Non-Invasive Stimulation of the Motor System: Methodology and Application for the Study of Motor Control

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Abstract

Transcranial magnetic stimulation (TMS) and peripheral nerve stimulation (PNS) are techniques used to study the neurophysiology of the motor system. The primary aim of this thesis is to investigate the reliability and validity of these techniques and their relevance to investigate the neural substrates of motor control in healthy individuals. When delivering TMS and PNS in combination, a method called TMS-conditioning of the monosynaptic reflex, it is possible to selectively assess the excitability of cortical, spinal and peripheral circuits. However, the intersession reliability of this method when stimulating forearm muscles was never investigated. In the first study, it was demonstrated that the method produced reliable results over the course of three sessions. In the second study, the effects of auditory activation and stimulus expectation on TMS motor-evoked potentials were examined. Masking ($P = 0.02$) or attenuating ($P = 0.004$) the sound produced by TMS and informing the participant of the time of stimulation ($P = 0.049$) decreased the responses recorded from forearm muscles. This suggests that part of the activity elicited by TMS is conducted through non-corticospinal pathways. Finally, the objective of the last experimental chapter was to investigate the acute effects of unilateral skill and strength training on the performance and neural circuits of the contralateral untrained limb. The results showed that a single session of unimanual skill (force-matching) training successfully increased skill in the untrained limb ($F_{1, 9} = 10.266$, $P = 0.011$), but a single session of unimanual strength training did not affect the untrained limb ($F_{1, 9} = 3.069$, $P = 0.114$). However, the excitability of the untrained motor cortex increased after strength and skill training ($F_{1,9} = 15.224$, $P = 0.004$), without any changes in spinal and peripheral excitability. This demonstrates that both training modalities induce long-lasting effects in the untrained motor cortex.

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List of Abbreviations

aMT	Active motor threshold
APB	Abductor pollicis brevis
BDNF	Brain-derived neurotrophic factor
Ca2+	Calcium ion
CI	Confidence interval
CMA	Cingulate motor area
cMEP	Cervicomedullary motor-evoked potential
CN	Cochlear nucleus
CNS	Central nervous system
COND_H	TMS-conditioned Hoffmann reflex
CPN	Common peroneal nerve
CS	Conditioning stimulus
cSP	Cortical silent period
CV	Coefficient of variation
DLPFC	Dorsolateral prefrontal cortex
ECRL	Extensor carpi radialis longus
EEG	Electroencephalography
EF	Early facilitation
EFD	Early facilitation delay
EMG	Electromyography
EMR	Evoked motor response
EPSP	Excitatory postsynaptic potential
FCR	Flexor carpi radialis
FCU	Flexor carpi ulnaris
FDI	First dorsal interosseus
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GLM	General linear model

$H_{M10\%}$	Hoffmann reflex at 10% of maximal motor wave
H_{max}	Maximal Hoffmann reflex
H-reflex	Hoffmann reflex
IPL	Inferior parietal lobe
ICC	Intraclass correlation coefficient
ICF	Intracortical facilitation
IHF	Interhemispheric facilitation
IHI	Interhemispheric inhibition
IPI	Interpulse interval
IPSP	Inhibitory postsynaptic potential
ISI	Interstimulus interval
LICI	Long intracortical inhibition
M1	Primary motor cortex
ME	Medial epicondyle
MEP	Motor-evoked potential
M_{max}	Maximal motor wave
MP	Motor practice
MRI	Magnetic resonance imaging
MSO	Maximum stimulator output
MT	Motor threshold
MVC	Maximum voluntary contraction
NMDA	N-methyl-D-aspartate
OC	Occipital cortex
PAD	Primary afferent depolarization
PAS	Paired associative stimulation
PET	Positron emission tomography
PmD	Dorsal premotor cortex
PMRF	Ponto-medullary reticular formation
PnC	Pontine reticular nucleus

PNS	Peripheral nerve stimulation
PSTH	Peri-stimulus time histogram
RC	Recruitment curve
RM	Repetition maximum
RMSE	Root mean square error
RS	Radial styloid
rTMS	Repetitive TMS
SD	Standard deviation
SE	Standard error
SICI	Short interval intracortical inhibition
SMA	Supplementary motor area
STDP	Spike-timing-dependent plasticity
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TS	Test stimulus
US	Ulnar styloid
α-MN	Alpha motor neuron

Conference abstracts arising from the thesis

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Chapter 1 – General introduction

1.1. Motor control and skill acquisition

"We have a brain for one reason and one reason only, and that's to produce adaptable and complex movements." Prof. Daniel M. Wolpert

The only mean by which humans are able to interact with the environment is by producing movements. As a field of research, motor control is the scientific study of the mechanisms by which movements are planned, executed and controlled. We complete movements with such simplicity and automaticity that it is difficult to appreciate the complexities our central nervous system (CNS) faces when performing them. The motor system must organize the timing and intensity of activation of the prime mover, antagonists and postural muscles (Rothwell, 2012). In addition, it must integrate proprioceptive (e.g. originating within the organism) information about body position and tactile information (Schmidt et al., 2018). At the same time, it receives exteroceptive (originating outside the organism) information through the other sensory systems such as visual and auditory inputs (Magill and Anderson, 2007) which can be used to adjust the movement *on the fly* and to evaluate it *a posteriori* (Utley and Astill, 2008).

The human ability to acquire, maintain and improve skilled movements has been fundamental in driving mankind's evolution. Across the lifespan, we learn how to modify our motor behaviour according to external stimuli. The interconnected processes through which we are able to learn new movement patterns is referred to as skill acquisition (Schmidt et al., 2018). Skills are sets of movements which are completed together to reach a specified goal. Repetition of skills, or practice, is essential in order to reach skilled performance. Practice results in higher probabilities of achieving the desired outcome, with greater consistency and more efficiency (Guthrie, 1935). Specific anatomical characteristics and neuronal adaptations may guide the acquisition of skilled behaviour with practice (Wolpert et al., 2001).

1.2. Why study motor control and skill acquisition

Already in the late 19th century scientists were investigating the basic neuroanatomical organization of the motor system in animal models (Hitzig and Fritsch, 1870, Luciani, 1891). However, their work was guided by the need of explaining the organization of

the CNS rather than by the necessity of understanding movements (Schmidt et al., 2018). The first scientific attempts to measure and describe skill acquisition were performed around the same years (Bryan and Harter, 1897, 1899). There was great interest at that time in understanding the optimal conditions under which workers were able to achieve better, faster performance in the work place (Clark and Oliveira, 2006), and the literature accumulated during these years reflects it (Bean, 1912).

The lack of contact between the study of behaviour and neurophysiology continued for much of the 20th century, until a renewed interest for motor behaviour and technological advances in the field of neurophysiology led to a new golden era for the study of motor control (Clark and Oliveira, 2006). Nowadays, the study of motor control finds application in many areas. For example, principles of skill acquisition such as the amount and timing of practice sessions and the feedback given to the performer are successfully applied in sport training (Kernodle and Carlton, 1992). Quantitative analysis of movement patterns can be used to guide performance (Hong and Bartlett, 2008). In addition, studying motor control and its neural substrates can help prevent the decline of movement abilities observed with aging and design appropriate interventions (Seidler et al., 2010). Assessment of motor neurophysiology after injury can help both the diagnosis of patients and the design of tailored rehabilitation protocols (Sherwood et al., 1996). Finally, methods and theories derived from the motor control literature have been successfully translated to clinical settings (Sattelmayer et al., 2016). These are particularly relevant for people who suffered an injury to the motor system and for whom the focus of rehabilitation is to re-learn how to perform specific movements (Onla-Or and Winstein, 2008). Many of the tasks we perform daily involve synergistic activity between muscles of the left and right side of the body. Bimanual movements are particularly important for skilled activities requiring fine coordination (Rokni et al., 2003). In this light, the processes by which bilateral movements are performed and the mechanisms by which information transfer occurs between one motor system and the contralateral one have received great attention.

1.3. Measuring motor control

Motor behaviour can be measured with different methods, the choice of which depends on the aspect of movement to analyse. The first and more intuitive way to measure goal-directed performance is to evaluate the outcome of the movement. The

complexity of this measure can range from the simple count of hits and misses to more sophisticated index of accuracy (Schmidt et al., 2018). Additionally, the object of measure can be the movement *per se*, as in the case of kinematic analysis which describe the motion of body parts during the task (Hall, 2014). A different approach is to measure how muscles are controlled by the central nervous system while moving. This can be achieved by recording surface electromyography (EMG) activity from the muscles of interest (Criswell, 2010). Measuring EMG permits to assess not only the relative activation of each muscle during multiple phases of a movement, but also temporal patterns of activity across multiple muscles (Murray et al., 1984). The reorganization of functional units while training is clearly revealed by measuring the emerging patterns of muscular activation via EMG analysis. Therefore, EMG analysis can help reveal whether the same muscles are being activated more efficiently with practice.

The involvement of cortical areas in movement can be measured with neuroimaging techniques such as electroencephalography (EEG), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Event-related potentials recorded through EEG are recorded during sport performance and used as a source of feedback to improve future performance (Petruzzello et al., 1991). Magnetic resonance imaging has revealed the structural (Freund et al., 2013) and functional (Herz et al., 2014) changes occurring in motor areas after injury and neurodegeneration. Lastly, a different approach to studying motor control is to measure the activity induced by electric/magnetic stimulation while or after people perform movements. These techniques, collectively named non-invasive stimulations, are discussed in the literature review chapter (Chapter 2.4 and 2.5). In more detail, the chapter will describe methods to activate the motor cortex via magnetic stimulation (transcranial magnetic stimulation, TMS) and peripheral nerves with electrical stimulation (peripheral nerve stimulation, PNS) in humans and how these can be used together to study descending neural pathways to spinal motoneurons with a method called TMS conditioning of the monosynaptic reflex.

1.4. Non-invasive stimulation in motor control

The techniques of TMS and PNS have been extensively used to investigate the mechanisms of motor control in healthy human participants. The topographic organization of the human motor cortex, which contains a representation of multiple

body muscles, can be revealed in healthy humans by stimulating it (Wilson et al., 1993). Manipulation of TMS parameters has permitted researchers to detail how intracortical and spinal circuits interact in shaping motor behaviour (Kujirai et al., 1993). Stimulating the corticospinal tract at the cortical and peripheral levels have uncovered the neural mechanisms which are responsible for the production, acquisition and retention of motor skills (Ljubisavljević, 2006, Meunier et al., 2007). Finally, by conditioning the monosynaptic reflex evoked in upper and lower limb muscles with cortical magnetic stimulation it is possible to differentiate between the monosynaptic and polysynaptic components of the descending volley, a method which can potentially reveal the organization of cortical and spinal interneuronal circuits which contribute to movement (Leukel et al., 2012). A detailed account of the applications of non-invasive stimulation to motor control and skill acquisition is provided in Chapter 2.6.

1.5. Reliability and validity of the methods

Measures derived from non-invasive stimulation of the motor system can be used to study the mechanisms underlying motor rehabilitation after injury (Saturno et al., 2008) and subsequently drive rehabilitation practices (Kumru et al., 2010). In order to do this, stimulation methods need to be able to produce stable and consistent results, a characteristic known as reliability. The most used measure of reliability in neuroscience is test-retest reliability, in which data collected under the same conditions over two (or more) sessions are compared (Weir, 2005). For quantitative variables, the relative consistency between measurements can be estimated via intraclass correlation coefficients (ICCs) (Bartko, 1966). ICCs measure the degree of similarity between multiple series of data (Koo and Li, 2016). ICCs have been used to assess how reliable parameters acquired with TMS and PNS are over multiple sessions. The motor threshold, which is the lowest intensity of stimulation at which a response can be elicited in the muscle of interest (Rossini et al., 2015), is highly reliable when measured from forearm muscles (Malcolm et al., 2006). The monosynaptic reflex induced by PNS, which measures the excitability of sensory afferents to the spinal cord and of spinal motoneurons, can be reliably recorded from forearm muscles as well (Christie et al., 2005). However, whether the conditioning effect of corticospinal descending activity on the monosynaptic reflex is a reliable phenomenon in the forearm has never been established. This issue was addressed by

measuring a series of neurophysiological parameters, among which TMS-conditioning of the monosynaptic reflex, recorded from the flexor carpi radialis (FCR) muscle in healthy participants over three sessions. Details of this study are reported in Chapter 4.

TMS has been commonly employed to study changes in corticospinal transmission occurring after training or damage to the CNS (Reis et al., 2008). However, the validity of the technique in measuring exclusively the excitability of the corticospinal system is hindered by its methodological limitations (Bolognini and Ro, 2010). The term validity refers to the degree to which an instrument or technique accurately measures a variable (Heale and Twycross, 2015). The finding that cortical magnetic pulses can excite all motor neurons supplying a given muscle (Magistris et al., 1998) proves that the method can measure the variable of interest. However, the activity induced by TMS can spread across multiple cortical areas and lead to unintended effects, which will not necessarily alter muscle responses through corticospinal pathways (Bestmann et al., 2015). This is a serious limitation to TMS construct validity, the extent to which a method measures the variable of interest. Some of the limitations to TMS validity are reported in Chapter 2.7, which gives a detailed description of the factors that can influence the outcome of cortical stimulation given on the motor cortex. In addition, in Chapter 5 it is described how two cognitive factors, namely auditory activation and stimulus expectation, can confound the results obtained when measuring motor evoked potentials (MEPs) induced by TMS.

1.6. Bilateral transfer

Once established that measures of motor excitability recorded upon cortical and peripheral stimulation are reliable and valid, these methods can be implemented to estimate which neural substrates are responsible for the behavioural changes occurring after motor training. Considering the organisation of the human motor system, in which each hemisphere controls muscles in the contralateral side of the body, the mechanisms by which information transfer between hemispheres is supported are still uncertain (Walsh et al., 2008). The two primary motor cortices are connected via commissural fibres which constitute the corpus callosum (Smith and Akelaitis, 1942). The importance of this structure in bimanual control is confirmed by the deficits in producing coordinated movements observed in people who underwent callosotomy surgery (Eliassen et al., 2000). TMS has been extensively used to study

interhemispheric transfer by delivering single pulse to one motor cortex at different times before stimulating the contralateral motor cortex. These studies revealed the existence of both excitatory and inhibitory pathways to the ipsilateral (related to cortical stimulation) spinal motoneurons. The literature on interhemispheric circuits studied with TMS is reviewed in Chapter 2.4.5.

The notion that activity elicited in one hemisphere can influence the excitability of the opposite hemisphere is confirmed by the phenomenon of bilateral transfer. Unilateral training of one limb can increase the performance measured in the untrained limb (Scripture et al., 1894). This effect has been observed over multiple muscles (Cook, 1933) and is of great relevance in rehabilitation practices after injury (Ausenda and Carnovali, 2011). Experimental evidence and possible neural mechanisms of bilateral transfer are discussed in Chapter 2.8. When the aim of training is to improve strength in the contralateral muscles, the behavioural effects of unilateral training on the untrained limb are usually measured after multiple training sessions (Lee and Carroll, 2007). However, there is neurophysiological evidence that some of the mechanisms which might guide strength increase are already occurring after a single session (Selvanayagam et al., 2011). In Chapter 6 it was assessed whether a single session of unimanual strength or skill training is sufficient to improve performance and strength of the opposite hand and induce changes in the excitability of the contralateral (non-training) hemisphere. In addition, the excitability of the contralateral (untrained) cortical, spinal and peripheral pathways assessed through non-invasive stimulations was measured before and after a single session of strength and skill training.

1.7. Summary

The overall objective of this thesis is to investigate the contribution of non-invasive magnetic and electrical stimulation to the understanding of the mechanisms of motor control in humans. The literature review chapter (Chapter 2) will start with introducing the fields of motor control and skill acquisition and describing two techniques used to study motor excitability in humans, namely TMS and PNS, and how these can be used in combination to study descending activity to spinal motoneurons. The focus will then shift on what the two techniques revealed about the neural organisation of the human motor system and motor control. The discussion will later move to the limitations that need to be addressed when measuring the results

obtained upon cortical stimulation of the motor cortex. Finally, the chapter will end by describing the phenomenon of bilateral transfer, the increase in strength and performance observed in untrained limbs after unimanual training of the opposite limb, from both a behavioural and neurophysiological prospective.

Methods which are common to the three experimental chapters described in the thesis are outlined in Chapter 3. In the first of the experimental studies, topic of Chapter 4, the intersession reliability of parameters collected via surface EMG from the FCR muscle after delivering TMS and PNS over three sessions was assessed. The second study (Chapter 5) explored the effects that two confounding factors, auditory activation and stimulus expectation, have on the motor-evoked potentials elicited by TMS in the forearm muscle. The third and final study (Chapter 6) was designed to test whether changes in behaviour and neural excitability can be observed after a single training session in the untrained limb. Measures of transfer included changes in strength and performance and in the excitability of the untrained cortical and spinal circuits. The findings of the three experimental chapters are summarized in Chapter 7 and discussed in light of their implications for the study of motor control and the issues that need to be addressed in the future.

Therefore, the aims of this thesis can be summarised as follows: (1) to measure the intrasession reliability of the TMS-conditioned monosynaptic reflex in the FCR muscle over the course of three sessions; (2) to determine the effects of the sound produced by TMS stimulation and of stimulus anticipation on the MEPs recorded upon TMS; (3) to assess the effects of a single session of unilateral strength training and skill training on the contralateral limb; (4) to compare changes in cortical, spinal and peripheral excitability occurring in the untrained motor circuits after one session of unilateral strength training and skill training.

Chapter 2 – Literature review

2.1. Introduction

The scope of this chapter is to provide a review of the literature which describes how non-invasive stimulation of the motor system has been used to study the mechanisms of motor control. The narrative starts by outlining basic principles of motor control and skill acquisition. Next, the stimulation techniques used to investigate the neurophysiology of the motor system are described, along with their contribution to our current understanding of movement production and skill acquisition. Confounding factors which limit the validity of cortical stimulation as a mean to assess motor excitability are presented. Finally, the chapter ends with describing the phenomenon of cross education of strength and skill, and how non-invasive stimulation has been used to evaluate which neural mechanisms underlie it in healthy humans.

2.2. Motor control and skill acquisition

Motor control is the study of how the CNS selects and applies muscular activation patterns to achieve purposeful movement (Gollhofer et al., 2013). The first fundamental problem the CNS faces is determining the appropriate motor commands to reach a specific end-goal. Before starting a movement, the person has to form a representation of what the task is and possible ways to achieve it. The person needs to have built an internal representation of the correct performance in order to interpret the externally induced error (Gollhofer et al., 2013). When performing a new movement, in the absence of such sensory-based representation, the person approaches the task by gathering information from environmental cues, what Gentile defined as “regulatory conditions” (Gentile, 1972). Once the performer accumulated enough evidence on what the task entails, the focus shifts to the possible ways in which the desired outcome can be achieved (Magill and Anderson, 2007). This phase was first described from a biomechanical perspective by Bernstein (1966). Because of the redundancy of the motor system, by which muscles and body parts can group together or move independently, when approaching a new movement we must solve the “degrees of freedom” problem. Bernstein suggested that performers initially limit the number of joints used and prevent as many body parts as possible to move independently. Independently moving degrees of freedom can be coupled together and act as functional synergies. An interesting corollary to this phenomenon was

recently proposed by Latash (2010). Rather than considering the many degrees of freedom as an issue, he posed that the abundancy of mechanical possibilities by which an endpoint can be reached is used by the central nervous system to explore the best synergies to adopt for a given task.

When the planning ends and the movement is initiated, a flow of information becomes available to the performer. Sensory receptors signal the position of the body in relation to the environment and/or to external objects, providing immediate feedback on the action performed. In addition, feedback is not limited to the proprioceptive domain but extend to incorporate visual, auditory and olfactory sensations (Magill and Anderson, 2007). This feedback can be used to modify the movement "on the fly" if sufficiently slow and to guide planning and execution of successive movements. The selection and reinforcement of a specific movement pattern is guided by the interpretation and correction of movement errors (Bernardi et al., 2015). Changes in the following actions occur if there is a mismatch between the sensory feedback and the "efferent copy" (von Helmholtz, 1963), a predictive internal representation of the movement. Our internal representations, or forward models, are first used to predict the consequences of our motor behaviour and then updated by incorporating the movement errors (Wolpert and Flanagan, 2001).

Through experience and practice, we learn to perform and refine in terms of temporal and spatial accuracy the desired movements and reach skilled performance (Willingham, 1998). Behaviourally speaking, the early phase of acquiring a new skill is characterised by a lot of errors and unstable performance (Magill and Anderson, 2007). While adjustments can be effective in reducing errors on a single trial, lack of consistency often renders improvements vain. Self-paced movements can be very slow, as the performer is actively engaged in interpreting environmental cues and refining their movement patterns (Bernardi et al., 2015). It is, however, during this phase that most of the improvements in accuracy occur (Utley and Astill, 2008). Participants become more and more capable of interpreting their errors, and of regularly selecting effective movement patterns. During this phase errors and feedback, both in terms of movement and performance outcomes, are fundamental and learning strategies are more effective. People transition from a phase in which the focus is on understanding what the task entails to one in which they explore how to better complete the task (Fitts and Posner, 1967). At the end of it, they should have

interiorised a movement pattern which will be refined with more practice (Gentile and Nacson, 1976).

A closer look at the movement patterns generated across the first practices reveals how the CNS starts exploring new combinations of multi-segmental units. The rigid movements observed at the beginning of the practice slowly give way to smoother actions as new synergies, or functional units, between muscles and joints are explored (Magill and Anderson, 2007). According to Bernstein, some of the degrees of freedom which were previously frozen by the notice are now freed as body parts become organised in synergies. In addition, releasing degrees of freedom allows greater adaptation to the environment and more flexibility (Bernstein, 1966). Bernstein's theories were corroborated by kinematic analysis showing that the range of motion of joints increases during the initial trials (Steenbergen et al., 1995), but at the same time new evidence showed how angular motion is reduced with practice on other tasks (Konczak et al., 2009). Such contradictory findings do not undermine the importance of Bernstein's work, but rather stress the challenge of reconciling the data accumulated under a unified framework (see Newell and Vaillancourt, 2001 for a comprehensive discussion of this issue).

EMG analysis reveals how, at the start of practice, the performer tends to activate more muscles than required by the task. After a few practice trials, EMG activity in unnecessary muscles decreases (Magill and Anderson, 2007). The remaining spatial patterns are further defined as temporal dynamics of activation change, increasing performance (Macpherson, 1991). Jaegers and his colleagues described changes occurring in three forearm muscles while learning to throw a dart: arm and shoulder muscles were activated both before and after dart release when participants started practicing; at the end of the first practice day a temporal order appeared in which different muscles were active at specific phases of movement (Jaegers et al., 1989). The emerging synergies depend on the geometrical and force generating capacity of the muscles, different neural strategies can be observed among people and synergies show great adaptation to task constraints (Carson and Riek, 2001).

With more practice, efforts become focused on stabilizing the acquired skill. Consistency is the main attribute differentiating between a novice and an expert (Magill and Anderson, 2007). This phase is characterised by low inter-trial variability in trajectory and performance and a progressive decrease in cognitive demands, such

that some movements can be performed without overt attention (Fitts and Posner, 1967). The movement pattern must be at the same time stable to ensure consistency and flexible enough to permit adaptation to unexpected changes in the regulatory conditions (Gentile, 1972). From a biomechanical standpoint, the coordination pattern selected and reinforced during early practice becomes more economical as the system uses the mechanical properties of muscles to reduce energy consumption. Following practice of fast arm movements to a target, participants learned how to exploit the intersegmental limb dynamic at the shoulder joint to maximise muscle moments and counteract gravity (Schneider et al., 1989). EMG analysis can help reveal whether the same muscles are being activated more efficiently across training sessions (Carson and Riek, 2001).

2.3. The neural substrates of movements

The goal of motor control is to understand not only the physical properties of movements, but also the neurophysiological mechanisms underlying them. It has been known for more than 50 years that the neural processes underlying the execution of a movement precede the start of the movement itself (Kornhuber and Deecke, 1965). The localisation and time course of pre-movement neural activation has been examined by high-resolution electroencephalography (EEG) recordings. A negative cortical potential is first observed from electrodes located on the supplementary motor area (SMA) and somatosensory areas (Ikeda et al., 1992). The earliest activity could be recorded up to 2 seconds before task initiation from SMA (Kornhuber and Deecke, 1965). For unimanual movements, a second marker of activity, the lateralised readiness potential, can be observed 500-800 ms before the movement when the activity localises to one hemisphere (Haggard and Eimer, 1999). This shift reflects the selection of a response site (left vs right).

With the advent of functional magnetic resonance imaging, which provides greater spatial resolution compared to the EEG, the neural network activated during movement preparation was extensively revisited (Lotze et al., 1999). Ball and his colleagues analysed EEG–fMRI–coregistered data while participants performed finger flexion movements (Ball et al., 1999). The pattern of neural activation started in the anterior cingulate motor area (CMA) and SMA area and then developed over M1 before movement release. In addition, they observed activity in inferior parietal lobe (IPL) during movement preparation. The authors suggested that the early

activation seen in higher-order areas are involved in pre-movement sensory awareness (IPL) and motor awareness (CMA) (Ball et al., 1999). Neural activity in SMA does not arise merely as a result of the increased attentional demands during motor preparation but reflects the nature of the movement to perform. The time-course of SMA activity during self-generated movements differs from the one recorded during movements generated in response to external stimuli, with the first showing an earlier (1.3 seconds) onset (Weilke et al., 2001) and greater overall activity (Wiese et al., 2004). Neural activation reflecting preparatory processes has been found in the lateral zone of cerebellum (Cui et al., 2000).

In humans, the primary motor cortex (M1) lies along the precentral gyrus anteriorly to the primary somatosensory cortex. Area M1 has a prominent role in all the stages of movements and motor skill acquisition (Dayan and Cohen, 2011). Electroencepalography studies show that M1 becomes active up to 400 ms before movement onset, when movements are prepared (Kornhuber and Deecke, 1965). When the action is initiated the first increase in cerebral activation is observed in M1 as revealed by functional magnetic resonance imaging (fMRI) (Weilke et al., 2001). M1 activity is modulated by the difficulty of the task even in situations in which the same number of muscles are required to perform complex and simple tasks (Kawashima et al., 1994). Kami et al. (1995) investigated neural activation changes occurring in M1 during skill acquisition by asking participants to train in performing specified sequences of finger movements. The trained movement resulted in a smaller area of activation early during practice but a larger area towards the end of the first session. They hypothesised that at this stage more M1 neurons are being recruited and become organised in a network which is activated by the sequence (Kami et al., 1995). Plastic adaptations in the motor cortex have been observed in rats (Kleim et al., 1998), primates (Nudo et al., 1997) and humans (Pascual-Leone et al., 1995). There is evidence that motor cortex reorganisation might support functional recovery after lesions to the spinal cord (Nishimura et al., 2007). The authors monitored the time-course of recovery of hand skills after lesions at the C4/C5 spinal level and the paralleling changes in activity of the motor cortex. They reported increased activity in the contralateral primary motor cortex in the early recovery stage (up to 45 days after surgery) while monkeys regained their ability to retrieve food using their fingers. Importantly, temporary inactivation of the primary motor cortex by microinjections of muscimol impaired food retrieval during this early stage (Nishimura et al., 2007).

Layer V of M1 contains large pyramidal neurons, whose axons project to brainstem structures and the spinal cord forming the corticobulbar and corticospinal tract (Bear et al., 2007). The majority (85%) of descending corticospinal axons decussate at the level of the medullary pyramids and enter the lateral columns in the spinal cord (lateral corticospinal tract) (Nathan et al., 1990). Most of the projections from pyramidal neurons synapse onto motor neurons in the ventral horn contralaterally (Nathan et al., 1990). In primates, monosynaptic connections exist between pyramidal neurons in the primary motor cortex and alpha motoneurons in the spinal cord (Bernhard and Bohm, 1954), and this has been linked to their ability to perform elaborate movements (Porter and Lemon, 1993). Indeed, even between primates, the number of cortical neurones with a monosynaptic projection to spinal motoneurons is higher in more dexterous species (Lemon, 2008). With the technique of retrograde tracing it has been possible to assess the extent of the cortico-motoneuronal projections to hand muscles in the macaque (Rathelot and Strick, 2006). The authors found a distributed network of neurons in the primary motor cortex projecting to motoneurons innervating finger muscles. Bennett and Lemon recorded the neural activity of cortico-motoneuronal cells while monkeys performed a precision grip task (Bennett and Lemon, 1996). The firing rates of these cells increased drastically during the movement time and in parallel with the electrical activity recorded from the hand muscles used in the task. This finding underlines the importance of the monosynaptic corticospinal pathways in producing hand movements (Bennett and Lemon, 1996).

The relevance of the corticospinal tract in producing finalised movements is confirmed by post-lesion studies (Lemon, 2008). A complete pyramidotomy leads to permanent loss of skilled hand functions in macaques (Lawrence and Kuypers, 1968). Bilateral transections at the level of the medulla were used to ensure that pyramidal tract (e.g. corticospinal) descending fibres were severed. Lesioned monkeys were able to walk and climb their cages shortly after recovering from the operation, abilities that rely mostly on the spared brainstem pathways. However, they were unable to use their hands and fingers independently to grasp food. After three weeks post-operation the animals began to recover the use of their upper limb extremities, but their dexterity never returned to pre-lesion quality (Lawrence and Kuypers, 1968). Studies of such kind demonstrate the incredible plastic properties of the motor system and underline the importance of M1 and the corticospinal tract in functional recovery. Given all these reasons, it is fundamental to develop techniques to measure activity in the motor

cortex and along the corticospinal tract in humans. In the following paragraphs, two methods developed to investigate the excitability of the motor system, namely TMS and PNS, are described.

2.4. Transcranial Magnetic Stimulation (TMS)

The first reported use of a magnetic field to stimulate the scalp is attributed to Barker and his colleagues at Sheffield University (Barker et al., 1985). The instrumentation they employed comprised a high-voltage capacitor capable of delivering a magnetic stimulus, nowadays commonly called pulse, through a flat coil (Figure 2.1 *A*). A major advantage of this method compared to electrical stimulation is that the magnetic field passes through high-resistance structures unchanged. According to Faraday's law of induction, the magnetic field induces a flow of electric current perpendicularly to it. The currents induced by the electric field are responsible for the activation of the neural elements located at the site of stimulation (Rothwell, 1997). The electric field causes ions to flow into tissues, altering the intracellular/extracellular electrical equilibrium and depolarising or hyperpolarising neurons (Rossi et al., 2009). If the induced current depolarises cortical neurons above threshold values, it will induce action potential discharge. When the coil is placed over the appropriate area of the motor cortex it is able to evoke a muscular action potential in a contralateral muscle similar to the one obtained with electrical stimulation of the scalp, which can be recorded from the muscle of interest by means of surface EMG (Figure 2.1 *B*). As opposed to electrical stimulation, TMS is painless for the subjects and does not require scalp electrode's placement (Barker et al., 1985). This method has since become the gold standard to assess the excitability of the corticospinal pathway non-invasively in humans (Hallett, 2007).

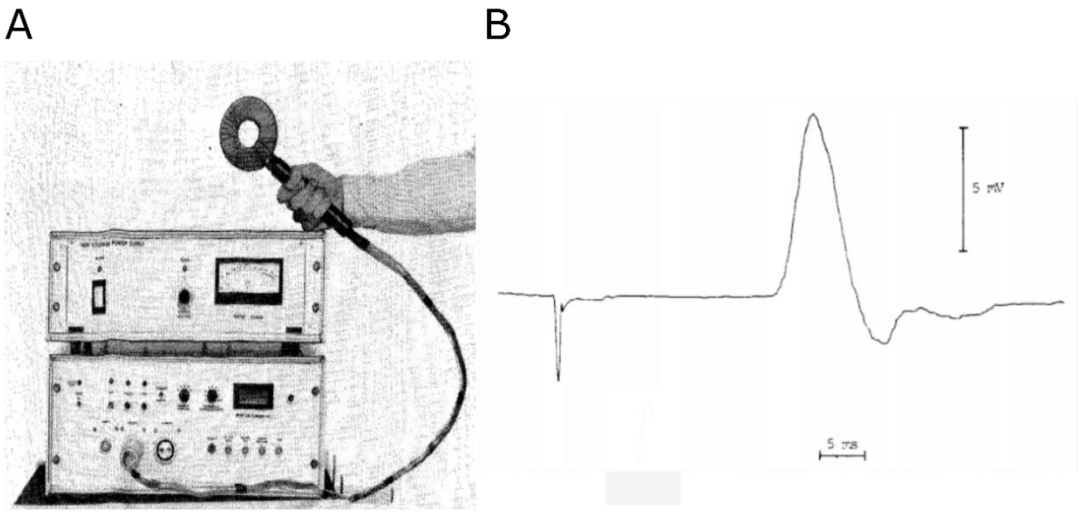

Figure 2.1. Magnetic stimulation of the motor cortex. *(A)* Magnetic stimulator and circular coil used in the original TMS study. *(B)* Muscle action potential recorded from the abductor digiti minimi muscle via surface EMG upon cortical stimulation. Adapted with permission from Barker et al. (1985).

2.4.1. The motor-evoked potential

When magnetic stimulation is delivered over the primary motor cortex, a clear motor output can be measured from contralateral muscles with surface electrodes, the motor evoked potential (MEP) (Hallett, 2007). The amplitude of the MEP increases with increasing stimulus intensity. However, the relationship between the two is not linear but is best described by a sigmoidal curve (Groppa et al., 2012a). MEP responses abruptly deviate from zero when the intensity reaches the threshold value and increase rapidly with higher intensities, reaching a plateau (Groppa et al., 2012a) (Figure 2.2). The size of the evoked MEP is linked to the number of the spinal motoneurons being recruited by the stimulation (Wassermann et al., 2008). TMS can recruit all the spinal motoneurons supplying the target muscle, but the peak-to-peak amplitude measured after supramaximal stimulation is smaller than the one obtained when directly stimulating the nerve innervating the muscle (Magistris et al., 1998). One of the explanations for the occurrence of this phenomenon is the disparate structure of pyramidal tract fibres. Fast, heavily-myelinated fibres are the first to reach the spinal motoneuron pools and are responsible for the first peak of EMG activity seen after TMS, while slower ones will constitute the later parts of the MEP (Wassermann et al., 2008). Cortical-induced MEPs are smaller, of longer duration and less synchronised

than muscle action potentials induced by peripheral nerve stimulation (Rossini et al., 2015).

2.4.2. The silent period

A pause in the ongoing EMG activity can be observed after a TMS pulse is given. This phenomenon is known as cortical silent period (cSP). Silent periods are longer in individuals affected by stroke and neurodegenerative diseases (Ahonen et al., 1998, Modugno et al., 2001), supposedly because of an unbalance between cortical inhibitory and excitatory circuits towards inhibition (Ahonen et al., 1998). The first part of the cSP is due to the refractoriness of spinal α-motoneurons (α-MNs), in that after-hyperpolarization inhibits further generation of action potentials in the same neurons (Inghilleri et al., 1993). The second part (which determines the length of the cSP) was originally considered to depend on intracortical mechanisms similar to the recurrent collateral inhibition of corticospinal neurons which was observed in the cat motor system (Stefanis and Jasper, 1964). Action potentials induced by the stimulation travel along axons collaterals which synapse into cortical interneurons and can inhibit pyramidal neurons (Inghilleri et al., 1993). However, recent evidence suggests that the spinal part of the silent period might be longer than originally observed (Yacyshyn et al., 2016). The authors recorded cervicomedullary motor-evoked potentials (cMEPs) delivered in isolation or after TMS while participants performed submaximal isometric elbow flexions. The intensity of the cortical pulse was set to induce a cSP of ~200 ms. The amplitude of the cMEP, which does not depend on cortical excitability, was reduced up to 150 ms after TMS delivery. The authors concluded that spinal circuits play a significant role in the latest phase of the cSP (Yacyshyn et al., 2016).

2.4.3. The motor threshold (MT)

The intensity of the magnetic pulse discharged by the stimulator is not presented in magnetic field units (e.g. Tesla) but rather as percentages of the maximum stimulator output (MSO), which can be manipulated by the experimenter. In research and clinical routines, it is customary to estimate for each participant a motor threshold (MT) defined as the minimum intensity necessary to evoke MEPs of given amplitude (e.g. 50 μV at rest. Precise MT estimates are essential because the intensity used in TMS protocols is often adjusted to participants' individual MT. The experimenter needs to

ensure that the position of the coil, recording electrode and of the participant itself is stable over time and no changes in the spontaneous firing rate of motoneurons (e.g. pre-activation) occur during the assessment (Groppa et al., 2012a). Even when experimental issues are controlled for, the calculation of a MT is not straightforward. Fluctuations in cortical excitability leads to substantial intertrial variability (Groppa et al., 2012a), with MT variability also being affected by anatomical differences, skull thickness and genetic components (Wassermann, 2002). Nevertheless, individual MT values showed good long-term reliability when MEPs are being recorded from the leg (Cacchio et al., 2011) and forearm (Ngomo et al., 2012) muscles.

2.4.4. Recruitment curves

Recruitment curves (RCs) can be built to describe the relationship between the intensity of the stimulus and the amplitude of the response (Devanne et al., 1997). The first of these experiments quantified the input-output properties of the first dorsal interosseus muscle (Devanne et al., 1997) (Figure 2.2). The intensities of the stimuli used to build recruitment curves started at 5% below threshold and were increased until reaching a plateau in the MEP. A sigmoid function was used to fit the data obtained with this procedure and accounted for more than 80% of the observed variance, showing the non-linearity of the input-output relationship. Additionally, two interesting features were observed. First, the discharge probabilities of single motor units increased linearly rather than sigmoidally with increasing stimulation. Furthermore, the steepness of the curve grew together with the baseline contraction level in both muscles. The corresponding decrease of the MT when muscles were in a contracting state was however modest. The authors considered the possibility that higher strength brought additional motor units with increasing electric field potentials into the discharge, rather than modulating the activity of the same group of motor neurons (Devanne et al., 1997). In addition to it, strong stimuli may synchronize the discharges of single motor neurons, and this will be reflected in an amplitude increase in the recorded EMG but not in change of discharge probability at the single unit level. A combination of the two mechanisms could explain the sigmoidal shape of the recruitment curve.

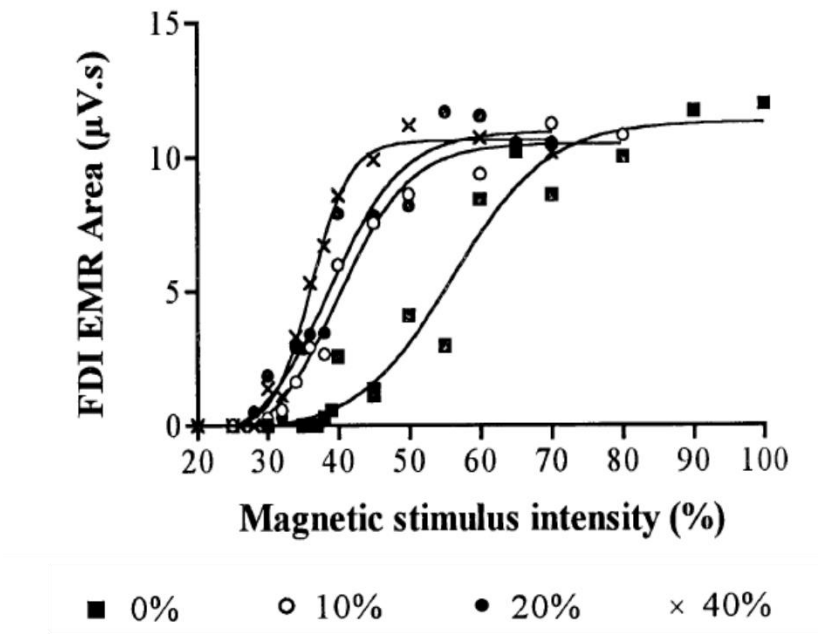

Figure 2.2. TMS recruitment curves. Area of the evoked motor responses (EMR) recorded with surface EMG from the first dorsal interosseous (FDI) muscle at multiple levels of contraction (0% to 40%). Adapted with permission from Devanne et al. (1997).

2.4.5. Facilitation and inhibition between the hemispheres

The brain is formed by two hemispheres which can be independently active (Gazzaniga, 2000). Muscles of the upper and lower limbs are controlled by the contralateral hemisphere (Brinkman and Kuypers, 1973). However, many of the movements produced require the coordinate activation of both arms, eliciting activity in both hemispheres (Geffen et al., 1994). The anatomical structure which connects the two hemispheres through nerve fibres is called corpus callosum. Information transfer between the two hemispheres, either directly through the corpus callosum or indirectly through cortical or subcortical areas, is essential in order to correctly perform bimanual movements (Geffen et al., 1994). Interhemispheric interactions can be tested by placing one TMS coil over each M1 area and manipulating the interstimulus interval (ISI) between two discharges (Ferbert et al., 1992). The first of the two stimuli (conditioning stimulus, CS) can either suppress or facilitate the MEP response induced by the second one (test stimulus, TS) in the contralateral muscle. Interhemispheric inhibition (IHI) is observed when using CS and TS of about 120% of MT of intensity, and is abolished when the intensity falls below threshold levels

(Ferbert et al., 1992). Suppression occurs at two different ISI: between 8 and 10 ms; at around 40 ms (Gerloff et al., 1998). The authors believed the first phase of inhibition to rely on intracallosal inhibition of one motor cortex on the other because of the short latency of the effects (Ferbert et al., 1992). Pathways involved in the inhibition observed at longer latencies may include brainstem and spinal interneuronal populations (Gerloff et al., 1998)

The existence of a similar facilitation mechanism between the two hemispheres was first observed in the same study (Ferbert et al., 1992), but was inconsistent among participants. This phenomenon was obtained at short ISIs (3-5 ms) between two TMS stimuli applied at both M1. The authors theorised that the only pathway which is short and rapid enough to explain this short latency would be the one connecting the two M1 areas through the corpus callosum (Ferbert et al., 1992). In a later study a reliable interhemispheric facilitation (IHF) was produced with a conditioning pulse at either 60% or 80% of the active motor threshold preceding the testing pulse by 6 ms (Ugawa et al., 1993). In humans, the majority of interhemispheric projections between motor areas are mediated by inhibitory interneurons (Asanuma and Okuda, 1962), and this may explain the discrepancies between the consistent IHI and the unstable IHF. Authors theorized the existence of a low threshold potential population of excitatory interneurons, which are activated by the subthreshold stimulus but whose effects are masked by the strongest inhibitory mechanisms when the intensity of the CS increased (Ugawa et al., 1993). This would explain the inconsistent facilitation observed in previous studies which have used conditioning stimuli of high intensities (Ferbert et al., 1992).

2.4.6. Facilitation and inhibition within hemispheres

Modern stimulators can discharge two pulses from the same coil at ISIs as short as 1 ms. Technical advances have made paired-pulse protocols easy to implement, and investigations of the effect of stimulating the same area repeatedly are now common for investigating motor control (Sohn and Hallett, 2004) and the neural changes occurring after lesions (Roy et al., 2011). Phases of inhibition and facilitation of a second pulse by the first are observed at different ISIs. In a relaxed muscle, TMS at motor threshold level depressed the response of following suprathreshold (MEPs of approx. 1.5 mV of amplitude) TMS on the same cortical region between 1-6 ms of ISI, a phenomenon known as short interval intra-cortical inhibition (SICI) (Kujirai et al.,

1993). The first phase of this phenomenon (at 1-2 ms ISI) may be due to refractoriness of corticospinal axons, by which after-hyperpolarization prevents neurons to fire again (Nakamura et al., 1997). Evidence of the cortical origin of the late phase were provided by recording MEPs and cervical epidural volleys on patients undergoing spinal surgery (Di Lazzaro et al., 1998b). The conditioning pulse suppressed the size of the I waves recorded at the cervical level with the exception of the first. Interestingly, a second peak of inhibition (long interval intracortical inhibition, LICI) is observed with suprathreshold stimuli spaced as far as 200 ms. In this scenario, the test MEP is not contaminated by the conditioning MEP, and yet its amplitude is modulated by it. Whether SICI and LICI are distinct mechanisms or depend on the same neural populations is still unclear (Reis et al., 2008).

Intracortical facilitation (ICF) is seen when a subthreshold CS precedes the TS by 10-15 ms (Kujirai et al., 1993). A cortical site of action was supported by the fact that the CS did not induce any discernible MEP and that the H reflex (considered to be an estimate of alpha motoneuron excitability) was not modulated by the CS (Ziemann et al., 1996). However, this explanation was challenged by data yielded from direct recordings of epidural volleys and motor evoked potentials (Di Lazzaro et al., 2006). Di Lazzaro et al. (2006) showed that facilitation of MEPs did not affect the descending volleys as neither the number nor the amplitude of I waves changed. The authors considered two possibilities: (1) the conditioning stimulus excite spinal interneuronal populations making motoneurons more responsive to the test stimulus. If this is the case, the conditioning stimulus might be strong enough to modulate the activity of spinal interneurons and decrease inhibition to alpha motoneurons; (2) epidural recordings represent only a part of the descending activity and smaller or "more dispersed" activity cannot be measured by this technique (Di Lazzaro et al., 2006).

2.4.7. TMS sites of activation

A long-standing debate in the literature regards which neural structures and at which site are being activated by the rapidly decaying magnetic pulse. Preliminary attempts to interpret the results were based on the observed differences between the latency of the responses evoked in the same muscle between electrical and magnetic stimulation (Day et al., 1989a). Day and his colleagues measured the effects of the two stimulation modalities on the discharge of motor units from the first dorsal interosseous muscle. Electrical stimulation elicited a first peak of activity at 25 ms from the stimulus

delivery when the intensity was at threshold level. With increasing stimulation intensities, two more peaks at intervals between 1-3.5 ms from the first could be observed. When giving magnetic stimulation through a round coil in which currents flowed clockwise from above, the first motor unit responses were observed around 27 ms after stimulation. Interestingly, magnetic stimulation intensities slightly higher than the threshold value could sometime elicit firing at multiple peaks in the same motor unit. This phenomenon was never observed with electrical stimulation. These results lent some support to the evidence accumulated from primate studies that had investigated descending activity induced by electrical stimulation. Electrically-induced corticospinal discharges were recorded from the lateral column of the cervical spinal cord, below the medulla, in anaesthetised monkeys (Patton and Amassian, 1954). Single shocks applied to the cortical surface induced a first peak of activation at the short latency of 1-2 ms from stimulation. This volley was named D-wave because it was likely due to direct activation of pyramidal axons (Patton and Amassian, 1954). It was "stable in contour and magnitude" and did not depend on the excitability of the cortex. The D-wave was followed by a series of more variable later responses, occurring at a periodicity of 1-1.5 ms and deemed I (indirect) waves.

From 1998, Di Lazzaro's research group published a series of studies providing the first direct evidence of the effects of descending volleys of activity (Di Lazzaro et al., 1998a) on spinal neurons in humans. The researchers had the opportunity to record activity from epidural electrodes implanted in the spinal cord (C1-C2 level) of patients being treated for chronic pain. In the first of those studies, they compared the results after applying transcranial electrical stimulation (TES) or TMS over the hand motor area. In line with animal work of a similar nature (e.g. Kernell and Chien-Ping, 1967), electrical stimulation given at threshold level for a muscular response produced a short-latency (onset between 2.4 and 2.6 ms) wave in the epidural electrode. Similar to the monkey's counterpart, this early D (direct) wave was thought to reflect direct activation of the corticospinal neurons and was observed both on participants at rest and when they spontaneously activated the muscle. Increasing the intensity of the current led to the occurrence of a late I (indirect) wave. The shortest wave induced by a magnetic pulse in a posterolateral-to-anteromedial direction was seen at latencies between 3.6-3.8 ms and was followed by a series of other I waves appearing with increasing stimulus intensity. Despite the lack of direct evidence, it is thought that indirect waves arise from trans-synaptic activation of corticospinal neurons

(Rothwell, 1997). I waves come at intervals of about 1-1.5 ms from each other and may reflect increasingly long polysynaptic activation. In a follow-up study, the level of baseline activity before stimulation was manipulated by asking the participants to perform an MVC while receiving the pulse. This pre-activation increased the amplitude of all the I waves, leaving their latencies unchanged (Di Lazzaro et al., 1998). The generation of the first I wave seems to follow a different mechanism that the one governing later waves, since inhibiting GABAergic activity by administering benzodiazepine had an effect only on the latter (Di Lazzaro et al., 2000).

Regarding the specific site at which excitation occurs, current models are mainly based on the latency of the evoked responses and the spatial distribution of the induced electric field. Amassian and his colleagues noticed that the onset of the D-wave evoked by electrical stimulation of corticospinal neurons in cats and monkeys was too short to be ascribed to transmission from the cortex, between 0.4 and 1.2 ms which is too short to include the synaptic delay necessary to stimulate cortical cell bodies (Patton and Amassian, 1954). The shorter chronaxie of axons supports the hypothesis that the brief electric pulse (<100 μs) produces stimulation in the initial segments of the pyramidal axon rather than cell bodies or dendrites, which require longer stimuli to be activated (Amassian et al., 1987). With TMS, the earliest activity is recorded 1-2 ms later than the D-wave seen with TES (Di Lazzaro et al., 1998a). The electric fields induced by TMS run parallel to the surface of the brain. Since the field strength dissipates with distance, excitation does not occur in the subcortical white matter but rather in the grey matter as stimulus strength is maximal in this region (Bijsterbosch et al., 2012). Stimulation is more likely to occur at sites where axons running parallel to the electric field bend, because the threshold of activation is lower where the axons curves (Amassian et al., 1987, Katz, 1966). Pyramidal axons lie perpendicular to the cortex and their excitability is unlikely to be affected by the field, while cortico-cortical fibres synapsing into pyramidal neurons will (Rothwell, 1997). The finding that changing the orientation of the coil to the scalp affects MEP latencies favours this hypothesis (Werhahn et al., 1994). The contribution of different descending waves to the shape of the MEP recorded with surface EMG is not a trivial issue. There is substantial agreement that the MEP onset represents activation of the monosynaptic component of the corticospinal tract. At longer latencies, there is the possibility that other spinal and supra-spinal circuits influenced the excitability of the tract and thereby the recorded signal. Potential disynaptic and oligosynaptic contributions

include activity from higher-order motor areas, transmission through propriospinal interneurons and spinal inhibitory pathways (Wassermann et al., 2008).

2.4.8. Limitations of the MEP as a measure of corticospinal excitability

Since its introduction, TMS greatly advanced our understanding of the human motor system physiology (Rothwell, 1997). However, the technological limitations of the technique are well known and hinder its research relevance. There is still not a conclusive answer on which neural elements, and at which time point, are activated by the electric field (Ziemann, 2000). When stimulating the cat visual cortex with strong magnetic pulses, a strong inhibitory phase precedes the excitatory phase (Moliadze et al., 2003). Similarly, stimulating the motor cortex can induce multiple inhibitory postsynaptic potentials (IPSPs) that will influence the recorded MEPs (Cowan et al., 1986). The potential role of inhibitory spinal/cortical interneurons in unknown. Even when using state-of-the-art TMS coils, activation is not local and can spread to the motor representation of contiguous muscles or, with higher intensities, to other cortical areas (Ziemann, 2000). For example, functional neuroimaging of the brain right after TMS delivery showed activation in the SMA, PmD and cingulate motor area, all of which can in turn modulate activity in motor interconnected structures (Bestmann et al., 2004). Adding to this inherent complexity, the lack of standardized routines to follow may be responsible for the paucity of replicable results observed across the years. Future studies need to describe in details the experimental setting and stimulation protocol in order for the results to be correctly interpreted (Ziemann, 2000). While the estimation of MT is reported in almost all studies, this has no physiological values if information about the stimulus-response curve is not available (Pierrot-Deseilligny and Burke, 2005).

A peculiarity of the primate motor system is the presence of monosynaptic corticospinal projections to motoneurons (Kuypers, 1981). The extent to which this system and the indirect spinal cord circuitries contribute to motor control is still a matter of debate. Most of the findings discussed so far seem to support the notion of a prominent role of the corticomotoneuronal component of the corticospinal tract in motor control. However, the interpretation of results in humans relies on the inconclusive assumption that the compound MEP evoked by a magnetic pulse reflects the activity of only the monosynaptic corticospinal pathway (Nielsen, 2016). It is

indeed likely that the MEP is influenced by the excitability of spinal neural populations. Ideally, any study aiming at demonstrating a cortical origin of a change in the MEPs should be validated by comparing the effects of TMS with the effects of cervicomedullary stimulation on the descending volley (Inghilleri et al., 1993).

A valuable alternative would be to measure the effect of TMS on the discharge probability of single motor units by means of needle electrodes. The participant is asked to contract the targeting muscle, the recorded electrical activity is amplified and a motor unit is then distinguished according to the shape of its response (Aimonetti and Nielsen, 2002). A peri-stimulus time histogram (PSTH) is generated by plotting the probability of firing of that particular unit at different time points preceding or following the stimulus (TMS pulse, in this specific case). With this technique, it has been possible to judge the relative contribution of monosynaptic and polysynaptic pathways to the motoneuronal response by measuring the latencies and durations of the peaks in increased firing probability across different tasks (Aimonetti and Nielsen, 2002) and different levels of activity (Palmer and Ashby, 1992a). Other studies demonstrated that cortical projections modulate the excitability of alpha motoneurons through a disynaptic route via spinal interneurons (Burke et al., 1984). Pairing cortical stimulation with stimulation of the peripheral nerve innervating the target muscle at different intervals may reveal the role of spinal circuits in determining motor unit discharge and thereby movement outcome (Petersen et al., 2003).

2.5. Peripheral nerve stimulation

It has been known since the late 18th century that passing an electrical current through the spine of dissected animals could elicit contractions in the target muscle (Galvani, 1791). Nowadays, electrical stimulation is widely used in medicine, sport and research. The apparatus used for research in humans typically comprises of a high-voltage stimulator which delivers brief pulses of direct current. Bipolar stimulating electrodes, made of a positive pole (anode) and a negative pole (cathode) spaced a few centimetres apart are frequently used to stimulate forearm nerves to avoid current spread to other nerves (Palmieri et al., 2004). For trans-cutaneous cathodal stimulation, negatively charged ions flow from the negative to the positive pole depolarising and inducing action potentials in the fibres beneath the cathode (Merrill et al., 2005). The maximum voltage supplied by the stimulator, in *mV* units, and the output current, in mA units, can be controlled by the experimenter. In addition, is it

often possible to manipulate the polarity and length of the pulses and hence favour the stimulation of different neural elements (Panizza et al., 1994). The two EMG responses most commonly studied after trans-cutaneous nerve stimulation are the motor wave (M-wave) and the monosynaptic reflex (H-reflex).

2.5.1. The motor wave

Many of the nerves routinely stimulated in research studies are mixed nerve, containing both motor (descending) and sensory (ascending) fibres. When stimulation reaches the threshold to cause depolarization in the motor axons, action potentials fire towards the neuromuscular junctions and produce muscle contraction (Palmieri et al., 2004). In the EMG trace, this phenomenon is observed as a deflection from baseline activity arising a few ms after the delivery of the pulse, the motor (M) wave. This compound action potential is an index of motor unit recruitment and its amplitude is independent from the excitability of spinal and supraspinal structures (Palmieri et al., 2004). A motor wave can be recorded in all limb muscles, preferentially in a position which avoid stretch of the muscle (Pierrot-Deseilligny and Burke, 2005). The strength-duration time constant for motor axons is shorter than for sensory axons and brief (0.3-0.5 ms) pulses are used to induce motor responses (Mogyoros et al., 1996). The intensity of stimulation is incremented until the motor wave reaches its maximum such that additional current does not increase the amplitude further. The EMG response obtained at this intensity is called M_{max} and represents the activation of the entire motoneuron pool (Pierrot-Deseilligny and Mazevet, 2000). The M_{max} can be used as a normalisation factor for the MEP (Marchand-Pauvert et al., 1999). MEPs calculated as a percentage of the M_{max} reflect motoneurons recruitment and the excitability of the corticospinal tract (Lackmy and Marchand-Pauvert, 2010).

2.5.2. The monosynaptic reflex

Muscle spindles contain sensory fibres that project to the spinal motoneurons innervating the homonymous muscle (Pierrot-Deseilligny and Burke, 2005). Electrical stimulation of the afferent fibres generates a response in the EMG called the monosynaptic reflex (Magladery and McDougal Jr, 1950). The monosynaptic reflex, or H-reflex, is the electrical analogue of the stretch reflex. In direct contrast to the stretch reflex, electrical stimulation bypasses the muscle spindles and the fusimotor activity to activate a ‘simple’ circuit between afferents and spinal

motoneurons (Knikou, 2008). The H-reflex was the first technique developed to measure nerve conduction and spinal excitability in humans. It is seen as a longer-latency (compared to the M wave) multiphasic response in the recorded EMG trace. The latency is consistent with a monosynaptic activation of alpha motoneurons (Magladery et al., 1951). The low variability in latency of motor unit activity after afferent stimulation supports the monosynaptic component hypothesis (Trontelj, 1973). Afferent stimulation recruits motoneurons according to the size principle (Henneman, 1957). Slow, small neurones are the first to be activated at threshold intensities. At higher intensities, bigger and faster neurons will start firing contributing not only to the increase amplitude but also to shortening of the latency. As the afferent volley needs to travel back to the spinal cord and then down the motor axons, the further away is the muscle from the spinal segment innervating it the longer the reflex latency will be (Palmieri et al., 2004). Ia afferents strength-duration time constant is longer than motor axons' one and pulses ranging from 0.8 to 1 ms are normally used for H-reflex stimulation (Mogyoros et al., 1996).

Monosynaptic reflex can be recorded at rest only in a few upper and lower limb muscles, but voluntary contractions of the target muscle might facilitate their occurrence (Burke, 2016). A strong determinant of the H-reflex amplitude at rest is the stimulation rate, the time between deliveries of consecutive stimuli (Burke, 2016). When the rate is high (above 0.2) consecutive responses are smaller than the first one because of neurotransmitter depletion (Curtis and Eccles, 1960). Interstimulus intervals of at least 5 seconds need to be used to address this issue. H-reflex testing is used to assess the effects of spinal and supraspinal conditioning volleys on spinal motoneurons (Pierrot-Deseilligny and Burke, 2005), for example by stimulating an antagonist muscle (e.g. tibialis anterior) before eliciting the H-reflex (in the soleus muscle) to assess reciprocal inhibition between pair of muscles (Iles, 1986). While traditionally considered to be an index of alpha motoneurons excitability, its underlying physiology has been recently reconsidered (more on section 2.8) (Burke, 2016).

2.5.3. Relationship between H and M recruitment curves

The motor wave and the monosynaptic reflex are mediated by partially overlapping (e.g. part of the motor pathway) pathways and a single electrical pulse can elicit both in the target muscle. However, the two volley recruit alpha motoneurons in a different

order: the afferent volley will recruit small motoneurons first, while the first axons to be directly stimulated will be the bigger, faster ones (Knikou, 2008). Sensory fibres have a lower rheobase, requiring stimuli of lower intensities to be activated, than motor axons and therefore the first response that can be recorded at low intensities is the H-reflex (providing that the pulse is sufficiently long) (Panizza et al., 1989). The produced afferent volley increases together with the intensity until the threshold to stimulate motor axons is reached and the M wave starts to appear. Increasing the intensity from this point has opposite effects in the two responses: the motor wave amplitude continues to increase until reaching its plateau, after which it stabilizes on the M_{max} amplitude value; the H-reflex decreases almost at the same rate of the M wave increase, and the response usually disappears at M_{max} intensity (Figure 2.3 *A* and *B*) (Pierrot-Deseilligny and Burke, 2005). In order to understand why the H disappears at high intensities it is important to consider the electrical properties of the two types of fibres. Although the threshold to activate the smaller Ia afferents is low, to recruit the whole pool of motoneurons intensities of about 400% of MT are necessary (Gracies et al., 1994). At this stimulus level the motor wave would have reached its peak too (Knikou, 2008). The strong electrical stimulus generates an antidromic volley in the motor axons which collides with the afferent volley at the motoneuron level and annihilates it, truncating the H (Pierrot-Deseilligny and Burke, 2005).

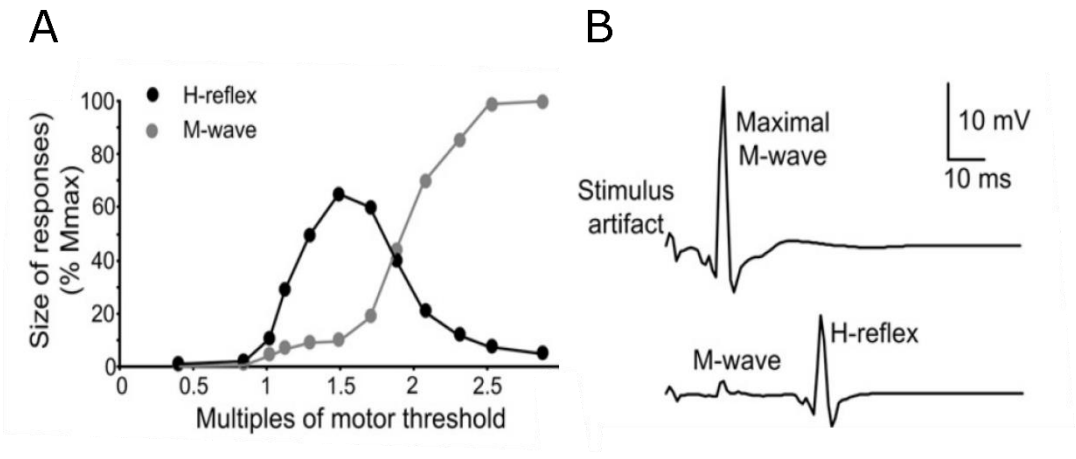

Figure 2.3. Relationship between the motor wave and the monosynaptic reflex. *(A)* Amplitude of the M wave and H-reflex evoked from posterior tibial nerve stimulation at increasing stimulation intensities. *(B)* Example of traces showing the disappearance of the H-reflex at M_{max} intensities (top) and the decrease of the M wave at H_{max} intensities (bottom). Adapted with permission from Knikou (2008).

For both clinical and research testing, it is essential to record M wave and H-reflex across multiple intensities to describe their input-output relationship (Knikou, 2008). A useful index to use as a baseline of reflex excitability is the ratio between H_{max} and M_{max} (Hugon, 1973). The ratio has clinical utility in the evaluation of spasticity caused by motor neurons lesions (Shemesh et al., 1977). In research settings, it is customary to record the reflex at a percentage of the M_{max} amplitude (Pierrot-Deseilligny and Burke, 2005). In this way it is possible to normalise the reflex values across participants and make sure that the same proportion of motoneurons is activated (Pierrot-Deseilligny and Burke, 2005).

The chosen percentage should be small enough to ensure H is still on the ascending limb at that intensity and there is not any collision with the efferent volley. Stimulation intensity should be adjusted such that a small M wave is present in order to monitor stability of stimulation (Pierrot-Deseilligny and Burke, 2005). If not possible, test stimuli should be alternated with control stimuli eliciting only the motor response (which should be stable throughout the testing). This protocol can be used to determine changes in spinal excitability occurring in response to therapies or after motor practice: if stimuli that evoked a certain reflex before practice become more/less effective after the practice, without any concomitant changes in the size of the M_{max}, changes in the spinal cord circuitry must have occurred (Palmieri et al., 2004).

2.5.4. Non-monosynaptic contributions to the H-reflex

The relatively short latency of the monosynaptic reflex onset and the evidence that Ia afferents have extensive (each afferent projecting to many motoneurons) monosynaptic projections to alpha motoneurons contributed to the historical view of the H-reflex as a simple spinal circuit (Burke, 2016). However, many studies have shown that an intricate system of interneurons contributes to its amplitude (Knikou, 2008).

Ib afferents. When an electrical pulse is delivered to the nerve, Ib afferents originating from Golgi tendon organs are activated together with Ia afferents (Burke, 2016). Ib afferents project to interneurons located in laminae VI and VII (Ib inhibitory interneurons) which inhibit alpha motoneurons (Pierrot-Deseilligny and Burke, 2005). Effects of this inhibitory volley will appear in the EMG recording around 0.5-1 ms (synaptic delay) after the onset and decrease the response (Burke et al., 1984).

Presynaptic inhibition. The motor system possesses a strong control mechanism which regulates the effects of incoming sensory feedback on the excitability of spinal motoneurons. This mechanism is called presynaptic inhibition. In cats, a conditioning pulse given to a flexor nerve can reduce the size of the extensor-induced excitatory postsynaptic potentials (EPSPs) in motoneurons without changing their membrane potentials (Skoglund, 1960). This inhibition is due to depolarization of the afferent terminals, which reduces neurotransmitter release at the afferent-motoneuron synapse (Willis, 2006). The pathway responsible for presynaptic inhibition has two primary afferent depolarization (PAD) interneurons. The last-order neurons are GABA-ergic and synapse into Ia terminals, reducing neurotransmitter release (Rudomin and Schmidt, 1999). In the context of H-reflex testing, presynaptic inhibition is a factor to consider when comparing reflex amplitudes obtained in different tasks and postures. For example, the H-reflex recorded from the soleus muscle decreased when participants went from walking at a natural speed to running (Capaday and Stein, 1987). This would imply that the excitability state of motoneurones is lower during running, but the EMG activity recorded from soleus muscle was actually increased compared to walking amplitudes at the same stimulation intensity (Capaday and Stein, 1987). The reflex gain decreased as a result of higher presynaptic inhibition and independently from the excitation of motoneurons (Knikou, 2008).

Reciprocal inhibition. Ia afferent fibres bifurcate in the spinal cord to innervate not only the homologous motoneurons but also a class of Ia inhibitory interneurons located in lamina VII. These interneurons monosynaptically inhibit the motoneurons antagonist to the muscle from which they receive Ia input (Pierrot-Deseilligny and Burke, 2005), a mechanism named reciprocal inhibition. Conditioning stimuli to the deep peroneal nerve facilitate (e.g. increase the size) the H-reflex recorded from the quadriceps muscle if the level of contraction is low (Marchand-Pauvert et al., 2002). When contraction increases, the facilitation is suppressed. Changes in PSTHs showed that the suppression of facilitation started around 0.8 ms after the onset, in line with a disynaptic inhibitory mechanism (Marchand-Pauvert et al., 2002). When interpreting results from H-reflex studies, it should be specified that only the onset of the response represents the excitability of the monosynaptic component (Pierrot-Deseilligny and Burke, 2005).

Descending tracts. The simplicity of H-reflex recording and stimulation has led over the decades to the erroneous assumption that it represents a spinal two-neurons

circuits (Burke, 2016). Changes in reflex modulations observed after experimental manipulations are often attributed to spinal mechanisms acting independently from the influence of descending activity. This is the result of an arbitrary dichotomous split between reflexes, historical domain of the spinal cord, and voluntary activity, represented by the brain. However, already in the early years of the 20th century, Sherrington cautioned that "all parts of the nervous system are connected together and no part of it is probably ever capable of reaction without affecting and being affected by various other parts" (Sherrington, 1952). In cats, the interneurons mediating presynaptic inhibition of afferents receive excitatory and inhibitory modulations from the sensorimotor cortex (Andersen and Eccles, 1962). Pyramidal tract volleys inhibit the transmission in the PAD interneurons mediating presynaptic inhibition with the total effect of facilitating the reflex response (Hultborn, 2006). The outcome of stimulation is reversed for Ib afferents, since cortical stimulation resulted in depolarisation of cutaneous and Ib afferents (Andersen and Eccles, 1962).

In humans, a protocol to study the convergence of cortical and peripheral volleys into spinal neurons has been proposed by Cowan and colleagues (1986). Electrical stimulation on the scalp was delivered at different time intervals from the peripheral stimuli. The conditioning (cortical) pulse was subthreshold for evoking activity in the recorded EMG. The descending volley was thereby too weak to elicit action potentials in the alpha motoneurons, but strong enough to modulate spinal excitability. When the interval between stimuli was manipulated such that these arrived synchronously at the motoneuron level, the net effect was a strong facilitation of the monosynaptic reflex. The facilitation depends on monosynaptic modulation of alpha motoneurons excitability by the corticospinal volley and was observed in flexor and extensor wrist and finger muscles and in the thenar muscle, but not in the soleus muscle. The facilitation was immediately curtailed by an inhibition. The authors proposed that the phase of inhibition depended on descending projections to Ia inhibitory interneurons responsible for the inhibition of the antagonist muscle (Cowan et al., 1986).

2.5.5. Conditioning the monosynaptic reflex with TMS

In this previous paragraphs, it was discussed how TMS is a valuable tool to estimate corticospinal excitability, but it does not permit to draw conclusions about which neural populations are being activated by the magnetic stimulus (Burke and Pierrot-Deseilligny, 2010). The excitability of the motor neurons, the 'common spinal

pathway' (Sherrington, 1925) is modulated by a series of descending tracts, by afferent inputs from the periphery and by spinal interneurons. Integration with spinal circuits could allow the online processing of sensory feedback signals, releasing the cortex from controlling muscle activity (Sinkjær et al., 2000). Similarly, the "purely monosynaptic" pathway which evokes the H-reflex is modulated by descending tracts activity (Andersen and Eccles, 1962).

Different techniques need to be used to assess spinal circuits independently. A common practice is to compare changes in MEPs occurring after motor practice or in response to lesions with changes in the amplitude of the monosynaptic reflex to estimate whether the effects are due to cortical or spinal events. Even if we assume that the two techniques activate the same motoneuronal pools (which is not entirely true at rest, see Morita et al., 1999), the two techniques alone cannot discern between changes occurring at a presynaptic level between Ia afferents and motoneurons and changes resulting from increased cortical drive (Burke and Pierrot-Deseilligny, 2010). A valid alternative would be to condition the monosynaptic reflex by giving subthreshold (not producing any compound MEP) TMS prior to the electrical stimulation of the desired nerve (Nielsen and Petersen, 1995). A facilitatory effect (increase of H amplitude) is commonly observed which is dependent on excitability changes at the cortical level, since it disappeared when magnetic stimulation was replaced with electrical stimulation (Petersen et al., 2003). Demonstrating that a manoeuvre influences the TMS-conditioned monosynaptic reflex without parallel changes in the H-reflex pathway indicates changes in cortical drive to motoneurons.

2.6. Application of non-invasive stimulation techniques for the study of motor control

2.6.1. TMS mapping of the motor cortex

The topographic organization of the motor cortex is revealed by moving the magnetic coil over the precentral gyrus and recording from different muscles of the body. It is thus theoretically possible to map every spot and obtain a "motor homunculus" representing the muscles of the body (Penfield and Boldrey, 1937). An attempt to map the areas of four functionally relevant arm muscles was first reported by Wassermann et al. (1992). Single stimuli were given to cortical positions 1 cm apart at 100% of stimulator output and the sum of the MEPs amplitudes at each site was calculated.

Motor-evoked potential could be elicited in each upper limb muscles when stimulating large areas of M1 (Wassermann et al., 1992). This finding is in line with the evidence derived from primate studies, in which large areas of M1 can innervate motoneurons of a single muscle (Rathelot and Strick, 2006). However, this approach was later revealed to overestimate the extent of each cortical representational area. At the intensity used to stimulate M1 (100% of stimulator output), the induced magnetic field spreads across the cortex and the magnetic pulse activated not only neurons located at the stimulation site but also neurons in other cortical areas (Wilson et al., 1993). More conservative methods were later preferred in which the stimuli were given at motor threshold level (Wilson et al., 1993). Mapping studies revealed two characteristics of motor cortical excitability: higher intensities are required in order to stimulate the leg area compared to the arm area; higher intensities are required in order to stimulate proximal rather than distal muscles in the arm (Wassermann et al., 2008).

2.6.2. The "virtual lesion" approach

Single pulses of magnetic stimulation, when given at the appropriate time and location, can disrupt the ongoing neural activity and interfere with the function of a given area (Pascual-Leone, 1999). This is referred to as the "virtual lesion" approach. Such experiments generally aim to demonstrate the contribution of a brain area to behaviour by observing how disrupting it influences performance. The first account of this procedure for the study of motor behaviour dates back to 1989 (Day et al., 1989b). A single, high-intensity magnetic pulse applied over the wrist motor area 100 ms after an auditory cue (informing the participants to start the movement) delayed motor performance by up to 150 milliseconds. Importantly, the effect was not observed after median nerve stimulation or in the arm ipsilateral to the cortical stimulation. In addition, the results could not be explained in terms of refractoriness of spinal motoneurons, since a second M1 electrical stimulus given during the delay period evoked a MEP in the wrist muscle. The authors speculated that the motor cortex may contribute to the execution of motor programs stored elsewhere within the brain, by running motor programs in a sequential way that can be interrupted by a stimulus given at the right time (Day et al., 1989b).

The involvement of another cortical structure important for movement production, the premotor cortex, was subsequently investigated in a similar experiment (Schluter et al., 1998). TMS was used to disrupt the performance of participants involved in a

choice reaction-time task. Single pulses were delivered on the motor and premotor left cortex at different time intervals from a visual cue which instructed participants to make a key press with their right hand. They used their middle finger to press the key if the visual cue was a big circle or a small rectangle and the index finger if the visual cue was a small circle or a big rectangle. Reaction times were longer when the contralateral premotor cortex was stimulated 100-140 ms after the visual cue. In comparison, reaction time were longer when the contralateral motor cortex was stimulated 300-340 ms after the visual cue. The authors concluded that the motor and premotor cortex have different roles in the performed movement: disrupting the premotor cortex at a time in which participants are selecting the movement led to longer reaction times, which point to a role of the area in movement selection; disrupting the motor cortex increased reaction time right before movement was initiated, indicating that movement preparation engages the primary motor cortex (Schluter et al., 1998).

2.6.3. Repetitive TMS

A TMS pulse influences the responses of subsequent TMS pulses given at different intervals because the excitatory inputs to motoneurons from the preceding stimulus summate with the potentials generated by the next stimulus (Valls-Solé et al., 1992) . When multiple pulses are given at certain intensities and frequencies, these might provoke strong and long-lasting changes in the excitability of the motor system. Pascual-Leone and colleagues employed different combinations of intensities and frequencies of cortical stimulation given on the motor cortex with participants at rest (Pascual-Leone et al., 1994b). Stimulating frequencies of 5 Hz and above induced progressively higher MEPs in the abductor pollicis brevis muscle. Increasing both the frequency and the stimulation intensities led to spread of activation as seen by recording MEPs from other muscles. In addition, the excitability of the corticospinal tract remained higher for 3 to 4 minutes after the end of the pulse train. It was later shown that high-frequency subthreshold TMS can enhance MEPs responses for up to 30 minutes after the end of stimulation (Quartarone et al., 2005).

Low-frequency TMS, where pulses are spaced by at least 1 second, is considered to have an inhibitory effect on the recorded MEPs (Wassermann et al., 2008). Trains of 0.9 Hz pulses at 115% of the resting motor threshold produce a decrease in the amplitude of the evoked responses lasting for 15 minutes (Chen et al., 1997). The

resulting after-effects were prolonged to 30 minutes when 1 Hz was chosen as stimulation frequency applied over the cortical representation of the flexor pollicis brevis muscle (Muellbacher et al., 2000b). The authors were able to prove that the excitability decreases were limited to the targeted muscle without spreading to adjacent muscles by showing that motor-thresholds for activating the biceps muscle did not change after low-frequency TMS compared to baseline values. This indicates that only the neural circuits repeatedly activated by repetitive TMS, and not cortical excitability in general, are affected by stimulation. However, the protocol did not cause participants' performance to deteriorate as the peak force and peak acceleration of the produced movements did not change after repetitive TMS. The authors believed this lack of effects to be dependent on the nature of the task used to measure performance, because maximal forces are produced by simultaneous activation of more muscles and not just the one which was targeted by the stimulation. Nevertheless, the casual link between stimulation-induced plasticity and performance has since been repeatedly observed (Luber and Lisanby, 2014). There is substantial agreement that the produced affects do not simply reflect repeated activation of corticospinal neurons but provide the neural substrate for inducing behaviour-relevant plasticity (Benito et al., 2012).

2.6.4. Paired Associative Stimulation

Multiple lines of research focused on designing protocols to induce long-lasting plastic changes in the excitability of the motor cortex. The effects of one such protocol, paired associative stimulation (PAS) (Stefan et al., 2002), seem to reflect mechanisms of associative plasticity (Markram et al., 1997). When an excitatory synaptic input repeatedly reaches the neuron before its discharge, the strength of the connection between the two neurons will increase. Presynaptic action potentials induce strong depolarization in the postsynaptic neuron, removing magnesium block and permitting Ca^{2+} influx which induce synaptic strengthening (Markram et al., 1997). Timing is essential in that if the input arrives after neural discharge, the postsynaptic neuron will be hyperpolarised and the strength of the synaptic connection will decrease. In the original PAS experiment, left median nerve stimulation was paired with a TMS pulse delivered to the right motor cortex (Stefan et al., 2000). The motor evoked potentials of the contralateral abductor pollicis brevis were measured before and after 90 pairs of peripheral-brain stimulation given at an interval of 25 ms.

An increase in the MEPs amplitudes was observed after the stimulation and persisted for at least 30 minutes. These findings were reproduced across different sites of peripheral stimulation (Stinear and Hornby, 2005). Long-lasting decreases of MEPs amplitude can as well be obtained when peripheral stimulation of the median nerve is paired with magnetic stimulation at the hand area at intervals of about 10 ms (Wolters et al., 2003). It has been suggested that PAS effects resemble a form of Spike Timing Dependent Plasticity as studied in cortical slice preparations (STDP) (Müller-Dahlhaus et al., 2010). First of all, the effects last for about sixty minutes after stimulation but the excitability of the circuits returns to baseline values after that (Stefan et al., 2000). Repetitive stimulation can induce long-lasting potentiation (Stefan et al., 2000) or depression (Wolters et al., 2003) depending on the interval between stimuli, an effect similar to the one observed when stimulating single pyramidal neurons (Markram et al., 1997). Pharmacological studies supported this hypothesis: blockage of NMDA receptors prevents increases of excitatory post-synaptic potential amplitudes in pyramidal neurons; when NMDA receptor antagonist dextromethorphan is administered before PAS it abolishes any excitability increase (Stefan et al., 2002).

2.6.5. Cortical excitability changes during movements

In order to understand how the CNS generates and control movements, it is important to characterise the neural processes occurring while humans perform movements (Reis et al., 2008). The excitability of the circuits generating MEPs while participants are preparing a movement was investigated using a reaction time paradigm (Rossini et al., 1988). The task consisted of ballistic thumb oppositions, produced as rapidly as possible, in response to an acoustic cue. The authors found that the probability of eliciting a response to transcranial magnetic stimulation started to increase around 100 ms prior to onset of EMG in the muscle of interest. At 40 ms before EMG onset, stimulus amplitudes below threshold for eliciting a response in the resting muscle produced an MEP in 100% of trials (Rossini et al., 1988). While interesting from an experimental prospective, the use of externally-paced task in which participants are instructed on when to start the movement might not be representative of the majority of task performed daily, which are self-paced and self-generated (Jahanshahi et al., 1995). In fact, there is evidence that the rise in cortical excitability occurring before self-paced movements is slightly different (Chen et al., 1998). When participants were

instructed to produce thumb movements whenever they wanted, MEPs induced by TMS were facilitated 20 ms earlier than MEPs recorded during a reaction-time protocol (Chen et al., 1998).

There is substantial agreement that the MEP amplitudes recorded from a target muscle increase when the muscle is active (Pascual-Leone et al., 2002), while at the same time the MEPs recorded from the contralateral homologous muscle are decreased (Leocani et al., 2000). MEPs recorded when a muscle is active are bigger because more spinal motoneurons are closer to their firing threshold and can be activated by the cortical stimulus (Rossini et al., 2015). At the onset of a unimanual thumb movement, the excitability of the contralateral homologous muscle decreases (Leocani et al., 2000). The authors proposed the existence of a mechanism by which unwanted contralateral movements are actively suppressed, perhaps mediated by transcallosal inhibition (Leocani et al., 2000). Mackinnon and Rothwell studied the relationship between the EMG patterns of triphasic wrist flexion movements and corticospinal excitability (MacKinnon and Rothwell, 2000). Subthreshold TMS stimuli elicited a response in wrist muscles when given up to 23 ms before the onset of the agonist burst. However, no changes in corticospinal excitability preceded the onset of the antagonist burst. The activity in agonist muscles seems to follow the time-course of the changes in excitability of the target muscle, at least in the time preceding EMG onset. The probability of evoking motor responses in an agonist muscle with subthreshold stimuli increases before the movement is initiated (Pascual-Leone et al., 1992).

Ballistic movements are often accompanied by postural adjustments in other muscles (Cordo and Nashner, 1982). Transcranial magnetic stimulation was used to investigate whether postural adjustments depend on compensatory spinal circuits or are pre-programmed together with the ballistic movement at a cortical level (Palmer et al., 1994). Left arm abduction produces three bursts of EMG activity: a first one in the deltoid, a second in latissimus dorsi and a third again in the deltoid muscles (agonist-antagonist-agonist pattern). Postural adjustments can be seen on the contralateral side by recording from the pectoralis major and abdominal muscles. A TMS pulse on the right motor cortex (contralateral to arm abduction) delays the triphasic EMG activity on the left side, but not the postural contractions. Conversely, left hemisphere TMS delayed only the onset of the EMG activity corresponding to the postural adjustments. The authors concluded that the contralateral postural reactions were centrally pre-

programmed and an integral part of the ballistic movement (Palmer et al., 1994). This hypothesis is corroborated by studies showing that postural reactions to heel raise (Iglesias et al., 2008) and finger flexion (Caronni and Cavallari, 2009) depend on activity in the primary motor cortex.

When a movement ends and the EMG activity in the muscle reverses to its pre-movement values, corticospinal excitability does not simply decrease to its baseline. Instead, MEP responses are increased up to 160 ms after EMG offset (Chen et al., 1998). It might be supposed that the membrane potential of spinal motoneurons is still altered during this phase but at subthreshold levels for generating descending action potentials (Chen et al., 1998). In addition, there is evidence that intracortical inhibitory pathways contribute to movement termination (Buccolieri et al., 2004). Buccolieri and his colleagues had participants perform bilateral isometric abduction movements of a pre-specified (2 seconds) duration. They found that intracortical inhibition (SICI) increases in the 30 ms before thumb relaxation, perhaps due to a intracortical mechanism which causes movement ending (Buccolieri et al., 2004).

2.6.6. Spinal excitability changes during movements

The time course of spinal excitability changes occurring before, during and after a movement has been intensely studied over the years. For lower limbs, participants were instructed to make plantar flexions in response to an auditory stimulus (Brunia and Vuister, 1979). In addition, they were presented with a warning stimulus given 4 seconds before the response stimulus. The amplitudes of the soleus H-reflexes were found to be increased in the 200 ms following the warning stimulus. Interestingly, the increase in soleus reflex excitability was seen even when the movement to be produced did not involve the soleus (e.g. index finger button press). This phenomenon was attributed to a generalised increase in corticospinal excitability. Immediately before the delivery of the second (response) stimulus, the responses in the involved muscle decreased while the responses from non-involved muscles remained stable. The authors suggested that this late phenomenon was due to the selective inhibition of movement-related Ia afferents at a presynaptic level (Brunia et al., 1982).

At the onset of a movement, reflex amplitudes recorded from the involved muscle increased (Brunia et al., 1982). The role of presynaptic inhibition in the observed increases was studied by Hutlborn and his colleagues with the use of conditioning stimuli to the homonymous and heteronymous muscles (Hultborn et al., 1987). A

vibratory shock to the tibialis anterior inhibits presynaptically the Ia volley which produces the monosynaptic reflex evoked in the soleus muscle (Morin et al., 1984). The vibration-induced inhibition disappeared at the onset of plantar flexions, which strongly activate the soleus muscle. At the same time, presynaptic inhibition of Ia terminals is increased in the muscles not involved in the contraction (Hultborn et al., 1987). These effects might be part of a greater mechanism by which the descending volley regulates the gain of afferent volleys according to the task demands (Ilmane et al., 2013).

Bimanual coordination is essential in order to perform many motor tasks. A unilateral arm contraction can influence the excitability of the pathway to the homologous, contralateral arm (Hortobágyi et al., 2003). Responses to TMS, cervicomedullary stimulation and PNS obtained upon stimulation of the right FCR muscle were recorded while the left forearm was contracted by the participant (voluntary contraction) or through stimulation (Hortobágyi et al., 2003). The authors found that MEPs evoked from the right forearm were higher when the left forearm was contracted. Responses to cervicomedullary stimulation remained unchanged by contraction, but the monosynaptic reflex evoked in FCR was suppressed by contralateral contraction. The authors concluded that the unilateral movement altered the excitability of the contralateral cortical neurons, which in turn acted upon segmental pathways by increasing the amount of presynaptic inhibition of Ia afferents (Hortobágyi et al., 2003). Similar changes of excitability are observed in the right FCR motor pathway when participants are instructed to perform rhythmic flexion and extension wrist movements with the opposite arm (Carson et al., 2004). Moreover, the authors found that changes in the amplitude of the MEPs and H-reflexes depended on the phase of the rhythmic movements. The effects of unilateral movement on the excitability of the homologous corticospinal pathway were more pronounced in the movement phases in which the left FCR was more engaged (Carson et al., 2004). The importance of contralateral projections for skill acquisition and strength increase will be discussed in more details in Chapter 2.8.

Spinal circuits are involved not only in the preparation and execution of movements, but also in their termination (Hultborn et al., 1996). The amplitude of the H-reflex is depressed up to 8 seconds after the offset of voluntary contractions (Crone and Nielsen, 1989a). Results obtained from recording motoneuron potentials in the cat showed that post-activation hyperpolarization only persists for about 100 ms after

discharge, and is thereby unlikely that the reflex depression depends on motoneuronal excitability (Brock et al., 1952). The post-activation depression is maintained even when passive movements (joint moved by an experimenter) are employed (Hultborn et al., 1996). Descending volleys induced by TMS were not suppressed during this period, indicating that cortico-motoneuronal transmission was not affected. The findings could not be explained in terms of increased presynaptic inhibition either, since its effects resolve in less than a second (Eccles, 1964). Instead, the evidence points to a reduction in neurotransmitter release from the previously activated fibres (Hultborn et al., 1996).

2.6.7. Skill acquisition and cortical plasticity

The central nervous system is intrinsically capable of reorganizing itself in response to external stimuli or events, a phenomenon known as neural plasticity. With TMS, it was shown that cortical reorganisation occurred in the primary motor cortex in patients with acquired of congenital peripheral lesions and CNS lesions (Cohen et al., 1991, Levy Jr et al., 1990). Two quadriplegic patients who practiced extensive training of biceps and deltoid muscles (most caudal muscles spared) were stimulated using TMS two years after the injury. The cortical area from which it was possible to elicit motor evoked potentials in the two trained muscles was enlarged when compared to healthy participants, which means that a higher number of cortical neurons projected to spinal motoneurons controlling these two muscles after training (Levy Jr et al., 1990). The topographic changes in the muscle representation are not merely the consequences of de-afferentation and axonal damage (Nudo et al., 2001). Instead, they represent a form of use-dependent plasticity, which is guided by the motor training and re-establishes functional activity (Nudo et al., 2001). Among the possible neural mechanisms supporting cortical reorganisation, strengthening of preserved synapses and collateral sprouting of corticospinal axons are likely to play a role (Levy Jr et al., 1990). Use-dependent plasticity can be observed even in the absence of CNS damage and at much shorter time-scales. Non-invasive stimulation was used to provide evidence that the same reorganisation occurs after motor training in the human motor cortex (Pascual-Leone et al., 1995). In this instance, a group of healthy participants trained on unimanual finger sequences playing the piano, 2 hours per day for 5 days. The cortical areas of the used fingers enlarged, and the motor threshold decreased steadily over the 5 days. No effects were observed in a group

which received TMS daily without practising on the piano (Pascual-Leone et al., 1995). In addition, a third group practiced on the piano without following any specific finger sequences. Participants in this group showed significantly smaller cortical enlargements and no performance increase at the end of day 5. The authors interpreted this finding as evidence that the induced plasticity represents the specific acquisition of a motor skill and not simply increased excitability. Results were replicated for muscles in the lower limb, when participants were instructed to perform ankle movements following the trajectories depicted on a computer screen (Perez et al., 2004). Two control groups were designed: one training on random dorsiflexions and plantarflexions; one receiving passive displacement of the leg. TMS recruitment curves recorded from the tibialis muscle were higher after training only in the skill training group (Perez et al., 2004).

Stimulating the human brain has provided new insights in the role of the primary motor cortex in motor control and motor learning. Muellbacher and colleagues investigated which motor aspects are specifically encoded in M1 according to its excitability (Muellbacher et al., 2001). Participants were instructed to produce either ballistic (as fast as possible) or ramp (increasing over 500 ms) index-thumb pinches. Peak force and peak acceleration increased after 60 minutes of training in the group performing ballistic movements but not in the one performing ramp movements. In parallel with the behavioural results, MEPs recorded from the flexor pollicis brevis increased after training only in the ballistic group (Muellbacher et al., 2001). These findings demonstrate a link between performance increase and cortical excitability, because only training in the task (ballistic movement) which increased brain activity led to stronger and faster movements (Ljubisavljević, 2006). The same research group exploited the disruptive properties of low-frequency rTMS to probe the involvement of M1 in the consolidation of the newly acquired motor skill (Muellbacher et al., 2002). Participants trained in generating faster and stronger finger pinches for five minutes, during which their peak force and peak acceleration were shown to increase. If, however, low-frequency rTMS (1 Hz) was given on the motor cortex after the practice session, participants' performance returned to baseline (pre-practice) levels (Figure 2.4). Conversely, low-frequency repetitive TMS given on the occipital and dorsolateral prefrontal cortex, did not interfere with the behavioural gains observed while training. This demonstrated that the primary motor cortex is necessary for the

early consolidation on newly acquired performance increases (Muellbacher et al., 2002).

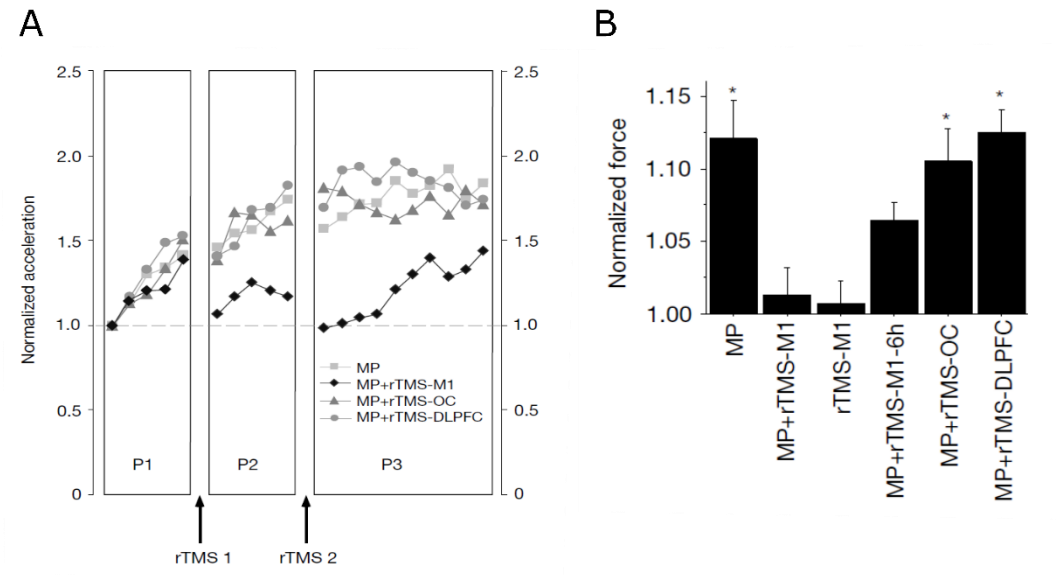

Figure 2.4. Effects of M1 rTMS on motor practice (MP). *(A)* Low frequency TMS (MP+ rTMS -M1) on M1 interfered with the improved acceleration during a ballistic task while stimulating the occipital (MP+ rTMS -OC) and dorsolateral prefrontal cortex (MP+ rTMS -DLPFC) had no effects. *(B)* Low frequency TMS given immediately after and 6 hours after (MP+ rTMS -M1-6h) training interfered with the improved strength, while stimulating the occipital and dorsolateral prefrontal cortex had no effects. Adapted with permission from Muellbacher et al. (2002).

Support for the exclusive role of M1 in encoding of motor memories post-training is far from unanimous. Indeed, there is evidence that the extent and time course of the neural changes depend on the characteristics of the motor experience (Adkins et al., 2006). People trained on a dynamic reaching task, where a force field generated by a robot produced changes in their desired trajectory (Baraduc et al., 2004). They progressively learned to compensate for the force field and to reach the target. Repetitive stimulation of M1 after the trained phase did not disrupt learning, evidence for the authors that the motor memory was encoded over a different network of neural structures. Learning to adapt to external perturbation might depend more on the subcortical and on associative cortical areas considering the high reliance on visual information to perform the task (Franklin et al., 2017).

There is controversy on whether resistance training induces plastic changes in the primary motor cortex (Carroll et al., 2002). Corticospinal responses to TMS and TES

were obtained before and after 4 weeks of finger abduction against a resistance. Training led to increased contraction strengths, but responses to both stimulation modalities remained unchanged after the 4 weeks. A similar study confirmed that strength training and skill training do not produce the same plastic changes on motor cortex excitability (Jensen et al., 2005). MEPs evoked from the biceps brachii muscles were higher after a single session and 4 weeks of visuomotor training compared to baseline. Conversely, strength training induced decreases in corticospinal excitability after 4 weeks and no short-term effects after a single session. Importantly, the authors reported how both types of training improved performance (Jensen et al., 2005). This finding indicates that changes in brain excitability are not always directly related to behavioural improvements and other context-related factors might contribute to its change (see Chapter 6 for more details on strength training and cortical plasticity). More recent works (Leung et al., 2015, Leung et al., 2017) suggest that training conditions are instrumental in modulating cortical excitability. Participants were allocated to: a skill training group in which they had to move their dominant hand according to the images displayed on a computer screen; a self-paced strength training consisting of 4 sets of biceps curls; a metronome-paced strength training in which biceps curls were timed to a metronome (Leung et al., 2015). MEP amplitudes increased and SICI decreased after a single session of skill training and metronome-paced strength training, but these effects were not observed after self-paced strength training. Similar effects were observed in a different study when the training protocol was extended to 4 weeks (Leung et al., 2017). Both these findings indicate that specific characteristics of the strength training protocol determine changes in corticospinal circuits occurring after training.

2.6.8. Skill acquisition and spinal plasticity

Whether neural plasticity occurs at different level of CNS or is limited to the neocortex has been a matter of debate over the last decades. The underrated role of the spinal cord in motor learning is the result of an arbitrary dichotomous split between voluntary activity, represented by the brain, and reflexes, domain of the spinal cord (Wolpaw and Tennissen, 2001). Nowadays, there is increasing evidence that plasticity can occur throughout life and across the whole CNS (Wolpaw, 2010). Spinal plasticity has been inferred by measuring changes in activity of the circuits mediating the monosynaptic reflex evoked upon nerve stimulation (Chapter 2.5). The

amplitude of the soleus monosynaptic reflex is reduced when moving from prone to standing positions in young people but not in an older population, which can reflect the difficulties in maintaining postural stability with ageing (Koceja et al., 1995). The ratios between H_{max} and M_{max} were shown to be significantly higher for athletes competing in various sports than for sedentary people (Nielsen et al., 1993a). However, the same measure of spinal excitability was smaller in professional ballet dancers. This finding indicates that the quality, rather than the amount, of exercise determines the direction of plasticity.

Long-term modulation of the excitability of the monosynaptic reflex pathway can be observed after motor training. Meunier and colleagues (Meunier et al., 2007) analysed whether training on a complex cycling task led to enduring changes in the amount of homosynaptic depression of the soleus monosynaptic reflex. Homosynaptic depression increases were observed at the end of the session in the experimental group cycling while pedal resistance was changed according to a pre-specified sequence, persisting one day after the training. No differences were observed in the control group performing constant cycling. They attributed the changes of homosynaptic depression to a persistent decrease in the probability of transmitter release at the synapses between Ia afferents and alpha motor neurons (Meunier et al., 2007). There are reasons to believe that the changes in the H-reflex pathway are independent from the amount of descending corticospinal activity (Ung et al., 2005). People trained over 16 days to walk backward on a treadmill showed spinal-specific effect of the training protocol (Ung et al., 2005). The soleus H-reflex evoked at different phases of the walking cycle decreased over consecutive sessions, without a concomitant change in the MEPs produced by TMS. A possible explanation is that the amount of presynaptic inhibition of afferent terminals exerted by non-corticospinal descending tracts increased with training (Ung et al., 2005).

The induction of plasticity in the monosynaptic reflex pathway has been relatively understudied for muscles of the upper limb, and there is a practical and a conceptual reason for this disparity: (1) while an H-reflex can be recorded after tibial nerve stimulation at rest in almost all people, difficulties in evoking it by stimulating other nerves are often reported (Burke, 2016); (2) because of the extent of direct corticomotoneuronal projections to hand muscles in humans (Lemon et al., 2004), the role of spinal circuits in the performance of skilled manual movements has always been considered to be restricted. Nevertheless, the flexor carpi radialis muscle, which

is activated during flexion, extension and radial deviation of the wrist, has been stimulated by targeting the median nerve (Christie et al. 2005). Monosynaptic reflexes evoked from this muscle were highly reliable over sessions, and could be evoked without muscle pre-activation in most participants (Christie et al., 2005).

A single session of arm cycling, a task designed to mimic locomotion movements by using the upper limbs, changes the excitability of motor pathways (Winkler et al., 2012). Participants were instructed to use an arm cycling device and, on a separate session, to flex and extend their right wrist following a 15 positions sequence. Intracortical facilitation and homosynaptic depression of the FCR H-reflex decreased after the locomotion-like training but not after the sequence training (Winkler et al., 2012). Roche and colleagues tested whether visuomotor adaptation in grip tasks produced spinal plasticity (Roche et al., 2011). In the visuomotor task, participants had to produce a target force between thumb and index finger and visual feedback informed them of each trial's outcome. The visual feedback was removed in the control task. Disynaptic inhibition, assessed with a conditioning electrical pulse to the radial nerve and a test pulse to the median nerve, decreased shortly after the end of both tasks but rapidly went back to baseline values. Similarly, the amount of presynaptic inhibition of FCR afferents was studied with a subthreshold conditioning pulse to the radial nerve delivered 13 ms before the test pulse to the median nerve. Presynaptic inhibition of FCR Ia afferents decreased following a similar time course, but only in the visuomotor task (Figure 2.5). The authors confirmed the result in another experiment in which it was shown that the amount of presynaptic inhibition remained unchanged on a second training session. This finding suggests that the circuits mediating presynaptic inhibition might be important for the acquisition, but not the retention of the new skill (Roche et al., 2011).

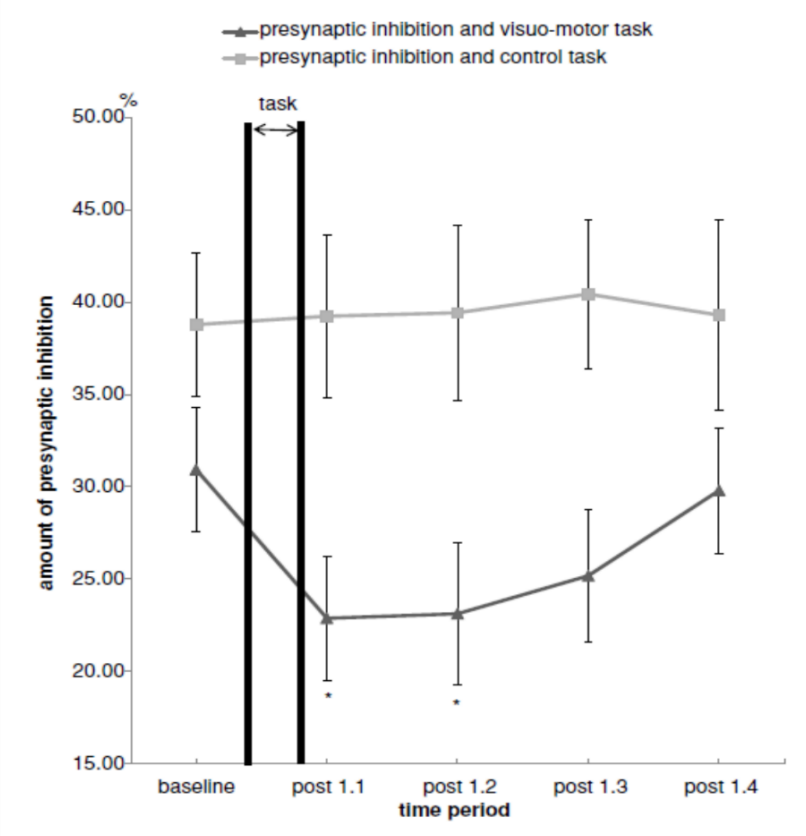

Figure 2.5. Changes in the amount of presynaptic inhibition after training with (visuo-motor) and without (control) feedback. Presynaptic inhibition was measured as the percentage difference between the control H-reflex and the conditioned H-reflex, divided by the control H-reflex. Consecutive testing (e.g. post 1.1 to post 1.2) were spaced by 4 minutes. From Roche et al. (2011), with permission.

2.6.9. Combining PNS and TMS for the study of motor control

Much of the work described in this chapter attempted to characterise which cortical and subcortical neural populations are activated at different phases of the movement. However, as noted in the previous paragraphs, there are two fundamental issues to consider when using such approach: (1) from the MEP alone it is not possible to discern between the monosynaptic component of the corticospinal tract and any indirect polysynaptic projection to the motoneuron pool (Pierrot-Deseilligny and Burke, 2005). The result of this methodological limitation is that changes in the MEP amplitudes occurring in response to experimental manipulations cannot be attributed entirely to an underlining effect on cortical excitability; (2) the H-reflex is commonly considered to reflect the excitability of the motoneuron pool at a given time. However, the primary motor cortex modulates the amount of presynaptic inhibition acting on

the Ia afferents, which will in turn affect the amplitude of the recorded monosynaptic reflex (Pierrot-Deseilligny and Burke, 2005). Thus, demonstrating that the amplitude of the H-reflex changes as a result of experimental manipulation (e.g. Ung et al., 2005) does not rule out the possibility that supraspinal structures guided these changes.

During the early 90s', multiple research labs started to explore protocols to selectively investigate the transmission in cortical and spinal pathways in humans (Nielsen et al., 1993a, Mazzocchio et al., 1994). These works typically employed a combination of cortical and peripheral stimulations delivered either alone or together at different time intervals. A protocol to study the convergence of cortical and peripheral volleys into spinal neurons has been proposed by Cowan and colleagues (1986). Electrical stimulation on the scalp was delivered at different time intervals from the peripheral stimuli. The conditioning (cortical) pulse was subthreshold for evoking activity in the recorded EMG. The descending volley was thereby too weak to elicit action potentials in the alpha moto neurones, but strong enough to modulate spinal excitability. When the interval between stimuli was manipulated such that these arrived synchronously at the motoneuron level, the net effect was a strong facilitation of the monosynaptic reflex. The facilitation depends on monosynaptic modulation of alpha motoneurons excitability by the corticospinal volley and was observed in flexor and extensor wrist and finger muscles and in the thenar muscle, but not in the soleus muscle. The facilitation was immediately curtailed by an inhibition. The authors proposed that the phase of inhibition depended on descending projections to Ia inhibitory interneurons responsible for the inhibition of the antagonist muscle (Cowan et al., 1986).

Subsequent studies modified the original protocol defined by Cowan and his colleagues by replacing the cortical electrical stimuli with the non-invasive TMS (Nielsen et al., 1993b). Monosynaptic reflexes were evoked in the soleus, tibialis anterior and FCR muscle both at rest and when participants contracted the muscles. The intensity of the TMS pulse was adjusted to be below the threshold to generate a MEP. In the soleus muscle at rest, an early facilitation was observed when the peripheral stimulus was delivered 2-5 ms before the cortical one. This was followed by an inhibition of the test reflex at -2 (peripheral first) to +1 (cortical first) ms. The facilitation was bigger if participants performed tonic plantarflexions, and the inhibitory effect disappeared. The earliest effect of cortical stimulation on the FCR H-reflex was seen at similar interpulse intervals (IPIs). According to the authors, the first peak of facilitation observed across all muscles is due to the synchronous arrival

at the motoneuron level of the afferent and descending volleys, and thereby mediated by the fast, monosynaptic corticomotoneuronal component of the corticospinal tract (Nielsen et al., 1993b). The interpretation of the later-observed inhibition in terms of neural populations involved is problematic, since multiple descending polysynaptic connections to motoneurons exist (Pierrot-Deseilligny and Burke, 2005). However, by stimulating at the same time the common peroneal nerve (n.b. tibialis anterior and soleus muscles are antagonists) and M1, an "extra" inhibition of the recorded soleus H-reflex was observed. This point to a role of Ia inhibitory interneurons responsible for the reciprocal inhibition of antagonist muscles in mediating the inhibition. To note, later studies on the effects of magnetic cortical stimulation on the FCR monosynaptic did not report any inhibition (Mazzocchio et al., 1994, Niemann et al., 2017). The finding that voluntary contraction increased the amount of H-reflex facilitation in all the muscles recorded is in line with an increase in cortical excitability compared to baseline values.

Studies in which the monosynaptic reflex evoked from leg muscles is conditioned with TMS provided valuable insights into the neural circuitry activated at different phases of movements. Conditioning-test pulses were delivered during quite standing, the stance phase of walking and tonic-dynamic plantar flexion in healthy participants (Petersen et al., 1998b). The intensity of the cortical stimulus was subthreshold for producing a motor response in the soleus muscle. In the stance phase and during dynamic plantar flexion, the TMS pulse increased the size of the H-reflex at stimulus intervals (-3 to -1 ms) compatible with the activation of monosynaptic corticospinal projection (Petersen et al., 1998b). The facilitating effect of TMS was smaller and occurred only when using higher cortical stimulation intensities if the participant was standing or performing tonic plantar flexion. According to the authors, their data suggested that cortical excitability is selectively increased during particular phases of human walking, to an extent which does not depend on the muscular activity (EMG recordings). This theory was corroborated by the observation that when the conditioning magnetic stimulus was replaced by TES, which activated corticospinal axons, the differences in the level of facilitation between the walking and the standing conditions disappeared (Petersen et al., 1998b).

There is evidence that voluntary contraction is preceded by changes in the excitability of spinal circuits. During a voluntary ankle dorsiflexion, the monosynaptic reflex evoked in the soleus muscle is depressed by reciprocal connections from dorsiflexor

muscles (antagonists to soleus) (Crone and Nielsen, 1989b). Surprisingly, however, MEP responses evoked in the soleus muscle are increased during dorsiflexion (Valls-Solé et al., 1994). The source of this facilitation was investigated by: comparing the MEPs obtained upon magnetic stimulation with the responses to cervicomedullary stimulation during and prior to dorsiflexion; studying the outcome of subthreshold stimulation on the size of the monosynaptic reflex evoked in the soleus muscle prior to dorsiflexion and plantarflexion. First, the amplitudes of both MEPs and cMEPs increased before dorsiflexion. Second, the short-latency (presumably monosynaptic) facilitation of the H-reflex observed with cortical stimulation did not differ before dorsiflexion. Both these findings suggest that part of the motor programme is processed at a subcortical level, possibly as part of a mechanism which ensure rapid adaptation between movement patterns (Geertsen et al., 2010).

Despite the growing body of evidence that motor training induces changes in the excitability of spinal circuits (Wolpaw, 2010), only a handful of studies employed cortical and peripheral stimulations in combination to test the effects of motor training. One of these works recently showed that the nature of the motor task is crucial in determining the occurrence of plastic changes in spinal circuits (Kubota et al., 2015). The designed tasks were a visuomotor task, in which people learned to perform ankle movements of pre-specified amplitude and durations in response to an auditory cue, and a control task, in which people simply contracted the ankle in response to an auditory cue. The amount of presynaptic inhibition to soleus motoneurons was determined by measuring monosynaptic reflexes conditioned by cortical stimuli given 5–10 ms prior to common peroneal nerve (CPN) stimulation. Presynaptic inhibition mediated through the corticospinal tract decreased after 20 minutes of visuomotor task compared to baseline (pre-task). These differences were not observed after 20 minutes of control task (Kubota et al., 2015).

The outcome of delivering cortical stimulation on the excitability of spinal circuits differs between the lower limb and the upper limb in humans (Meunier and Pierrot-Deseilligny, 1998). In this study, electrical stimulation of the CPN preceded stimulation of the posterior tibial nerve, which evoked the soleus H-reflex, by 20 ms. This method is employed to measure the amount of presynaptic inhibition of the terminals of Ia afferents on spinal motoneurons (Faist et al., 1996). Cortical magnetic stimuli delivered before (5 to 10 ms) and after (10 to 15 ms) CPN stimulation significantly decreased the amount of presynaptic inhibition acting on soleus

motoneurons. In wrist muscles, stimulating the radial nerve 10 ms before the median nerve inhibits the H-reflex recorded from the FCR muscle (Berardelli et al., 1987). TMS increased the inhibitory effect (bigger decrease of H-reflex values) if delivered 20 ms before and 10 ms after radial nerve stimulation. Thereby, the net effect of descending drive is a decrease of the amount of presynaptic inhibition in the lower limb and an increase of the amount of presynaptic inhibition in the upper limb (Meunier and Pierrot-Deseilligny, 1998). These differences may have arisen because of the greater extent of the monosynaptic component of the corticospinal tract in the upper limb (Meunier and Pierrot-Deseilligny, 1998), but their functional significance remains controversial.

An interesting protocol which permits to selectively evaluate which component of the descending volley is involved in modulating reflex excitability was recently developed by researchers at the University of Freiburg (Niemann et al., 2016). The method relies on the assumption that descending volleys evoked by TMS produce EPSPs in the targeted spinal motoneurons with a periodicity of 1- 1.5 ms. The first of these volleys, the D-wave, is conducted through monosynaptic corticomotoneuronal connections (Di Lazzaro and Ziemann, 2013). The stimulating procedure comprised of a subthreshold TMS pulse and an electrical pulse to the median nerve to elicit the H-reflex, delivered in isolation or combined at multiple time intervals between them. When analysing the data, for each participant the first ISI at which the amplitude of the conditioned H-reflex significantly differed from the unconditioned value was deemed “early facilitation”. Following intervals at which a facilitation/inhibition could be observed were termed according to their time delay from the early facilitation EFD (e.g. EFD + 1 ms). Under these definitions, the effects observed at EFD +1 ms are likely mediated through a disynaptic route from M1 to the spinal motoneurons (Niemann et al., 2016).

This approach was used to answer the question of how inputs from the motor cortex drive the activity of spinal motoneurons during movement preparation (Hannah et al., 2018). In particular, the authors tested why the increased activity observed at the cortical level at this stage of movement is not reflected in corresponding increased motor outputs. The task they designed was a reaction-time task in which a warning visual cue preceded an imperative visual cue, instructing participants to flex the wrist as fast as possible, by a fixed interval (Figure 2.6 *A*).

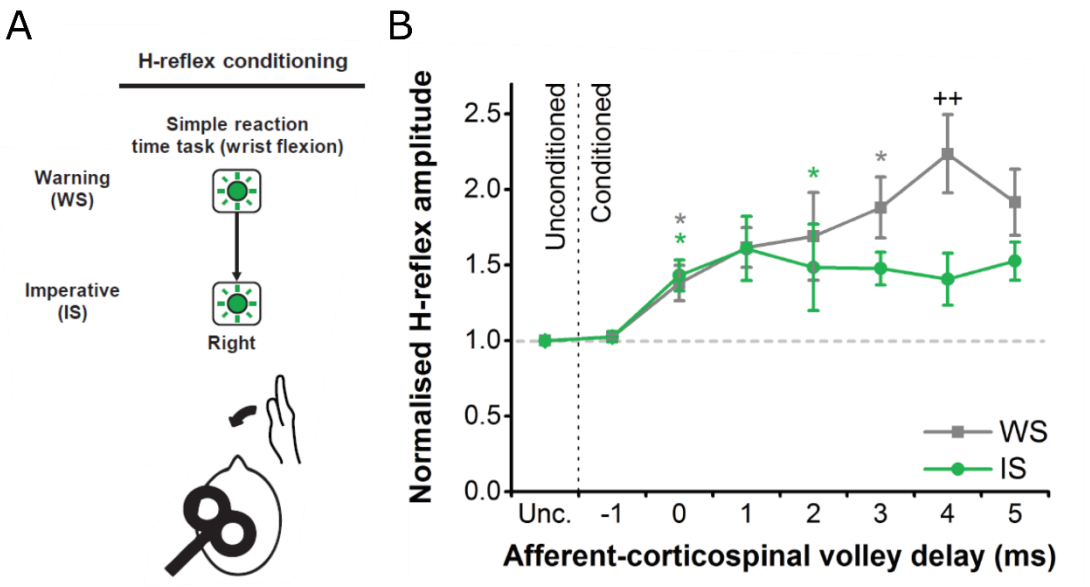

Figure 2.6. Interneuron circuits involved in movement preparation. (*A*) The reaction-time task consisted of a visual warning cue indicating the participant to prepare the moment (wrist flexion) and an imperative cue indicating the start the movement. (*B*) TMS and median nerve stimulation were delivered at the imperative and warning cue times at different delays between the arrival of the afferent volley and of the corticospinal volley at the spinal motoneurons level to produce conditioned H-reflexes. Adapted from Hannah et al. (2018).

Conditioned and unconditioned FCR H-reflexes amplitudes were compared between stimuli given at the warning signal time and stimuli given at the imperative signal time. At the ISI for which the corticospinal and afferent volleys arrived together at the spinal level (0 ms), H-reflexes were significantly facilitated both during the warning and the imperative periods (Figure 3.3 *B*). Nevertheless, a significant difference was found between the size of TMS-conditioned H-reflexes evoked during the warning and the imperative periods at 4 ms ISI, with the latter amplitudes being smaller. These results showed that, immediately before movement execution, a subset of the inputs to the targeted spinal motoneurons is suppressed white the others remain unaffected (Hannah et al., 2018).

Despite the continued interest in cortical stimulation as a mean to study the mechanisms of motor control in humans (Reis et al., 2008), the use of methods permitting to distinguish between the direct and indirect inputs to motoneurons is still minimal. It was argued that combining TMS with PNS constitutes a better option than cervicomedullary stimulation (Leukel et al., 2012) and paired-pulse TMS protocol (Burke and Pierrot-Deseilligny, 2010) when the object of interest is which specific

cortical-subcortical pathways constitute the neural basis for motor training and learning. Future studies need to complement the analysis of MEPs with stimulus paradigms testing motoneuron and spinal excitability across different conditions of training. There is little evidence of the effects of motor training on corticospinal excitability tested through the method of H-reflex conditioning. In addition, the intersession reliability of the method when used in forearm muscles (e.g. FCR) was never investigated. The aim of the first experimental chapter (Chapter 4) is to investigate whether TMS-conditioned H-reflexes recorded from FCR are a reliable measure of pathway-specific excitability over the course of three sessions.

2.7. Confounding factors influencing the outcome of TMS on the motor cortex

Motor-evoked potentials (MEPs) evoked by TMS delivered on the motor cortex are widely used electrophysiological parameters to assess the excitability and integrity of the corticospinal system (Bestmann and Krakauer, 2015). Despite the technical advancements achieved during the last few decades, the relevance of TMS as a technique to study the motor system is still limited by the high variability of MEP recordings both within and between participants (Schmidt et al., 2009). Intra and inter-participant variability can result due to physical and physiological factors. The former, which are discussed in Chapter 3, depend on technical aspects of magnetic stimulation and are often directly controllable by the experimenter. The latter depend on the design of the study and participants' characteristics and are described in the next paragraphs.

2.7.1. State-dependency

The excitability of the cortex and corticospinal tract before magnetic stimulation bias its output and therefore affects the neural response (Siebner et al., 2009). The clearest example of this relationship is the finding that the current required to elicit a muscular response is lower, and the MEP bigger, if the muscle is being contracted by the participant (Rossini et al., 1994). Kiers and his colleagues systematically varied intensities of stimulation and levels of muscle contractions to assess their impact on the MEPs recorded from the FDI muscle (Kiers et al., 1993). They observed that MEP variability decreased by more than half with increasing stimulation intensities from threshold intensities to 140% threshold intensities. When participants were instructed

to contract their FDI muscle prior to stimulus delivery, the amplitude of the recorded potential increased compared to resting values for the same stimulation intensities. Moreover, the coefficient of variation (CV), an index of trial-to-trial variability, decreased at all levels of muscle pre-contraction (Kiers et al., 1993). For example, on average the CV, which is defined as the standard deviation divided by the mean, decreased from 0.43 to 0.07 if participants were contracting their right FDI muscle at 30% MVC during stimulation. The facilitation induced by background activation occurred even during the generation of low force levels. This result demonstrates that pre-activating a muscle leads to stronger and more synchronised neural activation.

2.7.2. Fatigue

While contracting a muscle immediately before magnetic stimulation delivery can increase the obtained response, sustained contractions can have the opposite effect. The development of fatigue can be observed behaviourally as a decrease in the amount of force produced during the contraction (Carroll et al., 2017). More elaborated indices of spinal and supraspinal fatigue can be derived by delivering cortical and/or spinal stimulation during and after the contraction (Taylor et al., 2006). Participants were instructed to maintain a constant contraction level (60% of MVC) up to their endurance point, the point at which muscle force started to decline, while TMS was delivered throughout the contraction (Ljubisavljević et al., 1996). The authors observed that the MEP area decreased gradually up to the endurance point over the course of the contraction by about 20%. The authors were interested in finding whether this result was due to changes occurring at or distal to the neuromuscular junction, a peripheral phenomenon, or to a decrease in descending drive from cortical structures, a central phenomenon (Gandevia, 2001). Peripheral fatigue was measured by eliciting supramaximal motor waves with peripheral nerve stimulation at the end of the contraction. Surprisingly, it was found that only about half of the participants showed signs of peripheral fatigue, in that the maximal motor wave elicited by nerve stimulation decreased after the fatiguing contraction. However, MEPs decrements were observed in each participant which indicates the presence of central fatigue. It was proposed that corticospinal excitability decreases in parallel with the development of muscular (peripheral) fatigue (Ljubisavljević et al., 1996). MEPs collected immediately after the end of the contraction were significantly higher than

the ones collected during contraction, which the authors interpreted as a transient change in synaptic strength after movement (Ljubisavljević et al., 1996).

The time course of cortical excitability as measured by MEPs was measured as well during intermittent, short-duration maximal contractions (Taylor et al., 2000). Cortical pulses were delivered to the cortical area controlling elbow muscles during MVC lasting from 5 to 30 seconds, followed by 5 or 10 seconds rest. The MEP area increased over the course of the first contractions independently from their length. The MEP area fully recovered to his pre-contraction value only in the 10-seconds of rest condition (Taylor et al., 2000). According to the authors, the increase in size can be explained as a higher descending volley evoked by the magnetic stimulus (Taylor et al., 2006). In a different study, TMS was used to study the mechanisms of central fatigue as opposed to its peripheral counterpart (Liepert et al., 1996). It was demonstrated that in patients who sustained a CNS lesion the MEP recovery time (e.g. return to pre-exercise MEP amplitude values observed before contractions) after maximal voluntary contractions is prolonged compared to the healthy population, while no differences in peripheral measures of fatigue could be inferred. The effects of fatigue can be substantial in protocols aiming at testing levels of motor excitability post-exercise. Considering this evidence, TMS pulses should be delivered at least 2-3 minutes after the end of maximal contractions (Di Lazzaro et al., 2003).

2.7.3. Cerebral blood flow

It is commonly reported that**,** when multiple pulses are given spaced by a few seconds, the first MEPs recorded are usually the higher of the series (Brasil-Neto et al., 1994). The biological basis for this event was investigated by means of near infra-red spectroscopy analysis (Thomson et al., 2012). In this study, trains of two or four TMS pulses were delivered to the prefrontal cortex at IPIs of 5 seconds. Near infra-red spectroscopy measures the amount of oxygenated haemoglobin supplying oxygen to a certain region via capillary vessels (Edwards et al., 1993). After both pulse trains oxygenated haemoglobin levels dropped below the baseline values. Oxygen depletion might thus explain the reduction in neural excitability observed immediately after a pulse (Thomson et al., 2012). The after-effects of cortical stimulation on the hemodynamic response can persist for up to 10 seconds after stimulus delivery (Thomson et al., 2012). When short (4 seconds) and long (10 seconds) IPIs were used to measure MEPs in the same group of participants, it was confirmed that long IPIs

yielded significantly higher amplitude values when stimulation intensity is kept constant (Vaseghi et al., 2015). Despite the suggestion that cortical blood flow is reduced after TMS delivery (Thomson et al., 2012) a lot of TMS protocols continue to employ IPIs below 10 seconds (e.g. Latella et al., 2016), and a consensus on the optimal time interval between magnetic pulses has yet to be reached (see Chapter 5 for more information on this topic).

2.7.4. Arousal

The human ability to interpret and respond to incoming sensory information depends on the level of arousal, and the reduction in reaction time to an external stimulus as a function of arousal levels is an established phenomenon (Behar and Adams, 1966). Thus researchers have recently started to raise the concern that participants' arousal could contribute to the variability of outcome observed across trials and across participants (Furubayashi et al., 2000). Furubayashi et al. (2000) showed that a loud auditory stimulus (louder than 80 dB) given 30-50 ms, and for 50 ms or more, before the delivery of a cortical pulse resulted in a decrease in the response amplitudes (from 0.72 mV on average to 0.33 mV on average). To corroborate this results, Kühn et al. demonstrated that giving an auditory stimulus can inhibit the motor cortex if given 50 ms before TMS, but has no effect on subcortical excitability (Kühn et al., 2004). This was demonstrated by delivering TMS or subcortical electrical stimulation, which does not activate the motor cortex, 50 ms after acoustic stimulation at 100 dB. Responses recorded through EMG from the biceps brachii muscle were inhibited only if TMS was used as stimulation modality (Kühn et al., 2004). Taken together, Furubayashi et al. (2000) and Kühn et al. (2004) seem to point to a paradoxical reduction in motor system excitability at higher levels of arousal. However, further research showed that the effects of arousal on recorded MEPs are not always suppressive but rather are context-dependant. If, for example, brief acoustic stimuli are replaced with emotionally relevant auditory-visual stimuli (pieces of classical music and pictures), these can enhance motor responses to TMS (Baumgartner et al., 2007). However, in this latter study the combination of visual and auditory stimuli was presented continuously with, and not preceding, the delivery of brain stimulation. Marinovic and his colleagues reasoned that the discrepancies between studies might be due to the dissimilar level of participants' motor preparation at the moment of auditory stimulation (Marinovic et al., 2014). They manipulated this parameter and showed

that: (1) when the auditory-cortical stimulations were given in close temporal proximity but 2 seconds before the time when the participants were asked to start the movement, the corticospinal output was suppressed compared to control values (without auditory stimulation) and (2) when the auditory-cortical stimulations were given shortly before the participants should perform the movement (200 ms), the corticospinal output was enhanced compared to control values (Marinovic et al., 2014). This shows that, in conditions in which participants are preparing movements, the effect of auditory stimuli on corticospinal excitability is excitatory, but if the participants are relaxed at the time of acoustic stimulation the result is a decrease in corticospinal excitability (Marinovic et al., 2014).

2.7.5. Attention

The attentional state of the participant can influence its sensitivity to magnetic stimuli. Before describing the relevant literature on how attention can confound the outcomes of TMS delivery, it is worth underlining how the construct of attention overlaps with, but is distinct to, the previously discussed construct of arousal. Arousal is a stimulus-driven physiological response which arises spontaneously and does not require a cognitive component. Attention is the conscious attribution of cognitive resources to a given stimulus. In the previous section it was highlighted that arousal levels are commonly manipulated by using sudden auditory stimuli delivered before magnetic stimulation. In contrast, attention can be altered by asking participants to perform tasks of various nature (Izumi et al., 1995, Kiers et al., 1993). In one of the first reports on the variability of MEPs evoked by TMS, participants were asked to relax or to solve mental arithmetic while being stimulated (Kiers et al., 1993). Performing mental arithmetic had no impact on the amplitude of the recorded responses. The quality of the task people are asked to perform to raise attention seems to determine whether it will have an impact on motor excitability. Izumi and his colleagues compared MEPs obtained from the APB muscle: at rest; when participants produced a small (10% of MVC) contraction; when participants were thinking about contracting the thumb without any visible activity in the recorded EMG (Izumi et al., 1995). Amplitudes recorded when participants were thinking of contracting their thumb were significantly higher than the ones recorded at rest, but lower than the ones recorded at 10% of MVC. This suggests that attention influences the responses to TMS, but only if participants are focused on a task which directly engage the motor system.

Such studies have been the empirical basis for the theory that imagining a movement without generating it, the so-called motor-imagery, engages the same cluster of motor areas and neural populations as the actual movement (Jeannerod and Decety, 1995). Cortical and spinal excitability were assessed by recording MEPs and monosynaptic reflexes during mental imagery of wrist flexion movements and compared to resting values (Kasai et al., 1997). Results showed how MEPs were substantially enhanced by mental imagery while there were no changes in the amplitudes of the monosynaptic reflexes. The effect of mental imagery is muscle-specific and limited to the motoneuron pool engaged in the production of the actual movements, since the evoked responses upon TMS are larger in the flexor muscles while imagining wrist flexions and in extensor muscles while imagining wrist extensions (Hashimoto and Rothwell, 1999). Visual attention can modulate corticospinal excitability if the visual information is given in a task-specific context (Mars et al., 2007). Participants performed a delayed button-press task in which the instruction cue informed them on either the spatial information on where the imperative cue would appear or visual information on which hand to use for button-press. Corticospinal excitability was elevated during movement preparation only in the trials when participants knew which hand to use after the imperative cue (Mars et al., 2007). Despite this evidence, relatively little is known about the way participants' attentional state influence the data recorded with TMS at rest (see Chapter 5).

2.7.6. Auditory activation

It was previously described how a sudden auditory stimulus, which raises participants' arousal level, can influence the excitability of the motor system. The discharging of a TMS coil is accompanied by an abrupt clicking noise which increases with stimulation intensity and can reach 120 dB (Nikouline et al., 1999). This technically-induced sound might represent a confounding factor by itself in many TMS studies. Multiple behavioural studies have investigated how the "click" sound produced whenever a TMS pulse is discharged affects performance in a following motor task (Duecker and Sack, 2013). For example, Terao and colleagues employed subthreshold TMS, which does not elicit any activity in the recorded EMG, given prior (-50 to 0 ms) to the visual stimuli indicating the participants to extend their right wrist as quickly as possible (Terao et al., 1997). As expected, reaction times were found to be shorter in the condition in which subthreshold TMS preceded the visual cue. Surprisingly, this

phenomenon was observed even in a subgroup of participants which received TMS with the magnetic coil held off the scalp (Terao et al., 1997). The authors concluded that the discharging noise caused an intersensory facilitation, as the auditory stimuli which by itself does not require a response decreased the reaction time to the visual stimulus (Nickerson, 1973). This finding was replicated and extended by using sham TMS, which produce the same noise and scalp sensations as "normal" TMS but without generating magnetic currents (Duecker and Sack, 2013). Participants were instructed to respond with a button press as soon as they detected a visual target appearing on the screen. Firstly, sham TMS shortened reaction times by about 15-20 ms in a visual target detection task when delivered 250-150 ms before the visual stimulus. Secondly, reaction times were faster if sham TMS was delivered to the hemisphere ipsilateral to the hand used in the task. The authors suggested that the noise accompanying coil discharge resulted in a shift in visual attention towards the hemifield ipsilateral to the auditory stimulation, which reduced the time required to react to the target (Duecker and Sack, 2013).

Furthermore, TMS noise can represent a confounding factor even in single-pulse TMS studies probing the excitability of the motor system. A TMS study carried out in primates measured the activity recorded from neurons in the reticular formation after TMS and click stimuli delivered through a bone vibrator (Fisher et al., 2012). They found that TMS and click stimuli activated a similar subset of reticular neurons (Figure 2.7). This activity is independent from the current induced by the magnetic field and is thereby to be considered as an unwanted effect of TMS. Whenever interpreting the results of TMS studies in terms of corticospinal excitability, the possibility that other descending tracts are activated and modulate EMG activity has to be considered (Fisher et al., 2012).

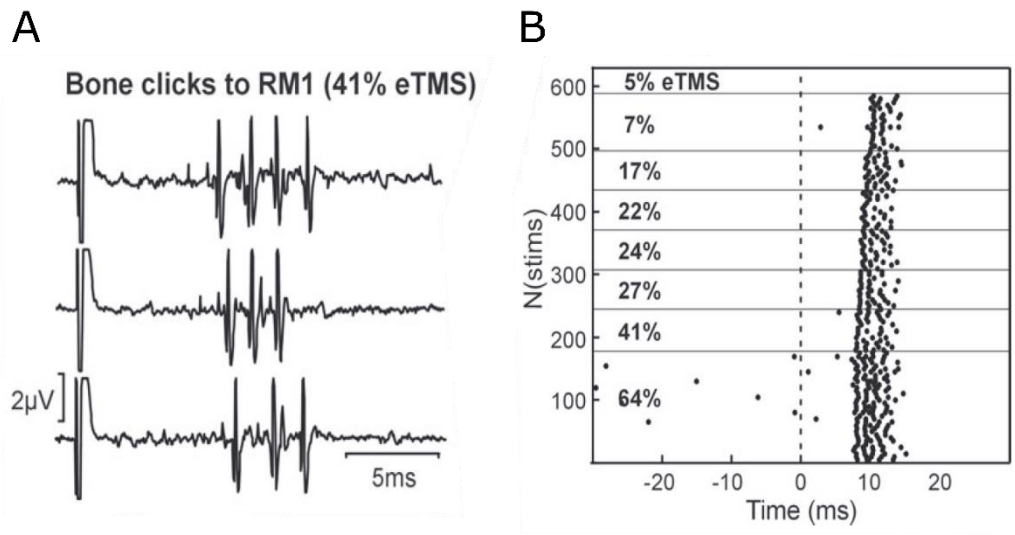

Figure 2.7. Reticular formation neuron activated by sound. *(A)* Recordings from a reticular cell after bone vibrator stimulation producing sound intensities comparable to TMS given at 41% of MSO (41% eTMS). *(B)* Raster plot of the responses of the same cell to increasingly higher sound stimuli. Adapted with permission from Fisher et al. (2012).

In light of this evidence, it is surprising that currently no firm guidelines exist on whether to employ methods to mask the sound accompanying TMS discharge. It is even more surprising given that doing so has shown to reduce auditory-evoked-potentials in electroencephalography (EEG) recordings (Nikouline et al., 1999), in single-pulse TMS studies. Confounding factors due to the noise produced by stimulation are experimentally addressed in Chapter 5.

2.7.7. Membrane potential

Even when carefully controlling for the factors described above, MEPs show great response variability in the same person under the same condition (Kiers et al., 1993). The membrane potential of cortical neurones oscillates at rest between up and down states (Amitai, 1994). The excitability of the corticospinal system at a given point correlates with the amplitude of the spontaneous occurring oscillation (Zarkowski et al., 2006). The obvious consequence is that pulses delivered at different excitability phase will induce different descending activity. In addition to this cortical-generated phenomenon, the pattern of spinal motoneurons discharging can influence the MEP size. When TMS-induced activity reaches the spinal cord, it causes motoneurons to

discharge in a desynchronised fashion such that the positive and negative phases of motor unit potentials cancel each other (Magistris et al., 1998).

2.7.8. Subject-specific factors

TMS pulses of the same intensity can elicit multi-phasic motor responses in some people but have virtually no effects on other individuals (Wassermann et al., 2008). A big factor in determining stimulation outcome is brain anatomy. In particular, the skull-to-cortex distance of the motor cortex is an important predictor of MT values (Herbsman et al., 2009). The folding structure of the central gyrus determines the spread and depth of the induced electrical currents and thereby the neural response (Thielscher et al., 2011). Among the possible genetic factors contributing to individual differences, it was shown that the increase in MEP amplitudes observed after motor training is reduced in individuals with polymorphism in the brain-derived neurotrophic factor (BDNF) gene (Kleim et al., 2006). MT values for the APB muscle were highly correlated between siblings (Wassermann, 2002). Motor responses evoked with TMS at threshold levels tended to be less stable in older participants (Pitcher et al., 2003). MEPs variability was higher in females compared to men when accounting for age-related difference, an occurrence that seems to depend on the ovarian hormones levels during different phases of the menstrual cycle (Pitcher et al., 2003). The sex hormone progesterone and its metabolites decrease neural excitability by binding to GABAergic receptors and hyperpolarising neuronal membranes (Smith et al., 2002). The role of hormone concentration in determining the responses to TMS and PNS was specifically addressed by Ansdell and his colleagues (2019). Women were tested at the early follicular, late follicular and midluteal phases of their menstrual cycle while performing fatiguing knee extensions. The authors found that MVC, M_{max} and MEP amplitudes were not affected by menstrual cycle phase. However, voluntary activation was found to be greater during the late follicular phase. SICI increased at the midluteal phase compared to the other phases. The authors concluded that oestrogen levels, which peak during the late follicular phase, have a neuroexcitatory effect which contributed to the higher voluntary activation (Ansdell et al., 2019).

While the delivery of magnetic pulses to the cortex is harmless to the participants, local discomfort can sometimes be reported. The levels of pain and discomfort reported by the participants at the end of the session were predictive of their reaction

times in a behavioural task (Meteyard and Holmes, 2018). Receiving TMS is a highly subjective experience, and factors such as arousal and attention can vary among people and even over the course of a single session in accordance with the level of familiarisation with the TMS setting (Cuypers et al., 2014). Psychological side effects can have an impact on the recordings obtained after motor cortex stimulation (Wassermann, 2000). As an example, the levels of intracortical inhibition were found to be correlated with neuroticism, a self-reported measure of anxiety, with more anxious participants showing less intracortical inhibition (Wassermann et al., 2001). The effects of participants' psychological state on the outcome of TMS on the motor cortex remains largely unknown (see Chapter 7.2.2).

2.8. Cross-education of strength and bilateral transfer of skill: behavioural evidence and neural mechanisms

2.8.1. Introduction

The scope of this section is to describe the phenomenon by which increases in strength and a more skilled performance are observed in the untrained limb following unilateral training of the contralateral limb (Ruddy and Carson, 2013). Traditionally, the effects of unilateral strength training and of unilateral skill training have been studied independently. The terms cross-education and bilateral transfer have been used specifically for describing the effects of unilateral strength training and skill training, respectively (Lee and Carroll, 2007). In line with this, the discussion will start with the behavioural evidence that resistance training in one limb affects the strength of the contralateral limb. It will then proceed by describing the sites of adaptation which could potentially represent the neural substrates that underlie the transfer of strength training to the untrained limb. The same order of discussion will be followed for studies investigating the bilateral transfer of skill, moving from the behavioural evidence to the proposed neural mechanisms supporting this phenomenon. Finally, a conceptual basis for the evidence accumulated in the years will be provided.

2.8.2. Strength training

2.8.2.1. Behavioural evidence

Since the late-19th century, researchers have noted that training-induced increases in performance can be observed in the contralateral, untrained limb (Scripture et al., 1894). In the first work directly assessing changes in skill and strength after multiple (10 and 9 respectively) sessions in the contralateral hand, Scripture and his colleagues measured the ability of participants in inserting a needle through holes of varying sizes and their muscular power in squeezing a bottle attached to a dynamometer. The author reported a 50% increase in successful reaching trials (needle successfully inserted in the hole) and a 43% increase in muscular power in the untrained hand (Scripture et al., 1894). The authors are also credited with coining the term "cross-education" to describe the observed effects. In the last few decades, many studies assessed the outcome of unilateral training on contralateral performance. Komi et al. (1978) recruited 3 pairs of homozygous twins, with one twin assigned to the experimental group performing unilateral maximal isometric knee extensions for 12 weeks, and the other twin to a control group, who undertook no training. At the end of training, the maximal strength produced when extending the trained leg increased on average by 20% when compared to the pre-training values (427 N to 512 N). In addition, the non-trained leg demonstrated an 11% increase in strength (386 N to 437 N). The control group did not demonstrate any increase in strength (448 N to 441 N). Carolan and Cafarelli complemented those findings by employing a similar isometric knee extensions protocol (Carolan and Cafarelli, 1992). Moreover, they monitored MVC increases in the test and control limbs on each week of the 8-weeks training schedule. Interestingly, they found substantial MVC increases already after a week (20 MVC per day, 3 days per week) in the trained limb. In contrast, the cross-education effect developed later and an increase in the MVCs when performed with the untrained limb was first observed after 2 weeks of training (Carolan and Cafarelli, 1992).

It has been suggested that cross-education depends on limb dominance and the potential for transfer is relatively limited when training the non-dominant limb (Farthing et al., 2005). The hypothesis is supported by neuroimaging studies showing that the area of the motor cortex which has projections to upper limb muscles is greater in the dominant hemisphere (left hemisphere in right-handed people) (Hammond, 2002). Coombs and his colleagues challenged this hypothesis by training two groups of right-handed participants in performing wrist flexion-extension with a dumbbell

either with the dominant or the non-dominant arm (Coombs et al., 2016). At the end of the three-weeks (9 sessions) training phase, participants were tested on their ability to complete a single repetition maximum (1RM) test with the heaviest possible dumbbell. Researchers reported a 22% increase in strength of the untrained arm in the group which trained with the dominant arm and an 18% increase in strength of the untrained arm in the group which trained with the non-dominant arm. Their conclusion was that the magnitude of cross-education does not depend on limb dominance and that both limbs can be strengthened by training the opposite one, which has important implications for the use of cross-education training in clinical populations, (Coombs et al., 2016).

The majority of the cross-education studies do not assess whether the effects of training extend after the training period. Green and Gabriel recently implemented a typical strength training protocol with an important addition: any cross-education effect which extended over the period of training was assessed by asking participants to take part in a retention session 6 weeks after the last training session (Green and Gabriel, 2018). The training included 6 weeks of dynamic contractions with a dumbbell or pulley cable and increases in strength were measured in either upper (FCR and ECR) or lower (tibialis anterior) limb muscles. At the post-training session, they found a 6% increase in strength in the untrained upper limb and a 13% increase in strength in the untrained lower limb. During the retention phase, participants were specifically asked not to train the wrist flexors and dorsiflexors muscle. Interestingly, when re-testing people after retention they observed that the cross-education effect continued to increase after the training reaching a 15% increase in strength in the upper limb and a 14% increase in strength in the lower limb. The authors concluded that the training regime had been successful in inducing increases in strength in both the trained and untrained limbs, and the neural processes supporting the behavioural increases continued to unfold after the training ended.

2.8.2.2. Neuromuscular adaptations

It was hypothesized that the muscular adaptations underlying cross-education are similar to the ones driving strength increase in the trained limb (Carroll et al., 2006). Unilateral strength training induces a cascade of muscular events in the used muscles (McDonagh and Davies, 1984). In contrast, repetitive attempts at demonstrating that similar mechanisms might support the cross-education effect have been unsuccessful

(Carroll et al., 2006). Participants trained on an isokinetic dynamometer performing flexions and extensions of the non-dominant forearm and leg for 8 weeks (24 sessions) (Housh et al., 1992). Strength was measured as increases in peak force values between pre-training and post-training, and the cross-sectional areas of forearm and leg muscle groups was measured through MRI scans before and after training. While hypertrophy was observed in all trained muscles, cross-sectional areas did not significantly increase in any untrained contralateral muscles. A more recent study (Farthing et al., 2009) demonstrated that performing isometric elbow flexions while the other arm was immobilized prevented muscle atrophy, which was indeed observed in a control group that did not train with the contralateral limb. Therefore, it has been suggested that unilateral training prevents muscular changes due to disuse but does not affect muscle mass in the homologous contralateral muscle under normal conditions (Hendy and Lamon, 2017).

However, it has been pointed out that much of the work studying neural adaptations after unilateral movements employ relatively short (3 to 8 weeks) training protocols, which may not be sufficient to induce permanent muscular changes (Hendy and Lamon, 2017). On the ground of these observations, the hypothesis that muscular mechanisms contribute to long-term increases in the performance of untrained limbs cannot be rejected, but it seems unlikely that these play a significant role in cross-education at the time-scales that are usually reported in the literature. Therefore, the focus of cross-education studies has shifted towards understanding which neural mechanisms are active at the acute (after a single session) and shorter-term (1-5 weeks) levels, before the change in muscular conformation are observed (Lee and Carroll, 2007).

2.8.2.3. Cortical mechanisms

There is no consensus on which neurophysiological substrates are responsible for the increase of contralateral strength after unimanual strength training. It is however plausible that some of these mechanisms are active already during the acquisition phase when unilateral movements are produced (Hortobágyi et al., 2011). The possibility that unimanual movements might influence the activity of homologous contralateral muscles have been repeatedly investigated over the years. The development of techniques enabling researchers to assess motor excitability in humans, such as TMS, has helped us understand the cortical mechanisms which might

support the cross-education process. EMG responses to TMS, electrical stimulation at the mastoid level (cervicomedullary stimulation) and to median nerve stimulation were collected from the right FCR muscle while participants contracted their left wrist (Hortobágyi et al., 2003). Right MEPs evoked with TMS were bigger in amplitude during left wrist flexions (at 25%, 50% and 75% of MVC), In contrast, cervico-medullary MEPs were not affected by the contraction. The conclusion was that the contralateral motor cortex, but not spinal motoneurons, is modulated by contralateral contractions (Hortobágyi et al., 2003).

In the context of strength training, similar effects of unilateral training on the excitability of the ipsilateral (untrained) motor cortex were reported with multiple training sessions (Lee et al., 2009). The authors employed the method of twitch interpolation, by which a supramaximal magnetic stimulus is applied to the motor cortex while the muscle is contracted (Todd et al., 2003). The rationale behind this method is that maximal voluntary contractions often fail to recruit all motor units, and an additional stimulation is needed in order to reach maximal forces (Todd et al., 2003). The force produced during an MVC with concurrent magnetic stimulation and the force produced during an MVC without concurrent magnetic stimulation are compared, and the amplitude of the superimposed twitch (difference between the two force values) is taken as an index that the voluntary contraction without additional stimulation was submaximal. Wrist extension MVC performed at the beginning and after 4 weeks (12 sessions) of unimanual contralateral training were compared, and voluntary activation was found to be increased (2.9 ± 3.5%) (smaller superimposed twitch). This finding suggest that one of the neural mechanism responsible for the strength increase observed in the untrained limb is an enhancement of cortical drive to the muscles (Lee et al., 2009).

There is contrasting evidence about the role of corpus callosum fibres (see Chapter 2.4.5) in cross-education (Ruddy and Carson, 2013). Direct interhemispheric connections between the two primary motor cortices via the corpus callosum are excitatory and contribute to the bilateral activation observed during unimanual movements (Donchin et al., 1999). In addition, each of the hemisphere can inhibit the activity in the other via local interneurons (Donchin et al., 1999). A group of volunteers trained for 20 sessions performing isometric index finger abduction at 80% of their MVC (Hortobágyi et al., 2011). The amount of IHI, measured by delivering a TMS pulse to each M1 area at 10 ms intervals, decreased both acutely after the end of

the first session (8.9%) and chronically after 20 sessions (30.9%) and strength increases were observed between the first and the last session (Hortobágyi et al., 2011). However, caution should be taken when interpreting data obtained when using a TMS paired-pulse technique if possible contributions from local circuits to the observed effect are not controlled for (Ruddy and Carson, 2013). If one of the two cortical stimuli (conditioning–test) is suprathreshold, its outcome on the EMG will depend on the excitability of spinal motoneurons too, which might have changed during training. In the context of IHI, a decrease in the activity produced by the test stimulus can arise because of changes in interneuronal circuits rather than in the intracallosal pathways activated by the conditioning stimulus.

2.8.2.4. Spinal mechanisms

The spinal cord possesses an extended network of circuits which control unilateral and bilateral movement execution (Pierrot-Deseilligny and Burke, 2005). Given this, it has been theorized that some of these circuits might support cross-education (Carroll et al., 2006). The consensus on the role of spinal circuits in the cross-education effect is far from unanimous. A group of participants attended five weeks (15 sessions) of unilateral plantar flexion training at MVC level (Lagerquist et al., 2006). Right and left tibial nerves were stimulated before and after training at increasing stimulation intensities in order to build H-reflex and M wave recruitment curves. In the trained limb, the amplitude of the H-reflex evoked at a stimulation intensity eliciting a motor wave of 5% of M_{max} significantly increased from pre-training to post training. The same result was not observed for the untrained limb despite a significant increase in the strength of this limb, with the latter being indicative of cross-education (Lagerquist et al., 2006). However, other evidence suggests that spinal circuits have a role in supporting the increase of strength observed in the trained limb. In a different study, Aagaard and his colleagues reported that the amplitude of the monosynaptic reflex elicited during maximal isometric ramp contractions increased after fourteen weeks of resistance training (Aagaard et al., 2002) suggesting that plasticity occurred at the level of the spinal cord with training. The excitability of the monosynaptic reflex pathway at rest did not change after training. According to the authors, data obtained during contraction better characterise the effects of training on cortical drive to spinal motoneurons while performing the movements. The data support the view that neural adaptation to strength training involves both cortical and spinal pathways, perhaps by

modifying the amount of presynaptic inhibition acting on Ia afferents and not affecting the excitability of spinal motoneurons at rest (Aagaard et al., 2002).

Research has shown that strong unilateral movements influence the activity of the contralateral limb through segmental pathways (Hortobágyi et al., 2003). During sustained (five seconds) left wrist contractions MEPs, cMEPs (obtained with electrical cervicomedullary stimulation) and H-reflexes were recorded from the opposite FCR muscle. MEPs amplitudes in the contralateral hand increased without a concurrent increase in cMEP amplitudes, pointing to a role of the motor cortex in facilitating activity in the resting arm. In addition, the monosynaptic reflex amplitudes in the right FCR decreased during contractions, which could be due to increases in presynaptic inhibition acting on afferents via spinal inhibitory interneurons (Hortobágyi et al., 2003). It is thereby possible that the same segmental pathways undergo long-lasting modification as a result of the repetitive use of the trained limb, thereby guiding cross-education. Unfortunately, because of the insensitivity of the monosynaptic reflex method to detect changes in spinal interneurons excitability, it has been challenging to validate this hypothesis experimentally (Lee and Carroll, 2007). Thus, the specific role of adaptations in spinal circuits in the cross-education phenomenon has yet to be established.

2.8.3. Skill training

2.8.3.1. Behavioural evidence

Repetitive practice of a new motor skill improves performance. Practice effects are task and effector-specific, but a certain degree of motor learning transfer can be observed over muscles and skills that were not specifically trained (Thorndike and Woodworth, 1901). Early experiments on the effects of practice on performance often employed mirror-tracing tasks, given their simplicity and automaticity (Latash, 1999). A series of experiments conducted by Cook (1933, 1934) investigated whether mirror-tracing a star-shaped maze with the right hand (or foot) increased mirror-tracing performance in the untrained hand (or foot). Training effects were extended to the contralateral limb, according to what is now known as the bilateral transfer phenomenon. Transfer of skills to the contralateral limb has since been demonstrated over multiple tasks. As opposed to cross-education after strength training, skill transfer is often observed after a single training session. Parlow and Dewey (1991) had participants practising a 5-keys finger tapping sequence on a typewriter. They

were asked to complete the sequence as many times as possible in 15 seconds. The effects of training on both (trained and untrained) hands were tested after every 10 practice trials. After 10 trials, performance when using the trained hand was greater (more sequences completed) than for the untrained hand. However, after 20 trials the performance difference between trained and untrained hand ceased to differ (Parlow and Dewey, 1991).

The amount of skill training which is transferable to the non-trained limb depends on the nature of the task (Lee and Carroll, 2007). As an example Teixeira (2000) used a simple task in which participants responded by pressing a button with the thumb anticipating the time of a luminous signal. The results showed that this anticipatory skill transferred completely to the other hand, without a significant effect of Group (hand used). However, as the authors discussed, the task employed had a strong perceptual component, and results might not depend on motor adaptation (Teixeira, 2000). In contrast, practice which requires precise manipulation of small objects do not transfer completely to the opposite limb (Gordon et al., 1994). Children and healthy adults were asked to grasp a grip instrument presented on a table in front of them and to maintain it lifted for 5 seconds at 10 cm from the table. The grip instrument had two strain gauge transducers which measure load force and grip force. The weight of the instrument could be changed by the experimenter without the participant being aware of it. Authors found that the knowledge about the object weight, and thereby the load force needed to perform the task, transferred from moving with the right hand after 21 trials to moving with the left hand. However, the weight-related information derived when training with the left hand did not transfer completely to the right hand as shown by a greater grip force rate to load force rate ratio. The authors concluded that bilateral transfer is asymmetrical for the designed task, possibly because of the greatest bilateral cortical activation observed when movements are performed with the dominant hand compared to the non-dominant hand (see the Cortical mechanism paragraph) (Gordon et al., 1994).

The possibility that tasks producing high levels of bilateral cortical activity induce greater amount of cross-education has been put forward (Hortobágyi et al., 2011). Carroll and his colleagues (2008) asked participants to train on a ballistic finger abduction task. They were instructed to move their right index finger as rapidly as possible while the rest of the hand was secured to exclude movements. The performance was measured as the peak acceleration produced during the finger

movements. This kind of task has a strong motor component, and potential confounds such as perceptual learning are minimised. As opposite to reaction time tasks (e.g. Teixeira, 2000) which require the acquisition of temporal relationships between the external stimuli and the movement to produce, ballistic movements do not produce activity in higher-order associative cortical areas (Ruddy and Carson, 2013). In addition, strong unilateral movements induce bilateral activity in the motor cortices which can facilitate transfer to the other limb (Muellbacher et al., 2000a). Participants performed an initial 10 trials with the left hand which were used as a baseline, then completed 150 trials with the right hand and finally post-training performance was measured on other 10 left-hand trials. Peak acceleration improved by 140% in the trained hand and by 82% in the untrained hand. EMG recorded from the FDI muscles showed that better performance was the result of a reduced interval from EMG onset to EMG peak amplitude after training, which is in line with the hypothesis that contralateral training induced changes in muscle activity patterns (Carroll et al., 2008). One of the adaptations which have been proposed to occur with ballistic training is a change in the order of motor unit recruitment towards selective and synchronous activation of fast-twitch type units (Moritani, 1993).

2.8.3.2. Cortical mechanisms

Functional neuroimaging data acquired with positron-emission tomography (PET) imaging show that during the execution of complex unimanual movements neural activity increases in a cluster of cortical motor areas including M1, SMA and cingulate gyrus in the ipsilateral (to the moving hand) hemisphere (Shibasaki et al., 1993). In light of this phenomenon, it seemed plausible that the repetitive activation of these areas due to practice leads to use-dependant plasticity, which could represent the neural structure of the bilateral transfer of skill effect. One study which specifically addressed the role of the effects of unimanual movements on the contralateral motor cortex through non-invasive stimulation dates back to 1998 (Tinazzi and Zanette, 1998). The authors measured EMG activity from the left APB muscle while participants: (1) tapped their right thumb over the right third finger; (2) tapped their right thumb on all fingers from second to last; (3) tapped their right thumb on all fingers following random sequences. MEPs were recorded by stimulating the right motor cortex at an intensity of 120% of the resting motor threshold. MEP values were enhanced were compared to rest values during sequence and random finger tapping.

However, MEPs amplitudes were unchanged if participants were tapping their right thumb over the right third finger. This finding indicates that the cognitive component of the task, which is the sequence introduced, and not the movement *per se* activates the motor cortex bilaterally. In addition, the motor cortex was stimulated with TES which as opposed to TMS stimulates corticospinal axons directly. The outcome of TES does not thereby depend on the excitability of the motor cortex. Responses to TES did not change during any movements, supporting the cortical origin of the change in descending activity. In conclusion, unimanual movements produced according to specified sequences induce bi-hemispheric interactions (Tinazzi and Zanette, 1998).

The last task described falls into the category of *procedural learning*, as the performer has to learn a predetermined sequence of movements. The involvement of the contralateral M1 in the transfer of such class of tasks was assessed by Perez and colleagues (Perez et al., 2007). Participants reacted to GO signals presented on a computer screen in four different locations by pressing the finger assigned to that specific location. The GO signals were presented either in a given sequence or a randomised order. Performance transfer was measured as changes in reaction times between pre-training and post-training values when using the untrained hand. Changes in the excitability of the trained and untrained neural circuits were assessed by recording EMG from the FDI muscles using multiple TMS-derived parameters including resting motor threshold, recruitment curves, IHI, and SICI. Reaction times decreased after training for both the random and specified sequence, which indicates that participants became faster in responding to the visual cue. Measures of intracortical (SICI) and interhemispheric (IHI) inhibition decreased after training in both hemispheres. In contrast, MEPs recorded with recruitment curves were higher only when stimulating the hemisphere contralateral to the trained hand. Taken together, these findings indicate that: training alters the excitation/inhibition balance in the training motor cortex in favours of excitation; training alters the connectivity between the trained motor cortex and the untrained one towards excitation. The resting motor threshold values did not change after training in both hemispheres. The increase in corticospinal excitability, measured via recruitment curves, in the trained hemisphere is a widely reported finding (e.g. Pascual-Leone et al., 1994a). However, this was the first study to demonstrate that interhemispheric inhibition is reduced after unimanual training in the untrained hemisphere (Perez et al., 2007).

Contrasting results were reported when the task involved ballistic movements (Carroll et al., 2008). First, training on ballistic finger abductions with one hand improved the performance in the untrained hand measured as an increase in peak abduction acceleration. In addition, and in contrast with the previous study, MEPs evoked from the untrained FDI were significantly higher after training when using stimulation intensities of 110%, 120% and 130% of MT (Carroll et al., 2008). This result proved that unilateral training affects directly the excitability of the ipsilateral motor cortex, and not just through its connectivity with the trained motor cortex. The importance of the untrained primary motor cortex in the cross-limb transfer phenomenon after ballistic training was confirmed by a study in which a "virtual lesion" was induced in the untrained M1 after unilateral training with repetitive-TMS (Lee et al., 2010). Participants were randomly assigned to a ballistic training or a control group which did not train. The task consisted of repeated index finger abductions following a visual cue. At the end of training, performance and corticospinal excitability (measured with TMS at 120% MT) were found to be increased for both hands and hemispheres. However, delivering TMS on the untrained M1 at low frequencies (1 Hz for 15 minutes) at the end of training interfered with the ongoing cross-education processes, disrupting the performance in the untrained hand and bringing the excitability of the untrained hemisphere back to its baseline values (Figure 2.8). As for the findings of Carroll and his colleagues, this indicates that processes occurring in the untrained M1 already while training support the development of cross-education. In addition, rTMS given on the trained hemisphere (contralateral to the hand performing movements) reduced the increases in performance observed after training in the trained hand but did not prevent cross-education to the other hand. The researchers suggested that the untrained motor cortex contributes to the emergence of bilateral transfer, possibly because strong unilateral movements induce bilateral descending drive (Lee et al., 2010). These findings are in contradiction with the ones reported by Perez and her colleagues (Perez et al., 2007), highlighting the difficulty in interpreting bilateral transfer observed after movements of different quality and of providing a unified theory of the neural mechanisms supporting it (Ruddy and Carson, 2013).

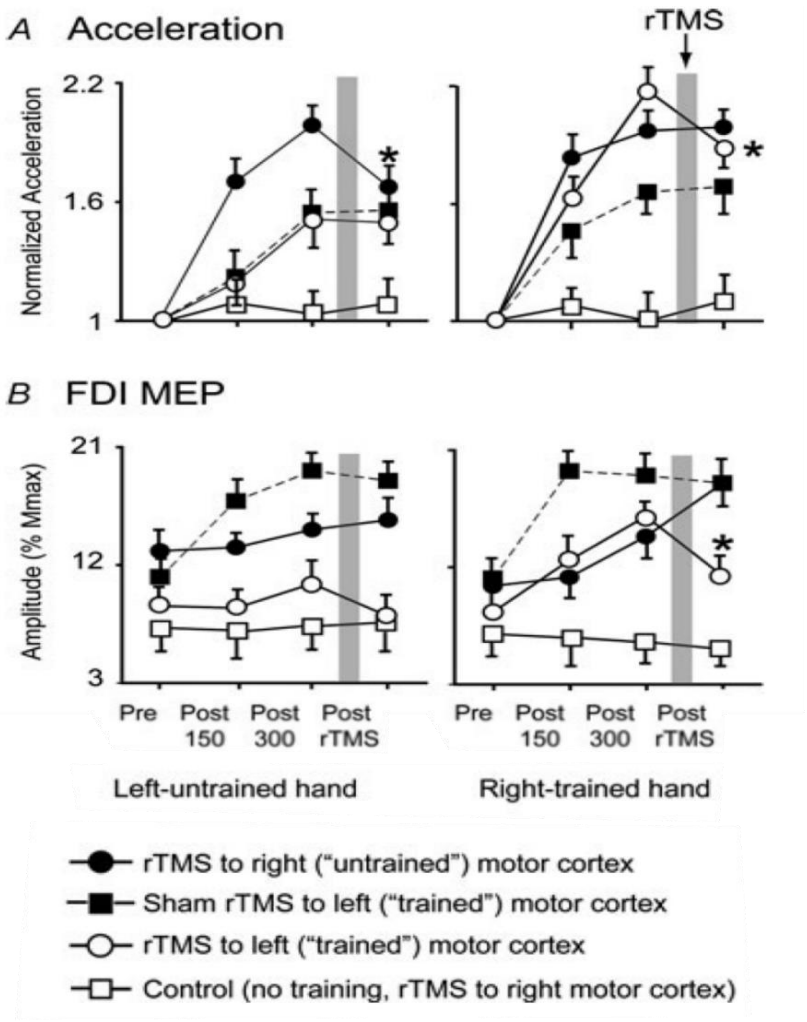

Figure 2.8 Involvement of the ipsilateral motor cortex in skill acquisition. *(A)* Performance changes during training (300 movements) and effects of rTMS on post-training performance. *(B)* MEP amplitudes during training and effects of rTMS on post-training MEP amplitudes. Adapted with permission from Lee et al. (2010).

2.8.3.3. Spinal mechanisms

The literature on the consequence of unimanual movements on the excitability of the contralateral monosynaptic reflex is contradictory. In the work by Tinazzi and Zanette (1998), none of the finger tapping protocols employed affected the amplitude of the monosynaptic reflex evoked in the untrained arm by median nerve stimulation. In contrast, a study by Carson and colleagues (2004) measured the modulation of the FCR H-reflex while the contralateral hand alternated flexion and extension of the wrist at a given frequency (2 Hz). The results showed that the right H-reflex was facilitated when the EMG activity of the left FCR was maximal during flexion (Carson et al., 2004). However, rhythmic movements were performed at high levels of strength (maximum contraction) and is therefore possible that a certain amount of strength is

necessary in order to alter spinal excitability of the contralateral limb. Indeed, a previous study by Muellbacher et al. (2000a) reported facilitation of the contralateral APB F-wave, which is a measure of spinal motoneurons excitability, only during unimanual movements >50% of MVC. It has been proposed that tasks with high complexity might engage extended bilateral motor pathways which fail to be activated by simple tasks (Hausmann et al., 2004), but no studies so far reported changes in untrained spinal circuits after complex tasks. Similarly, evidence that the monosynaptic reflex pathway of the untrained limb undergo long-lasting adaptation after contralateral training is lacking. To date, the involvement of segmental pathways in the bilateral transfer effect is purely speculative and based on clinical evidence and on the supposed role of spinal pathways in the control of bimanual movements (Lee and Carroll, 2007).

2.8.4. Similarities between strength and skill training

Throughout the chapter it was distinguished between studies conducted using tasks which maximize the improvements in strength and those in which skill performance is the main behavioural outcome. Recently, the terms cross-education and bilateral transfer have been used interchangeably (Barss et al., 2016). Carroll and his colleagues (Carroll et al., 2001b) suggested that resistance training represents a form of skill learning and that the mechanisms underlying cross education and bilateral transfer are overlapping. This theory is based on two experimentally-derived findings: first, strength training induces long-lasting neural and neuromuscular adaptation. Muscle recruitment patterns change with training paralleling the increase in strength (Carroll et al., 2001a). Training increases synaptic efficacy between the motor cortex and spinal motoneurons (Milner-Brown and Lee, 1975); second, strength changes extend to tasks which are qualitatively similar to the trained one (Fimland et al., 2009), a common characteristic of skill training protocols (Gagne et al., 1950). Furthermore, the increase in strength obtained after strength training extended to a sensorimotor task requiring the use of the same effectors (index finger in this specific case; Carroll et al., 2001a).

There have been numerous attempts at directly comparing the behavioural and neural effects of skill and strength training. Jensen et al. (2005) had participants perform heavy-load elbow flexions or a task in which they had to vary the position of the elbow joint according to a figure displayed on the computer screen in front of them. After

four weeks of training, performance and corticospinal excitability were measured for both groups and compared to pre-training values. Performance and corticospinal excitability increased in the skill training group. While strength increased in the strength training group, the maximal MEP evoked via TMS and the slope of the MEP recruitment curve significantly decreased after training. The authors argued that, while a learning component was present in the strength training group, its effects on motor excitability differed from skill learning (Jensen et al., 2005). However, they suggested that some of the factors which ultimately determined the outcome of the strength training could be related to its design: lack of visual feedback; lack of instruction to the participants; no elements of novelty (Jensen et al., 2005). Recent work (Leung et al., 2015) seems to endorse their intuition. Three tasks requiring different levels of strength and instructions to the participant were designed: a skill training task, in which participants had to replicate with their dominant arm the pattern of movements seen on a screen; a self-paced training at 70–80% of 1 repetition maximum intensity; a metronome-paced training at 70–80% of 1 repetition maximum intensity. Following training, corticospinal excitability and intracortical inhibition were facilitated in the ipsilateral (to the performing hand) hemisphere in the skill and metronome-paced training groups, but not in the self-paced training group (Figure 2.9) (Leung et al., 2015). Importantly, this study was one of the few which specifically addressed the mechanisms occurring at the early post-training stage of skill and strength training. Nevertheless, the authors did not include behavioural autcome variables and so could not prove that their training protocol succeeded in increasing both strength and skill in the contralateral hand. In the future, studies incorporating both the behavioural and neural measures could help elucidate the issue of whether cross-education of strength and bilateral transfer of skills can be observed after a single training session and whether these induce changes to the contralateral system through similar neural mechanisms (see Chapter 6).

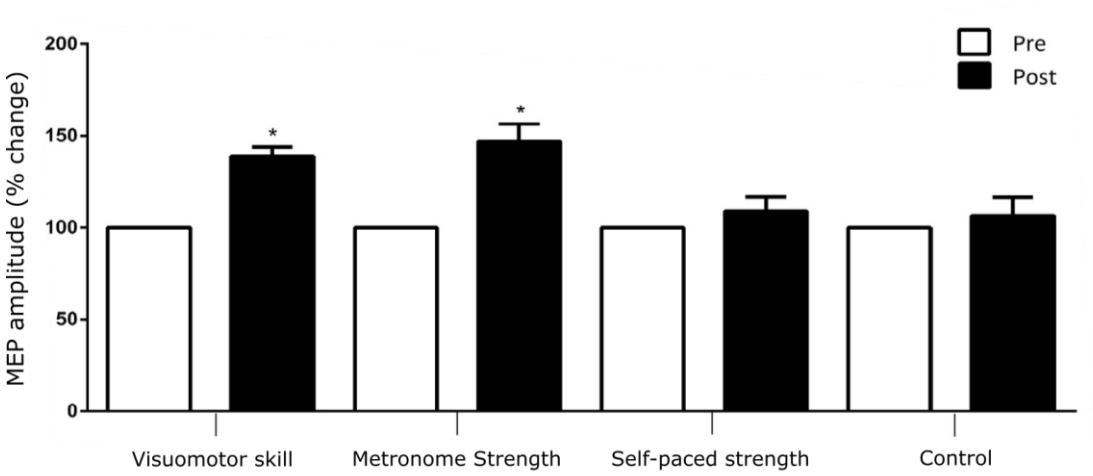

Figure 2.9. Effects of training on the excitability of the ipsilateral cortex. MEPs recorded with TMS delivered at 130% aMT on the right M1 from the left (untrained) biceps brachii muscle after different training protocols. Adapted with permission from Leung et al. (2015).

2.9. Conclusions

This chapter started with a description of the non-invasive stimulation techniques used to investigate neural activity in the motor system and of their contribution to our understanding of human control in humans. The method of TMS-conditioning of the monosynaptic reflex, used to study pathway-specific plasticity in the motor system, was discussed. The reliability of this method when EMG is recorded from FCR is the topic of the first experimental chapter of this thesis (Chapter 4). The validity of TMS as a measure of corticospinal excitability will be addressed in Chapter 5 by studying the effects of auditory activation and stimulus anticipation on the MEPs recorded from FCR. The behavioural and neural effects of a single session of unimanual strength training and skill training on the untrained limb will be assessed in the last experimental chapter (Chapter 6). Finally, the last chapter will present a general discussion of the experimental findings and of their relevance for future studies.

Chapter 3 – General methods

3.1. Introduction

The three studies which constitute the core of this PhD thesis have been conducted under similar experimental conditions and by using similar instrumentation. Thereby, aim of this chapter is to give a general description of the methods used throughout the thesis. The details of each experimental protocol will be described in the relevant chapter.

3.2. Participants

A grand total of 46 participants were enrolled in the three studies. Whenever possible, consecutive sessions for each participant were scheduled at the same time of the day to control for potential circadian rhythms confounding (Sale et al., 2007). Participants were in all cases blind to the experimental procedures. The experimental sessions were conducted at the School of Biomedical Sciences, University of Leeds. Participants' inclusion to the studies was determined according to their answers to the TMS pre-screening questionnaire and TMS acute screening questionnaire, administered at the beginning of the first session. These questionnaires are designed to minimise the risks arising from the use of transcranial magnetic stimulation in the healthy population, according to safety guidelines described by *The Safety of TMS Consensus Group* (Rossi et al., 2009). Exclusion criteria listed in the TMS pre-screening questionnaire included: having a familial history of epilepsy; ever having had a fainting spell; ever having had a seizure; having an implanted metallic device; having a heart disease; taking any medication that might affect the Central Nervous System. In addition, the TMS acute screening questionnaire was completed at the beginning of each session to ensure that the participant had not used alcohol or recreational drugs in the last twenty-four hours, had had enough sleep on the night before testing, defined as maximum two hours less than their average sleeping time, and enough to eat, defined as at least half of their normal consumption, in the last six hours before testing. Participants were asked not to take part in strenuous exercise in the 24 hours before the experiment or consume caffeine prior to the session. Inclusion criteria to participate in the studies were being aged between 18 and 40, being right-handed as self-reported (Chapter 4 and Chapter 5) or as assessed through the Edinburgh handedness inventory (Oldfield, 1971) (Chapter 6) and being in good health at the

time of testing. All participants were capable of following verbal instructions and to give written consent. Once the participants had completed the pre and acute screening questionnaires and had signed the consent form, they were introduced to the experimental protocols.

3.3. Dynamometer positioning

In order to keep the position of the participant controlled and constant through the session, an isokinetic dynamometer (Biodex Medical Inc, Shirley, NY, USA) was employed in all the studies. All experimental phases started with asking the participant to sit comfortably on the dynamometer. The participants were seated with hip and knees forming an angle of 90˚, feet resting on foot support and their head resting on the headrest (Figure 3.1 *A*).

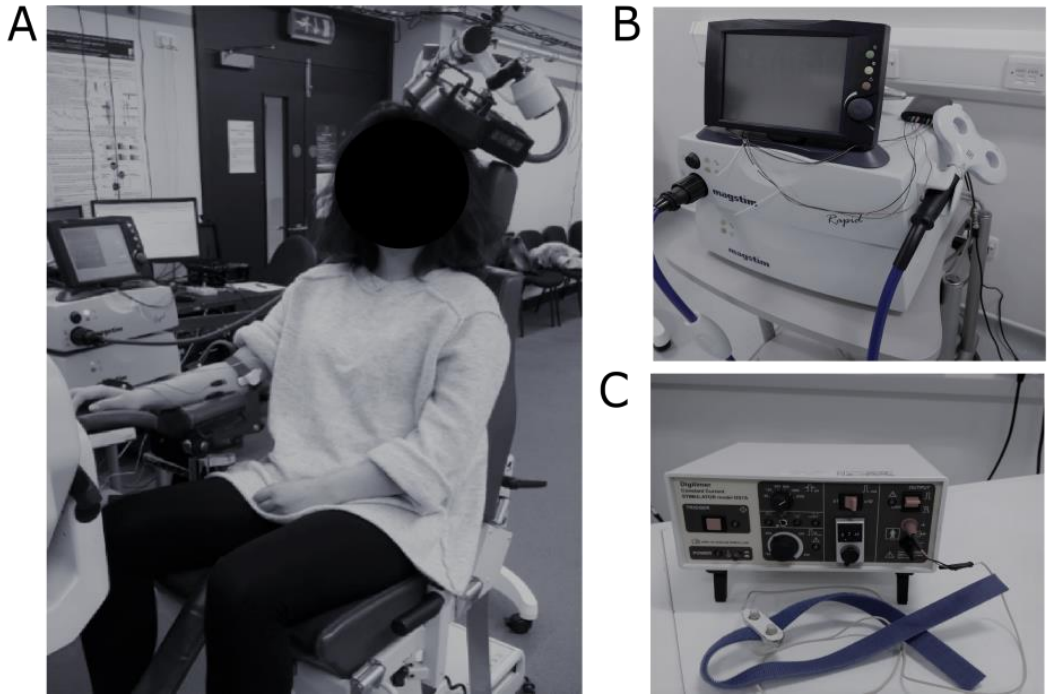

Figure 3.1. Setting and instrumentation. *(A)* Position of the participant while sitting on the dynamometer. *(B)* Magstim Rapid stimulator used for delivering TMS and *(C)* Digitimer DS7A used for delivering median nerve stimulation.

In study 1 and study 2, participants held the right forearm in full pronation and the elbow flexed at an angle of 120˚ supported by a dynamometer armrest. The position of the elbow was kept stable during the session. In study 3, the forearm was supinated, and participants were asked to grasp the handle on the dynamometer's wrist

attachment, with their elbow forming an angle of 140˚. Once found the most comfortable position, seat height, seat inclination and head height were recorded for future references.

3.4. Electromyography (EMG) Recordings

Surface EMG is a technique which permits the recording of myoelectric signals generated by contraction of skeletal muscles through electrodes placed on the skin (Merletti et al., 2004). A wireless system (Trigno, Delsys Inc., Natick, MA, USA) was used to record EMG activity in all the experiments (Figure 3.2). Each Trigno Avanti sensor consisted of four Ag/AgCl electrodes separated by a distance of 10 mm. Electromyography (EMG) activity was recorded from three muscles across the studies: flexor carpi radialis (FCR); flexor carpi ulnaris (FCU); extensor carpi radialis longus (ECRL). In study 1 (Chapter 4) and study 3 (Chapter 6), activity from flexor muscles was recorded by means of parallel-bar wireless mini sensors (2.5 × 1.2 cm) (Trigno, Delsys Inc., Natick, MA, USA). The ground electrode of mini-sensors was placed on the dorsum of the hand. For the extensor muscle and the FCR in study 2, bigger (3.7 × 2.6 cm) sensors were employed. The quality of EMG recordings depends on skin-electrode impedance (Day, 2002). The skin overlying the forearm muscles and lateral epicondyles was shaved when deemed necessary, then prepped using abrasive gel (Nuprep, Weaver and Company, USA) and 70% isopropyl alcohol swabs (Alcotip, Universal Hospital Supplies Ltd., UK) to remove dead skin secretions and lower skin-electrode impedance.

In study 2 and study 3, EMG activity was recorded from an antagonist muscle (ECRL) to ensure lack of pre-activation in this muscle which could alter the responses to magnetic and electric stimulation in the FCR muscle (Izumi et al., 2000). In addition, in study 1 activity from the FCU muscle was recorded via mini sensors to assess whether peripheral stimulation was selectively activating the median nerve and not the ulnar nerve. The optimal location to record activity from the FCR muscle is reported to be at one third of the distance between the medial epicondyle (ME) and the radial styloid (RS) (Christie et al., 2005), and at 10 cm from the medial epicondyle and to the ulnar styloid (US) to record activity from the FCU muscle (see Figure 3.2 *A*) (Gentili and Di Napoli, 2016). Similarly, the optimal location to record activity from the extensor carpi radialis longus was reported to be at $1/6^{th}$ of the distance from the lateral epicondyle to the estimated centre of origin of ECRL (Figure 3.2 *B*) (Riek

et al., 2000). In order to facilitate the electrodes' positioning, participants were asked to perform flexions, extensions, radial deviations and ulnar deviations of the wrist. Sensors were placed on the belly of the participant's muscle while the targeted muscle was contracted. Pictures of the electrodes position were taken on each session to ensure stability of recordings across days. Excessive background noise in the recordings indicate muscular pre-activation, which might alter the outcomes of stimulation. Baseline noise was visually checked after placing the sensors. The EMG signal was pre-amplified (gain = 909), recorded with a 20-450 Hz bandwidth and digitized at 2 kHz using data acquisition software (Spike2, Cambridge electronics Design, Cambridge, UK).

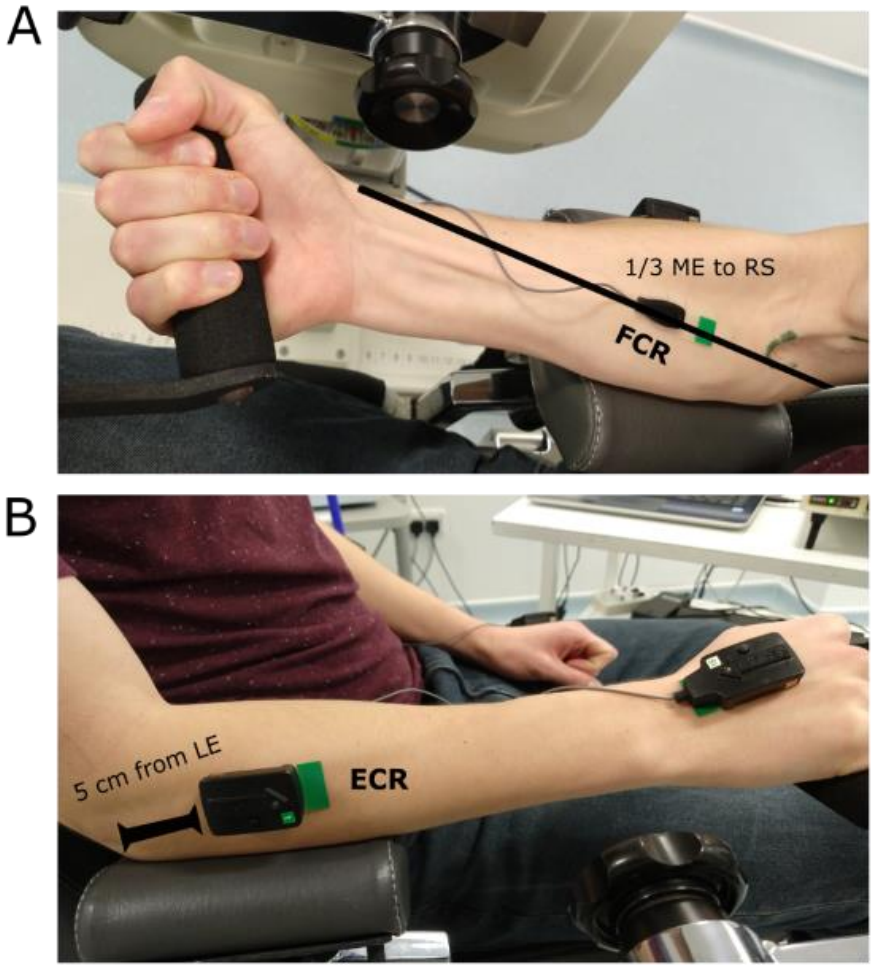

Figure 3.2. EMG sensor positioning. Sensor positioning to record activity from the FCR muscle *(A)* and ECR muscle *(B)*. ME = medial epicondyle; RS = radial styloid; LE = lateral epicondyle.

3.5. Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimulation (TMS) uses the principle of electromagnetic induction to stimulate cortical neurons. A magnetic field is produced from the coil tangential to the scalp, and this in turn induces an electric field running perpendicular to the magnetic one. The induced current causes activation in the neural populations under the stimulation site (Hallett, 2007). A typical capacitor can discharge a magnetic field of up to 2 Tesla which evolves rapidly (in the order of 100-200 μs) (Hallett, 2007). The current induced in the brain and the spread of neural activation, namely its focality (Deng et al., 2013), depend on the shape of the coil. When a certain coil is used, the intensity of the field decreases as a function of distance such that only a small part of the induced currents reaches deep structures in the brain (Rossi et al., 2009). For example, the first coils employed in the early years were circular (Figure 3.3 *A*), with outer diameters ranging from 8 to 15 cm (Barker et al., 1985). The coil current induces a current flow in the tissue below the coil which runs in the opposite direction according to Lenz's law of electromagnetism (Maccabee et al., 1991). Circular coils are often used to stimulate the brain bilaterally, because these can generate strong electrical fields (Pascual-Leone et al., 2002). However, circular coils have poor focality because their circumference cover a large part of the scalp and stimulation can occur at any point below the coil (Rösler et al., 1989). Significant improvements in terms of focality were made with the introduction of the figure-8 coil (Figure 3.3 *B*) which rapidly replaced the "original" round coils (Ueno et al., 1988). The figure-8 design includes two round coils whose currents flow in opposite directions and sum at their intersection, where the power will be maximal (Thielscher and Kammer, 2002). Simulation models estimated the field spread induced by 70 mm figure-8 coils to be as little as 5 cm^2 while the one induced by circular coils had a lower limit of 34 cm^2 (Deng et al., 2013). Other variants of shape include double-cone coils and H-shaped coils, producing powerful stimulation and mostly used to activate deeper brain regions (Hallett, 2007). However, none of the more elaborate coil shapes exhibit better depth/focality trade-offs than the figure-8 type (Deng et al., 2013) which is still the most widely used type in research and was used throughout this thesis.

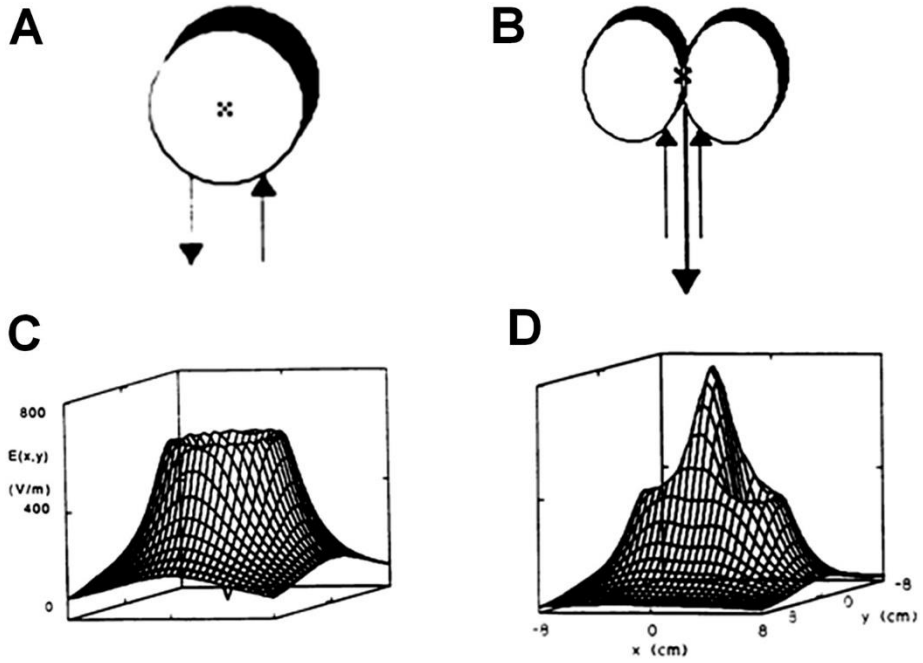

Figure 3.3. Electric fields produced by different magnetic coils. (*A*) Round coil and (*C*) resultant field. (*B*) Figure-8 coil and (*D*) resultant field. From Hallett (2007), with permission.

Taking into consideration that activation of nerve fibres is more likely to occur at locations where the fibre bends, it is not surprising that changing the orientation of the coil relative to the scalp affects the observed results. Mills et al. (1992) compared the effects of rotating the coil over the scalp while measuring electrical activity from the first dorsal interosseous (FDI) muscle. The largest and more consistent responses were observed when the current was induced from a posterolateral to an anteromedial direction, at 50° from the parasagittal plane and approximately parallel to the motor strip. This outcome was noted independently of background activity or stimulus intensity. The posterior-to-anterior orientation was used throughout this thesis. Accidental changes in the position and orientation of the TMS coil can compromise the data obtained when using TMS stimulation protocols because different neural elements will be stimulated if the coil is moved (Pierrot-Deseilligny and Burke, 2005). The anatomical reconstruction of the cortical surface obtained via MRI can be co-registered with TMS to guide coil navigation (Ettinger et al., 1996). This technique improves spatial precision of stimulation compared to traditional methods, but does not decrease response variability in the same participant (Gugino et al., 2001). Other methods to control for coil position and orientation such as the use of a reference grid

to mark the position on the scalp, and a coil holder to stabilise its position are commonly employed in research (Capaday, 1997), and were used in this thesis.

The first generation of stimulators delivered monophasic pulses, in which the current flow is stopped after the first quarter cycle and has a single polarity (Delvendahl et al., 2014). This pulse shape requires a long time for recharging the capacitor after each pulse, limiting the possibility of delivering pulses in close temporal proximity (Wassermann et al., 2008). Thus, nowadays it is common for most stimulators to produce biphasic coil current waveforms. Because the capacitor is not clamped, the voltage goes from positive to negative values and so the energy returns to the capacitor at the end of the cycle and another pulse can be rapidly discharged (Wassermann et al., 2008). There are, however, substantial differences when stimulating the motor cortex with different pulse shapes (Niehaus et al., 2000). MEPs recorded from two hand muscles (abductor pollicis brevis APB and FDI muscles) were compared when using monophasic and biphasic current pulses at the same stimulation intensities, which ranged from 68 to 142 Amp/μs. The biphasic stimuli induced MEPs at lower stimulation intensity levels and were thereby more effective than monophasic ones, probably because the second phase (second and third quarter cycles) is longer and more likely to generate action potential (Maccabee et al., 1998). Nonetheless, monophasic pulses induce more homogenous descending volleys as seen by epidural potentials recorded in patients with implanted spinal cord stimulators (Di Lazzaro et al., 2001). When building recruitment curves to study the input/output relationship of MEPs, employing monophasic pulses led to steeper curves when compared with biphasic pulses (Sommer et al., 2006). Again, this is consistent with the assumption that biphasic currents can activate more neurons at the same stimulation intensity (Sommer et al., 2006). Taken together this suggests that the second phase of current flow can stimulate different subsets of neurons and interneurons or the same neurons at different sites (Sommer et al., 2018). The stimulator employed in the experimental chapters described in this thesis (Magstim Rapid) delivers biphasic pulses and therefore requires lower stimulation intensities to activate neurons than monophasic ones (Sommer et al., 2006).

In this thesis, stimulation was delivered to the left M1 region by a Magstim Rapid stimulator (Figure 6.1 B) with the coil (70mm Double Air Firm coil in Chapter 4 and D70mm Alpha Coil in Chapter 5 and Chapter 6, Magstim Company, Whitland, Dyfed, UK) oriented at ~45° to the sagittal plane to produce a posterior-to-anterior current

flow across the motor strip (Day et al., 1989a). During all the interventions, the stimulation was controlled by Spike2 (Cambridge Electronic Design, Cambridge, UK) software. Before stimulation, head measurements were taken to estimate the region of M1 where the cortical representation of forearm muscles lies. First, a line was drawn with a non-permanent marker at the mid-point between the inion and nasion. Similarly, another line was drawn at the mid-point between the left and right ear lobules to find the vertex of the participant's skull. The cortical region located 2 cm anterior and 6 cm lateral to the vertex on the left hemisphere (Jasper, 1958) was marked for coil placement, and stimulation started around this region. TMS was first delivered at low (~30% of the maximum stimulator output, MSO) stimulation intensities which do not induce muscular responses, in order to make participants familiar with the scalp sensation and the 'click' noise typical of TMS (Dhamne et al., 2014). The magnetic coil was then moved across the left motor cortex while delivering stimulation in order to locate the optimal coil position to elicit MEPs in the FCR muscle, the so-named "hotspot" (Rossini et al., 1994). The position was marked with a non-permanent marker to ensure consistency of recordings over the session. Whenever more than one session was required (study 1 and study 3), the anatomical location of the hotspot in reference to the vertex was measured and used on the remaining sessions. In addition, pictures showing both the location and orientation of the coil were acquired on the first session. The position and orientation of the coil were monitored continuously, and if necessary adjusted to align with the scalp markings.

After establishing the hotspot, an individual motor threshold was estimated following the guidelines set by the International Federation of Clinical Neurophysiology (Rossini et al., 2015). The participant was asked to relax in order to prevent any pre-activation effect (Rossini et al., 2015). The resting motor threshold (rMT) was defined as the lowest TMS intensity, given as a percentage of the maximum stimulator output (MSO), which elicits MEPs with peak-to-peak amplitudes of 50 μV in at least 50% of trials (Figure 3.4 *A*). The experimenter sets the stimulator output at a low intensity (usually 30-35% of MSO) and increases it in increments of 1% until inducing a substantial response (50-100 μV) in the EMG in at least 5 out of 10 trials. This procedure was used in study 1 and study 2. In study 3, participants were asked to maintain a background muscular activation corresponding to 5% of their MVC (Hannah and Rothwell, 2017). This value was determined at the beginning of the

session by performing a maximum isometric contraction of the right wrist against the dynamometer handle. The active motor threshold (aMT) was defined as the lowest TMS intensity, given as a percentage of the maximum stimulator output (MSO), which elicits MEPs with peak-to-peak amplitudes of between 100 and 200 μV in at least 50% of trials (Rossini et al., 2015) (Figure 3.4 *B*). For study 1 and study 3, the IPI between two pulses was set at 5 seconds and 10 traces were recorded on each session. Details of the IPIs used for study 2 are described in Chapter 5.

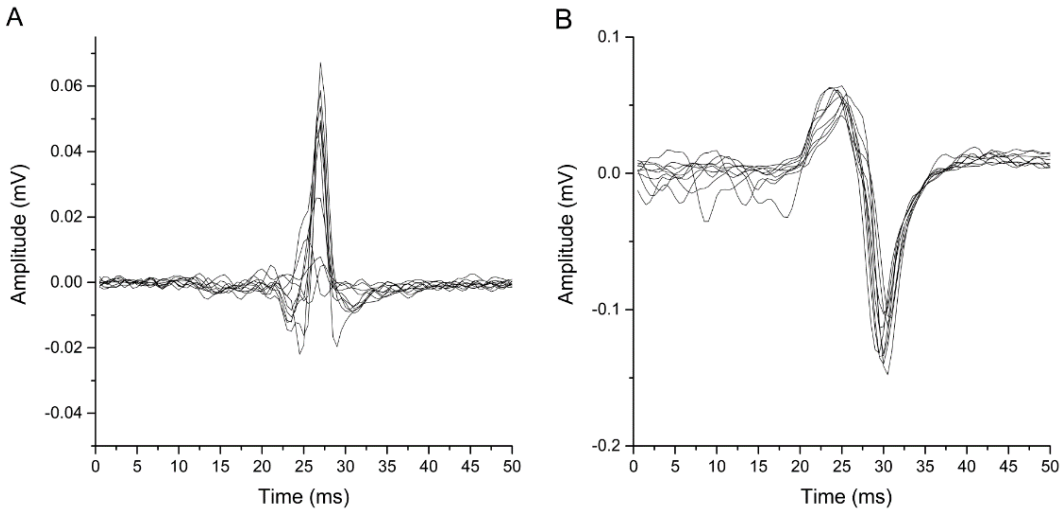

Figure 3.4. Resting and active TMS motor threshold. MT estimated from the resting *(A)* and active *(B)* FCR muscle with delivery of ten TMS pulses. Ten sweeps are superimposed in each picture. A muscle was deemed resting if the root mean square (RMS) of the background EMG recorded from FCR did not exceed 10 μV in the 50 ms preceding stimulus delivery.

3.6. Peripheral nerve stimulation (PNS)

The same procedures were used to derive parameters recorded upon median nerve stimulation in Chapter 4 and Chapter 6. PNS targeted the median nerve at the forearm level to induce muscular responses (M-waves) and monosynaptic reflexes (H-reflexes) in the FCR muscle (Figure 6.2). A bar stimulating electrode (E.SB010, Digitimer Ltd, Welwyn Garden City, UK) consisting of an anode and a cathode stainless steel electrodes of 8 mm diameter and spaced 25 mm was used. The felt pads were soaked in a saline solution before usage to increase conductivity. The stimulation was delivered through a constant-current stimulator (DS7A, Digitimer Ltd, Welwyn Garden City, UK) which was controlled by the acquisition software (Spike2, Cambridge Electronic Design, Cambridge, UK). Room temperature was maintained

constant (22 °C) in all the sessions to prevent any variation in skin surface temperature which might affect recordings (Dewhurst et al., 2005). The median nerve was stimulated using monophasic pulses of 1 ms of length. The stimulus width was chosen to maximize the difference in strength-duration properties of motor and sensory axons. The most reliable locus of stimulation for eliciting activity in FCR is described to be in the cubital fossa, medial to the tendon of biceps brachii, in parallel with the course of the nerve and with the cathode electrode proximal to the anode to prevent anodal block (Jaberzadeh et al., 2004). Once the optimal location to evoke motor wave was determined, the bar stimulating electrode was fixed with straps.

In study 1, PNS was delivered at rest with the right arm placed on an arm rest to prevent muscular activation. Repetition rates of 0.2 Hz were used in these sessions. In study 3, participants were asked to maintain a background muscular activation corresponding to 5% of their maximum voluntary contraction (MVC) by holding the dynamometer handle during stimulation. Contracting the muscle of interest is a widely used method to increase the probability of occurrence of a monosynaptic reflex and limit post-activation depression (Burke et al., 1989). The protocol used to record motor wave and monosynaptic reflex from the FCR muscle followed the guidelines of Burke (2016). The rate of stimulation was initially set to 1 Hz, to speed up the process of finding a good stimulation location at the cubital fossa level and to define whether an H-reflex was visible at varying intensities of stimulation. An additional criterion to ensure that stimulation was targeting the FCR was asking the participant to flex the wrist and then the index finger while being stimulated. An increase in the amplitude of the monosynaptic reflex should be observed only during wrist flexion when stimulating the median nerve FCR (Roche et al., 2011). Vertical cursors were placed on the screen to identify the time interval of 15 to 20 ms after stimulus delivery, which is the average latency time of the H-reflex (Hugon, 1973). The stimulation rate was then set at 0.2 Hz (every five seconds) for all recordings. The amplitude of the maximal motor wave (M_{max}) was measured by starting at low stimulus intensities (around 1 mA) and then increasing the intensity in steps of 0.3 mA until the peak-to-peak amplitude of the motor response reached a plateau and further increasing stimulation intensity had no effects on it. The plateau was visually confirmed by placing horizontal cursors at the positive and negative peaks of the recorded trace on the Spike2 interface. When increasing stimulus intensity did not cause any further increase in the M wave amplitude, the responses were deemed to be maximal (Figure

3.5). Ten traces were recorded at the intensity of stimulation at which the M wave is maximal. The amplitude of the M_{max} wave and the intensity at which a plateau was reached were recorded for future reference.

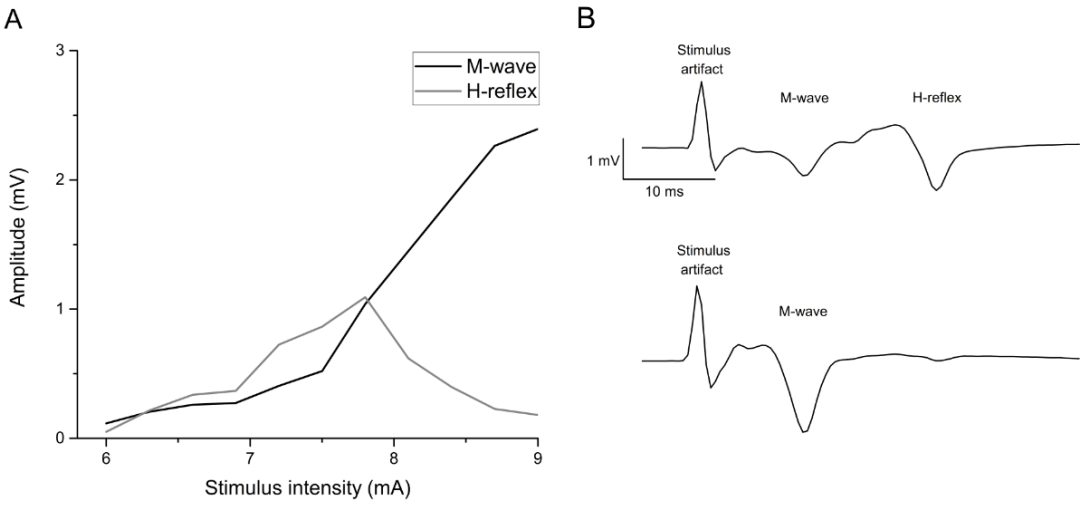

Figure 3.5. Recruitment curves and raw traces of motor wave and monosynaptic reflex. *(A)* Recruitment curves of the M-wave and H-reflex in a representative participant. Ten traces were recorded at each stimulation intensity, starting at 6 mA and increasing at rates of 0.3 mA until reaching M_{max} (around 9 mA). *(B)* Example of a trace recorded when the H-reflex was at peak (top) and when the M-wave was at peak (bottom).

The intensity at which to elicit monosynaptic reflexes was based on the peak-to-peak amplitude of the M_{max}. First, an intensity which would correspond to the 10% (Chapter 4) or between 10% and 15% (Chapter 6) were evoked. These percentages were chosen because stimuli given at higher intensities can produce antidromic volleys in the motor axons colliding with the monosynaptic reflex (Burke, 2016). In addition, it has been shown that at these intensities (up to 30% of M_{max}, Crone et al., 1990) the monosynaptic reflex is more susceptible to inhibition or facilitation by conditioning stimuli. Once the target H-reflex amplitude is elicited, the stimulus intensity is manipulated until the reflex induced matches the desired amplitude. Again, horizontal markers were placed on the Spike2 interface to ensure that the recordings corresponded to the right amplitude. Ten traces were recorded at this stimulation intensity. The method used to condition the monosynaptic reflex with TMS differs between Chapter 4 and Chapter 6 (Figure 3.6). Therefore, the specific details of each protocol are described in the relevant chapter.

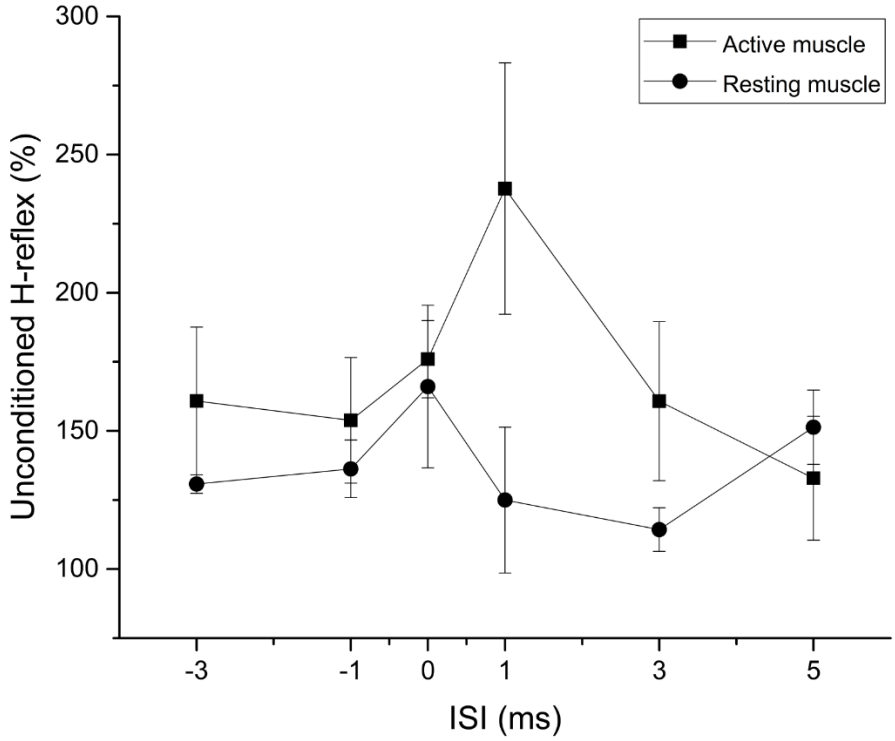

Figure 3.6. Comparison of the effects of TMS on the monosynaptic reflex evoked in resting and contracting muscles. The conditioning stimuli were subthreshold for evoking MEPs in the FCR muscle, and the H-reflexes were delivered at an intensity corresponding to 10% of M_{max}.

3.7. Data reduction

The raw data files produced by the Spike2 software were first processed inside the Spike2 environment. Custom-built scripts were run which identified each stimulus (TMS or PNS stimuli) on the continuously-recorded EMG traces and analysed only specific subsets of the data. The latency of a MEP is measured by taking the time between the stimulus artefact seen on the EMG and the onset of the response, reflecting the activation of the fastest corticospinal fibres (Fujiki et al., 1996). For forearm muscles at rest in a healthy individual, MEP onset is about 20 ms from stimulation (n.b. around 1-2 ms longer than the latency observed after scalp electrical stimulation) (Day et al., 1987). The amplitude of the MEP is measured between the two largest peaks of opposite polarity (peak-to-peak). For the MEPs evoked by TMS, the peak to peak amplitude of the EMG recorded in the FCR muscle was calculated for a time window between 13 and 50 ms after stimulus delivery. For the analysis of

maximum motor response, the peak to peak amplitude was calculated for a time window between 1 and 10 ms after stimulus delivery. For the monosynaptic reflex, the peak to peak amplitude was calculated for a time window between 13 and 50 ms after stimulus delivery. The latency (Figure 3.7) of the responses (study 1 and study 3) was determined by measuring the time interval between the stimulus delivery and the point when the EMG exceeded ±2 standard deviations (SD) of pre-stimulus EMG (Burke, 2016). More information of how the EMG activity recorded during MVCs and wrist flexion movements was analysed is presented in Chapter 6. Statistical analyses were performed using SPSS (Version 22.0) software with an a priori significance level of <0.05. Details of the statistical analyses performed for each study are given in the Methods section of the relevant chapter.

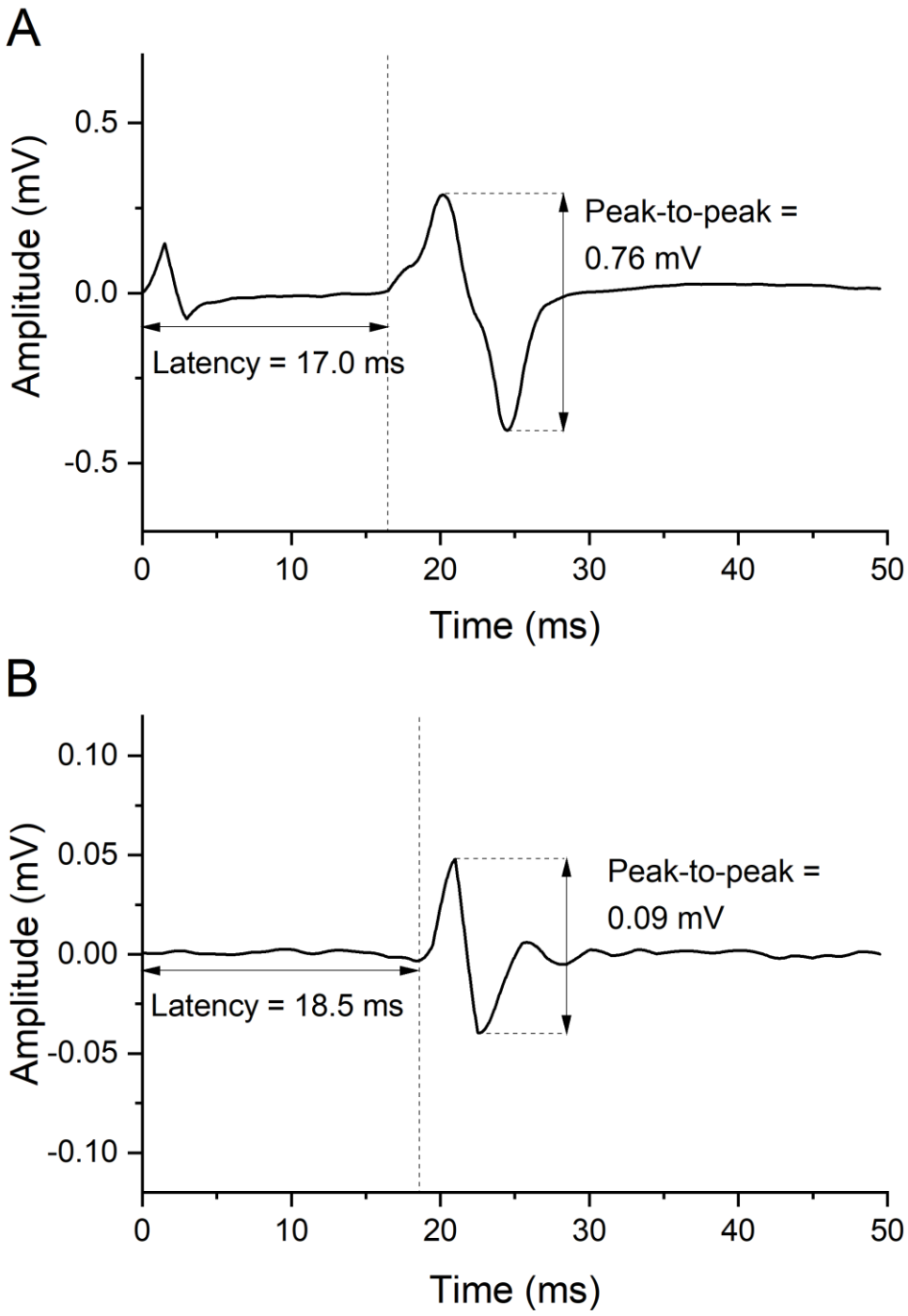

Figure 3.7. Measurement of latency and peak-to-peak amplitude. *(A)* H-reflex latency was measured as the time interval from the onset of stimulus artefact to the first deflection from baseline. *(B)* MEP latency was measured as the time interval from stimulus delivery to the point when the EMG exceeded ±2 standard deviations (SD) of pre-stimulus EMG. Peak-to-peak amplitude was measured as the absolute sum of the positive and negative peak of activation amplitudes.

Chapter 4 – Reliability of the TMS-conditioned monosynaptic reflex in the Flexor Carpi Radialis muscle

4.1. Introduction

Different types of motor training induce specific changes in the excitability of cortical and spinal circuits (Adkins et al., 2006) which may in part be responsible for the recovery of motor function observed after rehabilitative training (Nudo et al., 1996). Evidence shows that spinal and cortical circuits may all contribute to functional recovery (Raineteau and Schwab, 2001). For example, residual MEPs amplitudes are predictors of functional recovery in spinal cord injury (SCI) post-lesion stages (Curt et al., 1998), while H-reflex conditioning changes after training over the months following spinal injury (Penalva et al., 2010). Given this, it is of fundamental importance to develop robust methods which objectively measure the excitability of the motor system. The excitability of corticospinal circuits can be assessed in humans using different techniques. For example, TMS of the motor cortex induces an MEP in the muscles recorded as EMG (Hallett, 2007). The MEP amplitude can be used to estimate the excitability of the corticospinal tract (Leocani et al., 2000), but depends on motoneuron excitability (Pierrot-Deseilligny and Mazevet, 2000). Electrical stimulation of a peripheral nerve (PNS) produces multiple responses in the recorded EMG. Stimulation of the motor nerve fibres produces a short-latency response named M-wave, which reflects the excitability of motor axons (Palmieri et al., 2004). Stimulation of the sensory fibres produces an H-reflex. Studying the monosynaptic reflex provides the unique opportunity to test spinal motoneuron excitability without the influence of descending drive (McNeil et al., 2013). Finally, TMS can be used to evaluate descending influences on spinal reflex excitability through modulation of the H-reflex (Nielsen et al., 1993b), a method known as TMS-conditioning of the monosynaptic reflex.

Thus, when assessing changes in central nervous system excitability occurring after lesion or training, it is crucial to employ validated and reliable techniques (Gray et al., 2017). An evaluation of the test-retest reliability of parameters gives confidence that the observed effects are due to physiological factors rather than to the fluctuating nature of the metrics or to measurement errors (Christie et al., 2005). The conditioning effect of TMS on the monosynaptic reflex evoked in the FCR muscle has been previously investigated while participants were stimulated at rest and when

performing slight flexion of the wrist (Mazzocchio et al., 1994). The authors delivered subthreshold TMS and median nerve stimulation at different time intervals. An increase in the amplitude of the H-reflex when delivered preceding (2 to 4 ms) a cortical stimulus and one after (up to 2 ms) cortical stimulation was observed. Importantly, the amount of facilitation became larger with increasing stimulation intensities and when participants performed voluntary contractions (Nielsen et al., 1993b). This method of conditioning the H-reflex allows an evaluation of the effects of direct and indirect cortical pathways to spinal motoneurons (Nielsen et al., 1993b). Nevertheless, to the best of my knowledge, the intersession reliability of the TMS-conditioned H-reflex in forearm muscles (e.g. FCR) is yet to be established.

MEP responses induced by consecutive TMS pulses exhibit variability (Kiers et al., 1993). Sources of variability include technical factors such as coil orientation, position (see Chapter 3.5) and level of baseline excitability (Kiers et al., 1993). At rest, spontaneous fluctuations in motoneuron excitability occur and influence the outcome of stimulation (Darling et al., 2006). Response variability is inversely related to stimulus intensity, being maximal for lower stimuli (Pellegrini et al., 2018). When conditioning the monosynaptic reflex, cortical stimuli are often applied at subthreshold strengths (90% MT), such that they don't elicit any activity in the recorded EMG (Day et al., 1989a). However, stimulating with intensities based on a fixed threshold amplitude value assumes identical stimulus-response curves across participants (Burke and Pierrot-Deseilligny, 2010). Moreover, changes in excitability occurring throughout the experimental session may too affect the responses to subthreshold stimulation and add variability to the obtained conditioned H-reflex values (Burke and Pierrot-Deseilligny, 2010). Thereby, it was assessed whether changes in corticospinal excitability occurred throughout the experimental session by re-measuring the responses to subthreshold TMS after the conditioning phase.

The aim of the current project was to examine how reliable the responses obtained upon cortical and spinal stimulation are over days by measuring the intraclass reliability of a number of muscle activity parameters, recorded as EMG from the FCR muscle. The recorded measures were: (A) maximal motor wave amplitude (M_{max}), achieved by supramaximal peripheral stimulation, corresponding to the recruitment of all the motor units (Palmieri et al., 2004); (B) H-reflex amplitude to a corresponding stimulus at 10% of M_{max} ($H_{M10\%}$), on the ascending portion of the recruitment curve; (C) TMS-conditioned monosynaptic reflex (TMS – PNS). The effects of delivering

cortical stimulation at different ISIs from the peripheral nerve stimulation ranging from -7 ms (PNS first) to +7 ms (TMS first) was assessed. This range of ISIs was chosen based on previous studies assessing a conditioned H-reflex in the FCR muscle (Niemann et al., 2017). All these metrics were recorded on the same group of participants over 3 sessions.

4.2. Methods

4.2.1. Participants

Thirteen participants (mean age = 26.07, SD = 3.69, females = 6) were enrolled in the study. Consecutive sessions were separated by 24 hours in order to avoid any carry-over effects induced by the TMS protocol, and at the same time of day to control for any potential influence of circadian rhythms (Sale et al., 2007). Since sex hormones levels do not affect responses to TMS and PNS (Ansdell et al., 2019), female participants were recruited without controlling for the menstrual cycle phase. All participants gave written informed consent to procedures approved by the ethics committee of the Faculty of Biological Sciences at the University of Leeds. Participants were included in the study only if the H-reflexes recorded from their right FCR did not overlap with the motor waves recorded from the same muscle, rendering the interpretation of the recording difficult: two participants were excluded from the study for this reason.

4.2.2. Recording techniques

The participants were seated with hip and knees forming an angle of 90˚, feet resting on foot support, the right forearm in full pronation and the elbow flexed at an angle of 120˚ supported by a dynamometer (Biodex Corp., Shirley, NY). Electromyography (EMG) activity was recorded from the right flexor carpi radialis (FCR) and right flexor carpi ulnaris (FCU) to estimate cross-talk between the two muscles by means of parallel-bar wireless mini sensors (2.5 ×1.2 cm) (Trigno, Delsys Inc., Natick, MA, USA). The optimal location to record activity from the FCR muscle is reported to be at one third of the distance between the medial epicondyle and the radial styloid (Christie et al., 2005), and at 10 cm between the epicondyle and ulnar styloid to record activity from the FCU muscle (see Figure 4.1 A) (Gentili and Di Napoli, 2016). Pictures of the position of the electrodes were taken on each session to ensure the stability of recordings across days. The EMG signal was pre-amplified (gain = 909),

recorded with a 20-450 Hz bandwidth and digitized at 2 kHz using data acquisition software (Spike2, Cambridge electronics Design, Cambridge, UK). All measurements were performed at rest.

4.2.3. Stimulation techniques

Magnetic stimuli were delivered to the left motor area M1 by a Magstim Rapid stimulator with the coil (70mm Double Air Firm coil, Magstim Company, Whitland, Dyfed, UK) oriented at ∼45° to the sagittal plane to produce a posterior-to-anterior current flow across the motor strip (Rothwell, 1997). The optimal coil position to evoke MEPs in FCR was found by moving the coil over the scalp while delivering stimulation and by marking the position at which MEPs could be elicited with the lowest stimulation intensity. In order to ensure the stability of recordings across sessions, pictures of the coil position and orientation were taken and the distance from the vertex to the stimulation site measured. During all the interventions, the stimulation was controlled through Spike2 (Cambridge Electronic Design, Cambridge, UK) software.

Peripheral nerve stimulation (PNS) targeted the median nerve at the forearm (Figure 4.1). The stimulation was delivered through a bar stimulating electrode (E.SB010, Digitimer Ltd, Welwyn Garden City, UK) connected to a constant-current stimulator (DS7A, Digitimer Ltd, Welwyn Garden City, UK) which was controlled by the acquisition software (Spike2, Cambridge Electronic Design, Cambridge, UK). The median nerve was stimulated using monophasic pulses of 1 ms of length, to maximise the difference in strength-duration properties of motor and sensory axons. The most reliable locus of stimulation for eliciting activity in FCR is described to be in the cubital fossa, medial to the tendon of biceps brachii, in parallel with the course of the nerve (Jaberzadeh et al., 2004).

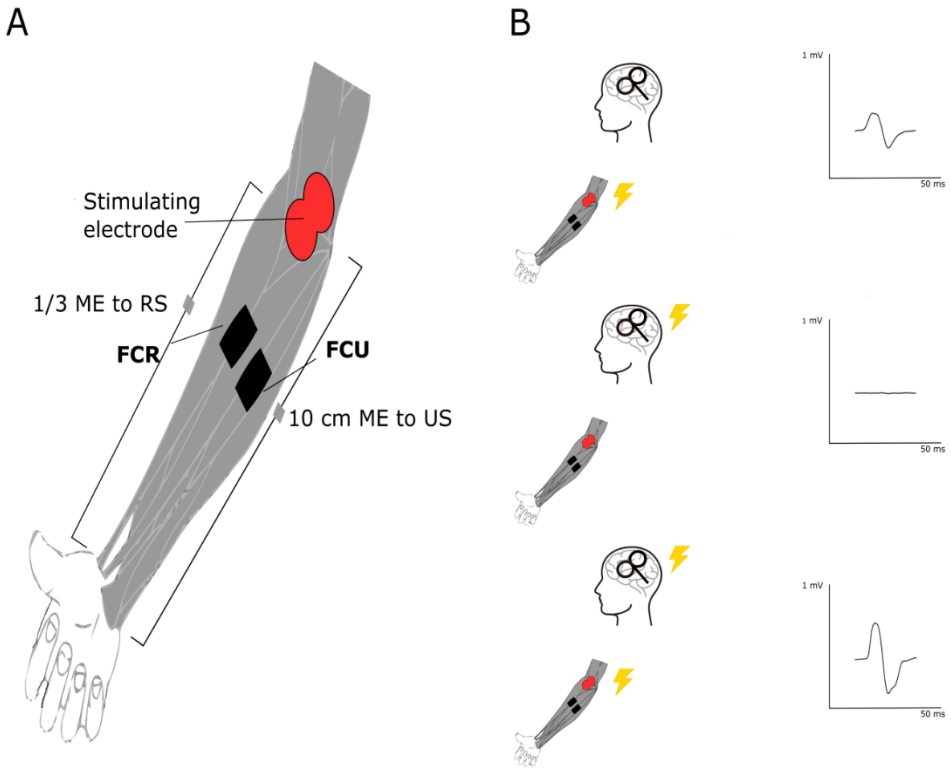

Figure 4.1. Recording and stimulation techniques. (*A)* Electrode positioning and location of median nerve stimulation. ME = medial epicondyle; RS = radial styloid; US = ulnar styloid. (*B)* Stimulation modalities and produced responses in a representative participant. PNS and produced H-reflex (top), subthreshold TMS and induced MEP (middle), TMS-conditioned monosynaptic reflex (bottom).

4.2.4. Experimental procedure

The recording procedure started with either TMS or PNS alone, in a randomized order. The TMS – PNS conditioning part always came later since the stimulation parameters used during this phase of the session are derived from the outcomes of TMS and PNS when given alone. EMG activity was recorded continuously during the experiment and baseline activity of the two recorded muscles was maintained below 10 μV RMS. Details of the experimental procedure are outlined in Figure 4.2.

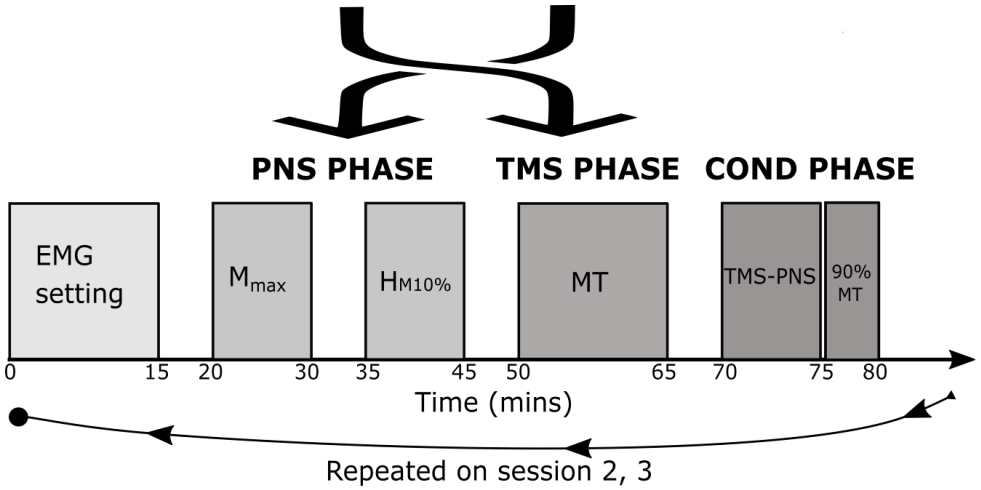

Figure 4.2. Time course of each experimental session. After EMG sensor placement, the recording part started with either TMS or PNS in a randomized order, always ending with the recording of TMS-conditioned monosynaptic reflexes and 10 traces of subthreshold TMS alone (90% MT).

PNS alone

The electrical stimulation started at low intensity (1.0 mA) and was then increased in steps of 0.2 mA until a monosynaptic reflex, the H-reflex, was discernible from the EMG recordings (Palmieri et al., 2004) (Figure 7.3 B). To estimate the M_{max}, the intensity of the stimulator output was incremented in steps of 0.3 mA starting from the intensity at which a monosynaptic reflex could first be evoked until the peak-to peak amplitude of the M wave reached its plateau. Ten traces were recorded at the intensity of stimulation at which the M wave was maximal. The second parameter was recorded by setting the intensity of median nerve stimulation to produce H-reflex amplitudes equivalent to 10% of M_{max} ($H_{M10\%}$) (Palmieri et al., 2004). Again, ten traces were recorded at a frequency of 0.2 Hz.

TMS alone

In the TMS phase, for each participant and in each session, an individual resting motor threshold was estimated. The resting motor threshold (MT) is defined as the lowest TMS intensity, given as a percentage of the MSO, which elicits MEPs with peak-to-peak amplitudes of >50 μV in at least 5 out of 10 traces. (Rossini et al., 1994). The ISI between two pulses was set at 5 seconds.

TMS – PNS conditioning tests

The conditioning TMS pulse was given at 90% MT intensity. The intensity of the electrical stimulation was the same used during the PNS phase to elicit H-reflexes at 10% of M_{max} ($H_{M10\%}$). In order to assess any changes of excitability occurring after the PNS and TMS phases, the protocol started with the acquisition of 8 unconditioned H-reflex traces. PNS stimulation intensity was adjusted if the amplitude evoked did not correspond to $H_{M10\%}$. The mean of the TMS-conditioned amplitudes at each ISI was normalised to control unconditioned H-reflexes and facilitation\inhibition quantified as a percentage of the baseline H-reflex (Palmieri et al., 2004). TMS and PNS were paired at ISIs ranging from -7 ms (PNS first) to +7 ms (TMS first) in steps of 2 ms, with an additional interval of 0 ms employed (Figure 4.3 *A*). Eight consecutive EMG traces were recorded for each ISI. At the end of each conditioning–test phase, ten traces were recorded while only subthreshold TMS (90% MT) pulses were delivered to check whether these produced any discernible MEPs in the FCR muscle.

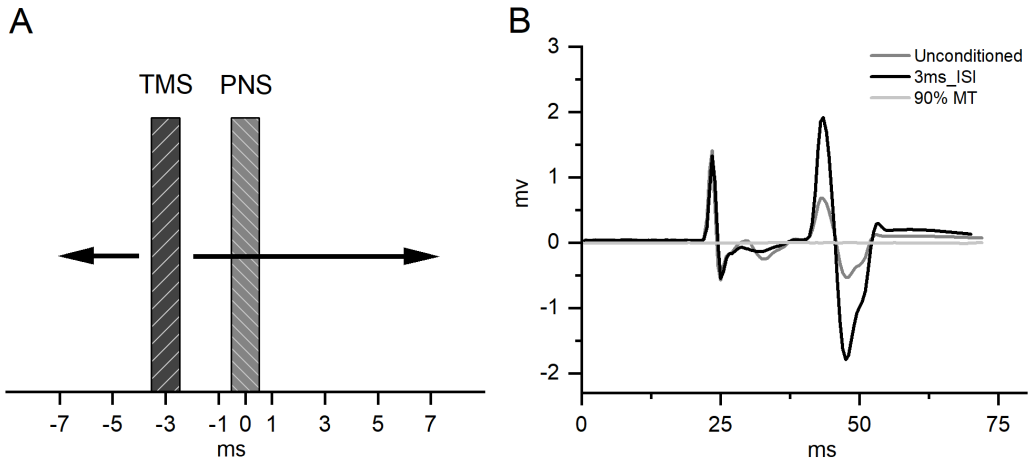

Figure 4.3. TMS-conditioned monosynaptic reflex protocol. *(A)* TMS was delivered at ISIs ranging from -7 to +7 ms (-3 ms in the example). A negative ISI indicates that the conditioning stimulus (TMS) was applied after the test stimulus (PNS). (*B)* Facilitation occurring at 3ms_ISI (TMS first) compared to the Unconditioned H-reflex in a representative participant (8 traces averaged). TMS alone (90% MT) did not produce any MEP.

4.2.5. Data reduction

The mean (peak-to-peak) amplitude of the 10 recordings (for M_{max}, $H_{M10\%}$ and 90% MT) or the 8 recordings (for the baseline unconditioned values and each ISI of the

COND_H phase) was analysed for each of the session. For the analysis of maximum motor response, the peak to peak amplitude was calculated for a time window between 1 and 10 ms after the stimulus artefact. For the monosynaptic reflex and for MEPs, the peak to peak amplitude was calculated for a time window between 10 and 50 ms after the stimulus artefact.

4.2.6. Data analysis

Statistical analyses were performed using SPSS (Version 22.0) software with an a priori significance level of <0.05. A two-way repeated-measures ANOVA with factors ISI (H, 1 ms, 3 ms, 5 ms, 7 ms, 0 ms, -1 ms, -3 ms- -5 ms, -7 ms) and SESSION (1, 2, 3) was conducted. Two of the thirty cells in the analysis design (3 sessions × 10 ISI) satisfied the conventional criterion ($p < 0.05$) that indicates deviations from normality. Therefore, the mean (peak-to-peak) of the natural logarithm transformed amplitude values obtained for each ISI in each session were calculated. Whenever the results of the Mauchly's test showed a violation of the sphericity assumption, Greenhouse-Geisser-corrected values were reported. Differences between conditioned and unconditioned reflexes obtained during the three sessions were assessed using post-hoc tests, and results from multiple comparisons were corrected with the Bonferroni procedure.

The ratio between conditioned and unconditioned H-reflex amplitude values obtained at each ISI was calculated and then used to estimate ICCs. The ICC ranges from 0 to 1 with 1 indicating perfect similarity (Koo and Li, 2016). A 2-ways mixed effects model was used to calculate ICCs following the equation given by Koo and Li (2016). Reliability analyses were performed for the following parameters: M_{max}, $H_{M10\%.}$, 90% MT, 1ms_ISI, 3ms_ISI, 5ms_ISI, 7ms_ISI, 0ms_ISI, -1ms_ISI, -3ms_ISI, -5ms_ISI, -7ms_ISI. ICCs values were interpreted as follow: 0.81 to 1, excellent; 0.61 to 0.80, good; 0.41 to 0.60, moderate; 0.21 to 0.40, fair; below 0.20, poor (Altman, 1990). In addition, to estimate the typical error across sessions, CVs were calculated for each measure (Hopkins, 2000). The RMS of the background EMG recorded from FCR in the 50 ms preceding stimulus delivery was calculated for each trial to ensure lack of changes in muscle pre-activation. Finally, a Pearson product-moment correlation coefficient was used to assess the relationship between Facilitation (3ms_ISI) and MEP amplitudes collected upon subthreshold TMS (90% MT).

4.3. Results

4.3.1. TMS-conditioned monosynaptic reflexes

A reliable H-reflex could be obtained at rest in 85% (11/13) of the participants. Mean values, SDs, range of values and baseline EMG of some of the parameters recorded are reported in Table 4.1. Results from the repeated-measures ANOVA revealed a significant effect of ISI ($F_{2.3, 23.4} = 12.81$, $P < 0.001$, $\eta^2 = 0.56$, achieved power 0.85). TMS significantly increased the size of the H-reflex when delivered at a range of ISIs from PNS, including: 1 ms ($P = 0.01$), 3 ms ($P = 0.014$), 5 ms ($P = 0.006$), 7 ms ($P = 0.03$) and 0 ms ($P = 0.012$) (Figure 4.4). Despite a significant effect of SESSION ($F_{1.5, 15} = 7.48$, $P = 0.009$, $\eta^2 = 0.43$), the ANOVA did not show any significant interaction effect between ISIs and sessions ($F_{5.8, 58.3.} = 0.98$, $P = 0.45$, $\eta^2 = 0.09$). Mean amplitude values for each session are presented in Figure 4.5.

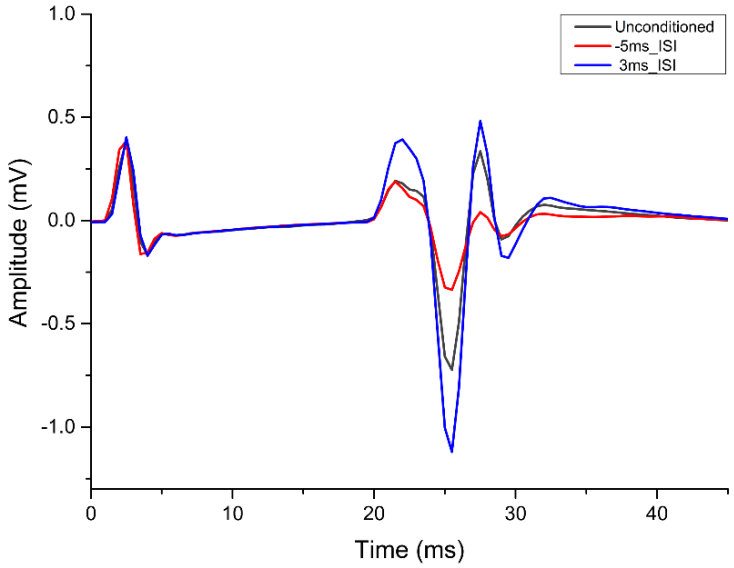

Figure 4.4. Effects of TMS on the monosynaptic reflex. Facilitatory (3ms_ISI) and Inhibitory (-5ms_ISI) effects of TMS on the monosynaptic reflex recorded from the FCR muscle in a representative participant. Each trace represents the mean of 8 sweeps.

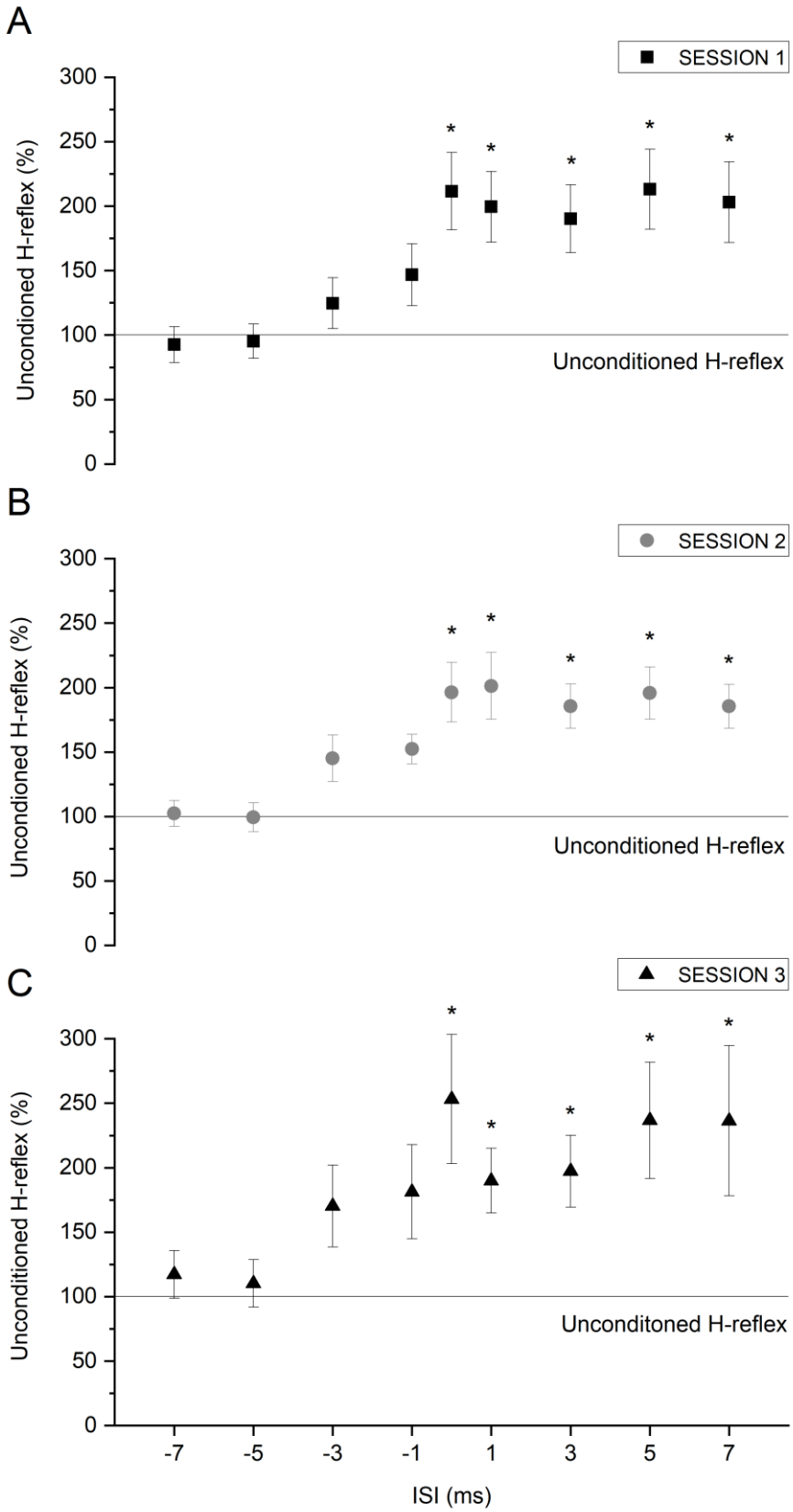

Figure 4.5. Mean (n = 11) ± SE of the conditioned H-reflexes values at different ISIs for SESSION 1 *(A)*, SESSION 2 *(B)* and SESSION 3 *(C)*. The horizontal line corresponds to the Unconditioned H-reflex value. Asterisks denote a significant difference from the Unconditioned mean value.

4.3.2. Reliability analysis

MT values, indicated as %MSO, were 61.9 (4.0) in the first session, 62.7 (4.6) in the second session and 61.4 (4.3) in the third session. Results from the ICCs analysis are reported in Table 4.2. Excellent between-session reliability was observed for $M_{max,}$ (ICC = 0.98, F = 274.95, *P* <0.001), $H_{M10\%}$ (ICC = 0.95, F = 60.01, *P* <0.001) and 3ms_ISI (ICC = 0.83, F = 15.37, *P* <0.001). Moderate to good (range 0.41 to 0.65) reliability was found for most of the other parameters except for -1ms_ISI (ICC = 0.37, F = 2.79, *P* = 0.02), showing fair reliability. In order to better visualize the spread of the recorded data over the 3 sessions in each participant, the mean amplitude values obtained at 3ms_ISI (Facilitation), 0ms_ISI and -5ms_ISI (Inhibition) are plotted next to each other (Figure 4.6). Figure 4.7 shows the raw traces collected on the first session when stimulating the median nerve alone (M_{max} on *A* and $H_{M10\%}$ on *B*) and 3 ms after the motor cortex (3ms_ISI on *C*). Figure 4.8 shows the mean traces collected on each session when stimulating the median nerve alone (M_{max} on *A* and $H_{M10\%}$ on *B*) and 3 ms after the motor cortex (3ms_ISI on *C*).

Table 4.1. Mean values, SD, range of values and pre-stimulus EMG (Base-EMG) obtained in each session for parameters M_{max}, $H_{M10\%}$ and 90% MT. All values are expressed in *mV* except for Base-EMG (μV).

	Parameter	Mean (SD)	Range	Base-EMG (SD)
SESSION 1	$M_{max,}$	4.66 (2.61)	1.92 - 9.13	7.34 (1.22)
	$H_{M10\%}$	0.47 (0.26)	0.05 – 0.93	4.13 (1.33)
	90% MT	0.024 (0.03)	0.004 – 0.099	3.12 (2.28)
SESSION 2	$M_{max,}$	4.95 (2.62)	2.17 – 9.36	7.36 (0.89)
	$H_{M10\%}$	0.49 (0.25)	0.05 – 0.89	4.46 (2.31)
	90% MT	0.020 (0.01)	0.006 – 0.031	3.16 (2.20)
SESSION 3	$M_{max,}$	4.77 (2.61)	2.12 – 9.04	7.42 (0.96)
	$H_{M10\%}$	0.48 (0.28)	0.06 – 1.02	4.40 (1.81)
	90% MT	0.019 (0.01)	0.006 – 0.047	3.40 (1.70)

Table 4.2. Intraclass correlation coefficient values (ICCs) and CVs of all the parameters recorded.

Parameter	ICC	*P*	*CV (%)*	*N*
$M_{max,}$	0.99	<0.001	6.04	11
$H_{M10\%}$	0.95	<0.001	14.77	11
1ms_ISI	0.51	0.004	10.29	11
3ms_ISI	0.83	<0.001	16.79	11
5ms_ISI	0.57	0.001	15.24	11
7ms_ISI	0.43	0.012	15.16	11
0ms_ISI	0.45	0.009	13.45	11
-1ms_ISI	0.37	0.024	20.76	11
-3ms_ISI	0.41	0.015	29.58	11
-5ms_ISI	0.56	0.002	21.86	11
-7ms_ISI	0.66	<0.001	23.82	11
90% MT	0.43	0.013	36.95	11

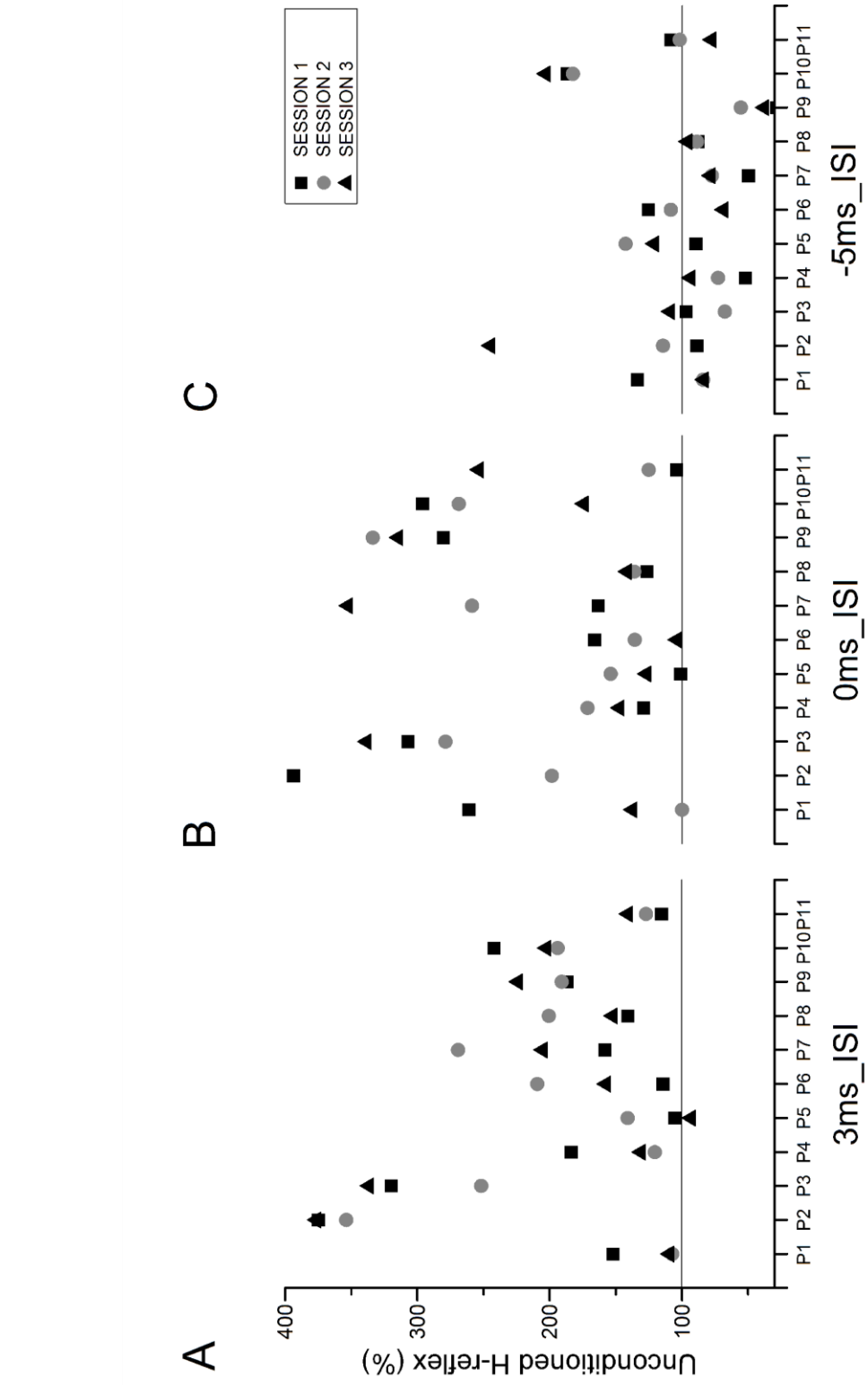

Figure 4.6. Reliability of the conditioned H-reflex. Mean amplitude values of the conditioned H-reflex at 3ms_ISI *(A)*, 0ms_ISI *(B)* and -5ms_ISI *(C)* for each participant (n = 11) and in each session.

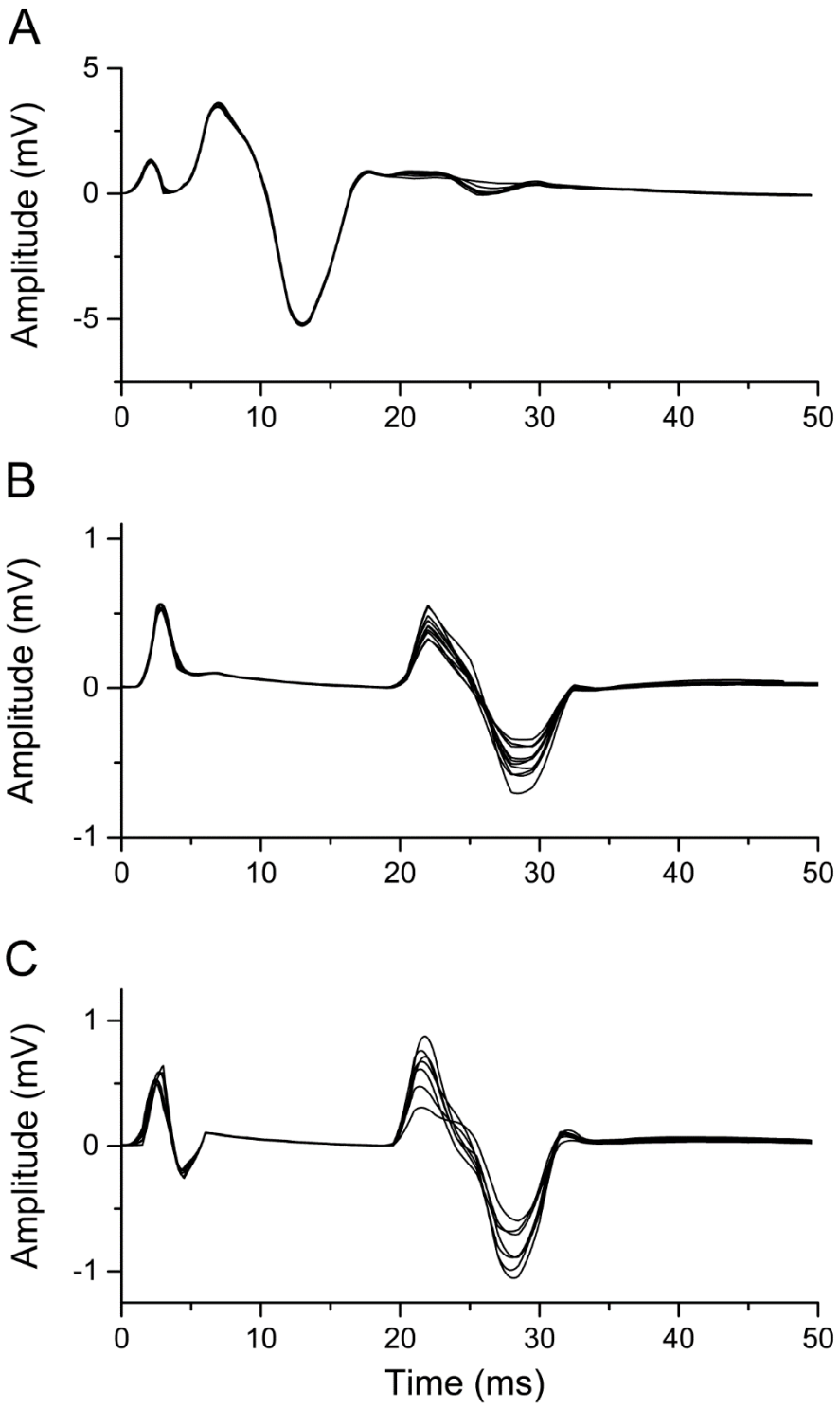

Figure 4.7. Intrasession stability of the recordings in a representative participant. *(A)* Stability of the M_{max} over 10 consecutive sweeps. *(B)* Stability of the unconditioned H-reflex over 10 consecutive sweeps. *(C)* Stability of the TMS-conditioned H-reflex (3ms_ISI) over 10 consecutive sweeps. Notice the scale difference between *A* and *B-C*. Stimulations were delivered at 0.2 Hz.

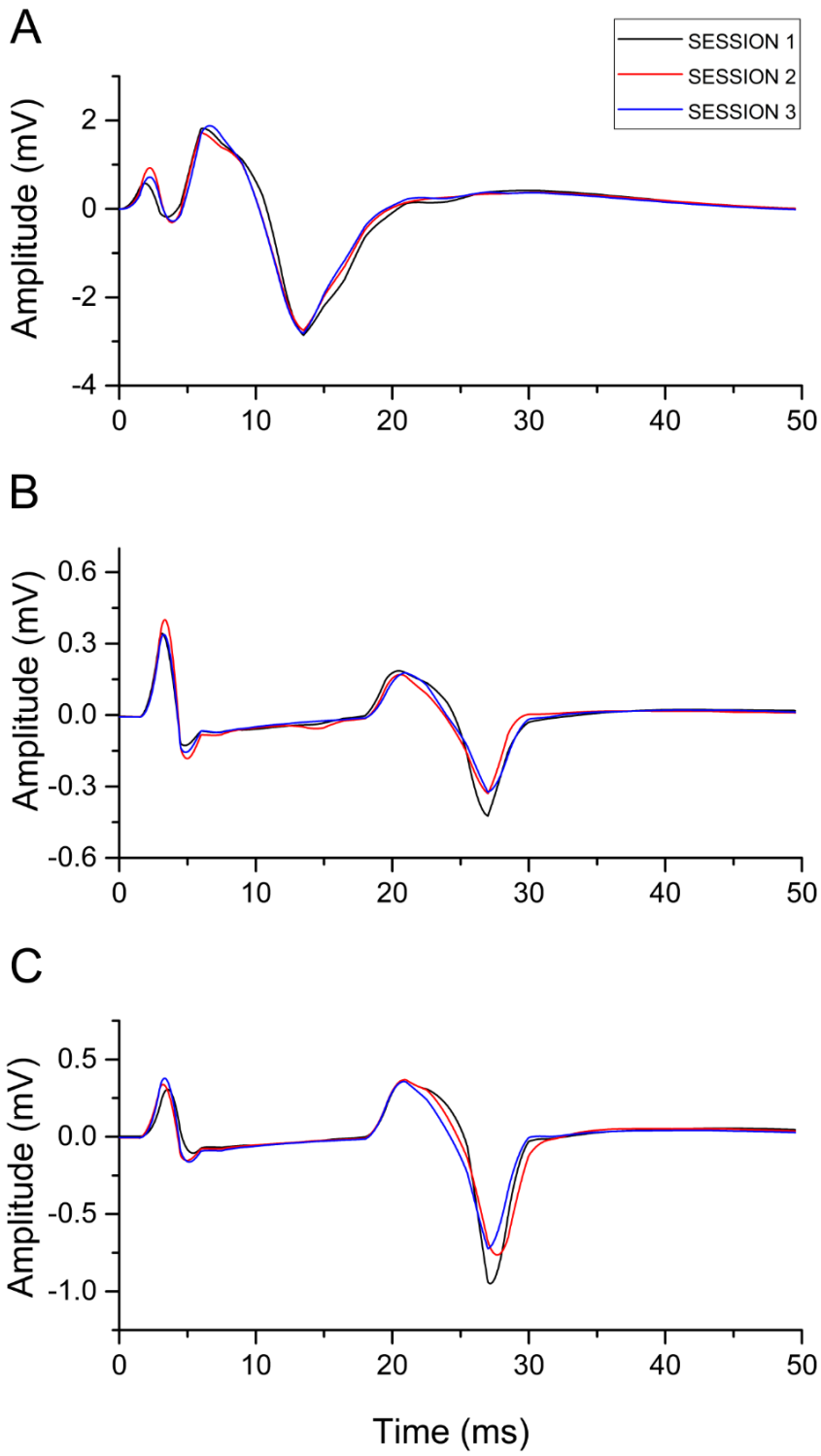

Figure 4.8. Intersession reliability of the recordings in a representative participant. *(A)* Stability of the M_{max} over 3 sessions. *(B)* Stability of the unconditioned H-reflex over 3 sessions. *(C)* Stability of the TMS-conditioned H-reflex (3ms_ISI) over 3 sessions. Notice the scale difference between *A*, *B* and *C*. Each trace represents the mean of 10 sweeps. Stimulations were delivered at 0.2 Hz.

Since subthreshold cortical stimulation induced a visible MEP in some participants and sessions (Figure 4.9 *B*), it was tested whether higher responses to 90% MT were paralleled by higher facilitation values. A strong correlation (r = 0.56, $P < 0.001$) was found between the two parameters (Figure 4.10).

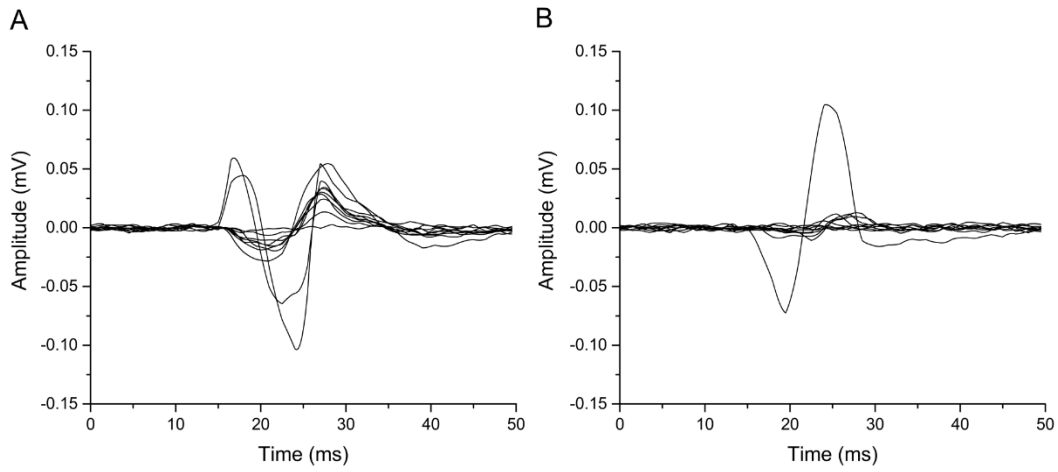

Figure 4.9. FCR motor evoked-potentials. EMG responses recorded from the FCR muscle upon stimulation at *(A)* 100% MT and *(B)* 90% MT. Ten sweeps are superimposed in each picture. Recordings in *B* were obtained after delivery of the conditioning protocol.

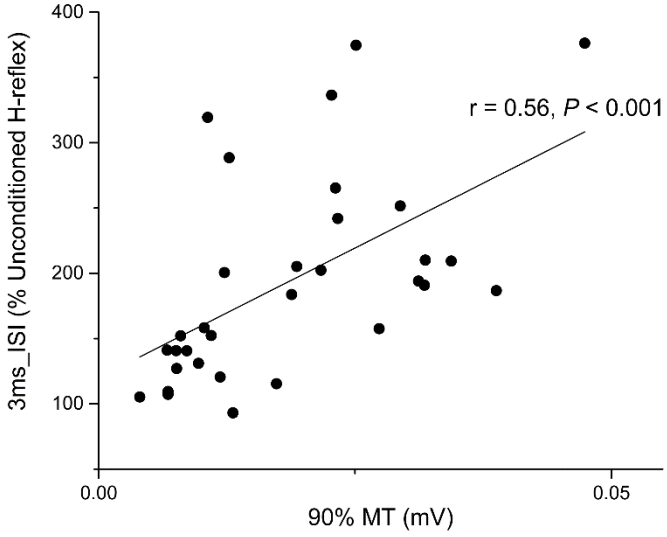

Figure 4.10. Correlation between subthreshold TMS and the amount of TMS-induced facilitation. Scatterplot showing the correlation between 90% MT and 3ms_ISI amplitude values recorded on each session ($n = 33$).

4.4. Discussion

The main aim of the current project was to determine the intersession reliability of a series of neurophysiological parameters recorded from the FCR muscle in the forearm, which may be useful to characterise changes in the excitability of corticospinal circuits occurring after lesions or during motor training. The finding (Nielsen et al., 1993b, Mazzocchio et al., 1994) that TMS increases the amplitude of the monosynaptic reflex evoked from FCR when given at a range of ISIs from the peripheral pulse (Figure 4.5) was replicated. Furthermore, it was shown that the intersession reliability of this phenomenon varied when using different ISIs, with the highest degree of reliability obtained when TMS preceded PNS by 3 ms (3 ms_ISI, ICC = 0.83) (Table 4.2). Finally, the results demonstrated that subthreshold TMS is not a reliable measure and can evoke descending activity, confounding the results obtained upon conditioning the monosynaptic reflex. It is not sufficient to ensure that the two stimulation modalities elicit the desired activity at the beginning of the session, but their outcomes need to be continuously monitored and if necessary adjusted to ensure that the variability does not depend on baseline changes in excitability of the motor system.

4.4.1. TMS-conditioned monosynaptic reflexes

Cortical stimulation significantly modulated the amplitude of the monosynaptic reflex when delivered simultaneously with (0 ms) and immediately before (from 1 to 7 ms) peripheral stimulation. This corresponds to earlier reports (Nielsen et al., 1993b) showing facilitation at a comparable range of ISIs. In particular, Mazzocchio and colleagues (1994) employed a similar experimental setting as described in the current study (use of a subthreshold TMS pulse and parameters recorded at rest) and reported facilitation of the evoked response by up to 130% of the unconditioned values. A potential explanation for the higher degree of facilitation observed in this investigation is the use of different stimulation parameters. The choice of stimulating the median nerve at an intensity which elicits a relatively small reflex (10% of M_{max}) in the muscle of interest was taken to preferentially activate low-threshold Ia fibres and minimize the potential collision with the motor wave (Pierrot-Deseilligny and Mazevet, 2000). Moreover, the employment of a coil orientation (posterolateral to anterio-medial) inducing an electrical field perpendicular to the central sulcus can explain the larger effects observed (Pierrot-Deseilligny and Mazevet, 2000). More

recent studies (Niemann et al., 2017) reported mean facilitation values as high as 200% with this coil orientation.

Multiple hypotheses have been advanced on the nature of the facilitation observed when the timing between peripheral and cortical stimulation is manipulated. Based on the difference in latencies between the recorded MEP and H-reflexes, Cowan and his colleagues (1986) first argued that the facilitation observed when peripheral stimulation preceded electrical stimulation of the scalp (e.g. -3 ms ISI) occurred because of a synchronous activation of spinal motoneurons brought upon by the two forms of stimulation. This facilitation is likely to be mediated by large-diameter corticospinal axons, which synapse directly on spinal motoneurons. When the cortical electrical stimulation was replaced by a magnetic stimulus, the onset of the early facilitation was reduced to -1/-2 ms ISI, reflecting the difference in MEPs onset timing observed between the two stimulation modalities (Mazzocchio et al., 1994). In another instance (Hannah et al., 2018), an ISI of 0 ms was shown to produce coincident arrival of the volleys at the spinal motoneurons level. An examination of the latencies of MEPs and unconditioned reflexes in the current study revealed how the onset response to magnetic stimulation was approximately 0-1 ms longer than the onset of the H-reflex.

Several mechanisms may be responsible for the long-latency facilitation reported in this study. Firstly, conditioning effects occurring at 3_ms ISI could be due to the late arrival of slow-conducting corticospinal tract neurons at the spinal motoneuron pools (Cowan et al., 1986). Different neural populations are potentially involved in the later stages of facilitation (5_ms – 7_ms ISI). Spinal motoneurons receive monosynaptic and polysynaptic inputs from other pathways like the reticulospinal, rubrospinal and vestibulospinal tracts, all of which may alter the excitability of spinal circuits and influence the amplitude of the monosynaptic reflex (Eccles and Lundberg, 1959). The spinal segmental interneuronal network too can modulate the conditioning effect. Interneurons mediating the presynaptic inhibition of Ia afferents are activated by cortical stimulation (Meunier and Pierrot-Deseilligny, 1998). Finally, it has recently been demonstrated (Niemann et al., 2017) that the time course of TMS-conditioning of the FCR H-reflex closely matches the late descending volleys (I waves) induced by TMS.

4.4.2. Reliability analysis

Any investigation of the reliability of TMS-conditioning effects on the monosynaptic reflex must start with the examination of its constituent parameters. Therefore, it is important to control for any intersession fluctuations in muscular, spinal and cortical excitability. The maximal amplitude of the motor wave (M_{max}) evoked in response to electrical stimulation of a peripheral nerve is elicited by recruitment of all motor axons (Palmieri et al., 2004). Monitoring changes in M_{max} is important to ensure that there were no changes in participants' position, location of the stimulating and recording electrodes or any muscular effects which may influence the recordings. This parameter showed excellent intersession reliability, in line with previous studies (Christie et al., 2005).

Difficulties in reporting a monosynaptic reflex in the FCR muscle at rest have been previously reported (Miller et al., 1995). Indeed, 2 participants were excluded from the total pool of 13 because a reliable reflex could not be evoked or was cancelled due to collision with the motor wave. A widely used method to increase excitability and facilitate the occurrence of a reflex is to ask participants to contract the muscle slightly while receiving stimulation (Pierrot-Deseilligny and Mazevet, 2000), but since the effects of TMS on the monosynaptic reflex differ during flexion (Nielsen et al., 1993b) it was preferred to simply exclude these participants. The intensity of the peripheral stimulus was chosen to evoke a reflex response in the ascending portion (10% of M_{max}) of the recruitment curve, at which the influence of Ib afferents is lower (Pierrot-Deseilligny and Mazevet, 2000). The mean amplitude of the $H_{M10\%}$ ranged widely across participants, reflecting the differences in M_{max} amplitudes. However, this parameter was highly stable across sessions (ICC = 0.95). At the beginning of the conditioning phase, 8 unconditioned H-reflexes elicited with the same stimulation intensity used during the $H_{M10\%}$ recordings were recorded to ensure that the stimulating electrode did not change position over the session. However, amplitude values did not always match with the previously recorded ones, indicating a potential shift in excitability between the two parts of the session. Nonetheless, unconditioned values were always elicited in the up-sloping part of the recruitment curve.

The H-reflex was conditioned with TMS pulses delivered at different ISIs relative to the peripheral stimulus (PNS). The ICC was highest (0.83) when TMS preceded PNS by 3ms. At this interval, the corticospinal volley may have reached spinal motoneurons slightly before the arrival of the afferent volley. This correlation

coefficient is higher than the one observed when TMS precedes the electrical stimulation of the soleus muscles (Gray et al., 2017). However, the response latencies of MEPs and H-reflexes evoked in the soleus muscle differ from the ones obtained in FCR and a comparison between the reported ICCs is not straightforward. Importantly, in the current study a range of intervals were tested while Gray and his colleagues only investigated long-latency effects (10 ms ISI).

Reliability was lower for all other conditioning ISIs recorded. A possible explanation for this decrease may be the polysynaptic nature of the conditioned responses. With longer ISIs, the chances of indirect descending pathways to influence the reflex increase and so does the variability of responses (Ribrault et al., 2011). Another factor which could contribute to the variability of facilitation is a change in the activation state of the participant (Nielsen et al., 1993b). This possibility was controlled for by monitoring any variation in the EMGs recorded from FCR and FCU muscles occurring immediately prior to any stimulation. Results from the repeated-measures ANOVA showed a significant main effect of SESSION on the amount of facilitation observed. A detailed examination of the mean facilitation percentages measured for each ISIs (Figure 4.5) revealed that the recordings from the third session tended to be higher than that from the first two sessions, especially at longer ISIs (5 to 7 ms) and 0 ms. This phenomenon may reflect a general excitability increases in the last session but could also represent long-lasting plasticity in the circuit, since the after-effects of pairing peripheral and cortical stimulation can be observed for days after the stimulation (McKay et al., 2002).

The importance of studies using TMS to assess motor excitability is hindered by the high variability of results and by the lack of reproducibility (Héroux et al., 2015). A series of issues need to be considered when measuring the outcomes of cortical stimulation (briefly reviewed by Burke and Pierrot-Deseilligny, 2010). The possibility that confounding effects not directly related to the stimulation protocol such as participants' attention and muscle pre-activation may affect the responses collected cannot be excluded. Nonetheless, the finding that pre-stimulus EMG did not change across sessions argues against a change in muscle pre-activation. In addition, the cortical pulse increased only the size of the H-reflexes with no effects on the M response, as would be expected if the changes occurred because of differences in muscle pre-activation or attention (Knikou, 2008).

A possible limitation of the study is the relatively small sample of participants. This is, however, in line with previous studies assessing intersession reliability of corticospinal parameters (Hoch and Krause, 2009). Moreover, reliability studies are often limited to measure the stability of parameters over 2 consecutive sessions. As clinical practices and rehabilitation protocols may require a higher number of sessions to be implemented (Gray et al., 2017), this study included a third session. The protocol implemented was chosen to be easily reproducible in a clinical setting and by considering time constraints which limit the usefulness of longer recording sessions. It was necessary to compromise between testing the effects at a wider range of conditioning ISIs and recording as many traces as possible for a single parameter. This could potentially explain the lower intersession reliability observed for parameters with a cortical stimulation component, given the big inter-pulse variability observed when delivering TMS (Chang et al., 2016).

4.4.3. Correlation analysis

The analysis of the correlation between the MEPs evoked by subthreshold TMS and the amount of facilitation obtained from each session yielded some interesting outcomes. It is indeed well documented (Niemann et al., 2017) that the TMS-conditioned values were higher when the intensity of conditioning stimulation changed from subthreshold to higher values. In addition, recent findings point to the evidence that subthreshold TMS is capable of inducing multiple descending volleys (Niemann et al., 2016). The huge inter-subject variability frequently reported when conditioning the H-reflex may arise from the difference in descending corticospinal activity produced by the conditioning pulse. It is not possible to conclude that the same intensity of stimulation (90% MT) will induce identical activation in all participants without building recruitment curves of the input-output relationship between intensity and MEP size (Burke and Pierrot-Deseilligny, 2010). This raises the question of whether the use of TMS thresholding protocols based on fixed response amplitudes (e.g. >50 μV) is the most appropriate in such instances when small differences in MEP amplitude might influence the outcome of the conditioning event. This is in sharp contrast with animal studies in which motor threshold is defined as the lowest intensity of stimulation generating a motor response, challenging the possibility of comparing animals' and humans' findings (Chakrabarty and Martin, 2011). Whenever subthreshold intensities are used, it is recommended to record at

least 20 traces showing that the chosen intensity never elicited a discernible MEP in the recorded muscle before and after conditioning. MT values are highly reliable over sessions (Dissanayaka et al., 2018), but the produced MEPs may vary across participants, leading to disparate responses to subthreshold stimuli among people. A better alternative would be to normalize the produced MEPs values to M_{max} intensity, so as to stimulate the same percentage of corticospinal axons between participants (Burke and Pierrot-Deseilligny, 2010). In addition, recruitment curves need to be built for each participant starting at intensities below the threshold and the values normalised to M_{max} to standardise the TMS intensities to be used.

4.5. Conclusions

Taken together, these findings indicate that the conditioning effect of TMS on the monosynaptic reflex evoked in FCR muscle is a reliable phenomenon. Its intersession reliability is higher when TMS preceded PNS by 3 ms and decreased at all other ISIs. TMS and PNS can be used in combination to assess changes in the excitability of the corticospinal tract occurring after lesions or rehabilitation. The relevance of the methods for studying the motor system is limited by the variability of the results obtained. Addressing the methodological issues of these techniques can reduce the variability and permit a better understanding of the neurophysiological mechanisms underlying motor control and motor learning. This study addressed the first aim of the thesis and demonstrated the reliability of the TMS-conditioning of the H-reflex method in FCR. Having established this, the following study (described in Chapter 5) focused on the validity of the MEP induced by TMS as a measure of corticospinal excitability.

Chapter 5 - The effects of sound and stimulus expectation on Transcranial Magnetic Stimulation-elicited motor evoked potentials

5.1. Introduction

Transcranial magnetic stimulation is a non-invasive technique that can be used to study changes in the excitability of the motor system in both experimental (Pascual-Leone et al., 1994a) and clinical settings (Hamzei et al., 2006). A single TMS pulse, when applied to the M1, can elicit a MEP in peripheral muscles induced by descending activity along the corticospinal tract (Hallett, 2007). The amplitude of the MEP is suggested to reflect excitability and integrity of local neural networks and their corticospinal projections (Merton and Morton, 1980). However, part of the descending activity constituting the MEP is conveyed through indirect (e.g. disynaptic and polysynaptic) cortical and subcortical circuits and is thereby impossible to study the monosynaptic corticospinal component in isolation via EMG recording (Burke and Pierrot-Deseilligny, 2010). Stimulating the motor cortex with TMS can induce unintended effects along with the site-specific, intended physiological effects, limiting the validity of the results in terms of corticospinal excitability.

TMS is often used to measure changes in the excitability of the corticospinal tract following experimental manipulation (Burke and Pierrot-Deseilligny, 2010). However, neuroimaging data show that magnetic stimulation, even when given at small intensities, induces bilateral activation in the auditory cortex (Bestmann et al., 2004). Fisher and his colleagues recorded responses from ponto-medullary reticular formation (PMRF) neurons in monkeys after TMS delivery (Fisher et al., 2012). They found that M1 stimulation produced responses in these neurons which are independent from the descending activity induced by the magnetic field, since the same neurons could be similarly activated by a click stimulus (Fisher et al., 2012). This constitutes a serious limitation for studies in which MEPs induced by TMS are used to assess activity in the corticospinal tract. Given this evidence, it is surprising that no studies to date have investigated the effects of discharging noise on the recorded MEPs.

The discharging of a TMS coil is accompanied by an abrupt clicking noise which increases with stimulation intensity and can reach 120 dB (Nikouline et al., 1999). The auditory response induced by stimulus discharging might disrupt or facilitate behavioural performance on visual discrimination tasks (Terao et al., 1997, Marzi et al., 1998). For example, recent work (Duecker and Sack, 2013) showed that when

delivered 250-150 ms before the appearance of a visual stimulus the sound produced by sham stimulation shortened reaction times to target detection. This suggests that the TMS pulse acts as a warning signal, possibly altering the attentional state of the participant and the resulting behaviour. Behaviourally speaking, the effects of sham TMS resemble the ones observed when startling acoustic stimuli are presented in the context of reaction-time tasks, the StartReact effect (Valls-Solé et al., 1995). Shortening of reaction times were observed during ballistic wrist flexions (Valls-Solé et al., 1999) and extension (Stevenson et al., 2014) and in both healthy participants and clinical populations (Valldeoriola et al., 1998). In the healthy population, a loud acoustic stimulus shortened reaction times in power and precision grips and in finger abduction tasks (Baker and Perez, 2017). However, in the SCI population the effect was preserved only in the power grip task. The authors concluded that both reticulospinal and corticospinal pathways contribute to the StartReact effect, but the loss of corticospinal function following SCI disrupted the effect in the tasks requiring fine finger manipulation (Baker and Perez, 2017). Despite this evidence, the possibility that the sound produced by TMS stimulation activates the corticospinal and reticulospinal tracts and therefore influences MEPs recorded from the muscle of interest was never assessed.

Little is known about how the interval between consecutive pulses (IPI) affects the induced activity (Vaseghi et al., 2015). It has recently been shown that the preceding stimulus may influence the size of the following one when given 3 seconds before it (Schmidt et al., 2009). Researchers compared the effect of IPI manipulation on single pulse stimulation (Vaseghi et al., 2015). MEPs given at 10 seconds IPI were significantly larger than the ones given at 4 seconds IPI. The authors explained this result in light of the drop in haemoglobin levels, which in turn reduces neural and muscular activation, observed following TMS and lasting up to 8-10 seconds (Thomson et al., 2012). While haemoglobin drop likely plays a role in this phenomenon, other studies suggest that stimulus anticipation affects the responses obtained when stimulating M1 (Grandjean et al., 2019). A loud, unexpected noise may facilitate or suppress EMG responses to TMS if given at an appropriate timing from it. The suppression is not observed when the interval between TMS pulses falls below 10 seconds, a phenomenon attributed to habituation to the auditory stimuli (Furubayashi et al., 2000). Whether habituation to the sound produced by the discharging coil influences the recorded MEPs remains an open question.

Given the above, the present study had two clear aims: (1) to investigate the effect of the attenuation or masking of the sound made by the TMS system at discharge on the amplitude of MEPs; (2) to determine whether it is possible to prevent stimulus expectation by increasing and "jittering" the IPI, and whether this phenomenon could be reversed by explicitly making the participants aware of the timing when they would receive the next stimulus. With respect to aim 1, it was expected to observe significantly lower MEPs amplitude values in the conditions reducing or masking the discharging sound compared to the condition where participants received stimulation without sound reduction/masking. The second hypothesis (aim 2) was that MEPs obtained when using long IPIs would be higher compared to a condition in which the IPI is shorter, but that this effect could be reversed when participants were aware of the time of stimulation, which would indicate that stimulus expectation reduced MEP amplitudes. In addition, the variability, measured by calculating coefficients of variation, of MEPs recorded in each condition and for each intensity of stimulation was measured. The reason for measuring it was that, if at least part of the intertrial variability observed in single trial MEPs amplitudes is due to confounding factors such as auditory activation and habituation, CVs should decrease in TMS protocols controlling for such factors.

5.2. Methods

5.2.1. Participants

A total of 23 healthy participants (M ± SD = 22.6 ± 4.2; females = 10) volunteered for the study. This number was decided after conducting a power calculation indicating 23 as the sample size necessary to detect an effect size of 0.3 f^2 with a power of $1-\beta = 0.80$ at level $\alpha = 0.05$. Inclusion criteria included being right-handed and aged between 18 and 40 years. Participants were excluded from the study if they had familial history of epilepsy or neurological disorders, were under any medication affecting the CNS, or had any contraindications to TMS (Rossi et al., 2009). Since sex hormones levels do not affect responses to TMS (Ansdell et al., 2019), female participants were recruited without controlling for the menstrual cycle phase. All participants gave written informed consent and the experimental procedures were approved Faculty of Biological Sciences Ethical Review Committee at the University of Leeds.

5.2.2. Electromyography (EMG) measures

Participants were tested while sitting on a dynamometer chair (Biodex Corp., Shirley, NY), with the right forearm in full pronation, the elbow and the head both fully supported. Electromyography (EMG) activity was recorded from two muscles: right flexor carpi radialis (FCR) and right extensor carpi radialis longus muscle (ECRL) using parallel-bar wireless sensor (3.7 × 2.6 cm) (Trigno, Delsys Inc., Natick, MA, USA). Raw EMG recordings were pre-amplified (gain = 909), recorded with a 20-450 Hz bandwidth and digitized at 2 kHz using data acquisition software (Spike2, Cambridge electronics Design, Cambridge, UK).

5.2.3. Stimulation techniques

Magnetic stimulation was applied to the left motor area M1 by means of a Magstim Rapid stimulator and a flat alpha coil (D70 Alpha Flat Coil, Magstim Company, Whitland, Dyfed, UK) being held by a support stand (Magstim AFC Support Stand, Magstim Company, Whitland, Dyfed, UK). The coil was oriented at ~45°, inducing a posterior-to-anterior current flow across the motor cortex, and moved across the left motor cortex while delivering stimulation in order to locate the optimal coil position to stimulate forearm muscles (Rossini et al., 2015). The position was marked with a non-permanent marker to ensure consistency of recordings over the session. The positions and orientations of the coil were monitored continuously, and if necessary adjusted to align with the scalp markings. During all the interventions, the stimulation was controlled through Spike2 (Cambridge Electronic Design, Cambridge, UK) software. Individual MTs were estimated for each participant as the smallest intensity of stimulation necessary to elicit peak-to-peak motor evoked potential (MEP) amplitudes of 50-100 μV in at least 5 out of 10 trials, following the relative frequency method (Rossini et al., 1994). MT values, expressed as %MSO, were on average 59.5 ± 3.2 across participants. MT values were used to calculate the intensities to be set during the recording phase according to the protocol described in Chapter 3.5 and used to calculate stimulation intensities.

5.2.4. Experimental design

Common to all experimental conditions, MEPs were recorded by delivering TMS at three intensities: 100% of MT; 120% of MT; 140% of MT. A total of 10 traces were recorded at each of the three intensities of stimulation. The order of delivery was

block-randomized across conditions and participants. All participants were unaware of the rationale of the study and the nature of each experimental condition. Neither the experimenter nor the participant could see the amplitude of the elicited MEPs at the time of stimulation. Five minutes of rest were introduced between the end of an experimental condition and the start of the next one. A total of six different stimulation conditions (outlined below) was completed in a randomized order and the respective MEPs recorded for each participant (Figure 5.1).

NORMAL condition

This condition was designed to mimic protocols commonly used to measure the excitability of the corticospinal tract. Participants were asked to relax throughout the stimulation, maintaining their eyes open but without paying attention to any visual cue. The inter-pulse interval (IPI) between successive stimuli varied between 4 and 6 s (20% jitter). A total of 30 (3 intensities × 10 traces) MEPs were collected during this phase. The total duration of the sequence was approximately three minutes.

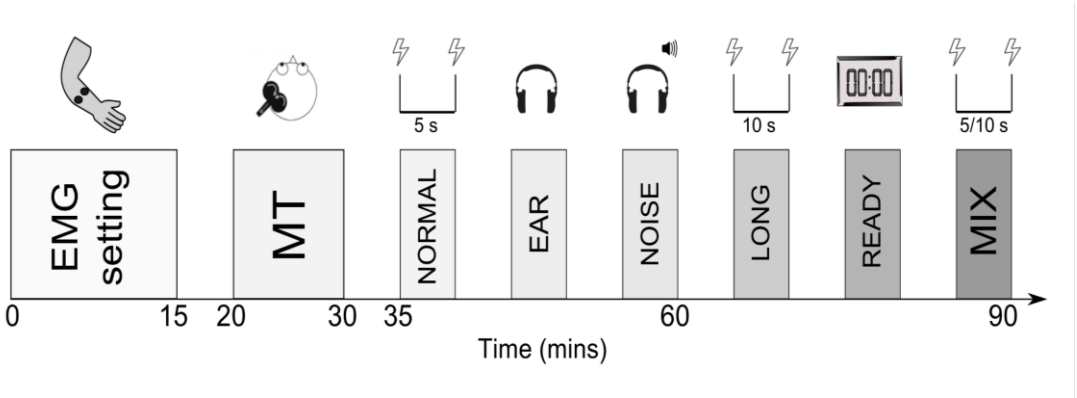

Figure 5.1. Time course of the experimental session. After electrode placement, an individual MT was estimated for each participant. The experimental conditions (see Methods for details) were then delivered in a randomised order, spaced by 5 minutes. Each experimental condition lasted between 3 and 5 minutes and the total duration of each session was approximately 90 minutes.

EAR condition

For this condition, participants were provided with sound-attenuating earmuffs (Peltor Optime, III, 3M, Maplewood, U.S.) to wear throughout the stimulation protocol. Wearing these attenuates the incoming “click” sound by an average 35 dB across all frequencies (single number rating). This condition was implemented to test whether

the amplitude of the noise produced by TMS delivery influenced the EMG response to stimulation of the motor cortex. The IPI was again jittered between 4 and 6 seconds, for a total session length of approximately three minutes (10 traces ×3 stimulation intensities).

NOISE condition

Participants were asked to wear headphones through which white noise (dB = 83) was played while stimulating M1 and recording MEPs. The amplitude was chosen to mask the sound produced by stimulation given at 60% of maximum stimulator output (Dhamne et al., 2014), as confirmed by participants. The position of the headband on the scalp was adjusted such that it didn't interfere with the coil to ensure consistent coil positioning across conditions. Ten traces (IPIs between 4 and 6 seconds) for each of the three stimulation intensities were recorded during this phase, lasting approximately three minutes.

LONG condition

This condition was designed to estimate the effects of increasing the IPI on the recorded MEPs. The IPI between successive stimuli varied between 8 and 12 seconds (20% jitter), to make it hard to anticipate the delivery of the next stimulus. Participants were asked to relax throughout the stimulation, maintaining their eyes open but without paying attention to any visual cue. A total of 30 (3 intensities × 10 traces) MEPs were collected during this phase. The total duration of the sequence was approximately six minutes.

READY condition

The same parameters used for the LONG condition were employed for the READY condition: IPIs varying between 8 and 12 seconds (20% jitter) and a total of 30 (3 intensities × 10 traces) collected responses. However, participants received visual feedback in the form of a stopwatch informing them on the time when they would receive the next stimulus (countdown to 00:00). The countdown to the next stimulus was shown through the TMS stimulator graphical interface. This feature was designed to prevent occurrence of the startling effect that a TMS pulse might induce when delivered unexpectedly.

MIX condition

In order to further assess whether the length of the IPI influences the amplitude of the recorded responses, a condition in which long (8-12 seconds) and short (4-6 seconds) IPSs were intermixed with a blocked randomisation was included. A total of 5 responses for each combination of IPIs and stimulation intensity were recorded (100_short; 100_long; 120_short; 120_long; 140_short; 140_long) for a total duration of approximately five minutes. Only a subset (n = 17) of participants completed this experimental condition. Results obtained from this condition were analysed independently.

5.2.5. Data analysis

Given that TMS amplitude data often reveal skewed distributions and deviations from normality (Nielsen, 1996), the mean (peak-to-peak) of the natural logarithm transformed amplitude values obtained for each intensity and in each condition was calculated. A GLM analysis was run using SPSS software (Version 22.0) with an *a priori* significance level of 0.05. Participant was included as a random factor, with Condition (NORMAL, EAR, NOISE, LONG, READY) and Intensity (100%, 120%, and 140% of MT) included as fixed factors. The normality of the distribution of residuals was assessed using the Kolmogorov-Smirnov test. No violation of normality could be inferred from the results (p = 0.20). The Levene's test of equality of error variances showed no violation (p = 0.69) of the assumption of homogeneity of variance. In addition, in order to ensure lack of changes in baseline excitability between conditions, an ANOVA was run between the RMSs of the background EMG recorded in each condition in the 50 ms pre-stimulus. A separate model was specified to estimate the effects of manipulating the IPIs on MEPs amplitudes with Participant as a random factor and IPI (MIX_LONG, MIX_SHORT) derived from the MIX condition and Intensity (100%, 120%, 140% of MT) as fixed factors. No violation of normality of the distribution of residuals could be inferred from the results (p = 0.20). The Levene's test of equality of error variances showed no violation of the assumption of homogeneity of variance (p = 0.26). For the analysis of inter-trials variability, CVs were calculated for each intensity, condition and participant. A GLM with Participant as a random factor and Condition (NORMAL, EAR, NOISE, LONG, READY) and Intensity (100%, 120% and 140% of MT) as fixed factors was used to examine the

effects of the different conditions and stimulation intensities on the variability of the recorded MEPs. Bonferroni corrections were applied to all pairwise comparisons.

5.3. Results

5.3.1. MEP amplitudes

Three participants could not tolerate the 140% MT stimulation intensity and therefore for these three participants MEP amplitudes elicited at this intensity were not collected (n = 20). The ln-transformed amplitude values were used for the GLM analysis. The average pre-stimulus RMS for each condition was 2.32 μV in the NORMAL condition, 2.32 μV in the EAR condition, 2.75 μV in the NOISE condition, 2.27 μV in the LONG condition and 2.15 μV in the READY condition. Pre-stimulus RMSs were not significantly different across conditions ($F_{4, 115}$ = 0.48, P = 0.75, η^2 = 0.017). Results from the GLM analysis (Figure 5.2) revealed a significant main effect of Intensity ($F_{2, 314}$ = 279.84, P < 0.001, η^2 = 0.641). Bonferroni-corrected post-hoc comparisons (Table 5.1) showed that MEP values increased from 100% MT to 120% MT intensities (P < 0.001) and from 120% MT to 140% MT (P < 0.001) (Table 5.1).

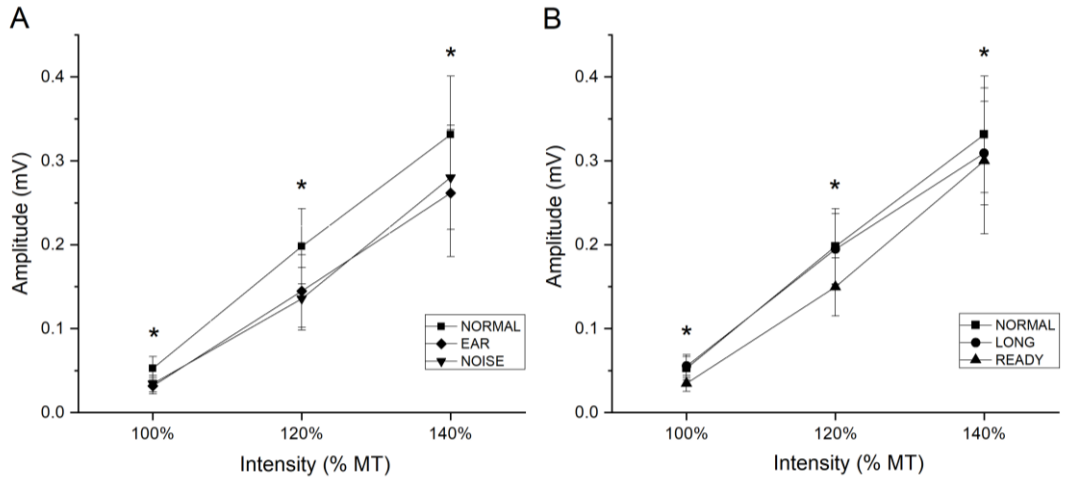

Figure 5.2. Effects of changing stimulation intensity on the MEPs amplitudes for different conditions. *(A)* Comparison between group mean MEPs values obtained with 100%, 120% and 140% MT intensities for the NORMAL, EAR and NOISE conditions and *(B)* for the NORMAL, LONG and READY conditions. The error bars represent the associated 95% confidence intervals. The asterisk denotes a statistically significant difference.

Table 5.1. Results of post hoc multiple comparisons.

Dependent variable	Group 1	Group 2	Mean difference	Sig.
MEP AMPLITUDES	NORMAL	EAR	0.408	0.004
	NORMAL	NOISE	0.356	0.020
	NORMAL	LONG	0.012	1.000
	NORMAL	READY	0.323	0.049
Dependent variable	**Intensity 1**	**Intensity 2**	**Mean difference**	**Sig.**
MEP AMPLITUDES	100% MT	120% MT	-1.430	<0.001
	120% MT	140% MT	-2.047	<0.001

A significant effect of Condition ($F_{4, 314} = 5.60$, $P < 0.001$, $\eta^2 = 0.067$) could be inferred from the GLM. Figure 5.3 depicts the mean MEP traces recorded in a representative participant over multiple conditions and intensities of stimulation. The interaction effect between Intensity and Condition on the amplitude of the MEPs was not significant ($F_{8, 314} = 0.69$, $P = 0.70$, $\eta^2 = 0.017$). Post-hoc comparisons revealed that MEP amplitudes were significantly higher in the NORMAL condition compared to the EAR ($P = 0.004$), NOISE ($P = 0.02$) and READY ($P = 0.049$) conditions, but no significant difference was found between NORMAL and LONG condition ($P = 1$) (Table 5.1 and Figure 5.4).

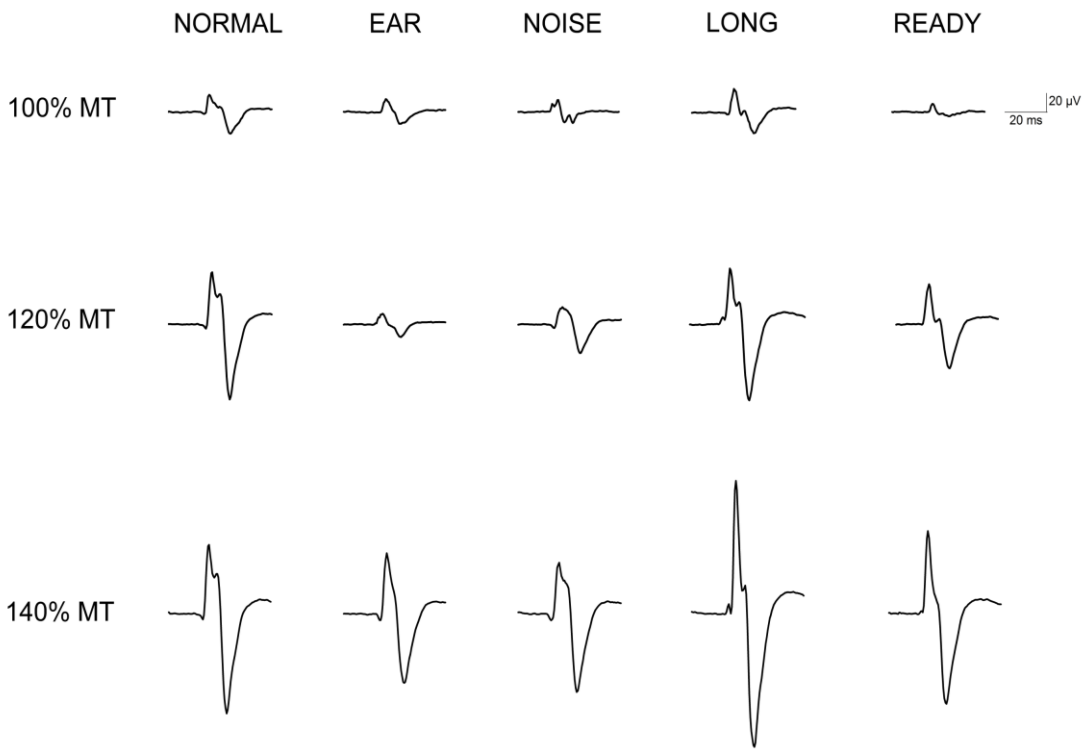

Figure 5.3. Examples of MEPs evoked by TMS under different conditions and intensities of stimulation in the right FCR muscle of a representative participant. Each trace represents the mean of 10 sweeps.

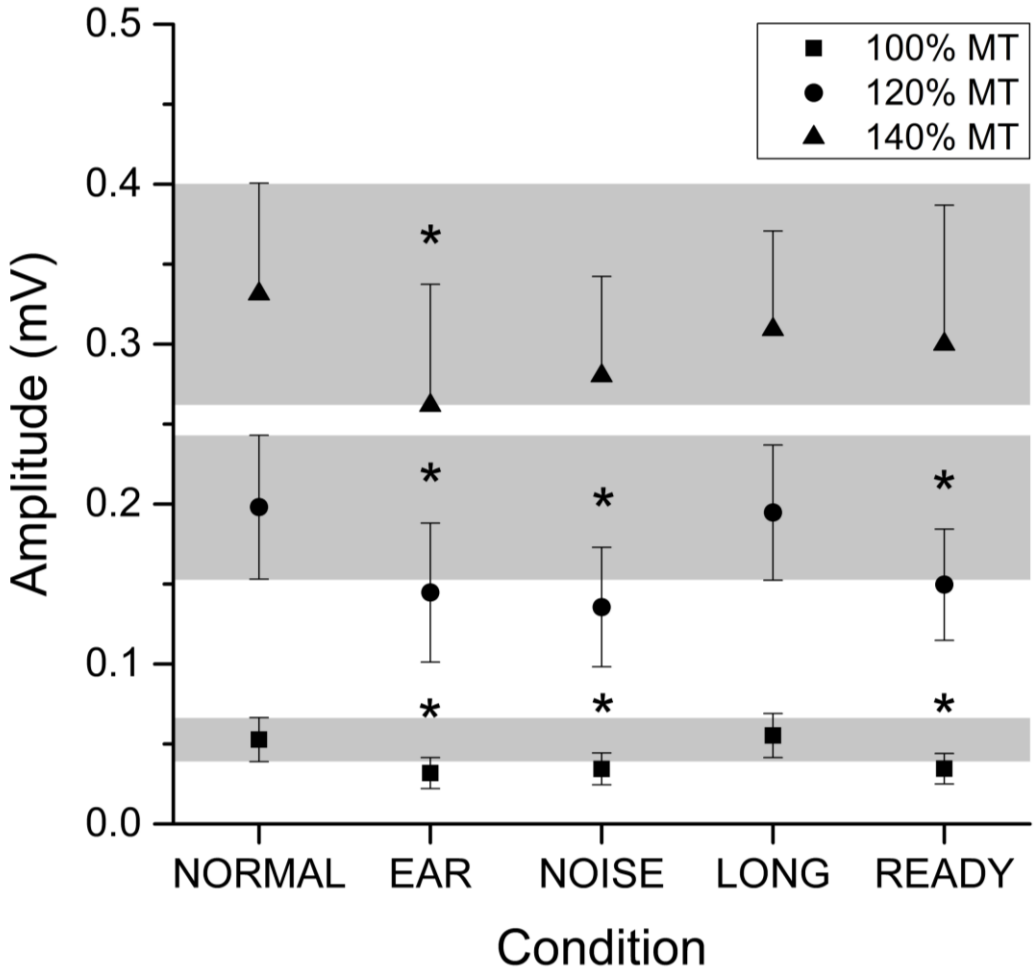

Figure 5.4. Effects of the experimental conditions on the MEPs amplitudes across different stimulation intensities. Comparison between groups mean MEP values obtained across five experimental conditions. The error bars represent the associated 95% confidence intervals. Asterisks denote a significant difference from the NORMAL condition.

A separate GLM was run to assess the effect of IPI (MIX_SHORT vs MIX_LONG) on MEP amplitudes recorded during the MIX condition (5 traces × 3 intensities × 2 conditions). While the main effect of Intensity was significant ($F_{2, 82}$ = 116.24, P < 0.001, η^2 = 0.739), no significant effect of IPI ($F_{1, 16}$ = 0.008, P = 0.93, η^2 = 0.000) was observed (Figure 5.5).

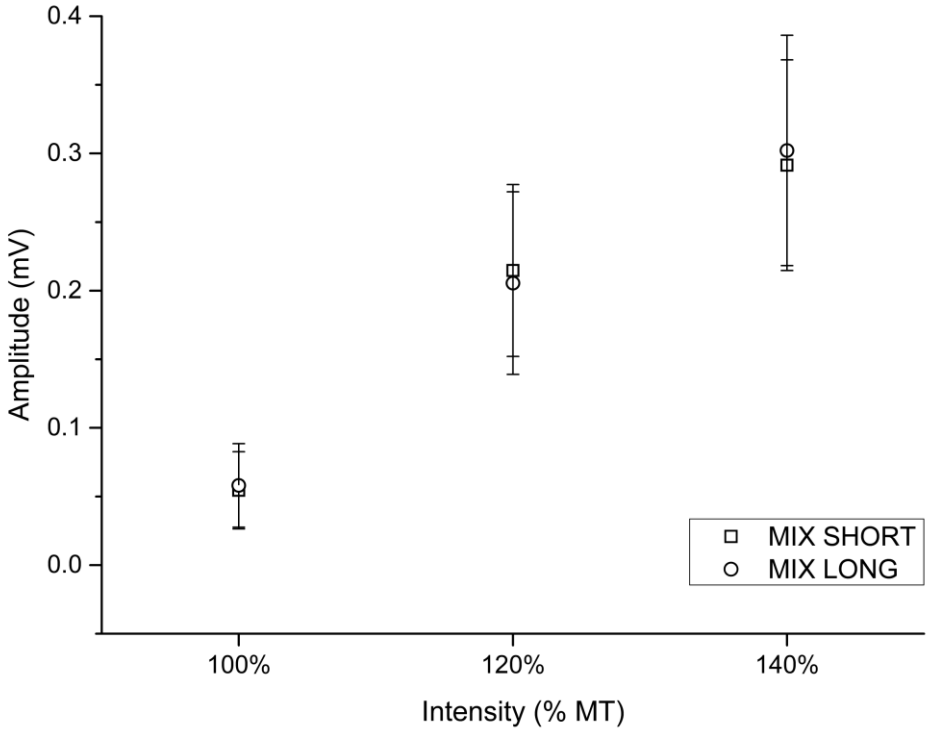

Figure 5.5. Effects of manipulating the IPIs on MEP amplitudes across different stimulation intensities. Comparison between group mean MEP values obtained with 100%, 120% and 140% MT intensities for the NORMAL, EAR and NOISE conditions. The error bars represent the associated 95% confidence intervals.

5.3.2. MEPs variability

No distribution of CV values showed deviation from normality according to the Shapiro-Wilks test (NORMAL: p = 0.14 – 0.74; EAR: p = 0.06 – 0.7; NOISE: p = 0.06 – 0.42; LONG: p = 0.09 – 0.26; READY: p = 0.22 – 0.40). Results from the GLM analysis revealed that the interaction effect between Intensity and Condition was non-significant ($F_{2, 298} = 0.96$, $P = 0.47$, $\eta^2 = 0.024$). There was a significant main effect of Intensity, indicating that variance significantly decreased with increasing stimulation intensities ($F_{2, 298} = 55.74$, $P < 0.001$, $\eta^2 = 0.263$) (Figure 5.6 *A*). Bonferroni-corrected pairwise comparisons showed that CV values diminished significantly from 100% MT to 120% MT intensities ($P < 0.001$) and from 120% MT to 140% MT ($P < 0.001$). There was no significant effect of Condition ($F_{4, 298} = 0.58$, $P = 0.68$, $\eta^2 = 0.007$) (Figure 5.6 *B*). Results from Levene's test (p = 0.37) showed no violation of the assumptions of homogeneity of variance.

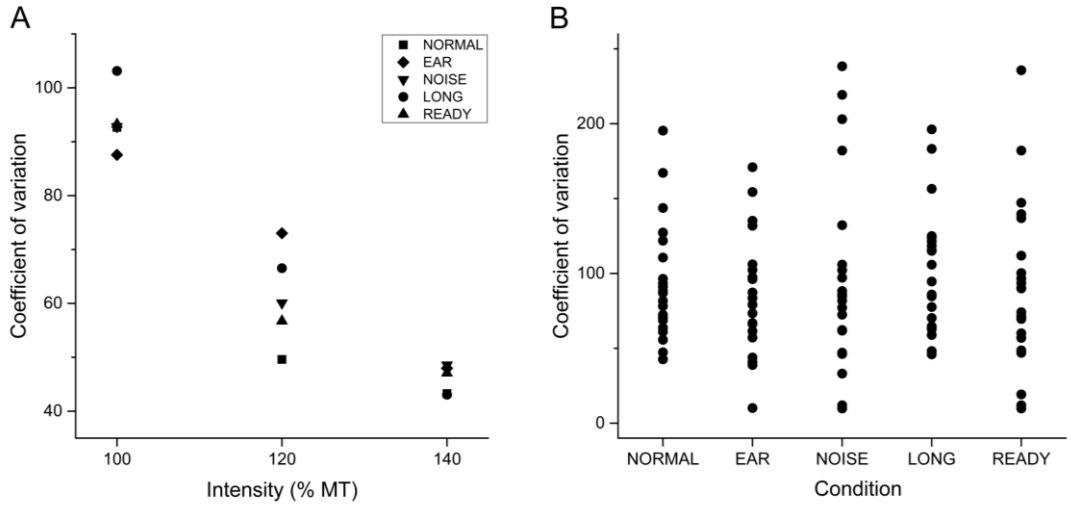

Figure 5.6. Effects of the experimental conditions on MEP amplitude variability across different stimulation intensities. *(A)* Group mean coefficient of variations across the three stimulation intensities for five experimental conditions. *(B)* Individual coefficient of variations for the 100% MT intensity across five experimental conditions.

As an additional observation, in order to visualise whether the effects of manipulating the conditions differed over participants, single participant data were plotted for each condition as percentages of the mean amplitude obtained in the NORMAL condition (Figure 5.7). The threshold for defining whether a difference occurred was set at 20% of changes from NORMAL. Use of white noise and earmuffs decreased MEP amplitudes in 15/23 participants. Knowledge of stimulation time (READY) decreased MEP amplitudes in 13/23 participants. Finally, longer IPIs decreased MEP amplitudes in 7/23 participants but increased them in 12/23 participants.

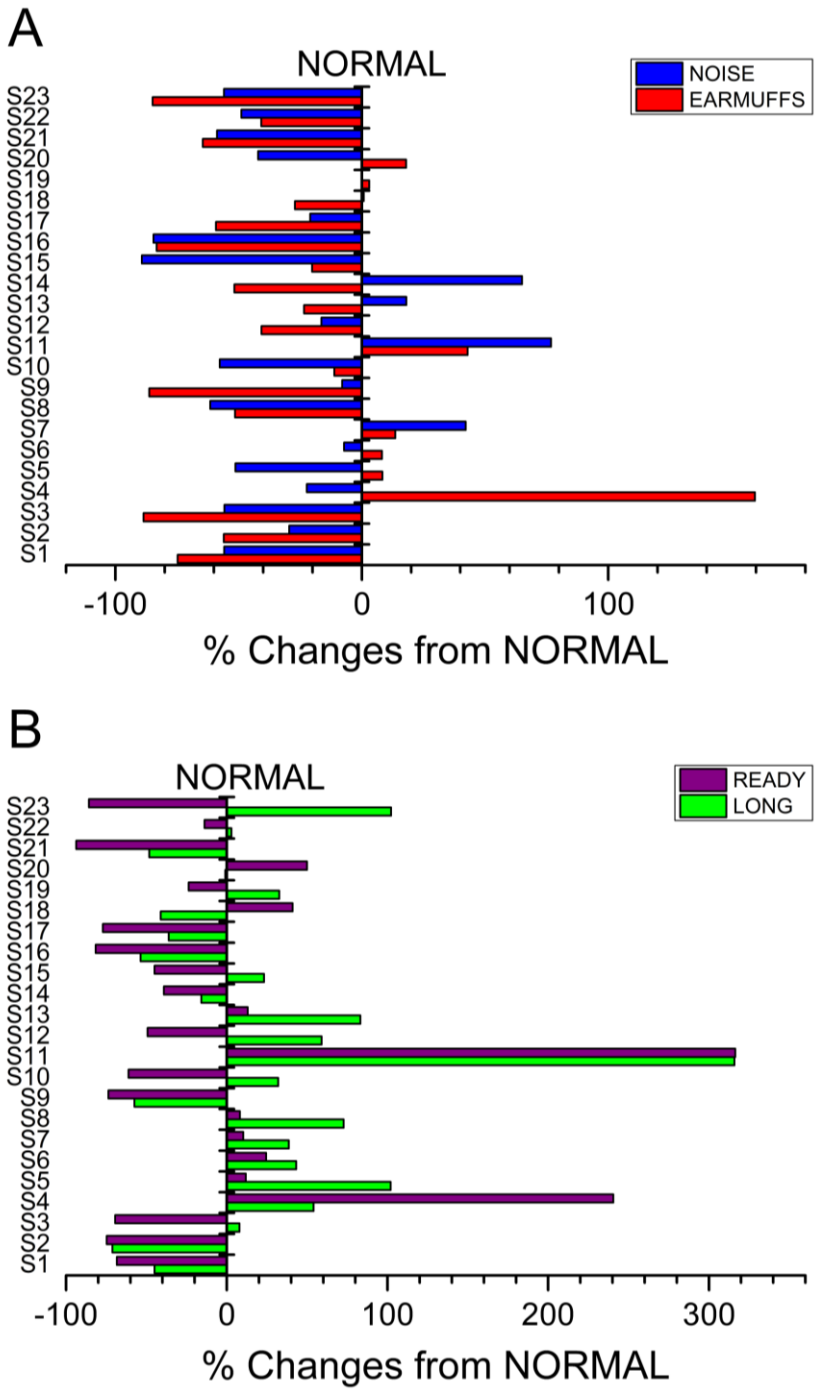

Figure 5.7. Breakdown of the effects of different conditions on MEPs recorded from each participant at MT stimulus intensity. Data are shown in reference to the amplitude values recorded under the NORMAL condition at an intensity of 100% MT. *(A)* Effects of sound and *(B)* stimulus anticipation on MEP amplitudes. Each bar represents the mean of 10 sweeps.

For visual representation purposes, all averaged MEPs recorded for each condition in a representative participant (30 sweeps for each condition) are displayed in Figure 5.8. This example helps to characterise the effects of different conditions on the shape and latency of the recorded MEPs. For this participant, EAR, NOISE and READY decreased the amplitude of the positive and negative peak of activation observed in the FCR EMG compared to NORMAL. In addition, LONG increased the amplitude of the first positive peak of activation observed in the FCR EMG and reduced the latency of the response compared to NORMAL.

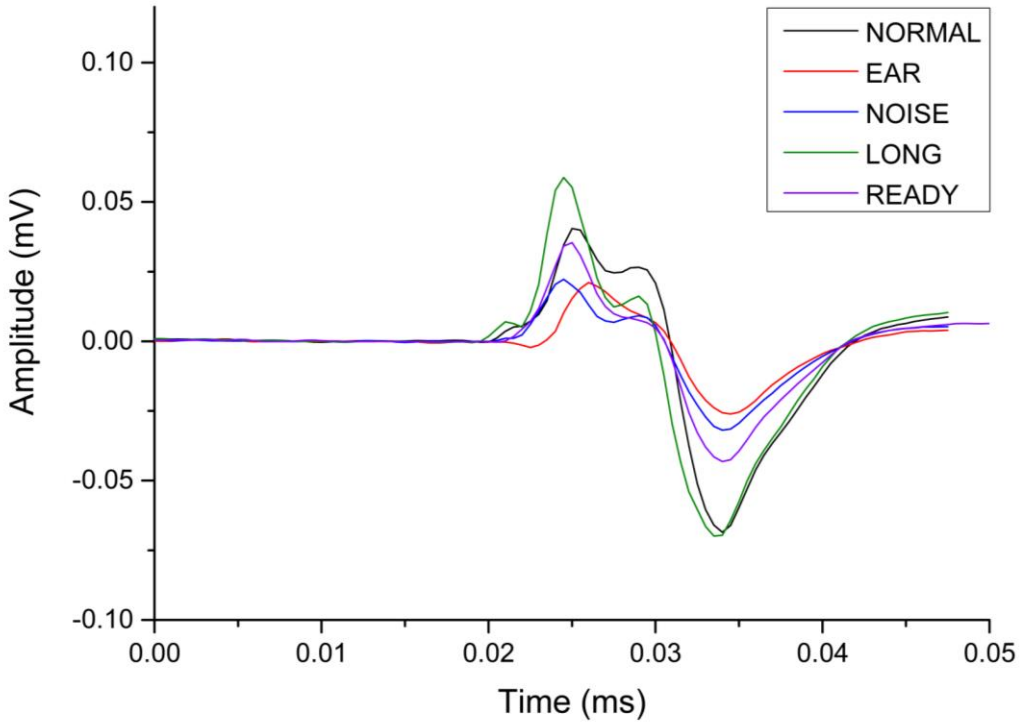

Figure 5.8. Mean MEPs recorded from a representative participant at the three stimulus intensity. Each trace represents the average of 30 (10 × 3 intensities) sweeps.

5.4. Discussion

The main aims of the presented study were to: (1) determine the outcomes of attenuating and masking the sound produced by TMS discharging on the MEPs recorded upon M1 stimulation; (2) investigate the effects of stimulus expectation on the MEPs recorded upon M1 stimulation. The data showed that MEP recordings were significantly higher on the NORMAL condition (routinely employed TMS protocol)

compared to the EAR (sound attenuating) and NOISE (listening to white noise) conditions. Increasing the IPIs (LONG) had no impact on the MEPs, confirmed by comparing traces recorded with long and short IPIs in the same condition (MIX condition). However, stimulus anticipation significantly decreased the activity elicited by TMS in the FCR muscle (READY < NORMAL). Finally, analyses of MEP CVs showed that the inter pulse variability did not significantly differ across all five conditions.

5.4.1. Attenuating/masking the sound

The significant effect of Condition showed that MEP amplitudes were significantly lower when using earmuffs compared with the normal condition. Similarly, MEP values were lower when participants listened to white noise. (Figure 5.4). Evidence derived from TMS studies on primates (Fisher et al., 2012) and the knowledge of distribution of corticoreticular and reticulospinal axons (Sakai et al., 2009) point to a role of reticular formation neurons in mediating this phenomenon. Reticular formation neurons can be activated by both TMS given on the motor cortex and acoustic stimuli delivered through a bone vibrator and have mono and disynaptic excitatory projections to spinal motoneurons (Baker, 2011, Fisher et al., 2012). Indeed, the StartReact protocol, consisting of a startling acoustic stimulus delivered before the movement, has been employed as a measure of reticulospinal activation (Dean and Baker, 2017) and to study the contribution of the reticulospinal tract to fine and gross hand movements (Baker and Perez, 2017). The hypotheses were that attenuating and masking the incoming sound would lead to a decrease in the number of activated motor neurons at all the intensity of stimulation. In this context, results obtained from the NOISE condition seem paradoxical. Considering that in the NOISE condition acoustic stimulation persisted during the whole protocol, one should expect reticular neurons to be repeatedly activated, which in turn would increase spinal excitability (Riddle et al., 2009). However, these neurons show habituation to repeated acoustic stimuli which reduce the synaptic response amplitudes (Yeomans and Frankland, 1995). The smaller MEPs measured in the NOISE condition may thus be explained by habituation to white noise.

5.4.2. Stimulus expectation

In this study the possible influence of stimulus anticipation/expectation on the recorded MEP amplitudes was also investigated. The data show no difference between traces obtained using short (5 s, NORMAL) and long IPIs (10 s, LONG) (Figure 5.4). These findings are apparently contradictory with the ones reported by Vaseghi and colleagues (2015). However, an important methodological difference in this protocol is the introduction of a 20% jitter around the IPI, effectively making the stimulus delivery time harder to predict. This finding was corroborated by designing a condition in which long and short IPIs were intermixed, showing no difference between MEPs recorded (Figure 5.5). Awareness of stimulation time (READY condition) diminished the responses to TMS when compared to the ones recorded with 5 seconds IPIs (NORMAL condition). The proposed interpretation of this conclusion is based mainly on studies showing that reaction times (Duecker and Sack, 2013) and corticospinal excitability (Fisher et al., 2012) are influenced by auditory stimuli delivered immediately prior an action release. Unexpected, loud sounds such as the TMS "click" elicit in mammals a characteristic multisensory response, the acoustic startle response (Davis, 1984). The response depends on physiological factors such as fear, attention and habituation (Wassermann et al., 2008). The effect is suppressed when participants are alerted of the stimulation (Hagemann et al., 2006), as in the condition where visual feedback instructed the participants about stimulus delivery.

It cannot be excluded that other correlated but independent factors, such as participant's attention, partially confounded the presented results. Nevertheless, participants were instructed to keep their eyes open throughout the session. The EMG activity in the pre-stimulus phase (50 ms before the MEP onset) did not change across conditions, indicating the lack of changes in baseline activity (e.g. pre-activation). Despite this, any subthreshold modulation of corticospinal excitability would go unnoticed by EMG recordings, and it could be that cortical structures may exert an inhibitory influence on downstream structures and reduce the descending corticospinal volley (Li, 2007). Designing a condition in which participants are both anticipating the stimulus arrival and habituating to the incoming auditory stimulus will help elucidate the theory that the two effects are not cumulative, but rather mediated by partially overlapping neural pathways.

5.4.3. Variability of the evoked MEPs

One of the objectives of the study was to assess whether the designed experimental manipulations would be effective in reducing MEP variability observed at rest. Comparing the CVs obtained for each participant and intensity did not reveal any significant effect of condition. A possible limitation of this study was the lack of an MRI-based navigation system to help coil positioning. The exact coil positioning was controlled throughout the duration of the experiment by marking the optimal location on the scalp, using a coil holder and headrest to keep head position in place. Even when controlling for experimental biases, MEPs exhibit high inter-individual and intra-individual variability which limit the utility of TMS (Kiers et al., 1993). Part of the pulse-to-pulse variance is due to spontaneous oscillation in the excitability of the motor system (see Chapter 2.7.7) (Brasil-Neto et al., 1992). Cortico-cortical excitability is correlated to the phase of EEG oscillatory activity at the time of the stimulus (Mäki and Ilmoniemi, 2010), and by inducing oscillatory activity in the motor system at specified frequencies it is possible to modulate corticospinal excitability (Schilberg et al., 2018). Multiple studies have successfully identified physiological factors generating individual differences such as brain anatomy, age and genetic factors (Wassermann et al., 2008). However, subject-specific features such as arousal, attentional state and discomfort are often overlooked and need to be reported and addressed in future studies assessing motor excitability.

5.4.4. Supplementary analyses

One participant was tested while wearing active noise-cancelling headphones (data not shown). As for the other conditions, ten stimuli were given at each of the three intensities and the MEPs produced were compared to the ones recorded in the NORMAL condition. Surprisingly, MEPs were found to be larger at 120% and 140% MT intensity with noise-cancelling headphones. The participant reported hearing the click sound every time the machine was discharging. A possible explanation for the inefficacy of noise-cancelling headphones is that the dominant frequency component of the TMS discharging noise is in the range of 2000 to 5000 Hz, which might be above the frequencies commercial headphones are capable of masking.

The outcome of delivering TMS on the motor cortex is highly variable both among trials and individuals (Marinovic et al., 2014). The effects of changing conditions of stimulation on each participant was visualised (Figure 5.7) to clarify whether the

significant differences observed reflected a universal phenomenon or were subject-specific. It is reported that masking and attenuating discharging sound was effective in reducing MEPs amplitudes in around 2/3 of the participants with little effects on the others. In contrast, only 56% of participants showed smaller responses in the trials in which they could anticipate stimulation time.

5.4.5. Practical implications for choosing experimental conditions

Given the different nature of the designed conditions, the choice of a TMS protocol to implement should be based on the specific research question. Use of white noise and earmuffs often pushed MT amplitudes below the value which would be considered threshold by definition (>50 μV). This issue must be considered when delivering stimulation at increasing percentages of MT value, as was the case in the current study, and interpreting results in terms of corticospinal excitability. MT values obtained when using earmuffs might better reflect the activity of corticospinal neurons, without the effects of the acoustic stimulus. Whether the two conditions designed (EAR and NOISE) successfully reduced the spread of activity to other pathways needs to be experimentally confirmed, but these constitute interesting alternatives to "classical" TMS protocols. The efficacy of white noise in masking the incoming sound seemed to deteriorate at 140% MT intensities (Figure 5.4). High stimulation intensities are often employed in diagnostic TMS studies requiring maximal corticomotor response (Rossini et al., 2015). Noise levels need to be changed according to the "click" produced to guarantee masking. While interesting from a theoretical point of view, giving visual feedback to participants to inform of stimulation time is likely to introduce many uncontrollable variables. Participants were instructed to focus their visual attention on the clock showing delivery time without producing any anticipatory reaction, but the attentional state induced by the instructions depended on individual characteristics and might constitute an additional source of variability.

5.5. Conclusions

The relevance of studies investigating motor system excitability with TMS is hindered by our lack of understanding of its methodological principles (Vaseghi et al., 2015). This study addressed the second aim of this thesis by assessing whether MEPs

recorded upon TMS, which are used as index of corticospinal excitability, are influenced by the sound produced by TMS discharging and stimulus anticipation. The present study demonstrated that the sound produced by TMS discharge influences the amount of activity recorded via EMG from the FCR muscle. Masking and attenuating the clicking sound might reduce unintended effects caused by auditory activation and provide a more valid measure of corticospinal excitability to contribute to diagnosis or ascertain efficacy of therapy. The current data show that participants' knowledge of discharging time decreased the amplitude of responses elicited by threshold and suprathreshold stimulation at rest (Figure 5.4). By using a randomized IPI instead of a constant IPI the possible confounding effect of habituation and anticipation can be minimized (Schmidt et al., 2009). Given that stimulus expectancy facilitated corticospinal excitability, delivering TMS at unexpected times might better characterise the excitability of the corticospinal tract at rest. A description of which neural pathways might be differentially activated by each experimental condition is provided in Chapter 7.2.2. Future studies could potentially address this issue by measuring the activity induced in different neural populations under various stimulation conditions directly in primates and indirectly in humans employing neuroimaging techniques. Methodological information such as instructions to participants and their prior experience of TMS need to be reported even in studies assessing motor excitability at rest. Together with the study described in Chapter 4, the first two experimental chapters assessed the reliability and validity of TMS and PNS when used to study excitability changes in the motor system. The findings of the current chapter guided the design of the last experimental chapter (Chapter 6), in which responses to cortical stimulation were obtained while participants performed background muscle activation and at lower stimulation intensities to limit the effects of the TMS-discharging sound on MEP responses.

Chapter 6 - The effects of strength and skill training on the neural circuits of the contralateral limb

6.1. Introduction

Unilateral strength training leads to strength gain not only in the trained limb, but also in the contralateral untrained limb. This phenomenon has been referred to as cross-education (Scripture et al., 1894) and multiple theories have been advanced on its functional significance and neural correlates (see Chapter 2.8). Cross-education is muscle (Davis, 1901) and task (Hellebrandt, 1951) specific, and has been shown to occur across a wide range of trained movements such as knee flexion and extension (Kannus et al., 1992), ankle plantarflexion (Lagerquist et al., 2006), elbow flexion (Kidgell et al., 2011) and wrist flexion and extension (Kidgell et al., 2015, Lee et al., 2009). Different studies have investigated the neural substrates of cross-education of strength by employing TMS (Ruddy and Carson, 2013). Lee and colleagues had participants performing forty right isometric wrist extension MVCs for twelve sessions spanning four weeks (Lee et al., 2009). MVCs were found to be increased in both arms after the training program. In addition, authors estimated the efficacy of the cortical drive in activating spinal motoneurons by applying TMS to the motor cortex while participants performed the MVC (Todd et al., 2003). They found an increase in neural drive, measured as a decrease of the superimposed twitch evoked by TMS during MVCs, to the untrained muscle (left extensor carpi radialis) after training. However, no changes in the excitability of spinal and cortical circuits of the untrained limb could be observed, since monosynaptic reflexes and MEPs recorded after training did not differ from baseline values (Lee et al., 2009).

Cross-education of strength is often assessed after multiple weeks of training, since the literature on resistance training shows that long protocols are necessary to induce changes in muscle structure (Hendy and Lamon, 2017). However, multiple studies support the hypothesis that cross-education is mediated by bilateral activation of the primary motor cortex and can be observed early on at the acute stage post-training (Carroll et al., 2008, Lee et al., 2010). The task employed in the first of such studies (Carroll et al., 2008) required participants to perform 300 ballistic right finger abductions. Practice increased peak acceleration of ballistic finger abduction movements in the trained and untrained hand. Corticospinal excitability, measured by delivering TMS at multiple supra-threshold intensities, increased in both hemispheres

after training. The second of such studies provided additional evidence for the role of the ipsilateral (to the moving hand) motor cortex in cross-education (Lee et al., 2010). Immediately after training, participants were administered low-frequency TMS, which inhibits cortical excitability (Chen et al., 1997), on the right M1 after right unimanual ballistic training. Cortical stimulation reduced both corticospinal excitability and the performance gains that were obtained with ballistic training. These findings point to a role of M1 in the acute effects of cross education, but to our knowledge no work has investigated the acute (within-session) outcome of unimanual wrist flexion training on the neural excitability of the contralateral corticospinal pathway.

Dragert and Zehr (2011) assessed changes in the excitability of the untrained spinal circuits after strength training. Participants completed 25 isometric dorsiflexion MVCs for 15 sessions over 5 weeks. The training protocol succeeded in increasing strength in both trained and untrained ankle dorsiflexors. The threshold for eliciting a monosynaptic reflex in the soleus and tibialis anterior muscle of the trained leg increased after training (from 6.1 to 16.6 % M_{max} in soleus and from 1.8 to 4.4 % M_{max} in TA). The maximal H-reflex which could be evoked by electrical stimulation in the soleus muscle decreased bilaterally after training (Dragert and Zehr, 2011), indicating that spinal excitability was reduced for both the trained and untrained legs. The possibility that spinal circuits are involved in the cross-education effect is supported by evidence that strong unimanual movements exert an influence over the spinal circuits of the contralateral hand (Hortobágyi et al., 2003). Monosynaptic reflexes and MEPs were recorded from the relaxed FCR muscle while participants flexed their left wrist at 50% and 75% of their MVC. Corticospinal excitability in the ipsilateral (to the trained arm) hemisphere was increased during both levels of contraction. Similarly, the amplitude of the H-reflex evoked in the resting FCR by stimulation of the median nerve decreased during contractions. The results showed that crossed effects alter spinal and cortical activity, but the possibility that such pathways support the behavioural improvements observed in the untrained limb remains unknown (Hendy and Lamon, 2017).

Transfer of motor skills to the contralateral untrained limb has been studied independently from strength training (Lee and Carroll, 2007). The effect, called bilateral transfer, has been reported using finger tapping (Parlow and Dewey, 1991), reaction-time (Perez et al., 2007) and precision grip tasks (Gordon et al., 1994). There

is controversy about which specific component of the motor task are transferred to the other limb (Teixeira, 2000). The timing component of the task, which is the time at which participants are required to respond with a movement, was shown to transfer from the trained to the untrained limb (Yao et al., 2014). However, force control was improved only for the trained hand (Yao et al., 2014). The authors speculated that the force required to perform the task was too low (10% MVC) to elicit bilateral activity in the motor cortex which, according to theories on the neural substrates of cross-education (discussed later in Chapter 7.2.3), supports the transfer phenomenon (Parlow and Kinsbourne, 1989). Using force-matching protocols requiring higher strength might help elucidate whether skills requiring control of force are transferred to the contralateral hand.

It has been proposed that strength and skill training might lead to performance improvements through similar mechanisms (Lee and Carroll, 2007). Two recent findings support this hypothesis. In a study by Green and Gabriel (2018), the training phase lasted for six weeks, during which participants performed wrist flexions (arm-training group) or ankle dorsiflexions (leg-training group) with their dominant limb at 80% of their MVC. At the end of training, strength increase was measured by re-assessing MVC for each participant, while skill acquisition was measured by asking participants to reproduce force traces displayed on the screen by flexing their wrist (for the arm-training group) or ankle muscles (for the leg-training group). In addition, strength increase and skill acquisition were measured even in the untrained non-dominant muscle. The results showed that training increased strength in the trained muscles and the effects transferred to the untrained contralateral muscles (Green and Gabriel, 2018). Interestingly, improvements in force-tracing performance were observed only in the untrained limbs and only when concurrent feedback was removed. Leung et al. (2015) assigned participants to either a visuomotor tracking group, a metronome-paced strength training, a self-paced strength training or a control group not performing movements. They measured cortical excitability with MEPs and SICI in both hemispheres after a single session of unimanual training. However, the authors did not measure behavioural outcomes of the training protocols on the non-trained arm performance. Corticospinal excitability increased and intracortical inhibition decreased after one session of visuomotor training and metronome-paced strength training but not after the other two trainings in both the trained and untrained arms. These results demonstrated that timed strength training and visuomotor training

modulate cortical excitability in both the trained and untrained hemisphere, a neural mechanism which might be important in cross-transfer effects (Leung et al., 2015). In addition, the authors concluded that some of the neurophysiological mechanisms which are responsible for the cross-education phenomenon after multiple training sessions are already occurring at the acute level after a single training session (Leung et al., 2015).

The aim of this study was to bridge the gap in the literature on contralateral transfer of skill and strength by: (1) measuring increases in strength (MVC) observed in the trained and untrained limb after a single session of isometric wrist flexion strength training; (2) measuring changes in fine force control in the trained and untrained limb after a single session of force-matching training; (3) compare changes in spinal and supraspinal neural excitability observed between baseline and after strength and skill training in the untrained hand. Measures of spinal and cortical excitability included recording of the monosynaptic reflex (H-reflex) evoked in the FCR muscle and MEPs induced by TMS at multiple suprathreshold intensity in the FCR muscle. In order to better characterize the neural pathways which are involved in the crossed effects, the monosynaptic reflexes evoked in the FCR muscle were conditioned by TMS delivered at multiple stimulus intervals following the protocol described in Chapter 4. TMS was delivered at intensities below the threshold to elicit MEPs in the FCR muscle and PNS was delivered at the intensity evoking monosynaptic reflexes of 10-15% of the maximal motor wave. This method permits to differentiate the monosynaptic component of the descending drive to motoneurons from other polysynaptic pathways contributing to the monosynaptic reflex (Leukel et al., 2012).

6.2. Methods

6.2.1. Participants

A total of 10 healthy participants (M = 23.5 ± 4.6; females = 4) volunteered for the study. Participants were included in the study if right-handed, as assessed by the Edinburgh Handedness Questionnaire (handedness scores ranging from 57.9 to 100, with 50 indicating mixed right handers and 100 indicating pure right handers) (Oldfield, 1971), aged between 18 and 40 and in good health at the time of testing. Participants were excluded from the study if they had any contraindications to TMS as detailed in the application guidelines of the Safety of TMS consent group (see Chapter 3.1) (Rossi et al., 2009). Since sex hormones levels do not affect responses

to TMS, PNS and MVC levels (Ansdell et al., 2019), female participants were recruited without controlling for the menstrual cycle phase. All participants underwent both experimental conditions over two sessions, and the order of allocation to conditions was counterbalanced across participants. The second session was scheduled at the same time of the day of the first to control for potential influences of circadian rhythms (Sale et al., 2007). The two sessions were separated by at least 7 days to avoid the influence of carry-over effects of stimulating the brain (Nitsche et al., 2008). All participants gave written informed consent prior to the start of the experimental session. The experimental procedures followed the Declaration of Helsinki and were approved by the Faculty of Biological Sciences Ethical Review Committee at the University of Leeds.

6.2.2. Electromyography (EMG) measures

Surface EMG activity was recorded via parallel-bar wireless sensors (Trigno, Delsys Inc., Natick, MA, USA) from four muscles: right flexor carpi radialis (FCR); left FCR; right extensor carpi radialis longus muscle (ECRL); left ECRL (see Chapter 3.4 for details on sensor positioning). Smaller sensors (2.5 × 1.2 cm) were used to record activity from the flexor muscles (lFCR and rFCR) to ensure that activity was recorded exclusively from these muscles (see Chapter 4) compared to the extensor muscles (lECRL and rECRL) (3.7 × 2.6 cm). Raw EMG recordings were pre-amplified (gain = 909), recorded with a 20-450 Hz bandwidth and digitized at 2 kHz using data acquisition software (Spike2, Cambridge electronics Design, Cambridge, UK).

6.2.3. Dynamometer assessment

At the beginning of the session, participants sat on a dynamometer (Biodex Medical Inc, Shirley, NY, USA) chair, with their right elbow and forearm forming an angle of 140˚ supported by the dynamometer armrest. The Biodex wrist attachment was fastened to the dynamometer, and participants grasped the handle on the wrist attachment which was parallel to the ground. The head was resting on a cushion and the feet were supported by a footrest. Before baseline testing, participants completed a warm-up phase which included producing random wrist flexion movements to ensure that the positions of the handle, chair and armrest were comfortable. Handle, chair and armrest positions were logged to ensure consistency of arm and body position over the session. At this stage, participants were introduced to the

dynamometer operation screen which was used to provide feedback of performance. They were shown that the torque they produced during a movement could be seen online as a red line on the screen, along with the peak torque produced at any time point during the movement and the degree of similarity between movements in a set (CV). The refresh rate of the operation screen was 2000 Hz.

6.2.4. Peripheral Nerve Stimulation

Motor waves and monosynaptic reflexes (H-reflex) were evoked in the FCR muscle by electrical stimulation of the median nerve at forearm. Stimulation was delivered at a rate of 0.2 Hz through a constant-current stimulator (DS7A, Digitimer Ltd, Welwyn Garden City, UK) controlled by the acquisition software (Spike2, Cambridge Electronic Design, Cambridge, UK). Stimuli consisted of square-wave pulses of 1 ms duration delivered at the level of the cubital fossa, medial to the tendon of biceps brachii, in parallel with the course of the nerve (Jaberzadeh et al., 2004). The optimal stimulation site was determined by moving the stimulating electrode until a reliable motor wave could be elicited in the target muscle. Increasing background activity in the FCR may be necessary in order to elicit the H-reflex (Bodofsky, 1999) in this muscle. Prior stimulation, participants were trained to maintain a background activation of the FCR muscle corresponding to 5% of their maximum voluntary contraction (MVC) via visual feedback of the EMG. Stimulation started at low intensities (~ 2mA) and was incremented until the peak-to peak amplitude of the motor wave reached its maximal amplitude (M_{max}). Ten traces were recorded at this intensity of stimulation. For recording of the H-reflexes, the stimulation intensity was set at an intensity which evoked reflexes of about 10-15% of the M_{max} amplitude ($H_{M10\%}$) on the ascending part of the recruitment curve (Burke, 2016). Ten traces were recorded at this intensity of stimulation. M_{max} and $H_{M10\%}$ were assessed before and after training. The amplitude of the motor wave produced at the intensity used to elicit $H_{M10\%}$ was monitored to ensure that the effects of training were limited to changes in the excitability of the afferent (H-reflex) pathway.

6.2.5. Transcranial Magnetic Stimulation

TMS was delivered to the left motor area M1 with a figure-8 coil oriented at ~45° to the sagittal plane, such that the current induced in the brain was perpendicular to the central sulcus (Janssen et al., 2015). The hotspot for inducing activity in the FCR

muscle was found by moving the coil over the scalp while delivering stimulation until a response could be seen in the EMG. Once located, the hotspot was marked to ensure consistency of stimulation site between pre and post-training. An IPI of 5 seconds was used for all the recordings. Participants maintained a background activation of the FCR muscle corresponding to 5% of their maximum voluntary contraction (MVC) while being stimulated. The active motor threshold (aMT) was defined as the minimum stimulation intensity at which MEPs of peak-to-peak amplitudes between 100 and 200 μV in at least 5 out of 10 trials could be elicited. Active motor threshold values, expressed as %MSO, were on average 54.8 ± 3.7 for the strength training session and 55.1 ± 3.8 for the skill training session. TMS was delivered at aMT and 120% aMT intensities to characterise changes in corticospinal excitability between before and after training. Only a sub-group (n = 7) of participants received TMS at 120% aMT intensities. Ten traces were recorded at each stimulation intensity.

6.2.6. TMS conditioning of the monosynaptic reflex

PNS and TMS were applied in combination in order to record TMS-conditioned monosynaptic reflex from the FCR muscle. The intensity of PNS was set to evoke FCR H-reflexes of 10–15% of the respective maximum M-wave. TMS intensity was given at subthreshold levels of 90% of aMT. The method was applied in accordance with a previous study in which the specific contribution of descending volleys to spinal motoneurons was assessed (Niemann et al., 2016). TMS was delivered at multiple ISIs ranging from -5 ms (PNS first) to +5 ms (TMS first) from the delivery of the electrical pulse targeting the median nerve. Each ISI was measured 10 to 15 times in a block-randomised order, and unconditioned H-reflexes and TMS at 90% of aMT were randomly recorded together with the conditioned responses to ensure that no changes in spinal and cortical excitability arose during the stimulation. The time interval between successive pairs of stimuli was set at 5 seconds. Offline, the latency of the MEPs evoked by TMS and of the H-reflexes evoked by PNS were estimated as the time interval between stimulus delivery and the onset of the response in the EMG (Chapter 3, Fig 3.7). The two latencies values were subtracted in order to assess the ISI at which the cortical-evoked volley and the peripheral-evoked afferent volley reach spinal motoneurons at the same time. The procedure was repeated after training, and the intensity of both stimulations (TMS and PNS) was adjusted to ensure that

magnetic stimulation was still subthreshold, and that unconditioned H-reflexes were of the appropriate amplitude.

6.2.7. Testing protocols

All participants (Figure 6.1) underwent two testing sessions (strength and skill) spaced at least by a week, and the order of sessions was randomised and counterbalanced across participants. Prior to start with the actual testing, participants completed a warm-up phase in which they attempted to produce wrist flexions with their hand supinated by grasping the dynamometer attachment (Figure 6.2 *A*). Participants provided feedback on the quality of the contraction produced and the handle's position was moved until they were comfortable that they could produce maximal contraction in that position. In addition, during this phase they were instructed to limit the contraction to the wrist flexor muscles and minimise the involvement of other muscle groups in the movement. The chair and handle positions were recorded for each participant on the first session and used on the following session. Sessions were divided in three phases: pre-training; training; post-training. Neurophysiological measures were obtained at the beginning of the session and at the end, after completing the behavioural part.

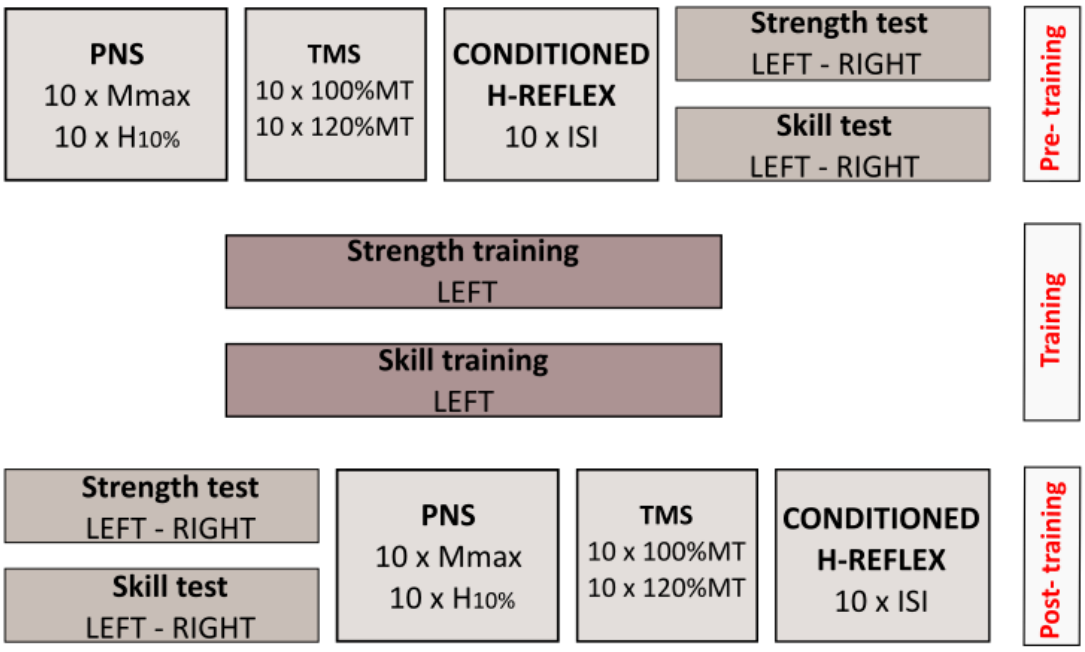

Figure 6.1. Time course of the two experimental sessions. The order of delivery of PNS and TMS was randomised across sessions and participants.

Strength testing

Participants performed three isometric wrist flexion MVCs, each lasting 5 seconds and with a 1-minute interval between them. The contralateral arm was kept relaxed during the unimanual MVC, as assessed via visual check of the EMG activity. A computer screen placed in front of the participants and running the Biodex software (Biodex Medical Inc, Shirley, NY, USA) instructed them on when to start the movement and how long to maintain it through a countdown timer. The criteria for accurate measurement of MVCs included: instructions to the participants; online feedback of performance given via the computer screen; standard verbal encouragements (Gandevia, 2001). Three MVCs were performed with the left and right arm, in a randomised order, before (Pre-training) and after (Post-training) training. The highest peak force value generated, which was calculated by the Biodex software at the end of the three movements for each hand, was used for comparison between PRE and POST training values.

Skill testing

Participants were asked to complete an isometric wrist flexion MVC with both hands at the start of the skill testing phase. The peak force value produced during the MVC was used to normalise the remaining contractions. Each participant performed three sustained contraction at 25% and 50% of their MVC with either the right or the left arm in a randomised order. The interval between consecutive movements was set at 30 seconds. When using the left arm, a computer screen displayed in front of them instructed participants on when to start the movement and how long to sustain it for. The target force to be produced was displayed as a line. Participants had to follow that line as closely as possible by contracting their wrist for three seconds, and their performance was displayed through another line providing concurrent knowledge of performance. Knowledge of performance was not provided while participants were being tested for the untrained (right) arm. This feature ensured that the cross-learning effects did not arise because participants learned to better interpret visual feedback from the screen and that corticospinal excitability in the untrained hemisphere did not increase as a result of the movements produced during testing, because practice without feedback does not impact corticospinal excitability (Muellbacher et al., 2001). Coefficients of variation for the task were calculated by the Biodex software and used as measures of skill control for comparison between PRE and POST values.

6.2.8. Acute training protocols

The acute effects of training were tested with a single session of strength training and force-matching training. All participants took part in one session of both training protocols spaced at least by a week and in a randomised order. Participants were given five minutes of rest after the baseline testing before starting the training protocol. The position of the dynamometer chair and wrist attachment relative to the participant was kept constant between the testing and training phases. Trainings were performed only with the left hand. All training protocols were delivered, recorded and stored in the Biodex Advantage Software program, version 3.44 (Biodex Medical Inc, Shirley, NY, USA).

Strength training session

In the strength session, participants were instructed to grasp the wrist attachment handle and follow the instructions on the screen. They were required to contract the left wrist as rapidly and as strongly as possible and to maintain the contraction for 2 seconds before relaxing (Selvanayagam et al., 2011). Each contraction was followed by 3 seconds of rest. The computer monitor showed real-time feedback of the force produced as a line on the screen (Figure 6.2 *B*). In addition, the peak torque produced across all the movements was numerically displayed on the screen. Four sets of ten contractions (each set lasting 50 seconds) were performed by each participant. Resting intervals between consecutive sets were set at three minutes. A countdown timer instructed the participants on when to rest and to start another contraction.

Skill training session

In the skill session, participants were instructed to grasp the wrist attachment handle and follow the instructions on the screen**.** They were required to produce a force matching 25% or 50% of their left MVC by producing wrist contractions for three seconds. In front of them, the computer monitor showed the target force as a red line, and the produced force as a purple line (Figure 6.2 *C*). The line representing the force produced had to be as close as possible to the target force red line. In addition, the degree of similarity between movements was numerically displayed on the screen, with lower numbers indicating better performance. Thirty seconds of rest were given after each 3 seconds contraction to prevent the training from becoming tiring and keep attentional levels high. Four sets of ten contractions (each set lasting 5 minutes) were performed by each participant.

6.2.9. Data reduction

For the analysis of strength, the highest muscular force output produced at any moment during each repetition was automatically generated by the Biodex software. Force was measured as torque, the moment of a given force on an object (the dynamometer handle in the specific case). The unit of measure was Nm, which is the torque of one newton applied to a one-meter moment arm. For the analysis of skill, the CV (given in % differences) which indicates the amount of variation between repetitions was automatically generated by the Biodex software. Torque data were sampled at a frequency of 100 Hz.

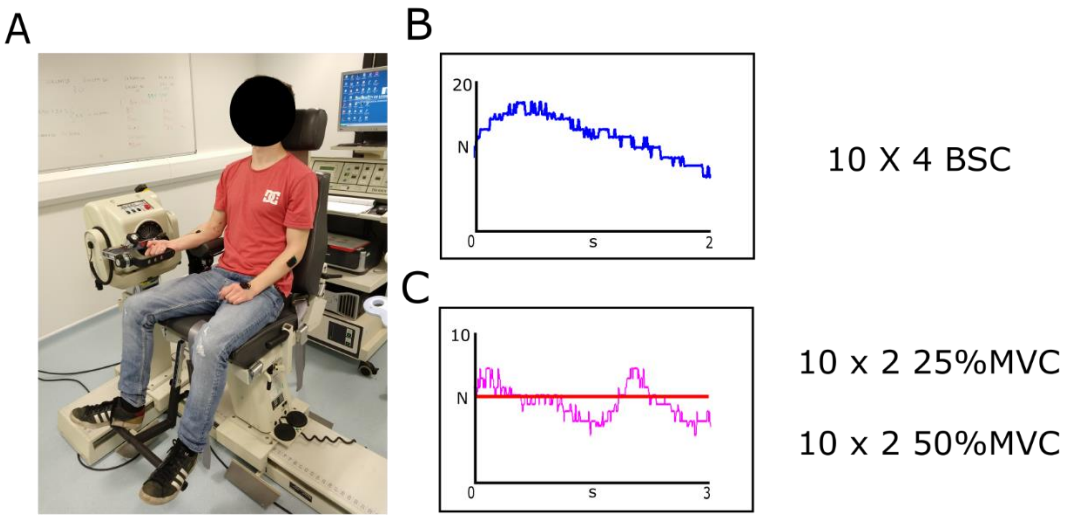

Figure 6.2. Training setting and details. *(A)* Position of the participant during the testing phase for the right hand. Examples of force traces produced during the strength *(B)* and skill *(C)* training protocols with total number of movements performed during training alongside the force trace.

CV values were computed as the standard deviation of the torque data divided by the mean average torque. For the analysis of maximum motor response, the peak to peak amplitude was calculated for a time window between 1 and 10 ms after the stimulus artefact. For the monosynaptic reflexes, TMS-conditioned H-reflexes and for MEPs, the peak to peak amplitude was calculated for a time window between 10 and 50 ms after the stimulus artefact. The mean (peak-to-peak) amplitude of the 10 recordings (for M_{max}, $H_{M10\%}$, aMT, and a120% aMT) was analysed for each session. Similarly, the mean (peak-to-peak) amplitude of the 10-15 recordings obtained at each ISI was analysed for each session.

6.2.10. Data analyses

Statistical analyses were performed using SPSS (Version 22.0) software with an a priori significance level of <0.05. Mean, SD and CV values were computed for all neurophysiological variables across participants. The outcome of specific training protocols on strength (MVC) and performance (CV_25%, CV_50%) was measured with two-way repeated-measures ANOVAs with factors TIME (PRE, POST) and HAND (RIGHT, LEFT). Separate two-way repeated-measures ANOVAs with factors CONDITION (SKILL, STRENGTH) and TIME (pre-training, post-training) were conducted for each of the parameter recorded from the right FCR muscle (aMT, 120% aMT, M_{max}, $H_{M10\%}$) acquired with cortical or peripheral nerve stimulation. In addition, to ensure lack of changes in the level of pre-activation, two-way repeated-measures ANOVAs with factors CONDITION (SKILL, STRENGTH) and TIME (pre-training, post-training) were conducted for the RMS of the background EMG recorded in the 50 ms preceding stimulus delivery in each parameter. In order to control for possible differences between conditions at baseline, paired t-tests were run between each parameter's values at pre-training in the two conditions. Whenever the results of the Mauchly's test showed a violation of the sphericity assumption, Greenhouse-Geisser-corrected values were reported. Results from multiple comparisons were corrected with the Bonferroni procedure.

The analysis of TMS-conditioned monosynaptic reflexes followed the protocol described by Leukel and his colleagues (2015). First, the mean amplitude value calculated at each ISI for each participant was divided by the mean of the individual unconditioned reflex value (Leukel et al., 2012). Using the control H-reflex as a reference for conditioned H-reflexes accounts for the differences in the amplitude of the H-reflex at baseline. The mean amplitude value obtained at each conditioning-test interval post-training was subtracted from the corresponding pre-training value. The first interval at which facilitation could be observed (EF) after the interval at which the peripheral and cortical volleys arrived at the same time at the spinal motoneurons (see Chapter 6.2.6) was assessed in each session as the time at which the conditioned mean amplitude increased by >20% compared to the unconditioned value. The EF ranged from -3 ms to 0 ms in all participants and sessions. A two-way repeated-measures ANOVAs with factors CONDITION (SKILL, STRENGTH) and TIME (pre-training, post-training) was conducted for the RMS of the background EMG recorded in the conditioning phase. To ensure the lack of differences in the amplitude

of the monosynaptic reflex recorded during this phase, unconditioned reflex amplitude values were compared between the two conditions using a paired Student's t-test. Similarly, paired t-tests were used to assess if the size of the unconditioned H-reflex differed from pre-training to post-training in each condition. A two-way repeated-measures ANOVA with the factors ISI (EF, EF+2. EF+4, EF+6) and CONDITION (SKILL, STRENGTH) was specified. Whenever the results of the Mauchly's test showed a violation of the sphericity assumption, Greenhouse-Geisser-corrected values were reported. Results from multiple comparisons were corrected with the Bonferroni procedure. In addition, because previous observations (see Chapter 4.3.2) suggest that EMG responses to subthreshold TMS might change during the experimental session, data obtained with TMS given at 90% aMT were analysed with a two-way repeated-measures ANOVA with factors TIME (PRE, POST) and CONDITION (SKILL, STRENGTH).

6.3. Results

6.3.1. Behavioural results

In the strength training sessions, mean MVC torques were 12.96 (SD = 4.37) Nm for the left hand before training and 13.9 (SD = 3.79) Nm for the right hand before training. Mean MVC torques were 13.12 (SD = 4.22) Nm for the left hand after training, corresponding to 101% of the pre-training value, and 15.11 (SD = 4.69) Nm for the right hand after training, corresponding to 109% of the pre-training value . For the strength training protocol, results from the repeated-measures ANOVA revealed no significant effect of TIME ($F_{1, 9} = 3.069$, $P = 0.114$, $\eta^2 = 0.254$) on the MVC torque data. The main effect of HAND was significant ($F_{1, 9} = 9.246$, $P = 0.014$, $\eta^2 = 0.507$), which indicate that participants produced higher MVCs with their right (dominant) hand. In addition, the interaction effect between TIME and HAND was non-significant ($F_{1, 9} = 4.481$, $P = 0.063$, $\eta^2 = 0.332$). Examples of the torques produced by a representative participant during the strength training protocol are presented in Figure 6.3. Examples of the MVC torque produced by a representative participant before and after strength training are presented in Figure 6.5. All raw torque traces were smoothed with a 5-point unweighted smooth to aid data visualization.

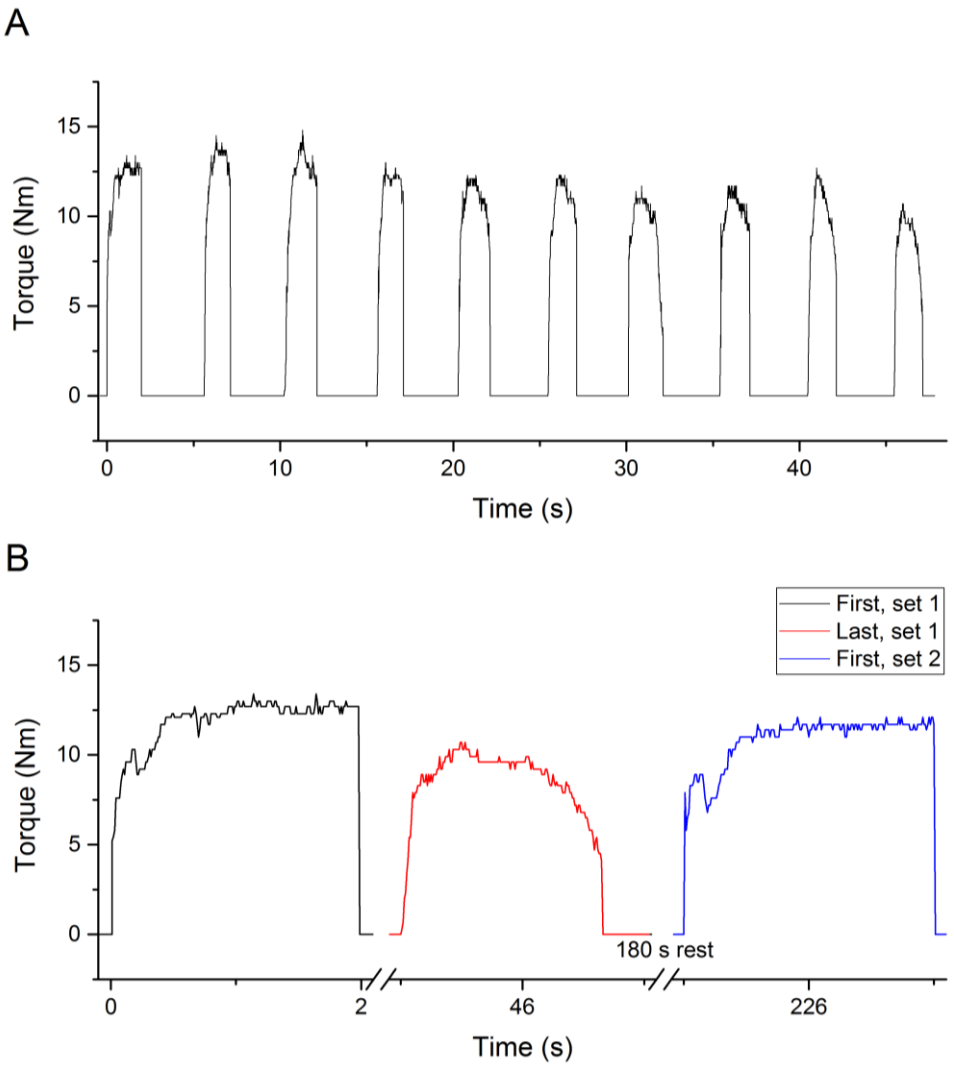

Figure 6.3. Strength training protocol. *(A)* Ballistic strength movements, lasting 2 seconds and spaced by a 3-seconds interval, performed by a representative participant with the left arm. *(B)* Comparison between the first movement of the first set, last movement of the first set and first movement of the second set (after 3-minutes rest).

Mean CVs and standard deviations measured during the skill testing phase are listed in Table 6.1. For the low force movements (25% MVC), CVs decreased by 28% in the left hand after training and by 65% in the right hand after training. Data analyses showed that force tracking performance increased between PRE and POST training (main effect of TIME; $F_{1, 9} = 10.266$, $P = 0.011$, $\eta^2 = 0.533$) as indicated by a significant reduction of CVs from PRE to POST in both hands. There was no statistically significant effect of HAND ($F_{1, 9} = 2.627$, $P = 0.140$, $\eta^2 = 0.226$). The interaction effect between TIME and HAND was non-significant ($F_{1, 9} = 3.879$, $P = 0.080$, $\eta^2 = 0.301$). For the high force movements (50% MVC), CVs decreased by

40% in the left hand after training and by 13% in the right hand after training. There was no significant effect of TIME ($F_{1, 9} = 0.832$, $P = 0.385$, $\eta^2 = 0.085$), HAND ($F_{1, 9} = 0.956$, $P = 0.354$, $\eta^2 = 0.96$) nor a significant interaction (TIME × HAND; $F_{1, 9} = 1.711$, $P = 0.223$, $\eta^2 = 0.160$). Examples of the torques produced by a representative participant during the skill training protocol are presented in Figure 6.4, and before and after skill training in Figure 6.6. All raw torque traces were smoothed with a 5-point unweighted smooth to aid data visualization.

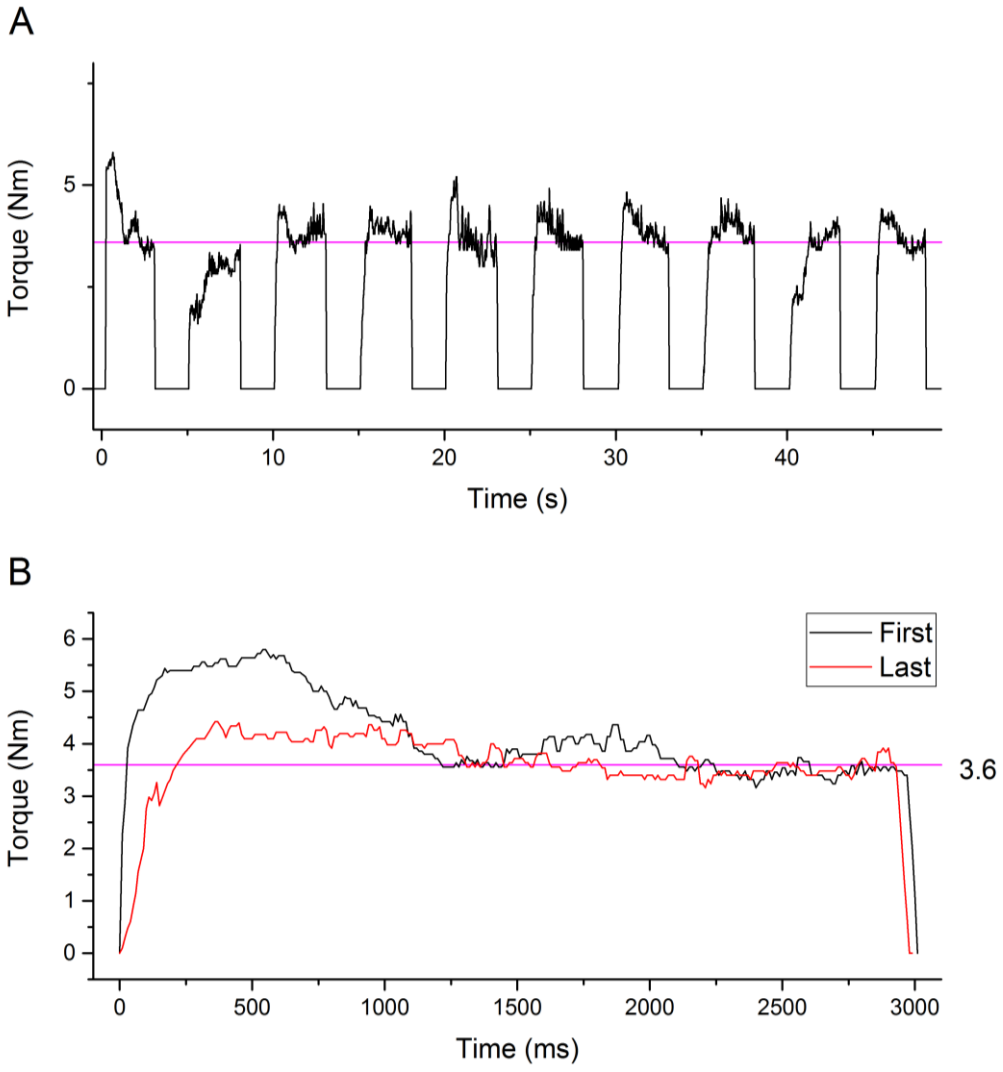

Figure 6.4. Skill training protocol. *(A)* Torques produced by a representative participant in the first skill training set. The target force was 3.6 Nm (25% MVC). Movements were performed with the left arm, lasted 3 seconds and were spaced by a 30-seconds interval (intervals not shown to help data visualisation). *(B)* Comparison between the first and last movement of the first set.

Table 6.1. Means and standard deviations of the CVs measured during skill testing. The right hand was assessed without visual feedback.

Parameter	Hand	MEAN ± SD (PRE)	MEAN ± SD (POST)
CV_ 25%	LEFT	15.26 ± 10.90	10.95 ± 8.82
CV_25%	RIGHT	34.26 ± 28.32	12.00 ± 9.53
CV_50%	LEFT	13.94 ± 10.97	8.28 ± 5.87
CV_50%	RIGHT	14.74 ± 12.72	12.85 ± 8.95

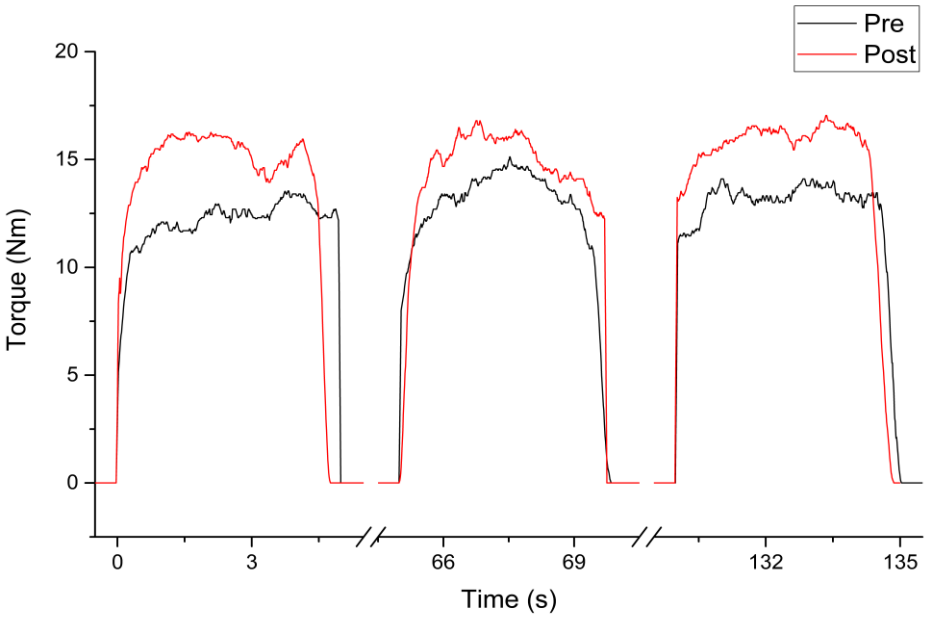

Figure 6.5. Strength testing. Comparison between the torques produced by performing three MVCs before (Pre) and after (Post) training. Movements were performed with the untrained right arm, lasted 5 seconds and were spaced by a 1-minute interval (time axis truncated).

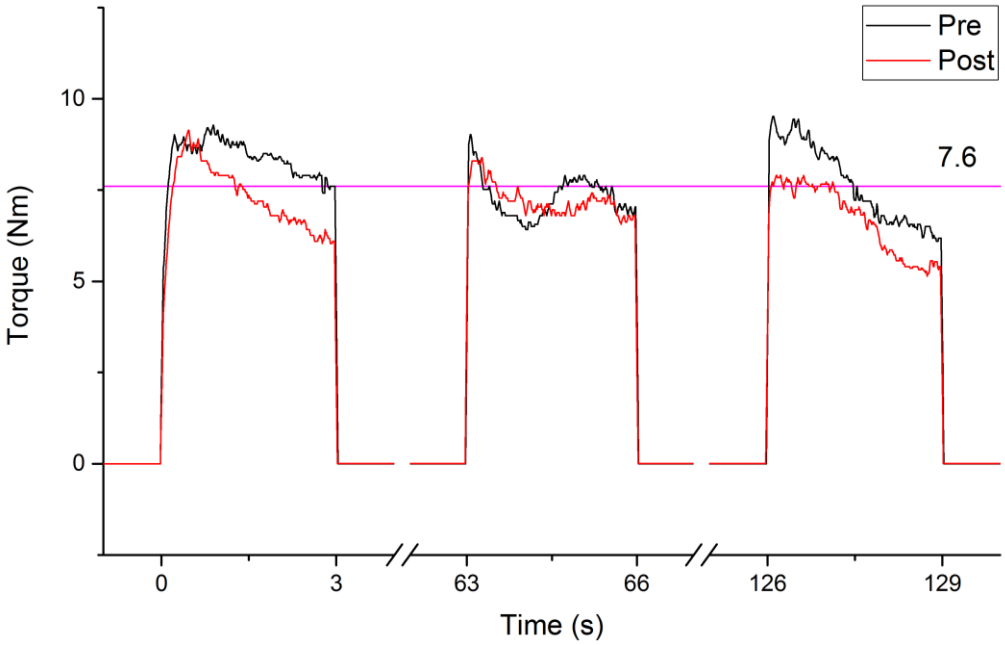

Figure 6.6. Skill testing. Comparison between the torques produced by performing movements at 50% MVC before (Pre) and after (Post) training. The target force was 7.6 Nm (50% MVC). Movements were performed with the untrained right arm, lasted 3 seconds and were spaced by a 1-minute interval (time axis truncated).

6.3.2. Neurophysiological parameters

Mean values and standard deviations of the parameters recorded (aMT, 120% aMT, M_{max}, $H_{M10\%}$) before and after (PRE, POST) both training protocols (STRENGTH, SKILL) are reported in Table 6.2. CVs and pre-stimulus EMG values are reported in Table 6.3. MEP values did not differ between the two conditions at the pre-training phase at aMT intensity ($t_{1,9}$ = -0.383, p = 0.710, d = 0.202) or 120% aMT intensity ($t_{1,9}$ = -0.415, p = 0.692, d = 0.226). Similarly, measures recorded with PNS did not differ before training in the two conditions ($M_{max;}$ $t_{1,8}$ = 0.827, p = 0.432, d = 0.113 and $H_{M10\%;}$ $t_{1,8}$ = 2.130, p = 0.066, d = 0.426) (data not measured in one participant).

Table 6.2. Means and standard deviations of the parameters recorded from the right FCR across time and conditions. M_{max} and $H_{M10\%}$ are expressed in mV, aMT and 120% aMT are expressed as $\%M_{max}$.

Condition	Parameter	MEAN ± SD (PRE)	MEAN ± SD (POST)
STRENGTH	aMT	2.74 ± 1.37	3.98 ± 3.38
	120% aMT	5.74 ± 1.58	6.23 ± 2.44
	M_{max}	4.82 ± 1.95	4.53 ±1.57
	$H_{M10\%}$	0.63 ± 0.39	0.61 ± 0.51
SKILL	aMT	3.10 ± 1.90	4.20 ± 2.57
	120% aMT	7.12 ± 5.01	8.20 ± 6.27
	M_{max}	4.59 ± 2.00	4.72 ± 2.24
	$H_{M10\%}$	0.50 ± 0.31	0.47 ± 0.30

M_{max} amplitudes did not change significantly across TIME ($F_{1,8}$ = 0.282, P = 0.610, η^2 = 0.034) or CONDITION ($F_{1,8}$ = 0.005, P = 0.947, η^2 = 0.001) and the interaction effect was non-significant (TIME × CONDITION; $F_{1,8}$ = 2.313, P = 0.167, η^2 = 0.224). Pre-stimulus EMG RMS did not change across TIME ($F_{1,8}$ = 1.237, P = 0.298, η^2 = 0.134) or CONDITION ($F_{1,8}$ = 0.508, P = 0.496, η^2 = 0.060) and the interaction effect was non-significant (TIME × CONDITION; $F_{1,8}$ = 1.897, P = 0.206, η^2 = 0.192). Similarly, there was no significant TIME ($F_{1,8}$ = 1.176, P = 0.310, η^2 = 0.128), CONDITION ($F_{1,8}$ = 0.821, P = 0.391, η^2 = 0.093) or TIME × CONDITION interaction ($F_{1,8}$ = 2.029, P = 0.192, η^2 = 0.202) effect for the $H_{M10\%}$ amplitudes (Figure 6.7 and 6.8). Pre-stimulus EMG RMS did not change across TIME ($F_{1,8}$ = 0.103, P = 0.756, η^2 = 0.011) or CONDITION ($F_{1,8}$ = 0.133, P = 0.724, η^2 = 0.015), and the interaction effect was non-significant (TIME × CONDITION; $F_{1,8}$ = 0.001, P = 0.974, η^2 = 0.000).

Table 6.3. Pre-stimulus EMG (base-EMG), expressed in μV, and CV of the parameters recorded from the right FCR across time and conditions.

Condition	Parameter	PRE		POST	
		Base-EMG (SD)	CV	Base-EMG (SD)	CV
STRENGTH	aMT	6.56 (2,61)	28.01	6.90 (2.36)	47.64
	120% aMT	8.00 (6.12)	31.48	7.60 (4.53)	42.66
	M_{max}	27.90 (6.84)	40.48	23.5 (10.35)	34.10
	$H_{M10\%}$	9.77 (4.88)	61.20	9.45 (5.07)	83.63
SKILL	aMT	6.10 (3.55)	31.41	5.42 (2.51)	44.83
	120% aMT	9.35 (8.32)	68.33	10.00 (6.25)	68.95
	M_{max}	21.4 (5.38)	43.53	23.8 (9.50)	47.40
	$H_{M10\%}$	9.38 (8.45)	61.25	9.0 (4.36)	65.22

For the MEPs recorded at aMT intensity, the two-way RM-ANOVA showed a significant effect of TIME ($F_{1,9} = 15.224$, $P = 0.004$, $\eta^2 = 0.628$, achieved power 0.93) but no significant effect of CONDITION ($F_{1,9} = 0.117$, $P = 0.740$, $\eta^2 = 0.013$) and a non-significant TIME × CONDITION interaction ($F_{1,9} = 0.007$, $P = 0.936$, $\eta^2 = 0.001$) (Figure 6.9 and 6.10). Pre-stimulus EMG RMS did not change across TIME ($F_{1,9} = 0.030$, $P = 0.866$, $\eta^2 = 0.003$) or CONDITION ($F_{1,9} = 1.442$, $P = 0.260$, $\eta^2 = 0.138$), and the interaction effect was non-significant (TIME × CONDITION; $F_{1,9} = 0.279$, $P = 0.610$, $\eta^2 = 0.030$). MEPs recorded at 120% aMT intensity did not differ across TIME ($F_{1,6} = 0.325$, $P = 0.590$, $\eta^2 = 0.051$), CONDITION ($F_{1,6} = 0.194$, $P = 0.675$, $\eta^2 = 0.031$) and the TIME × CONDITION interaction effect was non-significant ($F_{1,6} = 0.002$, $P = 0.967$, $\eta^2 = 0.000$). Pre-stimulus EMG RMS did not change across TIME ($F_{1,6} = 0.004$, $P = 0.954$, $\eta^2 = 0.001$) or CONDITION ($F_{1,6} = 0.344$, $P = 0.579$, $\eta^2 = 0.054$), and the interaction effect was non-significant (TIME × CONDITION; $F_{1,6} = 0.168$, $P = 0.696$, $\eta^2 = 0.027$).

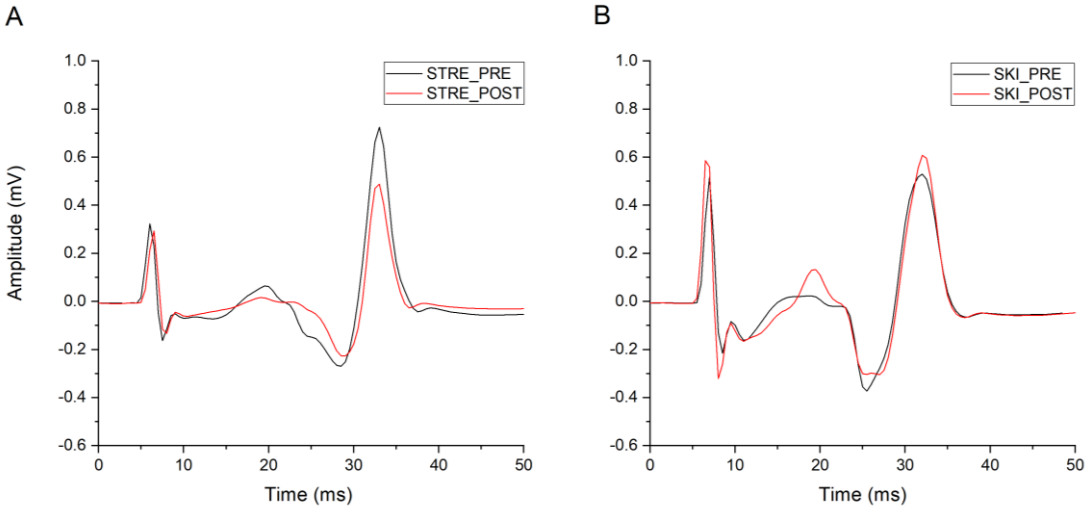

Figure 6.7. Examples of monosynaptic reflexes across time and conditions. *(A)* Mean of 10 monosynaptic reflexes ($H_{M10\%}$) evoked before (STRE_PRE) and after (STRE_POST) strength training in a representative participant. *(B)* Mean of 10 monosynaptic reflexes ($H_{M10\%}$) evoked before (SKI_PRE) and after (SKI_POST) skill training in a representative participant.

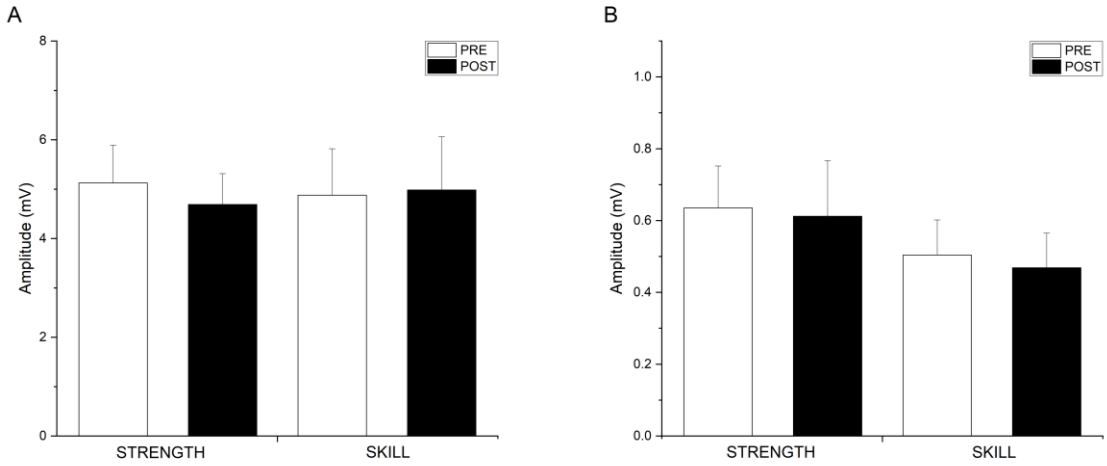

Figure 6.8. Results from peripheral nerve stimulation. *(A)* M_{max} (n = 9) and *(B)* $H_{M10\%}$ (n = 9) mean ± SE amplitudes recorded before (PRE, white bars) and after (POST, black bars) strength training and skill training.

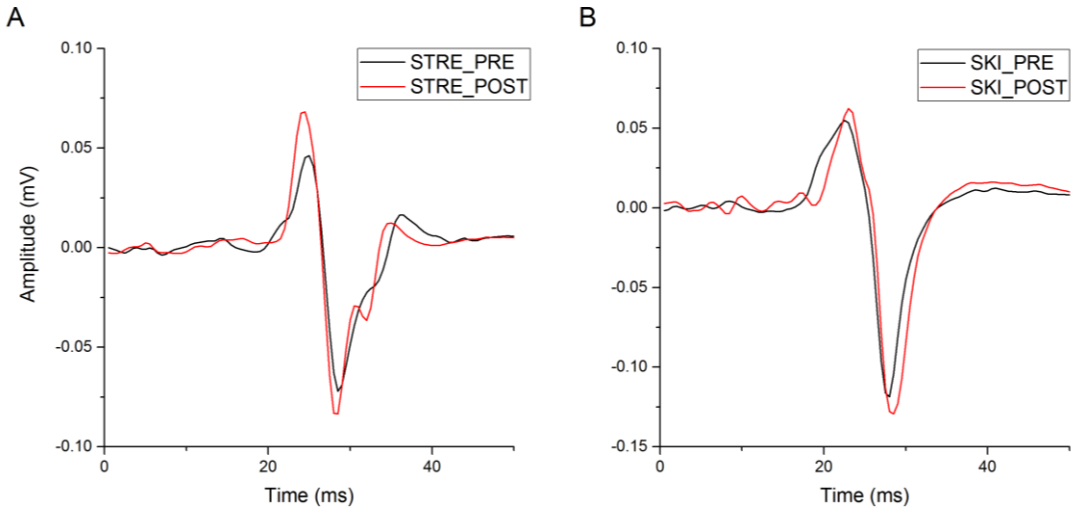

Figure 6.9. Examples of MEPs across time and conditions. *(A)* Mean of 10 MEPs evoked at aMT stimulation intensity before (STRE_PRE) and after (STRE_POST) skill training in a representative participant. *(B)* Mean of 10 MEPs evoked at aMT stimulation intensity before (SKI_PRE) and after (SKI_POST) strength training in a representative participant.

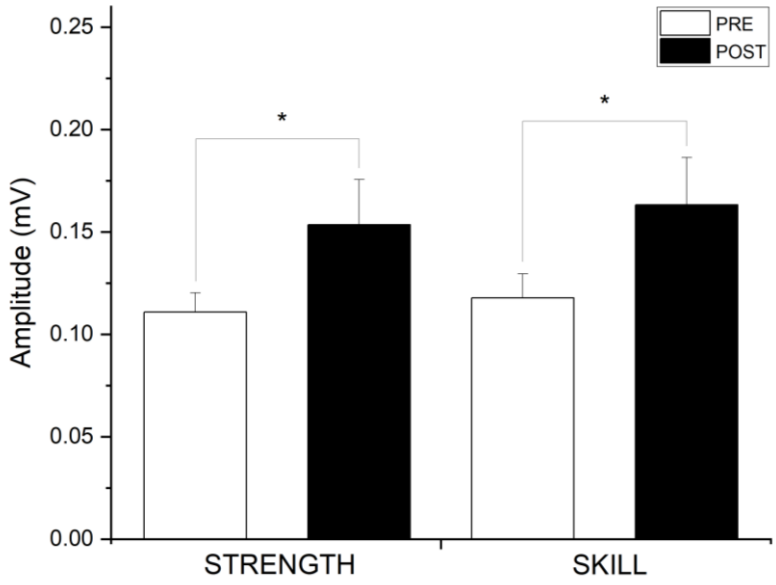

Figure 6.10. Results from TMS delivered at aMT stimulation intensity. Mean (n = 10) ± SE of the MEP amplitudes recorded before (PRE, white bars) and after (POST, black bars) strength training (left side) and skill training (right side) at aMT intensity. Asterisks denote a significant PRE vs. POST difference.

For the conditioning phase, the RMS of the background EMG did not change across CONDITION ($F_{1,9}$ = 0.147, P = 0.710, η^2 = 0.016) or TIME ($F_{1,9}$ = 0.041, P = 0.844,

η^2 = 0.005) and the interaction effect TIME × CONDITION was non-significant ($F_{1,6}$ = 0.277, P = 0.612, η^2 = 0.030). Results from paired sample t-tests showed that there was no difference in the unconditioned H-reflex values obtained during the conditioning protocol before and after training in the strength ($t_{1,9}$ = -1.037, p = 0.327, d = 0.313) and in the skill condition ($t_{1,9}$ = -0.663, p = 0.524, d = 0.148). Similarly, there was no difference at baseline between the unconditioned H-reflex values recorded in the strength condition and the ones recorded in the skill condition ($t_{1,9}$ = 0.652, p = 0.531, d = 0.185) (Table 6.3). The two-way repeated-measures ANOVA run to assess for changes in MEP amplitudes collected at 90% aMT intensity showed no significant effect of TIME ($F_{1,9}$ = 3.279, P = 0.104, η^2 = 0.267), CONDITION ($F_{1,9}$ = 0.055, P = 0.821, η^2 = 0.006) and no significant interaction effect ($F_{1,9}$ = 0.005, P = 0.945, η^2 = 0.001). Mean data recorded when PNS was delivered alone (Unconditioned- H) and TMS was delivered alone (Subthreshold TMS) are reported in Table 6.4. For the analysis of TMS-conditioned H-reflex, values representing the difference between PRE and POST amplitudes collected at each conditioning-test interval starting from EF up to EF+6 were entered into the repeated-measures ANOVA. The main effect of CONDITION was non-significant ($F_{1,9}$ = 0.019, P = 0.895, η^2 = 0.002). Similarly, there was no effect of ISI ($F_{3,7}$ = 1.550, P = 0.284, η^2 = 0.399) nor a significant interaction between CONDITION and ISI ($F_{3,7}$ = 3.426, P = 0.081, η^2 = 0.595). Mean H-reflexes amplitudes collected before and after both training protocols at multiple conditioning-test intervals are reported in Figure 6.11, expressed as percentages to the unconditioned mean value.

Table 6.4. Means and standard deviations of the Unconditioned H-reflexes and MEPs elicited by subthreshold TMS across time and conditions. All data are expressed in mV.

Condition	Parameter	MEAN ± SD (PRE)	MEAN ± SD (POST)
STRENGTH	Unconditioned- H	0.57 ± 0.29	0.73 ± 0.65
	Subthreshold TMS	0.02 ± 0.02	0.03 ± 0.03
SKILL	Unconditioned- H	0.51 ± 0.28	0.57 ± 0.45
	Subthreshold TMS	0.02 ± 0.04	0.03 ± 0.04

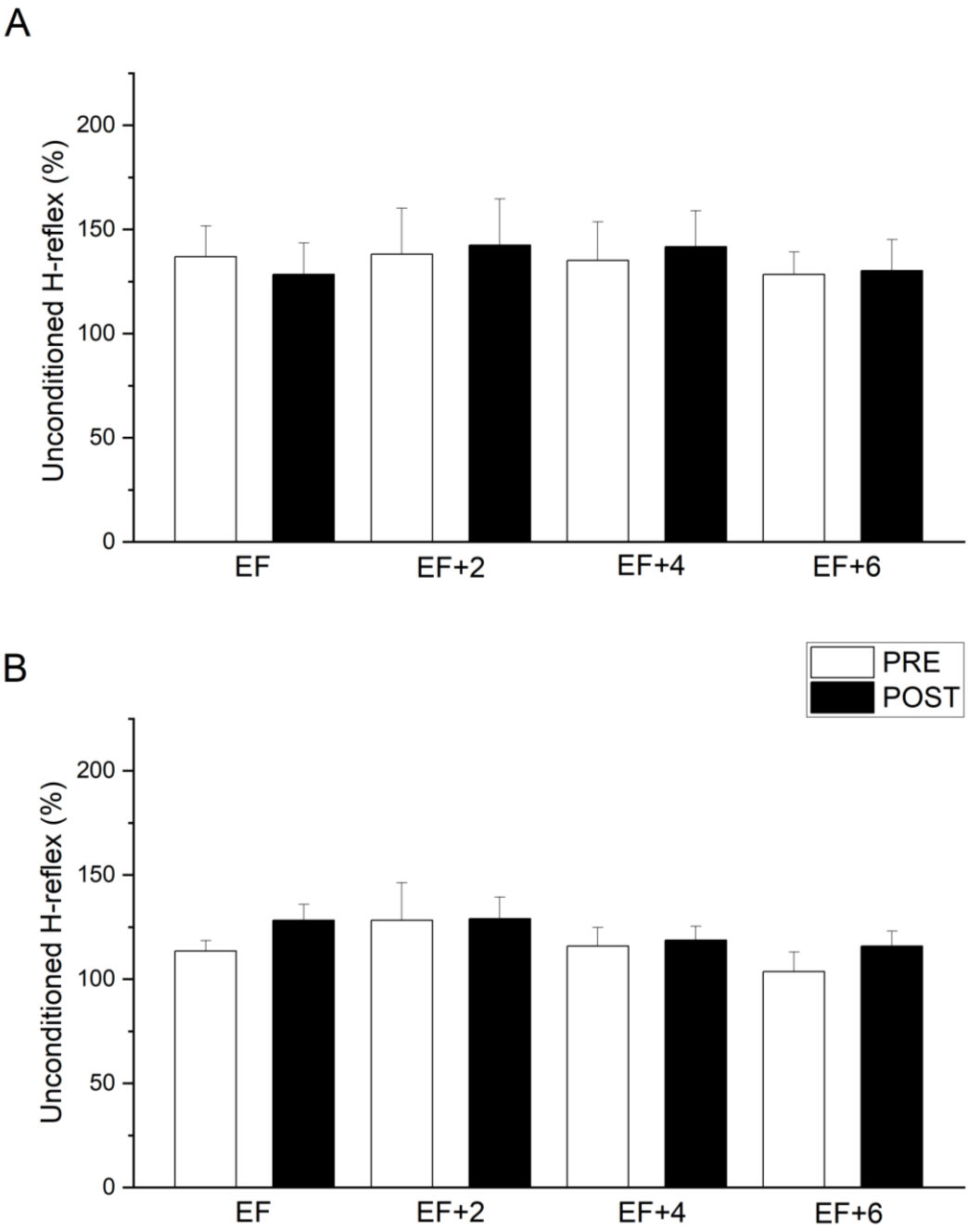

Figure 6.11. Mean conditioned H-reflex values across time and conditions. *(A)* Mean (n = 10) ± SE of the conditioned H-reflex expressed as a percentage of the unconditioned reflex recorded before (PRE, white bars) and after (POST, black bars) strength training at multiple conditioning-test intervals. *(B)* Mean (n = 10) ± SE of the conditioned H-reflex expressed as a percentage of the unconditioned reflex recorded before (PRE, white bars) and after (POST, black bars) skill training at multiple conditioning-test intervals.

6.4. Discussion

This study was designed to investigate the effects of a single session of unimanual strength training and skill training on performance and on the neural excitability of the contralateral hand. Three specific aims were described: (1) to measure changes in strength in both limbs after the training protocol; (2) to measure changes in skilled performance in both limbs after the training protocol; (3) to compare changes in excitability of the neural circuits of the untrained limb after skill and strength training. Changes in neural excitability were assessed by measuring MEPs elicited by TMS at two stimulation intensities, the amplitude of the monosynaptic reflex evoked upon electrical stimulation of the median nerve and the effects of cortical stimulation on the afferent volley produced by median nerve stimulation at different conditioning-test intervals. Each aim is discussed in the following section of the thesis, together with some consideration on the role of the primary motor cortex in mediating the cross-education of strength phenomenon.

6.4.1. Behavioural effects of strength training

Acute changes in strength between the baseline pre-training phase and the post-training phase were assessed by measuring the peak torque produced during three consecutive MVCs. The training protocol was not successful in increasing the maximal strength produced during wrist flexion in the trained nor the untrained hand. One possible explanation for this finding is that a single session is not sufficient to induce changes in peak force in the trained and untrained muscles. This is in line with the evidence that increased protein synthesis resulting from single bouts of resistance training develops within hours after training (Phillips et al., 1997). Indeed, similar lack of acute cross-education effects have been previously reported in the literature. Hortobágyi and his colleagues (Hortobágyi et al., 2011) measured changes in FDI peak force over the course of 20 sessions of a strength training protocol comprising five blocks of ten movements at 80% MVC on each session. The authors reported no significant effects of training on peak force until the 10^{th} session. In this light, it is worth mentioning that while the use of a single session can explain the lack of effects in the trained hand, the cross-education effect does not depend on muscular adaptations since training does not produce enough contralateral motor unit activation to drive muscular adaptation (Carroll et al., 2006). Another possibility is that the conditions of practice were not optimal to induce cross-education of strength (Ruddy

and Carson, 2013). It has been proposed (Howatson et al., 2013) that cross-education can be augmented by showing participants the movements performed by the exercising hand through a mirror. Zult and his colleagues (2016) tested this hypothesis by comparing the effects of 15 sessions of wrist flexions at 80% MVC between a mirror training group and a no-mirror training group. The results showed that after training cross-education was higher in the mirror tracing (61% increase in MVC) group than in the no-mirror training (34% increase in MVC) group (Zult et al., 2016).

An alternative explanation is that the total number of repetitions produced during training was too small to induce training effects (Selvanayagam et al., 2011). In support of this hypothesis, Nuzzo and colleagues (2016) observed acute increases in peak rate of force development in the trained arm after two blocks of 96 elbow flexor contractions. Finally, there is a possibility that the outcome measure used in the study (peak torque) is not an adequate measure of the changes occurring after training. Selvanaygam and colleagues (2011) observed acute effects of strength training on the movements produced with both the trained and untrained arm with a protocol (four sets of 10 ballistic contractions) similar to the one used in the discussed study. However, their mean outcome measures were TMS-induced twitch force resultant vectors rather than peak forces variables. Using more sensible outcome parameters such as changes in muscle recruitment patterns observed via EMG could potentially reveal hidden features of strength training unobserved in this study. Finally, there is a possibility that the strength did not increase in the untrained arm because the level of transfer from the non-dominant to the dominant hand is limited (Farthing et al., 2005). Right-handed participants were allocated to a left-training group, a right-training group or a control group. After 6 weeks of maximal isometric ulnar deviations, increases in strength were observed in the left hand for the right-training group, but not in the right hand for the left-training group nor in the control group which did not train (Farthing et al., 2005).

6.4.2. Behavioural effects of skill training

The efficacy of the skill training protocol in increasing performance in both limbs was measured by calculating coefficient of variations for each set of three movements and comparing baseline pre-training values with post-training values. Statistical analyses showed a significant effect of TIME for low-force movements, indicating that the variability of movements significantly decreased in both limbs after training. This

finding indicates that the trained skill (force-matching) transferred to the contralateral untrained limb. However, the variability of movements produced at 50% of MVC did not change after training in the trained or untrained hand. A close look at the CV values measured during 25% and 50% of MVC movements revealed that participants were substantially better at baseline in controlling the higher force. This is in line with the finding that wrist flexion movement variability decreases with increasing force (Salonikidis et al., 2009). It is thereby possible that performance errors at baseline were too low to benefit substantially from a single 40-movements session of practice.

Many of the studies reported in the literature of bilateral transfer employed variants of sequence learning task, in which participants learn to reproduce a given sequence (either implicitly or explicitly defined) of movements over time (Ruddy and Carson, 2013). However, a potential confounding factor of using such tasks is that the cognitive component (having to learn a specific sequence) is likely to generate activity along an extended network of cortical areas (Ruddy and Carson, 2013). At the other end of the spectrum, ballistic training tasks have been used to demonstrate that the acceleration component of motor learning transfers to the non-trained arm (Carroll et al., 2008). Tasks requiring strong unilateral contractions induce bilateral motor cortical activation (Lee et al., 2010), but there is still controversy on whether ipsilateral (to training hand) M1 activity during movement production is necessary to induce cross-education (Leung et al., 2015). The skill training task used in this study required participants to produce and maintain a certain amount of force (torque) for a given time. The intensities of the movements (25% and 50% of MVC) were chosen to prevent the effects of fatigue and to limit bilateral activation on M1 while training.

The conditions of practice were chosen to maximise both behavioural improvement and cortical engagement. First, short bouts of activity (3 seconds) were followed by longer intervals (30 seconds). This learning condition in which the time spent practicing is less than the time spent resting is called distributed practice and was shown to be beneficial for visuomotor training (Bourne Jr and Archer, 1956). Second, participants received visual feedback of the outcome of their movement (knowledge of performance) online, and learned to modify future behaviour accordingly (Hurley and Lee, 2006). This additional source of feedback augmented the information arising from proprioceptive feedback. The participants were instructed on when to start and end the movement by the dynamometer software. Multiple studies have shown that externally triggered movements, as opposed to self-generated ones, induce use-

dependent plasticity in motor areas (Perez et al., 2006). Leung and colleagues (2015) showed that metronome-paced strength training and skill training both successfully increased the excitability of the untrained motor cortex, while self-paced strength training did not. Unfortunately, the authors did not measure behavioural changes post-training in the untrained limb. The current study proved that the neural mechanisms were paralleled by performance increases.

A recent study assessed whether six weeks of force-matching training successfully increased force control in the untrained limb (Green and Gabriel, 2018). The authors reasoned that the amount of cross-education elicited by the training might have been under-estimated because the testing movements were qualitatively different from the training ones (isometric vs dynamic). This issue was resolved in the current study by keeping movement properties constant (isometric contractions) between the two phases. There is a possibility that the participants started learning how to better control force during the baseline testing performed in the untrained limb at the beginning of the session. This issue was controlled for by testing without concurrent visual feedback. Adding this feature also ensured that effects in the untrained limb did not depend on the increased ability of participants in interpreting feedback (a visual feature) but rather on the acquisition of a better force control strategy (a motor feature). Similar findings were reported in a study in which participants learned to reproduce a specified (35% MVC) pinch force output (Goodall et al., 2013). Errors in force production were reduced after training in the untrained hand, indicating that the knowledge about force production transferred to the contralateral hand. In the current study, the findings of decreased movement variability are indicative of adaptation of specific internal models related to the task, which became accessible to the untrained hemisphere after training (Kawato, 1999, Anguera et al., 2007). Finally, a limitation of the study is the use of CVs as measures of force control. CV values were computed as the standard deviation of the torque data divided by the mean average torque. This outcome parameter was chosen to maximise the importance of maintaining a constant force production irrespectively from the amount of force generated. The use of outcome measures which take into account the target force to produce, such as root mean square error (RMSE), could help understanding whether the participants also learned to better estimate the output force produced.

6.4.3. Changes in the monosynaptic reflex pathway

The role of spinal circuits in mediating the cross-education of strength phenomenon is largely unknown (Carroll et al., 2006). There is indeed evidence that unilateral strength training induces long-lasting changes in spinal circuits of the trained hand (Aagaard et al., 2002). When tested after 14 weeks of heavy-lifting training, the amplitudes of the H-reflex and of the V-wave (which represent the excitability of spinal motoneurons) evoked in the soleus muscle were found to be increased (Aagaard et al., 2002). The possibility that similar mechanisms might underlie the increase in performance of the untrained limb was assessed by Lagerquist et al. (2006). The authors first replicated the finding that strength training (maximal isometric plantar flexions in the specific case) increased the amplitudes of the H-reflex recorded in the training soleus muscle. Unilateral leg training was effective in increasing maximal muscle contractions on the untrained leg. However, they could not observe corresponding changes in the excitability of the untrained spinal circuits (Lagerquist et al., 2006). These findings are in line with the ones reported in the current study. The M_{max}, representing the response to excitation of the entire motoneuron pool, and the H-reflex, an index of motoneuron excitability, recorded from the FCR muscle were previously shown to exhibit excellent intersession reliability (Chapter 4). In the current study, neither the amplitude of the M_{max}, which was used to normalise H-reflex values before training, nor the amplitude of the monosynaptic reflex were affected by strength training. All this evidence suggests that a single session of unilateral strength training does not induce use-dependant plasticity in the untrained alpha motoneuronal pool. Nevertheless, there is a network of spinal circuits which could influence motor output and whose excitability cannot be selectively assessed by recording the monosynaptic reflex (Lee and Carroll, 2007). The possible role of circuits mediating presynaptic inhibition in the current study is discussed in the next paragraph.

The study of the plastic changes occurring in the motor system during the acquisition of motor skills has traditionally been focused on cortical and subcortical structures. The extensive work of Wolpaw and his colleagues at the State University of New York provided evidence that the monosynaptic reflex pathway undergoes use-dependent plasticity and this plasticity is manifested through different stages of skill learning (Wolpaw, 2001, Thompson and Wolpaw, 2014). The role of spinal circuits in the bilateral transfer effect, especially in upper limbs muscles, is largely unknown.

Tinazzi and Zanette (1998) did not observe changes in the H-reflex pathway while participant produced finger tapping movements with the other hand, independently of the difficulty of the task. When participants are asked to generate increasingly higher unilateral thumb abductions (from 10% to 90% MVC), the responses evoked upon cortical and peripheral stimulation in the contralateral resting APB muscle increased but only at high contraction intensities (Muellbacher et al., 2000a). In the current study, a force-generating component was added to the skill training protocol (force-matching) in order to assess whether it affected contralateral spinal circuits. While the force-matching protocol successfully elicited long-lasting changes in ipsilateral M1 excitability, it did not affect the monosynaptic reflex recorded from the trained FCR muscle. This adds to the evidence that bilateral transfer of skill does not depend on the untrained spinal motoneurons excitability.

6.4.4. Changes in the TMS-conditioned monosynaptic reflexes

During rhythmic unilateral wrist flexion, MEPs evoked from the ipsilateral M1 were increased and the afferent volley evoked by electrical stimulation of the median nerve was inhibited, but the excitability of the spinal motoneurons remained unchanged (Carson et al., 2004). Stronger increases in ipsilateral cortical excitability were observed in the phases of movement in which the left FCR was most active. The authors suggested that unilateral movements modulate the ipsilateral descending drive to the homologous muscle by presynaptic inhibition of the afferents according to the specific phase of the movement (Carson et al., 2004). The method of TMS-conditioning of the monosynaptic reflex employed in this study gives information about pathway-specific plasticity in the spinal cord (Leukel et al., 2012). It was previously demonstrated (Chapter 4) that the method exhibits moderate to excellent reliability across a range of ISIs. The protocol employed in Chapter 4 was replicated in the current study but participants were asked to produce a small (5% MVC) baseline contraction during stimulation to increase the excitability of the motoneuron pool (Knikou, 2008).

In line with early reports of the method (Mazzocchio et al., 1994, Nielsen and Petersen, 1995) TMS was delivered at intensities below threshold to elicit EMG responses in the FCR. This feature ensured that the only component of the cortical drive to spinal motoneurons was corticomotoneuronal (e.g. monosynaptic) (Nielsen and Petersen, 1995). At higher stimulation intensities, multiple descending waves

supposedly originating from intracortical circuits or higher motor structures in the brain contribute to MEP generation (Di Lazzaro and Ziemann, 2013).

If training elicits neural plasticity in the circuit mediating presynaptic inhibition of Ia afferents, this should be reflected in a modulation of the amount of facilitation observed when PNS is delivered at an interval such that the afferent volley reaches spinal motoneurons 1-2 ms after the corticospinal volley (Meunier and Pierrot-Deseilligny, 1998). Results from the current study showed that the conditioning effect of TMS on the monosynaptic reflex elicited in the untrained FCR did not change after contralateral strength or skill training. Together with the lack of peripheral changes (e.g. H-reflex), this suggests that spinal circuits are not involved in the cross-education and bilateral transfer effects (Leukel et al., 2012). Importantly, this finding does not exclude that changes in the amount of presynaptic inhibition on the afferents occurred in the trained hand. This possibility was not tested in the current study because that would have required longer sessions, and the after-effects of use-dependent plasticity could have vanished at these time-scales (Bütefisch et al., 2000). It does however indicate that the cross-education of skill does not depend on the modulation of corticospinal-mediated presynaptic inhibition acting on Ia afferents.

It still remains to be established whether the increase in MEP amplitudes observed after both training protocols results from higher cortico-motoneuronal drive or higher inputs to pyramidal neurons from other areas (Groppa et al., 2012b). This can be assessed by using the same TMS-conditioning protocol employed in the current study but with cortical stimulation above motor threshold, with the same rationale that the conditioning-test intervals after the early facilitation correspond to polysynaptic pathways to spinal motoneurons (Niemann et al., 2017). An important drawback of using the monosynaptic reflex, and conditioning it with TMS, to assess changes in spinal excitability is that the electrical pulse does not stimulate muscle spindles (Burke, 2016). Muscle spindles are mechanoreceptors which discharge according to muscle lengthening providing proprioceptive information to the CNS (Proske and Gandevia, 2012). The sensitivity of muscle spindles in detecting changes in lengthening is controlled through gamma (γ) motoneurons located in the spinal cord by regulating the tension of intrafusal fibres (Murthy, 1978). In forearm muscles, microneurography recordings showed that spindle afferent activity, and therefore fusimotor neurons, are modulated by stimulation of the primary motor cortex (Rothwell et al., 1990). This was confirmed by the finding that stretching a muscle

while active induces stretch reflexes at multiple latencies in the recorded EMG: the shorter-latency response which represents the monosynaptic spinal stretch reflex, partially overlapping with the H-reflex pathway; the longer-latency reflex which is conducted through a transcortical pathway (Palmer and Ashby, 1992b). The importance of the fusimotor system in postural control is well-established (Merton, 1953), but relatively little is known about its role in motor control and motor learning (Lan and He, 2012).

6.4.5. Comparing the effects of skill and strength training on the ipsilateral M1

The last aim of the study was to assess whether motor skill training and strength training induced different changes in the excitability of the untrained M1 as assessed with TMS. Results from the RM-ANOVA support the theory that the two training modalities facilitate cross-education through a similar mechanism, by modulating the excitability of the ipsilateral hemisphere. At a first view, this finding seems to contradict the one reported by Jensen and his colleagues (2005) on the effects of training modalities on corticospinal excitability. The authors tested whether 4 weeks (12 sessions) of heavy load strength training and visuomotor training differentially affected the trained M1 excitability. Surprisingly, the authors found that MEPs increased after skill training but decreased after strength training (Jensen et al., 2005).

While in the current study MEPs amplitudes were measured exclusively from the untrained (ipsilateral to training) hemisphere, it is unlikely that a protocol which decreased excitability in the trained hemisphere could induce the opposite effect on the untrained hemisphere. There are, however, important methodological differences which might explain the differences between these findings and the ones reported in the current study. First, the amplitude of MEPs evoked through M1 stimulation was measured at baseline (before training), after 2 weeks and after 4 weeks of training but no acute effects (within the first session) were tested. A recent study (Mason et al., 2020) assessed changes in corticospinal excitability observed after unilateral wrist strength training in the trained hemisphere. Contrarily to Jensen et al. (2005), the authors observed increases in corticospinal excitability, measured through MEP recruitment curves, after a single and multiple sessions of strength training. In addition, the strength training protocol designed by Jensen et al. (2005) included self-paced biceps curl rather than visually guided movements. Indeed, a later study

specifically tested whether the conditions of training could determine the outcomes of strength training in terms of cortical excitability (Leung et al., 2015). The authors tested whether unilateral training in a visuomotor tracking task, metronome-paced strength training and self-paced strength training modulated the excitability of the trained and untrained hemispheres. MEP amplitudes increased and SICI decreased after training in the visuomotor tracking task and metronome-paced strength training but not in the self-paced strength training. Importantly, the excitability of the ipsilateral (untrained) hemisphere increased as well for both the metronome-paced strength training and visuomotor tracking group (Leung et al., 2015). A series of studies from the same authors confirmed the finding that conditions of practice determine the increase in cortical excitability observed after training (Leung et al., 2017, Leung et al., 2018). Corticospinal excitability and SICI were recorded from the ipsilateral (untrained) hemisphere after slow-paced strength training, self-paced strength training and visuomotor skill training after 2 and 4 weeks of training (3 sessions for week) (Leung et al., 2018). In the slow-paced strength training, the eccentric and concentric phase of biceps curls were timed to a metronome. Cross education of strength was observed in both strength training groups, and bilateral transfer of tracking performance was observed in the skill training group. Nevertheless, MEP amplitudes increased and SICI decreased only in the slow-paced strength training and visuomotor skill training groups (Leung et al., 2018). All these findings point to the possibility that practice synchronised to acoustic or visual cues affects corticospinal excitability (Goodwill et al., 2012). An important addition of the study hereby discussed is the use of visual feedback when training for strength to provide participants with instantaneous knowledge of performance. Jensen and his colleagues speculated that visual feedback, which was lacking in their strength training, could be necessary to induce changes in the MEPs elicited from M1 (Jensen et al., 2005).

Future studies might address the specific role of conditions of practice in guiding the cross-education of strength phenomenon. In contrast with previous findings (e.g. Lee et al., 2010), the amplitude of MEPs evoked at 120% of MT did not change after either of the training protocols. Importantly, Lee and colleagues (2010) recorded responses at rest rather than during small baseline contractions, a state which yields substantially smaller responses compared to the ones hereby reported. In addition, EMG was collected from hand muscles rather than from forearm muscles as in the current study.

Since recruitment curves recorded from the FCR muscle show a plateau at relatively small intensities (around 130% rMT) (Suzuki et al., 2012), it is possible that 120% aMT stimulation at baseline was already capable of activating the corticospinal neurons which would become more excitable after training.

6.4.6. The relationship between strength training and M1 excitability

The lack of peak force improvements observed in this study after strength training despite a concurrent increase in the excitability of the untrained hemisphere raises an important question: what is the role of the motor cortex in mediating increases in strength and the cross-education of strength phenomenon? The first possibility is that the increase in MEP amplitudes is an epiphenomenon not directly related to strength. In support of this hypothesis, multiple studies reported a lack of correlation between the neurophysiological changes measured with TMS and the amount of cross-education (Carroll et al., 2008, Hortobágyi et al., 2011). In addition, there is still controversy on whether the net effect of strength training on the trained motor cortex is an increase (Beck et al., 2007), a decrease (Jensen et al., 2005) in excitability or it has no effect at all (Kidgell and Pearce, 2010). Regarding the trained hemisphere, it has been shown that strength gains are attenuated, but still significant, if the motor cortex is stimulated with low-frequency rTMS during training (Hortobágyi et al., 2009). Similarly, the improvements in peak acceleration observed after a session of ballistic finger abduction training in the untrained hand are decreased if rTMS is delivered to the untrained (ipsilateral to the movement) hemisphere, which indicates that the cross-transfer phenomenon partially relies on ipsilateral M1 (Lee et al., 2010).

The second possibility is that MEP amplitudes increased as a result of increased synaptic efficacy between corticospinal and motoneuronal synapses rather than as an increase in cortical excitability (Nuzzo et al., 2016). In the current study, changes in spinal excitability were assessed by measuring the amplitude of the monosynaptic reflex at 10% of M_{max}. However, this technique and TMS do not activate the same motor units (Morita et al., 1999). A better technique to selectively assess transmission through the pyramidal tract is electrical stimulation at the cervicomedullary level (McNeil et al., 2013). Increases in cMEP amplitudes have been observed after a single session of ballistic finger abduction (Giesebrecht et al., 2012) and ballistic isometric elbow flexion training (Nuzzo et al., 2016).

The final possibility is that the trained and untrained motor cortex can facilitate strength training and cross-education depending on the nature of the task and on learning conditions. Indeed, both the amount of cross-education and corticospinal excitability depend on the nature of the movement performed during training, with eccentric strength training significantly superior than concentric strength training in guiding ipsilateral neural activity and behavioural performance (Kidgell et al., 2015). In the future, the specific role of training characteristics rather than the behavioural outcome measure employed needs to be addressed to further characterize the link between neural adaptation and cross-education (Carroll et al., 2011).

6.5. Conclusions

This study was designed to assess the effects of a single session of unimanual skill training or strength training on movements performed with the trained and untrained hands and on the motor circuits of the untrained hand. First, the finding that unilateral skill training acutely increases both performance in the contralateral hand and neural excitability in the untrained motor cortex was replicated. However, a single session of ballistic strength training was not sufficient to increase the maximal force produced during isometric wrist flexion in the trained nor the untrained hand. The novel finding of the present study was that MEP amplitudes recorded from the untrained FCR muscle were found to be increased after a single session of strength training. This happened without any corresponding change in muscular excitability, as measured by the pre-stimulus EMG. It was argued that increased cortical excitability after strength training represents a learning component inherent to the task and which might promote the increment in maximal force observed over multiple sessions. The lack of modulation of the monosynaptic reflex pathway, both alone and when conditioned by TMS, suggests that spinal circuits have a limited role in mediating the cross-education of strength and bilateral transfer of skills effects. This study addressed the third and fourth aims of this thesis by assessing the behavioural and neurophysiological effects of a single session of unilateral strength training and skill training on the contralateral limb.

Chapter 7 – General discussion and conclusions

7.1. Introduction

In the last few decades, TMS and PNS became popular and promising non-invasive techniques to study the excitability of the motor system (Hallett, 2007). TMS is used in clinical settings as a diagnostic tool to investigate changes occurring after injuries to the motor system (Streletz et al., 1995) and more recently as a tool to augment rehabilitation (Benito et al., 2012). Despite these uses and applications, little is known about the neural pathways which are engaged when TMS is delivered to the motor cortex and which constitute the MEP recorded via surface EMG. In order to elucidate this issue, TMS has been used in combination with nerve stimulation in protocols that permit to differentiate between changes in the excitability occurring at the cortical level and the ones due to spinal and peripheral mechanisms (Hannah et al., 2018). However, no study hitherto has established whether this protocol, named TMS-conditioning of the monosynaptic reflex, can be measured reliably from the FCR muscle. Given this, the first objective of this thesis was to characterise the reliability of TMS when delivered by itself and in conjunction with nerve stimulation. In the first experimental chapter (Chapter 4) the intersession reliability of multiple parameters commonly recorded with TMS targeting the motor cortex and PNS of the median nerve, both alone and in conjunction, was evaluated in a population of healthy participants.

Another problem which limits the importance of the MEP recorded via TMS is its poor validity as a measure of corticospinal excitability. The aim of the second study (Chapter 5) was to assess the role of confounding factors such as noise and expectation on the recorded MEPs. This study investigated if and how the sound produced by the TMS system upon stimulation and the anticipating stimulus delivery affected MEPs measured in healthy participants. Non-invasive stimulation techniques can reveal the re-organisation occurring in the motor system in response to training. In addition, these can be used to reveal which neural pathways are activated when unimanual movements are performed (Carson et al., 2004). Therefore, aim of the third study (Chapter 6) was to investigate a novel application of non-invasive stimulation, as a method to study the spinal and cortical circuits which underline the behavioural phenomenon of bimanual transfer of skill and strength training. Moreover, this study assessed whether a single session of skill (force-matching) and strength training

modify performance of the untrained limb through the same neural mechanisms (Ruddy and Carson, 2013). This chapter will discuss the findings of these 3 experimental studies and their relevance for the future use of non-invasive stimulation of the motor system. Potential limitations of the designed protocols and the data produced through them will then be highlighted. A more general discussion on the issues that require to be addressed when using non-invasive stimulation techniques in human studies is also presented. Finally, the relevance of the presented results for future applications of the described techniques to the study of motor control will be discussed.

7.2. Main findings

7.2.1. Reliability of the TMS-conditioned monosynaptic reflex in the Flexor Carpi Radialis muscle

MEPs evoked by stimulation of the primary motor area and recorded with surface EMG are commonly used to measure the excitability of the corticospinal tract under different conditions (Chen et al., 1998). However, the outcome of stimulation does not depend only on the descending cortical volley but also on the excitability of spinal motoneurons, the final common pathway upon which segmental outputs converge (Burke and Pierrot-Deseilligny, 2010). Given this, MEP amplitudes alone cannot be used to discern between the monosynaptic component of the corticospinal volley and other polysynaptic contributions. This distinction is important in order to understand which neural populations are affected by injuries or interventions (Burke and Pierrot-Deseilligny, 2010). One method which permits differentiation between direct and indirect pathways to motoneurons is the use of conditioning the monosynaptic reflex with cortical stimulation, and measuring changes in amplitude compared to unconditioned values (Nielsen et al., 1993b).

TMS protocols often start with establishing an individual motor threshold (MT), defined as the minimum intensity of stimulation necessary to evoke MEPs of a chosen amplitude, for every participant (Rossini et al., 1994). MTs estimated with the Rossini-Rothwell method (Chapter 3.5) are highly reliable over multiple sessions (Malcolm et al., 2006). The method of TMS-conditioning of the H-reflex requires the use of cortical stimulation given at 90% MT, subthreshold for evoking activity in the EMG. The use of a stimulation intensity normalised to the MT value is based on the

assumption that the input-output TMS curves are identical for all participants (van der Linden and Bruggeman, 1993). Surprisingly, 90% MT showed poor reliability across sessions (ICC = 0.43). Such a feature can potentially explain the low ICCs found at multiple ISIs. Overall, the results indicate that the activity induced by subthreshold stimulation needs to be assessed and controlled for, for example by ensuring that a response is never produced before moving to the TMS-conditioning phase.

Recording of the H-reflex found wide application in animal neurophysiology studies as a model to assess pathway' specific plasticity (Chen and Wolpaw, 2002). In humans, most of the experimental work has focused on studying the H-reflex evoked in the soleus muscle upon tibial nerve stimulation (Burke and Pierrot-Deseilligny, 2010). The soleus H-reflex has been reported to be highly reliable over multiple sessions (Hopkins et al., 2000). In contrast, H-reflexes evoked in the FCR muscle at rest were considered too unstable to be used in clinical practice (Ioku et al., 1988). Christie and her colleagues (2005) were the first to report that the reflex could be recorded at rest in almost all participants (95% success rate) and was highly reliable over days (ICC = 0.89). The results of Chapter 4 were partially in line with these observations. Intersession reliability was high for both M_{max} and $H_{M10\%}$ (0.99 and 0.95) respectively. However, H-reflexes were present only in 85% of participants. Christie et al. (2005) attributed their high success rate to a difference in body position compared to previous studies during testing, with participants lying supine and FCR maintained at a resting length. This position was tested during the piloting phase of the described study, but no difference in the probability of recording H-reflexes was found when compared to sitting position (unreported data).

The primary objective of this study was to investigate whether the delivery of TMS at different time intervals from median nerve stimulation produces results which are reliable over time. The conditioning pulse was given at 90% MT and the test pulse induced H-reflexes of 10% of the M_{max} when delivered alone. A wide range of ISIs, between -7 ms (PNS first) to +7 ms (TMS first) was tested. The resulting effects closely resembled previous reports in terms of ISIs which were effective in facilitating the H-reflex (Mazzocchio et al., 1994) and size of the effects (Niemann et al., 2017). TMS significantly increased the amplitude of the monosynaptic reflex when given at 0, 1, 3, 5 and 7 ms before PNS. Regarding the reliability analyses, amplitudes measured at 3ms_ISI were highly reliable (ICC = 0.83) over sessions. Since the definition of ISIs was based on stimulus delivery time, and not on the time at which

the responses were first visible in the EMG (e.g. latency) it is not possible to give a conclusive answer on which component of the corticospinal volley was responsible for the seen effects. However, the finding that facilitation was observed at earlier (0 and 1ms) ISIs argues for a role of disynaptic or polysynaptic pathways at 3ms_ISI. Alternatively, facilitation at this interval could depend on the late arrival of slow-conducting corticospinal tract neurons at the spinal motoneuron pool (see Chapter 4).

Reliability was lower when TMS was delivered at the same time (0ms_ISI) and 1 ms after (1ms_ISI) PNS, which potentially means that the direct monosynaptic component of the descending volley does not always influence the activity of spinal motoneurons to the same extent (n.b. assuming that the efferent volley produces a stable H-reflex). As previously discussed, there is a possibility that the changing conditioning pulse (90% MT) caused a general decrease in reliability at all ISIs and thereby the variability does not lie in the facilitation *per se* but rather in the descending cortical volley. Other general limitations of the techniques used are discussed later (Chapter 7.3). The conclusions of the study are that TMS can reliably be used to condition the monosynaptic reflex evoked in the FCR muscle to study pathway-specific effects of experimental manipulations, but experimenters should ensure that the constituent elements of the protocol (subthreshold TMS and unconditioned H-reflex) are kept stable during the sessions.

7.2.2. The effects of sound and stimulus expectation on Transcranial Magnetic Stimulation-elicited motor evoked potentials

Having established the reliability of the methods used to measure cortical and spinal excitability via non-invasive stimulation, the next step was to assess the validity of the MEP as a measure of corticospinal excitability. Perhaps the greatest drawback of employing TMS to study the motor system is that our knowledge of the cortical areas and subcortical substructures activated by the magnetic pulse is still limited (Robertson et al., 2003). Fisher et al. showed that TMS pulses and click stimuli induced activity in reticular formation neurons of anaesthetised monkeys (Fisher et al., 2012). The study described in Chapter 5 was designed to assess whether two confounding factors, stimulation noise and stimulus expectation, can affect the amplitude and variability of the MEPs recorded from forearm muscles with TMS.

Attenuating and masking the noise significantly decreased the amplitude of the recorded MEPs. This effect was observed across increasing stimulus intensities and in the majority of the participants. Similarly, informing the participants of the time at which they would receive stimulation through visual feedback significantly reduced MEPs amplitudes. In addition, it was demonstrated that short and long IPIs did not produce significantly different MEPs. This last result contradicts the findings of Vaseghi et al., in which higher MEP responses were found with longer IPIs (Vaseghi et al., 2015). However, differences in the methods used can explain the divergent results. First, the use of IPIs was slightly different in the previous study (4 ms and 10 ms vs 5 ms and 10 ms). More importantly, in the study described in Chapter 5 a 20% jitter was introduced to make the stimulation time less predictable.

The use of TMS and surface EMG does not permit a detailed evaluation of which neural circuits are involved in the observed phenomena. Nevertheless, a purely speculative explanation based on these and recent literature's data and on the knowledge of the putative circuits engaged by TMS is provided. This explanation posits three independent but closely related mechanisms which explain the decrease in excitability observed in the study. The common assumption is that magnetic stimulation can activate neurons in the caudal pontine reticular nucleus (PnC), and this neural population is partially overlapping with the one activated by sudden acoustic stimuli and responsible for the startle response (Davis et al., 1982). Reticular neurons project monosynaptically and disynaptically to spinal motoneurons and can induce EMG responses at short latencies (Yeomans and Frankland, 1995). A simplified model explaining the pathways activated on each condition, which does not include interneuronal relays nor higher cortical descending volleys, is depicted in Figure 7.1.

First, in the NORMAL condition the descending corticospinal activity to spinal motoneurons could collide and summate with the reticulospinal signal producing bigger MEPs. The use of earmuffs to attenuate the sound caused by stimulus discharging successfully decreased EMG responses. It cannot be concluded that reticular neurons were not activated when using earmuffs, but it is conceivable that this population was excited to a lesser extend in this condition because reticular activation decreases at lower sound amplitudes (Fisher et al., 2012). A brief white noise stimulus at the intensity (83 dB) used in the study can elicit a startle reflex by itself (Blumenthal, 1988). However, in the NOISE condition, noise was not delivered

at a determined time point to cortical stimulation, but rather continuously while MEPs were recorded. Repeated activation of reticular neurons by sound induces habituation of the startle reflex, which might be due to decreases in neurotransmitter release from the presynaptic terminal (Lopiano and Montarolo, 1990). Again, the net effect on the descending volley would be a decrease in spinal excitability compared to the NORMAL condition.

In order to explain the last of the effects observed, the reduction in excitability observed when participants could anticipate the stimulation time, we need to draw on both the animal literature describing the neuroanatomy of the startle response pathway and behavioural research conducted with humans. First, the acoustic startle response is modulated by cortical areas in rats (Groves et al., 1974). Second, the effect of an acoustic startle stimulus on spinal excitability depends on task instructions, being facilitatory in situations in which motor preparation is high and a motor response needs to be exhibited (Kumru and Valls-Solé, 2006) and inhibitory at rest (Chen et al., 2016). In the absence of context-specific instruction, the effect of the startling noise will depend on subject-specific characteristics. Indeed, it has been shown that physiological state such as anxiety and arousal can alter the startle response (Ray et al., 2009). The hypothesis is that participants with higher arousal levels will be more affected by the startling component of the stimulation, because their cortical excitability will be at a higher level at the time of stimulation (Baumgartner et al., 2007). The ability of predicting what was perceived as a startling stimulus, which was provided in the READY condition, might have helped reducing arousal levels to a relaxed state. This hypothesis is supported by the observation than smaller responses in the READY conditions were not observed in all participants (Figure 5.9). It is also in line with recent reports (Maizey et al., 2013, Cuypers et al., 2014) that the level of anxiety of participants, especially in those who are naïve to TMS, correlates with the incidence of side-effects observed and might influence cortical excitability.

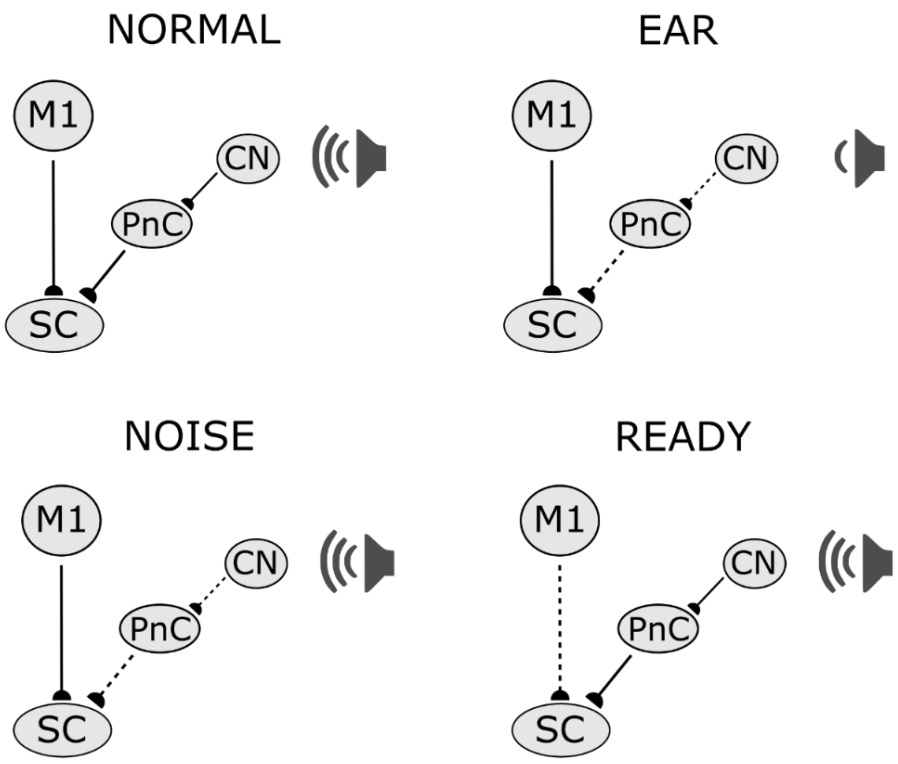

Figure 7.1. Model of the neural pathways activated under the different conditions employed in Chapter 5. The model assumes that the participant is in a high arousal state. Dotted lines represent decreased activity. Auditory stimuli activate cochlear nucleus (CN) neurons projecting to the caudal pontine reticular nucleus (PnC), which in turn synapse on spinal cord (SC) motoneurons via the reticulospinal tract. The reticular volley converges with the corticospinal volley (originating in the primary motor cortex M1) at the spinal level.

Participants for the study described in Chapter 5 were recruited from the students' population at the University of Leeds and many of them (76% as self-reported) had never experienced TMS before. In order to minimise confounding factors arising from differences in participants' state it has been suggested to include a familiarisation session when using TMS (Cuypers et al., 2014). Reporting the levels of anxiety and discomfort experienced by each participant and his/her personal history with receiving TMS constitutes a good practice to be implemented in future studies. Taken together, the results of this study add to the knowledge of non-physical factors which can influence the outcome of TMS on the motor cortex. Two potential solutions to limit the effects of discharging noise and stimulus expectation are reported, respectively by masking/attenuating the sound with the use of headphones or add variability to the interval between consecutive stimuli. The applications of these methods for future

works will depend on the rationale of the study and task demands and will be discussed later in this chapter (Chapter 7.4).

7.2.3. The effects of strength and skill training on the neural circuits of the contralateral limb

The first two experimental chapters described (Chapter 4 and Chapter 5) provided the methodological basis for extending the protocol to a third study. Unilateral motor practice improves strength and performance in the contralateral limb, a phenomenon known as cross-education or bilateral transfer. In the context of skill learning, acute (n.b. within-session) effects of unilateral training on contralateral performance have been reported (Gordon et al., 1994). There is no consensus on which network of cortical and subcortical structures might underlie this phenomenon (Ruddy and Carson, 2013). Some findings (Perez et al., 2007) indicate that neural adaptations occurring in the trained (contralateral to moving hand) motor cortex contribute to performance increases while others (Lee et al., 2010) show that changes in the excitability of the untrained motor cortex are necessary for cross-education to occur. Because the cross-education of strength is often measured over multiple weeks of training (Lee and Carroll, 2007), it is still unclear whether the acute effects of the two training modalities on the contralateral limb differ. In the current study, it was first assessed whether a single session of force-matching skill training increased performance in the trained and untrained limb. In addition, the spinal (via recording of the monosynaptic reflexes and TMS-conditioned monosynaptic reflexes) and cortical excitability (via MEPs) of the untrained limb-hemisphere was measured after training. Regarding strength training, increases in strength in the trained muscle after resistance training usually are measured after multiple sessions because the neuromuscular adaptations supporting it such as hypertrophy and hyperplasia develop over time and over multiple training sessions. However, it has been suggested that the contribution of morphological changes to cross-education of strength is limited (Lee and Carroll, 2007), and that neural mechanisms (see Chapter 2.8.2.3) which might already become active during the first training session contribute to it (Hortobágyi et al., 2011). This possibility was tested by measuring the maximal voluntary force produced during isometric wrist flexion before and after unilateral strength training in both limbs, and by measuring H-reflexes induced by median nerve stimulation, MEPs

recorded upon TMS and TMS-conditioned monosynaptic reflexes before and after training.

The results demonstrate that training increased skill (force-matching) in both the trained and untrained hand when movements were produced at 25% of MVC. The instructions to the participants while training were to produce a wrist flexion matching the target line displayed on the screen and maintain it constant for three seconds. When testing with the right untrained hand, visual feedback was removed to ensure that participants did not simply learn how to process visual information better during training (Muellbacher et al., 2001). During the training phase, participants learned how to integrate the visual information with proprioceptive feedback and developed an internal model of the task to be executed (Wolpert et al., 1995). This suggests that the internal model became accessible even to the contralateral untrained hemisphere, since performance increased in the untrained hand. Cross-transfer of force control has been previously assessed with different tasks (Teixeira, 2000, Yao et al., 2014). Teixeira (2000) tested whether tasks with strong perceptual (reaction-time) and motor (force control) components transfer to the untrained limb to the same extend. Results showed higher levels of transfer in the reaction-time experiment compared to force control, and no transfer at all when training with the non-dominant hand and testing the dominant one (Teixeira, 2000). The last finding is in sharp contrast with the results of Chapter 6 but there are important methodological differences that might explain this contrast. In Teixeira's study, participants had to launch a cursor to a specified target position. The target position was constant when using the dominant and non-dominant hand, independently of the maximal force which could be produced with that hand. A later study using the same task provided evidence that the errors in force production are bigger when the asymmetry of force between wrist flexor muscles increases (Teixeira and Caminha, 2003). As opposite to it, in the task employed in Chapter 6 the target force was normalised for each hand to the MVC produced by isometric wrist flexion, thus controlling for differences in the perceived level of force produced between the dominant and non-dominant hand.

In a different study, participants learned to produce pinch forces between the thumb and index finger of 35% of their MVC (Goodall et al., 2013). In line with our results, fifteen minutes of training reduced the errors in force output production in the untrained hand. However, it did not significantly affect the performance of the trained hand. There is a possibility that the difference in the target force (35% vs 25%) might

explain this discrepancy. Indeed, data from Chapter 6 show that training with higher force-matching targets (50%) did not lead to significant cross-transfer effects. Multiple studies (e.g. Salonikidis et al., 2009) proved that the variability of force production decreases with increasing strength. This was the case for the results of Chapter 6, in which smaller coefficient of variations were observed already at baseline between the 25% and the 50% conditions for both hands (see Table 6.1). It is then plausible that for both studies participants' performance at baseline was too good to be improved by a single practice session (see Chapter 6.4.2). In addition, the training protocol used in Chapter 6 differed from the one used by Goodall and his colleagues (2013) in that distributed practice was used to permit the participants to interpret their errors after each movement produced (Lee and Genovese, 1988).

The effects of a single session of strength training on peak muscular force produced during isometric wrist flexion was assessed in this study. The training protocol included four sets of ten movements lasting two seconds and followed by three seconds of rest. Participants were instructed to maximise the rate of force development and to use as much strength as possible in each movement. However, training was not successful in increasing peak torques in the trained or untrained hand. Possible explanations for the lack of a training effect have been presented in Chapter 6.4.1. One possibility raised, supported by similar findings (Hortobágyi et al., 2011), is that a single session is not sufficient to increase MVC in either of the limbs. However, this does not necessarily mean that motor behaviour did not change during training. Selvanayagam et al. (2011) employed a task requiring participants to maximise both speed and strength while performing thumb contractions. Twitch force resultant vectors induced by TMS changed directions after forty training movements. Changes in force-velocity relationships occurring during power training strongly correlate with the increase in muscle power (MacIntosh et al., 1993). In a different study, intramuscular EMG analysis showed that one of the effects of strength training is an increase in synchronised motor unit activation, perhaps due to higher recruitment of fast-twitch fibres (Moritani, 1993). This feature might have occurred in one or both hands but gone undetected in the current study. Multiple authors (Jensen et al., 2005, Green and Gabriel, 2018) suggested that strength increases as a consequence of multiple factors, some of which might depend on the acquisition of the new trained movement pattern. Indeed, even changes in muscle recruitment patterns have been considered as a learning strategy (Carroll et al., 2001b). The finding that the effect is

task-specific rather than muscle-specific (Rasch and Morehouse, 1957) supports the hypothesis of a learning component in strength training. In the context of cross-education, the increase in skill in the untrained hand following strength training (McGuire et al., 2014) suggested that skill acquisition in the form of better control of muscle activation has taken place. Force variability and strength were assessed in the untrained hand after participants practiced either maximal wrist flexions (control group) or alternating wrist flexions-extensions (experimental group) (McGuire et al., 2014). Strength training increased maximal isometric contractions and decreased torque variability in the untrained hand. Furthermore, strength increases and variability decreases transferred to a similar but untrained task (e.g. maximal wrist flexions for the experimental group and alternating wrist flexions-extensions for the control group), a phenomenon typical of skill training and indicating motor learning (McGuire et al., 2014). The possibility that force variability decreased after strength training in the study in Chapter 6 was not investigated but could support potential force improvements when using longer/multiple training sessions.

MEP amplitudes recorded at aMT intensity from the untrained hemisphere increased from pre-training to post-training after skill training and strength training. This happened in the absence of any changes in background excitability (pre-stimulus EMG). It was previously proved that a single session of procedural motor learning increases the excitability of the untrained motor cortex (Perez et al., 2007). Comparatively less is known about the transfer of force control to the untrained limb and the related modulation of activity in the untrained cortex. Camus et al. (2009) asked participants to complete a pinch force task with their dominant hand, producing specific forces at specific sequences. After 30 minutes of practice, TMS recruitment curves, SICI, ICF and IHI were measured bilaterally. Indices of cortical inhibition (SICI and IHI) decreased in the untrained hemisphere, but TMS recruitment curves did not change with training (Camus et al., 2009). Importantly, the task employed had a sequence learning component as opposed to the purely motor task employed in Chapter 6. In addition, authors did not determine movement strength according to participants' maximal contraction value. As the magnitude of crossed activation increases with the force of contraction (Perez and Cohen, 2008), it would be possible that movements were not sufficiently strong to elicit bilateral activity (Hortobágyi et al., 2003). Goodall et al. (2013) did not observe any increase in corticospinal excitability in the untrained hemisphere after a pinch force control task. Nevertheless,

the authors employed higher (140% MT) stimulation intensities compared to the current study. The finding that MEPs recorded at 120% aMT intensity were not modulated after training (Chapter 6.3.2) suggests that lower intensities need to be used to uncover the effects of training.

Selvanayagam and colleagues (2011) previously observed acute neural effects after a session of ballistic thumb movements, in the form of increased TMS-induced twitch force resultant vectors. However, this was tested in the trained hemisphere, and the resultant cross-education to the opposite thumb was not assessed. Similarly, Mason et al. (2020) observed acute and chronic increases in the trained M1 excitability after a strength training protocol comprising wrist flexion and extension movements. To the author's knowledge, no study has previously measured MEP changes in the untrained hemisphere after a single session of wrist flexion strength training. MEP amplitudes increased compared to the baseline after a single session in the current study. Importantly, the increase did not depend on changes in background activity of the muscle of interest, since pre-stimulus RMS did not differ between time and conditions. Hortobágyi et al. (2011) assessed if 20 sessions of submaximal strength training influenced the contralateral motor pathways. While inhibition from the trained M1 to the untrained M1 decreased after the first session and after all sessions, corticospinal excitability did not change in the untrained hemisphere. In contrast with it, Leung et al. (2015) showed that MEPs recorded from the untrained M1 increased after metronome-paced strength training. These discrepancies are best explained in terms of methodological differences such as the type of contraction or the conditions of training (Taube, 2011). Changes in strength observed at the early stages after training are considered to depend on the increased neural drive to muscle (Moritani, 1993). In the context of cross-education, the cortical plasticity observed in the current and other studies suggest the presence of a learning component in the strength task (see Chapter 6.4.6) (Jensen et al., 2005).

The present study was not the first to systematically compare contralateral changes in cortical excitability between strength and skill learning (Leung et al., 2015). Taken together, the evidence indicates that the two tasks promote changes in the contralateral limb through the same mechanism, by a long-lasting modulation of the untrained M1. Two hypotheses of the neural mechanisms underlying the crossed-effects of training have been proposed: (1) the bilateral access hypothesis posits that practice induces adaptation in the trained hemisphere at sites which become accessible to the untrained

hemisphere, for example via trans-callosal pathways; the cross-activation hypothesis states that unimanual training engages the motor cortex bilaterally, and plastic change occur already during training in both hemispheres (Lee et al., 2010). Importantly, the finding that MEP amplitudes increased in the untrained M1 does not falsify the bilateral access hypothesis, since these could change as a result of reduced inhibition from the other M1 (Hortobágyi et al., 2011). It was suggested that the cross-activation hypothesis is more plausible for tasks requiring strong contractions (Lee et al., 2010). In this light, the maximal contractions employed in the current study could have induced plasticity in both the motor cortices. Similarly, one could assume that skill training tasks including low-force production and more cognitive components engage both the motor cortex and higher-order motor areas in the trained hemisphere, and the knowledge derived from training is then transferred to the contralateral hemisphere (Lee et al., 2010). However, the force-matching task employed in this study required forces of up to 50% MVC, which renders the interpretation of findings problematic. A potential way to ascertain which hypothesis better fits the observed results is to test whether performance increases transferred to non-homologous muscles. If learning happened through bilateral transfer, the effects should be effector-independent (Ruddy and Carson, 2013).

7.3. Limitations

The first study (Chapter 4) was designed to be easily reproducible in a clinical setting and by considering the time constraints which limit the usefulness of longer recording sessions. An acceptable trade-off between testing the effects at a wide range of conditioning ISIs and recording as many traces as possible for a single parameter was made. This led to the collection of 8 traces for all the PNS and Conditioning parameters and 10 traces for the TMS parameter (90% MT). Recent works assessing the conditioning effects of TMS on the H-reflex included 10 to 15 (Niemann et al., 2016, Hannah et al., 2018) trials for each ISI. A possible drawback of the study design is the fact that the conditioning protocol was the last component of the experimental paradigm, and given this it could be that changes in muscle pre-activation might have affected spinal excitability during the session (Capaday, 1997). This was a necessary feature of the experimental protocol as the research question could only be answered if the stimulation intensities to be used were identified prior to the start of the conditioning protocol. However, this possibility was controlled for by monitoring the

background EMG recorded throughout the session. In addition, the motor wave recorded along the H-reflex was monitored to ensure that it was constant over the session, to exclude changes in spinal excitability (Knikou, 2008).

Another issue to consider is that the excitability of cortical and spinal neuron populations oscillate at rest (Keil et al., 2013). The baseline EMG activity before stimulation was constantly monitored, but subthreshold changes in motoneurons excitability could not be assessed and may contribute to the variability seen in the present experiment. The membrane potential of spinal motoneurons alternate between depolarized and hyperpolarized states (see Chapter 2.7.7) even in the absence of overt firing (Buzsáki and Draguhn, 2004) and a magnetic pulse delivered during the depolarized state might induce greater descending activity (Thut et al., 2017). In addition, large differences in the outcome of conditioning pulses are observed when changing stimulation parameters and participant position (Christie et al., 2005, Mazzocchio et al., 1994). Thus, the protocol used in this study, the data yielded from this protocol and the reliability of theses parameters may not extend to other experimental settings.

In Chapter 5, a total of six different conditions were tested on each participant and in the same session, with five minutes between them. Many studies demonstrate that an initial state of higher excitability causes the first few MEP amplitudes recorded in a series to be higher than the following ones (Brasil-Neto et al., 1994, Schmidt et al., 2009). This suggests that, when using multiple closely-spaced conditions and assessing the differences between them, it is possible that the first traces recorded in the first condition will be higher because of this effect rather than a real effect of the condition. This possibility was controlled for by randomising and counterbalancing the order of the conditions used across participants. The addition of earmuffs and headphones delivering white noise undoubtedly decreased the intensity of the sound reaching participants, but did not completely suppress it. The reason for this is that part of the noise induced by TMS discharging is conducted through bone and a low frequency component can still be perceived after masking (Conde et al., 2019). This constitutes an important disadvantage of using earmuffs and headphones. However, it does not impact on the assumptions nor on the importance of the findings of the study, which consider a reduction in the startle response evoked by TMS discharging as the primary mechanism responsible for the results. Finally, all recordings were conducted at rest during the study. It cannot be inferred that similar effects will be observed when

participants are asked to maintain a stable background contraction during stimulation, as was the case in Chapter 6, because the excitability of spinal motoneurons will in that case already be biased towards subthreshold activation (Rossini et al., 1994).

Many of the limitations of the protocol used to assess the excitability of motor circuits in Chapter 4 could be controlled for when designing the study described in Chapter 6. First, the number of traces recorded at each pair of conditioning-test interval was increased from 8 to 10-15 in order to provide a more accurate estimate of the excitability of each pathway. Second, during the delivery of the TMS-conditioning H-reflex protocol, control trials in which only TMS and only PNS were delivered to the participants were included, and control and conditioned reflexes were randomised (Pierrot-Deseilligny and Burke, 2005). This feature helped ensure that changes in spinal and cortical excitability did not occur during the stimulation. Finally, as opposed to the study described in Chapter 4, TMS-thresholding was completed while the participant performed a steady voluntary contraction of the wrist (aMT, see Chapter 3.5). There were multiple reasons for adding this feature: (1) background contraction have a facilitating effect on the occurrence of a monosynaptic reflex in FCR (Jaberzadeh et al., 2004). Indeed, a reflex could be recorded in all (10/10) participants in the presence of a voluntary contraction; (2) the variability of MEPs decreases during sustained contraction (Darling et al., 2006); (3) raising the baseline excitability of cortical neurons to an "active" state can potentially lower the impact of physiological factors such as participants' attention (the study reported in Chapter 5 was conducted with participants at rest).

Perhaps the greatest limitation of the study outlined in Chapter 6 is the lack of a control group performing random movements. Indeed, this issue was raised by Carroll et al. (2006) who conducted a meta-analysis on the effects of unilateral strength training on the contralateral limb. The main concern of the authors was the possibility that the effects were due to familiarisation with the apparatus and/or the task to perform and the pre-training measuring might be sufficient to increase performance. However, in this study participants had the opportunity to familiarise themselves with the training environment (positioning of the dynamometer, movement to produce, task to be performed) before the start of the baseline training session. Despite this, the possibility that skill learning started already when performing the three baseline movements pre-training could not be controlled for. Similarly, there is a remote possibility that the physiological assessment pre-training was effective by itself in raising the excitability

of the corticospinal tract when measured post-training. However, it has been demonstrated (Leung et al., 2015) that a baseline session of TMS followed by 30 minutes of rest has no effects on following TMS measures of excitability.

Another issue to consider is the possibility that fatigue developed while participants were performing the MVCs during the testing phases and the ballistic strength training, which could affect both behavioural and neural results. Regarding the changes in strength assessed with the MVC, the resting interval between consecutive MVCs was set at 1 minute during the testing sessions. Multiple reports (Weir et al., 1994, Matuszak et al., 2003) suggest that a 1-minute rest might be sufficient to minimise post-activation effects of fatigue when short bouts of training are employed. In the context of wrist flexors exercises, Ikai and Steinhaus (1961) showed no changes in the strength produced by three maximal efforts performed every 1 minute. The lack of a decrease in performance between the first and the third bursts of activity supports this notion (see Figure 6.5). The strength training protocol designed included a ballistic component followed by a sustained contraction, repeated for ten times in a very short period. Indeed, the development of fatigue, expressed as a reduction in the ability to maintain high contraction strength, was often observed over the course of the ten movements. However, sets of ten movements were followed by three minutes of rest in order to permit recovery to baseline. In addition, there were five-minutes of rest between the end of the training protocol and the start of the testing phase. For the neural measurements, analysis of the pre-stimulus background EMG confirmed the lack of changes in baseline excitability, which is a sign of the development of fatigue and would have resulted in an increase in MEP amplitudes (Søgaard et al., 2006). The small decrease in M_{max} amplitude observed after training (Table 6.2) could potentially represent a reduction in the efficacy of neuromuscular transmission. However, such an issue has been reported previously and occurred even after a period of rest (Crone et al., 1999). Crone and his colleagues observed reductions in the amplitude of M_{max} over the course of the same experiment, with maximal decreases observed around 40 minutes after the first M_{max} measure, grossly in line with the time passing between the pre and post-training assessment of M_{max} in the current study. For all the other parameters, the first measures were taken at least three minutes after training, which is past the time at which peripheral effects of post-activation fatigue are maximal (Selvanayagam et al., 2011).

The use of surface EMG to record electrical activity has limitations that can reduce its validity if not addressed. First, the quality and amount of activity recorded depends on the position of the electrodes (De Luca, 1997). The optimal position to record EMG activity from forearm muscle was chosen according to literature's guidelines (see Chapter 3). In studies where more than one session was required (Chapter 4 and Chapter 6), the position of the sensors was measured in relation to clear anatomical landmarks and pictures were taken to ensure reproducibility of position across days. For the first study, the influence of cross-talk from nearby muscles which could contaminate the signal was estimated by placing another sensor on the FCU muscle. This also helped ensuring that peripheral stimulation was targeting the median nerve and not the ulnar nerve. In addition, because spontaneous activity in the antagonist muscle can influence the excitability of the target muscle via reciprocal inhibition (Pierrot-Deseilligny and Burke, 2005), in studies 2 and 3 activity was recorded from the ECRL muscle and monitored previous to the delivery of stimulation. Similarly, coil position and orientation are important determinant of the outcome of TMS over the motor cortex. The TMS hotspot was marked on the scalp of each participants to ensure stability of recordings over the session. The use of a TMS support stand ensured that the orientation of the coil did not change over time. MRI-based navigation systems have been developed to help TMS coil positioning (Herwig et al., 2001). Nevertheless, there is evidence that by following standard and controlled procedures it is possible to reach accuracy levels without neuro-navigation systems close to the ones achieved with it (Jung et al., 2010).

A major problem in human neuroscience is the use of small sample sizes and the resulting low power of the studies reported (Barch and Yarkoni, 2013). For the study of Chapter 5, the number of participants was decided after conducting a sample size calculation indicating the sample size necessary to reach a power of $1-\beta = 0.80$ at level $\alpha = 0.05$ to be $n = 23$. The same calculation could not be performed for the study in Chapter 4 because of the nature (e.g. intersession reliability) of the analysis. Nevertheless, the final N was in line with previous studies assessing intersession reliability of corticospinal parameters (Hoch and Krause, 2009). Moreover, reliability studies are often limited to measure the stability of parameters over 2 consecutive sessions. As clinical practices and rehabilitation protocols may require a higher number of sessions to be implemented (Gray et al., 2017), a third session was included in the study. Finally, the issue of using a small sample size is an evident limitation of

the third reported study (Chapter 6). However, the use of a repeated-measure design provided greater statistical power compared to between-subjects designs, and the total number of participants is in agreement with other skill learning studies (e.g. Carroll et al., 2008, Suzuki et al., 2012). Perhaps more important but less considered in stimulation studies is the characterisation of how representative the participant sample is with respect to the general population. The inclusion criteria to participants in any of the experiments were quite broad, with the age range being between 18 and 40 years. Despite this, because of the academic setting of the studies, the main represented demographic was healthy, young (<30 years) university students. This general issue in human studies (see Henrich et al., 2010 for more details) needs to be addressed in consideration to the fact that many of the protocols initially tested in healthy young people are later extended to clinical populations, whose response to stimulation might vary according to their age (Bhandari et al., 2016).

7.4. Future directions

The findings arising from the experimental chapters described in this thesis suggest future directions for further research. For example, study 1 (see Chapter 4) was designed to address a clear methodological question: is the method of TMS-conditioning of the monosynaptic reflex reliable over sessions in the same sample of participants? The results showed that, for a certain range of conditioning-test intervals, the two techniques can be reliably used in combination. This indicates that the two techniques can be used to derive information about the effects of experimental manipulations on specific cortical and spinal circuits. Perhaps even more relevant for future applications is deciding the interval between stimuli according to the difference in latency of the two signals were generated alone, the method which was used in Chapter 6. Possible future studies will need to establish whether this method too demonstrates good intersession variability.

The most surprising finding of the study was the low reliability observed when measuring MEPs induced by subthreshold TMS. Importantly, this was paralleled by lack of changes in baseline excitability across sessions, as measured by the RMS of the background EMG in the 50 ms preceding the stimulus. The possibility that stimulation at this intensity might activate motor units and not only cortical circuits needs to be investigated in future studies (Niemann et al., 2016). Recent research has shown how intracortical facilitation (ICF), previously considered to depend on

cortical mechanism, is modulated through a subcortical pathway activated by the subthreshold conditioning stimulus (Wiegel et al., 2018). Similarly, the exclusive role of cortical circuits in mediating interhemispheric facilitation (IHF) and intracortical inhibition (SICI) needs to be re-addressed considering the recent findings. If subthreshold stimulation is capable of evoking descending activity by itself, it is erroneous to assume that a mechanism is of cortical origin based on the response to subthreshold stimulation (Wiegel et al., 2018).

The contribution of the reticulospinal tract to skilled control of forearm movements and to recovery of gross hand functions after injury is well established (Riddle and Baker, 2010, Baker and Perez, 2017). However, the reticular activation through sound discharged by TMS is an unwanted outcome and a serious issue when trying to interpret the results in terms of corticospinal excitability. The use of earmuffs and white noise to mask the sound, such as those used in Chapter 5, can be easily added to protocols testing MEP amplitudes at rest. TMS systems which produce substantially reduced noise (Goetz et al., 2014) can in the future replace the ones used nowadays. In addition, more studies directly recording the outcome of stimulation on the activity of cortical and subcortical neurons (e.g. Fisher et al., 2012) are needed in order to understand which neurons are being activated by the magnetic pulse.

Many participant-specific factors can confound the outcome of TMS (see Chapter 2.7.8), and the findings of Chapter 5 were discussed in light of these. In the future, TMS studies involving comparisons between data recorded under multiple conditions should estimate the percentage of participants in which an effect could be observed as was done in Chapter 5 (see Figure 5.7) rather than just the significance level. This will help understanding how common the effect is across the population and how strong in each participant. Researchers should aim to collect as much information as possible regarding the participants' state including level of stress, attention and previous exposures to TMS as these can be predictive of the individuals' response to TMS (Holmes and Meteyard, 2018). Understanding that receiving TMS is an highly subjective experience and that its efficacy depends on it is fundamental, especially since the technique became widely used in rehabilitation (Rossi and Rossini, 2004) and more and more data about the percentage of non-responders (Nettekoven et al., 2015) are accumulating.

In the third and last experimental study presented in this thesis the non-invasive techniques investigated in the first two studies have been applied to two forms of acute motor training: skill training and strength training. The results obtained in terms of performance changes and effects of training on the excitability of the ipsilateral motor circuitry raised open questions which need to be addressed in the future. For the training part, the findings seem to suggest that the development of use-dependent plasticity in the ipsilateral motor cortex after unilateral strength training is contingent upon the conditions of practice. For future work, much can still be done by applying the principles of motor learning to strength and skill training protocols. The use of distributed practice and augmented feedback in our task can be extended by including task variability into the practice session. The literature on skill acquisition shows that the use of variable practice, which involves performing different versions of the same skill, is positively linked with transfer of learning novel skills (Newell and Shapiro, 1976).

Isometric contractions were chosen because while performing dynamic eccentric or concentric movements the position of the surface electrode relative to the skin might change (Besomi et al., 2019). However, it has been shown that eccentric (muscle lengthening) contractions induce higher cross-education effects compared to concentric (muscle shortening) and isometric ones (Hortobágyi et al., 1997). Future studies might investigate the effects of a single session of eccentric strength training on the peak force produced by the untrained arm and on ipsilateral neural circuits. Ecological validity can be added to the training protocol by contextualising the performed movements to daily activities and showing how the improvements can extend beyond the single session and be applied to qualitatively similar tasks. Finally, unilateral training is beneficial in reducing motor deficit in the untrained limb after brain injury, spinal injury and neuromuscular disorders (Hortobágyi, 2005). The designed protocols can potentially be tailored for specific clinical populations such as cervical SCI patients with preserved but limited arm functionality.

The neurophysiological measures obtained after training raised important additional questions. First, what is the role of the increase in ipsilateral M1 excitability seen after strength training, if this does not lead to increases in strength? As previously (Chapter 6.4.6 and Chapter 7.2.3) discussed, the lack of performance increases does not preclude the possibility that changes in cortical drive to muscles or in functional patterns of activity between muscles occurred after training. The first possibility can

be disclosed in the future by using methods that quantify the amount of neural drive to the untrained muscle such as the twitch interpolation technique (Lee et al., 2010). Adaptations at the muscular level can be assessed by recording EMG activity from secondary movers and antagonist muscles and measure changes in recruitment patterns with training (Carson and Riek, 2001). In addition, the results suggest that the role of spinal circuits in mediating the bilateral transfer of skill is limited. However, the amount of presynaptic inhibition acting on Ia afferents to the FCR is decreased after a visuomotor task, as shown by eliciting a conditioning volley in the radial nerve prior to median nerve stimulation (Roche et al., 2011). Similar procedures can be adapted to the present study to resolve the role of presynaptic inhibition and disynaptic inhibition from the antagonist muscle (ECR) in the resting muscle during unimanual movements. As discussed in Chapter 6.4.4, with the monosynaptic reflex technique it is not possible to assess muscle spindle sensibility (Burke, 2016). The potential role of the fusimotor system can be addressed by conditioning MEPs evoked in the muscle of interest with muscle stretches given at multiple conditioning - test intervals from the cortical stimulus (Petersen et al., 1998a). Changes in the long-latency stretch reflex observed after skill or strength training would be indicative of a modulation of fusimotor activity through a transcortical pathway (Day et al., 1991).

Whether skill and strength training induce increases in cortical drive to the movement effectors can be assessed by measuring EEG activity throughout the session under the assumption that corticomuscular coherence, reflecting functional connectivity between cortical areas and muscles, should mediate the effect (Mima and Hallett, 1999). Finally, with the techniques employed in this study it is not possible to provide a conclusive answer on which of the two hypotheses of cross-education (Chapter 7.2.3) can explain the results. Tasks that require sensorimotor integration are more likely to generate bilateral activity in higher cortical motor areas and associative areas (Ruddy and Carson, 2013). The use of functional neuroimaging techniques during the training and testing phase can help understanding the network of cortical areas involved in the cross-education of skill and whether the same patterns of cortical activity are observed during and after unilateral strength training.

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