TRANSCRANIAL MAGNETIC STIMULATION AND ACTION

OBSERVATION: EXPLORING METHODOLOGICAL ISSUES

Michela Loporto

A thesis submitted in partial fulfilment of the requirements of the Manchester Metropolitan University for the degree of Doctor of Philosophy

Institute for Performance Research
Manchester Metropolitan University

October 2012

This research was funded, in part, by the Government of Malta
Acknowledgements

I would like to take this opportunity to thank a number of people who have helped me along the way. This thesis could not have been completed without their help.

First, I would like to thank my Director of Studies (DoS), Professor Paul Holmes, who has guided me every step of the way. Words alone cannot describe his commitment and dedication, always finding time, despite his very busy schedule. His help, advice, and guidance, both work-related and not, and have gone beyond his duties as DoS, and he has played a crucial role in my years at MMU. Without his encouragement and support, I would not be where I am today, and I will always look back on my PhD journey with very fond memories.

Second, a huge thank you goes to Dr Craig McAllister. His expertise has been instrumental in both the design and write up of this thesis. His advice has always been greatly valued and his patience has been a virtue. I truly appreciate all his hard work and dedication, and couldn’t have done it without him.

A big thank you goes out to the members of the technical team at MMU, in particular Grant Rockley, for his assistance in the lab.

I especially want to thank all the participants for giving up their time. Without their involvement, this thesis would not have been possible. A special thank you goes out to Zoe Franklin, who was a frequent volunteer, both in the pilot stages and experimental studies, and also lent a helping hand in the recruitment of participants.

I would also like to extend my gratitude to the Government of Malta who awarded me a scholarship to allow me to fund my studies. Without this award and funding I would not have been able to enrol on this course.

Finally, I don’t think I can ever truly express how grateful I am to my whole family, especially my parents. To say that they have been loving and supportive from the very start is an understatement. I am so appreciative of all their sacrifices to allow me to continue with my education, helping both financially and emotionally, always putting my needs above their own. I thank you with all my heart! I would also like to say a special thank you to my loving boyfriend David Wright. You have been so influential and important to me, and I cannot imagine ever doing any of this without you. Your friendship, love, help, support, and encouragement, to name a few, have kept me going when times were hard. You have been an inspiration to me and I look forward to a bright and happy future, with you by my side.
Publications and presentations associated with this thesis

Full peer review journal articles


*Based on the literature review section of this thesis*


*Based on research carried out within Study 2 of this thesis*


*Based on research carried out within Study 1 of this thesis*


*Based on research carried out within Studies 3 and 4 of this thesis*
Poster presentations


International academic conference presentations


Other national and international presentations


Abstract

This thesis explored a number of methodological issues present in motor cognition research using transcranial magnetic stimulation (TMS). The facilitatory effect of the corticospinal pathway during observation of simple hand actions was also investigated. TMS was applied to the motor cortex during action observation and the resulting MEP peak-to-peak amplitudes were analysed. A series of four studies were conducted to test whether a motor facilitation effect specific to the muscles involved in the observed actions were obtained, while simultaneously investigating five prominent methodological concerns in TMS research.

In Study 1 the issue of choosing the optimal control condition was investigated. The MEP facilitation obtained during action observation (ball pinch) was compared to two commonly used control conditions (fixation cross and static image). Consistent with published literature, the action condition resulted in larger MEP amplitudes than the controls. There was no statistical difference in MEP amplitude between the two resting conditions. It was argued, however, that the static image allows for more accurate comparison with the action condition by providing meaningful visual cues without the associated action. In Study 2, the effect of short-term physical execution on the relationship between observed actions and neural activity was explored. The motor facilitation effect was present during action observation. This was not enhanced following execution of the observed action which is in contrast with the literature that shows the observation-execution matching system tuned to familiarity with an action. In TMS studies, different stimulation timings are included in order to reduce anticipatory effects of the TMS pulse. While the different timings are usually analysed together, in Studies 1 and 2, the two stimulation timings were analysed separately. As a consequence, a motor facilitation effect was only evident for the earlier stimulation timing of 6250ms in Study 1. When participants executed the action prior to observing it in Study 2, there was no effect of stimulation timing, leading to speculation that the prior execution may have had some effect on the attentional demands during the subsequent observation. Studies 3 and 4 explored two general methods concerns regarding the motor hotspot and stimulation intensity. In Study 3, the muscle-
specificity notion was explored via observation of index finger and little finger movements versus observation of a static hand, with the corresponding muscles tested at their individual hotspots. This was a novel approach as one hotspot is typically used for all muscles under investigation. The choice of motor hotspot, however, did not significantly affect the muscle-specific findings, providing further support for the muscle-specific motor facilitation findings reported in the literature. Finally, Study 4 investigated the concept of stimulation intensity. TMS action observation studies differ in the stimulation intensities used, typically ranging from 110% to 130% of resting motor threshold. Since the motor response obtained through TMS may be affected depending on the stimulation intensity used, two stimulation intensities were employed (high vs. low) during observation of finger movements. A motor facilitation effect was reported in the low intensity stimulation, which was expected given that near threshold intensities are more representative of the ongoing level of cortical excitability. No motor facilitation effect was shown in the high intensity stimulation, possibly due to the nature of high stimulation intensities on the corticospinal pathway, or simply because the low intensity stimulations were always delivered before the high intensity stimulations. In light of the stimulation timing findings of Study 1, this may have resulted in participants getting distracted or fatigued, focussing their attention elsewhere (and therefore lowering MEP amplitudes) during the latter high stimulations.

From the results presented in these studies, it is clear that there is a muscle specific motor facilitation during action observation and its characteristics are influenced by many procedural, technical and cognitive and attentional factors. This thesis provides a much needed critical analysis into the methods and methodologies commonly adopted in this area of research. It is essential to continue to explore the methods employed in TMS motor cognition studies, making them accepted universally and scientifically rigorous.
# Table of Contents

Chapter 1: Introduction  
Chapter 2: Literature review  

2.1 Transcranial magnetic stimulation (TMS)  
2.1.1 History and basic principles  
2.1.2 Recording effects of TMS  
2.1.2.1 Motor evoked potentials (MEPs)  
2.1.2.2 Physiological basis of the MEP  
2.1.3 Different stimulating coils  
2.1.3.1 Coil positioning and orientation  
2.1.4 TMS safety  

2.2 Exploring the human motor resonance mechanism  
2.2.1 Discovery of mirror neurons  
2.2.2 Human mirror system  
2.2.2.1 Mirror neuron debate  
2.2.3 TMS in action observation research  
2.2.4 TMS in movement imagery research  
2.2.5 Comparing effects of observation, imagery, and execution using TMS  

2.3 TMS methodological issues  
2.3.1 Control condition issues  
2.3.2 Priming issues  
2.3.3 Stimulation timing issues  
2.3.4 Optimal scalp position issues
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3.5 Stimulation intensity issues</td>
<td>39</td>
</tr>
<tr>
<td>2.4 Aims and overview of the research programme</td>
<td>40</td>
</tr>
<tr>
<td>Chapter 3: TMS methods and methodology</td>
<td>46</td>
</tr>
<tr>
<td>3.1 General TMS procedure</td>
<td>46</td>
</tr>
<tr>
<td>3.1.1 Electromyography (EMG) skin preparation</td>
<td>46</td>
</tr>
<tr>
<td>3.1.2 Head measurement</td>
<td>47</td>
</tr>
<tr>
<td>3.1.3 Optimal scalp position</td>
<td>48</td>
</tr>
<tr>
<td>3.1.4 Resting motor threshold</td>
<td>49</td>
</tr>
<tr>
<td>3.2 General methods applied to all studies</td>
<td>52</td>
</tr>
<tr>
<td>3.2.1 Participant information</td>
<td>52</td>
</tr>
<tr>
<td>3.2.2 EMG recordings</td>
<td>52</td>
</tr>
<tr>
<td>3.2.3 TMS procedure</td>
<td>53</td>
</tr>
<tr>
<td>3.2.4 Experimental procedure</td>
<td>54</td>
</tr>
<tr>
<td>3.2.5 Data analysis</td>
<td>54</td>
</tr>
<tr>
<td>Chapter 4: Study 1: Motor facilitation during action observation: ‘controlling’ the controls</td>
<td>57</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>57</td>
</tr>
<tr>
<td>4.2 Aims and hypothesis</td>
<td>59</td>
</tr>
<tr>
<td>4.3 Methods</td>
<td>60</td>
</tr>
<tr>
<td>4.3.1 Experimental protocol</td>
<td>60</td>
</tr>
<tr>
<td>4.3.2 EMG profiles</td>
<td>61</td>
</tr>
<tr>
<td>4.3.3 Data analysis</td>
<td>62</td>
</tr>
<tr>
<td>4.4 Results</td>
<td>64</td>
</tr>
<tr>
<td>4.5 Discussion</td>
<td>67</td>
</tr>
</tbody>
</table>
Chapter 7: Motor facilitation during action observation: using different stimulation intensities

7.1 Introduction 109
7.2 Aims and hypothesis 111
7.3 Methods 112
  7.3.1 Stimulus-response curves 112
  7.3.2 Experimental protocol 113
  7.3.3 Data analysis 116
7.4 Results 116
7.5 Discussion 119

Chapter 8: General discussion 123

8.1 Summary of the research programme 123
8.2 Applications and implications of the research programme 127
  8.2.1 Design of action observation experiments 128
  8.2.2 The action-observation matching system 129
8.3 Future directions 131
8.4 Conclusions 138

References 140
Appendices

Appendix A: The TMS Safety Screen (TASS)

Appendix B: The Edinburgh Handedness Inventory (EHI)

Appendix C: Participant information sheet for Study 1

Appendix D: Participant information sheet for Study 2 (Experiment 1)

Appendix E: Participant information sheet for Study 2 (Experiment 2)

Appendix F: Participant information sheet for Study 3

Appendix G: Participant information sheet for Study 4

Appendix H: Informed consent form for Studies 1-4

Appendix I: Wire diagram of laboratory set-up

Appendix J: Discarded trials for Studies 1-4
List of abbreviations used throughout the thesis

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADM</td>
<td>Abductor Digiti Minimi</td>
</tr>
<tr>
<td>AMT</td>
<td>Active Motor Threshold</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FDI</td>
<td>First Dorsal Interosseus</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior Frontal Gyrus</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior Parietal Lobule</td>
</tr>
<tr>
<td>M1</td>
<td>Primary Motor Cortex</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
</tr>
<tr>
<td>OP</td>
<td>Opponens Pollicus</td>
</tr>
<tr>
<td>OSP</td>
<td>Optimal Scalp Position</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PMv</td>
<td>Ventral Premotor Cortex</td>
</tr>
<tr>
<td>RMT</td>
<td>Resting Motor Threshold</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary Motor Area</td>
</tr>
<tr>
<td>STS</td>
<td>Superior Temporal Sulcus</td>
</tr>
<tr>
<td>TASS</td>
<td>Transcranial Magnetic Stimulation Adult Safety Screen</td>
</tr>
<tr>
<td>tDCS</td>
<td>Transcranial Direct Current Stimulation</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
</tbody>
</table>
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Hotspot and threshold separation for FDI and ADM muscles for each participant</td>
<td>102</td>
</tr>
</tbody>
</table>

List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Diagrammatic illustration of TMS stimulation</td>
<td>18</td>
</tr>
<tr>
<td>2.2</td>
<td>A schematic representation of the motor evoked potential (MEP)</td>
<td>18</td>
</tr>
<tr>
<td>2.3</td>
<td>Two designs of stimulating coils</td>
<td>21</td>
</tr>
<tr>
<td>2.4</td>
<td>An illustration of postero-anterior coil orientation using a figure-of-eight stimulating coil</td>
<td>21</td>
</tr>
<tr>
<td>2.5</td>
<td>An illustration of the main areas of the human mirror system</td>
<td>25</td>
</tr>
<tr>
<td>3.1</td>
<td>An illustration of the 10-20 electrode placement</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>A functional map, illustrating the cortical representation from which the corresponding bodily responses are elicited</td>
<td>51</td>
</tr>
<tr>
<td>3.3</td>
<td>The laboratory set-up for Studies 1-4</td>
<td>56</td>
</tr>
<tr>
<td>4.1</td>
<td>The mean EMG maximal activity from the FDI and ADM muscles in Study 1</td>
<td>63</td>
</tr>
<tr>
<td>4.2</td>
<td>Video stimuli used in the observation condition in Study 1</td>
<td>63</td>
</tr>
</tbody>
</table>
4.3 The mean MEP amplitudes recorded from the right FDI and ADM muscles during observation of action, static and fixation videos at both 6250ms and 8750ms combined.

4.4 The mean MEP amplitudes recorded from the right FDI and ADM muscles combined during observation of action, static and fixation videos stimulated at 6250ms and 8750ms.

5.1 Video stimuli used in the observation experiments in Study 2.

5.2 The experimental design for Experiments 1 and 2 in Study 2.

5.3 Motor facilitation ratio data of 15 participants across 5 blocks, recorded from their right FDI and ADM muscles.

5.4 MEP facilitation ratio data of 15 participants across 5 blocks, recorded from their right FDI muscle at 6250ms and 8750ms after video onset for both Experiments 1 (observation) and 2 (combined).

5.5 Motor facilitation ratio data of 11 participants who performed both Experiments 1 and 2 across the 5 blocks, recorded from their right FDI muscle.

5.6 Maximal EMG activity recorded from the FDI and ADM muscles for all participants during the execution phase in Experiment 2.

6.1 Three different types of video clips used in Study 3: (i) a static hand, (ii) index-finger movements or (iii) little finger movements.

6.2 The different locations for the OSPs for the FDI and ADM muscles in the 13 participants in Study 3.

6.3 The mean MEP amplitudes recorded from the participants’ right FDI and ADM muscles during observation of index finger movement, little finger movement and static videos, recorded from the FDI and ADM OSP combined.

6.4 The MEPs recorded during observation of index finger movement, little finger movement and static videos, recorded from the both OSPs combined.

7.1 Stimulus-response curves from the 5 participants, showing the mean MEP values for 110% and 130% RMT.
7.2 Stimulus-response curves of the MEP amplitudes of the FDI and ADM muscles of all 5 participants 115

7.3 Two different types of video clips used in Study 4: (i) index-finger action or (ii) static hand 118

7.4 The mean MEP amplitudes recorded from the right FDI and ADM muscles combined during observation of action and static videos at high and low intensities 118
Chapter 1: Introduction

As human beings we interact with other individuals on a daily basis. Understanding the meanings of other people’s actions is therefore crucial to our communication, social cognition and interactions; we are usually able to predict what other people are doing and why they are doing it. We can also interpret the goals and intentions of others by observing their movements; this being crucial in order to carry out our social interactions. By observing others’ actions, we create an internal representation of that perceived action and we are able to use this information to predict future behaviours (Rizzolatti, Fogassi, & Gallese, 2001). This theoretical approach seems to underpin the majority of papers related to action understanding and learning.

From childhood to adulthood, we are constantly learning through observation. From learning to tie our shoelaces, to brushing our teeth, to more complex motor skills such as riding a bicycle, swimming, doing a gymnastic routine, or playing the piano, we are constantly trying to acquire new skills by watching people successfully perform those actions. Observational learning may be done in a variety of ways, such as via the modelling of a teacher or a sporting coach in order to demonstrate the skill, or by using videotapes or photos of skilled performers. Human skill learning has been thoroughly explored in psychology, and with the advent of neuroimaging and brain stimulation techniques, has drawn increasing interest in neuroscience research. Observational learning also forms part of many clinical rehabilitation programmes along with physical therapy, with patients trying to re-learn the motor skills that they may have lost as a result of illness or injury. So while it is commonly accepted that as
humans we do learn simply by watching other people perform, and we tend to understand their actions and intentions, the question that still remains to be answered is what brain activity underpins action observation and how are we best able to investigate these mechanisms?

Over the years there have been many attempts to investigate how humans understand the behaviours of others, and how they learn from such behaviours. Researchers from various fields of psychology and neuroscience have attempted to define the term ‘observational learning’. Gould and Roberts (1982) defined it as “the process whereby an observer reproduces, or attempts to reproduce, the actions exhibited by another person; the model” (p. 214). More recently Janelle, Champenoy, Coombes, and Mousseau (2003) described observational learning as, “the process by which individuals imitate the observed behaviour of others and potentially obtain performance proficiency with the observed behaviour by doing so” (p. 825). Early theoretical explanations for observational learning tended to be based in cognitive psychology, adopting an information processing approach (Sheffield, 1961; Bandura, 1969). Sheffield’s (1961) theory suggested that when a person observes another person performing a skill or action, the observer formulates a cognitive symbolic representation of the skill. This then acts as a ‘blueprint’ of the modelled act, to help guide overt reproduction of the skill, and is held in the observer’s memory. When attempting to perform the skill individuals then symbolically recall this blueprint of the modelled act and translate the sequence into the overt reproduction of the skill (Gould & Roberts, 1982). A major shortcoming of this work, however, was that it did not provide an explanation as to how the cognitive representations help the observer
reproduce the observed action, and neither did it explain the nature of the representations, or where in the brain they were supposed to reside.

Two propositions have been put forward to attempt to understand the mechanisms behind the ability to understand other people’s actions and intentions. The term ‘theory of mind’, derived initially from Premack and Woodruff (1978), refers to the inference or attribution of mental states (knowledge, beliefs, feelings, intentions, and desires) to others (Ward, 2012). This mentalising process, as has become known, recruits a network of cerebral regions that are outside the motor system, that include the superior temporal cortex, the temporoparietal junction, and the midline structures; posterior cingulate and medial prefrontal cortex (de Lange, Spronk, Willems, Toni, & Bekkering, 2008). Alternatively, and as a consequence of the advance of neuroscience and neuroimaging techniques, it has been proposed that the understanding of others’ actions is the result of a neural motoric simulation (Jeannerod, 1994), where covert actions can be elicited by observation of actions performed by others, where the observer puts himself “in the shoes of the agent” (Jeannerod, 2001, p. S104). Using techniques such as functional magnetic resonance (fMRI) and positron emission tomography (PET), brain locations that are activated both during observation and execution have been identified. This, along with the discovery of ‘mirror neurons’ (di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti, 1992), have been influential in providing support for this neural mechanism for motor simulation during action observation.

With the discovery of mirror neurons in the macaque monkeys (di Pellegrino et al., 1992) there has been strong support for a homologue observation-execution
matching system in humans where a set of neurons fire both when an individual observes an action as well as when they execute the same or similar action performed by someone else. It has been reported that mirror neurons are not only present in area F5, where mirror neurons were initially discovered in primates, but also in the inferior parietal lobule (IPL). This region receives input from the superior temporal sulcus (STS), whose neurons respond to observation of goal-directed movements but do not have motor properties (Rizzolatti & Fabbri Destro, 2007). The mirror neuron system, as it is preferably termed in humans, is therefore formed by the rostral part of the IPL, the STS, and the ventral premotor cortex (PMv).

The generalisation of theories from mirror neurons in primates to humans has received criticism in recent years (e.g., Dinstein, Thomas, Behrmann, & Heeger, 2008; Hickok, 2009). Many criticisms centre on the belief that mirror neurons are primarily involved in action understanding (e.g., Rizzolatti & Craighero, 2004). First, the definition of action understanding consists of two elements of ‘action’ and ‘understanding’ that seem to be conflicted. Different researchers often use the term to mean different things. For example, Uithol, van Rooij, Bekkering, and Haselager (2011) recently published a critical review highlighting the different meanings that have been attributed to both ‘action’ and ‘understanding’ over the years. Action meanings, goals, and kinematics can be found along a broad continuum which in turn effect the interpretation of ‘understanding’. Taking a ‘grasp’ of a cup handle action as an example, individuals can understand the basic kinematics of the actions as a form of grip; we can understand this as the goal of grasping the cup to drink, or to wash up, to pour out or refill, to hand the cup to someone else, or we may even understand the
action as the higher goal of quenching thirst. Another interpretation of motor understanding includes generating an appropriate response to the viewed action (Rizzolatti et al., 2001). Despite the many interpretations of the term ‘action understanding’, it is rarely clearly defined in mirror neuron literature. It is not the purpose of this thesis, however, to exhaust the possible definitions of the terminology of ‘action understanding’. However, these concerns are important to raise before discussing the research related to the mirror system, which is reviewed in Chapter 2. Despite their widespread acceptance, and intuitive appeal, the role that mirror neurons play in humans’ social communication, interactions, and understanding may not be as clear as many tend to, or want to, believe.

‘Motor resonance’ is another term used ambiguously in the mirror neuron system literature. The term ‘motor resonance’ has been frequently used when describing how an observer simulates an observed action in order to understand it (Decety & Grezes, 2006). Accordingly, individuals may understand actions by mapping the visual representation of the observed action onto our own motor representation of the same action, causing the motor system of the observer to ‘resonate’ after observation of that action (Rizzolatti et al., 2001). Two main interpretations have been postulated. Either the motor system of the action observer resonates with his or her own perceptual system, with both brain areas active in the motor resonance process (e.g., Rizzolatti et al., 2001), or with the resonance being between two different people; the motor system of the observer and the executor of the action (e.g., in Decety & Grezes, 2006). As highlighted previously with the notion of ‘action understanding’, motor resonance is another term where clear definitions need to be
provided to support the case for a motor resonance system within the action understanding process. While it is appealing to simply accept that individuals understand actions because of the activation of motor representations of that action in our brain (Rizzolatti et al., 2001), a motor representation is not enough to distinguish between the many goals, meanings, and intentions associated with each action. The focus of this thesis, however, was not to show whether a mirror neuron system exists in humans, or what its contribution to action understanding may be. For the purpose of this thesis, it will be accepted that a putative mirror system, in some form, plays a role in the activation of the human motor system during action observation. This concept was explored throughout the experimental studies presented in this thesis.

It is important to note that direct evidence for the existence of mirror neurons in humans has recently been provided by Mukamel, Ekstrom, Kaplan, Iacoboni, and Fried (2010), albeit in the supplementary motor area and medial temporal lobe. Much of the support for a mirror system in humans, however, has been indirect, by means of brain imaging and brain stimulation techniques such as fMRI and transcranial magnetic stimulation (TMS). TMS studies, in particular, have repeatedly shown activity in the motor cortex during action observation, as well as during imagery, often concluding that this activity is associated with mirror neuron activity (e.g., Fadiga, Buccino, Craighero, Fogassi, & Gallese, 1999; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Strafella & Paus, 2000). The rationale of TMS experiments exploring the mirror neuron system in humans was that if observation of an action resulted in an increase in motor cortex excitability, then the responses (motor evoked potentials; MEPs) recorded from the
muscles used to perform that action would increase. Stimulation of the cortex causes
discharge in corticospinal neurons and the peak-to-peak amplitude of the resulting
MEPs are measured. Since the pioneering work of Fadiga et al., (1995) who were the
first to use TMS to explore this action observation phenomenon, there has been a
plethora of positive research replicating an increased activation in primary motor
cortex during action observation. As mentioned earlier, the role and function of
mirror neurons have sparked critical debates (see Hickok, 2009, for extensive review).
The same cannot be said, however, for the methodology of TMS, by which these
indirect findings of mirror neuron activity have been consistently reported. As with the
many theories of the mirror neuron system, it is appealing to simply accept the
legitimacy of the many studies reporting positive findings. To date, there is no
published research questioning the validity of the methodology or methods used in
TMS experiments when exploring the excitability of the motor system during action
observation. This may be for a number of reasons. First, it may be the novelty of the
technique since it has only been used in motor cognition since 1985. Second, it may be
due to the ease with which TMS experiments can be carried out, making it both time
and cost efficient, especially compared to the high running costs of fMRI. Third, it may
be due to the ease with which the data can be analysed, compared to the more
complex analyses in, for example, electroencephalography (EEG). Interestingly, when
looking at the methods used in motor cognition TMS experiments, there does not
seem to be a consensus as to the best approach to carry out these experiments. This
makes comparisons across laboratories difficult and somewhat inconclusive.
There are well documented problems with the techniques of fMRI (e.g., inflated correlation (Yarkoni, 2009) and subtraction paradigm (Sartori & Umilta, 2000) issues), and EEG (spatial resolution issues; Srinivasan, 1999). With TMS still a relatively new technique, and currently being applied to much of the action observation research, it is critical to review the methodological limitations of the technique in the context of action observation. The main aim of this PhD therefore was to examine critically TMS as a technique to explore the effect of action observation on the motor system.

There were five concerns considered within this thesis.

1. Choosing the right control condition is of utmost importance when conducting an action observation study using TMS, as the amplitude of the motor responses obtained during action observation following the TMS pulse are compared to the non-action control conditions. Failure to use the right control conditions may bias the results, either by amplifying the motor responses and risking a type 1 error (false positive result), or reducing the effect and risking a type 2 error (false negative result). These issues were explored in Study 1.

2. It is important to check for priming effects when conducting action observation experiments; whether previous experience of the action being observed may prime the observer to perform that same action and lead to increased motor responses as measured by TMS. This was investigated in Study 2.

3. The timing of the TMS pulse is one aspect of the TMS action observation literature that has never been explored in relation to observation of repetitive
movements. In most studies, two or more stimulation time points are used during observation of a repetitive hand or finger action in order to reduce the predictability of the stimulus, as this has been shown to affect the size of the obtained motor response. The different time points used are assumed to reflect the same motor response, but this to date has not yet been explored. This was discussed in Studies 1 and 2.

4. In TMS action observation studies it is common for experimenters to record MEPs from a number of concurrent muscles. When stimulating over the motor cortex it is common practice to first locate the correct scalp position associated with the muscle of interest. When more than one muscle is being investigated this becomes problematic. One main finding which is reported consistently in the literature is a muscle specific effect during action observation; however this is usually reported without exploring the muscles separately. It is difficult to show that a motor response is muscle-specific without testing each muscle at its scalp location. This was examined in Study 3.

5. It is common practice in TMS action observation experiments to find each individual’s motor threshold before conducting any experiments; the level of intensity of the pulses delivered by the magnetic stimulator must be just high enough to get a motor response in 50% of the delivered pulses in a given number of trials. The experiments are then run at a percentage of the identified threshold. There is a wide range of intensities used in the literature, generally starting from 110% of the motor threshold, with some even as high as 150%. The physiological response to the TMS pulse, however, shows that
the motor response obtained through TMS may be affected depending on the stimulation intensity used, with the higher intensities being less representative of the ongoing level of cortical excitability than MEP amplitudes recorded using near threshold TMS intensities (Di Lazzaro et al., 2004). This was explored in Study 4.

To conclude, the main aim of this thesis was to address the validity of the methodology used in many TMS action observation experiments. The concept of the human mirror system was explored as the experimental basis through which the five methodological concerns were addressed. There were five main parts to the thesis:

- first, the technique of TMS was explained in detail: from its origins, to the different types of apparatus that can be used, to the physiology behind the motor responses evoked as a result of TMS;

- second, an assessment of the action observation research was presented, with particular emphasis on the work that has used TMS to explore the excitability of the motor system during action observation;

- third, some of the problems evident as a consequence of TMS action observation research were elucidated; along with a step-by-step review of the general methods that are adhered to when carrying out a TMS experiment;

- fourth, a series of studies were presented in an attempt to explore five fundamental methodological limitations in the TMS action observation literature, and to expand on the current findings relating to the mirror system and the excitability of the motor system; and finally
• the main findings of the studies were summarised, followed by a discussion of the implications and applications of this research programme.
Chapter 2: Literature review

2.1 Transcranial magnetic stimulation

2.1.1 History and basic principles

Transcranial magnetic stimulation (TMS) was developed at the University of Sheffield in 1985 by Anthony Barker and co-workers when they attempted to stimulate the brain by placing a coil on subjects’ scalps over the motor cortex and recorded twitch muscle-action potentials from contralateral finger muscles using skin-surface electrodes (Barker, 1996). TMS was the first painless and non-invasive method of investigating the cortical control of the human motor system. TMS is based on the laws of electromagnetic induction. A current passes through a coil of wire, and generates a magnetic field perpendicular to the current direction in the coil. The magnetic field can then induce a secondary electric current to flow in the neurons below the stimulation site, generating action potentials as they would when responding to environmental stimuli (Ward, 2006). Electrical stimulation of the brain is now rare. In its place, magnetic stimulation has found popularity as a clinical tool, and in research settings, due to the ease with which changes in resulting muscle activity can be measured through skin surface electromyographic (EMG) electrodes (see Figure 2.1 on p. 17). Its administration is also relatively pain free to participants.

TMS is used mainly to explore the motor cortex. Since the motor cortex has a large and direct projection to the spinal cord, each stimulus evokes a visible muscle twitch which results in an easy quantifiable measure of corticospinal conduction times (Jahanshahi & Rothwell, 2000). TMS is a non-invasive method for probing the
excitability of the human motor system. Stimulation of the cortex causes discharge in corticospinal neurons and produces both direct and indirect descending volleys into the spinal tract (Edgley, Eyre, Lemon, & Miller, 1990; Patton & Amassian, 1954). The motor responses recorded and measured using EMG are believed to be the result of activation of the corticospinal neurons (Lemon, 2002). TMS stimulation can temporarily excite or inhibit specific areas of the brain which allows for functional mapping of cortical regions (Hallett, 2000). TMS can either activate or suppress motor, sensory, or cognitive functions, depending on the brain location and parameters of its delivery (Anand & Hotson, 2002).

TMS also has a number of therapeutic uses. For example, TMS has been used in clinical settings to investigate treatment effects on the cortical plasticity of brain networks in patients with psychiatric disorder such as: depression (Paus & Barrett, 2004); attention deficit hyperactivity disorder (Acosta & Leon-Sarmiento, 2003); obsessive compulsive disorder (Mantovani et al., 2006); and addiction (Amiaz, Levy, Vainiger, Grunhaus, & Zangen, 2009). There has also been evidence showing the value of repetitive TMS in stroke rehabilitation (e.g., Kim et al., 2006). The details of these techniques are, however, beyond the scope of this thesis.

2.1.2 Recording effects of TMS

2.1.2.1 Motor evoked potentials (MEPs). When the TMS coil is placed over the region of the left motor cortex representing the hand muscles, then the subject undergoing stimulation may experience a sensation or involuntary movement in the right hand. The compound muscle action potential associated with the muscle
response is termed motor evoked potential (MEP). An MEP may be defined as the electrical muscular response elicited by artificially stimulating the motor cortex or motor pathway above the spinal motor neuron (Sandbrink, 2008). The MEP represents the firing of a portion of the spinal motoneurons projecting on a muscle and is evoked when the cortical stimulus produces descending volleys large enough to bring the spinal motoneurons to their firing threshold. Various parameters of MEP can be studied: the size of the MEP (amplitude, duration, and area); stimulation thresholds; silent period; and facilitation, amongst others (Rosler & Magistris, 2008). The latency of the MEP is defined as the time between the cortical stimulation and the onset of an evoked potential in the target muscle (Sandbrink, 2008). The size of the elicited MEP (peak-to-peak amplitude) is most commonly measured, and can be used to infer the excitability of the corticospinal motor system at the time of stimulation (see Figure 2.2 on p. 17).

MEP facilitation is a measure of corticospinal excitability, with a shortening of the latency, a decrease in motor threshold (discussed further on pp. 49-50), and an increase in peak-to-peak amplitude (Reid, Chiappa, & Cros, 2002). The silent period is an inhibitory phenomenon. If the target muscle is active at the time of stimulation then a variable period of EMG absence typically follows the MEP (Schnitzler & Benecke, 1994). The initial component of the silent period (<50ms) is generated by predominately spinal inhibitory mechanisms whereas the later components (>50 ms) reflect a long lasting inhibition that originates within the motor cortex (Inghilleri, Berardelli, Cruccu, & Manfredi, 1993). An important characteristic of MEPs is their spontaneous inter-individual and intra-individual variability in amplitude from one
stimulus to the next, even if the stimulation intensity is kept constant. The reason for this is currently unknown, but highlights that in order to obtain a reliable estimate of MEP amplitude, a large number of responses (approximately 10-15) should be obtained to control for this variability (Kiers, Cros, Chiappa, & Fang, 1993).

2.1.2.2 Physiological basis of the MEP. Currently there is a far from complete understanding of how TMS influences brain activity, due to the complexity of the cortical structures that are stimulated. The accepted mechanism, however, by which TMS activates the motor cortex to produce the MEP is termed the D- and I-wave hypothesis (Day et al., 1989). Briefly, this hypothesis proposes that the electrical current induced in the cortex exerts its effects by two different mechanisms. The electric current may excite corticospinal neurons and their axons directly, giving rise to D- (direct) waves, and/or excite the corticospinal neurons trans-synaptically, giving rise to I- (indirect) waves. Both forms of wave, termed descending volleys, are then transmitted down to the alpha-motoneurons in the spinal cord via the large diameter, fast conducting axons of the corticospinal tract (Edgley et al., 1990; Di Lazzaro et al., 2004). If these descending volleys, individually or via summation, are sufficiently strong, a synchronised discharge of the spinal alpha-motoneurons will lead to a subsequent muscle contraction.

The validity of TMS as a method for assessing changes in the excitability of the motor cortex is based on the implications of the D- and I-wave hypothesis. If TMS activates corticospinal neurons in a trans-synaptic manner, other processes that elicit a change in cortical excitability will also modify the extent to which the cortical stimulation excites the corticospinal neurons. These in turn, will influence the
amplitude of the MEP obtained in the target muscle. In contrast, if TMS activates corticospinal axons directly at sites downstream to synaptic input then the amplitude of the MEP will not reflect the overall balance of cortical excitability at the moment of stimulation. This is a valid reason for identifying each individual’s motor threshold (discussed further on pp. 49-50). The onset latencies of MEPs obtained using TMS of high intensity are typically 1-2 ms quicker than those obtained using threshold intensities. Epidural recordings in conscious humans have demonstrated that this is because threshold intensity TMS preferentially activates corticospinal neurons in an indirect trans-synaptic manner, whereas high intensity TMS activates the corticospinal axons directly at a site below the level of the motor cortex (Di Lazzaro et al., 2004). This finding indicates that the amplitudes of MEPs produced using high stimulation intensities will be less representative of cortical excitability levels than MEP amplitudes recorded using near threshold intensities of TMS. The practical implication is that it is important to use stimulation intensities that are close to motor threshold if the purpose of the experiment is to measure cortical excitability.

2.1.3 Different stimulating coils

The design, position, and orientation of magnetic stimulation coils are all central factors in focal stimulation of the nervous system (Barker, Jalinous, & Freeston, 1985). Different designs of stimulating coils exist and the coil shape determines the properties and the size of the induced magnetic field (see Figure 2.3 on p. 21). The original design of stimulating coil was circular and although it produces an effective activation of the motor cortex, it has a limited capacity to target specific muscles (Barker, 1996). This is because the strength of the induced electric field is minimal.
underneath the centre of the coil and maximum underneath its windings. As the coil diameters are generally large (e.g., 80-100mm), the windings span a considerable area of the skull surface. Figure-of-eight shaped coils that comprise two small circular coils aligned in the same plane have a maximum electric field strength underneath the central overlapping section. This allows for a more focal stimulation that is more suitable for mapping cortical representations of muscles (Wassermann, McShane, Hallett, & Cohen, 1992). It should be noted, however, that even when using the figure-of-eight coil, stimulation normally elicits MEPs in several muscles at a time. This reflects the considerable overlap of different muscle representations within the primary motor cortex (M1; Sanes & Donoghue, 2000).

2.1.3.1 Coil positioning and orientation. An important factor when stimulating the corticospinal system is the direction that the induced electric current flows in the motor cortex. Recordings of muscle responses following magnetic stimulation depend upon the orientation of the stimulating coil (Day et al., 1989). Boniface, Mills, and Schubert (1990) reported differences in MEP amplitudes as a result of varying the orientation of a figure-of-eight shaped coil on the scalp. Furthermore, coil orientation is crucial in determining whether the earliest corticospinal tract activation is due to direct or indirect activation (Kaneko, Kawai, Fuchigami, Shiraishi, & Ito, 1996). The electric current induced by the magnetic field exerts its effects by two different mechanisms. Following the ‘D- and I-wave hypothesis’, D-waves are produced by direct activation of the cortical tract neurons and I-waves are produced by indirect or trans-synaptic activation of the corticospinal tract neurons (Di Lazarro et al., 2004).
**Figure 2.1**: Diagrammatic illustration of TMS stimulation. The coil is held on the scalp and the current passes through the coil generating a magnetic field perpendicular to the current direction in the coil (a; retrieved from Siu On (Ed.), n.d., *Transcranial Magnetic Stimulation (TMS)*, http://www.neuro.hk). EMG surface electrodes record the compound muscle action potential associated with the muscle response (b).

**Figure 2.2**: A schematic representation of the motor evoked potential (MEP).
If the magnetic-induced current flows in a lateral-medial direction then corticospinal fibres are stimulated directly, whereas a postero-anterior current flow stimulates corticospinal fibres indirectly (Werhahn et al., 1994). It is when TMS activates corticospinal neurons in an indirect, or trans-synaptic, manner that the MEP amplitude obtained during stimulation reflects the overall balance of cortical excitability at the moment of stimulation. According to Brasil-Neto et al. (1992), a postero-anterior direction perpendicular to the central sulcus is the optimal orientation for achieving indirect trans-synaptic activation, with the stimulating coil held tangentially to the scalp with the handle pointing at 45° posterior-laterally with respect to the mid-sagittal axis of the head (see Figure 2.4 on p. 21). Even a slight positional change or rotation of the coil can alter the MEP significantly, especially when using a figure-of-eight shaped coil (Sandbrink, 2008).

2.1.4 TMS safety

When the single pulse stimulations are delivered once every few seconds, TMS is reported to be a safe and useful tool for investigating human neurophysiology. It is not known to carry any significant risk (Evans, 2007; Rossi et al., 2009; Wassermann, 1998). TMS in healthy adults appears to carry little risk beyond occasional transient headaches or local discomfort at the site of stimulation (Anand & Hotson, 2002; Rossi et al., 2009). In the event of either of these issues arising, the testing session is usually terminated immediately.

Prior to the testing session, participants are typically asked about a set of exclusion criteria before proceeding with TMS. They are required to complete a
Transcranial Magnetic Stimulation Adult Safety Screen (TASS; Keel, Smith, & Wasserman, 2001). This questionnaire includes items such as: ‘Do you or anyone in your family suffer from epilepsy?’, ‘Do you have any implanted devices such as cardiac pacemakers or medical pumps?’ and ‘Do you suffer from severe or frequent headaches?’ (see Appendix A). The purpose of the TASS is to alert investigators to factors in potential subjects that may predispose them to adverse events during TMS. A positive answer to any of the items in the TASS may indicate susceptibility to adverse effects of TMS and these participants are excluded from all TMS experiments. In the Magstim TMS safety document (Evans, 2007), it is reported that research into TMS has led to the understanding that any adverse effects linked with magnetic stimulation can be reduced or even eliminated through the choice of pulse frequencies, burst durations and amplitudes. When any adverse effects have been experienced, they reportedly end upon cessation of the stimulation procedures or within a few weeks of procedure completion. Research with TMS has also shown it to be safe to administer in children (Frye, Rotenberg, Ousley, & Pascual-Leone, 2008).
Figure 2.3: Two designs of stimulating coils; the figure-of-eight and circular coil (retrieved from Walsh & Pascual-Leone, 2003). The coil shape determines the properties and the size of the induced magnetic field.

Figure 2.4: An illustration of postero-anterior coil orientation using a figure-of-eight stimulating coil. This coil orientation provides optimal results when attempting to achieve trans-synaptic activation of corticospinal neurons, giving a good representation of their state of excitability. The coil handle should be held at approximately 45° postero-laterally with respect to the midsagittal axis of the head. The current induced in the coil flows toward the handle, which is in the opposite direction to the current induced in the cortex.
2.2 Exploring the human motor resonance mechanism

TMS has become a standard technique for the non-invasive investigation of motor cognition, used to explore motor activity during action observation. The discovery of a mirror neuron system is discussed in the following section. This system has provided researchers with a framework to how to interpret the understanding of motor actions. The term ‘motor resonance’ has been used frequently in this context, describing how an observer simulates an observed action in order to better understand it (Decety & Grezes, 2006).

2.2.1 Discovery of mirror neurons

Mirror neurons were first reported by di Pellegrino et al. (1992), using single cell recordings in the macaque monkey (discussed on pp. 3-4). Mirror neurons have motor properties and also discharge in response to observing object-related hand actions (e.g., grasping, tearing) and ingestive and communicative mouth actions (Ferrari, Gallese, Rizzolatti, & Fogassi, 2003). In addition, they also fire at the sound associated with the action (e.g., breaking a peanut) even when the action is not seen (Kohler et al., 2002). There are two main categories of mirror neurons depending on the type of congruence they exhibit between the visual actions they respond to and the motor responses they code: ‘strictly congruent’ neurons consist of about one third of F5 mirror neurons and fire for exactly the same action, whereas ‘broadly congruent’ mirror neurons represent two thirds of F5 mirror neurons and do not require observation of exactly the same action that they code motorically (Iacoboni & Mazziotta, 2007).
2.2.2 Human mirror system

The recent discovery of mirror neurons has provided some explanation for the underlying cortical processes behind fundamental behaviours such as action understanding and recognition (Umilta et al., 2001), intention (Iacoboni et al., 2005), and observational and imitation learning (Buccino et al., 2004). According to the ‘direct-matching hypothesis’, we understand actions by mapping the visual representation of the observed action onto our own motor representation of the same action, causing the motor system of the observer to ‘resonate’ after observation of that action (Rizzolatti et al., 2001). Motor resonance ‘is revealed either as an overt imitation or as a subliminal activation of the motor structures that would sustain the observed action’ (Montagna, Cerri, Borroni, & Baldissera, 2005, p. 1513). Neuroimaging studies have shown a complex network involved in observation of actions performed by others.

As shown by many brain imaging studies, the two main nodes of human mirror system (see Figure 2.5 on p. 25) are the inferior parietal lobule (IPL) and the ventral premotor cortex (PMv), the caudal part of the inferior frontal gyrus (IFG), and a region within the superior temporal sulcus (STS; for a review see Rizzolatti & Craighero, 2004). The first evidence of a human mirror system was provided by EEG studies in the early 1950s. Gastaut and Bert (1954) observed a desynchronisation of an EEG rhythm (mu rhythm) occur when the participants executed the actions as well as when they watched the actions being performed by someone else. Cochin, Barthelemy, Roux, and Martineau (1999) also reported a decrease in mu rhythm power while subjects observed and executed the same movement, indicating that observation and
execution of actions activate the same cortical areas. Other evidence for the existence of a matching observation-execution network comes from magnetoencephalographic (MEG) studies. Hari et al. (1998) and Muthukumaraswamy and Singh (2008) reported changes in event-related beta-band desynchronisation when subjects observed other individuals performing the action. This desynchronisation was similar to the activity seen in the motor cortex when subjects executed that same action, signifying activity of a mirror neuron system. Further evidence is provided by fMRI studies, offering support for the idea that the same neural areas that are active during execution are activated during observation of that action. For example, Buccino et al. (2001) reported that during the observation of object-directed actions using the hand (grasping a ball or a cup), mouth (biting an apple and chewing) and foot (kicking a ball or pushing a brake) different sectors of the premotor cortex were activated depending on the effector the action is performed with. TMS is another technique used to investigate the involvement of the motor system in humans during observation of others’ actions, by measuring cortical excitability during various phases of the action and discriminating the muscles involved in the motor replica, with good temporal resolution (Craighero, Metta, Sandini, & Fadiga, 2007). It is important to note, however, that only recently has direct electrophysical evidence been provided for the existence of mirror neurons in humans (Mukamel et al., 2010).
Figure 2.5: An illustration of the main areas of the human mirror system, corresponding closely with the mirror neuron system of primates (retrieved from Iacoboni & Dapretto, 2006).

2.2.2.1 Mirror neuron debate. The generalisation of theories from mirror neurons in primates to humans has received criticism in recent years (e.g., Dinstein et al., 2008; Hickok, 2009). Many criticisms focus on the belief that mirror neurons are primarily involved in action understanding via the motor resonance model (e.g., Rizzolatti & Craighero, 2004). Rizzolatti et al. (2001) have claimed that observed actions can be understood because, if they already belong to the observer’s motor repertoire, they are mapped onto the observer’s motor system causing it to ‘resonate’. This motor resonance results in an immediate understanding of the observed action. There are a few problems associated with this statement. First, the term ‘action understanding’ is never clearly defined in the mirror neuron system literature. The motor act of turning on a sink tap could be understood as cleaning up, filling a glass, washing hands, and so on. There could be a wide range of possible goals, meanings, and intentions involved in a single motor act; having a motor representation of that
act is not sufficient to distinguish between them. This was also discussed in Chapter 1. Second, the association between an observed action and the firing of motor neurons may simply reflect a Pavlovian association, where the mirror neuron response is purely a result of learned sensory-motor pairings (Press, Heyes, & Kilner, 2010). Third, individuals can understand actions that they have never performed (Gallese, Gernsbacher, Heyes, Hickock, & Iacoboni, 2011). This clearly presents a problem to the motor resonance theory of action understanding. In a recent mirror neuron debate, Iacoboni argues that mirror neurons would provide a ‘richer’ understanding of that action if the individual had internal motor knowledge of the observed action (Gallese et al., 2001). Having the action embedded in the motor repertoire would lead to a different ‘understanding’, while someone without previous experience would not be able to access the ‘enriched’ knowledge (Hickok, 2009). As discussed in Chapter 1, ‘motor resonance’ is another ambiguous term in the literature, where either the motor system of the action observer resonates with his or her own perceptual system, with both brain areas active in the motor resonance process (e.g., Rizzolatti et al., 2001), or with the resonance being between two different people; the motor system of the observer and the executor of the action (e.g., in Decety & Grezes, 2006).

The mirror neuron theory of action understanding via the notion of motor resonance is intuitively reasonable; however this proposal needs to be adequately tested to provide stronger evidence than is currently being proposed in the literature. Terminology is vital, and clearer definitions need to be provided in the future for the key concepts in this area, before further claims can be made regarding the contribution of mirror neurons to social and motor cognition.
2.2.3 TMS in action observation research

When TMS activates corticospinal neurons in an indirect, or trans-synaptic, manner it is then that the MEP amplitude obtained during stimulation reflects the overall balance of cortical excitability at the moment of stimulation. This is important for any study of action observation since proponents of the mirror system would hypothesise that the activity in the motor areas of interest, and related to the covert behaviour, are additive to the TMS stimulation indirectly-induced activity, thereby resulting in an MEP facilitation when compared to control conditions. Most TMS research in action observation has been applied over the primary motor cortex. Neuroimaging studies, however, suggest that the two main brain areas of the human mirror system are the IPL and the PMv, including the caudal part of the IFG. Fadiga, Craighero, and Olivier (2005), however, proposed a mechanism whereby robust cortico-cortico connections connect primary motor cortex and premotor cortex. It is believed, therefore, that primary motor cortex excitability is increased through activation of the premotor areas that connect to primary motor cortex (Rizzolatti, 2005). Also, previous work using a ‘virtual lesion’ TMS approach (e.g., Avenanti, Bolognini, Maravita, & Aglioti, 2007) have highlighted the role of the PMv-IFG complex in the encoding of observed actions in humans.

TMS has been used widely in research investigating corticospinal excitability during action observation. The data has shown that observation of an action performed by the self or others, in the absence of any recordable overt movement, modulates the excitability of the corticospinal pathway in humans (e.g., Fadiga et al., 1995; Gangitano, Mottaghy, & Pascual-Leone, 2001; Strafella & Paus, 2000).
modulation typically results in the increase of the amplitude of MEPs specific to the muscles involved in the observed action. It should be noted that to ensure that the MEPs obtained are a result of action observation, rather than residual muscle activity from actual physical movement, EMG should be constantly monitored. This is important since activation of the muscle of interest causes larger TMS-evoked MEPs (Kiers et al., 1993). Trials showing high EMG muscle activity should be removed from analysis.

In one of the first studies to use TMS in an action observation condition, Fadiga et al. (1995) applied single pulse TMS to participants’ primary motor cortex. They obtained MEPs from a variety of muscles known to be responsible for controlling the fingers while participants observed one of four conditions: (i) two action observation conditions consisting of an object-directed grasping action and the tracing of Greek alphabet letters in the air; and (ii) two baseline conditions where participants either observed the object alone or a dimming LED on a computer screen. The data showed that the MEP amplitudes obtained as the participants observed both action conditions were higher than those recorded during the two baseline conditions. A further point of interest was the specificity of the response as this modulation of the MEP amplitude was found only in those muscles of the hand that would have been used to physically perform the observed motor actions. That is, observation of both grasping actions and letter drawing increased the amplitude of the MEP obtained in the first dorsal interosseus (FDI) muscle, but only observation of the grasping action modulated the MEP obtained in the opponens pollicus (OP) muscle, as this muscle is involved in index finger and thumb grasping. Studies such as Strafella and Paus (2000) and Patuzzo,
Fiaschi, and Manganotti (2003) have also reported that observation of hand actions result in modulation of corticospinal excitability. Strafella and Paus applied TMS during a rest control condition, during observation of hand writing, and during observation of arm movements. The hand and arm movement conditions produced significantly larger peak-to-peak amplitude MEPs than those obtained in the resting condition. The results were muscle-specific, with higher MEPs only occurring in the muscles involved in executing the specific actions. Similarly, in the study by Patuzzo et al. (2003), participants observed hand movements, geometric objects, or a blank screen. Findings showed that observation of the hand movements resulted in greater MEP peak-to-peak amplitude responses, once again providing evidence for a mirror neuron system representation of action.

The use of TMS to understand human representation of action via action observation has increased. For example, Gangitano et al. (2001) examined whether the amplitude of the MEPs elicited in the FDI and OP muscles were modulated in relation to temporal aspects of an observed video of a reach and grasp movement. TMS was applied to the primary motor cortex (M1) while participants observed a hand reaching towards and grasping a ball using a precision grip. The stimulation was delivered at different time intervals corresponding to the following specific phases of the movement: (i) the initial stationary hand position; (ii) the beginning of the action when the hand was lifted from the table; (iii) during the increase of the grasp aperture; (iv) the time of maximal grasp aperture; and (v) when the hand closed on the ball. The data showed that the amplitude of the MEP recorded in the FDI muscle tended to increase throughout the movement with the largest MEP recorded at the
point of maximal grasp aperture. Gangitano, Mottaghy, and Pascual-Leone (2004) explored the effect further by asking participants to observe normal reaching and grasping actions compared to an unusual action, where the hand would close inappropriately and reopen prior to grasping the object. The data replicated that of Gangitano et al. (2001) for the observation of normal action, but showed the MEP facilitation only occurred during the early phase of the observed unusual movement, not during the second segment after the initial closure. It was proposed that this finding suggests that the motor representation ‘predicts’ the outcome of motor acts before they occur, rather than monitoring and matching the observed movements as they develop in an online fashion.

In another study, Borroni, Montagna, Cerri, and Baldissera (2005) examined the relationship between MEP amplitudes recorded from two antagonistically-paired forearm muscles (the flexor and extensor carpi radialis) while participants observed an experimenter performing a cyclic wrist flexion/extension movement. The amplitudes of the MEPs obtained in both muscles were facilitated in different periods of the observed movement; those recorded from flexor carpi radialis were facilitated when the observed movement was in a period of wrist flexion, and those recorded from the extensor carpi radialis were facilitated during observation of the corresponding wrist extension movement. These findings indicate that the more a muscle is active at a particular point in an observed movement, the greater the MEPs recorded from the muscle at that point. This study provides further evidence for the muscle and temporal specificity in the MEP facilitation effect whilst also demonstrating that the human
mirror system seems to respond to simple intransitive actions with no specific object-orientated goal.

Taken together, these studies indicate that familiarity with the observed action may play an important role in the motor facilitation effect. The ability to perform the observed movement, however, may not be essential to modulate corticospinal excitability. Romani, Cesari, Urgesi, Facchini, and Aglioti (2005) conducted a series of experiments examining the effects of observing biologically possible movements (such as abduction and adduction of the index finger) compared to biologically impossible movements created by shifting the position of the moving digit; the participant would effectively observe the same abduction/adduction movements occurring at points beyond the normally possible range of movement. Surprisingly, MEP facilitation occurred in the associated muscle when observing both the normal and impossible movements and this pattern of facilitation was consistent for a number of conditions involving possible and impossible movements. This suggests that facilitation effects are not limited to movements that are part of the observer’s motor repertoire, and neither is facilitation limited to ‘normal’ movements. One problem with this design is that the impossible movements were generated from normal finger movements and simply altered relative to the hand. Therefore, the effects could be explained as a response to local aspects of the observed normal kinematic action profiles. Certainly, more TMS-based studies are needed to test these effects, but the implications for the human movement sciences are evident; imaged and observed actions need not necessarily comprise those that are part of the individual’s contemporary history of experiences or present motoric ability. There may also be implications for
metaphorical imagery interventions where the imagined movements can frequently be impossible for humans.

2.2.4 TMS in movement imagery research

In contrast to the action observation literature, TMS research in movement imagery is limited, possibly because of the methodological difficulties in controlling the covert behaviour (see Holmes & Calmels, 2008, for a review). It is likely, however, that there is some shared neural substrate between motor execution, action observation and movement imagery (Holmes, Cumming, & Edwards, 2010). Therefore, in a similar way to the action observation studies discussed above, movement imagery also offers opportunities for TMS research and because of the shared circuitry between action observation and imagery, it is important to present the following imagery TMS research. Indeed, some studies have already demonstrated that engaging in movement imagery is associated with a measurable muscle specific change in corticospinal excitability. As with the action observation studies, EMG activity needs to be monitored throughout movement imagery experiments to control for non-experimental muscle activity at all times. In this way, MEP changes can be attributed to changes in the corticospinal system as a result of the movement imagery. In Fadiga, et al.’s (1999) study, participants imaged a forearm flexion and extension movement in time to an auditory stimulus whilst single pulse TMS was applied over the hand area of motor cortex. The amplitudes of the MEPs recorded from the biceps brachii during imagery of forearm flexion were significantly greater than those recorded during imagery of forearm extension. In contrast, the amplitudes of the MEPs recorded from the OP muscle were not affected by the movement imagery task. This suggests a
similar effect to that occurring during action observation in that corticospinal excitability was only found when the muscles involved in the physical task were imaged to perform as force-generating agonists. In a similar study, Facchini, Muelbacher, Battaglia, Boroojerdi, and Hallett (2002) applied TMS to the primary motor cortex whilst participants imaged thumb abduction movements. Consistent with Fadiga et al.’s (1999) findings, the results showed a rapid increase in motor cortex excitability during the motor imagery condition. In a more detailed study of imagery processes using TMS techniques, Stinear, Byblow, Steyvers, Levin, and Swinnen (2006), examined changes in corticospinal excitability during kinaesthetic imagery (imagining the feeling that the movement of the task creates) and third person perspective visual imagery (imagining seeing oneself performing the task) in a similar thumb movement task. The authors reported greater involvement of primary motor cortex in the movement imagery process during the kinaesthetic imagery condition. This is consistent with previous findings using other neurophysiological techniques (fMRI, e.g., Porro, Cettolo, Francescato, & Baraldi, 2000; EEG, e.g., Stecklow, Infantosi, & Cagy, 2010). Taken together, this evidence provides further support for a central mechanism to explain movement imagery’s effects rather than the now outdated peripheral ‘psychoneuromuscular’ theories.

2.2.5 Comparing effects of observation, imagery and execution using TMS

In one of the first attempts to compare MEP magnitudes in the three behavioural conditions, Clark, Tremblay, and Ste-Marie (2003) separated action observation into two further conditions of either passive observation, where the participant simply observed a movement, or in a condition requiring observation to
imitate. In this latter condition participants observed an action in order to perform it for themselves at a later time. All conditions showed a significant increase in MEP magnitude and significant decrease in MEP latency compared to the baseline conditions, mentally counting backwards and a post baseline activity check. While performing actions physically led to the greatest difference from the baseline conditions, there was no difference in the level of facilitation between passive observation, observation to imitate and movement imagery of the action. This is an interesting finding suggesting that not only do action observation and movement imagery show changes in corticospinal excitability, but confirming that both behaviours share at least some neural substrate with the physical execution of action. The possibilities for combining the two processes in multiple intervention strategies to support physical practice would seem sensible.

2.3 TMS methodological issues

Action observation research consistently shows a motor facilitation during action observation when compared to observation of a control condition. This increase in MEP amplitude during action observation has been increasingly reported across a number of laboratories, confidently reporting a muscle specific effect that supports motor resonance claims. The methods and methodology by which these results were attained are sometimes weak and have not been fully investigated. Addressing a number of concerns regarding the current TMS methodology in action observation research was the main aim of this thesis. The following section provides a critical review of some of these issues.
2.3.1 Control condition issues

An increasingly large number of research groups have used TMS to test the observation-execution matching system in humans. There have, however, been inconsistencies in the control conditions employed in these studies. For example, many TMS action observation studies (e.g., Alaerts, Heremans, Swinnen, & Wenderoth, 2009a; Gangitano et al., 2001, 2004; Leonard & Tremblay, 2007; Patuzzo et al., 2003) have compared the MEP amplitudes during action observation against observation of a blank screen, with results generally showing larger MEP amplitudes in the action observation condition. Control conditions need to be more rigorous, however, as it is not possible to determine whether the recorded effects are specifically due to the observation of the action *per se*. In this way, they could be due to the presence of an object on the screen irrespective of the type of action, muscles used, or meaning of the action. Whilst a small proportion of TMS action observation studies have compared action observation to static image controls (e.g., Catmur, Walsh, & Heyes, 2007; Lepage, Tremblay, & Theoret, 2010; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006), the inconsistencies in the control conditions make it difficult to compare findings across laboratories. Furthermore, by using a blank screen or a fixation cross control, researchers are unable to attribute the MEP changes to the observed action. It is, therefore, more revealing and informative to compare the MEP facilitation obtained during action observation to a static control of the same ‘action’ in order to represent a true facilitation; to report an increase (or decrease) from that static control. Using a static control condition clearly seems to have benefits over a blank screen in action observation studies and would seem to be the obvious control
choice. This was the focus of Study 1. There are, however, studies that have used other, novel, control conditions to explore the mechanisms of action observation further. Romani et al. (2005) compared TMS responses during the viewing of either biomechanically-possible or -impossible movements, the latter, in effect, acting as a novel control condition that allows normal movement comparisons beyond that which blank screen or static control conditions would allow. Other novel control manipulations should be explored further in the future.

2.3.2 Priming issues

The influence of action observation on execution has been widely explored (e.g., Brass, Bekkering, Wohlschlager, & Prinz, 2000; Brass, Bekkering, & Prinz, 2001; Kilner, Paulignan, & Blakemore, 2003) and has shown that initiation of movement execution was facilitated following movement observation (Brass et al., 2000). Taking the assumption that a mirror neuron system is present in humans and there are neural circuits that overlap which are involved in both observation and execution, the influence of one over the other is expected. Considerably few studies, however, have examined the effects of action execution on the possible priming effect on subsequent action observation. If action execution influences action observation then this information would show that the mirror system integrates observed actions of others with individuals’ personal repertoires, adding to the knowledge of how the human action observation-execution system works. The effects of long term practice and expertise on the action observation network have been investigated extensively over periods of weeks, months, and years. These cross-sectional studies focussed on the influence of expertise on the action observation-execution network (e.g., Aglioti,
Cesari, Romani, & Urgesi, 2008; Calvo-Merino, Glaser, Grezes, Passingham, & Haggard, 2005; Haslinger et al., 2005), with fewer studies showing the effects of short term practice of physical actions, on the responsiveness of the mirror system (e.g., Catmur et al., 2007; Sakamoto, Muraoka, Mizuguchi, & Kanosue, 2009). It is important to check for priming effects when conducting action observation experiments since previous experience of the action being observed may prime the observer to perform that same action and lead to increased motor responses as measured by TMS. Furthermore, executing the observed action should influence MEP amplitudes in the muscle performing the action but not in the unused muscle. Most studies have used a cross-sectional design to explore this (e.g., Aglioti et al., 2008; Calvo-Merino et al., 2005), with others such as Sakamoto et al., (2009) having had limitations which have been addressed and are the focus of Study 2.

2.3.3. Stimulation timing issues

In the majority of action observation studies, TMS pulses are delivered at various time points after video onset; with the timings usually corresponding to the same phase of the observed action. The rationale behind this is to remove the predictability of the stimulation onset. This is a commonly used approach (e.g., Alaerts, Swinnen, & Wenderoth, 2009b; Clark et al., 2003; Desy & Theoret, 2007; Romani et al., 2005; Sakamoto et al., 2009; Urgesi et al., 2006a). Since the TMS pulses are usually delivered during the same phase of the repetitive action, the general assumption is that the different timings incorporated in these studies will have no effect on the motor facilitation obtained during action observation.
The concept of the timing of an action has been explored in some studies, showing that MEP amplitudes are modulated in relation to temporal aspects of an observed reach and grasp action. For example, Gangitano et al. (2001) delivered TMS pulses at different time intervals corresponding to different phases of the observed movement. The data showed that the amplitude of the MEP recorded in the muscle specific to the observed action was largest at the point of maximal grasp aperture, showing that the motor facilitation follows the temporal course of the observed action. No research to date, however, has yet explored the effects of applying TMS at different time points during observation of a repetitive action. The MEPs obtained during two or more time points are usually combined following the assumption that this has no effect on the resulting MEP. Muscle resonance claims rely on the fact that MEP amplitude changes occur with similar time-course to EMG changes during action execution. Factors such as attention or eye gaze metrics, may affect the size of the MEP amplitude. Studies should therefore consider whether this may confound the muscle specific results. The effect of the stimulation timing on the MEP amplitude response was explored in Studies 1 and 2.

2.3.4 Optimal scalp position issues

In TMS action observation studies it is common for experimenters to record MEPs from a number of concurrent muscles (for example, when recording from various finger and wrist muscles during observation of a reach and grasp action). Researchers tend to determine the OSP for only one of the muscles under investigation (usually the main muscle of interest). Once that position is located, the coil is then positioned over that scalp site and stimulation occurs at that one site.
throughout the experiment. This is problematic because MEPs are recorded for other muscles which, as a result of this method, are not being stimulated at their respective OSPs. Often, despite testing muscles that are in close proximity with each other, they differ slightly in their positioning over the motor cortex. Stimulating a muscle at a site other than its OSP may also affect the muscle’s threshold intensity and, therefore, influence the stimulation intensity applied throughout the experiment. The significance of the importance of stimulation intensity is described further in section 2.3.5. Furthermore, to show a true muscle specificity effect, a double disassociation effect needs to be present. Studies often show an effect for the main muscle of interest with the secondary muscle simply acting as a control muscle, and without being tested. It is important to test the two separate muscles, and be able to report larger amplitude MEPs for both muscles, only when observing that muscle in action. Study 3 focussed on achieving this double disassociation effect, using separate optimal scalp positions for each muscle.

2.3.5 Stimulation intensity issues

In TMS action observation experiments, contrasting results are often obtained and this may be explained by the intensity used for stimulation. As explained on pp. 15-16, there are practical implications regarding the choice of the stimulation intensity used. It is therefore interesting to note the variety of intensities used by different researchers and laboratories to investigate similar phenomena. Some researchers have applied TMS over motor cortex with a stimulation intensity of 110% RMT (e.g., Borroni et al., 2005; Catmur et al., 2007; Gangitano et al., 2004; Montagna et al., 2005; Takahashi, Kamibayashi, Nakajima, Akai, & Nakazawa, 2008; Molnar-Szakacs, Wu,
Robles, & Iacoboni, 2007) with others stimulating at the higher intensities of: 120% RMT (e.g., Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002; Bufalari, Sforza, Cesari, Aglioti, & Fourkas, 2010; Fourkas, Ionta, & Aglioti, 2006; Patuzzo et al., 2003; Sakamoto et al., 2009); 130% RMT (e.g., Aglioti et al., 2008; Alaerts et al., 2009a, 2009b; Romani et al., 2005; Urgesi, 2006a); and some as high as 150% RMT (e.g., Li, Stevens, & Rymer, 2009). The range of intensities used in these experiments is of concern as TMS pulses can evoke different kinds of descending volleys depending on the intensity of the stimulation (Di Lazzaro et al., 2004), which will have an impact on the resulting MEPs obtained during action observation or movement imagery. It is likely that the studies that have stimulated at higher intensities are reporting a different effect to those stimulated at lower intensities, and this may have implications for the motor resonance hypothesis. Higher intensity stimulations may be activating corticospinal neurons in a different way to lower intensity stimulations, and may also be stimulating various muscles at a time. The issue of TMS intensity is not independent from the hotspot issue discussed in section 2.3.4 as changes in OSP also effect the TMS intensity. Study 4 focussed, therefore, on comparing high and low stimulation intensities on the modulation of MEP amplitudes during action observation.

2.4 Aims and overview of the research programme

This thesis presents a series of studies exploring methodological considerations when using TMS as a technique to investigate activity in the motor system during action observation. There were two main aims:
1. to address methodological issues in TMS action observation research and to make procedural recommendations and improve scientific rigor for future studies;

2. to explore the effects of action observation on the corticospinal system.

The majority of action observation experiments have shown an increase in corticospinal excitability during the observation of an action on screen. This effect has consistently been replicated. The methods, however, with which these experiments have been carried out has been inconsistent. The general hypothesis in most studies was that there would be an MEP motor facilitation effect when participants observed an action performed on screen, as opposed to a ‘non-action’ control condition. Since no data has been published exploring the methodological issues raised in section 2.3 of Chapter 2, it was unclear how the expected motor facilitation would be effected by each of the methodological concerns in this programme of research.

In Study 1, the ‘controls’ issue was examined. In TMS action observation experiments, participants usually observe someone else perform an action on screen, while TMS pulses are delivered at certain time points throughout the action video. In addition to the action video condition, there is usually a non-action control condition. MEPs are recorded and the mean peak-to-peak MEP amplitudes are compared across conditions. The typical finding is that the MEPs obtained during the action observation condition are larger than those recorded during observation of the control condition. Despite the importance of rigorous resting control conditions in this field of research, no studies have compared the effects of different controls in TMS action observation
studies. As discussed earlier, for the contrasts to be valid, the control must be appropriate for the theoretical design. The control conditions that were tested in Study 1 included a blank screen with a fixation cross in the middle of the screen, and a static image of a hand holding a ball. The action video consisted of a hand repeatedly pinching a soft ball. The fixation cross and static image are two common used controls in the TMS literature, which are often assumed to carry out the same function; acting as a condition with which to compare the size of the TMS responses obtained during observation of an action. Choosing the right control condition is important. Failure to use the right control conditions may bias the results, either by risking a type 1 or type 2 error. Not having a universal control condition when exploring the same hypothesis also makes it difficult to compare results across laboratories. It was hypothesised, therefore, that there would be an increase in MEP amplitude during the action observation condition when compared to both the static hand and fixation cross controls.

In Study 2, the same action video of a hand pinching a ball was used. As a result of the findings of Study 1, a static image control condition was used, as this was deemed to be the optimal control condition for action observation TMS research. This is because the observed stimuli on screen are more similar to those in the action condition, without the movement, and is a better test of the mirror neuron system predictions. In this study, priming effects were examined. Specifically, whether previous experience of the ball pinching action primed the observer to perform that action during action observation, leading to an increased motor response. Participants observed a series of action observation trials, similar to that carried out in Study 1.
This was then followed by a series of ball pinching execution trials where participants became more familiar with the ball pinching action. Participants then repeated the observation trials and the motor responses were recorded. The hypothesis was that action observation would be associated with a motor facilitation, as in Study 1, but that this effect would be enhanced after a brief period of action execution was performed prior to the observation trials.

Studies 1 and 2 also explored another methodological issue within the main experiments. In both studies, the TMS pulse was delivered at two set time points during the observation videos; one stimulation was delivered at 6250ms after video onset, and another stimulation was delivered at 8750ms after video onset. The timing of the TMS pulse is one aspect of the TMS action observation literature that has not been explored in relation to observation of repetitive movements. Two or more stimulation time points are often used during observation of a repetitive hand action in order to reduce the predictability of the stimulus, as this has been shown to affect the size of the obtained motor response. The different time point stimuli are assumed to reflect the same motor response. There has been no evidence to date which has supported this claim. As a result, it was unclear whether there would be a difference in MEP amplitudes between the two stimulation timings. This was examined in both Studies 1 and 2.

Studies 3 and 4 investigated two other important methodological issues, closely related to the general TMS procedure. It is common practice in TMS research to first measure the head of the participant and locate the optimal scalp position (also termed ‘motor hotspot’) over which the TMS coil is placed throughout the experiment. When
more than one muscle is being investigated, as is often the case, this becomes problematic. One main finding which is reported consistently in the literature is a muscle specific motor response during action observation; however this is usually reported without exploring the muscles separately. Study 3 investigated this issue in detail and was the first study to explore this by carrying out separate experiments for each muscle under investigation. Following the location of the motor hotspot, it is general practice in TMS experiments to identify the motor threshold for each participant in order to set the magnetic stimulator at an intensity of a given percentage of the motor threshold. Despite that, the motor response obtained through TMS may be affected depending on the stimulation intensity used. A wide range of intensities have been used in the literature. This is of concern for two reasons: i) higher intensities may be less representative of the on-going cortical activity, therefore the MEP response may be a reflection of something other than that which is being explored in the study, and (ii) it is difficult to compare and contrast results across laboratories. The effects of different intensities on the MEP response were investigated in Study 4.

The series of studies presented in this thesis are interlinked. The methodological issues described and explored throughout Studies 1-4 should continue to be investigated. More detailed analyses and investigations on how these experiments are implemented are warranted.
The main findings of this research programme are highlighted below. The implications and applications of these findings are discussed in Chapter 8.

- A control condition containing stimuli as close as possible to the observed action (minus the actual movement) should always be employed when investigating motor responses during action observation.

- It should not be assumed that the motor responses measured during action observation of a repetitive movement are not affected by the timing of the TMS pulse. The timing of the stimulation is important, due to attentional factors, and may influence the size of the MEPs obtained during action observation.

- Familiarity of executing an action has previously been shown to increase the size of motor responses obtained during action observation; however this may not be the case with simple everyday actions, as seen in Study 2.

- Despite each muscle having its own specific motor hotspot, stimulating two closely-located finger muscle representations in the same testing session using one mutual hotspot may not cause significant problems regarding the motor responses obtained during action observation.

- The intensity of the stimulation has been shown to affect the motor responses elicited by TMS. It is recommended that the intensity of the TMS pulse be as close to motor threshold as possible to be more representative of the ongoing level of cortical activity.
Chapter 3: TMS methods and methodology

TMS is still a relatively new psychophysiological technique and, whilst it is a valuable technique for measuring the excitability of a shared motor representation, it is not without its methodological concerns. Addressing some of these issues should allow future work in this area to be highly rigorous and is the main focus of the current research programme. This Chapter describes the general procedure that needs to be followed in order to carry out any action observation experiment using TMS.

3.1 General TMS procedure

3.1.1 Electromyography (EMG) skin preparation

Since the effects of TMS are monitored through EMG recordings it is essential to adequately attach the surface skin electrodes to the muscles of interest to measure the resulting muscle activity. The EMG signal is measured either by applying conductive elements or electrodes to the skin surface, or invasively within the muscle. Surface EMG is the more common method of measurement in TMS motor cognition research, since it is non-invasive and does not need to be conducted by medical doctors (Day, 2002).

Skin preparation is necessary to obtain good electrode-skin contact and get the best signal possible. The electrode site on the skin is therefore rubbed with an alcohol swab, to remove the outermost layer of skin, including dead skin material and oil secretions, and then allowed to dry. The area may first be shaved with a disposable razor. The electrode contacts should also be cleaned with an alcohol wipe. Once dry, the electrodes are then attached to the skin using adhesive skin interfaces. A small
amount of conductive gel is placed on the electrode contact to reduce the electrical impedance between the electrode contacts and the skin. In addition to the recording electrodes, a reference electrode is required and must be attached to electrically neutral tissue, such as a bony landmark (e.g., ulnar process of wrist), with the same degree of skin preparation given to the muscle sites (Burden, 2008).

3.1.2 Head measurement

Once the EMG electrodes are attached to the muscles, the next step is to measure the head in order to identify the correct positioning for TMS coil placement. The participant usually wears a tight fitting polyester cap to allow the researcher to easily mark the measurements on the head. The apex of the skull (termed Cz) is measured out using the International 10-20 System (Jasper, 1958). The 10-20 system is commonly used for EEG electrode placement and for correlating skull locations to underlying cortical areas (Herwig, Satrapi, & Schonfeldt-Lecuona, 2003). This system is now also being applied to coil positioning in TMS studies. The 10-20 system is based on anatomical landmarks proportional to the size and shape of the skull. The first measurement is taken in the anterior-posterior plane through the vertex, taken from the landmarks nasion to inion. The next measurement will be the lateral measurement of the central plane starting at the left preauricular point, passing through the previously marked centre point (nasion to inion measurement), ending at the right preauricular point (Klem, Luders, Jasper, & Elger, 1999). The point where these two marks intersect is termed Cz (see Figure 3.1 on p. 51).
3.1.3 Optimal scalp position

Having measured Cz, it is standard procedure to find the optimal scalp position (OSP) for stimulating the motor cortex area responsible for the muscles to be tested. Determining the stimulation site is primarily influenced by Penfield’s homunculus (see Figure 3.2 on p. 51), which illustrates cortical representation by means of a diagram of a cross-section of the cerebral hemispheres with solid bars at the periphery indicating the relative cortical areas from which the corresponding bodily responses are elicited (Schott, 1993). This map is more of a functional map of cortical output rather than a physiological or anatomical one. Once the approximate location has been determined (e.g., 4cm lateral, 1.5cm anterior to Cz for FDI muscle), it is then imperative to locate the OSP, and mark it to use for the duration of the experimental session for stimulating that muscle. The magnetic coil is then held over the OSP without any excessive coil or head movements, as even small changes in coil position or rotation can significantly influence MEP amplitudes (Boniface et al., 1990; Balslev, Braet, McAllister, & Miall, 2007). For this reason accurate monitoring of coil position is crucial. Fixing the coil to the head using a frame significantly aids this process as compared to when the coil is hand held. Newly available image-guided frameless stereotaxic neuronavigation systems, though expensive, reduce experimenter bias and offer the best solution for precise monitoring of coil position (Sparing, Hesse, & Fink, 2010).

The optimal scalp position is found by repeatedly stimulating around the neighbouring points (in steps of 0.5 to 1cm in all directions) of the approximate location on the scalp, using relatively high stimulation intensity (e.g., 60% stimulator
output for finger muscles and 70% stimulator output for wrist muscles). The OSP (or ‘motor hotspot’) is marked as the position that produces MEPs with the highest peak-to-peak amplitudes and shortest onset latencies in the target muscle. The next step is to determine the resting motor threshold.

3.1.4 Resting motor threshold

The resting motor threshold (RMT) needs to be determined in order to standardise the procedure across participants and establish the stimulation intensity in order to run the experiment for each individual participant. This is important as cortical excitability varies between people due to differences in the thickness of the skull, as well as on cortical network properties controlled by neuromodulators (Ziemann, Steinhoff, Tergau, & Paulus, 1998). A wide range of motor thresholds are found within the healthy population. Current evidence suggests that this variation is largely independent of age, gender and hemisphere but strongly reflects anatomical factors such as individual differences in the distance between scalp and the underlying cortical tissue (McConnell et al., 2001; Stokes et al., 2005). Individual RMTs are stable over time and show good reproducibility between sessions (Mills & Nithi, 1997). The stimulation intensity is commonly normalised to a given percentage of each participant’s RMT. RMT has been described as the lowest stimulator output intensity capable of producing MEPs of ≥ 50µv peak-to-peak amplitude in 50% of the stimulations (Rossini et al., 1994). This is achieved by sending a train of pulses over the OSP, starting from a set percentage intensity of the maximal stimulator output of the magnetic device being used, and gradually increasing or decreasing the output intensity in 1% - 5% increments until the RMT percentage is obtained. Active motor
threshold (AMT) can also be determined as the participant performs a small contraction of the target muscle (5-10% maximum voluntary contraction). For this measurement, 50% of MEPs greater than 100 µv are required. AMT values are considerably lower than RMT values. This reflects that the voluntary contraction leads to increased excitability at both the cortical and spinal motorneuron levels (Di Lazzaro et al., 1998). It is important for participants to maintain a constant limb position during any measures of motor threshold since variations in proprioceptive input can also modulate the excitability of the motor cortex (Lewis, Byblow, & Carson, 2001). Attention should also be paid to the inter-stimulus interval as the response to a given stimulus can be influenced by prior stimuli. An inter-stimulus interval of around five to ten seconds between each pulse is usually included to allow the effect of the previous stimulation to subside. This is also consistent with the safety guidelines for the length of inter-stimulus interval recommended by Chen et al. (1997).

To summarise, before starting a TMS experiment, it is standard procedure to apply the EMG electrodes to the muscles, measure out the head using the 10-20 system, locate the OSP for the target muscle, identify the RMT, and determine the preferred simulation intensity. The following section describes the general method that was applied to all the studies carried out in the thesis. Any exceptions or deviations from this method specific to any one study will be described fully in the methods section of that chapter.
**Figure 3.1:** An illustration of the 10-20 electrode placement based on anatomical landmarks used in EEG to correlate skull locations to underlying cortical areas. This system is also applied to TMS coil positioning (Adapted from Albino Eatpod, (2001). A Small Guide to a Brain Computer Interface – Parts I - V, now complete [Online forum comment] from http://arstechnica.com/civis/viewtopic.php?f=26&t=178288).

**Figure 3.2:** A functional map, illustrating the cortical representation from which the corresponding bodily responses are elicited (Adapted from “Theories of Phantom Limb Pain” (n.d.), from http://emedicine.medscape.com/article/1948621-overview).
3.2 General methods applied to all studies

3.2.1 Participant information

All participants were right-handed as assessed by the Edinburgh Handedness Inventory (EHI; Oldfield, 1971; see Appendix B). Participants were naïve to the purpose of the experiment. The TMS Adult Safety Screen (Keel et al., 2001; Appendix A) was used to identify and exclude any participants who may have been predisposed to possible adverse effects of the stimulation. No discomfort or adverse effects during TMS were ever reported by the participants either during or after the testing session. After reading an information sheet (see Appendices C-G) all participants provided written informed consent to take part in the studies (see Appendix H). The protocols were approved by a Departmental Ethics Committee at the Manchester Metropolitan University and conducted in accordance with the Declaration of Helsinki.

3.2.2 EMG recordings

EMG recordings were collected simultaneously throughout the experiments from the FDI and abductor digiti minimi (ADM) muscles of the right hand using bipolar, single differential, surface EMG electrodes (DE-2.1, Delsys Inc, Boston, MA). The electrodes comprised 10mm x 1mm silver bar strips, spaced 10 mm apart, recorded with a sampling rate of 2kHz, bandwidth of 20Hz to 450kHz, 92dB common mode rejection ratio, and >10^{15}Ω input impedance. Two electrodes were placed over the belly of the FDI and ADM muscles and a reference electrode was placed over the ulnar process of the right wrist. The EMG signal was received by a Micro 1401 (Cambridge Electronic Design (CED), Cambridge, UK) analogue-digital converter for the signals to be
stored on a computer using Spike 2 version 6 software (CED). Appendix I illustrates a wire diagram of the laboratory set-up.

3.2.3 TMS procedure

TMS was administered using a figure-of-eight coil (mean diameter of 70 mm) connected to a Magstim 200² magnetic stimulator (Magstim Co., Whitland, Dyfed, UK), delivering monophasic pulses with a maximum field strength of 2.2 Tesla. A mechanical arm (Monfrotto, Italy) held the coil in a fixed position over the OSP for stimulating the participant’s left motor cortex. The coil was orientated so that the flow of induced electrical current in the brain travelled in a posterior-anterior direction, perpendicular to the central sulcus. As mentioned on pp. 17-19, this is the optimal orientation for the resulting MEPs to reflect the overall balance of cortical excitability at the moment of stimulation (Brasil-Neto et al., 1992). The OSP was identified (following the procedure outlined on pp. 48-49) as the scalp location which produced MEPs of the greatest amplitude from the right FDI muscle with a stimulation intensity of 60% maximum stimulator output. The OSP was marked on a tightly fitting polyester cap on the participant’s head to ensure a constant location throughout the experiment. RMT was determined using the MEP amplitudes obtained from the FDI muscle and was defined as the minimum stimulation intensity that elicited peak-to-peak MEP amplitudes greater than 50 µv in at least 5 out of 10 trials (Rossini et al., 1994). The stimulation intensity was set at 110% RMT, except when intensity stimulation was an independent variable (in Study 4).
3.2.4 Experimental procedure

Participants were seated in a dimly illuminated room with their elbows flexed at 90° with their hands pronated in a relaxed position on a table directly in front of them. The participant’s head was comfortably supported on a chin and head rest to restrict movement. A 37 inch Panasonic LCD television screen (resolution, 1024 x 768 pixels; refresh frequency, 60Hz) was positioned one metre in front of the participant. Blackout curtains ran along either side of the table and behind the screen to eliminate any distracting visual stimuli in the room. Participants were requested to refrain from any voluntary movement and to attend to the different stimuli presented on the television screen. The experimental protocol for each study is discussed in the respective chapter. Figure 3.3 on p. 56 shows a photograph of the laboratory set-up for Studies 1-4.

3.2.5 Data analysis

A pre-stimulus recording of 200ms was used to check for the presence of EMG activity in the muscles before the TMS pulse was delivered. Individual trials in which the peak-to-peak amplitude of the baseline EMG activity was 2.5 SD higher than the mean baseline EMG activity of each participant were discarded from further analysis as the presence of EMG activity immediately prior to the stimulation may have influenced the amplitude of the subsequent MEP (see Appendix J for discarded trials information).

The peak-to-peak MEP amplitude was measured from both the FDI and ADM muscles during the observation trials in each study. The mean MEP amplitude was
then calculated and due to the large inter-participant variability in the absolute MEP amplitudes, these data were normalised using the z-score transformation. A z-score indicates by how many standard deviations each data point is above or below the mean. This is commonly applied in TMS action observation studies (e.g., Aglioti et al., 2008; Fadiga et al., 1995; Urgesi et al., 2006a). The normalised MEP amplitudes were then analysed. The level of statistical significance for all analyses was set at $\alpha = 0.05$, with Sidak corrections applied where necessary. Effect sizes were reported as partial eta squared ($\eta^2_p$) for main effects, and as the difference in z-scores (ES) for further comparisons.
Figure 3.3: The laboratory set-up for Studies 1-4, showing the: (a) television screen; (b) Delsys EMG kit; (c) Magstim magnetic stimulator; (d) EMG electrodes; (e) chin rest; (f) TMS figure-of-eight coil
Chapter 4: Study 1: Motor facilitation during action observation: ‘controlling’ the controls

4.1 Introduction

Research investigating primates by di Pellegrino et al. (1992) identified a class of visuomotor neurons in area F5 of the premotor cortex that discharge both when a monkey reaches for and grasps an object, and when it observes the experimenter perform the same or similar action. As discussed in the literature review in Chapter 2, Fadiga et al. (1995) first used TMS to investigate whether the excitability of the human motor system is influenced by the observation of another person’s actions. They reported that in comparison to non-action control conditions, the observation of hand and arm movements was associated with an increase in corticospinal excitability.

Many studies have since replicated this effect showing that, in the absence of overt movement by the observer, observation of an action can facilitate MEP amplitudes recorded from the muscles that would be used to perform the observed action (e.g., Borroni et al., 2005; Gangitano et al., 2001, 2004; Montagna et al., 2005; Strafella & Paus, 2000). This muscle-specific motor facilitation is typically interpreted as evidence of a neural system that matches both action execution and observation (Fadiga et al., 1995) similar to the mirror neuron activity as first reported in the macaque monkey (di Pellegrino et al., 1992) and more recently in humans (Mukamel et al., 2010). It is therefore believed that the MEP amplitude produced following the application of TMS over the motor cortex can provide an indirect marker of this action-observation matching system (as discussed in section 2.2.3, pp. 27-32).
As highlighted in section 2.3.1 on pp. 35-36, the resting control conditions employed across TMS action-observation studies have been inconsistent. For example, several studies (Alaerts et al., 2009a; Gangitano et al., 2001, 2004; Leonard & Tremblay, 2007; Sakamoto et al., 2009), have used the observation of a black background or a small fixation cross as their resting control condition against which to compare the MEP amplitudes obtained in the action condition. These studies consistently report larger MEP amplitudes for the action condition compared to the control. This choice of resting control, however, makes the motor facilitation effect difficult to interpret as it is not possible to determine whether it is specific to the observation of the moving action, or rather due to other attentional factors. With participants having to stare at a blank screen for relatively long periods of time, distraction, and other mental processes, may occur due to fatigue or boredom. Attention may then be directed internally (for example, on thoughts, somatosensory feelings and images) rather than externally (for example in this case, attending to the blank monitor in front of them). These attentional and cognitive processes may artificially inflate the difference between the experimental and resting control conditions. To determine if this is the case it is important to incorporate a control condition where the visual stimuli are as similar to the action condition as possible, excluding the movement.

By using a blank screen as a control condition, eye fixations are not controlled for and participants’ eyes may wander. Muscle activity and attentional foci are clearly not similar to eye movements and attention during an action observation condition. Including a central fixation cross on the blank screen allows eye movements to be
more controlled. This, however, does not address the attentional issues described above. In an attempt to further control these confounding variables, a static image control condition can be used. In this way, eye movements are more functionally related to those seen in action observation conditions. As such, eye movements and attention factors are optimally regulated, while still controlling for movement observation, which is the detrimental factor argued to access the mirror neuron system.

In some studies (e.g., Aglioti et al., 2008; Lepage et al., 2010; Romani et al., 2005; Urgesi et al., 2006a) a static image of the observed action has been used, with results showing significant differences between conditions. With the control condition similar to the observed action, it is more likely that any facilitation in MEP amplitude for the action condition is attributed to the direct consequence of the same image moving - the action observation. It is important to rigorously control the action observation and resting conditions employed during action observation research, as this could significantly affect the nature and magnitude of the results. To date, no studies have yet compared the effects of using different control conditions in TMS action observation research. All three conditions (blank screen, fixation cross, static image) could act as potential controls. This study attempts to look at two of these to explore how they influence MEP amplitudes.

4.2 Aims and hypothesis

The aim of the study was to compare the size of the MEP amplitude obtained during observation of a ball pinching action with two resting conditions; (i) a static
hand, and (ii) a fixation cross. It was hypothesised that the large difference in visual input between the action and fixation cross would produce increased MEP amplitudes in the action condition. If this effect was also present when comparing the action to the static hand, then this would lend greater support to the hypothesis that the motor facilitation effects are due to the observed action rather than other non-specific factors.

4.3 Methods

The methods employed in this study followed the procedure outlined in section 3.2 on pp. 52-55. Nineteen healthy female volunteers, aged 19 to 26 years (mean age 20.7 years), participated in this study. EMG recordings were collected as outlined on pp. 52-53 and the TMS procedure was identical to that reported on pp. 53-54.

4.3.1 Experimental protocol

Participants were requested to refrain from any voluntary movement and to attend to the stimuli presented on the television screen. Three different video clips, each lasting nine seconds, were presented during the experiment. The action video showed a right hand performing four pinching actions on a soft white ball (actual size 6.4cm in diameter) with the thumb and index finger at a frequency of 0.4 Hz. The static video was a still image that showed the same hand holding the ball between the thumb and index finger, without the pinching action. The choice of static hand was based on the least electromyographically-active frame (see time 0.0s in Figure 4.1 on p. 62) of the action condition to reduce any implied movement inherent in the static
condition (Kourtzi & Kanwisher, 2000). The fixation video consisted of a grey screen with a small black fixation cross in the centre.

The videos and protocol for the experiment are illustrated in Figure 4.2 on p. 62. Participants viewed 150 videos (50 action, 50 static, 50 fixation) split over five blocks. In each block 30 videos (10 action, 10 static, and 10 fixation), were presented in a random order. Two-minute rest intervals were provided between blocks. This was deemed sufficient as according to Balbi, Perretti, Sannino, Marcantonio, and Santoro (2002), MEP amplitudes return to control levels after one minute. A single pulse of TMS was applied over the OSP at either 6250 ms or 8750 ms after the video onset. These timings corresponded to the closing phase of the 3rd and 4th pinch in the action video. The variation in the TMS onset was to reduce the predictability of the stimulus. This is common in TMS research to avoid any priming effects which can reduce the size of the MEP (Takei, Hashimoto, Hagura, Matsumura, & Naito, 2005).

Once all five observation blocks had been completed, the participants were asked to perform a block of action execution trials, during which they pinched the same ball they observed in the action video, using their index finger and thumb. The purpose of these action execution trials was to establish the contribution of the FDI and ADM muscles to the performance of the pinching action. Participants performed thirty pinches to the beat of a metronome set at a frequency of 0.4Hz.

4.3.2 EMG profiles

An averaged EMG profile was created for the FDI and ADM muscles. Both muscles were active during the execution of the pinching action, however, a paired
samples t-test showed the maximal EMG activity of the FDI muscle was significantly higher than the ADM muscle, \( t \ (18) = 6.28, \ p < 0.001 \), indicating that the FDI was the prime mover in the observed action (see Figure 4.1, p. 62).

### 4.3.3 Data analysis

The data analysis followed the procedure outlined on pp. 54-55. The normalised MEP amplitudes were analysed using a three-way repeated measures analysis of variance (ANOVA) with 2 x muscle (FDI, ADM), 2 x stimulation time (6250, 8750) and 3 x video (action, static, fixation) as within-subject factors.

As with most TMS action observation studies, two different stimulation timings were used in order to address the anticipation of the TMS stimulation. As a consequence of this it was later realised that these two stimulation timings had in effect introduced a further independent variable. To address this, the data were reanalysed, with time as one of the within subject factors.

Significant interaction effects were then further explored through pairwise comparisons with Sidak corrections as appropriate. The level of statistical significance for all analyses was set to \( \alpha = 0.05 \). Effect sizes were reported as partial eta squared \( (\eta^2_p) \) for main effects, and as the difference in z-scores (ES) for further comparisons.
Figure 4.1: The mean rectified EMG maximal activity from the FDI and ADM muscles for all participants during the execution of the pinching action. The phase of the pinching action is illustrated in pictures below the time axis. The maximal EMG activity of the FDI muscle was significantly higher than the ADM muscle ($p < 0.001$).

Figure 4.2: Video stimuli used in the observation condition. Three different videos were presented which were termed action, static and fixation. The action video showed a right-handed pinch of a soft ball four consecutive times, with the thumb and index finger. The static video showed a static image of the same hand and ball without the pinching action. The fixation video showed a grey screen with a black cross in the middle. TMS was applied at either 6250 or 8750 ms after video onset.
4.4 Results

The aim of the experiment was to compare MEP amplitudes recorded from the FDI and ADM muscles during the observation of action, static and fixation videos for the two stimulation times of 6250ms and 8750ms. The repeated measures ANOVA showed a significant muscle x video interaction, $F(2,36) = 7.07, p = 0.003, \eta^2 p = 0.28$. In addition, an unexpected stimulation time x video interaction was found, $F(2,36) = 4.61, p = 0.02, \eta^2 p = 0.20$. There was no muscle x video x stimulation time interaction effect, $F(2,36) = 1.37, p = 0.27, \eta^2 p = 0.07$.

To examine the muscle x video interaction further, pairwise comparisons were conducted. The comparisons for the FDI muscle showed larger MEPs for the action video over the static video ($p = 0.04, ES = 0.19$), and over the fixation cross video ($p = 0.003, ES = 0.23$). There was no significant difference between the static hand and fixation cross controls ($p =0.75, ES = 0.05$). There were no significant differences between the action and static video ($p = 0.96, ES = 0.01$), the action and the fixation cross video ($p = 1.0, ES = 0.03$), or static and fixation cross ($p = 0.98, ES = 0.02$) for the ADM muscle (see Figure 4.3 on p. 65).

The stimulation time x video interaction could be seen as a serendipitous finding which was further examined by conducting pairwise comparisons. The MEP amplitudes were larger for the 6250ms stimulation timing than the 8750ms timing ($p = 0.003, ES = 0.2$) but only for the action video. The comparisons for the 6250ms stimulation timing data showed larger MEPs for the action video over the fixation cross video ($p = 0.001, ES = 0.22$), but not over the static video ($p = 0.15, ES = 0.15$).
There was no significant difference between the two control conditions ($p = 0.68$, $ES = 0.07$). For the 8750ms stimulation timing, there were no significant effects for the action video compared to the static hand ($p = 0.98$, $ES = 0.02$) or fixation cross ($p = 0.99$, $ES = 0.02$). There were no significant effects between the control conditions ($p = 1.0$, $ES = 0.004$; see Figure 4.4 on p. 65).
**Figure 4.3:** The mean MEP amplitudes recorded from the right FDI and ADM muscles during observation of action, static and fixation videos at both 6250 and 8750ms combined. The MEP amplitudes are presented as z-scores (mean ± SE). Significant differences are indicated by asterisks.

**Figure 4.4:** The mean MEP amplitudes recorded from the right FDI and ADM muscles combined during observation of action, static and fixation videos stimulated at 6250 and 8750ms. The MEP amplitudes are presented as z-scores (mean ± SE). Significant differences are indicated by asterisks.
4.5 Discussion

The study investigated whether the observation of a hand action was associated with a motor facilitation in relation to two common used control conditions (static image and fixation cross). The results presented a significant muscle x video interaction which showed an effect of video condition that was dependent on the muscle. There were significantly larger peak-to-peak MEP amplitudes in the FDI muscle during the observation of a ball pinching action compared to both control conditions, yet no such effect was found in the ADM muscle. When participants executed the observed action at the end of the experiment, the FDI muscle was significantly more active than the ADM muscle (see Figure 4.1 on p. 62). This suggests that the motor facilitation effect during action observation was specific to the muscle primarily involved in performing the observed action. This finding of a muscle-specific MEP facilitation during action observation is consistent with previous research in the area (e.g., Borroni et al., 2005; Fadiga et al., 1995; Gangitano et al., 2001, 2004; Strafella & Paus, 2000). Furthermore, an unexpected stimulation time x video interaction showed that the motor facilitation effect was influenced by the timing of the stimulation, with MEPs recorded at 8750ms after video onset failing to report a facilitation effect. This serendipitous finding has obvious implications for TMS methods and stimulation timing and whilst it remains important to control for participants’ anticipation factors, it is also important for experimenters to be aware that the later stimulation timings may cause participants’ attention to drift to non-specific factors.

MEP amplitudes recorded from the FDI muscle were facilitated during observation of the action video as compared to the fixation video, which is consistent
with the literature (e.g., Gangitano et al., 2001, 2004; Patuzzo et al., 2003; Sakamoto et al., 2009). Although it is appealing to claim, as others have, that this effect is a direct result of the observed hand action, there are many potential confounding attentional and non-specific visual factors that mean it is not possible to make this interpretation with any degree of certainty. For example, it could simply be the presence of a visual stimulus on screen that caused the increase in MEP amplitude, rather than the fact that participants were observing the movement of a limb.

The inclusion of the static hand video did control for some of the above factors and thus provided a more appropriate baseline condition. The results presented here showed that the MEP amplitudes recorded from the FDI muscle were also facilitated during observation of the action video as compared to the static video, providing further corroboration that the motor facilitation effect is due to the action observation, as is claimed in the mirror neuron system research. This finding is consistent with previous studies that have incorporated a static image as their control condition (e.g., Romani et al., 2005; Urgesi et al., 2006a). It is, however, in contrast with the results of Molnar Szakas et al. (2007) who did not detect a difference in MEP amplitudes recorded from the FDI muscle during the observation of hand gestures as compared to a static image condition. Results reported by Lepage et al. (2010) are particularly interesting as their motor facilitation effect was only present immediately (60-90 ms) after the onset of the observed index finger abduction, whereas no motor facilitation effect was present in the later 120-270 ms timing. The stimulation timings in the present study differed greatly from the Lepage et al. timings, with TMS pulses delivered at 6250ms and 8750ms from video onset.
The stimulation timings used in this study were chosen for two reasons: (i) both timings corresponded to the closing phase of the pinching action, and (ii) to remove the predictability of the stimulation onset. This is a common used approach in studies where TMS has been applied during observation of repetitive hand actions (e.g., Clark et al., 2003; Desy & Theoret, 2007; Romani et al., 2005; Sakamoto et al., 2009), however the different time points are usually combined and analysed together with the assumption that the different timings have no effect on the motor facilitation since they correspond to the same phase of the observed action. As reported in the results, the stimulation time x video interaction showed a difference between the data obtained from the 6250ms and the 8750ms, with the action condition producing significantly larger MEPs than the fixation cross only in the earlier stimulation timing (see Figure 4.4, p. 65). This highlights the fact that whilst most researchers stimulate at different time points, it may be incorrect to assume that this does not have an effect on the resulting MEPs, especially when videos are relatively long (> 5s). For example, Alaerts et al. (2009b) used stimulation timings varying between 3s and 9s, whilst Sakamoto et al.’s (2009) stimulation timings ranged between 10s and 20s. In both these studies it is unknown whether any differences would have been found between the earlier and later timings, with possibly only the earlier stimulations showing the motor facilitation effect. In addition, as shown in Figure 4.4 on p.65, larger MEP amplitudes were obtained in the action condition when stimulations were delivered at 6250ms, compared to 8750ms. Interestingly, this latter effect was not muscle-specific, since it was seen in both the FDI and ADM muscles.
One explanation for this non muscle-specific finding is that there may have been a drop in participants’ attention as they observed the pinching action. As there was no movement on screen, attention may have been low at both 6250ms and 8750ms during the observation of the static or fixation video conditions. This could explain why low MEP amplitudes were obtained at both timings. During the action condition, participants may have attended to the video at first, then lost focus and shifted their attention elsewhere as the video progressed, resulting in larger MEP amplitudes at 6250ms, compared to 8750ms. In support of this claim, it has been shown, that attentional factors do influence the size of the MEP. For example, Conte et al. (2007) investigated whether the size of MEP was influenced by attentional processes. Stimuli were delivered during three conditions that differed in attentional demand: ‘relaxed with eyes closed’, ‘looking at target hand of the repetitive TMS’, and ‘looking at non-target hand of the TMS’. They reported that larger amplitude MEPs were elicited when participants attended to the target hand as opposed to the non-target hand. This highlights the importance of monitoring participants’ attention during action observation research. One proposition may be to combine TMS action observation with eye-tracking devices. While monitoring participants’ gaze would not guarantee attentional focus, it would offer some support for the participants’ attentional fixations during the experimental conditions.

It is interesting to note that although the MEP amplitudes were slightly higher during observation of the static hand as compared to the fixation video this was not a statistically significant difference (see Figure 4.3 on p. 65). The difference in MEP amplitude (and effect size) between the action video and static video (ES = 0.19) was
smaller than the difference in MEP (and effect size) amplitude between the action video and fixation cross (ES = 0.23), especially with regards to the 6250ms data. There may, therefore, be a greater possibility of obtaining a significant difference between action and control observation conditions when a fixation cross control is used. Importantly, and as a consequence of the visual differences between action and fixation cross observation, it could be argued that using a fixation cross control may be more likely to produce a false positive result since no visual stimuli exist in this resting condition. TMS action observation studies typically have low power due to low participant numbers. Gangitano et al., (2001; 8 participants) and Sakamoto et al., (2009; 9 participants) are two examples of this. Baseline conditions which differ greatly from the action condition therefore increase the likelihood of obtaining a facilitation effect during action observation.

One reason why the two resting conditions may differ is because of the ‘implied movement’ perceived in the static image. According to Kourtzi and Kanwisher (2000), observers tend to extract dynamic information from static photographs which imply motion. In an fMRI study, the authors reported an increased blood oxygen level dependent (BOLD) response in the temporal/medial superior temporal cortex region during observation of static images of athletes, animals and nature scenes depicting implied movement in contrast to similar images with no implied movement. This effect has also been reported during observation of static images depicting human hand movements, compared to control images of a hand at rest. Urgesi, Moro, Candidi, and Aglioti (2006b) compared static images perceived to contain a high degree of implied movement with those that did not and reported a significant MEP facilitation during
the high implied movement images. In the current study, this effect was controlled for by presenting the hand in its least active form for this task (see time 0.0 in Figure 4.1 on p. 62) which may explain the difference in MEP amplitude obtained between the action observation video and the static hand. The static image of a hand holding a ball, however, likely implies greater movement than the fixation cross in the middle of a grey background. It is possible, therefore, that no significant difference was found between the static and fixation conditions because the chosen static image was associated with only a limited amount of implied movement. In addition, while static images may have implied movement, this is not a consequence of the action observation condition, but of imagined implied movements. These may access something other than the mirror neuron system. These theoretical problems remain to be addressed.

In conclusion, the data reported in this study is in line with the majority of TMS action observation research, demonstrating muscle specific MEP facilitation during action observation conditions compared to the resting control conditions. It could be argued that a static image is more suitable than a blank screen or fixation cross. Although there was no statistical difference between the two resting conditions, the static image allows for more accurate comparison with the action condition by providing meaningful visual cues without the associated action. The choice of control is essential to the accuracy of research in TMS and is one of a number of factors that should be delimited or controlled within rigorous experimental designs. Further studies should, therefore, expand on this current study by directly comparing and contrasting all non-action conditions that have been included as control conditions.
within the action observation literature. Finally, the lack of a motor facilitation effect for the later stimulation time of 8750ms highlights the importance of the choice of stimulation timing in TMS action observation experiments. This was explored again in Study 2.

Throughout the next Chapters, other factors relating to the methodology of TMS experiments are reported. Since TMS action observation research involves measuring the size of MEP obtained in response to observing an action on screen, it was important to investigate whether familiarity with that observed action had an increased effect on the recorded MEP. Long term experience of performing an action and its effect on the motor system has been widely explored in the literature, however few studies have investigated short-term effects of performance of an action prior to observing that same action. This possible priming effect was explored in the following study.
Chapter 5: Study 2: Motor facilitation during action observation: the priming effect of prior execution

5.1 Introduction

It is well established that observing the actions of another person has a strong influence on the observer’s motor performance (Brass et al., 2000, 2001; Kilner et al., 2003). As discussed in the literature review in Chapter 2, action observation experiments using TMS have shown a motor facilitation effect specific to the muscles involved in performing the observed action. This effect was demonstrated in Study 1 and this has been shown to be a consistent finding across numerous previous experiments (e.g., Fadiga et al., 1995; Gangitano et al., 2001, 2004; Montagna et al., 2005; Strafella & Paus, 2000).

While the effects of observation on execution have been widely explored (e.g., Brass et al., 2000, 2001; Kilner et al., 2003), considerably fewer studies have examined the effects of action execution on subsequent action observation. In an fMRI study, Cross, Hamilton, and Grafton, (2006) recorded BOLD signal magnitude from expert dancers. After learning and rehearsing novel dance sequences over a period of five weeks, the dancers observed and imagined performing the rehearsed dance sequences, as well as non-rehearsed control sequences. Results showed more pronounced BOLD activity in key components of the action simulation network, specifically the supplementary motor area (SMA), the superior temporal sulcus (STS), and the ventral premotor cortex (PMv), during observation of the rehearsed movements compared to non-rehearsed movements, highlighting the influence of
prior experience on action observation. Similarly, Calvo-Merino et al., (2005) showed the importance of personal motor repertoire when classical ballet experts, capoeira experts, and inexpert control participants, viewed videos of ballet and capoeira dance moves. The results indicated stronger BOLD responses in classical mirror areas such as the premotor, parietal cortices and STS, during observation of dance moves of their own expertise. Both studies indicate that motor training and/or visual familiarity of the observed movement influence the properties of the observation-execution network.

The effects of long term practice on the action observation network have been investigated extensively over periods of weeks, months, and years focusing on expertise and its influence on the action observation-execution network. For example, in a TMS study, Aglioti et al. (2008) examined the corticospinal excitability of elite and novice basketball players as they observed a series of basketball shots, soccer kicks, and static images. Results showed increased modulation of corticospinal excitability in the elite athletes, only when viewing basketball actions, suggesting activation of the motor system during observation is expertise-specific. This effect has also been explored using fMRI. Haslinger et al. (2005) compared BOLD activation in professional pianists with non-musician controls during observation of piano-playing related movements. Experienced pianists showed significantly greater BOLD activation within a cortical fronto-parieto-temporal network, demonstrating the importance of long-term training of the observed action. Two recent TMS studies, however, have shown that short term practice of physical actions, lasting only minutes, may be sufficient to modulate the responsiveness of the mirror system. Using a novel experimental approach, Catmur et al. (2007) trained participants to perform one action while
simultaneously observing another. Before training, participants showed a muscle-specific motor facilitation during observation of little and index finger movements. The effect was reversed following incongruent training where they performed one action while observing a different action; indicating that the properties of the human observation-execution network are modified by sensorimotor learning. In another training study, Sakamoto et al. (2009) explored the excitability of the corticospinal system during action observation where participants observed a hand repeatedly pinching a small soft ball across a series of five blocks. They initially found that observation of the finger pinch action did not lead to difference in motor facilitation across blocks. Following a period of action-execution training, however, the MEP amplitude increased on subsequent observation trials, with the MEP amplitudes in the fifth block being significantly larger than the MEP amplitudes of the first block of observation trials, suggesting that experience of action execution may produce an additional motor facilitation effect to that first described by Fadiga et al. (1995).

The current study is based in part on the experiment of Sakamoto et al. (2009), and addresses some methodological limitations from their study. First, the authors utilised a fixation cross as their baseline condition. Based on the findings reported in the previous chapter, a static image control was used throughout the current experiment as this provided similar visual features to the action condition, but without the overt movements. Second, rather than simply focussing on a comparison of MEP amplitudes across trials, as in Sakamoto et al. (2009), the action MEP amplitudes were calculated as a ratio of the static MEPs in order to report the modulation in MEP amplitude due to observation of the action per se. Third, as in Study 1, the TMS pulses
were delivered at two time points that corresponded to the closing phase of the pinching action, and these time points were analysed separately.

5.2 Aims and hypothesis

The study consisted of two experiments. The aim of Experiment 1 was to explore whether observation of an action increased the excitability of the observer’s motor system. The aim of Experiment 2 was to investigate whether brief periods of action execution would produce an additional facilitation of corticospinal excitability compared to observation alone. It was hypothesised that action observation would be associated with a muscle-specific motor facilitation and that this effect would be enhanced after a brief period of action execution was performed prior to the observation trials.

5.3 Methods

The methods employed in this study followed the procedure outlined on pp. 52-55. Nineteen healthy female volunteers, aged 19 to 26 years, participated in this study. Each experiment had fifteen participants. Eleven participants performed both experiments, whilst the remaining eight participants took part in only one experiment. The participants who completed both experiments attended the laboratory on two occasions, at least 24 hours apart, and performed the two experimental sessions in a randomised order. EMG recordings were collected as outlined on pp. 52-53 and the TMS procedure was identical to that reported on pp. 53-54.
5.3.1 Experimental protocol

Participants were requested to refrain from any voluntary movement and to attend to the stimuli presented on the television screen. Two different video clips, each lasting nine seconds, were presented during the experiment. The action video showed a right hand performing four pinching actions on a soft white ball (actual size 6.4cm in diameter) with the thumb and index finger at a frequency of 0.4 Hz. The static video was a still image that showed the same hand holding the ball between the thumb and index finger, without the pinching action (see Figure 5.1, p. 79).

The protocol for Experiment 1 is illustrated in Figure 5.2 on p. 79. Participants observed five blocks of trials each consisting of twenty videos (10 action and 10 static) which were presented in a random order. Each action video contained four repeated pinches. A single TMS pulse was applied over the OSP at either 6250 or 8750ms (3rd and 4th pinch respectively) after both the action and static video onsets, corresponding to the closing phase of the pinch in the action video (see Figure 5.1, p. 79). As in Study 1, the variation in the onset of the TMS pulse was to remove the predictability of the stimulus. A total of 10 MEPs were collected per video condition per block, resulting in a total of 100 MEPs (50 MEPs for action video and 50 MEPs for static video condition). Two-minute rest periods were provided between blocks.

In Experiment 2 the participants first observed a block of 20 videos (10 action and 10 static) identical to those used in Experiment 1. During the observation, TMS was applied at the same time points as in Experiment 1. Participants then performed an action execution block, during which they performed the same pinching action with
the same ball that they observed in the videos. Prior to the first execution block, participants pinched the ball between five to ten times, to ensure they were using the correct finger muscles for the pinching action as assessed by EMG traces (index finger and thumb). They performed thirty pinches to a metronome set at a frequency of 0.4Hz. A two-minute rest period was then provided before the start of the next observation block. The experiment consisted of five action observation and four action execution blocks (see Figure 5.2, p. 79). 120 pinches were recorded throughout the action execution blocks, along with a total of 100 MEPs (50 MEPs for action video and 50 MEPs for static video condition) collected during the five observation blocks.

5.3.2 Data analysis

The data analysis followed the procedure outlined on pp. 54-55. A pre-stimulus recording of 200ms was used to check for the presence of EMG activity in both the right and left hand before the TMS pulse. EMG activity from the FDI and ADM muscles in the left hand was recorded as an additional control to eliminate the possibility of any left hand movements influencing the right hand MEP amplitudes as a result of inter-hemispheric connections (see Sohn, Jung, Kaelin-Lang, & Hallett, 2003).

The main aim of the current study was to test whether MEPs recorded from the FDI and ADM muscles were modulated during observation of an action in relation to observation of a static hand. The peak-to-peak MEP amplitude during the action and static conditions was measured from both the FDI and ADM muscles. MEP amplitude differences between the two conditions were then compared using separate paired samples t-tests for each muscle.
Figure 5.1: Video stimuli used in the observation blocks. Participants observed two different videos, each of 9s duration. The action video showed a right-handed pinch of a soft ball four consecutive times, with the thumb and index finger. The static video showed a static image of the same hand and ball without the pinching action. TMS was applied at either 6250 or 8750 ms after video onset.

Experiment 1

<table>
<thead>
<tr>
<th>1&lt;sup&gt;st&lt;/sup&gt; Obs</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Obs</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Obs</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Obs</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
</tr>
</tbody>
</table>

Experiment 2

<table>
<thead>
<tr>
<th>1&lt;sup&gt;st&lt;/sup&gt; Obs</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Exec</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Obs</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; Exec</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Obs</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Exec</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Obs</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; Exec</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
<td>2 min rest</td>
</tr>
</tbody>
</table>

Figure 5.2: The experimental design for Experiments 1 and 2. In Experiment 1, participants observed 10 action and 10 static videos per block, while MEPs were recorded. There were five observation blocks (Obs), with two-minute rests between blocks. In Experiment 2, participants completed an observation block, as in Experiment 1. During the execution blocks (Exe), participants performed 30 ball pinches. After a two-minute rest period, participants then completed an observation block, while MEPs were recorded. There were a total of 5 observation blocks and 4 execution blocks.
After establishing a motor facilitation effect for action observation, the aim was to investigate whether action execution prior to action observation of the same action would result in an additional motor facilitation. In order to explore the stability of the corticospinal facilitation effect, the MEP (action/static) ratio was calculated for each participant per observation block by dividing the mean amplitude of the action MEP by the mean amplitude of the static MEP. This was performed separately for both the FDI and ADM muscles.

To test the effects of action observation on corticospinal excitability in the absence of prior execution, the MEP ratios collected from the fifteen participants during Experiment 1 were analysed using a two-way repeated measures analysis of variance (ANOVA) with muscle (FDI, ADM) and block (1,2,3,4,5) as within-subject factors. To test the effects of action observation on corticospinal excitability following action execution in Experiment 2, the MEP ratio collected from the 15 participants were analysed using a two-way repeated measures ANOVA with muscle (FDI, ADM) and block (1,2,3,4,5) as within-subject factors. To evaluate the effects of action execution further, a second analysis was conducted in order to compare the results of Experiments 1 and 2 directly. The MEP ratios recorded from the FDI muscle in the eleven participants who completed both experiments were analysed using a two-way repeated measures ANOVA with experiment (observation, combined) and block (1,2,3,4,5) as within-subject factors.

As a follow up from the analysis in Study 1, a third analysis was conducted to explore the effect of stimulation time on the MEP ratio. In this analysis, the MEP ratios recorded from the FDI muscle in the 15 participants who took part in Experiments 1
and 2 were analysed using a two-way repeated measures ANOVA with stimulation time (6250ms, 8750ms) and block (1,2,3,4,5) as within-subject factors.

During Experiment 2, EMG activity of the FDI and ADM muscles in the right hand was recorded as participants executed the observed pinching action. For each participant, an averaged EMG profile was created for each of the four action execution blocks. The EMG activities from individual trials, aligned according to the onset of the metronome beat, were rectified and then averaged. To test whether the participants executed the pinching action in a similar way across the execution blocks, the peak value of each participant’s EMG profile was submitted to a two-way repeated measures ANOVA with muscle (FDI vs. ADM) and block (1,2,3,4) as within subject factors.

The level of statistical significance for all analyses was set at α = 0.05 and effect sizes were reported as partial eta squared ($\eta^2_p$) for main effects, and as the difference in z-scores (ES) for further comparisons.

5.4 Results

5.4.1 Experiment 1

Experiment 1 investigated whether observation of an action produced a motor facilitation in relation to observation of a static hand control. The paired samples t-test for the FDI muscle showed larger amplitude MEPs in the action condition, compared to the static condition, indicating the presence of a motor facilitation, $t (14) = 2.10, p = 0.05$. However, there was no motor facilitation in the ADM muscle as MEP amplitudes between the two conditions were not significantly different, $t (14) = -0.53, p = 0.60$. 


The repeated measures ANOVA using the MEP (action/static) ratio indicated that there was a main effect of muscle, $F(1, 14) = 4.77$, $p = 0.05$, $\eta^2_p = 0.3$, with a larger motor facilitation occurring in the FDI muscle as compared to the ADM muscle. There was no main effect of block, $F(4, 56) = 0.36$, $p = 0.8$, $\eta^2_p = 0.03$, and there was no interaction between muscle and block, $F(4, 56) = 0.94$, $p = 0.5$, $\eta^2_p = 0.06$. This indicates that the motor facilitation ratios were similar throughout all five observation blocks (see Figure 5.3 – Experiment 1 on p. 85).

5.4.2 Experiment 2

Experiment 2 investigated whether a period of repeated action execution performed prior to observation of an action would produce an additional motor facilitation to that reported in Experiment 1. As in Experiment 1, the paired samples $t$-test showed the presence of a motor facilitation in the FDI muscle, $t(14) = 3.82$, $p = 0.002$, but not in the ADM muscle, $t(14) = 1.77$, $p = 0.1$, during the observation of the pinch action as compared to the static hand. The repeated measures ANOVA indicated that there was a main effect of muscle, $F(1, 14) = 8.16$, $p = 0.01$, $\eta^2_p = 0.4$, with a larger motor facilitation occurring in the FDI as compared to the ADM muscle. There was no main effect of block, $F(4, 56) = 1.43$, $p = 0.2$, $\eta^2_p = 0.09$, or interaction between muscle and block, $F(4, 56) = 0.61$, $p = 0.7$, $\eta^2_p = 0.04$. This indicates that the motor facilitation ratios were similar throughout all five observation blocks, suggesting that prior execution of the observed action did not produce an additional increase in MEP amplitude (see Figure 5.3 – Experiment 2 on p. 85).
5.4.3 Stimulation timing analysis

Following the effect of stimulation timing on the action MEPs in Study 1, another analysis was carried out to test whether a similar timing effect would be present during Experiments 1 and 2 (see Figure 5.4, p. 86). The repeated measures ANOVAs for both experiments, however, did not show any significant effects of stimulation timing across the five observation blocks. The results of Experiment 1 showed that effect of stimulation time just failed to reach statistical significance, $F(1,14) = 4.28, p = 0.06, \eta^2_p = 0.23$, and there was no time x block interaction, $F(4,56) = 0.43, p = 0.79, \eta^2_p = 0.03$. The results of Experiment 2, showed that there was no effect of stimulation time, $F(1,14) = 1.67, p = 0.22, \eta^2_p = 0.11$, and there was no stimulation time x block interaction, $F(4,56) = 0.37, p = 0.83, \eta^2_p = 0.03$.

5.4.4 Comparison of Experiments 1 and 2

After analysing the results of Experiments 1 and 2 separately, it was important to directly compare them, to test whether the prior action execution added an additional motor facilitation during action observation, compared to action observation alone. The results of the comparison analysis between Experiments 1 and 2 showed that MEP ratios obtained in the FDI muscle were similar in both experiments (see Figure 5.5 on p. 87). The repeated measures ANOVA indicated that there was no main effect of experiment, $F(1, 10) = 0.98, p = 0.4, \eta^2_p = 0.09$, or block, $F(4, 40) = 0.78, p = 0.5, \eta^2_p = 0.07$ or experiment x block interaction, $F(4, 40) = 0.7, p = 0.6, \eta^2_p = 0.07$, demonstrating that the action execution blocks did not enhance further the motor facilitation effect that was already present during action observation trials.
5.4.5 EMG analysis

EMG activity was recorded from both the FDI and ADM muscles during the execution of the pinching action in Experiment 2 (see Figure 5.6, p. 87). Both muscles were active during the execution of the pinching action, however, the ANOVA showed that there was a main effect of muscle, with the FDI muscle being more active than the ADM muscle, $F(1, 13) = 32.5, p < 0.001, \eta^2_p = 0.7$. This result is consistent with the EMG data reported in Study 1. Furthermore, there was no difference in the maximal EMG activity recorded across blocks, for both the FDI, $F(1.8, 25.1) = 1.9, p = 0.17, \eta^2_p = 1.2$, or the ADM muscle, $F(2, 28) = 1.4, p = 0.26, \eta^2_p = 0.1$. 
Figure 5.3 Motor facilitation ratio data of 15 participants across 5 blocks, recorded from their right FDI and ADM muscles. For both observation (Experiment 1) and combined (Experiment 2) experiments, there was a main effect of muscle (Experiment 1: $p = 0.05$; Experiment 2: $p = 0.01$) showing a facilitation in the FDI muscle as a result of action observation. There was no main effect of block (Experiment 1: $p = 0.8$; Experiment 2: $p = 0.2$) or interaction between muscle and block (Experiment 1: $p = 0.5$; Experiment 2: $p = 0.7$). Error bars represent standard error.
Figure 5.4 MEP facilitation ratio data of 15 participants across 5 blocks, recorded from their right FDI muscle at 6250ms and 8750ms after video onset for both Experiments 1 (observation) and 2 (combined). There was no significant effect of time for both experiments, approaching significance at p = 0.06 for Experiment 1, with a greater MEP ratio for the 6250ms data as compared with the 8750ms data. The error bars represent standard error.
Figure 5.5: Motor facilitation ratio data of 11 participants who performed both Experiments 1 and 2 across 5 blocks, recorded from their right FDI muscle. There was no main effect of experiment \((p = 0.4)\), or block \((p = 0.6)\). There was no interaction between experiment and block \((p = 0.6)\), showing that the execution prior to the action observation did not influence the motor facilitation effect further. The error bars represent standard error.

Figure 5.6: Maximal EMG activity recorded from the FDI and ADM muscles for all participants during the execution phase in Experiment 2. Each data point represents the mean data combined across all four action execution blocks.
5.5 Discussion

This study was designed to test whether a relatively short period of repetitive action execution was sufficient to alter the observation-execution system responses during action observation. Based on the results reported by Sakamoto et al. (2009), it was predicted that corticospinal excitability during action observation would be influenced by the observer’s visual and tactile familiarity with the observed action.

The results from Experiment 1 replicated the findings of Study 1 and confirmed the typical TMS action observation finding (e.g., Fadiga et al., 1995; Gangitano et al., 2001, 2004; Strafella & Paus, 2000), that passive observation of an action increased corticospinal excitability, compared to control conditions. This effect was only present for the FDI muscle which was the main muscle involved in the pinching action. MEP amplitudes recorded from the ADM muscle, which was minimally involved in executing a pinch (see EMG activity in Figure 5.6 on p. 87), were not significantly modulated during action observation. The results of the current study are consistent with previous studies that have reported motor facilitation effects which correspond to EMG profiles recorded during execution of the observed action (e.g., Fadiga et al., 1995; Montagna et al., 2005; Romani et al., 2005; Strafella & Paus, 2000). These results support the concept of an observation-execution matching system which acts to prime the excitability of the observer’s motor system during action observation (Fadiga et al., 1995; Rizzolatti & Craighero, 2004).

In Experiment 2, where participants executed the action prior to the observation trials, it was hypothesised that there would be an increased motor facilitation in the FDI
muscle. This effect was not detected in the results of Experiment 2 (Figure 5.3 – Experiment 2, p. 85) or when comparing the results of both experiments (Figure 5.5, p. 87). These findings indicated that a short period of action execution prior to observation of the same action did not enhance the motor facilitation effect further. The discrepancy with Sakamoto et al. (2009) was unexpected since the same action and frequency of action repetition was used during both the observation and execution conditions. Much of the literature supports the notion that the human observation-execution matching system is tuned to previous experience and familiarity with an action (e.g., Calvo-Merino et al., 2005; Cross et al., 2006; Haslinger et al., 2005). It is important to note that the studies discussed here used participants who were experts in their field, and recorded cortical activity during the observation of a complex skill, rather than the simple ball-pinching action used in this study. The experts in the music and sport-related studies would have practiced specific movement patterns over a number of years, developing and refining those actions into skilful movements. Participants in the current study simply pinched a ball over the course of one testing session, executing the pinch action 120 times, and observing the same action a further 50 times. The results reported in this study suggest that increased familiarity with the observed action during the course of one testing session did not enhance the motor facilitation effect (see Figure 5.5 on p. 87). It is important to note that the action presented to the participants may have been one with which they were already familiar, and was not sufficiently complex to lead to further corticospinal modulation through repetition. This is in contrast to Sakamoto et al. (2009), where the authors did report a change in corticospinal excitability for the combined observation and execution phase of their experiment. These differences may be a result
of methodological differences with Sakamoto et al.’s study rather than any additional corticospinal facilitation.

The current study was designed to counteract a number of limitations that were present within the Sakamoto et al.’s (2009) study. First, rather than using a fixation condition as the baseline condition, MEP amplitudes were recorded during the observation of a static hand. This allows for more effective control of visual attention and any motor facilitation effects are more likely to be a direct result of the observed action per se (as discussed in Chapter 4). The second limitation that was addressed was to intersperse the static control trials with the action trials as opposed to presenting them in separate blocks. This is important because although the stimulating coil was fixed in position using a mechanical arm, as opposed to a hand held coil, slight movements of the coil, especially between blocks, are still possible. The method of analysis incorporated in this study, which was to calculate the MEP action/static ratio across each block of observation trials, minimises the effects of coil movement as they will be common to both action and static trials. This analysis method also controlled for any unwanted effects of muscle fatigue (see Balbi et al., 2002; McKay, Tuel, Sherwood, Stokic, & Dimitrijevic, 1995) which may have resulted from the action execution trials. The effects of muscle fatigue were also controlled by including two-minute rest periods between action execution and observation blocks.

A further difference between the current study and that of Sakamoto et al. (2009) was the choice of stimulation intensity. The stimulation of the motor cortex can evoke different activity depending on the intensity of the stimulation. This is discussed in detail in Chapter 7. In the current study, a stimulation intensity of 110% RMT was used in
contrast to Sakamoto et al. (2009) who used a higher intensity of 120% RMT. By stimulating with an intensity close to RMT (here, 110% RMT), the motor response recorded was more likely to be representative of the ongoing level of cortical activity. The increased intensity used in the Sakamoto et al. study may reflect a different neural mechanism to that tested in the current study. This may account for differences between the two studies. The concept of stimulation intensity and its effect on the action observation-execution matching system were explored in Study 4.

By training participants to perform one action while observing another, Catmur et al. (2007) demonstrated that it is possible to modify the excitability of the observer’s motor system by executing actions during observation, revealing that the properties of the observation-execution network are not innate or permanent, but the product of sensorimotor learning (for extensive reviews see Gallese et al., 2011; Heyes, 2010; Hickock, 2009). It is important to note that the Catmur et al. (2007) study explored congruent versus incongruent action observation and execution, fundamentally different to the current study where participants executed an action prior to observing the same action. Despite this, the finding reinforces the hypothesis postulated in this study that repeated execution of an action should enhance the MEP facilitation obtained during subsequent action observation. The action presented to the participants in the current study was a highly familiar pinching action. As shown in Figure 5.3 on p. 85, MEP action facilitation was evident in block 1, which may already have reached a ceiling, with no additional facilitation possible across the later blocks. This finding was, again, in contrast with that of Sakamoto et al. (2009), where a significant facilitation effect was reported between the first and final block of trials. It would therefore be interesting to investigate
whether the observation and execution of unfamiliar, or incongruent, actions have an effect on the action observation-execution network.

The results of the stimulation timing analysis reported no significant effect of time between the MEPs recorded at 6250ms and 8750ms. The lack of difference between stimulation time points was unexpected following the results reported in Study 1. As shown in Figure 5.4 (p. 86), neither the results of Experiment 1 or 2 revealed significant differences in the MEP amplitudes recorded from the FDI muscle when comparing the 6250ms and 8750ms stimulation time trials. It is important to note, however, that in Experiment 1, which uses the same protocol as Study 1, the difference in stimulation time trials approaches significant at $p = 0.06$, with the larger motor facilitation ratio for the earlier stimulation timing of 6250ms, which is in line with the findings of Study 1. The lack of significant time difference may be a consequence of three main differences in the analysis between Study 1 and Experiment 1 of the current study: (i) there was no fixation cross data included in the present study; (ii) Study 1 utilised normalised $z$-scores, whilst the current study used an action/static MEP ratio in order to control for coil movement and fatigue across the five blocks; and (iii) the stimulation timing in Study 1 was not muscle specific, with both muscles showing larger MEPs in the action condition as opposed to the static hand, whereas in Experiment 1, the MEP ratio is presented for the FDI muscle only. If the ADM data been included, the effect of time may have reached significance. In Study 1, the proposed reason for the lower amplitude MEPs in the 8750ms data was an attentional deficit during the latter stages of the action observation trials. In Experiment 2, participants repeatedly executed the ball pinching action prior to observing it, which may have led to increased attention throughout the trials, resulting in the null
effect of stimulation timing for Experiment 2. These findings are still inconclusive and more research is warranted in this area in order to fully understand the effect of stimulation timing on the MEPs obtained during repetitive hand actions.

In conclusion, the data from this study demonstrated that simple action observation results in a facilitation of the corticospinal pathway irrespective of whether the same simple action is performed prior to observation. Action execution in this study did not have a significant effect on the subsequent observation in terms of corticospinal excitability, although this may be a consequence of the simplicity of the task. This finding is in contrast to Sakamoto et al.’s (2009) results, but differences between the two studies could be explained by differences in the methods used. The findings reported here provide further support for action observation and its influence on the corticospinal system, whilst also illustrating the limited effect of prior physical movements on the corticospinal circuit for simple tasks. Future studies should investigate the effect of short-term action execution of familiar/unfamiliar actions and congruent/incongruent actions followed by a period of action observation on the action-execution network, taking into account the stimulation timing. In the following study, the concept of muscle-specificity was explored. While both Studies 1 and 2 showed a significant motor facilitation effect only for the muscle involved in the observed action, the TMS coil was placed over the motor hotspot for the specific muscle, which may have contributed to the results. In the next study, this new approach was explored by using the hotspots for both the muscles under investigation on two separate testing sessions.
Chapter 6: Study 3: Motor facilitation during action observation: using different
‘hotspots’ to investigate muscle specificity

6.1 Introduction

In TMS motor cognition studies exploring mirror neuron activity during action observation, it is common for experimenters to record MEPs from a number of concurrent muscles, for example finger and wrist muscles during observation of reach and grasp actions (e.g., Gangitano et al., 2001; Montagna et al., 2005). At the start of each TMS experimental session it is standard practice to find the optimal scalp position (OSP) for eliciting responses in the target muscle (see section 3.1.3 on p. 48). Researchers tend to determine the OSP for only one of the muscles under investigation (usually the main muscle of interest). Once that position is located, the coil is then positioned over that scalp site and stimulation occurs at that site throughout the experiment.

The concept of a somatotopic organisation of the primary motor cortex has been reported (Schieber, 2001). A cortical region is organised to control movements of different body parts. Through techniques such as electrical stimulation, it has become clear that the different body part representations in the cortex are not as specific as first described by Penfield’s homunculus, but rather the representations of smaller body parts overlap within their respective sections (Schieber, 2001). MEPs are therefore being recorded for other muscles which, as a result of the current method, may not be stimulated at their respective OSPs. In addition, despite the figure-of-eight stimulating coil (used in all experiments in this thesis) allowing for more focal stimulation than for example the circular coil, stimulation normally elicits MEPs in several muscles at the one
time. This reflects the considerable overlap of different muscle representations within M1 (Sanes & Donoghue, 2000), but also the relatively large area of the motor cortex being stimulated with every TMS pulse. Despite being in close proximity with each other, the finger muscles differ slightly in their positioning over the motor cortex. By not conducting separate experiments, each time using a different muscle’s OSP, the argument of muscle specificity during action observation should be treated with caution.

The TMS action observation literature, including Studies 1 and 2 presented in this thesis, has shown a selective motor facilitation of the muscle that would be involved in the actual execution of the observed action (e.g., Fadiga et al., 1995; Romani et al., 2005; Urgesi et al., 2006a). This is evident through greater facilitation of MEP size in, for example, the FDI muscle during observation of index finger movements compared to the ADM muscle, and vice versa for observation of little finger movements. In addition, stimulating a muscle at a site other than its OSP may affect the muscle’s threshold intensity and influence the stimulation intensity applied throughout the experiment. For example, at a given scalp location, one participant, whose data was presented in Study 2, had motor thresholds for the FDI and ADM muscle at 43% and 52% respectively. In order to guarantee obtaining MEP responses from both muscles, the experiment would have to be run using the higher threshold of the ADM muscle. If the experiment were then run at 110% of motor threshold, the muscles would both be stimulated at 57% of the stimulator output, resulting in the FDI being stimulated at approximately 130% rather than 110% of motor threshold. As discussed on pp. 15-16, the MEP amplitudes recorded using high stimulation intensities are less representative of the ongoing level of cortical excitability than MEP amplitudes recorded using near threshold TMS intensities (Di Lazzaro et al.,
2004). This highlights the practical implications regarding the choice of intensity used, which is different for each of the muscles under investigation, with their own OSP, and stimulation intensity threshold. The effect of stimulation intensity was explored further in Study 4.

To explore the effect, if any, of the chosen OSP on the muscle-specific motor facilitation reported consistently in the literature, as well as in Studies 1 and 2, two finger muscles (FDI and ADM), were tested on two occasions (once for each muscle’s OSP) while participants watched videos of (i) a static hand, (ii) index finger movement, and (iii) little finger movement.

### 6.2 Aims and hypotheses

The aim of the current study was to expand on current TMS studies by exploring the muscle-specific effects of action observation, taking into account the different OSPs (and therefore stimulation thresholds) for each muscle under investigation. The main hypothesis was that there would be a muscle specific facilitation, with (i) higher MEPs obtained for the FDI muscle during observation of index finger movements and (ii) higher MEPs for the ADM muscle during observation of little finger movements. It was also hypothesised that higher MEPs would be obtained for both muscles during action observation compared to the MEPs obtained during the static condition. Finally, the effect on the resulting MEP for the FDI muscle tested at the ADM muscle’s OSP and vice-versa was unknown.
6.3 Methods

The methods employed in this study followed the procedure outlined on pp. 52-55. Twelve healthy volunteers (three males) aged 18 to 43 years (mean age 24.2 years), participated in this study. EMG recordings were collected as outlined on pp. 52-53 and the TMS procedure was identical to that reported on pp. 53-54, with the exception that the OSP for the FDI and the ADM muscles were found separately, one in each testing session. Resting motor threshold (RMT) was determined using the MEP amplitudes obtained either from the FDI or the ADM muscle (depending on the testing session).

6.3.1 Experimental protocol

Participants were requested to refrain from any voluntary movement and to attend to the stimuli presented on the television screen. Three different types of video clips, each lasting five seconds, were used throughout the experiment. Each video consisted of the dorsal view of either a male or female right hand: (i) static hand; (ii) five cycles of right index finger abduction/adduction and; (iii) five cycles of right little finger abduction/adduction (see Figure 6.1 on p. 98). Participants viewed a total of 3 blocks. Each block contained 36 videos with: (i) 12 index finger actions; (ii) 12 little finger actions; and (iii) 12 static hand videos. The experimental procedure was carried out on two separate occasions, once using the FDI OSP and once using the ADM OSP.
Figure 6.1: Three different types of video clips used in this study: (i) a static hand, (ii) index-finger movements or (iii) little finger movements. One TMS pulse was delivered per video at either 2500 or 3500ms after video onset. Participants viewed a total of three blocks, with each block containing 36 videos.

Figure 6.2: The different locations for the OSPs for the FDI and ADM muscles in the 12 participants. The shape and colour determine the muscle and frequency of scalp position respectively, as described in the key above. Cz represents the apex of the skull.
6.3.2 Data analysis

The data analysis followed the procedure outlined on pp. 54-55. The normalised MEP amplitudes were submitted to a 2x2x3 repeated measures (ANOVA) with muscle (FDI, ADM), OSP (FDI, ADM) and video (index finger action, little finger action, static) as within-subject factors.

For post-hoc comparisons, multiple pairwise t-tests with Sidak’s correction were performed. The level of statistical significance for all analyses was set to $\alpha = 0.05$. Effect sizes were reported as partial eta squared ($\eta^2$) for main effects, and as the difference in z-scores (ES) for further comparisons.

Due to relatively low participant numbers (although normal for TMS research) and the high number of independent variables, a follow up analysis was performed to increase statistical power. The mean MEP values for observation of both finger movements were each divided by the MEP values obtained for observation of the static hand. This created an index finger/static and little finger/static ratio which was entered into a repeated measures ANOVA with OSP (FDI, ADM), muscle (FDI, ADM), and ratio (index/static, little/static) as within subject factors.

6.4 Results

The aim of the current study was to test whether MEPs recorded from the FDI and ADM muscles were modulated during observation of the different video stimuli presented, using both the FDI and ADM OSPs on two separate testing sessions.
6.4.1 Influence of OSP

The OSP for each muscle was measured on the scalp guided by Jasper’s 10-20 electrode placement system (Jasper, 1958). The most common OSP for the FDI muscle was 4cm lateral and 1.5cm anterior, relative to Cz, compared to 4cm lateral from Cz for the ADM muscle. This is illustrated in Figure 6.2 (p. 98), where triangles represent ADM OSP locations and circles represent FDI OSP locations. The colours represent the number of participants, where blue represents 1 participant, red represents 2, orange represents 4, and green represents 5 participants. As explained in the legend of Figure 6.2, for example, the orange triangle shows the OSP location of four participants for their ADM muscle, while the green circle shows the OSP location of five participants for their FDI muscle. The mean resting motor threshold for the FDI muscle was 48.9%, with the ADM muscle slightly higher at 51.2%. Table 6.1 shows the hotspot separation and motor threshold for the FDI and ADM muscles for the individual participants.

Figure 6.3 (on p. 103) shows the group means of the MEP amplitudes, converted into normalised z-scores, in the FDI and ADM muscles, during the video observation conditions. The repeated measures ANOVA indicated that there were no significant interaction effects of OSP for either OSP x muscle interaction $F(1,11) = 0.84, p = 0.38, \eta^2 = 0.07$, or OSP x video interaction, $F(2, 22) = 0.27, p = 0.77, \eta^2 = 0.02$, showing that the OSP location had no significant effect on the MEPs recorded during observation of the three video conditions for both the FDI and ADM muscles.
Table 6.1: The hotspot separation and motor threshold percentages for the FDI and ADM muscles for the individual participants. The mode value is included for the hotspot location, and the mean value is calculated for the motor threshold.

<table>
<thead>
<tr>
<th>Participant</th>
<th>FDI</th>
<th>ADM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hotspot</td>
<td>Threshold</td>
</tr>
<tr>
<td>1</td>
<td>4cm, 1.5cm</td>
<td>45%</td>
</tr>
<tr>
<td>2</td>
<td>4cm, 1cm</td>
<td>39%</td>
</tr>
<tr>
<td>3</td>
<td>4cm, -1cm</td>
<td>44%</td>
</tr>
<tr>
<td>4</td>
<td>4cm, 1.5cm</td>
<td>67%</td>
</tr>
<tr>
<td>5</td>
<td>4cm, 1cm</td>
<td>42%</td>
</tr>
<tr>
<td>6</td>
<td>4cm, 1.5cm</td>
<td>43%</td>
</tr>
<tr>
<td>7</td>
<td>4cm, 1.5cm</td>
<td>56%</td>
</tr>
<tr>
<td>8</td>
<td>4cm, 0cm</td>
<td>57%</td>
</tr>
<tr>
<td>9</td>
<td>4cm, 1.5cm</td>
<td>37%</td>
</tr>
<tr>
<td>10</td>
<td>3cm, 1.5cm</td>
<td>55%</td>
</tr>
<tr>
<td>11</td>
<td>4cm, 0.5cm</td>
<td>38%</td>
</tr>
<tr>
<td>12</td>
<td>4cm, -1cm</td>
<td>55%</td>
</tr>
</tbody>
</table>

Mode: 4cm, 1.5cm | 4cm, 0cm | Mean: 48.9% | 51.2%

6.4.2 Influence of muscle

The repeated measures ANOVA showed a significant muscle x video interaction, F(2,22) = 10.5, p < 0.001, $\eta^2_p = 0.49$, demonstrating that a change in MEP amplitude across video conditions was dependent on the recorded muscle. Figure 6.3 shows larger MEPs in the FDI muscle for index finger observation compared to both little finger and static hand observation. Pairwise comparisons using Sidak’s corrections, showed no significant differences between the index finger movement and little finger movement observation ($p = 0.15, ES = 0.21$), or index finger movement versus static hand observation ($p = 0.2, ES = 0.2$). There was also no difference between the static hand and little finger observation ($p = 1.0, ES = 0$). For the ADM muscle, MEPs recorded during
observation of little finger movements were significantly larger than MEPs recorded during index finger movement ($p = 0.02$, ES = 0.2) and fell just short of significance compared to static hand observation ($p = 0.06$, ES = 0.2). There was no significant difference between the index finger movement and static hand observation ($p = 1.0$, ES = 0.01). In addition, irrespective of OSP, observation of index finger movement resulted in larger amplitude MEPs for the FDI muscle than the ADM muscle ($p = 0.001$, ES = 0.2), and larger amplitude MEPs for the ADM muscle than FDI muscle during observation of little finger movements ($p = 0.006$, ES = 0.2).

### 6.4.3 Ratio analysis

Figure 6.4 (p. 103) presents the results of the MEP ratio analysis of the FDI and ADM OSP combined, with the finger movement MEPs divided by static MEPs. The repeated measures ANOVA showed a significant muscle x ratio interaction, $F(1,11) = 12.3$, $p = 0.004$, $\eta^2_p = 0.51$. Pairwise comparisons with Sidak’s correction showed significantly higher ratio for index/static compared to little/static ($p = 0.05$, ES = 0.3) for the FDI muscle, and significantly higher ratio for little/static compared to index/static ($p = 0.007$, ES = 0.2) for the ADM muscle. There was a significant OSP x ratio interaction ($p = 0.05$, $\eta^2_p = 0.3$), however pairwise comparisons showed no further significant effects. It may be that the OSP did have an effect on the MEP ratio, however the effect was not large enough due to low statistical power. In addition, there was no OSP x muscle x ratio interaction, $F(1,11) = 0.04$, $p = 0.84$, $\eta^2_p = 0.003$. 
Figure 6.3: The mean MEP amplitudes recorded from the participants’ right FDI and ADM muscles during observation of index finger movement, little finger movement and static videos, recorded from the FDI and ADM OSP combined. The MEP amplitudes are presented as z-scores (mean ± SE). Significant differences are indicated by asterisks.

Figure 6.4: The MEPs recorded during observation of index finger movement, little finger movement and static videos, recorded from the both OSPs combined. The MEP ratios (action/static) are presented (mean ± SE) for both FDI and ADM muscles. Significant differences are indicated by asterisks.
6.5 Discussion

This study was designed to explore (i) whether observation of an action, as opposed to a static image, resulted in an increase in corticospinal facilitation, and (ii) whether the corticospinal facilitation was muscle-specific, by testing two muscles and using two OSPs on separate occasions. While Figure 6.3 showed the hypothesised increase in MEP amplitude during action observation as opposed to the control condition of a static image, this did not reach statistical significance for either muscle. Interestingly, irrespective of the hotspot tested, observation of index finger movement resulted in larger MEPs for the FDI muscle, with higher MEPs for the ADM muscle during little finger observation, highlighting the muscle-specific effect. Furthermore, during observation of the static hand control, there was no significant difference in MEP amplitude between the FDI and ADM muscle.

Many studies have reported muscle-specific facilitation effects during action observation (e.g., Fadiga et al., 1995; Romani et al., 2005; Urgesi et al., 2006a). The data presented here is the first study to explore this effect taking into account the OSP for each muscle under investigation. Since it is common practice to determine the OSP for the main muscle of interest it has been assumed that all tested muscles will consequently be stimulated sufficiently at that single scalp site. This method also affects the stimulation intensity applied throughout the experiment. This may lead to a bias in results. It may show a strong muscle-specific effect, which may actually be the result of the secondary muscles not being stimulated as strongly as the main muscle of interest. In the current study, there was high variability between participants’ OSPs for both muscles; with the most common FDI OSP being 1.5cm anterior to the most common ADM OSP (see Figure
6.2 on p. 98). Despite the difference in hotspot location, the data reported no significant effect of OSP. This finding is encouraging for two reasons: (i) it allows researchers to test certain muscles during a single experimental session, with participants able to undergo less stimulation sessions, possibly leading to lower participant dropout rates; and (ii) the interpretations of the muscle-specific findings reported in previous TMS action observation studies remain valid. One reason for the lack of OSP effect is that there is considerable overlap of finger muscle representations within the primary motor cortex (Melgari, Pasqualetti, Pauri, & Rossini, 2008; Sanes & Donoghue, 2000), with TMS normally eliciting MEPs in several muscles at a time. As reflected in this data, stimulating two closely-located finger muscle representations in the same testing session will not cause significant problems. Investigating the corticospinal representations of a combination of arm and finger muscles, however, may be considerably more difficult as their motor cortex representations are further apart (Melgari et al., 2008).

The lack of difference between video conditions for the FDI muscle was surprising. Figure 6.3 (on p. 103) shows considerably larger MEP amplitudes for observation of index finger action, compared to both little finger and static hand observation. A reason for the lack of significance may lie in the statistical power of the data, since only twelve participants were tested and a reasonable number of variables were analysed. In an attempt to correct for this, a second analysis was carried out, by creating MEP ratios for the action videos against the static hand for both the FDI OSP and ADM OSP in order to reduce the video factor by one. Therefore, the MEPs recorded during observation of index and little finger movements were divided by the MEPs recorded from static hand observation, resulting in an index/static MEP ratio and little/static MEP ratio for both the
FDI and ADM muscles. A positive MEP ratio value indicated higher action (index finger and/or little finger) excitability compared to the static hand control condition. By comparing the ratios against each other it was possible to investigate the muscle-specificity phenomenon. Once again, there was no main effect of OSP, however there was a significant OSP x ratio interaction. Further comparisons of this interaction showed no significant effects, which indicates that the effect may have been too small and the statistical power was not high enough to detect further differences. Therefore, whilst the different OSPs may not have resulted in large significant differences in the data for the two muscles, further research should continue to explore this phenomenon with larger sample sizes. With the FDI and ADM OSP data combined, results showed significantly higher index/static ratio compared to little/static ratio for the FDI muscle, whilst showing significantly higher little/static ratio compared to index/static ratio for the ADM muscle. It may be incorrect to use the term ‘motor facilitation’ to describe the effect presented here, since that would indicate that the action MEPs were significantly higher than the static MEPs, which were not evident in the first analysis above. Taken together, the ratio analysis provides further support for muscle specific responses induced by the movement observation.

The current study was the first study to explore the argument of muscle specificity by carrying out separate experiments for each muscle under investigation. This was important for two reasons: (i) coil location affects the magnitude of the resulting MEP response, and (ii) coil location affects the motor threshold of the muscle under investigation and as a result affects the stimulation intensity used throughout the experiment. Stimulation intensity is another important methodological issue that has not
yet been explored in the literature. A wide range of intensities has been used in previous research. In the following study, the motor facilitation obtained during action observation when stimulated at a low or a high intensity was investigated.
Chapter 7: Study 4: Motor facilitation during action observation: using different stimulation intensities

7.1 Introduction

TMS is used primarily to explore the motor cortex. Since the motor cortex has large and direct projections to the spinal cord, each stimulus results in a quantifiable measure of corticospinal activity (Jahanshahi & Rothwell, 2000). If the current passed through the stimulating coil is of sufficient intensity, stimulation of the cortex will cause discharge in corticospinal neurons and produces descending volleys into the spinal tract (Edgley et al., 1990; Patton & Amassian, 1954). The motor responses recorded using EMG are believed to be the result of activation of the corticospinal neurons (Lemon, 2002). TMS has been used widely in research investigating corticospinal excitability during action observation. As described extensively in Chapter 2, in response to primary motor cortex stimulation, observation of an action in the absence of overt movement modulates the excitability of the corticospinal pathway (e.g., Fadiga et al., 1995; Gangitano et al., 2001; Strafella & Paus, 2000). This modulation results in an increase in the amplitude of MEPs specific to the muscles involved in the observed action.

Before conducting a TMS action observation experiment, it is important to first establish a participant’s resting motor threshold (RMT). This is often identified as the lowest stimulator output intensity capable of producing MEPs of ≥ 50µv peak-to-peak amplitude in 50% of the stimulations (Rossini et al., 1994). The experimenter then conducts the experiment at a certain percentage of the participant’s resting motor threshold (RMT). Some researchers have applied TMS over the motor cortex with a
stimulation intensity of 110% RMT (e.g., Gangitano et al., 2004; Montagna et al., 2005), with others stimulating at the higher intensities of 120% RMT (e.g., Patuzzo et al., 2003; Sakamoto et al., 2009), 130% RMT (e.g., Aglioti et al., 2008; Romani et al., 2005), and some as high as 150% RMT (e.g., Li et al., 2009). Although these experiments have all reported motor facilitation during action observation, the range of intensities used in these experiments is of concern. First, due to the relatively large size of the TMS coil, stimulating at a high intensity will stimulate large areas of the brain, rather than just the motor cortex site responsible for projections to the muscles being tested. Second, when stimulating near motor threshold, the TMS pulses induce the already excited neurons (due to neural activity during action observation), just above threshold, resulting in descending volleys. Third, TMS pulses can evoke different kinds of descending volleys depending on the intensity of the stimulation (Di Lazzaro et al., 2004), which may have an impact on the resulting MEPs obtained during action observation.

As described in Chapter 2 on pp. 15-16, the accepted mechanism by which TMS activates the motor cortex to produce MEPs has been termed the D- and I-wave hypothesis (Day et al., 1989). When the axons of corticospinal neurons are stimulated directly they give rise to D- (direct) waves, and when they are excited trans-synaptically they give rise to I- (indirect) waves. Both forms of descending volleys are then transmitted down to the spinal cord via the corticospinal tract (Edgley et al., 1990; Di Lazzaro et al., 2004). If of sufficient strength to activate spinal motor neurons, these descending volleys will then lead to a subsequent muscle contraction. If TMS activates corticospinal neurons in a trans-synaptic manner, other processes that elicit a change in the excitability of corticospinal neurons will also modify the extent to which the cortical stimulation excites
the corticospinal neurons. These in turn, will influence the amplitude of the MEP obtained in the target muscle. In contrast, if TMS activates corticospinal axons directly at sites downstream to synaptic input then the amplitude of the MEP will not reflect the overall balance of cortical excitability at the moment of stimulation. This is a valid reason for identifying each individual’s motor threshold as the amplitudes of MEPs produced using high stimulation intensities will be less representative of cortical excitability levels than MEP amplitudes recorded using near threshold intensities. The practical implication is that it is important to use stimulation intensities that are close to motor threshold if the purpose of the experiment is to measure cortical excitability.

Despite the range of stimulation intensities used in action observation research, no research has yet compared the effect of stimulating at a near threshold intensity to a higher intensity. This study aims to address this gap in the literature.

7.2 Aims and hypothesis

The aim of the study was to compare the size of the MEP amplitude obtained during observation of an index finger abduction/adduction movement compared to a static hand control using two different stimulation intensities: low (110% RMT), and high (130% RMT). Based on previous studies, it was hypothesised that there would be a motor facilitation during action observation for both stimulation intensities.
7.3 Methods

7.3.1 Stimulus-response curves

Following some pilot work, it was decided that a more appropriate way to standardise the procedure, and reduce large inter-participant variability, would be to obtain stimulus-response curves based on the stimulation intensities of 100% RMT, 105% RMT, 110% RMT, 115% RMT, 120% RMT, 125% RMT, and 130% RMT. Average MEP peak-to-peak values were obtained at each stimulation intensity. The MEP values obtained for the 110% RMT and 130% RMT would then be used as a marker on which to base the two stimulation intensities (low, high) used throughout the experiment.

The methods employed in this phase of the study followed the procedure outlined on pp. 52-55. Five participants (1 female), aged 18-28 years, volunteered to take part. EMG recordings were collected as outlined on pp. 52-53 and the TMS procedure was identical to that reported on pp. 53-54, with the exception that seven different stimulation intensities (stated above) were used. Participants were requested to refrain from voluntary movement as they observed a blank television screen (resting condition). There were four blocks of trials, each consisting of 5 trials at each intensity, resulting in a total of 20 trials per intensity. Two-minute rest intervals were provided between blocks. The stimulus-response curves for both the FDI and ADM muscles are presented in Figure 7.1 (on p. 113).

When looking at the stimulus-response curves for each of the five participants, it is apparent that the MEP amplitudes, shown as a function of stimulus intensity, varied substantially between individual participants (see Figure 7.2 on p. 114). Taking the FDI
muscle as an example, since it was the main muscle of interest, all participants recorded an MEP amplitude of approximately 100µv at RMT (with a standard deviation of 45µv). An intensity increase of 15% resulted in a high variability of MEP amplitudes, ranging from 334µv to 1021µv (with a standard deviation of 295 µv). Figure 7.2 clearly shows that the MEP amplitude increases as the stimulation intensity increases, however the rate at which this occurs differs between individuals. This suggests that using a percentage of the RMT may not always be an adequate way for standardising the TMS procedure across participants.

7.3.2 Experimental protocol

The main experiment used the data provided by the stimulus-response curves to adjust the percentage value of the stimulator output at the start of the experiment, so that the 110% RMT at rest for each participant would be approximately 380 µv, and the 130% RMT at rest would be approximately 1250 µv (as shown in Figure 7.1). The methods employed here followed the procedure outlined on pp. 52-55. Seventeen healthy volunteers (4 females), aged 18 to 24 years (mean age 19.6 years), participated in this study. EMG recordings were collected as outlined in on pp. 52-53 and the TMS procedure was identical to that reported on pp. 53-54, with the exception that two intensities were used throughout the study; high intensity and low intensity. For the purpose of this study, 110% was chosen as the low intensity and 130% was chosen as the high intensity. The aim was to record clear MEP amplitude differences between two intensity conditions. For the purpose of this study, since 110% and 130% RMT are two intensities used frequently in action observation literature, it was deemed appropriate to base the two intensities on these values.
Participants were requested to refrain from any voluntary movement and to attend to the stimuli presented on the television screen. Ten stimulations at both 110% RMT and 130% RMT were first delivered at rest and the mean MEP amplitude was obtained. When the mean value differed considerably (more than +/- 100 µv) from the corresponding mean value obtained in the stimulus-response curves data, then the stimulation intensity was adjusted accordingly and 10 further stimulations were delivered. This was repeated until an acceptable mean value was obtained. Two different videos, each lasting five seconds, were used throughout the experiment. Both videos consisted of the dorsal view of a male right hand: (i) a static hand and; (ii) five cycles of index finger abduction/adduction (see Figure 7.3 on p. 117).
Figure 7.2: Stimulus-response curves of the MEP amplitudes of the FDI and ADM muscles of all 5 participants
One TMS pulse was delivered per video at either 2500 or 3500ms after video onset. Participants viewed a total of 4 blocks. Each block contained 20 videos with: (i) five action videos stimulated at a low intensity; (ii) five action videos stimulated at a high intensity; (iii) five static hand videos stimulated at a low intensity and; (iv) five static hand videos stimulated at a high intensity. In each block, the low intensity stimulations were delivered before the high intensity stimulations in order to reduce any residual activity from the high intensity trials in the low intensity trials. The two video conditions were presented in a random order.

7.3.3 Data analysis

The data analysis followed the procedure outlined on pp. 54-55. The normalised MEP amplitudes were submitted to a 2x2x2 repeated measures ANOVA with muscle (FDI, ADM), intensity (low, high) and video (action, static) as within-subject factors. For post-hoc comparisons, pairwise comparisons with Sidak’s corrections were performed. The level of statistical significance for all analyses was set to $\alpha = 0.05$. Effect sizes were reported as partial eta squared ($\eta^2_p$) for main effects, and as the difference in z-scores (ES) for further comparisons.

7.4 Results

The aim of the current study was to test whether MEPs recorded from the FDI and ADM muscles were modulated during observation of the different video stimuli presented (action, static), using two different stimulation intensities (low intensity, high intensity). The repeated measures ANOVA revealed a significant video x intensity interaction, $F(1,16) = 7.26, p = 0.02, \eta^2_p = 0.31$. No other interactions were significant.
Pairwise comparisons showed larger MEPs during action observation than static observation ($p = 0.001$, $ES = 0.2$) only for the low intensity stimulation (see Figure 7.4, p. 117). No significant effects were reported for the high intensity stimulation ($p = 0.82$, $ES = 0.02$).
Figure 7.3: Two different types of video clips used in this study: (i) index-finger action or (ii) static hand. One TMS pulse was delivered per video at either 2500 or 3500ms after video onset. Participants viewed a total of 4 blocks, with each block containing 20 videos (10 delivered at a low stimulation intensity, and 10 delivered at a high stimulation intensity).

Figure 7.4: The mean MEP amplitudes recorded from the right FDI and ADM muscles combined during observation of action and static videos at high and low intensities. The MEP amplitudes are presented as z-scores (mean ± SE). Significant differences are indicated by asterisks.
7.5 Discussion

This study was designed to explore (i) whether observation of an action, as opposed to a static image, resulted in an increase in corticospinal facilitation, and (ii) whether the obtained facilitation was modulated depending on the stimulation intensity used. The results showed that, consistent with Studies 1 and 2, and with the published research in the area (e.g., Romani et al., 2005; Lepage et al., 2010), there was an increase in MEP amplitude during action observation as opposed to the control condition of the static hand. This was only the case, however, when the stimulations were delivered at the lower intensity of approximately 110% RMT. No significant differences between the action and static observation were obtained when stimulations were delivered at the higher intensity of approximately 130%. In addition, no significant muscle effects were reported in his study. This was an unexpected finding given the data from the previous three studies reported in this thesis. This potentially weakens the action observation effect reported in the earlier low intensity stimulation of this study since the task and participant demographics were the same. The lack of a repeated specific muscle effect is difficult to explain and highlights the variability that can be found in human biological signals, and especially TMS MEP data. This said, however, the initial hypothesis of this study suggested that a motor facilitation effect would be evident for both stimulation intensities. In contrast to this hypothesis, the action MEPs were higher than the static MEPs only in the low intensity condition. This finding conflicts with the various studies that have stimulated at 130% RMT (e.g., Aglioti et al., 2008; Romani et al., 2005; Urgesi et al., 2006a) and have all reported a motor facilitation during action observation compared
to the observation of a static image. In addition, the stimulation timing of the TMS pulse used in these studies was similar to the timing used in the current study.

As discussed on pp. 15-16 and pp. 39-40, there are implications regarding the choice of stimulation intensity, since TMS pulses can evoke different kinds of descending volleys depending on the intensity of the stimulation (Di Lazzaro et al., 2004). If the axons of corticospinal neurons are stimulated directly then they give rise to D-waves, whereas if they are stimulated trans-synaptically they give rise to I-waves (Day et al., 1989). It is likely, therefore, that the MEP facilitation obtained at the low intensity of 110% RMT would be different to that obtained at the higher intensity of 130% RMT (illustrated in Figure 7.3, p. 117). This does not explain why researchers who have stimulated participants at 130% RMT have reported a motor facilitation effect for action observation, in direct contrast to the findings of this study. It is clear that further research needs to be carried out to explore the differences in motor facilitation effects using different stimulation intensities before any firm conclusions can be made.

One reason why there was no motor facilitation effect for the high intensity stimulations may have been due to the order with which the stimulations were delivered. In each block of trials, whilst the video conditions were presented in a random order, the high intensity stimulations were always delivered after the low stimulations. This was done to reduce any possible residual effects from the high intensity stimulation on the low intensity stimulation MEPs. In light of the results obtained from the stimulation timing data in Study 1, where no motor facilitation was reported for the later stimulation timing possibly due to attentional deficits, this may have confounded the high intensity MEP results. Therefore participants may have lost attentional focus in the latter stages of
each observation block, irrespective of whether they were watching a static hand or finger movement, resulting in similar MEP amplitudes for both video conditions.

Another aspect of this study focused on an alternative method for determining the stimulation intensity based on a percentage of the RMT. Typically, the motor threshold is first established (as discussed on pp. 49-50) in order to standardise the procedure across participants. The experiment is then run at a pre-determined percentage of that threshold value. If an individual’s RMT was at 40% of the stimulator output, and the experiment was to be conducted at 110% RMT, then the stimulator output’s percentage throughout the experiment would be at 44%. This method, however, may result in high inter-participant MEP variability. To counterbalance this, therefore, stimulus-response curves (as shown in Figure 7.1 on p. 113) could be first obtained, using mean values to adjust the chosen intensity. Rather than simply basing the percentage output values on individuals’ motor thresholds, they could be based, in part, on the mean scores recorded in the stimulus-response curves. This approach may provide another way of standardising the procedure, which may be more suitable to lower standard deviations across participants.

To conclude, it was unclear, in part, why a motor facilitation was found at low intensity stimulation but not at high intensity. It has been reported that stimulation intensity affects the nature of the corticospinal descending volleys; therefore it was not unexpected that the results of the two intensities used in this study would differ. The lower intensity (approximately 110% RMT), which did provide a motor facilitation for action observation, was the same intensity as was used throughout this research programme, where a motor facilitation was constantly reported. It can be concluded,
therefore, that stimulating the motor cortex at 110% RMT does result in a motor facilitation for action observation when compared to a static control. Based on these findings, the same cannot be concluded for higher intensities, with stimulation intensities at 130% RMT seeming unsuitable. The implications of this in terms of how the motor cortex is thought to be influenced by mirror neuron activity is important. At 130%, researchers cannot be confident that the MEP is representative of the mirror neuron activity. In contrast, at 110%, and with a greater expectation that the MEP is a consequence of I-wave activity, the association with mirror neuron activity is more compelling. Additional research is warranted in order to reach an accord for the optimal stimulation intensity applied in TMS motor cognition research.
Chapter 8: General discussion

This chapter brings together the findings of the four main studies. The key findings from the research have been discussed and summarised. The potential implications and applications of the research have then been presented, followed by recommendations for future research.

8.1 Summary of the research programme

The main aims of this research programme were to provide a more detailed understanding of the motor facilitation effect in action observation. In addition, some of the methodological concerns related to TMS were addressed, as TMS is one technique frequently used in action observation research.

The data from Study 1 emphasised the importance of choosing the most appropriate control condition when conducting an action observation study using TMS. The data showed a significant difference in corticospinal excitability between the action condition and both controls. There was a stronger effect size for the comparison with the blank screen, which may in part have been due to the magnitude of the differences in visual stimuli between the two conditions. In action observation studies, the amplitude of the motor responses obtained during action observation following the TMS pulse are generally compared to non-action control conditions. Failure to optimise control conditions may, therefore, bias the results, either by amplifying the delta motor response and risking a type 1 error (false positive result), or reducing the overall effect and risking a type 2 error (false negative result). The inclusion of a static image control for addressing the attentional and non-specific visual factors associated with using a blank screen
control, would theoretically allow more accurate comparisons with the action observation condition by providing meaningful visual cues without the associated action. This contrast is especially important when testing for mirror neuron system responses since mirror neurons discharge during observation of an action performed by someone else (di Pellegrino et al., 1992). Following the results of Study 1, a static image was incorporated as the control condition for all subsequent experiments in this thesis.

The effects of observation on subsequent action execution have been widely explored (e.g., Brass et al., 2000, 2001; Kilner et al., 2003) and are clearly important to motor learning. Few studies, however, have examined the effects of action execution on subsequent action observation, which is another important condition for skill learning and especially relearning. In Study 2, therefore, priming effects were examined. Previous experience of a ball pinching action primed the observers to perform that same action during the action observation, leading to increased motor responses. Participants observed a series of action observation trials, similar to those carried out in Study 1, followed by a series of ball pinching execution trials where participants were predicted to become more familiar with the action. Participants then repeated the observation trials and the motor responses were recorded. The motor facilitation effect obtained in Study 1 was again present in Study 2, however the effect was not enhanced after a brief period of action execution. This may have been due to the action being a highly familiar every day action. The latency of the action-observation priming effect and the effect of familiarity of the action may be important; however, this remains to be tested. Recently, Higuchi, Holle, Roberts, Eickhoff, and Vogt (2012) investigated neural changes, using fMRI, during observation learning and physical practice and how this modulation is associated with
improvements in performance. The activity recorded in the dorsolateral prefrontal cortex during observational practice positively correlated with changes in guitar chord response time. The authors also reported decreased neural activity as the learners became more skilled at the task. Investigating cortical modulation during observation leads to interesting advances in our knowledge of the neural underpinnings of observational learning, however exploring the accompanying changes in behaviour or performance are equally important. It would be interesting to replicate aspects of this study using TMS to examine the relationship between observation learning and execution over a longitudinal period.

Another methodological concern explored within Studies 1 and 2 was that of the timing of the TMS pulses that were delivered during action observation conditions. It is generally assumed that the motor responses measured during action observation of a repetitive movement are not affected by the choice of the timing of the TMS pulse. The timing of the stimulation, however, may be important due to attentional factors, and may influence the size of the MEPs obtained during action observation. The results of Study 1 showed a noticeable difference between the data obtained from the two stimulation points of 6250ms and 8750ms. There was a significant motor facilitation effect for the action observation condition that was only evident in the 6250ms data when compared to the fixation cross control. This effect was almost replicated in the first experiment of Study 2. During Experiment 1 (observation only), there was a higher motor facilitation ratio for the 6250ms data as compared to the 8750ms data, but this only approached significance at $p = 0.06$ for the FDI muscle. The disparity in the results between Experiments 1 and 2 of Study 2, and Study 1, may have been a consequence of the
maintenance of attention in the execution of the action and its continued priming effect on the corticospinal system. This is still a supposition at this point and further research is necessary to continue to explore the effect of stimulating at different time points during observation of a repetitive action, using actions that are both highly familiar and novel to the observers. Following the results of Studies 1 and 2, shorter videos of less than 5000ms were employed in the final two studies.

As described on pp.48-50, when stimulating over the motor cortex, it is common practice to first locate the ‘motor hotspot’ associated with the muscle of interest, then find each individual’s motor threshold and set the magnetic stimulator intensity to a percentage of that motor threshold. Studies 3 and 4 explored these important procedural concerns in further detail. In TMS action observation studies it is common for experimenters to record concurrently MEPs from a number of muscles. One main finding consistently reported in the literature, and also found in Studies 1 and 2 here, is a muscle specific effect during action observation. This is, however, usually reported without testing each muscle at its own scalp location. In Study 3 this was explored by using the motor hotspots of two separate muscles, tested on two occasions. The results reinforced the notion of muscle specificity despite the lack of statistical significance in the FDI muscle, with the results of both the FDI and ADM muscles showing a trend for a specific motor facilitation effect for the observed matching action. In addition, when the MEP action/static ratio was presented for both muscles, the muscle-specific effect was statistically significant. Furthermore, there was no significant effect of hotspot, which was encouraging for the validity of the interpretations of the muscle-specific findings reported in previous TMS action observation studies and those reported in the studies in this
thesis. Once the motor hotspot has been identified, it is important to establish a participant’s motor threshold. Typically, researchers conduct action observation experiments at a stimulation intensity of between 110% and 130% RMT. Despite the range of stimulation intensities used in TMS action observation research, no research has compared the effect of stimulating at a near threshold intensity to stimulating at a higher intensity. Study 4 addressed this gap in the literature. The results showed a motor facilitation effect at relatively low intensity stimulation but not at the higher intensity stimulation. The lack of motor facilitation for the higher intensity was in contrast with previous action observation research where participants were stimulated at 130% of RMT. A reason for this may be that in each experimental block, the higher stimulations were always delivered after the lower stimulations in order to reduce any residual effect that higher intensities may have on the subsequent lower intensity MEPs. In the light of the effect that stimulation timing has on MEPs, this may have confounded the results. The data for this study was, therefore, inconclusive and more research is required to explore the possible effects of different stimulation intensities on the corticospinal pathway.

8.2 Applications and implications of the research programme

Since the discovery of mirror neurons in the macaque monkeys (di Pellegrino et al., 1992) there has been strong support for a homologue observation-execution matching system in humans where a set of neurons fire both when individuals observe an action as well as when they execute the same or similar action performed by someone else (for reviews see Rizzolatti et al., 2001; Rizzolatti & Craighero, 2004). In recent years, however, there have been debates on the nature of the mirror neuron system in humans
and their potential involvement in action understanding (for a review see Hickok, 2009).

TMS research in action observation has provided indirect evidence for a mirror neuron system in humans, with a plethora of positive research showing larger peripheral muscle MEPs during action observation (e.g., Fadiga et al., 1995; Gangitano et al., 2001; 2004; Strafella and Paus, 2000). Prior to the research conducted in this thesis there had been no published research questioning the validity of methods used in TMS experiments when exploring the excitability of the motor system (and hence the putative mirror neuron system) during action observation. As such, the legitimacy of the studies reporting positive findings was simply accepted at face value. It was therefore important to address this gap in the literature to review critically the TMS methods that have been applied to action observation research to either provide support, as well as extend previous findings, and/or discuss alternatives for more rigorous methodological approaches to action observation research using TMS.

8.2.1 Design of action observation experiments

There are well documented issues with the methods used in techniques such as EEG and fMRI (see p. 8). In TMS, however, critical method-based research is limited. The technique’s methodological limitations in the context of action observation have not yet been fully explored. Throughout this thesis, the main aim was to examine critically the technique of TMS, as well as offer alternate methods for exploring the observation-execution matching system in humans. Much of the mirror neuron research using TMS has been accepted without challenge, with mirror neurons being credited for a number of social and cognitive behaviours that, arguably, go beyond the actual data. The scope of
this thesis was, therefore, to take a methodological ‘step back’ and explore the methods employed behind the recording of this ambitious and intuitively appealing data.

With the MEPs obtained through magnetic stimulation being so variable and unstable (see p. 14), it is imperative to consider carefully how they are being obtained and what other factors might be causing or contributing to MEP modulation without using the default mirror neuron system explanation. In addition, without a consensus as to the best approach to carry out motor cognition experiments using TMS, it makes comparisons across laboratories difficult and inconclusive, which is unhelpful to research generally. Designing TMS action observation experiments as scientifically rigorous as possible is vital. The studies presented in this thesis were aimed at tackling some of the major methodological concerns that were evident in the literature but had not yet been explored. Future studies should incorporate these findings into their experimental designs, especially when choosing the control conditions, stimulation timings and stimulation intensity to be applied to their research.

8.2.2 The action-observation matching system

Whether mirror neuron systems are involved in action understanding or not, it is evident that there is some form of observation-matching system in humans which plays a role in activating the corticospinal circuit during observation of an action, and this has been illustrated in all four of the studies explored in this thesis. Using the technique of TMS, MEPs of larger amplitudes were recorded when participants observed actions on screen, in contrast to control conditions, showing increased corticospinal excitability during action observation. This finding has often been associated with a human mirror
neuron system. The ambitious claim has been that it is these mirror neurons that allow individuals to ‘understand’ the viewed action, and learn, imitate, and simulate that observed action. A focus of this thesis has been to investigate the observation-execution matching system as a whole. Specifically, the research has focussed on whether there is increased corticospinal excitability when individuals observe a conspecific perform an action, since this is the main prediction associated with a human mirror system. Showing increased MEP amplitudes and corticospinal facilitation when individuals observe actions has important implications for observational learning and development, as well as having clinical and sporting applications. The ability to learn without having to practice is essential to human development. From childhood to adulthood, individuals learn a range of motor and social skills simply by observing others around them. Investigations into the neural underpinnings of action observation has demonstrated that when physical training may not be possible, watching the action may still activate the neurons involved in those specific actions, and this has been reported throughout this programme of studies.

The benefits of observational learning can be extended to clinical settings. Research, for example observation-based and imagery interventions, have been applied to stroke rehabilitation (e.g., Celnik, Webster, Glasser, & Cohen, 2008; Ertelt et al., 2007; Holmes, 2007). Research has shown increased cortical excitability similar to that reported throughout Studies 1-4. This provides theoretical support for incorporating observation along with physical therapy during patient rehabilitation. When an individual is no longer able to perform an action, e.g., following a stroke or injury, then watching another person perform that movement may activate the action-observation network in a similar way to when they used to perform the movement pre-stroke/injury. Holmes and Ewan (2007)
have reported that observation-based therapy, post-stroke, can be used to motivate physical training, support the re-acquisition of lost movement patterns, and may also allow patients to take some control over their rehabilitation process. These changes were associated with a better recovery. The research implications of action observation in clinical therapy are still in their infancy. The opportunities for further work are clear, the work presented in the current studies provides a solid framework for which to explore the neural underpinnings of action observation and apply the findings to clinical settings.

8.3 Future directions

Research into the cortical processes during action observation is still at a relatively early stage. The studies in this thesis have attempted to examine a number of methodological issues. There are, however, still gaps in the literature that need to be addressed. With many publications in mirror neuron research using TMS showing the hypothesised motor facilitation, it is important to challenge the methods that have been employed. It is essential that future research verify claims made here and continue to address these methodological concerns. One approach may be to combine various techniques, such as TMS, fMRI, MEG, and EEG, in order to better triangulate the data. This method may provide a more complete understanding of the effects of action observation on cortical modulation in different areas of the brain. Combining TMS with other neuroimaging methods allows for further investigations into whether contributions of “a specific brain area to task performance may reflect mostly local modular processes, or rather functional interactions with interconnected cortical regions” (Ruff, Driver, & Bestmann, 2009, p. 1048). In addition, a recent paper by Miniussi and Thut (2010) provided a detailed description of the advantages of integrating TMS and EEG and how
this can provide invaluable information about brain functioning, beyond which either technique can do alone.

In TMS action observation research it is generally assumed that the participants remain attentive to the actions displayed on the screen throughout the experiment. It is possible, however, that participants may lose concentration and/or shift their attention elsewhere during data collection. Equipment is now available which can monitor participants’ eye-gaze to provide an indication of what participants are looking at and thereby predictions can be made about visual attention. Future action observation research should combine TMS with eye-tracking devices in order to monitor participants’ eye gaze. Whilst this does not guarantee attentional focus, it could provide an indication of whether participants were following or attending what was being displayed on the screen. Trials where participants were not looking at the action on screen could then be discarded from the analysis. Future research should seek to address this gap in the literature as no research has yet combined these two techniques.

The studies in this thesis were a first attempt at evaluating TMS methods critically, in particular with regards to exploring the stimulation timing (explored in Studies 1 and 2), motor hotspot (explored in study 3), and the stimulation intensity (explored in Study 4) issues. During the analyses of the first two studies, it became apparent that the two time points used to stimulate during the observation trials resulted in differences in MEP amplitudes, which may have been the result of a reduction in participants’ attention. This finding was unexpected since the action being observed was repetitive, with both time points corresponding to the same phase of the observed action. It became apparent, however, that using varied stimulation timings may have confounded the results.
Typically, different time points have been combined in a single analysis. This can be seen as problematic. Future research into repetitive observed actions needs to explore this further by stimulating at a number of different time points to see whether significant differences would be obtained. Should this be the case, then the results of published studies, especially those using longer video durations, may need to be reconsidered. A combination of TMS and eye-tracking research should be beneficial to explore the concept of stimulation timing and attentional variations.

The results of Study 3 provided support for the muscle-specific effect. This was the first study to explore this issue by using different motor hotspots for each muscle under investigation. While the different hotspots did not significantly alter the MEP data for the two muscles, further research should continue to explore this phenomenon. Larger sample sizes are warranted, as well as investigations exploring the different motor hotspots for other limb muscles, such as wrist and finger muscles combined.

With regards to the choice of stimulation intensity, the results from Study 4 remain inconclusive. As discussed on pp. 15-16 of Chapter 2, and in Chapter 7, the currents elicited from TMS may excite corticospinal neurons either synaptically or trans-synaptically, which in turn will affect the MEPs obtained in the peripheral muscles. If the neurons are activated at sites downstream to the synaptic input, then the MEPs will not reflect the cortical excitability. This highlights the importance of choosing the appropriate stimulation intensity when exploring motor cognition indirectly through TMS. Stimulation intensities close to motor threshold are more representative of the cortical excitability levels at the time of stimulation. In Study 4, low intensity TMS was compared with high intensity, resulting in a motor facilitation evident only for the low stimulation during
action observation. While this is consistent with the hypothesis that different intensity TMS affects the corticospinal excitability differently, it is in direct contrast with other action observation literature that has reported a motor facilitation effect at higher motor thresholds (e.g., Aglioti et al., 2008; Li et al., 2009; Romani et al., 2005). There is a gap in the literature exploring this phenomenon. Future work should address this by comparing and contrasting MEP amplitudes obtained during action observation at a number of different intensities. In addition, while it is common practice to run the experiment at a set percentage of an individual’s motor threshold, it may be more effective to obtain stimulus-response curves first (as in Study 4), in order to further reduce inter- and intra-participant variability, and standardise the procedure across participants.

A further avenue for new research would be to explore modulation of the corticospinal pathway during observation of more complex or more ‘contextually-embedded’ movements. The studies in this thesis, as well as the majority of published action observation research, used simple hand or finger movements, such as reach and grasp or pinching actions, or finger abduction/adduction movements. This research was important in order to be able to isolate the muscles involved in the action, and to consider the mechanisms of the observation-execution matching system. Once the methods have been tested, and the effects of observation on the corticospinal pathway explored critically, future research should consider task demands thereby increasing the ecological validity of such studies. This could be done by incorporating observation of full-bodied skills, or movements embedded in real-life contexts, in order to engage participants more fully, allowing researchers to apply the findings to complex learning and sporting environments. To date, only a few studies have explored these ideas using
TMS. For example, Aglioti et al. (2008) delivered TMS pulses as basketball players observed free shots while having to anticipate the fate of the shot. This research into the neural underpinnings of professional basketball players’ anticipatory mechanisms provided an advancement in this area, but is not without limitations. Future research needs to be conducted to continue to apply lab-based research to real-life contexts. To expand on improving the ecological validity of action observation TMS studies, another future direction could be to incorporate observation of actions performed by live models, rather than videos. This presents challenges, such as the reliability of the model accurately performing the action similarly each time. However, in most learning and sporting contexts individuals usually first observe the skill or task performed by a teacher or sporting coach in front of them during a practice session. Therefore, exploring the modulation of the corticospinal pathway during live observation would advance the scientific knowledge of cortical processes during observation, as experienced in real-life situations, thus adding to the ecological validity of the study.

The experiments presented in this thesis, as well as the majority of referenced articles, have used single-pulse TMS. This approach has its limitations, as the increase in corticospinal excitability (represented by the MEPs obtained in the peripheral muscles) may have occurred through different neuronal pathways. The paired pulse method (Kujirai et al., 1993), which provides two TMS pulses through a single stimulating coil, allows stronger claims to be made for the effects being due to changes at a cortical rather than spinal level. With regards to action observation, Fadiga et al. (2005) suggest at least two mechanisms by which the facilitatory effect could occur. Data from primates shows a strong interconnectivity between premotor area F5, and primary motor area M1; a similar
potential cortico-cortical mechanism may also be present in humans to allow the activity of mirror neurons in the premotor cortex to increase the excitability of motor cortex. Similarly, the facilitation effect could be due to connections between the premotor cortex and the spinal cord. To address this important methodological and mechanistic concern, the paired pulse TMS method has been proposed (Kujirai et al., 1993). This method offers the potential to provide two TMS pulses, at short inter-stimulus intervals, through a single stimulating coil. The ability to change pulse intervals, and to control the power level of each stimulus allows for the experimenter to evaluate the effects of an initial conditioning stimulus on the amplitude of the MEP elicited by a subsequent test stimulus. One form of the paired pulse technique is to use a sub-threshold conditioning stimulus and a supra-threshold test stimulus. If the intensity of the first conditioning pulse is set to 80% of the motor threshold and the inter-stimulus interval is set between 1-5 ms then this pulse will act to reduce the MEP elicited by the subsequent test pulse and is a valid approach for probing intra-cortical excitability (Kujirai et al., 1993). A few paired-pulse TMS studies (e.g., Patuzzo et al., 2003; Strafella & Paus, 2000) have investigated the effects of action observation on corticospinal excitability and intra-cortical inhibition, resulting in significant increases in MEP amplitude in the observation conditions compared to baseline conditions, and a modulation in intra-cortical inhibition and facilitation. As a result of the paired pulse TMS technique, stronger claims for the effects being due to changes at a cortical rather than spinal level can be made.

The reason why the studies presented in this thesis used single-pulse was that since the thesis was based on exploring methodological issues embedded in the TMS action observation literature, it was important to first focus on the single-pulse technique,
which is more commonly used. Future TMS work should continue these methodological investigations using the paired-pulse technique in order to certify that any modulation in MEP size is a result of cortico-cortical projections. Throughout this thesis, the motor facilitation effect obtained is always referred to as a ‘corticospinal’ facilitation. Using paired pulse TMS, it would be possible to show with more certainty that motor facilitation effects during action observation are the direct result of cortico-cortical modulation rather than corticospinal.

Another avenue for non-invasive investigation of the motor cortex is by the application of weak direct current through the scalp via small electrodes, by means of transcranial direct current stimulation (tDCS). The current flow either increases (by anodal stimulation) or decreases (by cathodal stimulation) neuronal excitability in the specific area being stimulated. The excitability changes are controlled by the current duration and intensity of the stimulation (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche & Paulus, 2000). In the last few decades, as a result of the emergence of techniques such as TMS, and neuroimaging techniques such as fMRI and PET, tDCS has been re-evaluated as a reliable method to induce and modulate neural changes in the motor cortex (Nitsche et al., 2008). For testing cortical excitability in the primary motor cortex, tDCS can be combined with single pulse TMS, by exploring the effects of anodal and cathodal tDCS on the MEP amplitudes. For example, Uy and Ridding (2003) reported that tDCS modulated MEP amplitudes in the FDI muscle that persisted for up to one hour after stimulation. Whether therapeutic changes can endure for weeks or months remain to be determined (George & Aston-Jones, 2010). To conclude, TMS offers greater spatial and temporal resolution than tDCS. Importantly, however, tDCS is currently less expensive, more
portable, well-tolerated, and associated with fewer safety concerns (Hamilton, Messing & Chatterjee, 2011). By applying tDCS to the primary motor cortex, prior to TMS, further information can be attained about the excitability of the motor cortex during action observation, and the application of such techniques for rehabilitation after injury or stroke.

8.4 Conclusions

This thesis explored methodological issues in TMS motor cognition research, while exploring the effects of the corticospinal pathway during observation of simple actions. Some of the findings presented here offer further support for the muscle-specific motor facilitation effect reported consistently in the action observation literature. In all four studies, MEPs recorded from the muscles involved in the observed action were larger than the MEPs recorded during the control conditions. This main finding adds to the literature that supports the existence of a mirror neuron system in humans. It must be stressed, however, that TMS can only provide indirect evidence for the putative mirror neuron system. TMS stimulation occurs over the motor cortex, which is believed to be strongly connected to the premotor cortex, where mirror neurons are located (Fadiga et al., 2005). The resulting MEPs are a measure of corticospinal projections from the motor cortex to the peripheral muscles, which includes possible spinal involvement. Future research should take advantage of the paired-pulse TMS technique, which may help determine the cortical or spinal origin of corticospinal facilitation during action observation.
TMS is a relatively new technique used to explore the observation-execution matching system in humans, and its possibilities have yet to be fully explored. This thesis provided a first attempt into analysing critically a number of important methodological concerns within TMS action observation research. Some results, however, proved inconclusive. This thesis has contributed to highlighting the importance for future research to continue to explore the methods employed in TMS motor cognition studies, especially in relation to the choice of stimulation timing and the appropriate stimulation intensity.

Action observation research benefits a number of sectors, including sporting and clinical settings. Observational learning has long been acknowledged in the field of psychology to be a cognitive and motivational tool. Recent advances in neuroscience, with the aid of techniques such as fMRI, EEG, and TMS, have provided researchers with evidence of cortical activation during observation of actions. This has allowed clinical practitioners, and sporting coaches alike, to apply the knowledge gained from lab-based research and incorporate it into their rehabilitation settings for patients who have lost their motor ability, as well as injured athletes, respectively. Action observation research has been applied to various sectors and environments. It is therefore imperative to continue to refine these methods and make them universally accepted and as scientifically rigorous as possible.
References


George, M. S., & Aston-Jones, G. (2010). Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). *Neuropsychopharmacology*. doi: 10.1038/npp.2009.87


frequency and muscle specificity. *Brain Research, 900*(2), 282-294. doi: 10.1016/S0006-8993(01)02369-1


Premack, D., & Woodruff, G. (1978). Does the chimpanzee have a ‘theory of mind’? *Behavioral and Brain Sciences, 1*, 515-526. doi: 10.1017/S0140525X00076512


Sparing, R., Hesse, M. D., & Fink, G. R. (2010). Neuronavigation for transcranial magnetic stimulation (TMS): where we are and where we are going. *Cortex, 46*(1), 118-120. doi:10.1016/j.cortex.2009.02.018


Appendix A: The TMS Safety Screen (TASS; Keel et al., 2001)

If you agree to take part in this study, please answer the following questions. The information you provide is for screening purposes only and will be kept completely confidential.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever suffered from any neurological or psychiatric conditions?</td>
<td>YES / No</td>
</tr>
<tr>
<td>If YES please give details (nature of condition, duration, current medication, etc).</td>
<td></td>
</tr>
<tr>
<td>Have you ever suffered from epilepsy, febrile convulsions in infancy or had recurrent fainting spells?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Does anyone in your immediate or distant family suffer from epilepsy?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>If YES please state your relationship to the affected family member.</td>
<td></td>
</tr>
<tr>
<td>Do you suffer from migraine?</td>
<td>YES/ NO</td>
</tr>
<tr>
<td>Have you ever undergone a neurosurgical procedure (including eye surgery)?</td>
<td>YES/ NO</td>
</tr>
<tr>
<td>If YES please give details.</td>
<td></td>
</tr>
<tr>
<td>Do you currently have any of the following fitted to your body?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Heart pacemaker, Cochlear implant, Medication pump</td>
<td></td>
</tr>
<tr>
<td>Surgical clips, Metal plates</td>
<td></td>
</tr>
<tr>
<td>Are you currently taking any unprescribed or prescribed medication?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>If YES please give details.</td>
<td></td>
</tr>
<tr>
<td>Are you currently undergoing anti-malarial treatment?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Have you drunk more than 3 units of alcohol in the last 24 hours?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Have you drunk alcohol already today?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Have you had more than one cup of coffee, or sources of caffeine, in the last hour?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Have you used recreational drugs in the last 24 hours?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Did you have very little sleep last night?</td>
<td>YES / NO</td>
</tr>
<tr>
<td>Have you already participated in a TMS experiment today?</td>
<td>YES / NO</td>
</tr>
</tbody>
</table>

Date of Birth

___/___/

Name (in CAPITALS) _________________________________________________

Signature ___________________________ Date _________________________
Appendix B: The Edinburgh Handedness Inventory (EHI; Oldfield, 1971)

Please indicate your preference in the use of hands in the following activities by putting a + in the appropriate column. Where your preference is so strong that you would never try to use the other hand, unless absolutely forced to, put ++. If you are really indifferent put a + in both columns.

<table>
<thead>
<tr>
<th>Name:</th>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drawing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throwing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scissors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toothbrush</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knife (without fork)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hammer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screwdriver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennis racquet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knife (with fork)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cricket bat (lower hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golf club (lower hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broom (upper hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Striking match (match hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opening box/jar (lid hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dealing cards (card dealing hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Which foot do you prefer to kick with?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Which eye do you use when using only one?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix C – Participant information sheet for Study 1

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Participant Information Sheet

Title of Study:
Motor facilitation during action observation: ‘controlling’ the controls

1) This is an invitation to take part in a piece of research.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?

The purpose of the study is to investigate whether observation of a repetitive ball pinch movement increases the activity in the area of the brain that controls hand movements.
3) Why is the study being performed?

It has recently been discovered that a sub-set of brain cells termed ‘mirror neurons’ are involved in processing information during both the execution of self-performed actions and the observation of other people’s actions. It has been proposed that these mirror neurons are important for understanding the actions that other people make. The current study is using a non-invasive method of brain stimulation to test whether brain activity is influenced when observing others performing familiar pinching actions.

4) Why am I being asked to take part?

You and approximately fourteen other people will be invited to take part in this study. The study requires normally, healthy individuals to take part. Additionally you must be right handed and have normal vision, or corrected-to-normal vision.

5) Do I have to take part?

You are under no obligation to take part in this study. If after reading this information sheet and asking any additional questions you do not feel comfortable taking part in the experiment you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect the standard of care you receive in any way, nor will it affect your relationship with any of the staff at the Manchester Metropolitan University.

If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be provided with a copy of this together with this information sheet for your records. In addition to this you will be asked to fill in a copy of the Transcranial magnetic stimulation Adult Safety Screen (TASS) which will confirm your eligibility to participate.

6) What will happen to me if I agree to take part?

If you agree to take part in the study you will be asked to come to the Psycho-physiology laboratory in the Department of Exercise and Sport Science at the Manchester Metropolitan University for a test session. Whilst there you will be asked to sit at a desk and watch a series of different video clips, during which, on some occasions TMS will be applied to measure brain activity.

The TMS equipment used comprises a figure-of-8 shaped coil held against the side of the head. When stimulated it causes the nerves in the scalp and the brain to become briefly activated. The sensation caused by this stimulation is not unpleasant and will cause you no pain. Stimulation to the area of the brain we are interested in will cause a muscular twitch to occur in one hand. The muscle twitch will be recorded using electromyography (EMG) surface
electrodes. These will record very small electrical signals emitted during muscle activity. The surface electrodes used to record these signals will require self-adhesive pads to be attached to the skin over the muscle. The recording of EMG signals and stimulation with TMS is a completely safe and painless procedure.

The testing session will last approximately 1½ hours. This will provide enough time to fully explain the procedures, prepare you for EMG recording and TMS stimulation, and conduct the experiment. In recognition of the time you are being asked to give up to take part in the study, you will be reimbursed to the sum of £10 in cash which will be given to you at the end of the experiment.

7) Are there any disadvantages or risks in taking part?

TMS is a non-invasive technique for delivering electrical stimulation to humans through the scalp. Whilst research has concluded that TMS is a safe research method that carries no significant risk of long-term side-effects, there is a minimal risk of mild headaches and slight discomfort at the site of stimulation. The safety screening questionnaire (TASS) should exclude participants who are susceptible to these side-effects, however, in the unlikely event that either of these occurs, please alert the investigator and the experiment will be stopped immediately.

8) What are the possible benefits of taking part?

In addition to being paid £10 in cash, your involvement may help further our understanding of the human motor system.

9) Who are the members of the research team?

The principal investigator conducting the study is Miss Michela Loporto. Dr Paul Holmes (Director of Studies) and Dr. Craig McAllister (Supervisor) are the additional members of the research team. If you require further information on the study before taking part please feel free to contact the principle investigator, Miss Michela Loporto via email: m.loporto@mmu.ac.uk.

10) Who is funding the research?

This is a self-funded project with programme fees provided by the Malta Government Scholarship Scheme.

11) Who will have access to the data?

All data collected during the course of the research will remain confidential and will only be used for the purposes of the study. The data will be stored in coded form and the principal investigator and supervisory team will have access to the data. The data will be kept stored for five years before being destroyed. The data is being collected as part of the principle investigator’s PhD project; therefore the results of the study will be reported in the final thesis. Any information linking your identity to the study will not be included in this. It is also likely that
the findings will be communicated in scientific journals or conferences in the future, however, in this event, your name or identity will not be disclosed. Should you wish to obtain a copy of the summary of the study’s findings please feel free to contact the investigator via email: m.loporto@mmu.ac.uk

12) Who do I contact if I feel my rights have been violated?

If at any point during the study you feel that your rights as a participant have been violated and you wish to make a complaint regarding your involvement in the study please contact:

The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400,

Thank you for considering participation in this study.
Appendix D – Participant information sheet for Study 2 (Experiment 1)

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Participant Information Sheet

Title of Study:
Investigating motor facilitation during action observation

1) This is an invitation to take part in a piece of research.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?

The purpose of the study is to investigate whether observation of a repetitive ball pinch movement increases the activity in the area of the brain that controls hand movements.

3) Why is the study being performed?

It has recently been discovered that a sub-set of brain cells termed ‘mirror neurons’ are involved in processing information during both the execution of self-performed actions and the observation of other people’s actions. It has been proposed that these mirror neurons are important for understanding the actions that other people make. The current study is using a
non-invasive method of brain stimulation to test whether brain activity is influenced when observing others performing familiar pinching actions.

4) Why am I being asked to take part?

You and approximately fourteen other people will be invited to take part in this study. The study requires normally, healthy individuals to take part. Additionally you must be right handed and have normal vision, or corrected-to-normal vision.

5) Do I have to take part?

You are under no obligation to take part in this study. If after reading this information sheet and asking any additional questions you do not feel comfortable taking part in the experiment you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect the standard of care you receive in any way, nor will it affect your relationship with any of the staff at the Manchester Metropolitan University.

If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be provided with a copy of this together with this information sheet for your records. In addition to this you will be asked to fill in a copy of the Transcranial magnetic stimulation Adult Safety Screen (TASS) which will confirm your eligibility to participate.

6) What will happen to me if I agree to take part?

If you agree to take part in the study you will be asked to come to the Psycho-physiology laboratory in the Department of Exercise and Sport Science at the Manchester Metropolitan University for a test session. Whilst there you will be asked to sit at a desk and watch a series of different video clips, during which, on some occasions TMS will be applied to measure brain activity.

The TMS equipment used comprises a figure-of-8 shaped coil held against the side of the head. When stimulated it causes the nerves in the scalp and the brain to become briefly activated. The sensation caused by this stimulation is not unpleasant and will cause you no pain. Stimulation to the area of the brain we are interested in will cause a muscular twitch to occur in one hand. The muscle twitch will be recorded using electromyography (EMG) surface electrodes. These will record very small electrical signals emitted during muscle activity. The surface electrodes used to record these signals will require self-adhesive pads to be attached to the skin over the muscle. The recording of EMG signals and stimulation with TMS is a completely safe and painless procedure.
The testing session will last approximately 1½ hours. This will provide enough time to fully explain the procedures, prepare you for EMG recording and TMS stimulation, and conduct the experiment. In recognition of the time you are being asked to give up to take part in the study, you will be reimbursed to the sum of £10 in cash which will be given to you at the end of the experiment.

7) Are there any disadvantages or risks in taking part?

TMS is a non-invasive technique for delivering electrical stimulation to humans through the scalp. Whilst research has concluded that TMS is a safe research method that carries no significant risk of long-term side-effects, there is a minimal risk of mild headaches and slight discomfort at the site of stimulation. The safety screening questionnaire (TASS) should exclude participants who are susceptible to these side-effects, however, in the unlikely event that either of these occurs, please alert the investigator and the experiment will be stopped immediately.

8) What are the possible benefits of taking part?

In addition to being paid £10 in cash, your involvement may help further our understanding of the human motor system.

9) Who are the members of the research team?

The principal investigator conducting the study is Miss Michela Loporto. Dr Paul Holmes (Director of Studies) and Dr. Craig McAllister (Supervisor) are the additional members of the research team. If you require further information on the study before taking part please feel free to contact the principle investigator, Miss Michela Loporto via email: m.loporto@mmu.ac.uk.

10) Who is funding the research?

This is a self-funded project with programme fees provided by the Malta Government Scholarship Scheme.

11) Who will have access to the data?

All data collected during the course of the research will remain confidential and will only be used for the purposes of the study. The data will be stored in coded form and the principal investigator and supervisory team will have access to the data. The data will be kept stored for five years before being destroyed. The data is being collected as part of the principle investigator’s PhD project; therefore the results of the study will be reported in the final thesis. Any information linking your identity to the study will not be included in this. It is also likely that the findings will be communicated in scientific journals or conferences in the future, however, in this event, your name or identity will not be disclosed. Should you wish to obtain a copy of the summary of the study’s findings please feel free to contact the investigator via email: m.loporto@mmu.ac.uk
12) Who do I contact if I feel my rights have been violated?

If at any point during the study you feel that your rights as a participant have been violated and you wish to make a complaint regarding your involvement in the study please contact:

The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400,

Thank you for considering participation in this study.
Title of Study:

Investigating the excitability of the human motor system during action observation

1) This is an invitation to take part in a piece of research.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?

The purpose of the study is to investigate whether repetitive ball pinching actions prior to observation of the same action increases the activity in the area of the brain that controls hand movements.
3) Why is the study being performed?

It has recently been discovered that a sub-set of brain cells termed ‘mirror neurons’ are involved in processing information during both the execution of self-performed actions and the observation of other people’s actions. It has been proposed that these mirror neurons are important for understanding the actions that other people make. The current study is using a non-invasive method of brain stimulation to test whether brain activity is influenced when observing others performing familiar pinching actions.

4) Why am I being asked to take part?

You and approximately fourteen other people will be invited to take part in this study. The study requires normally, healthy individuals to take part. Additionally you must be right handed and have normal vision, or corrected-to-normal vision.

5) Do I have to take part?

You are under no obligation to take part in this study. If after reading this information sheet and asking any additional questions you do not feel comfortable taking part in the experiment you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect the standard of care you receive in any way, nor will it affect your relationship with any of the staff at the Manchester Metropolitan University. If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be provided with a copy of this together with this information sheet for your records. In addition to this you will be asked to fill in a copy of the Transcranial magnetic stimulation Adult Safety Screen (TASS) which will confirm your eligibility to participate.

6) What will happen to me if I agree to take part?

If you agree to take part in the study you will be asked to come to the Psycho-physiology laboratory in the Department of Exercise and Sport Science at the Manchester Metropolitan University for a test session. Whilst there you will be asked to sit at a desk and watch a series of different video clips, during which, on some occasions TMS will be applied to measure brain activity. You will also be required to repeatedly pinch a soft white ball in time with a metronome. The TMS equipment used comprises a figure-of-8 shaped coil held against the side of the head. When stimulated it causes the nerves in the scalp and the brain to become briefly activated. The sensation caused by this stimulation is not unpleasant and will cause you no pain. Stimulation to the area of the brain we are interested in will cause a muscular twitch to occur in one hand. The muscle twitch will be recorded using electromyography (EMG) surface electrodes. These will record very small electrical signals emitted during muscle activity. The surface electrodes used to record these signals will require self-adhesive pads to be attached to
the skin over the muscle. The recording of EMG signals and stimulation with TMS is a completely safe and painless procedure.

You will only be asked to attend one testing session which will last approximately 1½ hours. This will provide enough time to fully explain the procedures, prepare you for EMG recording and TMS stimulation, and conduct the experiment. In recognition of the time you are being asked to give up to take part in the study, you will be reimbursed to the sum of £10 in cash which will be given to you at the end of the experiment.

7) Are there any disadvantages or risks in taking part?

TMS is a non-invasive technique for delivering electrical stimulation to humans through the scalp. Whilst research has concluded that TMS is a safe research method that carries no significant risk of long-term side-effects, there is a minimal risk of mild headaches and slight discomfort at the site of stimulation. The safety screening questionnaire (TASS) should exclude participants who are susceptible to these side-effects, however, in the unlikely event that either of these occurs, please alert the investigator and the experiment will be stopped immediately.

8) What are the possible benefits of taking part?

In addition to being paid £10 in cash, your involvement may help further our understanding of the human motor system.

9) Who are the members of the research team?

The principal investigator conducting the study is Miss Michela Loporto. Dr Paul Holmes (Director of Studies) and Dr. Craig McAllister are the additional members of the research team. If you require further information on the study before taking part please feel free to contact the principle investigator, Miss Michela Loporto via email: m.loporto@mmu.ac.uk.

10) Who is funding the research?

This is a self-funded project with programme fees provided by the Malta Government Scholarship Scheme.

11) Who will have access to the data?

All data collected during the course of the research will remain confidential and will only be used for the purposes of the study. The data will be stored in coded form and the principal investigator and supervisory team will have access to the data. The data will be kept stored for five years before being destroyed. The data is being collected as part of the principle investigator’s PhD project; therefore the results of the study will be reported in the final thesis. Any information linking your identity to the study will not be included in this. It is also likely that the findings will be communicated in scientific journals or conferences in the future, however, in this event, your name or identity will not be disclosed. Should you wish to obtain a copy of
the summary of the study’s findings please feel free to contact the investigator via email: m.loporto@mmu.ac.uk

12) Who do I contact if I feel my rights have been violated?

If at any point during the study you feel that your rights as a participant have been violated and you wish to make a complaint regarding your involvement in the study please contact: The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400,

Thank you for considering participation in this study.
Appendix F – Participant information sheet for Study 3

MANCHESTER METROPOLITAN UNIVERSITY
MMU Cheshire
Department of Exercise and Sport Science
Information Sheet for Participants

Title of Study:
Motor facilitation during action observation: using different ‘hotspots’ to investigate muscle specificity

Participant Information Sheet

1) This is an invitation to take part in a piece of research.
You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?
The purpose of the study is to investigate whether observation of a hand action increases the activity in the area of the brain that controls the specific muscle involved in the action.
3) Why is the study being performed?

Previous research has reported that there is an increase in brain activity when observing another person perform an action. This increase has been reported in areas of the brain responsible for performing the observed movements. Researchers have speculated that this increase in activity is specific to the muscles involved in performing the observed action. However, there have been methodological limitations to these studies, whereby several muscles have been tested at the same time. For example, it is common for researchers to stimulate only one position on the scalp yet record responses from several muscles. However, it is known that each muscle has its own optimal position for stimulating on the scalp. Therefore, in this study, we hope to address these limitations by testing each muscle separately on different testing sessions.

4) Why am I being asked to take part?

You and approximately fourteen other people will be invited to take part in this study. The study requires normal, healthy individuals to take part. Additionally you must be right handed and have normal vision, or corrected-to-normal vision.

5) Do I have to take part?

You are under no obligation to take part in this study. If after reading this information sheet and asking any additional questions you do not feel comfortable taking part in the experiment you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect the standard of care you receive in any way, nor will it affect your relationship with any of the staff at the Manchester Metropolitan University. If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be provided with a copy of this together with this information sheet for your records. In addition to this you will be asked to fill in a copy of the Transcranial magnetic stimulation Adult Safety Screen (TASS) which will confirm your eligibility to participate.

6) What will happen to me if I agree to take part?

If you agree to take part in the study you will be asked to come to the Psycho-physiology laboratory at Manchester Metropolitan University for a test session. Whilst there you will be asked to sit at a desk and watch a series of different video clips during which TMS will be applied to measure brain activity.

The TMS equipment used comprises a figure-of-8 shaped coil held against the side of the head. When stimulated it causes the nerves in the scalp and the brain to become briefly activated. The sensation caused by this stimulation is not unpleasant and will cause you no pain. Stimulation to the area of the brain we are interested in will cause a muscular twitch to occur in
one hand. The muscle twitch will be recorded using electromyography (EMG) surface electrodes. These will record very small electrical signals emitted during muscle activity. The surface electrodes used to record these signals will require self-adhesive pads to be attached to the skin over the muscle. The recording of EMG signals and stimulation with TMS is a completely safe and painless procedure.

You will be asked to attend two testing sessions which will last approximately one and a half hours each. This will provide enough time to fully explain the procedures, prepare you for EMG recording and TMS stimulation, and conduct the experiment.

7) Are there any disadvantages or risks in taking part?

TMS is a non-invasive technique for delivering electrical stimulation to humans through the scalp. Whilst research has concluded that TMS is a safe research method that carries no significant risk of long-term side-effects, there is a minimal risk of mild headaches and slight discomfort at the site of stimulation. The safety screening questionnaire (TASS) should exclude participants who are susceptible to these side-effects, however, in the unlikely event that either of these occurs, please alert the investigator and the experiment will be stopped immediately.

8) What are the possible benefits of taking part?

Your involvement may help further our understanding of the human motor system during action observation.

9) Who are the members of the research team?

The principal investigator conducting the study is Miss Michela Loporto. Dr Paul Holmes (Director of Studies) and Dr. Craig McAllister (Supervisor), are the additional members of the research team. If you require further information on the study before taking part please feel free to contact the principle investigator, Miss Michela Loporto via email: m.loporto@mmu.ac.uk.

10) Who is funding the research?

This is a self-funded project with programme fees provided by the Malta Government Scholarship Scheme.

11) Who will have access to the data?

All data collected during the course of the research will remain confidential and will only be used for the purposes of the study. The data will be stored in coded form and the principal investigator and supervisory team will have access to the data. The data will be kept stored for five years before being destroyed. The data is being collected as part of the principle investigator’s PhD project; therefore the results of the study will be reported in the final thesis. Any information linking your identity to the study will not be included in this. It is also likely that the findings will be communicated in scientific journals or conferences in the future, however,
in this event, your name or identity will not be disclosed. Should you wish to obtain a copy of the summary of the study's findings please feel free to contact the investigator via email: m.loporto@mmu.ac.uk.

12) Who do I contact if I feel my rights have been violated?

If at any point during the study you feel that your rights as a participant have been violated and you wish to make a complaint regarding your involvement in the study please contact:

The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400,

Thank you for considering participation in this study.
Appendix G – Participant information sheet for Study 4

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire
Department of Exercise and Sport Science

Participant Information Sheet

Title of Study:
Motor facilitation during action observation: using different stimulation intensities

1) This is an invitation to take part in a piece of research.
You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether or not you wish to take part.

2) What is the purpose of the research?
The purpose of the study is to investigate whether observation of a repetitive finger movement increases the activity in the area of the brain that controls hand movements and whether this is influenced by the TMS intensity.
3) Why is the study being performed?

It has recently been discovered that a sub-set of brain cells termed ‘mirror neurons’ are involved in processing information during both the execution of self-performed actions and the observation of other people’s actions. It has been proposed that these mirror neurons are important for understanding the actions that other people make. The current study is using a non-invasive method of brain stimulation to test whether brain activity is influenced when observing others performing familiar pinching actions.

4) Why am I being asked to take part?

You and approximately fourteen other people will be invited to take part in this study. The study requires normally, healthy individuals to take part. Additionally you must be right handed and have normal vision, or corrected-to-normal vision.

5) Do I have to take part?

You are under no obligation to take part in this study. If after reading this information sheet and asking any additional questions you do not feel comfortable taking part in the experiment you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect the standard of care you receive in any way, nor will it affect your relationship with any of the staff at the Manchester Metropolitan University.

If you do decide to take part you will be asked to sign an informed consent form stating your agreement to take part and you will be provided with a copy of this together with this information sheet for your records. In addition to this you will be asked to fill in a copy of the Transcranial magnetic stimulation Adult Safety Screen (TASS) which will confirm your eligibility to participate.

6) What will happen to me if I agree to take part?

If you agree to take part in the study you will be asked to come to the Psycho-physiology laboratory in the Department of Exercise and Sport Science at the Manchester Metropolitan University for a test session. Whilst there you will be asked to sit at a desk and watch a series of different video clips, during which, on some occasions TMS will be applied to measure brain activity.

The TMS equipment used comprises a figure-of-8 shaped coil held against the side of the head. When stimulated it causes the nerves in the scalp and the brain to become briefly activated. The sensation caused by this stimulation is not unpleasant and will cause you no pain. Stimulation to the area of the brain we are interested in will cause a muscular twitch to occur in one hand. The muscle twitch will be recorded using electromyography (EMG) surface
electrodes. These will record very small electrical signals emitted during muscle activity. The surface electrodes used to record these signals will require self-adhesive pads to be attached to the skin over the muscle. The recording of EMG signals and stimulation with TMS is a completely safe and painless procedure.

You will only be asked to attend one testing session which will last approximately 1½ hours. This will provide enough time to fully explain the procedures, prepare you for EMG recording and TMS stimulation, and conduct the experiment.

7) Are there any disadvantages or risks in taking part?

TMS is a non-invasive technique for delivering electrical stimulation to humans through the scalp. Whilst research has concluded that TMS is a safe research method that carries no significant risk of long-term side-effects, there is a minimal risk of mild headaches and slight discomfort at the site of stimulation. The safety screening questionnaire (TASS) should exclude participants who are susceptible to these side-effects, however, in the unlikely event that either of these occurs, please alert the investigator and the experiment will be stopped immediately.

8) What are the possible benefits of taking part?

Your involvement may help further our understanding of the human motor system.

9) Who are the members of the research team?

The principal investigator conducting the study is Miss Michela Loporto. Dr Paul Holmes (Director of Studies), Dr. Craig McAllister (Supervisor), and Dr. David Wright (assisting with data collection) are the additional members of the research team. If you require further information on the study before taking part please feel free to contact the principle investigator, Miss Michela Loporto via email: m.loporto@mmu.ac.uk.

10) Who is funding the research?

This is a self-funded project with programme fees provided by the Malta Government Scholarship Scheme.

11) Who will have access to the data?

All data collected during the course of the research will remain confidential and will only be used for the purposes of the study. The data will be stored in coded form and the principal investigator and supervisory team will have access to the data. The data will be kept stored for five years before being destroyed. The data is being collected as part of the principle investigator’s PhD project; therefore the results of the study will be reported in the final thesis. Any information linking your identity to the study will not be included in this. It is also likely that the findings will be communicated in scientific journals or conferences in the future, however, in this event, your name or identity will not be disclosed. Should you wish to obtain a copy of
the summary of the study’s findings please feel free to contact the investigator via email: m.loporto@mmu.ac.uk

12) Who do I contact if I feel my rights have been violated?

If at any point during the study you feel that your rights as a participant have been violated and you wish to make a complaint regarding your involvement in the study please contact:

The University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400.

Thank you for considering participation in this study.
Appendix H – Informed consent form for Studies 1-4

Department of Exercise and Sport Science
MSc Sport and Exercise Science
Informed Consent Form

Name of Participant:

Supervisor/Principal Investigator: Dr. Paul Holmes/ Michela Loporto

Project Title: TMS and Action Observation: Methodological Concerns

Ethics Committee Approval Number: 14.10.09(i)

Participant Statement

I have read the participant information sheet for this study and understand what is involved in taking part. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without giving a reason. Any concerns I have raised regarding this study have been answered and I understand that any further concerns that arise during the time of the study will be addressed by the investigator. I therefore agree to participate in the study.

It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the University Secretary and Clerk to the Board of Governors, Manchester Metropolitan University, Ormond Building, Manchester, M15 6BX. Tel: 0161 247 3400 who will undertake to investigate my complaint.

Signed (Participant)  Date

Signed (Investigator)  Date
Appendix I: Wire diagram of laboratory set-up

The wire diagram illustrates the laboratory set-up, including the equipment used and the EMG electrodes placement. Computers 1 and 2, and the Magstim, are connected to the Micro 1401 analogue-digital converter. The EMG skin electrodes are attached to the finger muscles of the participant and muscle activity is recorded using the EMG Delsys system. The EMG signal is amplified and fed into the Micro 1401. The data is analysed on Computer 1 using Spike and Signal software programs. The participant views a series of video clips presented on the television LED screen and is stimulated by the TMS at selected time points.
Appendix J – Discarded trials for Studies 1-4

A pre-stimulus recording of 200ms was always used to check for the presence of EMG activity in the muscles before the TMS pulse was delivered. Individual trials in which the peak-to-peak amplitude of the baseline EMG activity was 2.5 SD higher than the mean baseline EMG activity of each participant were discarded from further analysis as the presence of EMG activity immediately prior to the stimulation may have influenced the amplitude of the subsequent MEP. The table below shows the percentage of deleted trials for Studies 1-4.

<table>
<thead>
<tr>
<th>Study</th>
<th>Discarded Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3%</td>
</tr>
</tbody>
</table>
| 2     | Right hand: Experiment 1 – 3.3%, Experiment 2 – 1.6%  
       | Left hand: Experiment 1 – 1.1%, Experiment 2 – 1.0% |
| 3     | FDI OSP – 3.3%  
       | ADM OSP – 3.9%  |
| 4     | 110% RMT – 4.7%  
       | 130% RMT – 3.4% |