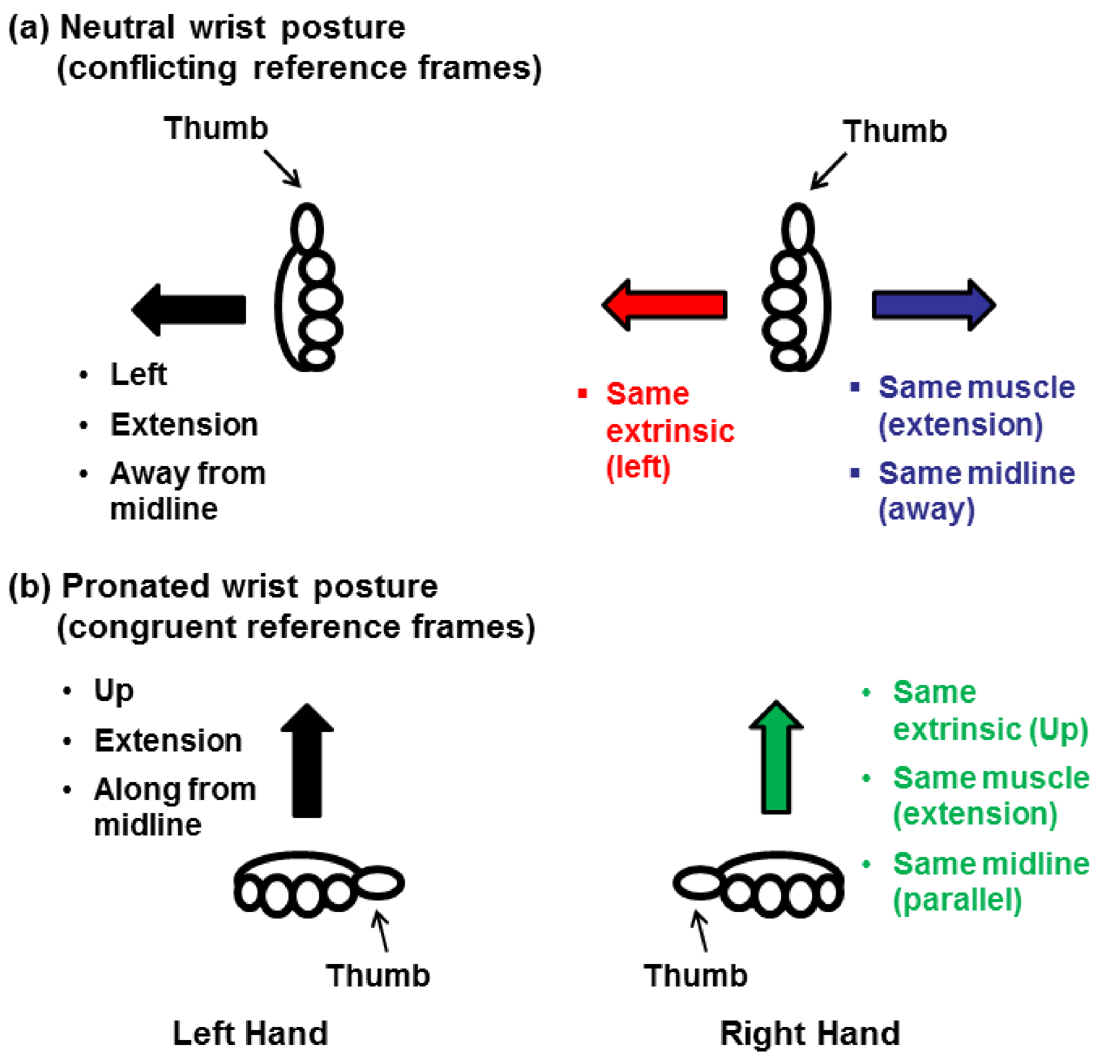


Figure 3.1. A schematic illustration of movement representation between two limbs defined in (a) conflicting reference frames in neutral wrist posture, (b) congruent reference frames in pronated wrist posture.

In this study, we hypothesized that use-dependent learning could be defined in four possible coordinate frames, i.e. extrinsic, muscle-based, midline, and/or training axis. The extrinsic reference frame defines direction in external space independent of limb posture. A muscle-based reference frame defines direction of movement by the muscles involved in generating the same movement as the training direction. A midline reference frame defines direction in relation to the body midline, i.e. toward or away from the body midline. The training axis refers to the line of the axis about which training is executed. For example if the training direction is an upward extension movement in a pronated position, the training axis is along the line of motion which stretches in both directions, i.e. upward and downward directions in the example. To test our hypothesis that aiming performance was biased toward training direction in the untrained limb, transfer of aiming bias was tested under postural manipulations that varied whether or not the training direction (TD) was aligned for the two limbs according to extrinsic, muscle-based, midline and/or training axis reference frames.

We chose to train participants in pronated and neutral (midway between supination and pronation) wrist postures so that the training direction defined according to extrinsic, muscle-based, midline, and/or training axis reference frames could be manipulated to be either congruent (pronated wrist posture) or conflicting (neutral wrist posture) conditions. To account for all possibilities with respect to the aiming bias in the right wrist as a result of training with the left wrist, we designed four experimental conditions that combined one of two possible hand positions with one of two possible training directions to study the effects of ballistic training. The four experimental conditions were termed Pronated Extension (PE), Pronated Flexion (PF), Neutral Extension (NE) and Neutral Flexion (NF) (see Figure 3.2 and Figure 3.3).

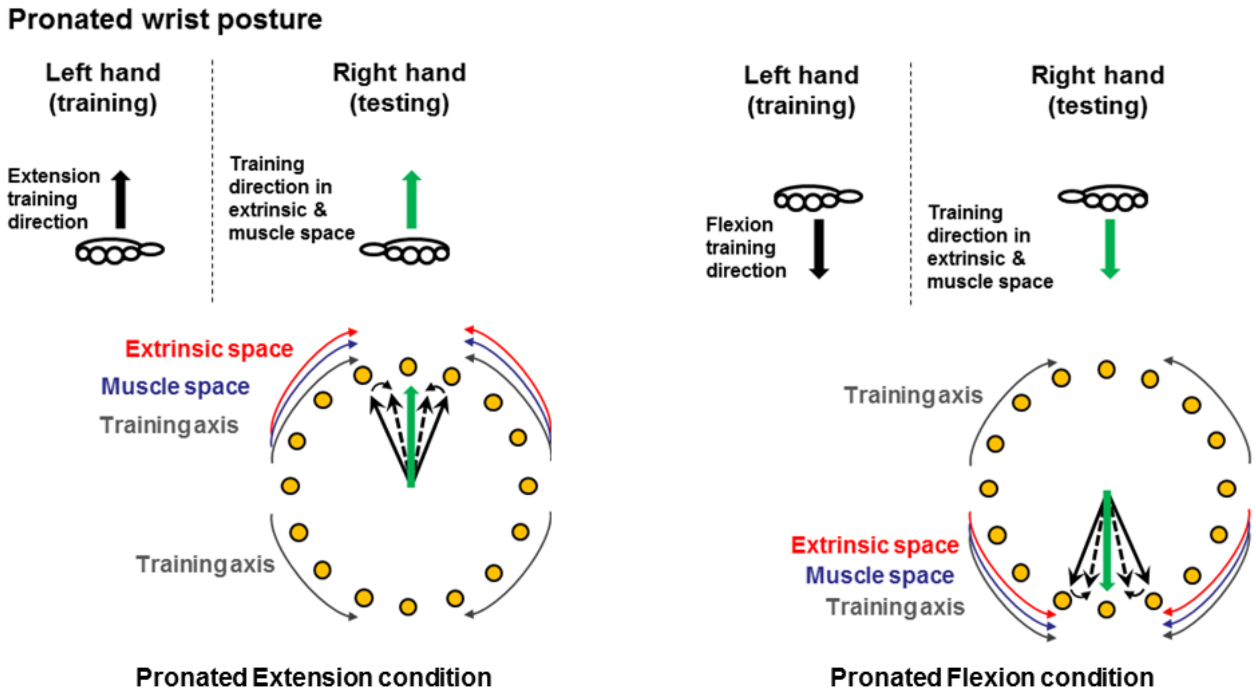


Figure 3.2. Schematic illustrations of the Pronated Extension and Pronated Flexion conditions. Black thick arrows show the extension and flexion training directions for the left (trained) hand. Green thick arrows show the matching training direction defined according to extrinsic and muscle space for the right (testing) hand. Yellow circles depict the 16 radial targets in the aiming task for the right hand. Red, blue and grey thin arrows depict the direction of aiming bias in the testing limb if use-dependent learning is defined according to extrinsic, muscle and training axis respectively in the trained limb. Black thin arrows show the initial aiming path toward the target near to the training direction in the testing limb. Black thin dash arrows depict the aiming path bias toward the training direction after training in the testing limb. Midline axis is not involved because the training direction is aligned with the midline of the body.

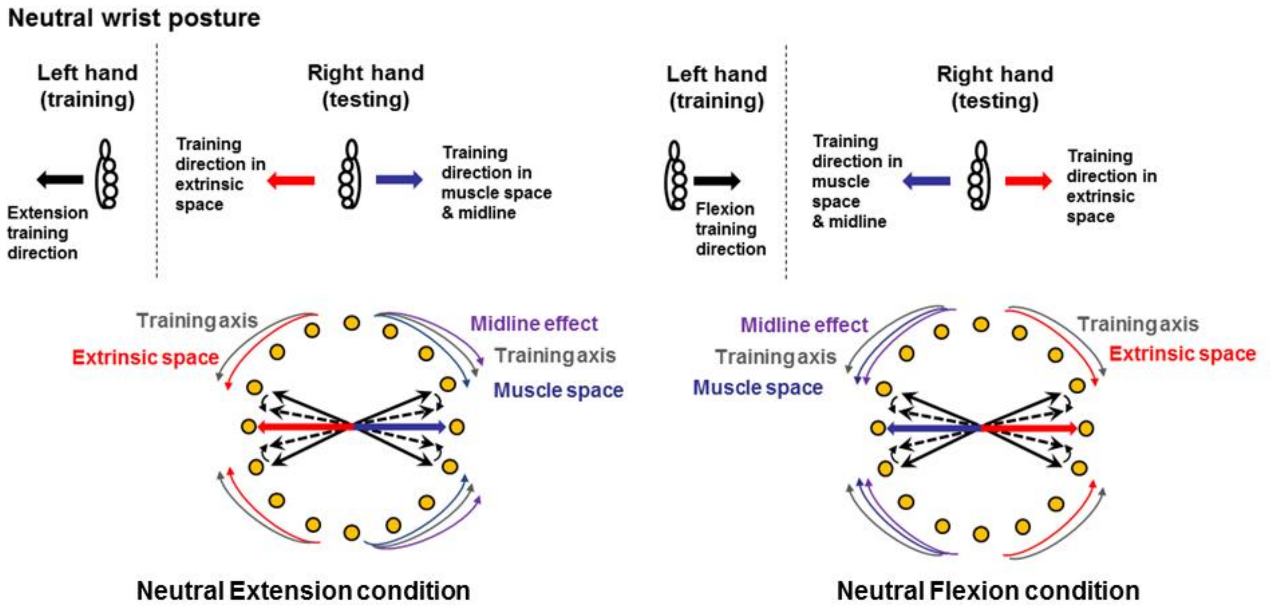


Figure 3.3. Schematic illustrations of the Neutral Extension and Neutral Flexion conditions. Black thick arrows show the extension and flexion training directions for the left (trained) hand. Red thick arrows show the matching training direction defined according to extrinsic space for the right (testing) hand. Blue thick arrows show the matching training direction defined according to muscle space for the right (testing) hand. Yellow circles depict the 16 radial targets in the aiming task for the right hand. Red, blue, purple and grey thin arrows depict the direction of aiming bias in the testing limb if use-dependent learning is defined according to extrinsic, muscle, midline and training axis respectively in the trained limb. Black thin arrows show the initial aiming path toward the target besides the training direction in the testing limb. Black thin dash arrows depict the aiming path bias toward training direction after training in the testing limb.

Participants were randomly assigned to one of the experimental conditions and trained to make ballistic contractions to a single target with their left wrist, and aiming bias was then probed with their right wrist via an aiming task that required weak forces to acquire 16 radial targets. In the pronated wrist posture, training directions consisting of extension/flexion contractions in the left hand matches extension/flexion of the right hand, i.e. training direction defined according to extrinsic, muscle, midline and/or training reference frames between both limbs are congruent (see Figure 3.2). For example, a left wrist extension contraction corresponds to an upward movement in extrinsic space, and the right wrist also requires an extension force for upward aiming. In contrast, extension/flexion training directions with the left wrist when in a neutral position would have a conflicting representation for the same extension/flexion movements in the right wrist, i.e. training direction defined according to extrinsic and training axis reference frames versus muscle, midline and training axis reference frames (see Figure 3.3). For example, a left wrist extension contraction corresponds to a contraction of homologous extensor muscles defined in muscle space in the right wrist would result in rightward aiming instead. Regardless in which reference frame that use-dependent learning might be defined, we speculated that each reference frame would have its own weighting on the extent of aiming bias transferred. Therefore for conditions whereby the training direction was congruent according to all reference frames between two limbs, we would expect a higher degree of bias transfer compared to the conditions in which the training direction conflicted in various reference frames.

Here, we illustrated some possible examples for the relative weightings of individual reference frames in use-dependent aiming bias. We described the relative weightings for each reference frame according to wrist postures (i.e. pronated and neutral postures) since the direction of bias for both wrist postures would be of similar direction for the extension and flexion training directions. We assigned positive weightings to represent bias toward the training direction expressed in extrinsic space, whereas negative weightings represent bias away from the training direction expressed in extrinsic space.

Prediction of weightings in pronated wrist posture

When both hands are pronated, the training direction defined according to extrinsic, muscle, and training axis reference frames for both limbs are congruent. The effect of a midline centred reference frame is negligible in a pronated wrist posture because extension and flexion training direction are parallel with the midline axis (Figure 3.4). If use-dependent learning in the trained limb is defined according to an extrinsic reference frame, we should see a positive bias toward training direction defined according to the extrinsic reference frame in the untrained limb. Positive bias toward training direction in the untrained limb would be also expected if use-dependent learning in the trained limb is defined according to muscle and/or training axis reference frames. As cardinal directions are reported to be the most frequent movement directions made by humans (Slijper et al., 2009), we might expect a negative bias away from the training direction if use-dependent learning is also defined according to the training axis, i.e. bias toward the opposite direction of the training direction. Therefore the predicted net effect for use-dependent aiming bias in the untrained limb would be a greater positive bias since the training direction defined according to extrinsic, muscle, and training axis reference frames for both limbs are congruent.

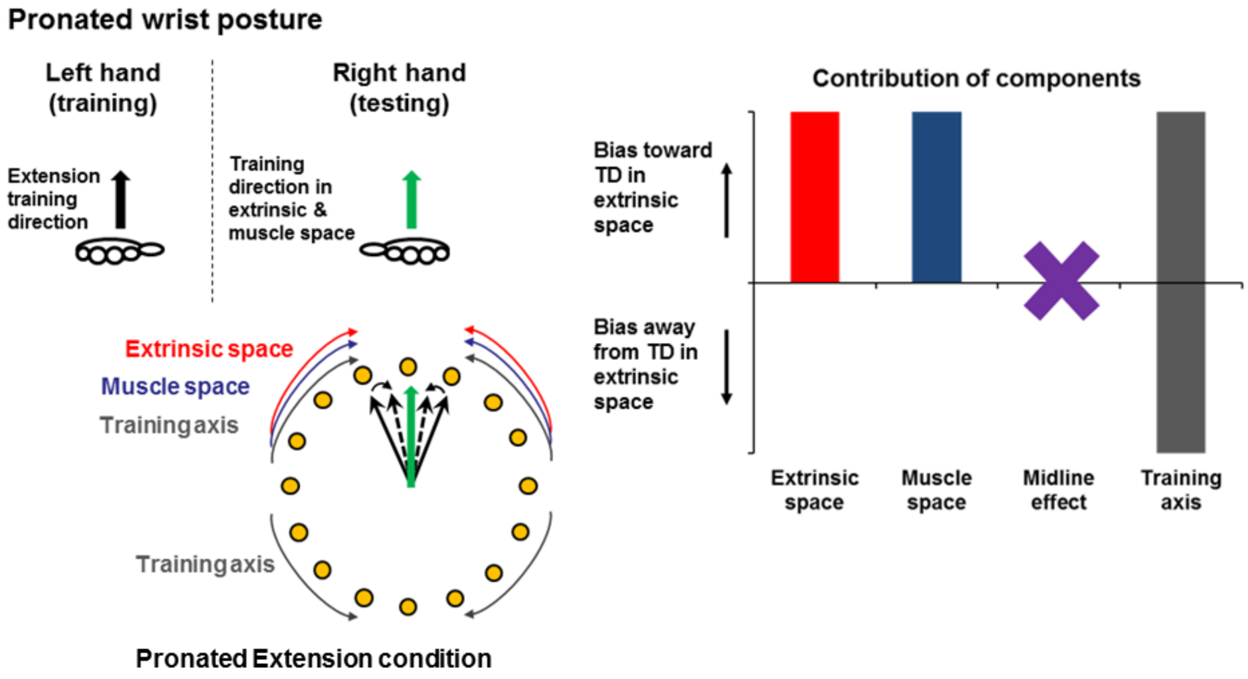


Figure 3.4. Predicted weightings for each coordinate frame in the pronated wrist posture. For illustration purposes, only Pronated Extension condition (details as described in Figure 3.2) is shown. The graph on the right shows the possible weightings from the extrinsic, muscle, midline and training axis reference frames for the use-dependent aiming bias in the testing (right, untrained) limb. Positive bias represents bias toward the training direction expressed in extrinsic coordinates, whereas negative bias represents bias away from the training direction expressed in extrinsic coordinates. If use-dependent learning is defined according to the extrinsic, muscle and/or training axis reference frames in the trained limb, positive bias toward training direction would be expected in the untrained limb. Midline effect is negligible because extension and flexion training direction are parallel with the midline axis. A greater positive bias toward training direction would be the predicted net effect for use-dependent aiming bias in the untrained limb in pronated wrist posture.

Prediction of weightings in neutral wrist posture

The training direction defined according to extrinsic, muscle, midline and training axis reference frames are conflicting when both limbs are in a neutral hand posture, i.e. extrinsic and training axis reference frames versus muscle, midline and training axis reference frames (Figure 3.5). If use-dependent learning is defined according to extrinsic and training axis reference frames in the trained limb, we would expect to see positive bias toward training direction in extrinsic space in the untrained limb. If use-dependent learning is defined according to muscle, midline and training axis (opposite direction of training direction) reference frames in the trained limb, we would expect to see negative bias away from training direction in extrinsic space in the untrained limb. However, as there is currently no way of knowing the relative weighting of all these reference frames, it is not possible to predict with certainty what the net effect for use-dependent aiming bias when the training direction defined according to extrinsic, muscle, midline and training axis reference frames between two limbs are conflicting.

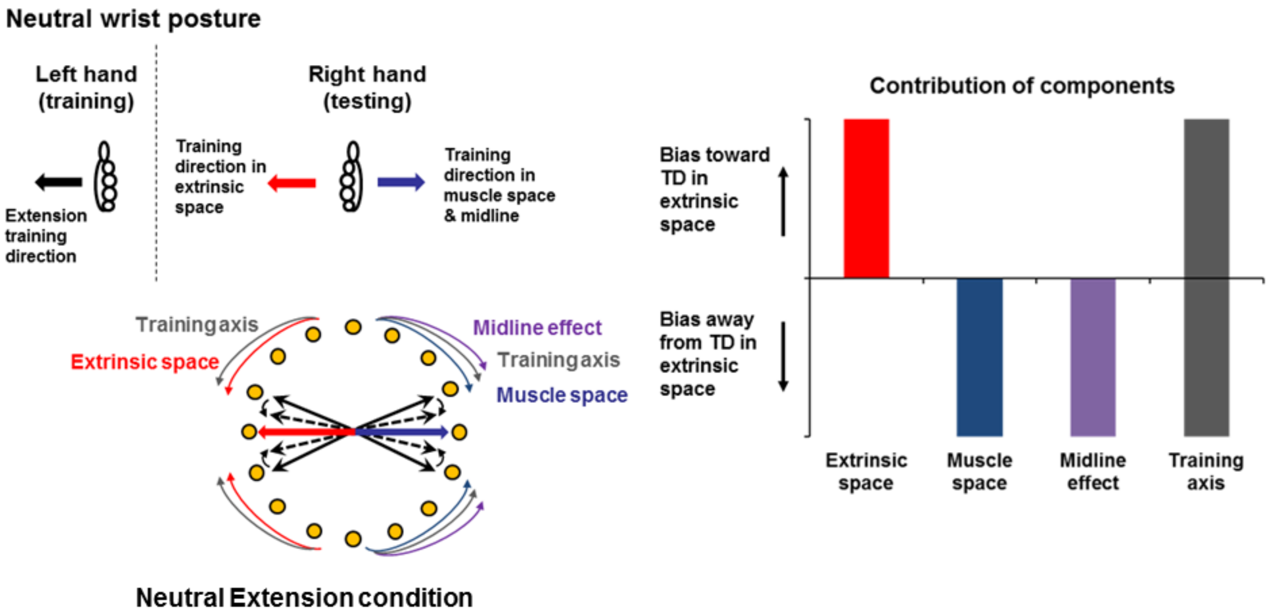
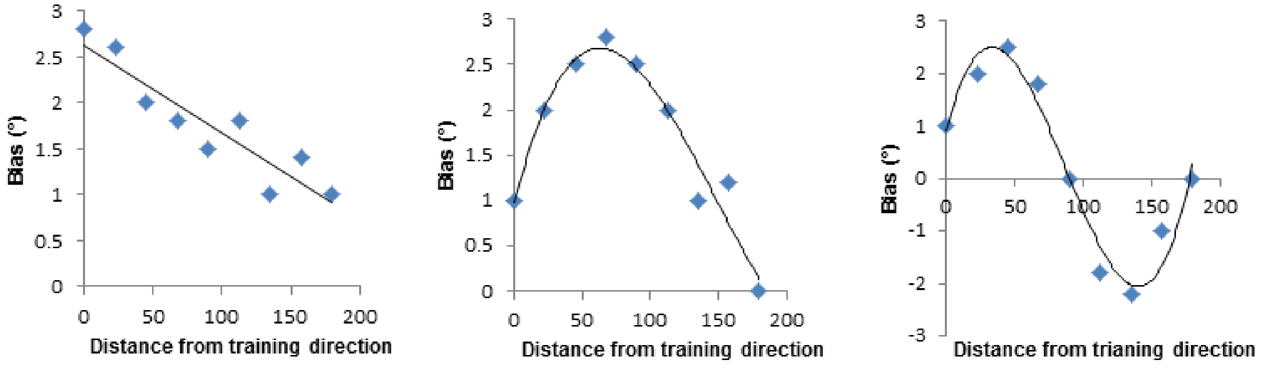


Figure 3.5. Predicted weightings for each coordinate frame in the neutral wrist posture. Left figures show Neutral Extension condition (details as described in Figure 3.3). Right graph shows the possible weightings of extrinsic, muscle, midline and training axis reference frames for the use-dependent aiming bias. Positive bias represents bias toward the training direction expressed in extrinsic space, whereas negative bias represents bias away from the training direction expressed in extrinsic space. If use-dependent learning is defined according to extrinsic and training axis reference frames, positive bias toward training direction would be seen. If use-dependent learning is defined according to muscle, midline and training axis (opposite direction of training direction) reference frames, negative bias away from training direction would be seen. The net effect for use-dependent aiming bias is difficult to predict when the training direction defined according to

extrinsic, muscle, midline and training axis reference frames between two limbs are conflicting.

Predicted possible aiming bias trend

With the predicted weightings of each reference frame, we could expect to see linear, quadratic and/or cubic trends for the aiming bias (Figure 3.6). To illustrate the possible bias trends, aiming biases for each target are plotted as a function of distance from training direction in extrinsic space, i.e. the nearest target distance to the training direction would be labelled 0°, while the target furthest away from the training direction would be labelled 180°. A linear trend indicates that targets closer to training direction would have more positive biases toward training direction, and that targets opposite to the training direction would have more negative biases away from training direction (Figure 3.6a). A quadratic trend would display a monophasic curve with aiming bias increasing toward training direction with increasing target distance from training direction to a peak and gradually decreases to baseline for targets opposite to the training direction (Figure 3.6b). A cubic trend would display a biphasic curve with a positive and negative peak. Aiming biases increase toward the training direction with increasing target distance until the first peak and then decrease negatively toward the midline opposite to training direction reaching the second peak before returning to baseline at the opposite targets from the training direction (Figure 3.6c).



(a) Linear **(b) Quadratic** **(c) Cubic**

Figure 3.6. Figures illustrate the possible trends for aiming bias after ballistic training plotted in extrinsic coordinates. Positive angles represent bias toward the training direction, whereas negative angles represent bias away from the training direction.

Therefore, the purpose of this study was to examine whether reference frame is a factor that influences the transfer of use-dependent aiming bias. We addressed this by manipulating wrist postures in pronated and neutral (midway between supination and pronation) positions so the training direction defined according to extrinsic, muscle-based, midline, and/or training axis reference frames in both limbs are in congruent and conflicting manner respectively. We hypothesized that transfer of aiming bias would be greater when the training direction defined according to extrinsic, muscle-based, midline, and/or training axis reference frames in both limbs are congruent.

3.2 Methods

Fifty-nine right-handed participants (35 males, 24 females; aged between 18 and 55 years old) with no recent shoulder, arm or wrist injuries volunteered for the study. Five participants took part in two of the experiments which were conducted more than three months apart. Their results were not different from those of the other participants in the same experimental conditions. Right handedness was confirmed with the Edinburgh Handedness Inventory (Oldfield, 1971). The study was approved by the Medical Research Ethics Committee of The University of Queensland. All participants were briefed on the experimental procedures and gave written informed consent before the experiment which conformed to the Declaration of Helsinki. All participants were kept naïve about the purpose of the experiment.

3.2.1 Experimental protocol

Participants were randomly assigned to one of the four experimental conditions according to hand position and training direction, namely Pronated Extension (PE), Pronated Flexion (PF), Neutral Extension (NE) and Neutral Flexion (NF). For the pronated wrist posture, the training direction in extension/flexion movement with the left hand matched the extension/flexion direction of the right hand defined according to extrinsic, muscle, midline and/or training reference frames (see Figure 3.2). For the neutral wrist posture, extension/flexion movement with the left wrist in neutral position would have a conflicting representation of extrinsic and training axis reference frames versus muscle, midline and training axis reference frames in the right wrist for the same extension/flexion movement (see Figure 3.3).

Participants performed two blocks of familiarisation aiming tasks (Fam 1 - 96 trials and Fam 2 - 160 trials respectively) with their right hand (Figure 3.7). Participants were required to make a centre-out movement from the origin toward the targets. In the first block of familiarisation trials, participants received real-time feedback with a cursor that corresponded to their force from target appearance until target acquisition. Probe trials were introduced in the second block of familiarisation aiming trials. The online cursor was replaced by an expanding circle whereby the radius of the circle corresponded to the magnitude of force. In this manner, the directional information regarding the movement was thus removed. Target acquisition during probe trials was defined as intersecting the target radius with the radius of the circle controlled by the force exerted by the participants. Only probe trials were used in actual experiment to examine the aiming behaviour.

The experiment began with 48 probe trials (16 targets x 3 trials per target) with the right hand as a baseline measurement. Subsequently, five sets of 10 ballistic sustained contractions were performed with the left hand toward the assigned training direction. A rest period of 3 minutes was given in between each set of contractions to avoid muscle fatigue. At the beginning of each 3-min rest period between the 2nd and 5th set of ballistic contractions, participants aimed toward 4 of the 16 radial targets in randomised order with the right hand. The transition time between left hand training and right hand aiming were within 30 s to minimise any time-dependent memory decay during learning. The experiment ended with a post training aiming measurement of 48 probe trials (16 targets x 3 trials per target) 5 minutes from the last set of ballistic contractions.

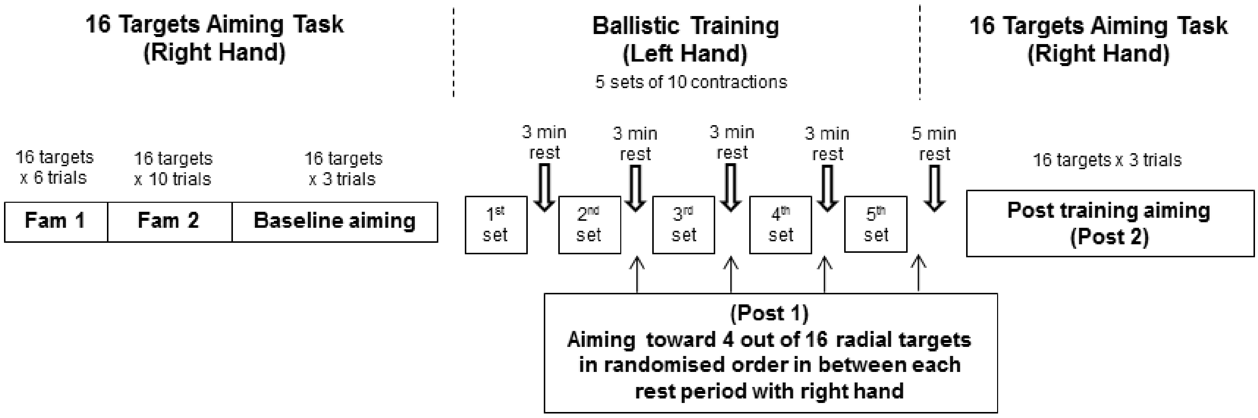


Figure 3.7. Schematic illustration of the experimental protocol. The protocol comprises 2 blocks of familiarisation aiming blocks (Fam 1 and Fam 2), a baseline and a post training measurements for the right hand, and 5 sets of 10 ballistic contractions for the left hand.

3.2.2 Experimental setup

Participants were comfortably seated in front of a computer screen placed approximately 1.2 m away at eye level (Figure 3.8). Each forearm was secured into a custom-made hand manipulandum, described previously (de Rugy, et al., 2012). Both elbows were kept at 110° with the forearm parallel to the table and supported by the manipulandum. The wrists were secured by a series of twelve metal clamps contoured around the metacarpal-phalangeal joints and around the wrist proximal to the radial head. The manipulandum could be rotated along the longitudinal axis of the forearms and fixed either in a pronated position or in a neutral position without the need for readjustment of the clamps. Wrist forces in radial-ulnar deviation and flexion-extension axes were recorded with a six degree-of-freedom force transducer (JR3 45E15A-163-A400N60S, Woodland, CA) attached at the end of the manipulandum. The forces were sampled at a rate of 2 kHz with two 16-bit National Instruments A/D boards (NI BNC2090A, NI USB6221, National Instruments Corporation, USA). The online forces exerted in flexion-extension and abduction-adduction directions were displayed as a cursor in two dimensional space (x = flexion-extension, y = abduction-adduction) on the computer screen via a custom written Labview program (LabView2009, National Instrument, USA).

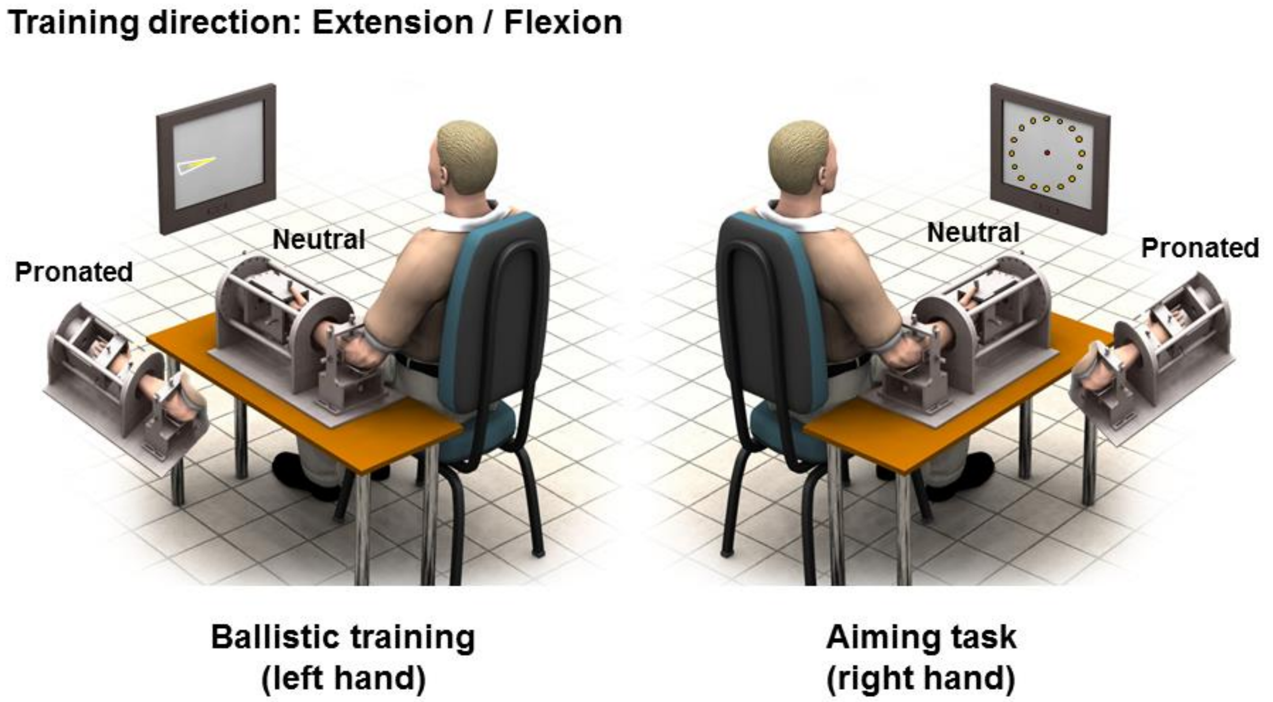


Figure 3.8. Experimental setup. Participants are trained to make ballistic contractions toward an extension or flexion direction with their left hand, and aiming bias is probed with their right hand via an aiming task that required weak forces to acquire 16 radial targets.

3.2.3 Isometric aiming task with right hand

The isometric aiming task involved moving a cursor to acquire one of 16 radial targets, which were spaced 22.5° apart, located 75 % of the distance from the origin to the edge of the computer screen (11.5 cm). The targets appeared once only in random order in every cycle. The participants were required to acquire the target within a movement time between 150 ms and 250 ms with 15 N of force. The movement time was defined as the time taken for the cursor to move from 10 % to 90 % of the target distance. The cursor gain was set so that 20 N was required to reach the edge of the display for all participants. A successful acquisition of target was registered when the cursor was within 10 % of the radius distance from the centre of the target for 10 ms. Two audio tones were sounded to indicate the successfulness of target acquisition. The first tone indicated whether the target was acquired (high-pitched tone: 500 ms, 800 Hz sinusoid) or not (low-pitched tone: 500 ms, 300 Hz sinusoid). A second tone indicated whether movement time was achieved (high-pitched tone: 500 ms, 800 Hz sinusoid) or not (low-pitched tone: 500 ms, 300 Hz sinusoid).

3.2.4 Ballistic contractions with the left hand

Prior to beginning the experiment, maximum voluntary contractions (MVC) were determined in the training direction with a training gain that required 300 N to reach the edge of the screen. The training gain was subsequently readjusted to 1.5 times of each participant's MVC for training in the main experiment. The training direction was presented as a 4° wide wedge-shaped target to ensure contraction was produced within the vicinity of the training direction. Participants had to start the contraction as quickly and as hard as possible upon appearance of the target which was accompanied an audio cue (0.25 s, 200 Hz). A 2-second continuous audio cue of 900 Hz was played immediately following the low frequency trigger cue, to prompt participants to sustain their contractions before relaxation. A yellow real-time force feedback line from the origin corresponding to the force exerted was displayed on the screen. Participants had to maintain their contraction within the width of the wedge-shaped target. The participants were trained for five blocks of 10 ballistic contractions with a rest period of 3 minutes between each block of contractions.

3.2.5 Data analysis

The force traces were recorded on disk and analysed offline via a custom-written Matlab program (Mathworks, Natick, USA). Movement directions for both hands were calculated from the x- and y-axis force channels of the force transducers. The force exerted with the left hand during training was expressed in percentage of individual's MVC. The mean force of the first set of contractions was compared with the overall mean force of five sets contractions in each experimental condition group using paired t-tests to examine whether force decreased with training.

The aiming angles toward each target were calculated at the 90 % of movement distance for all probe trials at baseline, immediately post training and at 5 minutes post training. Baseline aiming angles which deviated by 45° or more from the target locations were excluded from analysis (Neutral Extension condition: 3 %; Neutral Flexion condition: 3 %; Pronated Extension condition: 4 %; Pronated Flexion condition: 2 %). Aiming bias for each target immediately post training and at 5 minutes post training was defined as the difference between baseline aiming angle and post training aiming angle. Positive angles indicate an aiming biased toward the training direction in extrinsic space. Negative angles indicate an aiming biased away from the training direction in extrinsic space. No visual online feedback of the right wrist was provided in the main experiment. Hence, a drift in perceived limb position could occur when visual feedback of the limb is absent during movement (Wann & Ibrahim, 1992). In order to account for this possibility, as well as for small additional physical rotations in the hand manipulandum, we subtracted the mean post training bias across all targets from the bias observed at each target. This effectively removed the effect of any uniform bias common to all targets, allowing us to focus on bias in relation to the training direction.

For each experimental condition, aiming bias was plotted for all targets as a function of absolute angular distance from the training direction (i.e. between 0° to 180°). For this study, we were interested in the predicting whether ballistic training in one limb would influence the aiming bias into fitting a linear, cubic or quadratic trend in the opposite untrained limb. The effects of ballistic training on aiming bias was examined by a 16 x 2 ANOVA (target x time) with polynomial contrasts for significant trend at immediately post training and 5 minutes post training of each experimental condition and for the pooled data according to hand positions. All data were presented in mean ± SEM, unless stated otherwise. Statistical significance level was set at $p < 0.05$.

3.3 Results

3.3.1 Force effort of left hand

The mean force exerted on the first set of contractions for all participants was above 76 % of MVC in each experimental condition. The mean force decreased significantly between the first set and the overall mean contractions in all experimental conditions, presumably due to fatigue (Table 3.1, $p < 0.05$).

Table 3.1. Percentage of mean force for the first set of contractions and the mean force for a total of five sets of contractions. Force data are presented in mean ± SD.

Experimental Conditions	First set of mean force (N) (10 contractions, % of MVC)	Overall mean force (N) (50 contractions, % of MVC)	p value
Neutral Extension (n = 16)	77 ± 7	73 ± 8	<0.001
Neutral Flexion (n = 16)	80 ± 9	74 ± 8	0.001
Pronated Extension (n = 16)	80 ± 5	78 ± 6	0.039
Pronated Flexion (n = 16)	80 ± 7	77 ± 5	0.013

3.3.2 Aiming bias of right hand with congruent reference frames

Pronated Extension and Pronated Flexion conditions

For both PE and PF conditions, targets closer to training direction exhibited more positive biases toward the training direction in extrinsic space. There was an increasing bias away from training direction (negative bias) for targets further away from the training direction (Figure 3.9 and 3.10).

Polynomial contrasts for both PE and PF conditions showed significant trends at immediately post training and 5 minutes post training. PE condition showed a near significant linear trend ($F_{(1,11)} = 4.69$, $p = 0.053$, $\eta p^2 = 0.29$) immediately post training and 5 minutes post training showed both linear and cubic trends (linear: $F_{(1,15)} = 11.13$, $p = 0.005$, $\eta p^2 = 0.43$; cubic: $F_{(1,15)} = 8.79$, $p = 0.01$, $\eta p^2 = 0.37$). Significant linear trends were observed for PF condition at immediately post training ($F_{(1,15)} = 10.92$, $p = 0.005$, $\eta p^2 = 0.42$) and 5 minutes post training ($F_{(1,15)} = 6.48$, $p = 0.022$, $\eta p^2 = 0.30$).

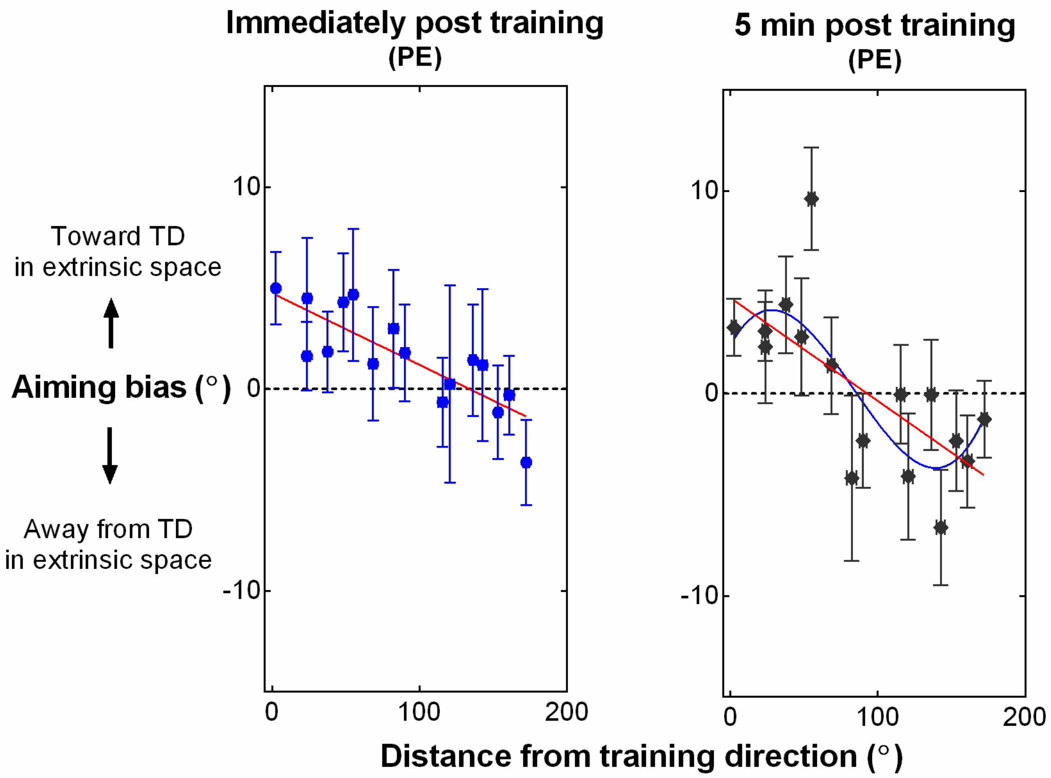


Figure 3.9. Aiming biases for the Pronated Extension condition immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The TD defined according to extrinsic, muscle and/or training axis reference frames are aligned in the pronated hand position. Positive angles indicate biases toward training direction. Negative angles indicate biases away from training direction. Trend lines are fitted into the graphs for illustration purposes; red lines indicate the linear trend and blue line indicates the cubic trend.

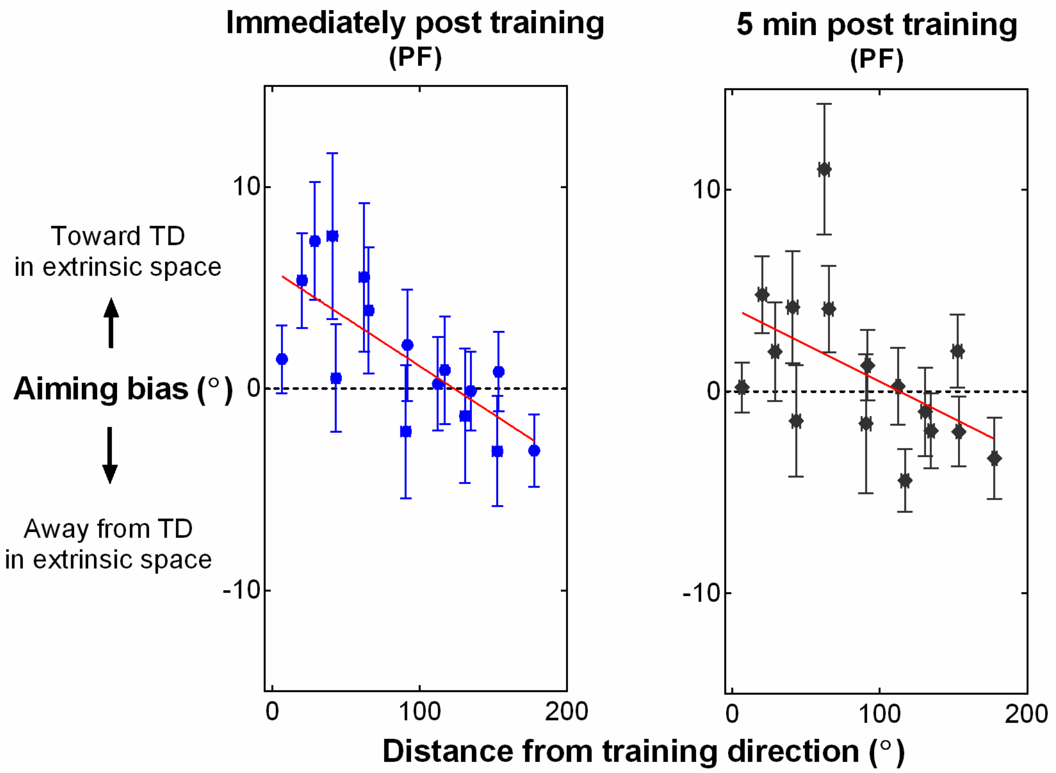


Figure 3.10. Aiming biases for the Pronated Flexion condition immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The TD defined according to extrinsic, muscle and/or training axis reference frames are aligned in the pronated hand position. Positive angles indicate biases toward training direction. Negative angles indicate biases away from training direction. Trend lines are fitted into the graphs for illustration purposes; red lines indicate the linear trend.

Aiming biases in congruent reference frames

When results were pooled for both conditions in the pronated forearm orientation, significant trends were observed immediately post training (linear trend: $F_{(1, 27)} = 15.75$, $p < 0.001$, $\eta p^2 = 0.37$) and 5 minutes post training (linear trend: $F_{(1, 31)} = 17.7$, $p < 0.001$, $\eta p^2 = 0.36$; cubic trend: $F_{(1, 31)} = 9.45$, $p = 0.004$, $\eta p^2 = 0.23$). Therefore, ballistic training with the left hand resulted in systematic aiming bias for the right hand when the training direction defined according to extrinsic, muscle and/or training axis reference frames were congruent for both hands (Figure 3.11).

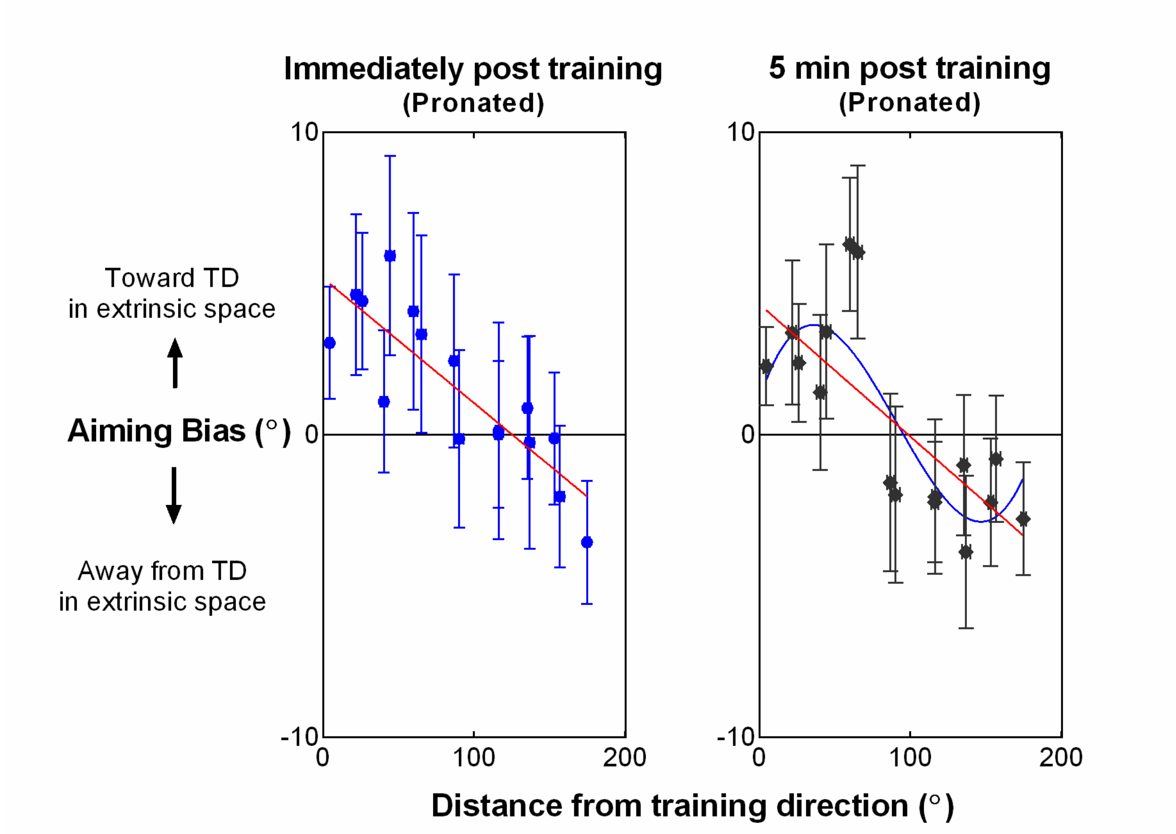


Figure 3.11. Aiming biases for pooled data in the pronated hand position immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The TD defined according to extrinsic, muscle and/or training axis reference frames are aligned in the pronated hand position. Positive angles indicate biases toward training direction. Negative angles indicate biases away from training direction. Trend lines are fitted into the graphs for illustration purposes; red lines indicate the linear trend and blue line indicates the cubic trend.

3.3.3 Aiming bias of right hand with conflicting reference frames

Neutral Extension and Neutral Flexion conditions

There was no trend observed for the aiming bias after ballistic training at immediately post training and 5 min post training for both NE and NF conditions ($p > 0.05$, Figure 3.12 and 3.13).

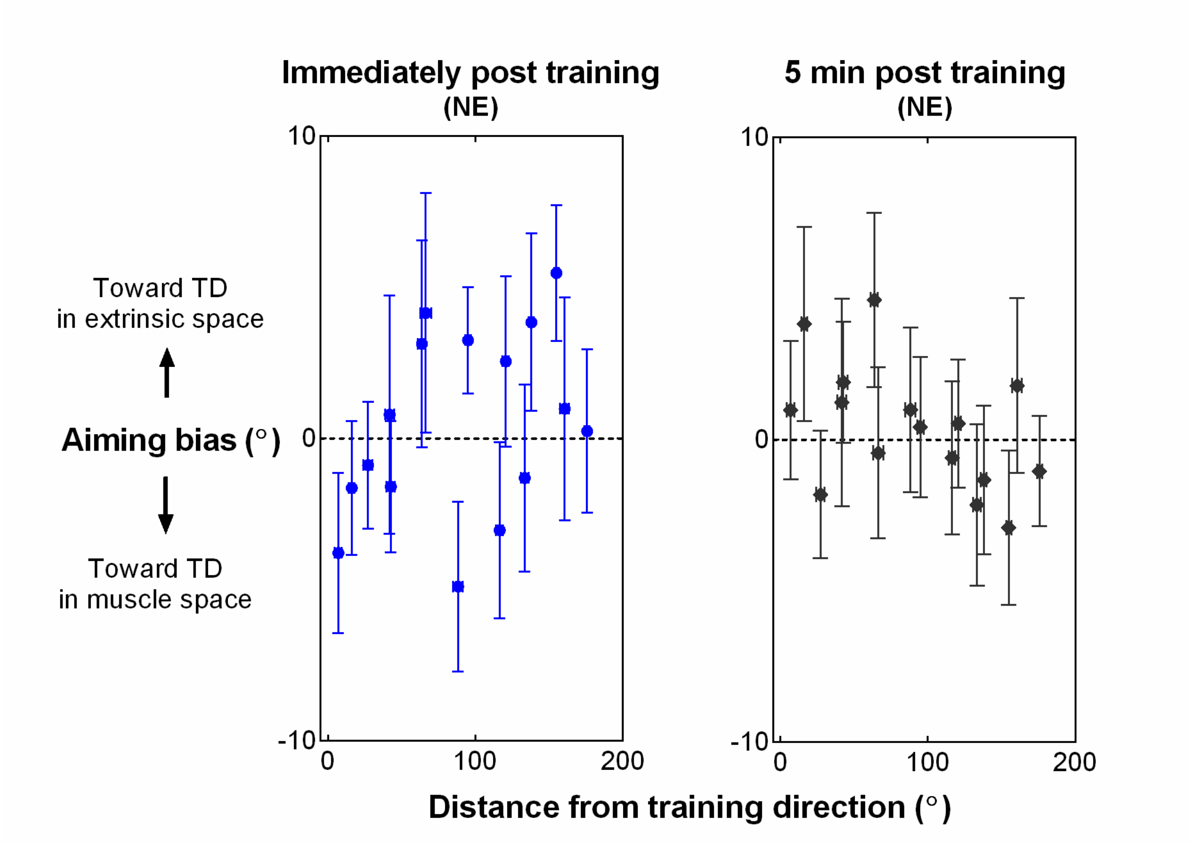


Figure 3.12. Aiming biases for the Neutral Extension condition immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The reference frames in which the training direction is defined for both limbs in the neutral hand position are conflicting, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames. Positive angles indicate biases toward training direction in extrinsic space. Negative angles indicate biases toward the training direction in muscle space. No trend is observed for the aiming bias after ballistic training.

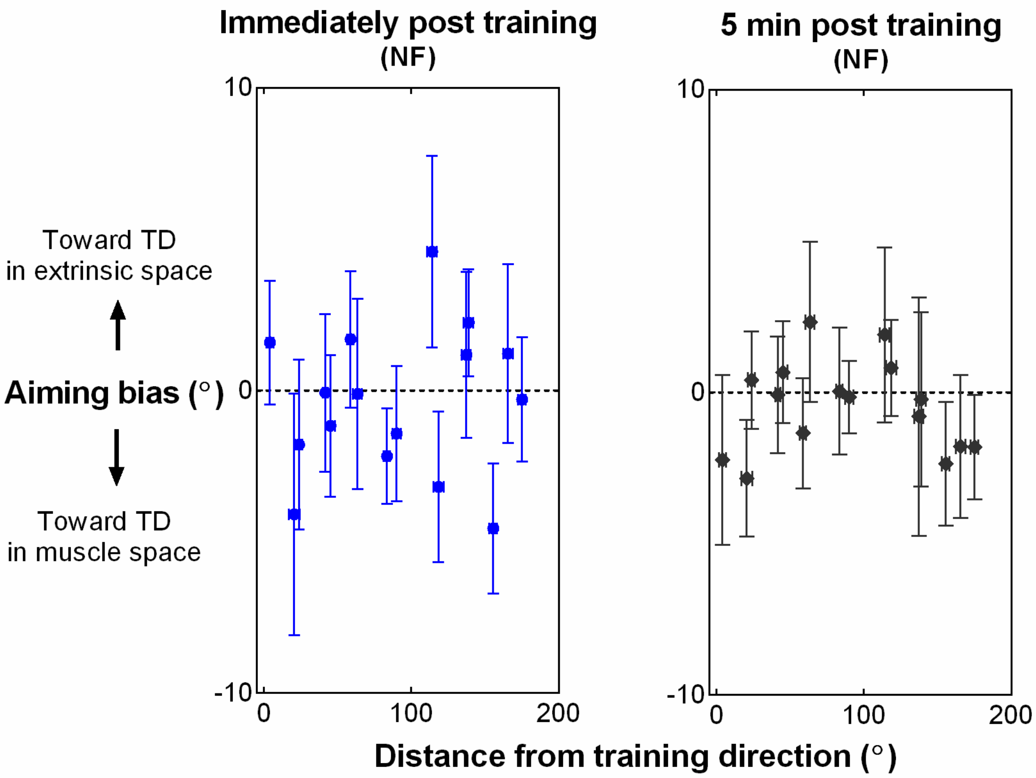


Figure 3.13. Aiming biases for the Neutral Flexion condition immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The reference frames in which the training direction is defined for both limbs in the neutral hand position are conflicting, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames. Positive angles indicate biases toward training direction in extrinsic space. Negative angles indicate biases toward the training direction in muscle space. No trend is observed for the aiming bias after ballistic training.

Aiming biases in conflicting reference frames

When results were pooled for both conditions in the neutral forearm orientation, no trend was observed for the aiming bias immediately post training and 5 min post training (p>0.05, Figure 3.14). Hence, ballistic training with the left hand did not result in any systematic aiming bias for the right hand when the reference frames in which the training direction is defined for both limbs was conflicting, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames.

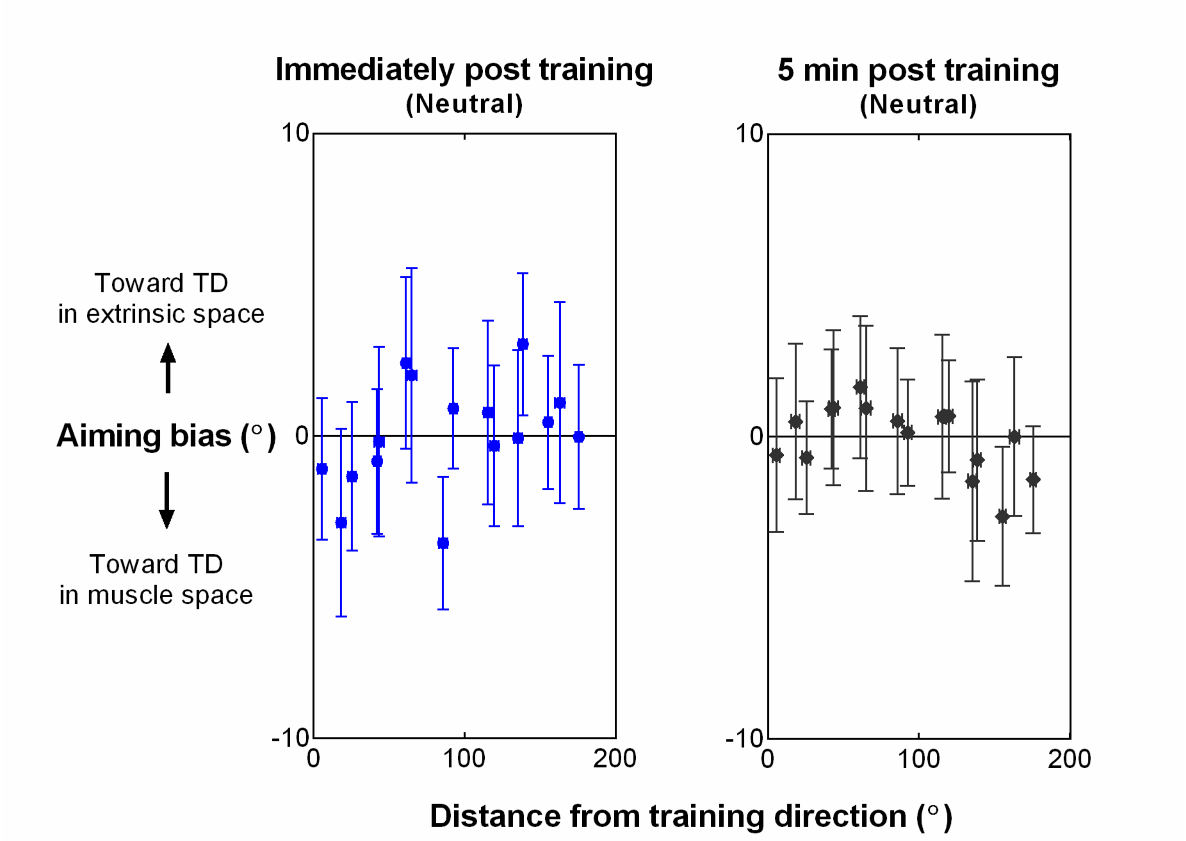


Figure 3.14. Aiming biases for pooled data in the neutral hand position immediately post training and 5 minutes post training. Aiming biases are plotted for each target as a function of distance from training direction (TD) in extrinsic space. The reference frames in which the training direction is defined for both limbs in the neutral hand position are conflicting, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames. Positive angles indicate biases toward training direction in extrinsic space. Negative angles indicate biases toward the training direction in muscle space. No trend is observed for the aiming bias after ballistic training.

3.4 Discussion

The results show that use-dependent aiming bias can transfer between limbs. A systematic aiming bias is evident when the training direction defined according to extrinsic, muscle, and training axis reference frames were congruent for both limbs in the pronated wrist posture. There is no specific bias trend seen when the training direction defined according to extrinsic, muscle, and training axis reference frames were conflicting for both limbs in the neutral wrist posture, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames. The aiming bias for the untrained limb is most likely influenced by the training direction defined in relation to the extrinsic space, muscle space, midline effect, and training axis with varied wrist postures. The trends of aiming bias observed in the untrained hand allow us to infer the reference frames in which use-dependent learning could be defined.

When both limbs were oriented in pronation, weightings from the midline effect were negligible because vertical extension and flexion training directions in a pronated position aligned with the midline axis of the body. Systematic biases after ballistic training suggested that use-dependent learning might be defined according to extrinsic, muscle space and/or training axis reference frames. A linear trend was observed immediately after ballistic training (Figure 3.9 to 3.11), such that aiming for target distances ±90° from training directions were positively biased toward the training direction, and target distances more than ±90° from the training direction were more negatively biased away from the training direction. Interestingly, the aiming biases at 5 minutes after training exhibited a cubic trend (Figure 3.11). The biphasic curve indicates aiming for targets closer to training direction had increasing positive biases from baseline toward a peak and decreased to baseline with increasing target distance. The aiming bias continued negatively to another peak at the opposite target from the training direction before returning to baseline along the midline. We speculate that the positive biases observed in both linear and cubic trends might be contributed by the effects from the extrinsic, muscle and/or training axis (same direction as the training direction) reference frames, while negative biases for the targets ±90° opposite to the training direction might be contributed by the effects from the training axis that is pulling at the opposite direction. Nevertheless, both linear and cubic trends portray a similar basic pattern of bias, given that the aiming data have a degree of variability involved. The cubic trend is also consistent with our theoretical predictions where no bias is expected at the training direction (0°) and at the target opposite to the training direction (180°). Therefore, our results supported the concept of weighted summation from the effects of extrinsic, muscle and/or training axis reference frames.

The lack of significant bias observed for the pooled data when the wrist was in a neutral wrist posture might be due to the opposing effects of the reference frames defined for the training direction for both limbs, i.e. extrinsic and/or training axis reference frames vs muscle, midline and/or training axis reference frames. Positive biases that might be contributed by the effects from extrinsic and training axis (same direction as the training direction) reference frames could be competing with the effects contributed by the muscle, midline and training axis (opposite to training direction) in negative opposite direction. Furthermore, the extent of weightings from the midline for the Neutral Extension and Neutral Flexion conditions is unknown. We could speculate that the midline effects might be greater for the Neutral Flexion condition because movements toward the midline axis with upper limbs are known to be easier and more stable to execute (Carson, 2005; Swinnen, 2002), and the flexor muscles are also more sensitive to plasticity changes than the extensors muscles (Vallence et al., 2012). As there is a systematic bias reported with the pronated wrist posture conditions, the lack of effect in the neutral posture might reflect a situation in which use-dependent aiming bias effects were eradicated by the conflicting reference frames.

The different extent of transfer in aiming bias that we observed with aligned and conflicting reference frame conditions suggest that use-dependent learning might be represented according to multiple coordinate frames. We also showed that reference frame alignment of movement representations between limbs influenced the transfer of aiming bias. To date, no study has directly investigated cross limb transfer from the perspective of reference frames with use-dependent learning. However, we could refer to previous visuomotor adaptation studies on cross limb transfer. Cross limb transfer is reported to be generalised according to extrinsic coordinates in visuomotor adaptation studies. For example, after training the right arm with a leftward shift in the visual feedback, the left arm showed rapid learning for leftward shifts by a reduction of direction error from the second trial (Sainburg & Wang, 2002; Taylor, et al., 2011). Wang & Sainburg (2004) also demonstrated that performance was facilitated when both arms were trained with identical altered visual feedback defined according to extrinsic reference frame. Most of the visuomotor studies were mostly performed in horizontal plane reaching contexts, there was a conflict between the required visuomotor recalibration defined according to intrinsic (joint- and muscle-based) versus extrinsic coordinates for the two different limbs for any given target location. Recently, Carroll et al. (2014) reported an immediate transfer of visuomotor adaptation, regardless of the hand trained, when the altered visual feedback had identical effects in the eye- (extrinsic) and joint-based (intrinsic) coordinates for both hands in the sagittal plane. These visuomotor adaptation studies suggested that the alignment of the extrinsic and intrinsic reference frames in which the learning is defined between limbs affects the transfer of error driven adaptation between hemispheres.

Although we could not directly compare our results (use-dependent learning) with these error-based learning studies, we could also suggest that the issue of reference frames is important for the transfer of use-dependent aiming bias based on our data. We suggest that use-dependent learning might be represented according to multiple coordinate frames and the access to the shared information arises from unilateral training is affected by the alignment of reference frames that represent the movement. This was supported by our data whereby systematic bias occurred only when the reference frames in which the training direction was defined for both limbs were congruent, but not when the reference frames in which the training direction was defined for both limbs were conflicting. There is a possibility that conflicting reference frames deter the transfer of use-dependent learning to the untrained limb due to opposing representations of the training direction. Therefore, this study suggests that the alignment of reference frames is important for effective interhemispheric interactions that induce cross limb transfer.

3.5 Conclusion

Our findings show that use-dependent aiming bias can be transferred between limbs and is represented according to multiple coordinate frames. The extent of aiming bias transfer is possibly a net result of the summation of weightings from extrinsic, muscle, midline and training axis reference frames, and is therefore affected by the alignment of these reference frames. Transfer of aiming bias is obvious when the training direction defined according to extrinsic, muscle, and training axis reference frames are congruent for both limbs in pronated wrist posture, but not in neutral wrist posture where these reference frames are conflicting. Our study thus suggests that the alignment of reference frames is important for effective interhemispheric interactions that induce cross limb transfer. In order to understand the role of reference frame conflicts in transfer of use-dependent learning, the representation of learned movements within the trained hemisphere with TMS should be examined.

CHAPTER FOUR

TMS-evoked twitch directions change with hand posture according to intrinsic coordinates

4.1 Introduction

Measurements of neural activity in the motor cortex of primates have revealed that individual neurons have broadly tuned preferred directions, such that their firing rates correlate with the direction of movement (Georgopoulos et al., 1982). The neurons fire rapidly for movements toward their preferred directions, and firing rates decrease progressively as movement directions depart from their preferred directions (Georgopoulos et al., 1986). Therefore, the actual movement direction can be predicted from a population vector extracted from a sample of active neurons in the motor cortex. The population vector is obtained by summation of vectors corresponding to individual neurons, where the direction components are the preferred directions of each neuron, and the magnitude components are the firing rates of each neuron, (Georgopoulos et al., 1988; Schwartz et al., 1988). Recent neuroimaging studies have shown that the human motor system exhibits similar preferred direction coding to those observed in primates during reaching tasks (Cowper-Smith et al., 2010; Eisenberg et al., 2010; Truccolo et al., 2008). However, neuroimaging methods have not been able to dissociate the reference frames in which the preferred direction is represented.

In the late 90s, Kakei and colleagues demonstrated that the reference frames of wrist movement can be dissociated into extrinsic versus muscle and joint coordinates by altering wrist postures between pronation and supination (Kakei, et al., 1999). For example, an upward movement is produced by the wrist extensor muscles when the wrist is in pronation, but by the wrist flexor muscles when the wrist is in supination. In their study, neuronal activities recorded from the primary motor cortex displayed extrinsic-like and muscle-like properties. The preferred direction of neurons with extrinsic-like properties remained relatively stable while the preferred direction of neurons with muscle-like properties shifted about 46° to 90° with the wrist. In the current study, we used Kakei's wrist paradigm to explore the movement representation at the primary motor cortex with transcranial magnetic stimulation (TMS). TMS has been widely used to assess the neurophysiological changes of the excitability in the human primary motor cortex (Hallett, 2007). The magnetic field from the coil induced a current underneath the scalp which both excites and inhibits the cortical neurons indirectly via synaptic inputs. TMS recruits several muscles and the resultant direction of force exerted at the limb endpoint provides information about the combination of muscles activated. Therefore, the force vectors allow us to study the reference frame in which the neurons activated by the TMS pulse in motor cortex collectively represent movement. In this study, we examine whether the muscles activated by stimulation of the same part of motor cortex changes as a function of posture, and whether the neurons activated by TMS represent extrinsic or intrinsic movement direction.

4.2 Methods

Twelve participants (10 males and 2 females; aged between 20 and 37 years old) with no recent shoulder, arm or wrist injuries were recruited for the study. Right handedness was confirmed by Edinburgh Handedness Inventory (Oldfield, 1971). Participants were screened for neurological disorders and contraindications in relation to the application of TMS with a medical history questionnaire. The study was approved by the Medical Research Ethics Committee of The University of Queensland. All participants were briefed on the experimental procedures. Written informed consent was given prior to the experiment which conformed to the Declaration of Helsinki.

4.2.1 Experimental protocol

The experiment involved participants to be quietly seated while single-pulse TMS was applied to the left motor cortex to elicit muscle twitches from the right wrist (Figure 4.1). Participants' right wrist was passively rotated between neutral and pronated postures in a randomised order. Four blocks of 20 TMS were applied to the left motor cortex in each respective hand position, i.e. 4 blocks of TMS in pronated posture and 4 blocks of TMS in neutral posture, for the experimental session.

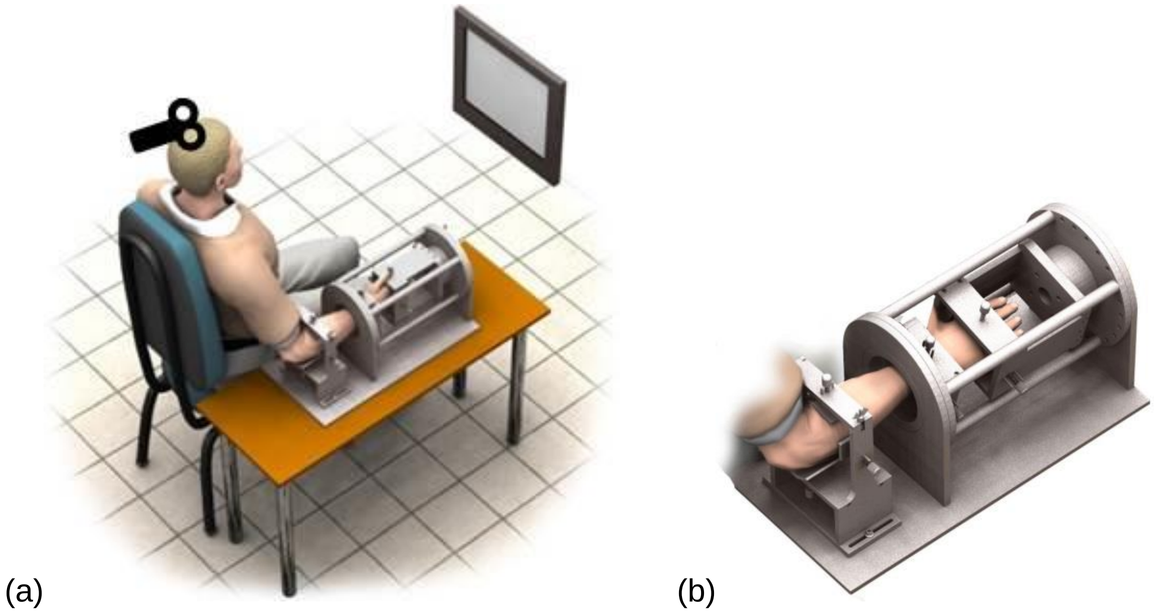


Figure 4.1. Experiment setup. Participant’s right forearm is secured in a custom-made hand manipulandum which allows passive rotation of wrist between (a) neutral (midway between supination and pronation) and (b) pronated postures.

4.2.2 Experimental setup

Participants seated with their right forearm secured in a custom-made hand manipulandum, described previously (de Rugy, et al., 2012), which allowed passive rotation of wrist between neutral (Figure 4.1a, midway between supination and pronation) and full pronation (Figure 4.1b). The elbow was kept at 110° with the forearm parallel to the table and supported by the custom-made hand manipulandum. The wrist was fixed by a series of adjustable metal clamps contoured around the metacarpal-phalangeal joints and around the wrist joint proximal to the radial head. Wrist forces in radial-ulnar deviation and flexion-extension directions were recorded by a six degree-of-freedom force transducer (JR3 45E15A-163-A400N60S, Woodland, CA) attached to the manipulandum. The neutral wrist posture corresponded to flexion/extension in horizontal plane and radial/ulnar deviation in vertical plane while the pronated wrist posture corresponded to flexion/extension in vertical plane and radial/ulnar deviation in horizontal plane (see Figure 4.2). Force data were sampled at a rate of 2 kHz with via a 16-bit National Instruments A/D boards (NI BNC2090A, NI USB6221, National Instruments Corporation, USA). A cursor that corresponded to the wrist forces were displayed in two-dimensions (x = flexion-extension, y = abduction-adduction) on the computer screen approximately 1.2 m away at eye level via a custom written Labview program (LabView2009, National Instrument, USA).

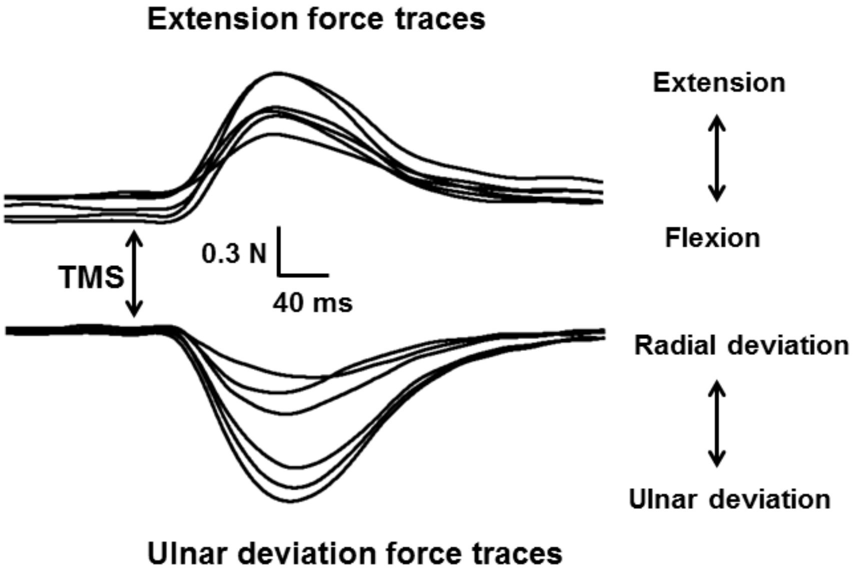


Figure 4.2. Example of raw force traces. Upper force traces represent extension-flexion directions. Lower force traces represent radial-ulnar deviation directions.

4.2.3 Transcranial magnetic stimulation

Single-pulse TMS was delivered via a 70 mm diameter figure-of-eight magnetic coil (Magstim 200, Magstim, UK) over the forearm area of the left motor cortex. The magnetic coil was held tangentially on the scalp with the handle pointing backwards and 45° away from mid-sagittal axis. The coil was moved to locate a hotspot whereby the strongest and most consistent twitches were identified online for each participant. The hotspot location for each participant was marked on the scalp to ensure the consistent coil placement throughout the experiment. The testing intensity was selected to elicit a muscle twitch of resultant magnitude between 0.5 N to 1 N.

4.2.4 Data analysis

The forces traces were recorded on disk and analysed offline via a customised Labview analysis program. The peak value of individual x and y force traces was manually selected in each trial for each participant. The resultant vectors of these x and y force traces were used to compute the twitch angles and training directions for each trial. Circular statistics, as described by Burgess-Limerick et al (1991), was used to calculate the twitch angles for each participant. The consistency of the twitch directions was described by a uniformity value, which was expressed between the values of 0 and 1, by dividing the sum of unit vectors by the total number of vectors. The closer the uniformity values to 1, the greater the consistency of twitch directions between trials. Uniformity values close to 0 indicate that twitch directions were randomly distributed. The uniformity of the twitches for each posture was compared to a Chi-square distribution to determine whether the mean direction was statistically reliable for each posture and each participant.

To check for twitch consistency across 4 blocks of TMS trials, i.e. 40 trials, across participants in each hand posture, the twitch angles of the same hand posture were normalised to the first block of TMS trials. One-way repeated measures using circular statistics were using to check for any difference across the 4 blocks of trials for each hand posture. The twitch angles were then pooled according to the same hand posture to increase the power of analysis.

To examine whether twitch direction rotated with the wrist joint, pairwise comparisons were made between the pronated and neutral postures with circular statistics. Due to the differences in the twitch responses across the participants, all twitch responses were normalised to their pronated posture for meaningful group comparisons. The differences in twitch angles between the pronated and neutral postures suggested the reference frame that the twitch direction was represented. Positive twitch angle deviation indicates twitches rotated with wrist joint according to joint- and muscle-based reference frame. All data were presented as mean ± SD, unless otherwise stated. Statistical significance was set at the 0.05 level.

4.3 Results

One-way repeated measures using circular statistics revealed that there was no significant difference found across the 4 blocks of trials for each hand posture ($p > 0.05$ for each hand posture). The twitch angles were pooled according to the same hand posture for subsequent group analysis to increase power.

4.3.1 Group data

The group mean twitch direction rotated 106 ± 51° ($n = 12$, uniformity = 0.61) as the wrist joint changed from the pronated to the neutral posture ($F_{1,22} = 33.28$; $p < 0.0001$; Figure 4.3). This suggests that the neurons activated by TMS represent movement direction according to joint and/or muscle-based reference frames.

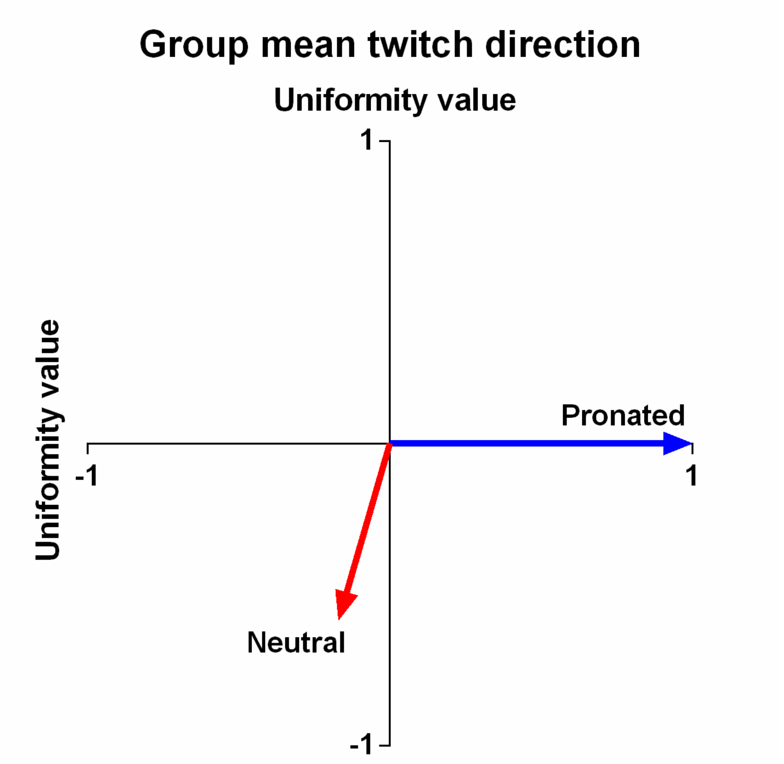


Figure 4.3. Group mean twitch direction plotted in extrinsic coordinates ($n = 12$). Normalised twitch direction in the pronated posture is shown with a blue arrow. Normalised twitch direction in the neutral posture is shown with a red arrow. The magnitude of the arrows indicates the uniformity of the twitches. The closer the uniformity value to 1 indicates the twitches between trials are consistent. Uniformity value closer to 0 indicates the twitches between trials are variable.

4.3.2 Single subject data

Typical twitches recorded from a participant in pronated and neutral wrist postures during the experiment were shown in Figure 4.4. Twitch directions recorded in pronated posture (twitches in blue) were different from the direction recorded in neutral posture (twitches in red).

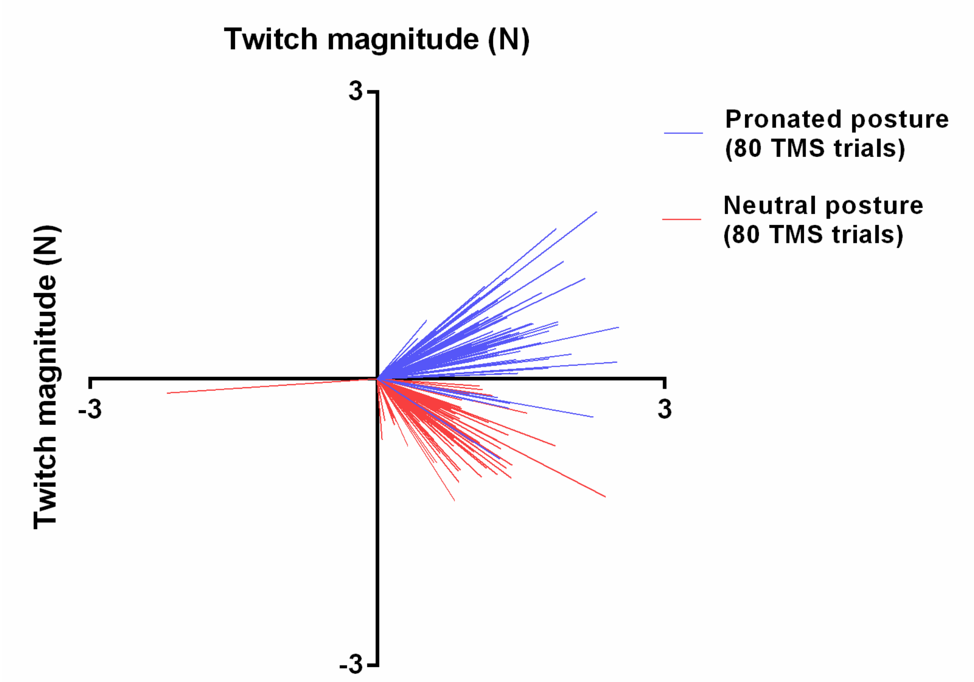


Figure 4.4. An example of TMS-evoked twitches plotted in extrinsic coordinates recorded from one participant. Twitches recorded in pronated posture are shown in blue. Twitches recorded in neutral posture are shown in red. Twitch directions rotated with the wrist joint between postures.

As neural representation may be variable between people, we examined resting twitch directions in pronated and neutral postures for each individual (Table 4.1). The differences in the twitch angles between pronated and neutral postures showed whether the twitches rotated with the wrist joint. A positive twitch angle difference, up to a maximum of 180°, would indicate that twitches rotated with the wrist joint according to joint and/or muscle coordinates. Twitch angle differences close to 0° indicate that twitches were consistent in extrinsic space in both wrist postures. Negative twitch angle differences between -90° and -180° render interpretation of twitch direction difficult because twitch directions align neither with the extrinsic nor muscle-based direction with the wrist posture.

Twitches from nine of twelve participants rotated significantly with the wrist joint according to a joint- and/or muscle-based reference frames when the wrist was passively rotated from pronated to neutral position (rotation range between 57° and 130°, highlighted in blue, Table 4.1). TMS-induced twitches from Subject 10 remained invariant in the extrinsic space (~9° of change between postures, Table 4.1, highlighted in pink). The twitches from Subjects 11 and 12 were inconsistent in both pronated and neutral postures (Table 4.1, highlighted in grey). In addition, the twitch directions from Subjects 11 and 12 in the neutral position pointed toward the quadrant furthest from that midway between the predicted muscle and extrinsic representations; such that changes in twitch angle from the pronated posture are uninterpretable.

Table 4.1. Normalised twitch directions in the pronated and neutral wrist positions for individual participants. Twitch angles are expressed in extrinsic coordinate frame.

Subject	**Pronated position**		**Neutral position**		**Twitch angle change (°)**	**Reference frame of twitches**
	Angle of twitch (°)	**Uniformity of twitch**	**Angle of twitch (°)**	**Uniformity of twitch**		
1	0	0.94^{+}	-84	0.97^{+}	84*	**Joint / Muscle**
2	0	0.94^{+}	-102	0.91^{+}	102*	
3	0	0.96^{+}	-129	0.83^{+}	129*	
4	0	0.94^{+}	-96	0.94^{+}	97*	
5	0	0.97^{+}	-57	0.94^{+}	57*	
6	0	0.85^{+}	-104	0.94^{+}	104*	
7	0	0.85^{+}	-102	0.67	102*	
8	0	0.77	-130	0.90^{+}	130*	
9	0	0.87	-89	0.69	89*	
10	0	0.59	9	0.90^{+}	-9	**Extrinsic**
11	0	0.65	-139	0.10	-139*	**Indeterminate**
12	0	0.48	-152	0.59	-152*	

Twitch angles are normalised to the pronated posture and expressed in the distance between 180° and -180°

$^{+}$ denotes a significant uniformity of twitches in each posture ($p < 0.05$). Uniformity value closer to 1 indicates the twitch directions are consistent. Uniformity value closer to 0 indicates the variability of twitch directions is high.

* denotes a significant shift in twitch direction from the pronated to neutral position ($p < 0.05$). Positive angle change indicates the twitches rotated with the wrist joint according to joint and/or muscle reference frames.

4.4 Discussion

The present study demonstrated that twitch directions rotated 106 ± 51° with the wrist according to a joint- and/or muscle-based reference frames from pronated to neutral posture. Single unit recordings showed the preferred directions of neurons in the motor cortex shifted as the posture of the wrist changed during voluntary reaching task (Kakei, et al., 1999; Kakei et al., 2003). Here, we showed that the involuntary muscle twitches evoked by TMS at the motor cortex changes as a function of wrist posture as well.

Kakei, et al. (1999, 2003) measured neuronal activity from primates' motor cortex during voluntary reaching and found three different types of neurons with namely extrinsic-like, extrinsic-like with gain and muscle-like properties that are approximately equally represented in the primary motor cortex. Extrinsic-like neurons encode movement direction in space independent of wrist muscle activity or joint movement. Extrinsic-like with gain neurons have similar encoding of movement direction in space but the magnitude of the firing is dependent on the forearm posture. Muscle-like neurons fire in relation to the activity of muscles, regardless of limb posture. The neuronal recordings showed that when the primates were trained to reach for targets at different wrist postures, these neurons fired according to their preferred direction. For example, when the wrist was rotated from pronation to supination, the preferred direction of the extrinsic-like neurons remained relatively stable while the muscle-like neurons shifted by about 46° to 90° with the wrist. While there is a limitation in measuring the neuronal activities in humans, TMS is used to activate cortical neurons directly and indirectly via the induced current underneath the coil to reflect the excitability of both cortical and spinal synapses (Kernell & Hultborn, 1990; Rothwell, 1997; Terao & Ugawa, 2002; Weber & Eisen, 2002). TMS could therefore activate both the extrinsic-like and muscle-like neurons identified by Kakei and colleagues. Our TMS results showed that involuntary muscle force twitches followed the changes in the wrist posture, i.e. a rotation between 61 % and 174 % for a 90° wrist rotation from pronated to neutral posture. We suggest that the neurons activated by TMS that contributed to the change in twitch directions during wrist rotation are the muscle-based neurons recorded by Kakei, et al. This speculation is most likely possible because the peripheral responses to TMS are primarily due to activation of corticospinal output cells with the limb at rest. The peripheral responses are not modulated at the spinal level during rest because there is no firing of corticospinal output cells that correlate with the extrinsic direction. Hence, we could suggest that the extrinsic-like neurons that Kakei recorded could be projecting to other places.

4.5 Conclusion

The direction of involuntary muscle twitches evoked by TMS closely followed the wrist according to a joint and/or muscle reference frames when the wrist was altered between pronated and neutral posture. Although TMS could, in theory, activate both extrinsic-like and muscle-like neurons, our data suggest that the neurons activated by TMS at rest are most likely the muscle-based neurons. We suggest that the extrinsic-like neurons identified by Kakei et al could be projecting to other areas. However, such representation of movement at rest could change after training. A shift of the TMS-evoked twitches training could provide us with more understanding of the role of motor cortices in movement representation.

CHAPTER FIVE

TMS-evoked twitches can shift toward ballistic training direction either according to extrinsic or muscle-based coordinates depending on the individual

5.1 Introduction

A classic example of use-dependent learning is demonstrated by a shift in the twitch direction evoked by Transcranial Magnetic Stimulation (TMS) toward the training direction after repetitive thumb ballistic movements (Classen, et al., 1998; Giacobbe, et al., 2011; van Elswijk, et al., 2008). Apart from a change in the direction of artificially-evoked twitches, use-dependent learning arising from repetitive aiming also reduces the variability of voluntary movements (Verstynen & Sabes, 2011) and biases subsequent voluntary movements toward the repeated direction (Selvanayagam, et al., 2012a; Verstynen & Sabes, 2011). For example, Selvanayagam et al. (2012a) demonstrated that a single session of 40 ballistic isometric contractions repeated in one direction caused movements aimed toward target locations which were more than 90° away to bias toward the practiced direction.

Use-dependent learning effects are likely to be mediated by the strengthening of synapses in the network of neurons contributing to the movement (Bütefisch et al., 2004; Carroll et al., 2001; Selvanayagam, et al., 2012a; Verstynen & Sabes, 2011). However, it remains unclear whether use-dependent learning within the corticospinal tract is represented according to the extrinsic coordinates of the training direction in external space, or according to the intrinsic coordinates of the joint torques or muscle activations involved in the training.

To explore the reference frame in which use-dependent learning occurs, subjects performed an isometric training task with the wrist in a given forearm posture, and we tested the resulting use-dependent adaptation by measuring the direction of twitches evoked by TMS with the wrist in different postural orientations. Dissociation of reference frames into extrinsic and intrinsic coordinates can be achieved by rotating the wrist about the supination-pronation axis (de Rugy, et al., 2012; Kakei, et al., 1999). For example, an upward movement is produced by the wrist extensor muscles when the forearm is in pronation, but by the wrist flexor muscles when the forearm is in supination. We have shown that resting twitch directions rotated with passive changes in the orientation of the wrist joint according to a joint and/or muscle reference frame in Chapter 4. In other words, the extrinsic direction of resting twitch directions was different for different wrist postures, whereas the muscle-based twitch directions were consistent irrespective of posture.

In this chapter, we oriented the wrist in two separate postures, a posture used for training and the posture used for generalisation, and examined resting twitch directions in both postures before and after a session of ballistic training. We chose the two wrist postures so that a shift in twitches towards one direction would imply an extrinsic representation of use-dependent learning, whereas a shift in the opposite direction would imply a muscle-based representation. The purpose was to characterise the reference frame in which the neural elements activated by TMS (i.e. the corticospinal tract) represent use-dependent learning.

5.2 Methods

Thirteen right-handed participants (7 males and 6 females; aged between 20 and 37 years old) with no recent shoulder, arm or wrist injuries volunteered for the study. Right-handedness was confirmed with Edinburgh Handedness Inventory (Oldfield, 1971). A medical questionnaire was used to screen all participants for neurological disorders and contraindications in relation to the application of TMS. The study was approved by the Medical Research Ethics Committee of The University of Queensland. All participants were briefed on the experimental procedures and written informed consent was given prior to the experiment which conformed to the Declaration of Helsinki.

5.2.1 Experimental protocol

In this study we were primarily interested in the reference frames in which adaptation occurs after ballistic training by examining the resting TMS-evoked twitches in training and testing wrist postures. Our previous unpublished data (refer to Chapter 4) revealed that resting twitches rotated with the wrist joint. Therefore, if a baseline twitch evoked in the training (pronated) position was along the horizontal axis (i.e. 0° as depicts in Figure 5.1a), the direction of twitches should rotate to approximately -135° in the testing (semi-supinated) position (refer to Figure 5.1a). To allow the dissociation of reference frames in the testing position, ballistic training was carried out in a pronated posture and the training direction was set at 45° counter-clockwise from each individual’s mean baseline twitch direction in the semi-supinated posture. Note that this direction was estimated online during data collection on the basis of an automatic algorithm, and differed slightly from the true twitch direction measured post hoc via manual twitch peak selection (Figure 5.1b).

When ballistic training was performed in the pronated posture, there was a single training direction defined according to both extrinsic and muscle-based reference frames. However when the wrist was rotated to test generalisation, the training direction defined according to an extrinsic reference frame differed from the training direction defined according to a muscle-based reference frame (see Figure 5.1c). The training direction defined according to muscle space rotated with the wrist joint, whereas the training direction defined according to extrinsic space remained unchanged in both wrist positions. If training caused a counter-clockwise change in the direction of twitches evoked in the generalisation testing position (i.e. semi-supinated posture) toward the extrinsic training direction, it would imply that use-dependent learning was represented in an extrinsic reference frame. In contrast, if ballistic training induced a clockwise rotation towards the direction in which the trained muscles pull, it would suggest that use-dependent learning was represented in a muscle-based reference frame (see Figure 5.1c).

The experiment began with the measurement of resting TMS-evoked twitches from the right wrist, in both training (pronated) and testing (semi-supinated) positions (Figure 5.1a). The order of wrist positions was fully randomised for each participant. Two blocks of 20 resting twitches were recorded for each wrist posture (i.e. 40 trials per posture) with a variable inter-stimulus interval between 4s and 6s. After the baseline twitch data collection, an isometric ballistic training protocol which consisted of four blocks of ten ballistic contractions toward a single training direction was performed in the training (pronated) position (Figure 5.1b). A rest period of three minutes was provided between each block of contractions to avoid muscle fatigue. Immediately after the training (approximately 30 s after the last block of ballistic training), resting twitch directions were again measured in the training and testing positions (Figure 5.1c). Similarly, two blocks of 20 resting twitches were recorded for each wrist posture with variable inter-stimulus interval between 4 s and 6 s, and the order of wrist postures was fully randomised and counterbalanced for each participant. The total duration of post training twitch recording for both wrist postures was approximately six minutes.

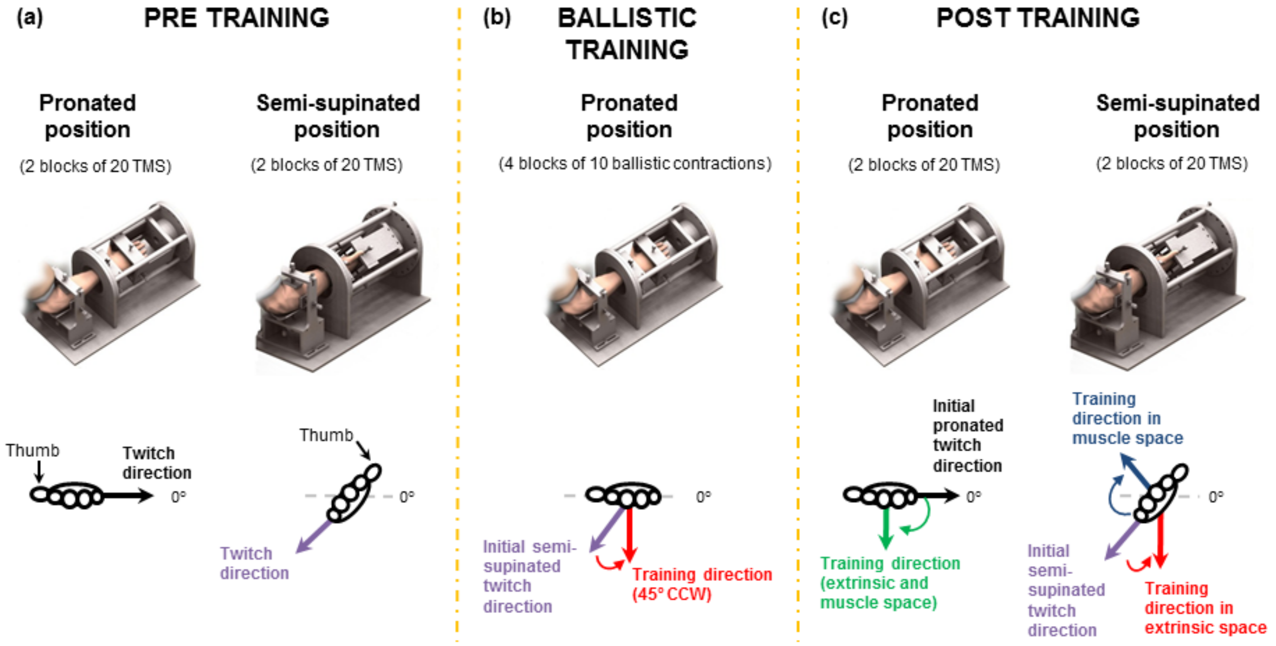


Figure 5.1. Experimental protocol. The upper halves of the figures show the wrist postures in the custom-made hand manipulandum. Lower halves of the figures show schematic illustrations of the twitch directions at different wrist postures. (a) Baseline twitch directions were measured in training (pronated) and testing (semi-supinated) positions. (b) The training direction was set at 45° counter-clockwise (CCW) from the estimated mean baseline twitch direction in the semi-supinated position, but training was performed in the pronated position. (c) Resting twitches were recorded immediately after the ballistic training in both wrist postures to examine the shift in twitch direction.

5.2.2 Experimental setup

Participants sat approximately 1.2 m in front of a computer screen displayed at eye level. The right forearm was secured in a custom-made hand manipulandum, described previously (de Rugy, et al., 2012), which allowed passive rotation of wrist postures between full pronation (Figure 5.2b, training position) and semi-supination (Figure 5.2c, testing position, a difference of 135° from full pronation). The elbow was kept at 110° with the forearm parallel to the table and supported by the custom-made hand manipulandum as shown in Figure 5.2a. The wrist was fixed by a series of metal clamps contoured around the metacarpal-phalangeal joints and around the wrist proximal to the radial head. Wrist forces in radial-ulnar deviation and flexion-extension directions were recorded via a six degree-of-freedom force transducer (JR3 45E15A-163-A400N60S, Woodland, CA) attached to the manipulandum. Force data were sampled at a rate of 2 kHz with via a 16-bit National Instruments A/D boards (NI BNC2090A, NI USB6221, National Instruments Corporation, USA). A cursor that corresponded to the wrist forces was displayed in two-dimensions (x = flexion-extension, y = abduction-adduction) on the computer screen via a custom written Labview program (LabView2009, National Instrument, USA).

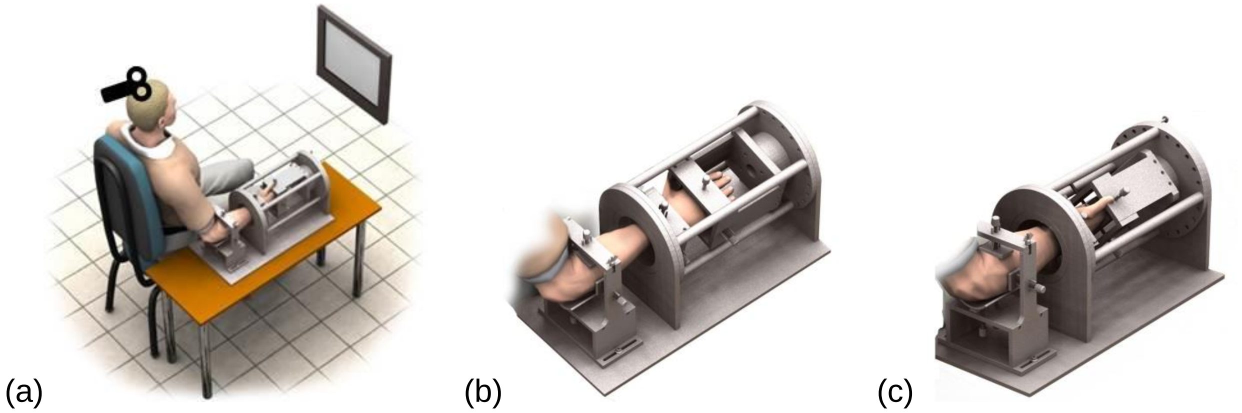

(a) (b) (c)

Figure 5.2. Experimental setup. (a) Typical experimental setup in which a participant’s right forearm is secured in a custom-made hand manipulandum. (b) Participants’ right forearm in pronated (training) position and (c) semi-supinated (testing) position.

5.2.3 Transcranial magnetic stimulation

Single-pulse TMS was delivered via a 70 mm diameter figure-of-eight magnetic coil (Magstim 200, Magstim, UK) over the forearm area of the left motor cortex. The magnetic coil was held tangentially on the scalp with the handle pointing backwards and 45° away from mid-sagittal axis. The coil was moved to locate a hotspot whereby the strongest and most consistent twitches were identified online for each participant. The hotspot location for each participant was marked on the scalp to ensure the consistent coil placement throughout the experiment. The testing intensity was selected to elicit a muscle twitch of resultant magnitude between 0.5 N to 1 N.

5.2.4 Ballistic contractions

Prior to beginning the experiment, participants performed ten familiarisation trials, to encourage them to produce their maximum voluntary contractions (MVC) toward the designated training direction. A standardised target, which required 225 N of force to acquire, was set for all participants. Each contraction trial began with a white visual reference line from the origin to the target force level depicting the training direction on the computer screen together with an audio cue (0.25 s, 400 Hz). The audio cue acted as a signal for the participants to start the contraction toward the training direction. Participants had to start the contraction as quickly and as hard as possible to the cue. A red real-time force feedback line from the origin corresponding to the force exerted was displayed on the screen. Participants had to maintain their contraction with minimum possible deviation between the white reference line and the force feedback (i.e. the red line). A 2-second continuous audio cue of 900 Hz was played immediately following the low frequency trigger cue, to prompt participants to sustain their contractions before relaxation. The cursor gain was set so that 300 N was required to reach the edge of the display for all participants.

5.2.5 Data analysis

The force traces were recorded on disk and analysed offline via a customised Labview analysis program. The peak value of individual x and y force traces was manually selected. The resultant vectors of these x and y force traces were used to compute the twitch angles, training directions and training force magnitudes for each trial. Circular statistics as described in Burgess-Limerick et al (1991) was used to calculate the twitch responses for each participant. The consistency of the twitch directions was described by a uniformity value, which was expressed between the values of 0 and 1, by dividing the sum of unit vectors by the total number of vectors. The closer the uniformity values to 1, the greater the consistency of twitch directions between trials. Uniformity values close to 0 indicate that twitch directions were randomly distributed. The uniformity of the twitches for each posture was compared to a Chi-square distribution to determine whether the mean direction was statistically reliable for each posture and each participant. Positive angles were defined in a counter-clockwise direction from the horizontal axis. Likewise, negative angles indicated clockwise direction in relation to the horizontal axis.

To check for twitch consistency across 2 blocks of TMS trials, i.e. 40 trials, across participants in each hand posture, the twitch angles of the same hand posture were normalised to the first block of TMS trials. Pairwise comparisons using circular statistics were using to check for any difference between 2 blocks of trials for each hand posture. The twitch angles were then pooled according to the same hand posture to increase the power of analysis. Due to the differences in the twitch responses across the participants, all twitch responses were normalised to the pronated posture for meaningful comparisons. All data were presented as mean ± SD, unless otherwise stated. Statistical significance was set at the 0.05 level.

Pre-training data

Pre-training twitch angles were calculated trial-by-trial in both training (pronated) and testing (semi-supinated) positions for each participant. Pairwise comparisons using circular statistics were used separately for the pronated (training) and semi-pronated (testing) postures to determine whether there were significant differences between the first and second block of twitch angles. The two blocks of twitch angles would be pooled for subsequent analysis if there was no significance found between blocks. In order to establish whether the twitch direction rotated with wrist joint, pairwise comparisons were made between the pronated and semi-supinated postures.

Exclusion criteria for training

The characteristics of the baseline twitches from three of the thirteen participants (see Table 5.1, Subject 11 to 13) made it impossible for us to draw inferences about the coordinate frame of adaptation due to training for these people. According to the design of the study, the differences in baseline twitch direction between training (pronated) and testing (semi-supinated) positions had to be at least 45° in order to dissociate the reference frame of adaptation. Subjects 11 and 12 had twitch angle changes between wrist postures that were less than 45°. The twitches of Subject 11 were invariant in extrinsic space regardless of wrist posture, whereas Subject 12 exhibited inconsistent twitch directions (uniformity below 0.5) in both wrist postures (see Table 5.1). The twitches from Subject 13 in the semi-supinated position pointed toward the quadrant furthest from that midway between predicted muscle and extrinsic representations; such that changes in twitch angle from this baseline direction with training would be uninterpretable. Data from these three subjects were therefore not included in the analysis to determine the coordinate frame of adaptation.

Post training data

The twitch angles from the remaining ten of thirteen participants were then analysed as a group and individually post training. The shift of the twitch angles after ballistic training was examined in a group level and individually. Pairwise comparisons were made within each hand posture, i.e. 1) between the pronated (training) in the pre training and the pronated (training) in the post training, and 2) between the semi-pronated (testing) posture in the pre training and the semi-pronated (testing) in the post training.

For each participant, the differences between pre-training and post training twitch directions for each posture were calculated. The percentage change in twitch direction before and after ballistic training was calculated by expressing the twitch angle change in relation to the angle between the training direction and the baseline twitch angle in each posture (Figure 5.3). The polarity of twitch angle changes suggested the reference frame according to which the twitches had shifted. Positive angle change percentages indicate that the twitches shifted toward the training direction for the training wrist posture, and that twitches shifted toward the extrinsic training direction in the generalisation testing posture.

Negative angle change percentages indicate that the twitches shifted away from the training direction for the training wrist posture and toward the intrinsic training direction in the generalisation testing posture.

(a)

$$\%\ \text{of angle change} = \frac{\text{Pre-training twitch angle} - \text{Post training twitch angle}}{\text{Pre-training twitch angle} - \text{Training direction angle}} \times 100\%$$

(b)

$$\%\ \text{of angle change} = \frac{\text{Post training twitch angle} - \text{Pre-training twitch angle}}{\text{Training direction angle} - \text{Pre-training twitch angle}} \times 100\%$$

Figure 5.3. Formulas for calculating the percentage of angle change after ballistic training for (a) training (pronated) position and (b) testing (semi-supinated) position. Differences in the twitch direction change were calculated with the reference to the training direction. The polarity of twitch angle changes after training suggests the reference frame in which adaptation was represented. Positive angle change percentages indicate that the twitches shifted toward the training direction for the training wrist posture, and that twitches shifted toward the extrinsic training direction in the generalisation testing posture. Negative angle change percentages indicate that the twitches shifted away from the training direction for the training wrist posture and toward the intrinsic training direction in the generalisation testing posture.

Training force exerted and training directions

The force exerted for each block of training and across four blocks of training was averaged for each participant (n = 10). One-way repeated measures ANOVA was used to determine whether the average force exerted differed between each training block. The group mean training direction was also calculated and expressed in both extrinsic and muscle coordinates.

5.3 Results

Pairwise comparisons revealed that there were no significant differences between the first and second block of resting twitches measured within each hand posture in the pre and post training ($p > 0.05$ for all comparisons in each hand posture). Therefore, the two blocks of resting twitches for each wrist positions in pre and post training were pooled for subsequent analysis to increase the power.

5.3.1 Group baseline data

Twitch direction changes between training and testing positions (n=13)

The overall group mean twitch direction rotated 109 ± 50° (n = 13, uniformity = 0.61) with the wrist joint from the training (pronated) to testing (semi-supinated) position according to joint- and/or muscle-based reference frames (Figure 5.4, $F_{1,24} = 38.23$; $p < 0.0001$).

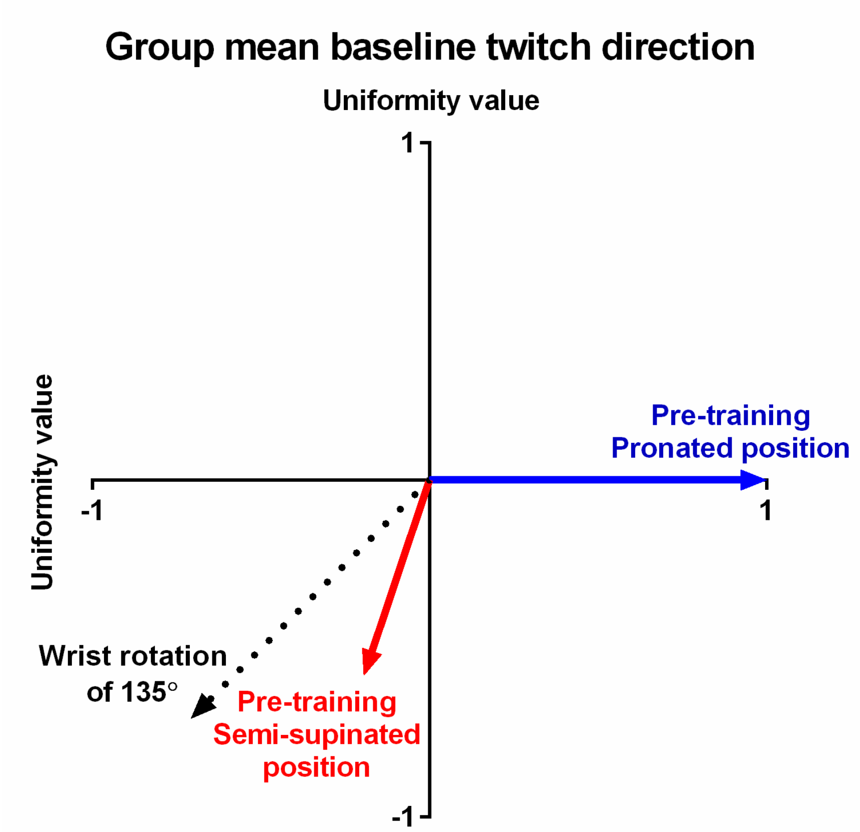


Figure 5.4. Normalised group mean baseline twitch directions plotted in extrinsic coordinates (n = 13) before ballistic training. Twitches rotated 109 ± 50° with the wrist joint from the training (pronated) position to testing (semi-supinated) position according to joint- and/or muscle-based reference frames. Black dotted line depicts the wrist rotation of 135° from training to testing position.

Twitch direction changes between training and testing positions for subjects included in the main experiment

After excluding the three participants who could not be studied for the main experiment, the group mean baseline twitch direction rotated 118 ± 30° (n = 10; subject 1 to 10, uniformity = 0.86) with the wrist joint from the training (pronated) to testing (semi-supinated) position according to joint and/or muscle reference frames (Figure 5.5, $F_{1,18} = 117.63$; $p < 0.001$).

5.3.2 Pre-training - Single subject data

Baseline twitch direction changes between training and testing positions

As neural representation may be variable between people, we examined individual pre-training twitch directions in training (pronated) and testing (semi-supinated) positions. The differences in the twitch angles between training and testing positions showed whether the twitches rotated with the wrist joint. A positive twitch angle difference, up to a maximum of 180°, would indicate that twitches rotated with the wrist joint according to the joint and/or muscle coordinates. Twitch angle differences close to 0° indicate that twitches were consistent in extrinsic space in both wrist postures. Negative twitch angle differences between -90° and -180° render interpretation of twitch direction difficult because twitch directions align neither with the extrinsic nor muscle-based direction in the training wrist posture.

Twitch directions from ten participants rotated significantly with the wrist joint according to joint and/or muscle reference frames when the wrist was passively altered from the training (pronated) to the testing (semi-supinated) position (rotation range between 73° and 179°, Table 5.1, highlighted in blue). The twitch directions of the excluded participants (refer to section 5.2.5) were shown for reference in Table 5.1.

Table 5.1. Normalised baseline pre-training twitch directions in the training (pronated) and testing (semi-supinated) positions for individual participants. Twitch angles are expressed in extrinsic coordinate frame.

Subject	Training position (Pronated)		Testing position (Semi-supinated)		Twitch angle change (°)	Reference frame of twitches
	Angle of twitch (°)	Uniformity of twitch	Angle of twitch (°)	Uniformity of twitch		
1	0	0.97^+	-146	0.87^+	146*	Joint / Muscle
2	0	0.95^+	-151	0.95^+	151*	
3	0	0.94^+	-95	0.91^+	95*	
4	0	0.87^+	-98	0.84^+	98*	
5	0	0.83^+	-118	0.90^+	118*	
6	0	0.96^+	-96	0.76	96*	
7	0	0.79	-124	0.95^+	124*	
8	0	0.82	-105	0.62	105*	
9	0	0.76	-73	0.79	73*	
10	0	0.69	179	0.90^+	-179*	
11	0	0.81	-12	0.89^+	12	Extrinsic
12	0	0.49	-43	0.48	43*	Indeterminate
13	0	0.63	101	0.77	-101*	

Twitch angles are normalised to pronated posture and expressed in the distance between 180° and -180°

$^+$ denotes a significant uniformity of twitches in each posture ($p < 0.05$). Uniformity value closer to 1 indicates the twitch directions are consistent. Uniformity value closer to 0 indicates the variability of twitch directions is high.

* denotes significant shift in twitch direction from the pronated and semi-supinated posture (p <0 .05).

Positive angle change indicates the twitch direction rotated with the wrist joint according to joint and/or muscle reference frames.

5.3.2 Ballistic training force and direction

The overall mean force exerted by all of the participants (n = 10) for 4 blocks of ballistic training was 66 ± 27N. There was no difference in force exerted between each training block ($F_{3, 24} = 0.29$; $p = 0.84$), suggesting that training produced minimal muscle fatigue.

The group mean training direction was 40 ± 30° counter-clockwise from the baseline twitch direction recorded in semi-pronated posture. Because the training direction was estimated online during data collection on the basis of an automatic algorithm, the actual training direction was slightly different after calculation from the true twitch direction measured post hoc via manual twitch peak selection.

5.3.3 Post training

Group data

Ballistic training induced a significant shift of 25 ± 27° (uniformity = 0.89, 32 % shift) toward the training direction with the wrist in the training (pronated) position ($F_{1, 18} = 7.38$; $p = 0.014$). In this case, the training direction defined according to extrinsic and muscle spaces are aligned, so it is not possible to distinguish the coordinate frame representations of adaptation. When the wrist posture was altered to the generalisation testing (semi-supinated) position, the training direction defined according to extrinsic and muscle reference frames were dissociated. However, there was no significant shift in the twitch direction from baseline (Figure 5.5, mean shift = 4 ± 32°; 9 % shift, $F_{1, 18} = 0.05$; $p = 0.86$).

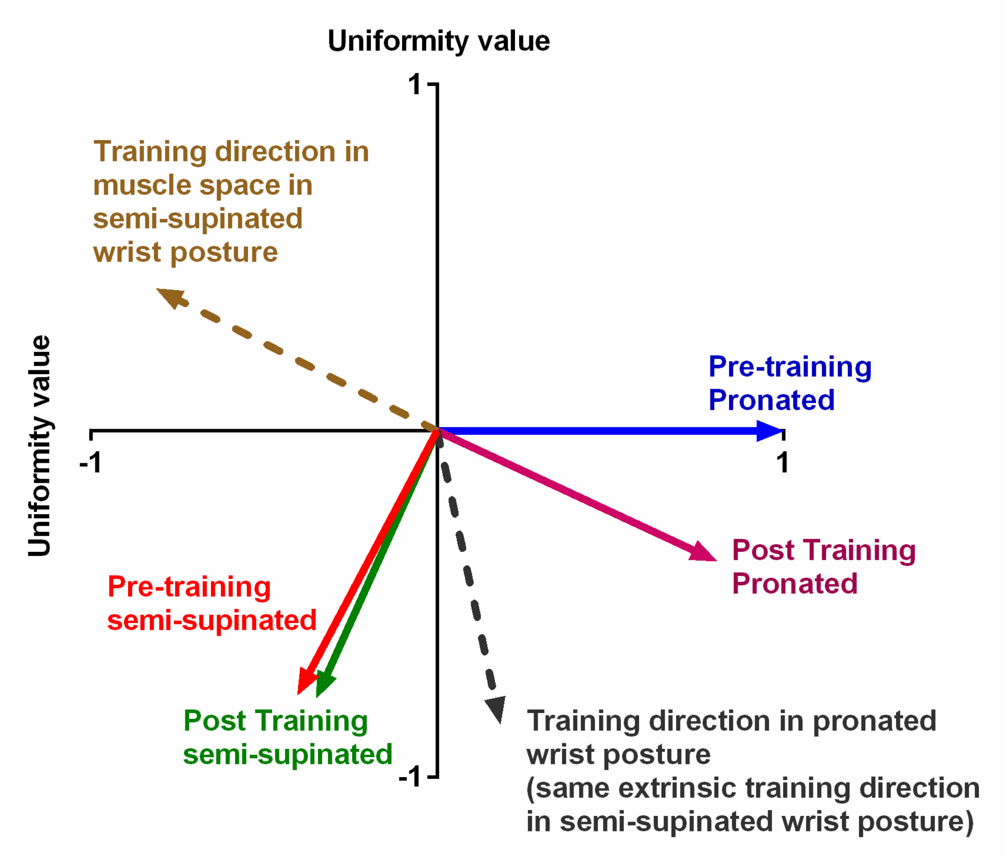


Figure 5.5. Normalised group mean twitch directions plotted in extrinsic coordinates (n = 10). Training direction is plotted in both extrinsic (black dashed line) and muscle-based (brown dashed line) reference frames for semi-pronated wrist posture. Ballistic training induced a significant shift toward the training direction in the training (pronated) position, but no significant shift in the testing (semi-pronated) position.

Single subject data - Post training twitches in pronated training position

Ballistic training induced significant shifts in twitch directions toward the training direction for seven out of ten participants in the training (pronated) posture (Table 5.2, highlighted in blue, significant shifts between 17 % and 71 %). Twitch directions from three of ten participants did not show a significant shift after ballistic training, with twitches that shifted by 4 % and 15 % toward training direction for two participants, and twitches that shifted away (12 %) from the training direction for one participant (highlighted in pink).

Table 5.2. Normalised post training twitch direction in the training (pronated) position for individual participants. Twitch angles and training direction are expressed in extrinsic coordinate frame.

Subj	Pronated (Pre-training)		Pronated (Post Training)		Training Direction (°)	% of angle change	Direction of shift
	Angle of twitch (°)	Uniformity of twitch	Angle of twitch (°)	Uniformity of twitch			
2	0	0.95^+	-29	0.95^+	-101	28*	**Toward training direction according to extrinsic and muscle space**
3	0	0.94^+	-13	0.95^+	-74	17*	
4	0	0.87^+	-17	0.97^+	-51	34*	
6	0	0.96^+	-40	0.97^+	-67	60*	
1	0	0.97^+	-34	0.36	-116	29*	
5	0	0.83^+	-35	0.81	-75	46*	
10	0	0.69	-98	0.88^+	-137	71*	
7	0	0.79	-3	0.80	-79	4	
9	0	0.76	-4	0.93^+	-28	15	
8	0	0.82	7	0.90^+	-55	-12	**Away from training direction**

Twitch angles are normalised to pronated posture and expressed in the distance between 180° and -180°.
$^+$ denotes a significant uniformity of twitches in each posture ($p < 0.05$). Uniformity value closer to 1 indicates the twitch directions are consistent. Uniformity value closer to 0 indicates the variability of twitch directions is high.
* denotes significant shift in twitch direction before and after ballistic training in the pronated posture ($p < 0.05$).
Positive % of angle change indicates the twitch direction shifted toward the training direction according to both extrinsic and muscle space.
Negative % of angle change indicates the twitch direction shifted away from the training direction.

Single subject data - Post training twitches in semi-pronated testing position

Ballistic training induced an adaptation that was either represented in an extrinsic reference frame (n = 5) or in a muscle-based reference frame (n = 5) for different subjects (see Table 5.3). For the five participants who showed adaptation consistent with representation in an extrinsic reference frame, three participants showed a significant shift in twitches toward the training direction while the shift in twitches for the other two participants did not reach a statistically significant level (Table 5.3, significant shift highlighted in pink). Likewise, for the other five participants who had adaptation consistent with a muscle-based reference frame, three participants showed a significant shift in twitch direction while the shift in twitches for the other two participants also did not reach a statistically significant level (Table 5.3, significant shift highlighted in blue).

Table 5.3. Normalised post training twitch direction in the testing (semi-supinated) position for individual participants. Twitch angles and training direction are expressed in extrinsic coordinate frame.

	Semi-supinated (Pre-training)		**Semi-supinated (Post Training)**		**Training Direction (°)**	**% of angle change**	**Direction of shift**
Subj	**Angle of twitch (°)**	**Uniformity of twitch**	**Angle of twitch (°)**	**Uniformity of twitch**			
1	-146	0.87^{+}	-130	0.86^{+}	-116	51*	**Toward training direction according to extrinsic space**
2	-151	0.95^{+}	-114	0.90^{+}	-101	75*	
3	-95	0.91^{+}	-81	0.84^{+}	-74	67*	
6	-96	0.76	-91	0.70	-67	19	
8	-105	0.62	-101	0.63	-55	7	
5	-118	0.90^{+}	-135	0.93^{+}	-75	-41*	**Away from training direction according to muscle space**
7	-124	0.95^{+}	-134	0.97^{+}	-79	-22*	
10	179	0.90^{+}	163	0.98^{+}	-137	-35^{*}	
4	-98	0.84^{+}	-104	0.97^{+}	-51	-12	
9	-73	0.79	-77	0.86^{+}	-28	-9	

Twitch angles are normalised to pronated (training) posture. Twitch angles in the testing position are calculated in relation to the training position and expressed in the distance between 180° and -180°.

$^{+}$ denotes a significant uniformity of twitches in each posture ($p < 0.05$). Uniformity value closer to 1 indicates the twitch directions are consistent. Uniformity value closer to 0 indicates the variability of twitches is high.

* denotes significant shift in twitch direction before and after ballistic training in semi-supinated posture ($p < 0.05$).

Positive % of angle change indicates the twitch direction shifted toward the training direction according to extrinsic space.

Negative % of angle change indicates the twitch direction shifted away from the training direction according to muscle space.

5.4 Discussion

Pre-training twitch directions

The mean baseline twitch direction changed by 109 ± 50° when the wrist was passively rotated by 135° between pronated and semi-supinated postures. The mean twitch direction therefore shifted according to joint and/or muscle reference frames between wrist postures. Our findings are consistent with our previous results reported in Chapter 4, where twitch direction rotated closely with the wrist joint.

Post training twitches in the training posture

When twitches were evoked by TMS in the training (pronated) posture, the ballistic training direction defined according to both extrinsic and muscle coordinates was identical. A training related adaptation was shown by a significant shift of 25 ± 27° (uniformity =

0.89) in the group mean baseline twitch direction toward training direction. Our results are consistent with previous data from our laboratory, where twitch direction shifted significantly after training was performed at a distance of 90° away from the baseline twitch direction (Selvanayagam, et al., 2011). While some previous TMS studies, which involved repetitive ballistic thumb movements, reported that twitch directions shifted toward the training direction after 30 minutes of training at 180° away from baseline (Bütefisch et al., 2000; Bütefisch, et al., 2004; Classen, et al., 1998; Giacobbe, et al., 2011; Kaelin-Lang et al., 2005), we showed early neural adaptation occurred with a session of just 40 ballistic wrist contractions at 90° distance away from baseline (Selvanayagam, et al., 2011). The shift in twitch directions is likely to reflect the reweighting of connectivity among the forearm muscles to favour net force production in the training direction (Selvanayagam, et al., 2012a).

Despite the fact that TMS-evoked twitches are possibly amenable to adaptation occurring at subcortical levels such as muscle and spinal effects, a cortical contribution is evident (Bütefisch, et al., 2000; Bütefisch, et al., 2004; Classen, et al., 1998). This is supported by the findings from Classen, et al. (1998) whereby the directional change of transcranial electrical stimulation (TES) evoked movements was substantially smaller than TMS-evoked movements after ballistic repetitive thumb movements. TMS is known to evoke trans-synaptic responses that occur at the cortical level while TES evokes a direct activation of the pyramidal tract axons just below the grey matter and is attributed to the subcortical level (Di Lazzaro et al., 2004; Di Lazzaro et al., 1998; Nakamura et al., 1996). Therefore, it is likely that the shifts in twitch direction reflect use-dependent plasticity following ballistic training occurs at least partly at the cortical level.

Furthermore, pharmacological evidence points toward the possible involvement of long term potentiation (LTP) as an underlying mechanism for the use-dependent effects in motor cortex (Bütefisch, et al., 2000). Bütefisch, et al. (2000) reported that the shift of TMS induced thumb movements toward training direction was abolished with the inhibition of the N-methyl-$_{D}$-aspartate (NMDA) receptors and facilitation of ϒ-aminobutyric acid (GABA)$_{A}$ receptors. Since LTP is mediated by the NMDA receptor and is facilitated by the inhibition of GABAA receptor (Bütefisch, et al., 2000; Ziemann et al., 2006), LTP is a potential candidate for the shift of twitch direction following ballistic training in our study.

Post training twitches in the testing posture

When twitches were evoked by TMS in the testing (semi-supinated) position, the ballistic training direction defined according to extrinsic and muscle-based reference frames were dissociated. The group mean twitch direction remained unchanged from baseline after ballistic training (mean shift = 4 ± 32°). However, individual results showed that twitch directions from three participants shifted significantly toward the training direction in extrinsic space, while twitch directions from another three participants shifted significantly towards the training direction defined according to a muscle-based reference frame (see Table 5.3). As twitch responses for each participant were consistent between different blocks of TMS trials in each wrist position, our results suggest that use-dependent learning following ballistic training is represented either in extrinsic or in muscle-based reference frames for different subjects.

There are mixed reports about the reference frames according to which motor learning (such as error-based learning and use-dependent learning) generalises depending on the context of the motor behaviour tasks. For example, error-based learning with force field adaptation during arm reaching movements was reported to generalise to different postures according to intrinsic coordinates (Bays & Wolpert, 2006; Malfait & Ostry, 2004; Malfait et al., 2002; Shadmehr & Mussa-Ivaldi, 1994), and to the opposite limb according to extrinsic coordinates (Burgess et al., 2007; Criscimagna-Hemminger, et al., 2003), whereas error-based learning with visuomotor perturbation showed generalisation toward extrinsic space in relation to the target location (Sainburg & Wang, 2002; Taylor, et al., 2011; Wang & Sainburg, 2004). However, more recent error-based learning studies, i.e. adaptation to force field or visuomotor perturbation, have suggested that motor learning could be represented in a combination of extrinsic and intrinsic coordinate frames (Berniker et al., 2014; Brayanov et al., 2012; Carroll, et al., 2014).

In a recent force field adaptation study by Berniker et al. (2014), patterns of generalisation could not be explained by representation in any single coordinate system when intralimb reaching was tested with different shoulder, elbow and wrist angles. Likewise, Brayanov, et al. (2012) demonstrated that intralimb visuomotor adaptation during horizontal plane reaching generalises to a combination of extrinsic and intrinsic coordinate frames when tested across a range of different movement directions and arm configurations brought about by changes in the shoulder angles. The idea that generalisation of error-based learning involves a combination of extrinsic and intrinsic reference frames is also consistent with the transfer of adaptation observed between limbs. Indeed, an immediate transfer of visuomotor adaptation was recently reported, regardless of the hand trained, when the altered visual feedback had identical effects in the eye- (extrinsic) and joint-based (intrinsic) coordinates for both hands in the sagittal plane (Carroll, et al., 2014).

In use-dependent learning, our previous unpublished laboratory data showed that the voluntary behavioural wrist aiming biased movements toward the training direction in extrinsic space after generalisation was tested in different wrist postures (Selvanayagam, et al., 2012b). Here, we showed that TMS might be able to activate a visuomotor network where we demonstrated that use-dependent learning represented by involuntary muscle twitches is generalised according to either extrinsic or muscle-based reference frames depending on subjects. Neurons in the primary motor cortex are known to exhibit muscle-like and extrinsic-like properties (Kakei, et al., 1999, 2003), thus TMS could activate these neurons in our study, as mentioned in Chapter 4. Therefore, we suggest that use-dependent learning most likely changes the excitability of the extrinsic-like and muscle-like neurons and is represented in multiple reference frames, but that extrinsic reference frame is more heavily weighted during voluntary task (Selvanayagam, et al., 2012b).

5.5 Conclusion

This study provided evidence of the generalisation of use-dependent learning in the corticospinal pathway via TMS-evoked twitch directions after isometric ballistic training in the training wrist. TMS-evoked twitches rotated with the wrist joint according to the joint and/or muscle reference frames at rest, and ballistic training induced an adaptation that generalised according to either to an extrinsic or a muscle-based coordinates for different subjects.

CHAPTER SIX

General Discussion

The main goals of this research were to enhance our understanding of the reference frames in which movements are represented in both primary motor cortices during unilateral movements, and how the issue of reference frames influence the hemispheric interactions that underlie cross limb transfer of motor skills. The key findings of the thesis provide evidence that alignment of spatial reference frames is important for effective functional interhemispheric interactions that induce cross limb transfer.

6.1 Summary of Results

Existing literature reported that primary motor cortex ipsilateral ($M1_{ipsi}$) to the active limb is active during unilateral movement (Bütefisch, et al., 2014; Chiou, et al., 2014; Hinder, et al., 2010; Howatson, et al., 2011; Kim, et al., 1993; Lee, et al., 2010; McMillan, et al., 2006; Verstynen & Ivry, 2011). To understand the movement representation of the activity in $M1_{ipsi}$, we first examined the timing and the coordinate frames of movement that is represented in the $M1_{ipsi}$ to the active limb in Chapter 2. The $M1_{ipsi}$ was probed with TMS at regular time intervals during motor preparation, up to the onset of movement. The time course of changes in the TMS-evoked twitch directions showed that twitch directions and corticospinal excitability remained similar to that observed at baseline in early motor preparation (>100 ms preceding movement onset). It was only late in motor preparation (~20 ms preceding movement onset) that we observed a sharp deviation of the TMS-evoked twitch direction toward muscle and/or midline reference frames in the $M1_{ipsi}$, at a time that presumably coincided with the release of motor commands to the active limb. The deviation of the TMS-evoked twitch directions suggest an excitability change in the corticospinal pathway that innervates the muscles, which in turn was reflected by a directional change of force vector. Indeed, our MEP results showed that there was an increase in excitability of the homologous muscles in both limbs. Although we did not specifically test for inhibitory processes in this experiment, evident changes in TMS-evoked twitch parameters just before movement onset might be associated with decreases in interhemispheric inhibition and/or in intracortical inhibition.

The lack of significant changes in the twitch parameters during early motor preparation (>100 ms preceding movement onset) reported in Chapter 2 prompted the possibility that conflicts in the reference frames of movement representation for the two limbs might have prevented the functional expression of interhemispheric interaction. Hence in Chapter 3, we sought to examine the role of alignment among various reference frames on interhemispheric interaction in the context of use-dependent biases. We studied the effects of ballistic training with the left wrist on the isometric aiming task using weak forces with the right wrist, while the postures of both limbs were varied in order to manipulate the degree of congruence between the spatial coordinates for both limbs. For example, for training in an upward direction (extension movement) in a pronated wrist posture, the training direction defined according to both extrinsic and muscle-based representations are congruent for the two limbs. In contrast, for the same extension training action in a neutral wrist posture, the training direction represented in extrinsic and muscle coordinates are in conflict for the two limbs. We found that ballistic training in the left hand induced a systematic bias in the right hand when the direction of training was congruent according to intrinsic and extrinsic reference frames in both limbs, but not when there were reference frame conflicts. Our results imply that congruent reference frames of movement representation enhance interhemispheric interactions, whereas conflicting reference frames of movement representation interfere with the interhemispheric interaction. Although the exact mechanisms responsible for the systematic bias remain unclear, we suggest that interhemispheric interaction is influenced by a combination of factors related to movement representation, such as reference frames (extrinsic and muscle-based), midline effects and training axes. As a result, the systematic bias in the untrained limb might have been explained by the weighted sum of these factors - reference frames (extrinsic and muscle), midline effect and training axis. For example, when the training direction was equivalent for both limbs when defined in extrinsic and muscle-based reference frames, the extrinsic and muscle-based effects would sum to induce a greater bias toward the training direction.

The results of Chapter 3 provide evidence that use-dependent learning bias is represented in multiple reference frames. In order to understand the role of reference frame conflicts in transfer of use-dependent learning, we further assessed the neural representation of use-dependent learning by characterising the coordinates in which the TMS-evoked twitches are represented within the trained limb (Chapter 5). However, we first sought to determine whether resting twitches were represented consistently according to extrinsic or muscle space when these reference frames were dissociated by changes in posture (Chapter 4). Our results show that twitch directions follow the wrist according to joint and/or muscle reference frames when the wrist posture was altered between pronated and neutral positions. The joint and/or muscle reference frame representations at rest provided us with baseline behaviour without adaptation and subsequently enabled us to examine adaptations induced by use-dependent learning. This allowed the design of Chapter 5, which involved manipulation of the wrist posture between training and testing positions in order to dissociate intrinsic and extrinsic learning representations. We found systematic shifts in the evoked twitch directions toward the training direction in the training posture, but variable twitch direction changes in the generalisation posture, indicating that adaptation could be represented in either extrinsic or muscle-based reference frames for different subjects.

6.2 Implications of the studies

The findings of this thesis have implications for advancing our knowledge of cross limb transfer from the perspective of reference frames associated with use-dependent learning effects. We provided evidence that use-dependent learning is represented in multiple reference frames in both trained and untrained limbs (Chapter 3 and 5) and that use-dependent aiming bias could be transferred between limbs (Chapter 3). The transfer of use-dependent learning is enhanced when the representation of movement between limbs are in congruent reference frames, which suggests that the alignment of reference frames has important implications for the efficacy of unilateral training in eliciting cross limb transfer.

Currently, no study has directly investigated cross limb transfer from the perspective of reference frames with use-dependent learning. However based on the evidence from error-based learning studies, we could infer that reference frame issues indeed influence the efficacy of cross limb transfer. For example, for visuomotor adaptation studies with horizontal reaching contexts, the transfer of error driven adaptation occurred for the untrained arm was not immediate when the direction and degree of visual rotation defined in extrinsic space was matched for both arms (Lei & Wang, 2014; Wang & Sainburg, 2004). However, another visuomotor adaptation study reported an immediate and strong transfer of error driven adaptation (about 60 % to 86 % of transfer) when the altered visual feedback of a reaching task was defined in identical eye- and joint-based coordinates for both limbs in the sagittal plane (Carroll, et al., 2014). These visuomotor adaptation studies suggested that transfer is affected with the alignment of reference frames between limbs, and the extent of transfer is greater when the coordinate frames between both limbs are matched extrinsically and intrinsically.

The results of this thesis suggest that movements are represented in multiple reference frames in both primary motor cortices, and the alignment of reference frame between both limbs influence the interactions between hemispheres during unilateral movements. In order to better harness the benefit of cross limb transfer, we should consider manipulating the spatial target direction and limb configuration so that the extrinsic and intrinsic reference frames for these components are matched between limbs. Further research is necessary to investigate whether such a manipulation of reference frames during unilateral training has the potential to improve current rehabilitation programs for individuals with unilateral motor disorders.

6.3 Possible mechanisms for transfer of use-dependent learning effects

This thesis focused on the examination of the reference frames in which ballistic motor adaptation is represented and how the issue of reference frames influence the interaction between hemispheres during unilateral training. We did not seek to identify the exact mechanisms underlying interhemispheric interactions during unilateral training from the perspectives of reference frames. However, we could speculate from our findings that the transfer of use-dependent learning might arise from either the ‘cross activation’ or the ‘bilateral access’ models previously suggested for cross limb transfer.

Unilateral ballistic training, which required strong descending drive, has been shown to increase corticospinal excitability bilaterally (Carroll, et al., 2008; Lee, et al., 2009; Lee, et al., 2010). Neural adaptation induced through experience by unilateral training could be encoded in a neural network that is responsible for use-dependent learning in both hemispheres and is accessible by the untrained limb (Carroll, et al., 2014; Verstynen & Ivry, 2011; Verstynen & Sabes, 2011). Accessibility of the information by the untrained limb could be brought about via the corpus callosum because of massive connections between hemispheres (van der Knaap & van der Ham, 2011; Wahl & Ziemann, 2008). Neuroimaging and transcranial magnetic stimulation (TMS) studies showed activity in both hemispheres during unilateral movements (Bütefisch, et al., 2014; Chiou, et al., 2014; Hinder, et al., 2010; Howatson, et al., 2011; Kim, et al., 1993; Lee, et al., 2010; McMillan, et al., 2006; Verstynen & Ivry, 2011). Many different brain areas, such as parietal and prefrontal regions, have been identified to be involved in motor planning and these areas receive multiple representations of targets in visual guided movements (Cohen & Andersen, 2002; McGuire & Sabes, 2009; Sabes, 2011). Primary motor cortex is one of the sites that combine the extrinsic and intrinsic representations of movement before the release of motor commands (Cohen, et al., 2010; Kakei, et al., 2003; Yanai et al., 2008). This evidence may explain our results whereby adaptation in the corticospinal pathway with TMS and behaviour aiming biases in use-dependent learning are represented by multiple coordinate frames. Therefore, we could speculate that the accessibility of information between two hemispheres is enhanced when some of these coordinate frames are matched. This is supported by our findings whereby systematic bias was found during cross transfer effects of use-dependent learning when the training direction defined according to extrinsic and muscle reference frames for both limbs are congruent.

6.4 Future directions

This thesis increases our understanding of the importance of spatial reference frames in which movement is represented between both limbs during unilateral movements. Many additional questions related to reference frame issue could be explored, and a number that I could suggest for future studies are listed below.

- Do TMS-evoked twitches in the passive limb shift during early motor preparation when the movement directions defined for both limbs are in congruent reference frames?

We showed that TMS-evoked twitches in the passive limb remained at baseline during early motor preparation, and there was a sharp deviation of twitch direction toward the muscle and/or midline reference frames during late motor preparation when the active limb was preparing a movement whose representation according to extrinsic and muscle reference frames are conflicting for the passive limb. Using a prepared movement that is defined in conflicting reference frames between limbs might have eradicated any behavioural effect during motor preparation, i.e. no change in twitch direction during early motor preparation. Therefore we are interested to know whether by aligning the reference frames of the movement direction for both limbs, twitch directions would display an earlier change during early motor preparation. If the movement direction defined according to extrinsic and muscle reference frame are congruent for both limbs promotes the behavioural effects of movement planning, we would expect to see an earlier shift in the twitch direction and/or a greater effect (shift) in the twitch direction during late motor preparation.

- Does lateralisation affect the transfer of use-dependent aiming bias?

We have shown the transfer of use-dependent bias was obvious by training the non-dominant hand and aiming with the dominant hand when the training direction defined according to the extrinsic and intrinsic reference frames was congruent in both limbs. However, it is not known whether lateralisation affects the extent of bias transfer. We could answer this question by training the dominant hand and aiming with the non-dominant hand. If lateralisation occurs for transfer from dominant to non-dominant hand, we would expect to yield a greater use-dependent aiming bias transfer by training the dominant right hand, especially in the limb configurations where the training direction defined in extrinsic and intrinsic reference frames between limbs are congruent. Therefore by replicating the use-dependent aiming bias study (Chapter 3), we could decide whether lateralisation affects the transfer of use-dependent aiming bias.

- Does use-dependent learning adapt differently for individuals?

We showed that use-dependent learning is represented in multiple reference frames and generalised to either extrinsic or muscle coordinates for different subjects in Chapter 5. We are interested to find out whether these effects are consistent across time for individuals, and whether there are factors that might influence the reference frames in which the twitches are expressed. If the effects are consistent across time, individuals' twitch direction would generalise to the same coordinate frame when tested at different days. Since twitch directions for some subjects are generalised to extrinsic coordinate frame, we could manipulate the visual feedback, such as removing visual feedback during training to explore whether visual feedback affects the adaptation to be represented in extrinsic space. If vision is an important factor for subjects who generalised use-dependent learning in extrinsic space, we would expect to see a generalisation toward intrinsic space.

6.5 Practical applications

Use-dependent learning is defined according to multiple reference frames. The issue of reference frames in relation to cross-limb transfer can be considered during the planning or modifying existing training or rehabilitation programs to maximise skills transfer, increase or preserve strength of the opposite untrained limb. Three possible examples of how the reference frame issue might be applicable to cross-limb transfer are:

1) the reaching movement in a stroke rehabilitation program could be performed in a manner whereby the trained reaching direction defined in extrinsic or muscle-based reference frame are congruent with the opposite limb to maximise the cross training effects.

2) to increase or preserve strength in the opposite limb during a unilateral injury, the posture of limbs could be manipulated so that the training direction defined according to extrinsic or muscle-based reference frame is congruent to increase strength or in conflict to preserve strength of the opposite limb in the strength training program.

3) in learning of a new skill, such as playing a musical instrument, both limbs could be oriented parallel to the midline and learning the key sequences in muscle-based reference based for more efficient transferred to the opposite limb.

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