The effect of manipulating action observation variables on corticospinal excitability using transcranial magnetic stimulation

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Abstract

Action observation interventions have been shown to contribute to improvements in motor performance and (re)learning. This thesis examined the effect of manipulating action observation variables on corticospinal excitability (CSE) using transcranial magnetic stimulation (TMS), with the aim of informing interventions for motor (re)learning. Eye-tracking and interview techniques were employed in combination with TMS to provide novel explorations for how screen position, visual context, and emotional valence influence CSE, visual attention, and individual experience during action observation. The Pilot Experiment (Chapter 5) tested the appropriateness of both single- and paired-pulse TMS techniques during action observation. Results determined that single-pulse TMS was appropriate for the subsequent experiments included in this thesis. Experiment 1 (Chapter 6) investigated the effect of screen position during action observation on CSE. The results demonstrated greater CSE during action observation on a horizontal, compared to a vertical, screen position, but only once each individual's viewing preference had been taken into account. Experiment 2 (Chapter 7) investigated the effect of congruent and incongruent contexts on CSE. The results indicated that congruent context during action observation facilitates CSE more than control conditions in contrast to an incongruent visual context. Experiment 3 (Chapter 8) explored the effect of each participant's most preferred, least preferred, and neutral preference food items involved in an observed reach and grasp action on CSE. The results showed no significant differences between the control condition and observing a reach and grasp of each participant's personalised least preferred and neutral preference food items. Significant inhibition of CSE was shown during observation of a reach and grasp of each participant's most preferred food item. The three main experiments in this thesis provide novel contributions to action observation literature by incorporating eye-tracking and interview techniques in combination with TMS to better determine the nature of CSE modulation. Taken together, these findings directly inform both future research and practice in motor (re)learning by highlighting the importance of meaning and context during action observation.

Research output

Thesis publications

Riach, M., Holmes, P. S., Franklin, Z. C. and Wright, D. J. (2018) 'Observation of an action with a congruent contextual background facilitates corticospinal excitability: A combined TMS and eye-tracking experiment.' *Neuropsychologia*, 119 pp. 157-164. doi: 10.1016/j.neuropsychologia.2018.08.002

Based on research carried out within Experiment 2 of this thesis

Riach, M., Wright, D. J., Franklin, Z. and Holmes, P. S. (2018) 'Screen position preference offers a new direction for action observation research: preliminary findings using TMS.' *Frontiers in Human Neuroscience*, 12(26). doi: 10.3389/fnhum.2018.00026

Based on research carried out within Experiment 1 of this thesis

Additional publications

Wright, D. J., Wood, G., Franklin, Z. C., Marshall, B., Riach, M. and Holmes, P. S. (2018) 'Directing visual attention during action observation modulates corticospinal excitability.' *PLoS One*, 13(1). doi: 10.1371/journal.pone.0190165

Not based on research carried out within this thesis

Eaves, D., Riach, M., Holmes, P. and Wright, D. (2016) 'Motor imagery during action observation: a brief review of evidence, theory and future research opportunities.' *Frontiers in Neuroscience*, 10(514). doi: 10.3389/fnins.2016.00514

Not based on research carried out within this thesis

Thesis oral conference presentations

Riach, M., Holmes, P. S., Franklin, Z. C. and Wright, D. J. (2018) '*Action observation with a congruent contextual background facilitates corticospinal excitability: A TMS and eye-tracking experiment.*' Research in Imagery and Observation. Bielefeld: Germany, April 12-13.

Based on research carried out within Experiment 2 of this thesis

Riach, M., Holmes, P. S., Franklin, Z. C., and Wright, D. J. (2017) '*Screen position and viewing preference effect corticospinal activity during action observation.*' Research in Imagery and Observation. Roehampton: England, May 18-19.

Based on research carried out within Experiment 1 of this thesis

Riach, M., Holmes, P. S. and Wright, D. J. (2016) '*Transcranial magnetic stimulation in action observation: A comparison of single versus paired-pulse techniques*.' Research in Imagery and Observation. Manchester: England, April 6-7.

Based on research carried out within the Pilot Experiment of this thesis

Thesis poster conference presentations

Riach, M., Holmes, P. S., Franklin, Z. C., and Wright, D. J. (2017) '*Screen angle and viewing preference effect corticospinal excitability during action observation*.' International Brain Stimulation Conference. Barcelona: Spain, March 6-8.

Based on research carried out within Experiment 1 of this thesis

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List of acronyms and abbreviations

ADM – Abductor digiti minimi

ANOVA – Analysis of variance

AOI – Area of interest

BOLD – Blood oxygen level dependent

CS – Conditioning stimulation

CSE – Corticospinal excitability

Cz – The central midline point of the International 10-20 system

EHI – Edinburgh handedness inventory

EMG - Electromyography

FDI – First dorsal interosseous

fMRI – Functional magnetic resonance imaging

ICF – Intracortical facilitation

ICI – Intracortical inhibition

ISI – Interstimulus interval

LQ – Laterality quotient

MEP – Motor evoked potential

ms – Millisecond

MSO – Maximal stimulator output

OSP – Optimal scalp position

ppTMS – Paired-pulse transcranial magnetic stimulation

RMT – Resting motor threshold

rTMS – Repetitive transcranial magnetic stimulation

spTMS – Single-pulse transcranial magnetic stimulation

TMS – Transcranial magnetic stimulation

TS – Test stimulation

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Chapter 1. Introduction

Neural activity has shown to be present in overlapping regions of the brain during both simulated (i.e., observed and imagined) and executed actions (Hardwick et al., 2018). This is thought to be the reason behind the efficacy of action observation and motor imagery interventions for motor (re)learning (Buccino, 2014). As such, considerable research has focussed on exploring the effects of action observation and motor imagery on cortical activity. This has been tested through a variety of techniques, with transcranial magnetic stimulation (TMS) arguably being the most common. When used to stimulate activity in the motor regions of the brain, TMS generates a muscle contraction corresponding to the stimulated area. The amplitude of this muscle contraction provides a measure of corticospinal excitability (CSE) (Bestmann and Krakauer, 2015; Ruffino et al., 2017; see Chapter 2.3). TMS research exploring action observation demonstrates CSE facilitation during action observation compared to static controls consistently (e.g., Fadiga et al., 1995; see Loporto et al., 2011; Naish et al., 2014 for reviews). Similar facilitation of CSE has also been demonstrated during motor imagery (e.g., Fadiga et al., 1999; see Grosprêtre et al., 2016 for a review). As such, given the assumption that a more active extended motor system is beneficial for motor (re)learning (Buccino, 2014; Eaves et al., 2016), both techniques have been considered as useful adjuncts to physical therapy in rehabilitation and sporting settings (e.g., Collet and Guillot, 2012; Buccino, 2014).

1.1. The (human) mirror neuron system and simulation theory

One of the postulated mechanisms thought to provide a partial shared neural substrate between simulated and executed actions is the (human) mirror neuron system. Mirror neurons are a type of neural cell in the brain that discharge during both the observation and execution of an action. Di Pellegrino et al. (1992) provided the initial evidence for the existence of mirror neurons in Macaque monkeys. They found similar activation during both the observation and execution of object manipulations in individual neurons in the F5 area of the premotor cortex. Supporting research has indicated the existence of a broader mirror neuron system in the monkey brain, with areas such as the inferior parietal lobule (Fogassi et al., 2005) and superior temporal sulcus (Allison et al., 2000) shown to be involved.

Evidence of a human mirror neuron system has been inferred from experiments demonstrating activity in similar regions of the brain during both observation and execution of an action (Hardwick et al., 2018). The majority of evidence for a human mirror neuron system is indirect, with few experiments able to demonstrate direct evidence from single-cell recordings (see Mukamel et al., 2010 for one such experiment). There are alternative explanations for the research findings in this area. These include the possibility that the reported activity could result from associative learning through previous experiences of observing and executing actions, rather than the reflection of an innate ability to “mirror” another’s actions (Press et al., 2011). Also, Hickok (2009) argues that the widely accepted role that mirror neurons have in action understanding is lacking in evidence in both the monkey and human brain. Given the limited direct evidence for the existence of

mirror neurons in humans, and alternative explanations for many of the findings, both the existence of mirror neurons and the exact role of the mirror neuron system in the human brain are contested.

Jeannerod (2001) proposed an alternative mechanism, whereby an observer understands the actions of another by relating to the experience of the agent. This involves a simulated (either observed or imagined) action providing information regarding the kinematic profile (e.g., Hardwick et al., 2018), feasibility (e.g., Borroni et al., 2011), and meaning (e.g., Enticott et al., 2010) of observed actions. The resulting activity in the postulated action observation-execution matching mechanism does not require action execution. This observation-execution matching mechanism enables the preparation of the motor regions to execute the observed action in a similar way. Specific cortical regions involved in such a mechanism have been demonstrated using functional magnetic resonance imaging (fMRI; Hardwick et al., 2018; Chapter 2.2). In addition, the involvement of the motor regions is commonly demonstrated using TMS, through increases of CSE in the muscles involved in executing an observed action (Naish et al., 2014; Chapter 2.4). The evidence supporting the Simulation Theory can also be considered as complementary to the human mirror neuron system. Considering the uncertainty with the human mirror neuron system, however, and the necessity for further research to understand its mechanisms, only Jeannerod's (2001) Simulation Theory and an action observation-execution matching system have been used to inform the experiments in this thesis.

1.2. Action observation and motor imagery

Traditionally, action observation and motor imagery have been considered as separate simulation techniques. An increased focus on combining the two techniques, however, is reflected in recent research (Vogt et al., 2013; Eaves et al., 2016). This originates from neuroimaging research which indicates that action observation and motor imagery share partial neural substrate both with one another and with motor execution (Grèzes and Decety, 2001; Hétu et al., 2013). In addition, TMS research has shown that action observation and motor imagery both facilitate CSE independently in comparison to control conditions (Naish et al., 2014). Combining the two motor simulation techniques has been shown to facilitate CSE to a greater extent than both action observation (Ohno et al., 2011; Wright et al., 2016) and motor imagery (Tsukazaki et al., 2012; Mouthon et al., 2015) individually.

Despite the recent increased interest in combined action observation and motor imagery, several issues exist with the use of motor imagery, and simply combining action observation and motor imagery does not circumvent them all. A major factor is that not everyone can produce clear and vivid motor imagery (Isaac and Marks, 1994). The ability of an individual to image has been linked to CSE modulation, as individuals with lower imagery ability demonstrate lower CSE during motor imagery compared to those with higher imagery ability (Guillot et al., 2008; Williams et al., 2012), potentially affecting the efficacy of imagery interventions for motor (re)learning. The ability to image may even be worse in patient populations utilising motor (re)learning interventions. For example, some stroke patients suffer lesions to the cortical regions necessary for the formation of motor imagery. This can result

in disruption to their image generation and management abilities (Gaggioli et al., 2004; Holmes, 2007). In addition, the imagery perspective, timing, and modality cannot be wholly controlled, as participants may switch between different imagery perspectives (Ewan et al., 2010), alter the timing of the imaged action (Decety et al., 1989), and utilise a variety of modalities (Holmes, 2007). Some of these problems with motor imagery are reduced when combined with action observation, but aspects of the participant's image still cannot be controlled.

Action observation offers several benefits that overcome the inherent issues associated with motor imagery, such as being able to control the visual image the participant produces (Holmes and Calmels, 2008). Unlike motor imagery, videos of action execution can be manipulated to provide the desired perspective (see Chapter 6), context (see Chapter 7), and preferences (see Chapter 8), enabling tight methodological design for exploring CSE. Furthermore, action observation has been shown to facilitate CSE (Naish et al., 2014; Chapter 2.4) and benefit motor (re)learning (Buccino et al., 2018; see Chapter 2.1) independently of motor imagery. It also enables the use of eye-tracking techniques to measure visual attention to explore the participants' experiences and better understand the nature of CSE modulation (see Chapter 3.4). As such, this thesis focusses on the use of action observation alone.

1.3. Thesis outline

The series of experiments included in this thesis expanded on existing literature exploring CSE during action observation. TMS was utilised to explore the additional meaning observers attribute to an observed action and the effect this has on CSE.

Additional measures such as eye-tracking and one-to-one interviews were employed to expand the understanding of any modulation of CSE during action observation.

Initially, in a pilot experiment (Chapter 5), the use of paired-pulse TMS was explored in comparison to single-pulse TMS during action observation. This experiment was used to determine the TMS technique used in the following experiments included in this thesis. Based on the results of the pilot experiment, it was determined that single-pulse TMS would be used to maintain the focus of informing action observation interventions for motor (re)learning.

Experiment 1 (Chapter 6) explored screen position preference using TMS combined with a post-experiment questionnaire and interview. Participants observed index finger-thumb pinches of a sponge ball presented on a horizontal and a vertical screen position. The results suggest that ensuring anatomical and perceptual congruency with the physical task, as on the horizontal screen position, alongside consideration of participants' screen position viewing preferences, may be an important part of optimising action observation interventions. Particularly, this experiment demonstrates the importance of the individual meaning that each observer attributes to the observation task (see Chapter 2.4.4.2), and how such individual experiences may need to be accounted for when exploring CSE. Once this was determined, it became apparent that the entire observer experience should be explored. For example, the visual information, and subsequent meaning, provided by the action observation stimuli could also be an important consideration.

Experiment 2 explored the meaning of an observed action through background objects providing visual context (Chapter 7). In addition to TMS, eye movements were recorded to provide a measure of visual attention (see Chapter 3.4). Participants observed a static hand, and an index finger-thumb pinch of a sponge presented against a blank background, a background context that was incongruent to the observed action, and a background context that was congruent to the observed action. CSE was shown to be facilitated in comparison to a control condition when the visual context was congruent to the observed action. When the action was presented against a blank background, or the background context was incongruent to the observed action, then no facilitation of CSE was found. Furthermore, participants' visual attention was drawn to the background scene when observing actions with either a congruent or incongruent context. This, alongside the modulation of CSE, indicated that only the congruent background context provided information pertinent to understanding the goal and intention of the observed action. Taken together, it is suggested that contextual cues may be useful inclusions for observation interventions for motor (re)learning. Contextual cues relating to a common experience were provided in Experiment 2, though the meaning of some contextual cues may differ between each observer. As such, personalised contextual cues should be explored to provide a detailed insight into the effects of meaning on CSE.

Experiment 3 (Chapter 8) further demonstrated the effect that meaning relating to food preference, and the individual experiences associated with this, may have on CSE. This experiment provided an in-depth exploration of the observers' experiences using eye-tracking alongside TMS, and a post-experiment interview.

This was particularly appropriate as each participant observed a wholly individualised set of videos based on food items that they indicated they most preferred, least preferred, and for which they had a neutral preference. Results of this experiment were inconclusive. Specifically, observing a reach and grasp of pleasant food items inhibited CSE in relation to the control condition. Also, observing the same action with unpleasant food items only facilitated CSE in comparison to the pleasant food item. Eye-tracking results indicated that the unpleasant food item might have been perceived as more of a threat compared to the other food items. This was supported by the interview data, revealing stronger affect associated with the unpleasant food item. Including food items, or other items of strong emotional valence, may not be suitable for action observation interventions, though further TMS research is required to determine the nature of the effects shown.

The series of experiments included in this thesis provide novel action observation findings using TMS, eye-tracking, and interviews. Importantly, novel approaches to explore the experiences of each observer are utilised; the result of which indicate their importance in determining the nature of CSE modulation during action observation. This thesis provides both considerations for designing and delivering action observation interventions for motor (re)learning based on TMS, eye-tracking, and interview data, and arguments for the continuing use of these additional measures to complement the use of TMS in action observation research.

Chapter 2. Literature Review

2.1. Action observation and motor learning

The actions of others are key to learning, social organisation and survival (Rizzolatti and Craighero, 2004). Observation of another's actions is used to acquire new skills and is considered an important human developmental ability (Ewan et al., 2010; Loporto et al., 2011). Humans have been shown to learn through observation of another performing an action, without having to actively perform that action (Cross et al., 2009). This can also improve the performance of already learned actions (e.g., Edwards et al., 2003; Hardwick and Edwards, 2011).

In a key experiment demonstrating the effect of action observation on motor performance, Brass et al. (2000) measured response times as participants were instructed to imitate an observed action as fast as possible. Reaction times were faster when the observed action was identical to the movement that was to be subsequently executed by the participant compared to when the observed action was different. This result demonstrates that observing an action can prime the execution of the action and facilitate performance, but only if the observed and executed actions are identical. In addition, Edwards et al. (2003) instructed participants to observe the reach and grasp of an object immediately prior to executing the same reach and grasp of an object that was either the same or different in size. The findings of this experiment supported Brass et al.'s (2000) findings by demonstrating faster reach execution when the objects involved in both observation and execution were of the same size compared to when the objects were of different sizes. The priming that action observation provides, therefore, can

be considered to only be effective when the same action is subsequently executed by the observer (Edwards et al., 2003; Bähr et al., 2018).

Existing movement patterns may also be modified as a result of observing the same action with different kinematics. Hardwick and Edwards (2011) demonstrated that the kinematic profile used in an observed action could be reflected in the observer's own movement. For example, observing a reaching action with a high wrist height increased the wrist height of the observer during the execution of a reach. The ability to learn actions through observation, including the effect it has on movement kinematics, is one of the bases for using action observation as an intervention for motor (re)learning in clinical populations such as stroke, Parkinson's disease, and cerebral palsy patients (Buccino, 2014).

Impaired function of the upper limb is common in stroke patients. This can affect their ability to perform activities of daily living and their participation in activities they undertook prior to the stroke (Ewan et al., 2010; Mottura et al., 2015). Relearning the motor skills necessary to perform these activities with the affected limb is, therefore, of high importance. Traditionally, such relearning interventions focus on repetitive action execution with the affected limb (Ertelt et al., 2007). Although the efficacy of repetitive action execution is demonstrated consistently (e.g., Dombovy, 2004; Vecchio et al., 2017), with the demonstrated efficacy of observation on motor skill learning in healthy populations, action observation interventions are considered to be a useful adjunct to benefit traditional physical therapies to facilitate motor relearning (Buccino, 2014).

Ertelt et al. (2007) conducted one of the first systematic studies to investigate the efficacy of action observation for motor (re)learning. They tested an action observation intervention within a chronic stroke population. Over 18 sessions, participants observed videos of a healthy limb performing activities of daily living before performing the same actions with their affected limb. Participants in the control group received the same amount of care as the other participants but performed the action execution repetitions without prior observation of the actions. Ertelt et al.'s (2007) findings demonstrated significant motor function improvements in the affected limb during the Frenchay Arm Test (De Souza et al., 1980) and the Wolf Motor Function Test (Wolf et al., 1989) for the patients that completed the combined physical therapy and action observation intervention. This effect was not only present immediately after the intervention was completed, but was sustained for at least eight weeks post-intervention. It can be suggested, therefore, that the motor function improvements were not temporary, but may be a result of long-term neuroplastic changes (Ertelt et al., 2007). Franceschini et al. (2012), who utilised an observation intervention immediately prior to physical therapy of the same movements, demonstrated similar findings. Improvements of motor function were seen after the four-week intervention and were sustained for at least four to five months. Sugg et al. (2015), Fu et al. (2017), and Adhikari et al. (2018) have reported similar findings, indicating that combined action observation and physical therapy interventions may benefit relearning in stroke populations.

The utilisation of action observation interventions has also been explored in Parkinson's disease and cerebral palsy populations. For Parkinson's disease patients, action observation interventions combined with physical therapy have

been shown to increase autonomy in activities of daily living (Buccino et al., 2011) by improving motor function and reduce freezing of gait (Pelosin et al., 2010). Child cerebral palsy patients were also shown to improve motor function that was sustained, and in some cases improved, two months post-intervention (Buccino et al., 2018). It is thought that the action observation intervention provided the participants with visually-guided movement strategies that they could continually apply to movement execution, resulting in sustained motor function improvements (Buccino et al., 2018).

Although these results show that action observation interventions can benefit motor (re)learning when used as an adjunct to physical therapy, the optimal method for delivering such interventions is yet to be established. Action observation interventions have the flexibility to be presented in different modalities, agencies, and perspectives (Holmes, 2011). These are important factors that should be considered whilst designing the intervention. The underlying physiological mechanisms must first be understood before attempting to optimise such interventions. Observing a healthy limb perform actions may not just be providing accurate visual feedback (Hartman and Altschuler, 2016), but may influence neural mechanisms such as motor preparation (Pomeroy et al., 2005). It is understandable, therefore, that research has explored neural mechanisms in the motor regions of the brain during action observation in an attempt to inform and optimise subsequent interventions.

2.2. Neural mechanisms during action observation

The same areas of the brain necessary to execute an action are recruited whilst observing the same action performed by another (Buccino et al., 2018). This action observation-execution matching mechanism forms the basis of action observation research and subsequent interventions. Early research utilising electroencephalography demonstrated desynchronization of the mu rhythm in the motor cortex (Gastaut and Bert, 1954). This reflects increased activity during both the execution and observation of actions, a finding which has been replicated (Cochin et al., 1999; Altschuler et al., 2000).

There is some contention, however, as to where the activity in the motor regions originates. Evidence indicating the involvement of the motor regions of the brain during action observation was demonstrated in early electroencephalography work (e.g., Gastaut and Bert, 1954; Cochin et al., 1999), and is a consistent finding using TMS (e.g., Fadiga et al., 1995; Gangitano et al., 2004; see Naish et al., 2014; Chapter 2.4). It was thought initially that the primary motor cortex was involved (Jeannerod, 2001), though positron emission topography research contended this (Grèzes and Decety, 2001). Later fMRI research findings indicated that the primary motor cortex is only involved during action execution (Hardwick et al., 2018), and as such is not a part of the observation-execution matching mechanism. The findings of TMS research, therefore, appear to be the result of premotor cortex involvement, increasing activity via cortico-cortical or cortico-subcortical pathways (Fadiga et al., 2005). It is possible to explore individual corticocortical pathways using paired-pulse TMS, whilst single-pulse explores an overall level of activity across the motor

regions. This is discussed further, with an exploration of the appropriateness of each technique, in Chapter 5.

Specific regions have been shown to be involved in the observation-execution matching mechanism using fMRI. This technique provides a blood oxygen level dependent (BOLD) image; an indication of which cortical regions are either more or less active based on changes in blood flow (Chaminade et al., 2005). An increased BOLD signal, and therefore increased activity, in premotor regions and somatosensory networks is elicited during both action observation and execution (Hardwick et al., 2018). This includes the bilateral ventral premotor cortex and the supplementary motor area (Hardwick et al., 2018). In addition, regions such as the inferior parietal lobule, pars opercularis, posterior parietal lobe, and premotor cortex demonstrate increased activity during observation of upper-limb movements compared to control conditions (Iacoboni et al., 1999; Buccino et al., 2004; Iacoboni et al., 2005; Ferri et al., 2015; Biagi et al., 2016).

Each region appears to have a different involvement in the observation-execution matching mechanism. The premotor cortex, for example, is involved in action preparation (Hoshi and Tanji, 2007) and the coordination of fine motor movements (Hardwick et al., 2018). In addition, the pre-supplementary motor area is associated with both motor tasks and non-motor cognitive processes (Leek and Johnston, 2009), providing links between stimuli and actions (Nachev et al., 2008). The observation-execution matching mechanism also possesses the ability to process rich multisensory information available during action observation in regions such as the inferior parietal lobule (Block et al., 2013). The inferior parietal lobule is

involved in the execution of complex, rather than simple, actions (Weiss et al., 2008), but maintains an important role in action understanding during observation. It enables the processing of tactile information and a greater understanding of observed tool use (Orban, 2016; see Hardwick et al., 2018).

As each region is involved in specific information processing, the exact cortical regions recruited during action observation appear to vary based on the observation task. A greater BOLD signal was demonstrated in the ventral premotor cortex, for example, when the observed actions contained objects providing context, compared to when the action was presented without context (Iacoboni et al., 2005). The ventral premotor cortex is more typically associated with action execution than action observation, though there are overlaps in the BOLD signal indicating some involvement during action observation (Hardwick et al., 2018). Activity in the motor regions of the brain is inferred consistently using TMS, with greater CSE demonstrated during action observation compared to control conditions (see Naish et al., 2014; Chapter 2.4 for reviews).

2.3. TMS

One of the most common techniques utilised to explore activity in the motor regions of the brain underlying action observation is TMS. Its conception by Barker et al. (1985) was seen as a significant improvement from transcranial electrical stimulation, which resulted in severe participant discomfort (Horvath et al., 2010). As a painless and non-invasive technique, TMS quickly became a common research, diagnostic, and therapeutic tool (Horvath et al., 2010; Noohi and Amirsalari, 2016).

TMS works on the principle of electromagnetic induction proposed by Faraday in 1831 (Rossini et al., 2015). Faraday discovered that an electrical current generates a corresponding magnetic field that can induce currents in a secondary circuit. Barker et al. (1985) demonstrated that this principle could be used transcranially to induce secondary electrical currents in the brain. When the induced secondary current stimulates the motor regions of the brain, an involuntary muscle contraction occurs (Rossini et al., 2015). This muscular contraction is referred to as a motor evoked potential (MEP) and is measured using surface electromyography (EMG) electrodes. The amplitude of the muscular contraction provides a marker of CSE (Bestmann and Krakauer, 2015; Ruffino et al., 2017).

When applied to the motor regions of the brain, TMS evokes multiple descending volleys, either D- (direct) or I- (indirect) waves, in the pyramidal tract (Day et al., 1989). If the number or strength of the descending volleys is sufficient following the stimulation, a muscular contraction specific to the stimulated region of the motor cortex will occur (Day et al., 1989). Motor region excitability is only reflected, however, if the induced current flow is in a posteroanterior direction. This current flow results in the descending volleys having a trans-synaptic origin (i.e., I-waves), which is necessary to reflect the overall excitability of the stimulated region at that time (Sakai et al., 1997; Opitz et al., 2013).

The principles discussed above enabled TMS to be used as a diagnostic tool initially (Horvath et al., 2010), with research exploring its use to identify disorders such as myelopathy (spinal cord injury), amyotrophic lateral sclerosis (a degenerative nerve disease), and multiple sclerosis (a demyelinating disease; see Chen et al., 2008).

Progressions were made toward therapeutic applications, especially with the emergence of repetitive TMS (rTMS): a rapid, continuous train of pulses applied to the same area. Specifically, the cognitive effects that rTMS could produce (e.g., Pascual-Leone et al., 1991), and the lasting nature of such cognitive effects (e.g., Pascual-Leone et al., 1994) indicated the potential usefulness of rTMS as a therapeutic intervention. Kolbinger et al. (1995) demonstrated the potential efficacy of rTMS as a treatment for drug-resistant depression. All patients reported improvements in their symptoms following the rTMS treatment, whereas no such improvements were seen in the participants following sham stimulation. Subsequent research exploring the therapeutic effects of rTMS on depression have replicated Kolbinger et al.'s (1995) findings (e.g., O'Reardon et al., 2007; George et al., 2010), resulting in the approval of the treatment in countries such as United States of America, Canada, and Australia (see Horvath et al., 2010).

Alongside the diagnostic and therapeutic uses, wider TMS research has explored cortical functions in different regions of the brain. The motor regions are a common stimulation site during TMS research, as the resulting MEPs provide an opportunity for simple quantification of CSE at the point of stimulation (Bestmann and Krakauer, 2015). Smaller amplitude MEPs indicate lower CSE, and greater amplitude MEPs indicate greater CSE. This paradigm has been used to explore modulation of CSE before, during, and after motor behaviour (Bestmann and Duque, 2015), and specific mechanisms such as intracortical inhibition (ICI; Kujirai et al., 1993) and intracortical facilitation (ICF; Nakamura et al., 1997).

The different applications of TMS often require different TMS techniques. The initial technique developed by Barker et al. (1985) was single-pulse TMS. This provides a marker of CSE when the induced current is of trans-synaptic origin. Single-pulse TMS can induce such a current and evoke I-waves, but the large area underneath the TMS coil simultaneously stimulates a large number of cortical neurons, resulting in the reflection of both inhibitory and facilitatory responses in the MEP (Nakamura et al., 1997). This makes it difficult to identify whether any CSE modulation was a result of changes in cortical or spinal excitability, or both (Ni and Chen, 2008; Rothwell et al., 2009).

Paired-pulse TMS was subsequently developed to explore the individual ICI and ICF networks in the brain. By combining the test stimulation used in single-pulse TMS with a conditioning stimulus immediately prior to a test stimulus, researchers can obtain a better indicator of corticocortical excitability at the point of stimulation (see Chapter 5.1). This technique, however, does not necessarily overcome the single-pulse TMS issue of not obtaining data from individual mechanisms, as ICI modulation is also likely to result from the summation of both ICI and ICF mechanisms (Peurala et al., 2008). It also adds an additional source of variability compared to single-pulse TMS with the addition of the conditioning stimulus (Du et al., 2014). As such, more research is required to understand and optimise the use of TMS (see Chapter 5). Single-pulse TMS is still considered a useful measure of CSE, however, and is a commonly used technique in action observation research.

2.4. Action observation and TMS

The first experiment to use TMS to explore CSE during action observation was performed by Fadiga et al. (1995). Participants were instructed to observe reach and grasp actions, an arm tracing shapes in the air and static objects, and to complete a light dimming detection task. Their results indicated greater MEP amplitudes and, therefore, facilitated CSE during the action observation conditions compared to both the static object observation and light dimming detection control conditions. This effect, however, was only present in the muscles that would be involved in the execution of the observed action. Such a muscle-specific effect is a key element of the observation-execution matching mechanism, and a consistent finding in action observation research (e.g., Strafella and Paus, 2000; Loporto et al., 2013; see Naish et al., 2014 for a review).

CSE modulation during action observation not only reflects the muscles used to execute the observed action, but also the extent of muscular contraction necessary to perform the observed action (e.g., Gangitano et al., 2001; Alaerts et al., 2010). Gangitano et al. (2001) compared MEP amplitudes across different phases of a reach and grasp action, including the point of maximal finger aperture, the finger-thumb closure phase, and intermediate phases. Greater MEP amplitudes were recorded during the point of maximal aperture, showing that CSE was optimally facilitated at the point of maximal contraction. This has since been replicated, with increases of CSE also demonstrated as a result of observing actions utilising greater force (Alaerts et al., 2010). Again, these findings were specific to the muscles involved in executing the observed action.

Facilitated CSE, and the inference of a more active extended motor system during action observation, is implicitly assumed to be more effective for motor (re)learning (Eaves et al., 2016). Many of the variables shown to modulate CSE during action observation have not been tested in motor (re)learning settings, although early research has demonstrated the efficacy of observation interventions in healthy, stroke, and Parkinson's populations (see Chapter 2.1). There is a rich body of research exploring activity in the motor regions of the brain during action observation using TMS (see Loporto et al., 2011; Naish et al., 2014). More research is required, however, to identify variables that may optimise the design and delivery of such interventions (Holmes and Wright, 2017). Such variables that require consideration during observation interventions include perspective, context, and emotional valence. These topics are discussed in the following subsections.

2.4.1. Perspective

Visual perspective has been suggested to modulate CSE during action observation. The two main types of visual perspective, first- and third-person, relate to the vantage point taken by the observer. A first-person perspective involves attributing an observed action to one's own body and space, whereas a third-person perspective involves the attribution of an observed action to another person (Vogeley and Fink, 2003). To explore the effect of visual perspectives on CSE, Maeda et al. (2002) instructed participants to observe an index-finger and a thumb abduction and adduction with the orientation of the hand representing either a first- or third-person perspective. They reported that CSE was facilitated to a

greater extent when participants observe actions from a first-person visual perspective compared to third-person visual perspective, even when both visual perspectives show movements made by another individual. Kaneko et al. (2007) and Alaerts et al. (2009) both supported and expanded on these findings by demonstrating that CSE is facilitated when the hand presented in the first-person perspective could be considered to be the observer's own, compared to the third-person perspective demonstrating the hand of another individual. They proposed that the previous experience of executing the observed action is required to modulate CSE, which only the first-person perspective provides (Kaneko et al., 2007). They also propose that the third-person perspective directed away from the observer's body is not sufficient to facilitate the sense of previous execution and, therefore, CSE (Kaneko et al., 2007).

The CSE facilitation during first-person visual perspective is thought to reflect the observer being better able to relate the observation to the self. A first-person perspective aims to create an action observation condition that mimics a 'self-based' movement in contrast to a third-person visual perspective which tends to be 'other-based' and, therefore, perspective is conflated with agency. Importantly, increasing the perception of observing a self-based action by combining a first-person visual perspective with an egocentric spatial reference frame (e.g., by matching the observed action with the observer's spatial positioning) has been shown to evoke stronger neural activity in the observer's extended motor system (Vogeley and Fink, 2003) and is consistent with Jeannerod's Simulation Theory (Jeannerod, 2001). This is not a consistent consideration, however, as most research presents observation stimuli on a vertical screen, rotated 90° away from

anatomical congruency. Even if the video is filmed from a first-person perspective, presenting an action on a vertical screen could result in the perception of observing a third-person perspective and, therefore, another person. This creates a perspective-agency confound that requires controlling. Results from visual perspective-based research suggest that action observation interventions filmed from a first-person visual perspective may be more effective at promoting functional neural change than interventions filmed from third-person visual perspectives regardless of screen position (see Ruffino et al., 2017 for a recent review). More research is required, however, controlling for the perspective-agency confound to explore testing the effect of visual perspective on different screen positions.

Also, and in a similar way to the visual perspective-agency confound, presenting an action on a vertical screen provides a different reference frame to the observer's own. This presents an action with reduced visual cues for self-agency and a sense of ownership (Jeannerod and Pacherie, 2004) despite the action being presented in a first-person visual perspective and aiming to represent the viewer's limb. Therefore, in order to represent the action with the more congruent egocentric reference frame, and thereby retain a greater perception of self-agency and ownership with the presented action, the observer may make mental adjustments to the observed action, termed 'ego-relative remapping' by Filimon (2015:2). Specifically, it can be proposed that the observer may have to use concurrent coordinative motor imagery (Vogt et al., 2013) during the action observation condition to rotate the observed action and reinstate an anatomically-accurate

egocentric reference frame that is more congruent with the physical action characteristics.

It appears beneficial, therefore, that to optimise both access to motor representations and subsequent observation interventions for motor (re)learning, a first-person visual perspective promoting ownership and self-agency is provided (Jeannerod, 2001). A first-person visual perspective video, observed on a horizontally angled screen located in the observer's peripersonal space should provide a more accurate egocentric reference frame during action observation, though this requires testing (see Chapter 6). The efficacy of such stimuli presentation, however, may be dependent on each individual observer's preference. Perspective preference is a common manipulation variable within motor imagery (Hall, 1997; Calmels et al., 2006), and a preference for viewing angle has shown to be present during action observation (Ustinova et al., 2010). Each individual's viewing experience, therefore, should be considered during action observation (see Chapter 2.4.4.2).

2.4.2. Context

Another variable that may be important to consider within action observation research is the context in which an action is presented. The visual context in which the action is presented for observation has shown to modulate activity in the motor regions of the brain. For example, Iacoboni et al. (2005) used fMRI to compare cortical activity during observation of grasping a cup against a blank background with two background scenes that provided context that was congruent with the observed action (i.e., a reach and grasp to either drink from or clean a cup).

Iacoboni et al. (2005) reported a significant increase in activity in the premotor cortex and the inferior frontal gyrus when the observed action occurred within a contextually relevant background scene that provided additional visual information regarding the goal and intention of the movement, compared to the blank background that was devoid of additional visual information. This initial finding suggests that activity in the motor regions of the brain during action observation can be modulated by providing visual contextual information regarding the goal and intention of the observed action.

The importance of context during action observation has been explored further by research using TMS. For example, in a similar experiment to Iacoboni et al. (2005), Amoruso et al. (2016) demonstrated that context provided via the objects involved in an observed action modulates CSE. They presented participants with videos that involved object interactions, such as pouring water from a bottle into a glass. The object states in the videos were manipulated to be either congruent (e.g., a bottle full of water and an empty glass) or incongruent (e.g., an empty bottle and a glass full of water) to the observed action. CSE was facilitated when the state of the objects were congruent compared to when the states of the objects were incongruent. Enticott et al. (2010) provided further support for the relevance of visual context by instructing participants to observe a grasping action that either was mimed or involved a hand-object interaction. Their results indicated that observing the hand-object interaction facilitated CSE to a greater extent compared to when there was no object present to provide context to the observed action. In addition, Donne et al. (2011), who presented participants with meaningless (i.e., a thumb tap), goal-directed (e.g., pen grasp), and social (e.g., handshake) actions,

have also demonstrated similar findings. Both the goal-directed and social actions facilitated CSE compared to the meaningless action. The authors suggest that the provision of additional contextual information regarding the goal and intention of the movement contributed to the increase of CSE (Donne et al., 2011). Taken together, this research indicates that CSE is facilitated when observing actions within a scene that provides the observer with accurate visual contextual information about the goal and intention of the observed action. As such, the inclusion of this type of priming information within action observation interventions may benefit motor (re)learning.

Despite evidence that the context in which an action is observed can modulate activity in the motor regions of the brain, some aspects of the relationship require further investigation. For example, Iacoboni et al. (2005) only examined the effect of action observation with a blank background compared to action observation with background scenes that were related to the observed reach and grasp. Based on this, the authors claimed that action observation with a meaningful visual context modulates activity in the brain. It is possible, however, that the reported changes in neurophysiological activity were due to the presence of additional visual information in the background, rather than the congruence of that information to the observed action. Amoruso et al. (2016) provided some initial evidence that the congruency of the context contributes to the facilitation of CSE during action observation, but did not include the additional background information that was included by Iacoboni et al. (2005), so this still requires further exploration.

2.4.3. Emotional valence

Objects used within action observation videos require careful consideration, as the emotional valence attributed to them by the observer may alter CSE. Modulated CSE as an emotional response to objects would suggest that widely distributed neural networks influence the activation of motor representations. By the age of 9 months, humans can associate emotional responses with objects (Watson and Rayner, 1920) and demonstrate an understanding of an object's conceptual knowledge (Hunnius and Bekkering, 2010). This enables appropriate responses to objects from an early age that could be critical to survival (Darwin, 1872). These responses are reflected in the extended motor system relative to the emotional valence (i.e., the strength of positive or negative emotion elicited) of an observed object. This is reflected as modulated physiological responses, such as spinal tendinous reflex (Both et al., 2003) and CSE (Nogueira-Campos et al., 2016). The effect is also reflected through modulated behavioural responses, such as response times (Pereira et al., 2006; Coombes et al., 2007; Ferri et al., 2010) and completion times (Ferri et al., 2010) of an action.

The emotional valence of an object has been shown to modulate CSE during an object viewing task (e.g., Coelho et al., 2010; Van Loon et al., 2010). Commonly, participants have been presented with a static image or object considered to be either unpleasant, neutral, or pleasant, whilst a TMS pulse is delivered and subsequent MEP amplitudes recorded. This research typically demonstrates that observation of static unpleasant and pleasant images facilitates CSE compared to neutral stimuli (e.g., Hajcak et al., 2007; Van Loon et al., 2010), or that observation

of static unpleasant images facilitates CSE compared to static pleasant images (e.g., Coelho et al., 2010; Van Loon et al., 2010; Enticott et al., 2012). CSE modulation relating to emotional valence suggests the influence of neural networks such as limbic systems, indicating a widely distributed neural mechanism influencing motor behaviour (Mallet et al., 2007), and has also shown to be present during tasks relating to action observation.

Enticott et al. (2012) expanded on previous findings by utilising static unpleasant or pleasant images from the International Affective Picture System (Center for the Study of Emotion and Attention, 1999) to prime participants' emotional valence immediately prior to action observation. TMS pulses were applied during observation of the action, rather than the static image, to explore the priming effect of emotional valence on the observation-execution matching mechanism. Priming action observation with unpleasant images significantly facilitated CSE when compared to action observation primed with pleasant images. These findings were proposed to indicate an adaptive avoidance of perceived threat, by priming the motor regions of the brain for withdrawal behaviour. Nogueira-Campos et al. (2014) demonstrated similar findings whilst exploring the effects of observing emotionally valent objects on CSE recorded during movement preparation. Participants were instructed to either perform or not perform a reach and grasp of an unpleasant, pleasant or neutral object. TMS pulses were delivered immediately prior to a go signal, indicating whether the participant should perform the movement or not. Their results demonstrated that preparing to execute a reach and grasp of an unpleasant item compared to a pleasant item resulted in a significant facilitation of CSE. This increased activity when preparing to execute a

reach and grasp to an unpleasant item may indicate the activation of withdrawal networks, similar to the findings of Enticott et al. (2012). Taken together, these findings suggest that activity in the motor regions of the brain is affected by the emotional valence of images and that this may affect subsequent action observation and execution by preparing to withdraw or avoid the unpleasant stimuli.

Whilst the effect of the emotional valence of images on CSE is well established, only one study has explored this effect during action observation itself. Nogueira-Campos et al. (2016) instructed participants to observe a reach and grasp of unpleasant and pleasant objects. They reported greater CSE during observation of a reach and grasp of unpleasant compared to pleasant objects. These findings support and expand on the previous research using static images (e.g., Hajcak et al., 2007; Coelho et al., 2010; Enticott et al., 2012), indicating that the emotional valence of an object modulates CSE during action observation (Nogueira-Campos et al., 2016). Nogueira-Campos et al. (2016) propose that the facilitation of CSE during observation of interactions with unpleasant stimuli compared to pleasant stimuli could be a result of the need to overcome aversive-like circuits in order to execute the observed action with unpleasant stimuli, and/or the need to prevent overt execution of the observed action whilst observing the pleasant stimuli.

The effect of emotional valence on CSE has been demonstrated multiple times during image viewing, and once during action observation. Further research is required, however, to address some of the limitations of the research area. For example, only Nogueira-Campos et al. (2016) have explored the emotional valence

of an object during action observation, but the presented objects were not individualised based on participant preference. The extent of the emotional valence experienced by each participant for each object may vary, so this approach may even result in participants viewing an object that they perceive to be neutral or unpleasant that has been categorised as pleasant (see Chapter 2.4.4.2). In addition, as a static control was not included either, further research is required to enhance the understanding of emotional valence during action observation.

Based on the assumption that a more active extended motor system during action observation may result in more effective motor (re)learning (Buccino, 2014; Eaves et al., 2016), it could be assumed that presenting unpleasant stimuli during action observation interventions would benefit motor (re)learning. However, patients may not want to observe intervention videos containing unpleasant stimuli, which could adversely affect their motivation during action observation interventions and influence the motor response (Cheng et al., 2006; Naish et al., 2014). This modulation on the motor response during observation interventions may subsequently affect the efficacy of the intervention, so it is important to explore each participant's experiences whilst observing emotionally-valent stimuli.

2.4.4. Understanding corticospinal excitability modulation

The measure of CSE provided by TMS is a useful measure of activity in the motor regions of the brain during action observation, though the cause of the modulation is not always apparent. Additional measures, such as eye-tracking and interview discussed below, may be useful to determine the nature of any CSE modulation present during action observation.

2.4.4.1. Visual attention

Possible explanations for modulated CSE during action observation could lie within visual attention. Visual attention is the shift of focus to a relevant location either with (overt) or without (covert) moving the eyes (Carrasco, 2011). Changes in visual attention has shown to be associated with changes in CSE (Conte et al., 2007), and a growing body of research has begun to explore eye movements, a useful measure of visual attention, alongside TMS during action observation (Donaldson et al., 2015; D'Innocenzo et al., 2017). This research typically involves the simultaneous recording of MEPs and eye movements whilst participants observe action stimuli, enabling researchers to establish how visual attention influences CSE. For example, CSE is facilitated in a muscle-specific manner when actions are observed in central compared to peripheral vision (Leonetti et al., 2015). A movement presented within peripheral vision results only in the recognition of general aspects of the movement. The detailed kinematic information required to understand how to perform the observed movement, therefore, is not available, preventing muscle-specific CSE modulation. In comparison, observing an action in central vision enables detailed analysis of a visual scene. A movement presented within central vision, therefore, does provide detailed kinematic information of an observed movement, enabling muscle-specific CSE modulation. This is demonstrated further with facilitated CSE when there are more fixations on hand-object interactions compared to non-action-related areas of the video, indicating the importance of the detailed visual analysis provided by central vision (Donaldson et al., 2015).

In addition, directing participants' visual attention explicitly towards task-relevant features of an observed action has been shown to modulate CSE to a greater extent than passive observation conditions (D'Innocenzo et al., 2017; Wright et al., 2018). This effect is present as the task-relevant feature passes through central vision whilst fixating on a fixation cross (D'Innocenzo et al., 2017), and whilst the task-relevant feature is fixated on, being maintained in central vision (Wright et al., 2018). As visual attention during action observation appears to modulate CSE, it is important to consider the use of eye-tracking measures alongside TMS. For example, including objects in the background of an observation video may modulate visual attention, diverting attention away from the movement. This may modulate CSE by either providing a distraction or additional relevant meaning to the stimulation.

The affect of an observed action and object may also modulate visual attention and, as a result, CSE. Visual attention can be drawn towards either threatening or rewarding visual stimuli based on affect (e.g., Tamir and Robinson, 2007; Ford et al., 2010), which could result in modulated CSE. In general, negative emotions divert visual attention towards negative stimuli, and positive emotions divert visual attention towards positive stimuli (see Ford et al., 2010). With the possibility of both visual and emotional variables influencing visual attention during action observation, it is important to include eye-tracking measures to provide a detailed understanding of any CSE modulation.

2.4.4.2. Individual experience

The individual experience of each participant is an essential consideration within action observation research. It is often overlooked, however, due to methodological considerations. Participants tend to be instructed to observe the same videos, including the same objects and movements, so that kinematic equivalence and object size and shape is identical. This approach maintains tight methodological control, but does not take in to account individual preferences. These preferences may be important for both modulating CSE and motor (re)learning interventions, but are not currently explored within action observation research.

Preference, particularly relating to the use of a specific visual perspective, is an accepted methodological manipulation variable within motor imagery research (Hall, 1997; Calmels et al., 2006). Participants tend to maintain a preference for the imagery perspective that they are best at utilising (Callow and Roberts, 2010). As such, participants' visual perspective preference has become an important consideration when designing imagery interventions, with imagery interventions often employing the participant's preferred imagery perspective (Ewan et al., 2010). Given that motor imagery and action observation share partial neural substrate and elicit some common activity in the motor system (Hétu et al., 2013), individuals may also have viewing preferences during action observation in a similar way to imagery perspective preference. For example, in one of the few studies to consider action observation viewing preferences, Ustinova et al. (2010), using a third-person visual perspective video, manipulated the viewing angles of an avatar

during a reaching action. Following viewing, participants indicated a preference for observing the movement at greater angles (i.e., 45° or higher). If action observation viewing preference is evident within a third-person visual perspective, preference may also exist for a first-person visual perspective action observed from different reference frames and may reveal CSE differences during different action observation conditions.

One element of individual experience that has been shown to modulate CSE is the emotional valence of objects during observation of static images or object interactions (e.g., Hajcak et al., 2007; Nogueira-Campos et al., 2016 as discussed in Chapter 2.4.3). With preferences shown to exist during action observation, and the affect associated with observed stimuli, it appears necessary to explore elements relating to each individual participant's experiences during action observation. Such elements may modulate CSE, yet most research does not consider them. This can be explored through interviews and questionnaires following the observation procedure, specifically designed to explore each participant's experiences throughout the observation. This could assist in determining the nature of any demonstrated modulation of CSE.

2.4.5. Aims of the thesis

As discussed in this chapter, there is a consistent finding of CSE facilitation during action observation compared to static control observation (Loporto et al., 2011; Naish et al., 2014). With this facilitation occurring in similar regions of the brain to those involved in the execution of the same action, action observation is recommended as a useful adjunct intervention to physical therapy in motor

(re)learning settings (Buccino, 2014). More research is necessary, however, to identify optimal methods for the design and delivery of such interventions (Holmes and Wright, 2017).

The overall aim of the thesis was to identify potential variables that could be applied to optimise observation interventions for motor (re)learning. The experiments included in this thesis explore different facets of action observation, specifically relating to the meaning of the observed action to the observer. As such, the experiments aimed to:

1. explore the effect of different screen positions on CSE during action observation (Experiment 1, Chapter 6);
2. explore the effect of background context on CSE during action observation (Experiment 2, Chapter 7); and
3. explore the effect of emotional valence of objects on CSE during action observation (Experiment 3, Chapter 8).

In addition to the use of TMS, measures of paired-pulse TMS, eye tracking, and deductive interviews were utilised where appropriate. The use of these additional measures respectively aimed to:

4. compare the use of single-pulse TMS with paired-pulse TMS, and subsequent CSE and corticocortical excitability during action observation (Pilot experiment, Chapter 5);
5. explore visual attention during action observation, specifically relating to background context and emotional valence (Experiments 2 and 3, Chapters 7 and 8); and

6. explore individual experiences during action observation, specifically relating to screen position viewing preference and emotional valence (Experiments 1 and 3, Chapters 6 and 8).

Chapter 3. Methodology

This chapter provides information regarding the methodology for the techniques used in each of the subsequent studies included in this thesis. This initially includes information regarding pre-experimental participant considerations, such as TMS safety, susceptibility to adverse effects, and handedness. Procedural considerations within the EMG, TMS, eye-tracking, and interview techniques are then discussed, along with considerations prior to, and during, the data analysis.

3.1. Participant considerations

3.1.1. TMS safety and adverse effects

Single-pulse TMS is a safe and useful tool for investigating CSE and is usually a well-tolerated and painless experience for participants (Keel et al., 2001; Anand and Hotson, 2002; Rossi et al., 2009). It is widely perceived as a safe procedure, though it does come with some risk of adverse effects. These risks can range from mild discomfort, such as headaches, to syncope and seizure. These adverse effects are rare (Rossini, 2014), with approximate occurrence rates of 5% for mild adverse effects (Maizey et al., 2013) and 0.1% for seizure (Dobek et al., 2015), with no occurrence rates known for syncope, possibly due to the possible confusion of seizure and syncope symptoms (Zaidi et al., 2000; Epstein, 2006). Syncope episodes during TMS are thought to be more common than seizure (Epstein, 2006), but the occurrence is still rare with few incidents reported (e.g., Hadar et al., 2012; Sczesny-Kaiser et al., 2013; Gillick et al., 2015). Adverse effects are more common in susceptible participants (Anand and Hotson, 2002), however, so safety recommendations should be adhered to (Rossi et al., 2009; Rossini et al., 2015), and

participants should be screened for risk factors increasing their susceptibility to experiencing adverse effects (Keel et al., 2001).

The most common adverse reactions to single-pulse TMS are mild levels of pain and discomfort at the site of stimulation, and transient headaches (Anand and Hotson, 2002; Evans, 2007; Maizey et al., 2013), though most participants rarely report experiencing any issues (Wassermann, 1998; Groppa et al., 2012; Maizey et al., 2013). The causes of these reactions are unclear and most likely vary across participants. One cause could be unavoidable stimulation of the nerves and muscles under and around the stimulating coil (Anand and Hotson, 2002; Groppa et al., 2012). This is more likely to be an issue during rTMS due to the increased number of stimulations over a short period, but it can still occur over the period of a single-pulse TMS experiment and can result in discomfort for the participant. An additional cause could be the design of the experimental protocol. For example, chin- and head-rest restraints are used to reduce head movements and maintain coil position. This is a necessary experimental factor, even though the restrictive setup may cause discomfort through forced posture (Anand and Hotson, 2002). These effects are rare, and such issues can be reduced through screening for participants who suffer from migraine (Keel et al., 2001) and familiarising participants with the procedure (Groppa et al., 2012 see Chapter 4.2.1). Participants, however, should be made aware of the risk before taking part.

The most severe side effects, seizure and syncope, are also the rarest (Evans, 2007; Groppa et al., 2012). TMS can induce hypersynchronisation of groups of neurons (Pasley et al., 2009), which, in turn, can result in seizure (Rossi et al., 2009), though

very few cases of TMS-induced seizure have been reported (Rossi et al., 2009; Dobek et al., 2015). Most cases of TMS-induced seizure have been during rTMS when procedures have exceeded previously published guidelines (Rossi et al., 2009), and less than 5% of reported seizures have been during single-pulse TMS (Rossi et al., 2009). Often, the participants who experienced seizure were under medication, were predisposed to epilepsy, or suffered from central nervous lesions or disease (Evans, 2007; Rossi et al., 2009). It has even been shown to be relatively difficult to intentionally trigger seizures in epileptic participants using single-pulse TMS (Loo et al., 2008), with stimulation intensities substantially greater than those commonly used required to induce seizure reliably (Evans, 2007). As such, single-pulse TMS is not thought to be a high-risk procedure for seizure (Evans, 2007; Rossi et al., 2009).

Another possible adverse reaction to TMS is syncope (Groppa et al., 2012). It is thought that it is not caused by the TMS itself (Evans, 2007), but through anxiety and discomfort during the TMS procedure which can result in vasodepressor syncope (Groppa et al., 2012). Syncope can result in similar behavioural responses to seizure such as stiffening and jerking (Rossi et al., 2009; Groppa et al., 2012), but participants recover full consciousness within seconds rather than minutes (see Groppa et al., 2012). The likelihood of syncope can be reduced by familiarising participants with the TMS procedure to reduce anxiety, and by screening those who have previously suffered from syncope episodes (Groppa et al., 2012; Gillick et al., 2015).

Adverse effects experienced during TMS are rare. The incidence can be reduced by screening potential participants who may be at an increased risk (Keel et al., 2001). For example, screening for and excluding participants who suffer from migraines may aid in reducing the incidence of headaches (Keel et al., 2001). In addition, the incidence of seizure can be reduced by screening for participants who: are taking seizure threshold-lowering medication; are sleep deprived; are under the influence of alcohol; or have, at one time, had neurological disorders (Wassermann et al., 1996; Anand and Hotson, 2002; Loo et al., 2008; Dobek et al., 2015). As such, the use of a safety screen, such as the TMS adult safety screen (Keel et al., 2001; Appendix 1), is commonplace in TMS experiments (Craighero et al., 2014; Naish et al., 2016; Wright et al., 2018). This questionnaire requires yes or no responses to questions such as: “Have you ever suffered from any neurological or psychiatric conditions?”; “Have you ever suffered from epilepsy, febrile convulsions in infancy or had recurrent fainting spells?”; and “Did you have very little sleep last night?” A “yes” response to any of the questions indicates the necessity for further investigation by the experimenter and does not necessarily act as an absolute contraindication to TMS (Keel et al., 2001; Groppa et al., 2012). Such investigation can involve checking the individual participant’s history and extent of psychiatric conditions, the location of the target effect of medication, and the consistency of sleep levels. Only the presence of implanted devices, such as metal plates in the skull and pacemakers, should act as an absolute contraindication to participation (Rossi et al., 2009; Groppa et al., 2012), with the risk-benefit of the procedure being carefully considered on an individual basis (Groppa et al., 2012).

There is still a possibility of experiencing adverse effects during a TMS experiment, even though the risk of their occurrence can be reduced through screening and familiarisation. Familiarising the participants with the procedure can help to reduce any anxiety they may have regarding the procedure, reducing the likelihood of adverse effects (Groppa et al., 2012; see Chapter 4.2.1 for the familiarisation procedure used in this thesis). Each participant should be made aware of the risks of participation in TMS experiments, and, in the incidence of any adverse effect, testing should be terminated immediately (Rossi et al., 2009). Single-pulse TMS, however, causes only transient changes in neural activity and does not appear to carry any long-term risk (Anand and Hotson, 2002). The available evidence, including the rarity of adverse effects, points towards TMS being a safe procedure when previously published safety guidelines are adhered to (Evans, 2007; Rossi et al., 2009).

3.1.2. Participant handedness

Hand preference, or handedness, manifests as the preferential use of one limb more than the other for manual activities (Corey et al., 2001). This results in not only behavioural asymmetry, but also a functional asymmetry of brain organisation (Yahagi and Kasai, 1999). The emerging asymmetry could potentially result in the modulation of MEP amplitudes depending on whether the observed hand action is relative to the dominant or non-dominant hand of the observer (Yahagi and Kasai, 1999). As such, it has become commonplace to use handedness as an inclusion criterion in action observation research utilising TMS, with most studies only including right-handed participants (e.g., Gangitano et al., 2001; Alaerts et al., 2009;

Loporto et al., 2012). The existing research on the effect of handedness during motor simulation, however, presents limited and inconsistent findings.

Due to functional asymmetry (Yahagi and Kasai, 1999), it is thought that motor simulation of the non-dominant hand would not facilitate CSE to the same extent as motor simulation of the dominant hand. This has shown to be the case in right-handed participants during motor imagery of index finger flexion, where significantly greater CSE was demonstrated in the right first dorsal interosseous (FDI) compared to the left FDI muscle (Yahagi and Kasai, 1999). The left-handed participants, however, showed no significant differences of CSE in the right and left FDI muscles. This demonstrates partial support for handedness-related functional asymmetry but indicates that this may be more prominent in the right- than left-handed population. The effects found in right-handed participants have been replicated during first-person perspective observation of right- and left-handed index finger flexion (Aziz-Zadeh et al., 2002). In this experiment, right-handed participants showed significantly greater CSE in the right compared to the left FDI muscle. This was not tested with left-handed participants, but the findings begin to indicate functional asymmetry of CSE facilitation during action observation of hand movements.

During observation of both right- and left-handed whole-hand grasps, Sartori et al. (2013) found that left-handed participants demonstrated greater CSE in the left abductor digiti minimi (ADM) compared to the right ADM. This is in contrast to the imagery findings of Yahagi and Kasai (1999), indicating that the observed action patterns triggered optimal motor representations in the observer by mapping them

onto the representation for their preferred hand, providing support for handedness-related functional asymmetry during motor simulation. As the observed action was of a third-person perspective, however, this may have been a consequence of ego-relative remapping (mental translation of a stimulus to line up relative to the self; Filimon, 2015). This would allow the observer to relate the action to the egocentric reference frame, enabling an anatomically-accurate representation of the physical pattern of movement (Filimon, 2015).

In contrast to Sartori et al. (2013) findings of remapping, Borroni et al. (2008) showed bilateral excitability during the observation of both right- and left-handed movements, with equal excitability demonstrated in both hemispheres. This resulted in the postulate that 'motor programmes' may not encode laterality, but instead an abstract representation of movement. In partial support of this, left-handed participants have demonstrated a bilateral pattern of activation in areas of the motor system to a greater extent than right-handed participants (Rocca et al., 2008). Together, these findings indicate that CSE may not be modulated in left-handed participants as a result of observing a right- instead of a left-handed movement.

Research indicates functional asymmetry through handedness, though this has only been demonstrated consistently among right-handed participants. This could be a consequence of different experiences of sensorimotor learning between the right- and left-handed populations. It is possible that a facilitated MEP profile is a result of observing an action of which the observer has previous sensorimotor experience (Catmur, 2013). As the left-handed population have greater opportunity to acquire

skills with their non-preferred right hand (Yahagi and Kasai, 1999), they may be more likely to have previous sensorimotor experience with using both hands than the right-handed population. The observed actions presented in the studies included in this thesis are simple, non-skill-based movements, which may not require sensorimotor learning, which may also explain why left-handed participants appear likely to demonstrate a similar CSE profile to right-handed participants whilst observing a right-handed movement (e.g., Rocca et al., 2008; Borroni et al., 2008).

With the findings discussed, and the success of previous action observation experiments utilising TMS that included left-handed participants, participants in the experiments reported in this thesis were not excluded based on handedness. However, to account for possible issues due to differences in sensorimotor experience, right- and left-handed participants were presented with a right-handed movement. In addition, handedness was measured using the Edinburgh Handedness Inventory (EHI; Oldfield, 1971; see Appendix 2) to provide clarity of the methods and complete descriptive information of the participants. This questionnaire requires participants to indicate which hand they prefer to use with tools and in a series of tasks, such as writing, a hammer, and the lower hand on a cricket bat. It also allows participants to indicate the strength of their preference to generate a single laterality quotient (LQ), ranging from -100 (strong left-handedness) to +100 (strong right-handedness (Oldfield, 1971).

3.2. Electromyography

The recording of electrical potential changes in a muscle is enabled using EMG electrodes (U.S. National Library of Medicine, 2018). Recording muscular activity resulting from TMS provides a method of measuring of MEPs; the amplitude of which enables inference of CSE modulation (Bestmann and Krakauer, 2015; Ruffino et al., 2017). Such recordings can be taken from multiple muscles simultaneously, allowing for comparisons between experimental and control muscles. EMG can also be used to ensure that changes are not due to physical movement (Kiers et al., 1993), and enable more accurate measures of optimal scalp position (OSP) and resting motor threshold (RMT; Westin et al., 2014).

The electrodes and participant's skin at the electrode sites must be adequately prepared to ensure that the EMG signal is as high quality as possible. Alcohol wipes are used to remove any dead skin and oil over the muscles of interest and the bony landmark used for the reference electrode site (Day, 2002; Burden, 2017). Non-invasive surface electrodes are also cleaned using alcohol wipes, before having adhesive stickers attached and conductive gel applied to the electrode contacts. This ensures consistent contact with the skin and reduced electrical impedance respectively. The electrodes are then attached to the skin over the muscle belly of the middle of the muscles of interest (Day, 2002). To ensure this process has been enabled high-quality EMG signals, baseline measurements during both activity and inactivity of the muscles of interest are taken (see Chapter 4.2.2 for detailed baseline EMG procedure).

By recording EMG signals throughout a TMS experiment, it is possible to determine that the recorded changes are a result of the CSE modulation and not a result of physical movement. Activation of the muscle immediately prior to a TMS pulse can result in greater MEP amplitudes than would be recorded from the same muscle during inactivity (Hess et al., 1987; Kiers et al., 1993; Devanne et al., 1997; Darling et al., 2006). This would affect the measure of CSE, potentially confounding the overall results of the experiment. As such, it is important to remove individual trials that may have been affected by physical movement (Loporto et al., 2012), the procedure for which is discussed in Chapter 4.2.2.

3.3. TMS

3.3.1. Coil

Three main TMS coil shapes are used for treatment and research: circular; conical; and figure-of-eight (Magstim, 2018). Each shape has different stimulating properties that must be considered before their use (Figure 3.1). The circular coil is the original design used by Barker et al. (1985). This coil can effectively activate the motor cortex, but it is not able to deliver a focal stimulation, resulting in difficulty when it comes to targeting specific muscles(Cohen et al., 1990). Like the circular coil, the conical coil is not able to deliver stimulation as focal as the figure-of-eight coil but is better suited to deliver deep-penetrating stimulations that can be used to target leg and back muscles (Lu and Ueno, 2017). The most focal coil, the figure-of-eight coil, generates a smaller area of maximum electric field strength under the overlapping section (Ueno et al., 1988). Converging two current flows in opposite directions increases the current density in the target area, enabling a focal

stimulation with a resolution of 5mm (Ueno et al., 1990). This does allow specific muscles to be targeted during stimulation, although multiple muscles are usually stimulated simultaneously due to the overlap of motor representations in the motor cortex (Sanes and Donoghue, 2000).

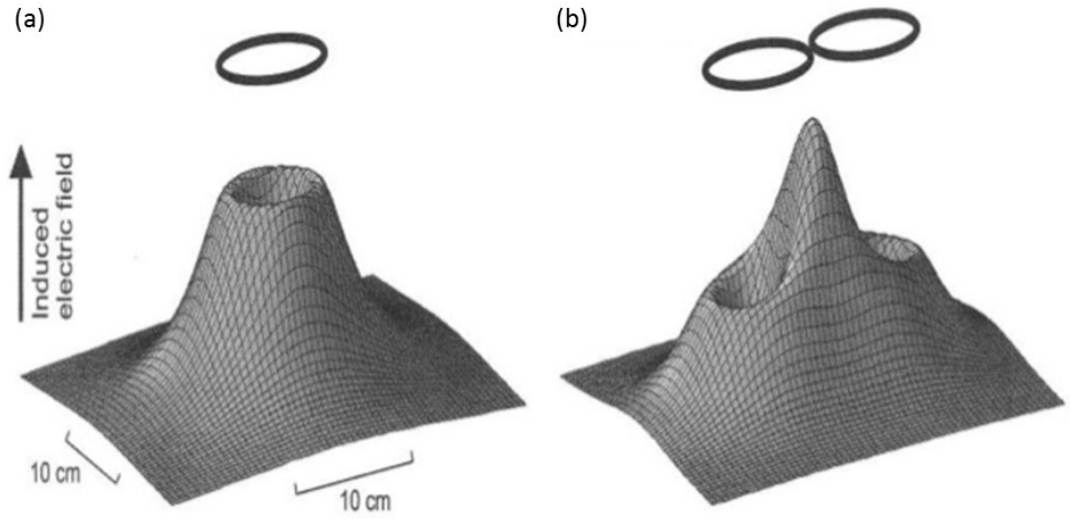


Figure 3.1. The electrical field induced by a circular (a) and a figure-of-eight (b) coil: the two most commonly used TMS coils (adapted from Hallett and Chokroverty, 2005)

With the figure-of-eight coil being highly focal, it is highly susceptible to positional and rotational changes. A change in the position of the coil can result in stimulation outside of the intended area, whilst a change in the rotation will alter the direction of the induced current in the motor cortex (Opitz et al., 2013). By inducing a current flow in a poster-anterior direction, the motor cortex is activated in an indirect manner (Werhahn et al., 1994). These I- (indirect) waves have a trans-synaptic origin, enabling a reflection of motor cortex excitability (Sakai et al., 1997; Opitz et al., 2013). The coil should be positioned at a 45° angle to the midline between nasion and inion landmarks of the skull to achieve an induced current flow in a poster-anterior direction (Brasil-Neto et al., 1992). Changing this orientation can

result in a more lateral-medial flow direction, triggering D- (direct) waves and reflecting motor cortex excitability to a reduced extent, even at the same stimulation intensity (Werhahn et al., 1994). These changes in position can result in significantly different MEPs (Sandbrink, 2008; see Supplementary material 1 for a demonstration of this effect), so correct and consistent coil placement is imperative. This is achieved by tracing around the coil to mark the coil location on a tightly-fitting cap worn by the participants to ensure consistent placement across blocks. Even with the susceptibility to positional and rotational issues of the figure-of-eight coil, due to the benefit of having focal stimulations to test the OSP consistently (see Chapter 3.3.2), a figure-of-eight coil was used in all of the studies included in this thesis.

3.3.2. Optimal scalp position

The OSP for the experimental muscle is found to ensure the highest possible MEP amplitudes during testing (Loporto et al., 2013; Rossini et al., 2015). Penfield's homunculus (Figure 3.2) demonstrates how motor representations are mapped across the motor cortex, and provides an initial guide on determining the OSP. More specifically, research has shown that the modal location of the OSP for the FDI muscle of the right hand, one of the muscles used in the studies presented in this thesis, is 4cm lateral and 1.5cm anterior from the central point of the midline of the skull (Loporto et al., 2013); measured by the International 10-20 System (Jasper, 1958). With variation across participants, however, it is important to determine each participant's OSP individually, using the modal location as a starting point only.

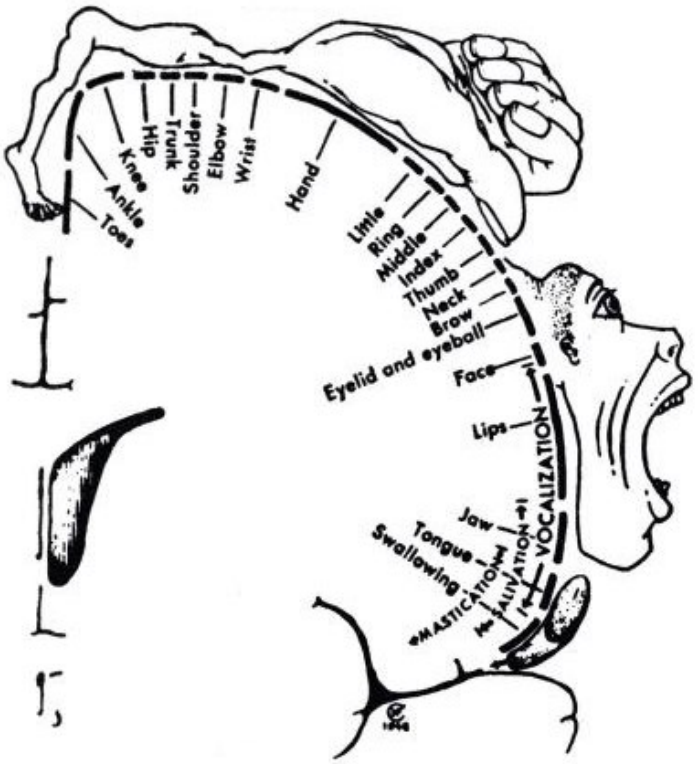


Figure 3.2. Penfield's homunculus provides an initial guide on OSP determination (adapted from Covington Jr., no date)

The OSP is found by first stimulating the modal location of the right-hand region of the motor area. The coil is then moved in 1cm steps around this location until the OSP is found based on MEPs of maximal amplitude in the experimental muscle, and consistent amplitude in the control muscle (e.g., Rossini et al., 2015; Wright et al., 2016). Stimulating outside of this area can result in lower MEP amplitudes and incorrect stimulation intensities (Boniface et al., 1990; Balslev et al., 2007), so it is important that an accurate OSP is found and maintained consistently. This can be achieved by the careful marking of the coil location on a tightly fitting cap on the participant's head. Accurate and consistent coil placement is especially important as most experiments record MEPs from at least two muscles concurrently (Naish et al., 2014).

Testing two or more different muscles simultaneously creates difficulty in determining an appropriate OSP. It is possible to stimulate multiple muscles simultaneously due to the overlap of motor representations (Sanes and Donoghue, 2000), but the OSPs differ (Loporto et al., 2013) so that one, or more, muscle is not stimulated optimally. Previous research tends to determine the OSP whilst attempting to stimulate maximum and consistent MEP amplitudes from both muscles to take this into account. This appears problematic, but no differences have been found in motor resonance effects whilst stimulating either the experimental or control muscle OSP (Loporto et al., 2013). As such, the commonly adhered to determination of the OSP appears to be best-practice at this time and is used in all studies included in this thesis.

3.3.3. Resting motor threshold

The motor threshold is defined as the minimal stimulation intensity required to elicit an EMG response of approximately 50μV in the target muscle, with the RMT being performed whilst the target muscle is at rest (Rossini et al., 1994; Rossini et al., 2015). This is the most commonly used method in TMS research (e.g., Patuzzo et al., 2003; Ohno et al., 2011; Hyde et al., 2017). Another commonly used method of determining the motor threshold, especially during paired-pulse TMS, is whilst the target muscle is active (e.g., Paus et al., 2001; Wagle-Shukla et al., 2009; Di Lazzaro et al., 2016); referred to as the active motor threshold. This method requires a contraction of approximately 20% maximal muscle strength to be maintained in the target muscle during the delivery of TMS (Rossini et al., 2015). Threshold stimulation of an active muscle produces more variable MEP amplitudes than

stimulating a relaxed muscle (0-1mV compared to 0-0.5mV respectively; Rossini et al., 2015). In addition, as the stimulation interferes with a participant's ability to maintain a muscle contraction of consistent strength, measuring active motor threshold is more difficult than measuring RMT (Rossini et al., 1994). As such, the studies included in this thesis measured the motor threshold with the target muscles at rest (RMT).

Using EMG enables a reliable measure of the RMT, with alternatives, such as visual observation of muscle twitch, resulting in significantly higher motor thresholds (Westin et al., 2014). Aside from being a safety issue by causing stimulations beyond recommended guidelines (Westin et al., 2014), higher stimulation intensities encourage direct wave activation and confound the MEP amplitudes (Di Lazzaro et al., 2004).

Stimulation intensities closer to the RMT encourage indirect, trans-synaptic, activation of the motor cortex. It is intuitive, therefore, that these lower intensities should be used when testing CSE (Di Lazzaro et al., 2004). There is not a set value, however, that can be applied to all participants. As there are individual differences within the excitability of corticospinal pathways, skull thickness, and other anatomical features (Ziemann et al., 1998; McConnell et al., 2001; Stokes et al., 2005), it is important to normalise the experimental stimulation intensity for each individual participant. This is achieved through determination of each participant's RMT individually by gradually reducing the stimulation intensity until responses of approximately 50μV are found in the target muscle, and setting the experimental intensity to a standardised transformation.

Normalisation of the experimental stimulation intensity in previous research has ranged from 110% (e.g., Gangitano et al., 2004; Catmur et al., 2007; Wright et al., 2018) to 150% (e.g., Li et al., 2009) of the RMT. Such variation between published research prevents accurate comparisons of results, as the higher intensities may have reflected direct wave stimulation, rather than the targeted indirect waves to reflect CSE (Di Lazzaro et al., 2004). A direct comparison of experimental intensities has shown that, when compared to 130%, only 110% of the RMT was sufficient at reducing the chance of direct wave stimulation to reflect CSE accurately (Loporto et al., 2013). A comparison to 120% of the RMT may be required, but consistent utilisation of a standardised normalisation of the RMT is required to enable accurate comparisons of results. As such, all the studies included in this thesis used an experimental stimulation intensity of 110%.

3.3.4. TMS data analysis

Multiple considerations are required prior to analysing the MEP data produced during an action observation experiment utilising TMS. For example, a key element of TMS research during action observation is to explore CSE during the observation of an action whilst at rest. It is problematic, therefore, if the muscle(s) being tested in a participant are active immediately prior to and at the point of the stimulation. This would affect the validity of the experiment and is reflected in the profile of the MEP (van den Bos et al., 2016).

Voluntary contraction of the target muscle has been shown to significantly increase the MEP amplitude compared to when it is at rest (Ugawa et al., 1995; Kojima et al., 2013; van den Bos et al., 2016). The MEP amplitude can be increased from as little

as 5% maximal voluntary contraction (Taylor et al., 1997), and can be increased further with contractions of greater strength (van den Bos et al., 2016) and duration (Sacco et al., 1997). Previous research has accounted for muscular contraction in the target muscle(s) by recording a baseline EMG signal immediately prior to the stimulation, and removing any trials that contain increased EMG activity (e.g., Fourkas et al., 2006; Alaerts et al., 2009; Loporto et al., 2012). One such example of this, and the procedure used in the TMS data analysis in this thesis, is to remove any trials that had an EMG baseline greater than 2.5 standard deviations above the mean (Loporto et al., 2012).

Another important consideration is the inherent variability of the MEP. Intra-individual MEP amplitudes are highly variable (Kiers et al., 1993; Julkunen et al., 2009; Choudhury et al., 2011), despite attempts to refine and standardise TMS method (e.g., Loporto et al., 2013; Rossini et al., 2015). Coil orientation, location, and stability have all been shown to produce variability in the MEP amplitude (Hashemirad et al., 2017), as has the planning and selection of actions, medication, and neurological disorders (see Bestmann and Duque, 2015). Controlling for such methodological issues, however, does not eradicate variability (Hashemirad et al., 2017). This appears to imply it is the result of underlying neurophysiological mechanisms (Kiers et al., 1993; Bestmann and Duque, 2015).

The neurophysiological cause of MEP amplitude variability is unknown. It has been described to be the result of both intra-cortical and long-range cortico-cortical pathways generating the stimulation-induced I-waves (Bestmann and Duque, 2015), and also the spontaneous fluctuations of CSE (Kiers et al., 1993). Such variability can

be controlled to an extent by stimulating during slight muscular contraction (5-10% maximal contraction; Darling et al., 2006), and at higher stimulation intensities (Pitcher et al., 2003). This introduces different methodological issues, however (see Chapters 3.2 and 3.3.3 respectively). Considering this, and the potential increase in variability during action planning and selection (Bestmann et al., 2008), there appears to be no method at present to satisfactorily reduce variability in the MEP amplitude during action observation experiments utilising TMS. It appears that the best option at present is to account for the variability, by normalising the data over a greater number of trials; the recommendation of which is 30 trials per condition (Cuypers et al., 2014; Goldsworthy et al., 2016; Hashemirad et al., 2017). The most common normalisation options are percentage change and *z*-score transformations. Percentage change involves calculating the difference of MEPs during experimental conditions to a control image, such as a fixation cross or a static hand. This approach, however, is based on the assumption that control conditions provide an absolute baseline value, which may not be the case. For example, research has indicated that static images can imply movement, which can influence the observer's perception of the stimulus as if it was moving (Pavan et al., 2011). *Z*-score transformations are perhaps a more appropriate method of normalisation, as they the standardise the highly variable data onto a scale indicating the number of standard deviations each data point is around the mean. As such, this is frequently used within action observation experiments utilising TMS (e.g., Fadiga et al., 1995; Urgesi et al., 2006; Aglioti et al., 2008), including those within this thesis.

3.4. Eye-tracking

Acquiring visual information is considered a necessary part of human function, including movement understanding and control (Causer et al., 2012). A greater understanding of the visual information pertinent to understanding and controlling movement is obtained by recording each participant's eye movements. By projecting infrared lights onto both of the participant's eyes, eye-tracking glasses can record eye movements via in-built cameras. These eye movements can be calibrated against a visual scene recorded via the glasses' front-facing camera, to enable a measure of where a participant is looking, accurate to 0.5° visual angle (ETG 2w; SensoMotoric Instruments, Teltow, Germany). These recordings can indicate decision-making and attention throughout observation and performance of a movement (Vickers, 2009). Pairing measures of eye movements with other techniques, such as TMS, enables in-depth explorations of how changes in visual attention can alter CSE during action observation (D'Innocenzo et al., 2017; Wright et al., 2018).

3.4.1. Eye-tracking equipment

Eye-tracking equipment can come in two main forms: static and mobile. Static eye-trackers place the infrared illumination and eye-camera(s) in front of the participant, either near the visual stimulus for remote eye-trackers, or built-in to the head-rest for tower-mounted eye-trackers. Tower-mounted eye-trackers require head- and chin-rests to immobilise the participant's head (e.g., SR Research, 2018), but, as a result, records eye-tracking data with high precision and accuracy (Holmqvist et al., 2011). Remote eye-trackers are capable of recording eye-

movements from a distance with the lack of head restriction enabling additional measuring equipment to be added to the participant's head (Holmqvist et al., 2011). Compared to tower-mounted eye-tracking, however, remote eye-trackers typically record lower quality data in terms of sampling frequency (e.g., 30-60Hz compared to 1000Hz capabilities of tower-mounted eye-trackers), accuracy when the head is in a less-than-optimal position, and increased data loss if the participant moves too much (Niehorster et al., 2018).

Mobile, or 'head-mounted', eye-trackers place the infrared illumination and eye-camera(s) on the head of the participant, usually on a helmet or pair of glasses, whilst a front-facing scene camera records the visual stimulus (Holmqvist et al., 2011). Even with lower quality data than static eye-trackers (30-60Hz compared to capabilities of up to 1000Hz), mobile eye-trackers still enable sufficient quality data of most eye-tracking metrics and allows the participant much greater mobility (Holmqvist et al., 2011; Niehorster et al., 2018). This enables the recording of eye-tracking metrics during activities such as driving and buying food in a supermarket (Holmqvist et al., 2011).

3.4.2. Eye-tracking data capture

Eye-tracking equipment can be used to record either monocular or binocular data. Most research uses monocular data capture, recording data from one eye only, as both eyes tend to look in roughly the same position (Holmqvist et al., 2011). There can be, however, a disparity of up to 2° visual angle between a participant's eyes (Cornell et al., 2003), making this assumption problematic. Binocular data capture accounts for this by recording from both eyes simultaneously and can considerably

increase the accuracy and precision of eye-tracking data (Cui and Hondzinski, 2006) by calculating averages between data collected from the two eyes (Holmqvist et al., 2011). In addition, binocular data capture enables depth-calibration, to the benefit of mobile eye-tracking (Holmqvist et al., 2011).

With multiple options available, it is important to determine the eye-tracking sampling frequency prior to testing. Recent technologies have sampling frequencies of 60 or 120Hz, with some static eye-tracking equipment being capable of around 1000Hz (Leube et al., 2017). A sampling frequency of 1000Hz records a data point every millisecond, providing precise eye movement data. Such data can be noisy, however, with a large number of data points recorded. Lower frequencies are less noisy and are still able to provide acceptable precision for eye movement metrics. A sampling rate of 120Hz records a point approximately every 8ms, providing enough temporal precision to detect fixations (a period where the eye is relatively still) and saccades (rapid eye movements between fixations; Leube et al., 2017). 60Hz, however, with one data point recorded approximately every 17ms, significantly under-estimates the detection of saccades (Leube et al., 2017). 60Hz sampling is still highly capable of recording fixations, with no significant difference in fixation detection between 60 and 120Hz sampling frequencies (Leube et al., 2017). As fixations were the preferred metric for the studies included in this thesis, mobile eye-tracking equipment with a sampling frequency of 60Hz was used throughout.

3.4.3. Eye-tracking stimuli

Additional considerations for obtaining high-quality eye-tracking data include equipment calibration and stimulus location. Correct and frequent calibration is

essential for obtaining adequate eye movement data for analysis (Duchowski, 2007). This is performed by instructing the participant to fixate on a visual landmark and aligning the cursor with this landmark in the eye-tracking software. This is often achieved by calibrating against multiple visual landmarks (Duchowski, 2007; Leube et al., 2017), with the latest eye-tracking technologies only requiring either one or three points to calibrate the equipment accurately (SensoMotoric Instruments, 2016). The calibration should be checked frequently and, if necessary, re-calibrated to ensure precise data collection (Duchowski, 2007). Even the most accurate and precise calibration can be disrupted, however, if there are imaging problems with the participant's eyes. For example, when participants look down, the upper eyelid lowers, preventing accurate imaging of the eyes (Duchowski, 2007).

3.4.4. Eye-tracking data analysis

A useful method of analysing eye-tracking data is to first define areas of interest (AOI) within the visual scene (e.g., Holmqvist et al., 2011; Blascheck et al., 2014; Hessels et al., 2016). Multiple considerations are required, however, to utilise AOI correctly (Holmqvist et al., 2011). For example, the positioning of AOI is a key consideration. Initially, it is the hypotheses that should determine the AOI, but also the location and shape of the AOI need to be decided with consideration to the hypotheses, visual scene, and subsequent analysis (Holmqvist et al., 2011). For example, the location of an AOI is not necessarily restricted to one place. When a stimulus moves across a screen, the stimulus will move away from a static AOI, rendering it useless. In such instances, dynamic AOI that follow the location, size and shape of the relevant stimulus throughout the movement are utilised (Kang et

al., 2016). This can be performed both with stimuli that are presented on a screen and during natural environments in which a participant will be moving.

It is thought that AOI should not overlap, as a single data point within an overlapping AOI could be counted twice, and issues will arise in calculating dwell time (time spent looking in an AOI, from entry to exit) as it becomes difficult to determine to which AOI the dwell time belongs (Holmqvist et al., 2011; Kang et al., 2016). It can also make calculating eye movements between AOI difficult, as it is unclear whether such a transition is a movement inside an AOI, or a movement from one AOI to another (Holmqvist et al., 2011). It can be necessary to have overlapping AOI (Holmqvist et al., 2011), however, especially within visual stimuli containing multiple objects and a movement. Dwell time and transitions were not key metrics for the studies using eye-tracking techniques in this thesis, and fixations were coded manually to avoid inaccuracies within AOI.

Once the AOI have been defined and data points assigned to the appropriate AOI, several metrics could be used to analyse the participant's eye movements. This can include fixation count and duration, dwell time, saccades, and pupil dilation. The eye-tracking metrics to be used for analysis are usually determined beforehand, as this will influence the positioning, location and shape of AOI (Holmqvist et al., 2011). As with the AOI, there are multiple considerations for determining which metrics to use for subsequent analysis.

The most commonly used metrics relate to fixations; where the eye is relatively still for a period of time. The number of fixations and the duration of fixations are utilised to indicate attentional processing (Vickers, 2009; Holmqvist et al., 2011). An

increase in the number of fixations can indicate how important (Jacob and Karn, 2003), noticeable (Poole et al., 2004), or semantically informative (Henderson et al., 1999) a stimulus is. It can also indicate poor search efficiency (Goldberg and Kotval, 1999), or difficulty in interpreting the fixated information (Ehmke and Wilson, 2007) as participants fixate more to find relevant visual information. An increase in fixation duration indicates deeper information processing as participants focus on critical (Harris Sr and Christhilf, 1980), less frequent (Dambacher and Kliegl, 2007), and more complex (Rayner et al., 2012) visual information. There are issues with using fixations as a measure of attention; these include attention shifting prior to eye movement (Deubel, 2008) and increased fixation durations as a result of daydreaming (Chapman and Underwood, 1998). It is considered that fixation metrics, however, are a sound and useful indirect measure of attention (Holmqvist et al., 2011). As attention to specific stimuli was a key variable within the studies included in this thesis, fixation count and the percentage fixation duration were used.

Other metrics include saccades, the diameter of the pupil, and dwell time. A decreased saccadic rate can reflect higher mental workload (Nakayama et al., 2002) and fatigue (Van Orden et al., 2000), whilst an increase can indicate arousal (Morris and Miller, 1996). When presenting visual stimuli containing both static and dynamic items, however, it can be unclear what the saccadic rate reflects (Holmqvist et al., 2011). In addition, recording saccades requires a sampling rate of at least 120Hz (Leube et al., 2017). The eye-tracking equipment available for the experiments in this thesis had a maximum sampling rate of 60Hz, which would result in a significant under-estimation of saccadic data (Leube et al., 2017).

Considering both the lack of clarity and the equipment available, saccades were not recorded in the experiments included in this thesis.

It can also be unclear what causes any changes seen in pupil diameter during an experiment, as potential experimental confounds such as mental workload (Van Orden et al., 2000), emotion (Partala and Surakka, 2003) and anticipation (Kang et al., 2009) all increase pupil dilation, whilst fatigue can decrease pupil dilation (Hopstaken et al., 2015). Attempts to reduce anticipation towards the procedure, such as familiarising each participant with the procedure, were implemented for the studies included in this thesis (see Chapter 4.2.1), but some participants still experienced anticipation of the stimulation. With this, and findings that fatigue could influence pupil dilation data throughout the duration of the experiment (Hopstaken et al., 2015), pupil dilation was not used as an eye-tracking metric in the studies included in this thesis. Finally, dwell time can present a measure of interest in a visual stimulus (Pieters et al., 1996), but can be difficult to determine when overlapping AOI are used, resulting in low data validity. Although not overlapping, the close proximity of AOI in the studies included in this thesis was taken into account, so dwell time was not used either.

3.5. Qualitative methodology

3.5.1. Interviews

Research interviews are purposeful conversations regarding a specific theme, with the intention of exploring the meaning of a participant's experience(s) from their own point of view (Ryan et al., 2009; Brinkmann and Kvale, 2015). These can take different forms based on the nature of the study, methodological requirements,

and research questions (Ryan et al., 2009; Purdy, 2014). The form of a research interview tends to be developed in either an unstructured, structured, or semi-structured manner (Ryan et al., 2009; Purdy, 2014). This relates to how the questions and topic of an interview are determined and adhered to both prior to and during the interview process.

An unstructured interview allows for a flexible, spontaneous approach to the conversation, with no specific line of questioning (Ryan et al., 2009). Some key areas of the interview are pre-determined, such as key themes of the interview, but the focus is to follow the direction of the participant's responses (Ryan et al., 2009; Purdy, 2014). These are useful to employ in follow-up interviews; to build on the first interview and, potentially, newly obtained knowledge (e.g., Cushion and Jones, 2006; Purdy and Jones, 2011). In contrast, a structured interview is more rigid, with predetermined questions that do not change throughout the course of the conversation. This tends to generate quantitative data as there is little room for deviation from the interview script (Ryan et al., 2009; Purdy, 2014), and so is only appropriate for research with a focussed research question. A semi-structured interview provides a middle ground between the unstructured and structured interviews. Similar to structured interviews, semi-structured interviews also tend to use predetermined questions, but, as with unstructured interviews, maintain an element of flexibility to enable deviation from the script to explore additional emerging topics of interest (Ryan et al., 2009; Purdy, 2014; Brinkmann and Kvale, 2015). This tends to encourage issues to be explored from an individual perspective, providing richer data than a structured interview (Ryan et al., 2009).

Interviews can take place one-to-one, or as a focus group. One-to-one interviews involve only the interviewer and participant and enable in-depth exploration of attitudes, opinions and beliefs of personal experience(s) (Purdy, 2014). This can provide a narrow, but personal, viewpoint on the experience, and can provide greater control over the topics discussed (Ryan et al., 2009). Focus groups enable a broader discussion by involving more participants in the same interview, with discussion led by a moderator (Brinkmann and Kvale, 2015). This can provide a wider range of attitudes, opinions, and beliefs relating to experience(s) through discussion amongst participants (Purdy, 2014), as the focus is on generating different viewpoints, rather than coming to a consensus (Brinkmann and Kvale, 2015). With focus groups, however, the course of the interview is harder to control (Brinkmann and Kvale, 2015), especially as some participants may either dominate or not contribute to the conversation (Purdy, 2014), and others may conform rather than express honest opinions (Stewart and Shamdasani, 2014). A one-to-one interview approach was used in the experiments in this thesis to obtain individual accounts of specific experiences during action observation. The one-to-one approach also enabled the interview to take place immediately after the experiment, so the likelihood of forgetting and attrition as a result of arranging a second session would be reduced.

Once the form of the interview has been decided, multiple considerations need to be made regarding the design of interview questions. One such consideration is whether to employ an inductive or deductive approach. An inductive approach tends to be driven by the interview data. This requires the researcher to approach the study without preconceived ideas of the nature of the findings to result in the

generation of general theories (Creswell, 2013). In contrast, a deductive approach uses pre-existing theory and hypotheses to guide the design of questions and probes. This ensures that the data gathered can be used to explore specific, deductive, hypotheses (Creswell and Plano Clark, 2017). As the interviews used in this thesis were employed to enrich quantitative data collected from TMS and eye-tracking techniques, a deductive, hypothesis-driven, design was chosen to be more appropriate.

Additional considerations lie within the design of the questions themselves. The questions must be designed in such a way as to not lead the participant to the response (Purdy, 2014), be that with its content, structure, or wording (Moser and Kalton, 2017). They should also contain simple language, with the avoidance of ambiguous words, and technical terms and jargon, unless appropriate for the target population (Purdy, 2014; Moser and Kalton, 2017). Additional considerations include the avoidance of presuming questions, questions that contain more than one question, and embarrassing questions (King and Horrocks, 2010; Purdy, 2014; Moser and Kalton, 2017).

Probes can be employed to expand further on the initial questions to ensure a thorough consideration and response from each participant (Pope and Mays, 2006; Brinkmann and Kvale, 2015). The use of probes can range from specifically designed follow-up questions to spontaneous follow-up questions and the use of silence. Designing probes prior to the experiment enables a thorough consideration of all elements that are predetermined to be appropriate, whilst spontaneous probes can be used to encourage participants to elaborate on specific, unplanned, lines of

discussion. In addition, silence can be used to encourage the participant to continue and elaborate on their discussion (Purdy, 2014; Brinkmann and Kvale, 2015). Such probes enable the pre-existing theory to be covered entirely, whilst still giving each participant opportunity to discuss their own individual experiences. Most of the probes used in the interviews included in this thesis were predetermined, with the freedom for the use of both silence and spontaneous probes where appropriate to ensure a thorough and elaborate consideration of the discussed topics.

3.5.2. Interview data analysis

The qualitative data analysis procedure is usually determined prior to the interviews, as the method of analysis will inform the design and development of the interview guide, process, and transcription method (Brinkmann and Kvale, 2015). Most commonly used methods of interview analysis involve qualitatively assigning codes to relevant passages of the interview. The aim of analysing these codes is to provide a detailed account of an individual's experience. Grounded theory and thematic analysis are two such examples that start similarly with the coding but result in the development of theory (McLeod, 2011) or a description of the commonly occurring patterns (Braun and Clarke, 2006) respectively.

As grounded theory aims to generate a theory, the researcher does not adhere to a preconceived theoretical framework (Allan, 2003). As such, a research question may not be required as the theory develops throughout the interview process (Brinkmann and Kvale, 2015). This can result in high ecological validity due to the theory being grounded in the collected data (McLeod, 2011), though it is considered impossible to completely disregard pre-existing theoretical frameworks

(Thomas and James, 2006). As thematic analysis does not require anchoring to a pre-existing theoretical framework either (Braun and Clarke, 2006), the researcher's own pre-existing theoretical frameworks may be inadvertently applied (Thomas and James, 2006). This is an issue with an inductive approach to qualitative analysis, where the results should be driven from the data, and not the researcher's own experiences and beliefs (Braun and Clarke, 2006).

Thematic analysis does not only have to be used to analyse qualitative data inductively, but can instead be used deductively to test specific hypotheses (Braun and Clarke, 2006). This still results in a rich description of occurring patterns but is driven by the researcher's theoretical interest (Braun and Clarke, 2006). It also avoids the issue relating to pre-existing theoretical frameworks, as the researcher's theoretical framework is both acknowledged and accepted as a fundamental methodological component of the analysis (Thomas and James, 2006). This results in a less rich description of the overall data, but a more detailed analysis of specific elements related to the research question (Braun and Clarke, 2006). A deductive, thematic approach was deemed most appropriate for the qualitative analysis, considering the specific research questions used in the studies included in this thesis due to the quantitative elements.

Chapter 4. General Methods

This chapter provides an overview of the general methods used in each of the subsequent studies included in this thesis. This includes descriptive participant information, TMS, eye-tracking, and interview procedures, and methods of data analysis. Specific details and methods unique to each experiment are discussed in the respective chapters.

4.1. Participant information

A priori power analyses were conducted using G*Power 3.1.9.2 to determine the necessary participant size for each experiment included in this thesis. Calculations used accepted alpha ($\alpha = 0.05$) and power parameters ($\beta = 0.80$), and effect sizes (*f*) individual to each experiment that were calculated based on previous research. Effect sizes are not commonly presented in the relevant literature, but were determined to be 1.79 for Experiment 1 (based on Maeda et al., 2002), 0.61 for Experiment 2 (based on Amoruso and Urgesi, 2016), and 1.21 for Experiment 3 (based on Nogueiro-Campos et al., 2014 and Nogueira-Campos et al., 2016). Due to the large effect sizes, power analyses indicated necessary participant sizes much smaller than those commonly used for TMS research. These small participant sizes may not be sufficient to overcome the commonly occurring inter-individual differences (see Chapter 3.3.3 and 3.3.4). As such, sample sizes were calculated with a medium effect size (*f*) of 0.25 (Cohen, 1988), resulting in participant sizes of 24 for all experiments.

The EHI (Oldfield, 1971; see Appendix 2) was used in each experiment to measure participants' handedness. Research has indicated that a participant's handedness

may be a factor in altering MEP amplitudes (Yahagi and Kasai, 1999; Sartori et al., 2013), although action observation research using TMS continues to demonstrate modulated CSE whilst including both right- and left-handed participants (e.g., Romani et al., 2005; Enticott et al., 2010; Wright et al., 2018). Participants were not excluded, therefore, based on handedness, though handedness was recorded to provide clarity of the methods and complete descriptive information of the participants. The Transcranial Magnetic Stimulation Adult Safety Screen (Keel et al., 2001; Appendix 1) was used to ensure that no participants were predisposed to the possible adverse effects of the stimulation. Volunteers indicating susceptibility to adverse effects were excluded prior to the experiment, and no participants reported discomfort or negative reactions during any of the experiments. After reading an Information Sheet for Participants (see Appendix 3), participants provided full written consent by signing an Informed Consent Form (see Appendix 4) prior to participation. The protocols were granted ethical approval by the Manchester Metropolitan University local ethics committee and were conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013).

4.2. General TMS procedure

4.2.1. Familiarisation

Prior to the experimental setup and procedure, each participant was individually familiarised with the laboratory, equipment, and procedure. The purpose of this was to provide the participants with sufficient information to enable them to provide informed consent and aid in reducing any anxiety linked to participation.

The aim was that, in turn, this might improve participant experience and reduce the likelihood of adverse effects (Groppa et al., 2012).

First, during the recruitment phase, each prospective participant was provided with an Information Sheet for Participants (see Appendix 3), and were encouraged to ask any questions they may have. Immediately prior to taking part in the experiment, participants were guided around the laboratory, shown where they would be seated during the experiment, and where the experimenter would be sat in relation to them. They were then shown the head- and chin-rest and explained how the restrictive setup was necessary to ensure accurate coil placement, but could cause possible discomfort. At this stage, participants were reassured of the maximum duration that they would be in the setup at any one time (8-10 minutes), that they would be permitted breaks at regular intervals during the experiment, and that they could withdraw at any time without having to state a reason.

The TMS coil was then shown to the participant, with an explanation of how it works, what it does, and what they would experience as a result of the stimulation. To demonstrate the muscular contraction that TMS triggers, a video was shown of a previous participant in the complete setup, including head- and chin-rest, and a TMS-induced muscular contraction in the right hand. The experimental procedure was then explained verbally, including the nature and number of videos that would be seen, and the number of phases of the experiment. Each participant was then asked if they understood and had any questions and if they were still willing to participate in the experiment. Finally, participants were reminded of their right to withdraw, which no participant exercised.

4.2.2. Electromyography procedure

EMG recordings were collected simultaneously from the mid-point of the muscle belly of the FDI and ADM of the right hand using bipolar, single differential, surface electrodes (DE-2.1, Delsys Inc, Boston, MA). A reference electrode was attached over the ulnar process of the right wrist. All electrodes and electrode sites were cleaned using alcohol wipes prior to electrode attachment. A double-sided sticker was attached to each electrode along with a thin layer of conductive gel along the metal strips to maintain a sound contact with the skin (Figure 4.1). Baseline EMG recordings were collected using Signal 4.11 following electrode attachment during rest, and during the abduction and adduction of the right index and little finger to test the FDI and ADM muscles respectively. Correct and clean electrode placement was determined to be when peak-to-peak amplitudes were less than 30μV at rest and greater than 1500μV during activity of the FDI and ADM (Figure 4.2). During the experiment, the EMG signal was recorded using Spike2 6.18 software (Cambridge Electronic Design, Cambridge, UK) via a Micro 1401-3 analogue-to-digital converter (Cambridge Electronic Design, Cambridge, UK), with a sampling rate of 2kHz, bandwidth of 20Hz to 450kHz, 92dB common mode rejection ratio and $>10^{15}\Omega$ input impedance.

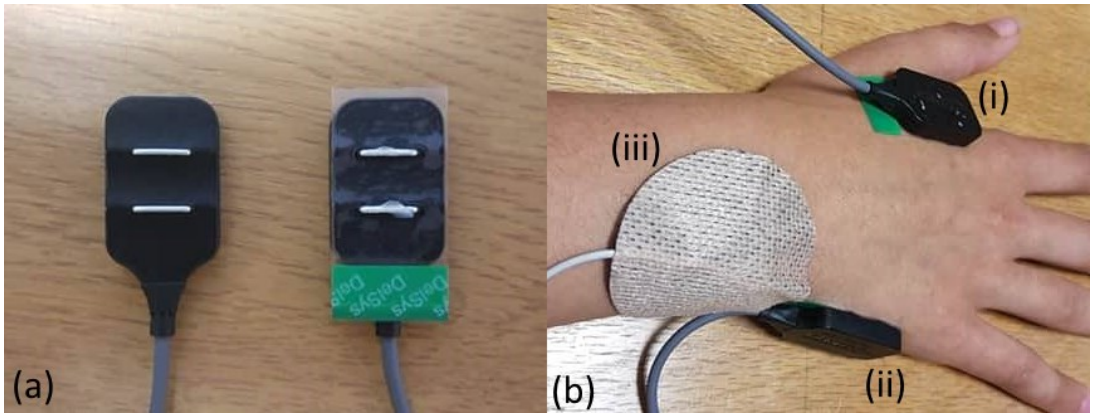


Figure 4.1. Electrode preparation (a), showing a clean electrode (a, left) and an electrode prepared with a double-sided sticker and conductive gel (a, right), and subsequent electrode placement (b) on the FDI (i) and ADM (ii) muscles, with a reference electrode over the ulnar process (iii)

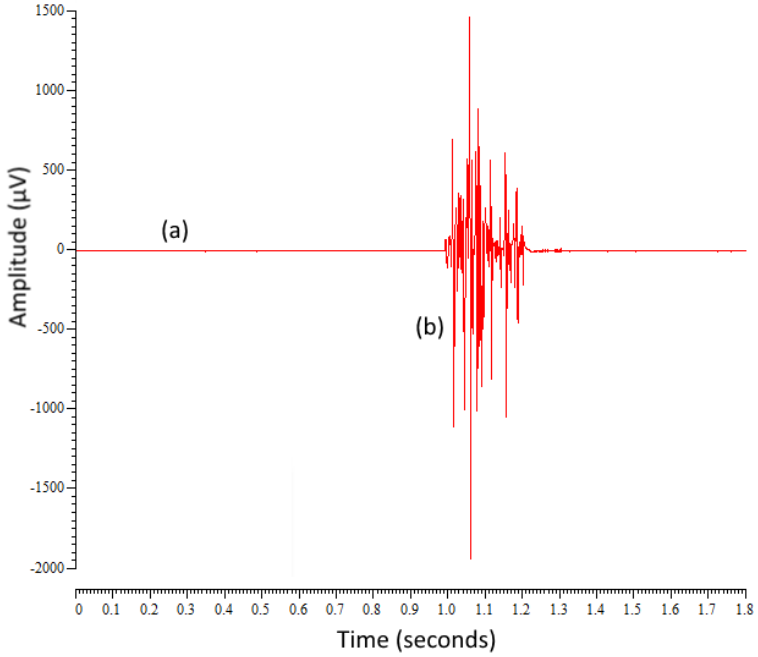


Figure 4.2. Baseline EMG procedure, demonstrating the FDI muscle at rest (a; 7.02µV) and during contraction (b; 3406.52µV)

4.2.3. TMS procedure

Following the EMG procedure, the remaining TMS procedure involved delivering monophasic pulses with a maximum field strength of 2.2 Tesla through a figure-of-eight coil (two 70mm diameter loops) connected to a Magstim 200^2 magnetic stimulator (Magstim Co., Whitland, Dyfed, UK). The TMS procedure followed the

published guidelines of Loporto et al. (2011). The coil was fixed in place over the hand representation of the left motor cortex throughout the experiment with a mechanical arm (Manfrotto™, Cassola, Italy) and was orientated for the induced current to flow in a poster-anterior direction by positioning the coil at a 45° angle to the midline between nasion and inion landmarks of the skull (Figure 4.3). This orientation was used to achieve indirect trans-synaptic activation and optimal MEP amplitudes (Sakai et al., 1997; Opitz et al., 2013). Maintaining consistent coil positioning is important, as slight movements can alter the positioning and rotation of the TMS coil, potentially influencing MEP amplitudes significantly (Boniface et al., 1990; Balslev et al., 2007; Chapter 3.3; Supplementary material 1). To ensure this, the mechanical arm was used to maintain coil position and a chin- and head-rest was used to ensure minimal head movement by the participant.

4.2.3.1. Optimal scalp position

To determine the area of the brain to be stimulated during the experiment, each participant's OSP needed to be identified. The participant was asked to wear a tightly fitting polyester cap, so that head measurements could be marked easily and clearly without drawing on their scalp. The participant's head was then measured to identify the approximate location of the right-hand region of the motor area. The central midline (Cz) point was measured using the International 10-20 System (Jasper, 1958; Figure 4.4). This was calculated by first measuring the distance between the participant's nasion and inion landmarks across the anterior-posterior plane of the skull. The midpoint was measured and marked on the cap. A following measurement was taken perpendicular to and directly through the previous mark,

from the left to the right preauricular point. Again, the midpoint was measured, and the intersecting point of the two marks was used as Cz.

The modal location of the motor area for the right hand FDI muscle OSP is 4cm left lateral and 1.5cm anterior from Cz (Loporto et al., 2013; Figure 4.5). This was used as the starting point for determining a participant's OSP. The TMS coil was fixed against this starting point and used to deliver four stimulations at 60% maximal stimulator output (MSO; Loporto et al., 2013). The coil was moved in 1cm steps around the starting point, stimulating each area four times at intervals of approximately seven seconds, until the area achieving the highest peak-to-peak amplitudes in the experimental muscle (i.e., the FDI), and consistent amplitudes in the control muscle (i.e., the ADM) was found. The outline of the coil at the OSP was then traced on the cap to allow for correct and consistent coil positioning throughout the experiment (Figure 4.5).

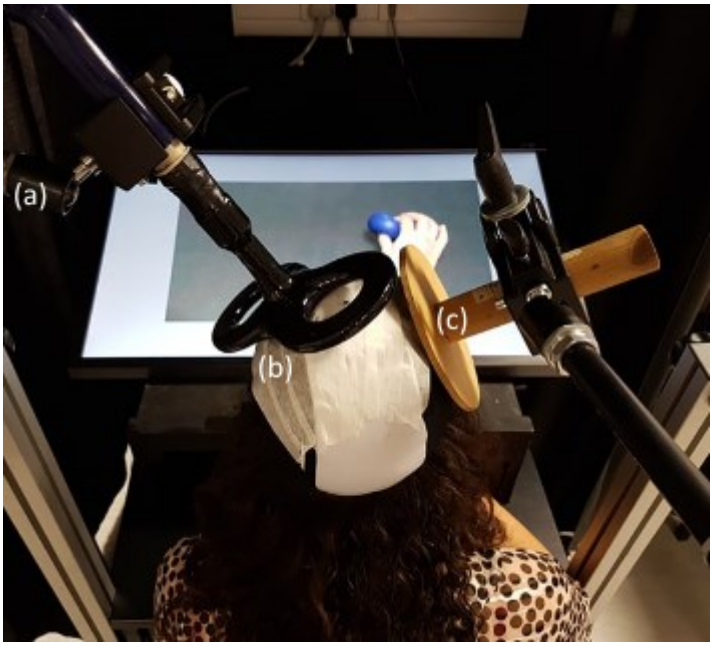


Figure 4.3. The mechanical arm (a) used to maintain the TMS coil position and orientation (b) and the head-rest position (c)

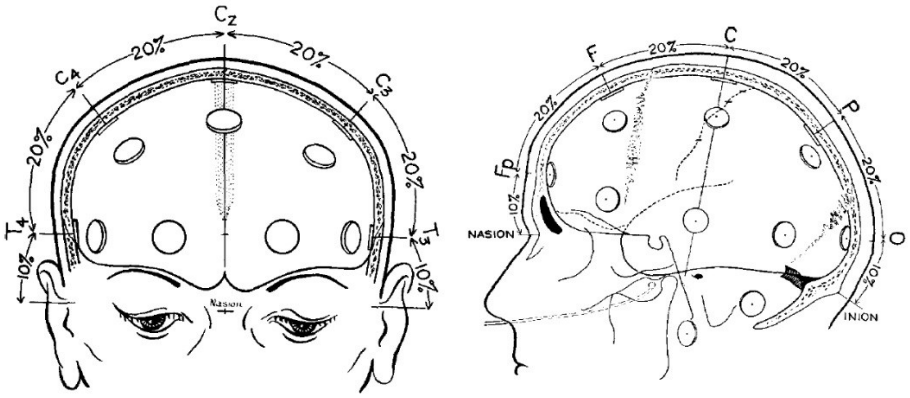


Figure 4.4. The International 10-20 system (Jasper, 1958) used to position the TMS coil

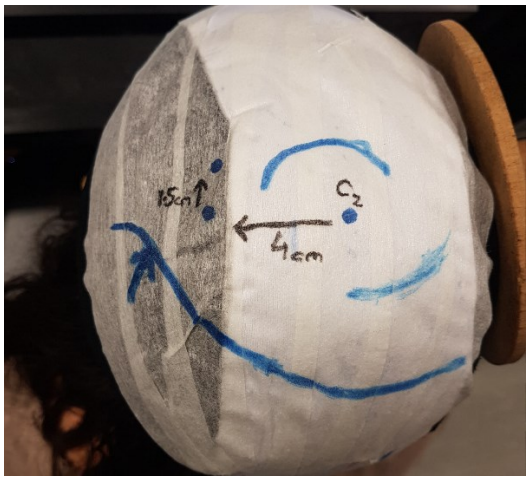


Figure 4.5. The OSP starting point of 4cm lateral and 1.5cm anterior to Cz, and the coil outline marked on a tightly fitting cap

4.2.3.2. Resting motor threshold

As there are individual differences within the excitability of corticospinal pathways, skull thickness, and other anatomical features (Ziemann et al., 1998; McConnell et al., 2001; Stokes et al., 2005), it is important to standardise the experimental stimulation intensity for each individual participant. This was accomplished by setting the experimental stimulation intensity at a predetermined percentage of each individual participant's RMT.

After determining the OSP, the coil was kept in place to determine the RMT. The stimulation intensity was gradually reduced in steps of 1-3% MSO until peak-to-

peak MEP amplitudes of 50μV or more were found in 5 out of 10 trials (Rossini et al., 1994). This stimulation intensity plus 1% MSO was defined as the RMT (Rossini et al., 2015). The experimental stimulation intensity was then set at 110% RMT to reduce any chance of direct wave stimulation (Loporto et al., 2013).

4.3. Eye-tracking procedure

Where possible (i.e., Experiment 2 and Experiment 3), participants' eye movements were recorded alongside the TMS protocol using iView ETG 2.7 software (SensoMotoric Instruments, Teltow, Germany) at a sampling rate of 60Hz. The eye-tracking glasses (ETG 2w; SensoMotoric Instruments, Teltow, Germany) contained two cameras and projected six infrared lights onto both of the participants' eyes to record eye movements. A circular cursor indicated the location of gaze in the visual scene recorded from a forward-facing camera to an accuracy of 0.5°. To calibrate the glasses, a three-point calibration was used on a five-point grid prior to testing. Calibration was monitored throughout the experiment and, if necessary, adjusted before the onset of the following block.

4.4. Interview procedure

Where individual meaning was explored (i.e., Experiment 1 and Experiment 3), a one-to-one deductive semi-structured interview was conducted with each participant on the completion of the TMS protocol to explore their experiences of the action observation and its components. An interview script was created to ensure that all necessary descriptors of motor imagery and action observation were covered with each participant. Each question was designed to be open-ended and with a number of possible probes. This, alongside the use of spontaneous probes

and silence, was to ensure a thorough consideration and response from each participant, and a thorough understanding of each individual's experiences (Pope and Mays, 2006; Purdy, 2014).

The questions and probes were deductively designed to explore both the elements related to the specific variables manipulated in each experiment and the commonly recurring themes in action observation and motor imagery research (see Holmes and Calmels, 2008 for a review of these themes). Using pre-existing theory and hypotheses to guide the questions and probes ensured that the data would be specific enough to explore specific, deductive, hypotheses (Creswell and Plano Clark, 2017) and, in the case of this thesis, enrich the quantitative TMS and eye-tracking data.

4.5. General procedure applied to all studies

General experimental procedures and data analyses are discussed below. However, with the exact parameters of each experiment differing slightly, the specific protocols and statistical analyses used are discussed in the respective chapters.

4.5.1. Experimental procedure

Participants were seated at a distance of 1m in front of a 32" television screen (Pilot and Experiment 1: LCD, DGM Model LTV-3203H; Experiment 2 and 3: LED, Samsung U32E850R) with their elbows flexed at 90° and their hands pronated in a relaxed position on a table directly in front of them. A chin- and head-rest was used to reduce head movements. Participants were asked to refrain from any voluntary movement during each condition and to attend fully to the stimuli presented on the

television screen. Blackout curtains were drawn alongside the television and table setup to reduce distracting visual stimuli. The exact laboratory set-up for each experiment is demonstrated in the respective chapters. One stimulation was delivered per trial at the point of maximal FDI contraction of the observed action. Two stimulation timings were used in each experiment where possible (i.e., Pilot, Experiment 1, and Experiment 2) to reduce the predictability of the stimulation (Loporto et al., 2012). Participants were given a break of approximately two minutes between each block. The specific experimental protocols for each experiment are discussed in the respective chapters.

4.5.2. Data analysis

4.5.2.1. TMS

A pre-stimulus recording of 200ms prior to the TMS pulse was used to identify trials influenced by muscle activity immediately prior to the stimulation. Individual trials in which the baseline peak-to-peak amplitude was 2.5 standard deviations greater than the mean baseline were discarded from further analysis (Loporto et al., 2012). This resulted in 4.59% of MEP data being discarded across all experiments (see Appendix 5 for further information on discarded MEP data). The number of rejected trials were analysed using a two-way repeated measures ANOVA to check for differences between the number of rejected trials per block. In addition, a two-way repeated measures ANOVA was used to check for differences in the remaining pre-stimulation EMG activity across blocks.

To account for both inter- and intraindividual variability in TMS-induced activity, raw MEP data were transformed into *z*-scores. *Z*-score normalisation indicates the

number of standard deviations each data point is around the mean. This method is common practice amongst TMS action observation studies (e.g., Fadiga et al., 1995; Urgesi et al., 2006; Aglioti et al., 2008). The data was then analysed using repeated measures ANOVAs and post-hoc pairwise comparisons (see Chapter 4.5.2.3 and the respective experiment chapters).

4.5.2.2. Eye-tracking

Individual trials were analysed by drawing AOI around different elements of the video, such as the hand, object, and background. A process of manual fixation-by-fixation mapping of fixation locations to the different AOI on a reference image was utilised to ensure that fixations were assigned to the appropriate AOI. The number of fixations and percentage fixation duration were calculated within each AOI throughout the experiment using BeGaze 3.7 software (SensoMotoric Instruments, Teltow, Germany). A fixation was defined as any gaze that remained within 1° ± 0.5° of visual angle for a minimum duration of 100ms (Salvucci and Goldberg, 2000). Once fixation count and percentage fixation duration were calculated, the data were analysed using repeated measures ANOVAs and post-hoc pairwise comparisons (see Chapter 4.5.2.3 and the respective experiment chapters).

The purpose of the eye-tracking data analysis was to understand where participants directed their visual attention under free viewing conditions during action observation, and how the inclusion of background context and emotional valence influenced this. As such, regression analyses were not used to explore fixations as a predictor of MEP amplitude. Had the purpose been to explore the effect directing attention on CSE such analyses would be useful (e.g., Wright et al., 2018). Within

the experiments that utilised eye-tracking in this thesis, however, each condition was observed under free-observation conditions, rendering any conclusions regarding how direction of visual attention modulates CSE inconclusive. Instead, the eye-tracking metrics were used to enable a greater understanding of any CSE modulation during the observation of a movement as a complete visual experience.

4.5.2.3. General quantitative analysis

All quantitative data were analysed using IBM SPSS Statistics 22. Repeated measures ANOVAs were used to explore the MEP and eye-tracking data. Significant effects were explored further using post-hoc pairwise comparisons with Bonferroni corrections. Where Mauchly's test indicated that the assumption of sphericity was violated, corrections of the degrees of freedom were applied following the recommendations of Girden (1992). The Huynh-Feldt correction was applied when epsilon exceeded 0.75, whilst the Greenhouse-Geisser correction was applied when epsilon was less than 0.75. The alpha level for statistical significance for all analyses was set at $\alpha = 0.05$. ANOVA effect sizes are reported as partial eta squared (η_p^2) and pairwise comparison effect sizes are reported as Cohen's *d*. The specific analyses used are discussed in the respective chapters.

4.5.2.4. Interview

A deductive thematic analysis following the procedure outlined by Braun and Clarke (2006) was used to analyse the qualitative data. The data analysis procedure appears linear, but it is important to clarify that it was a recursive procedure, with constant reflection and returning to the data to clarify and develop each step (Braun and Clarke, 2006).

The first stage involved transcribing the data, before reading and re-reading to become completely familiar with the data. Braun and Clarke (2006:87) describe this as 'reading the data in an active way' and involves searching for meaning within the data whilst reading and taking notes for initial coding ideas. These initial coding ideas then form the basis of the interview codes. The codes identified interesting or key features within the data and were specific to typical descriptors of motor imagery and action observation, such as visual perspective and movement agency (see Holmes and Calmels, 2008). At this stage, the third supervisor (ZCF) coded a portion of the interviews independently using the same descriptors of motor imagery and action observation. The independent codes generated by the two authors were discussed within the experimental team until agreement was reached to ensure reliable coding and consistent use of terminology. This was to ensure the validity of the interview data and the lead experimenter's interpretations.

The next phase involved collating all relevant codes into potential themes and sub-themes, considering relationships between codes, sub-themes, and themes. Initial thematic maps were developed at this stage to benefit the visualisation of the themes (Braun and Clarke, 2006). These initial themes were refined by taking into consideration the specific coded data extracts within each theme, and how these relate to the sub-themes, themes, and entire data set. As the reviewing and refinement continued, the thematic maps were adapted until a satisfactory thematic map had been developed.

The themes were then defined, with reports developed to identify its story, and how it fits into the wider story of the data as a whole. The report-writing stage was

then treated as the final opportunity to analyse the data (Braun and Clarke, 2006). Appropriate data extracts were found and provided within the detailed report, finally to be related back to the hypotheses and literature. Throughout the qualitative analysis, coding and data management were facilitated using NVivo qualitative data analysis software (version 11).

The methodological considerations discussed in Chapter 3 and the general methods outlined in this chapter were utilised in the experiments included in this thesis. The coming chapter outlining the Pilot Experiment (Chapter 5) was the final methodological consideration. Single- and paired-pulse TMS were compared during an action observation experiment to inform the TMS technique to be used in the subsequent experiments. As determined by the Pilot Experiment, single-pulse TMS was utilised across all experiments (outlined in Chapters 6-8), alongside combinations of the additional techniques outlined in this chapter.

Chapter 5. Pilot experiment

Exploration of single- and paired-pulse transcranial magnetic stimulation techniques during action observation.

5.1. Introduction

Since the seminal work of Fadiga et al. (1995), who demonstrated facilitated CSE during action observation compared to control conditions, single-pulse TMS (spTMS) has become a popular technique to test activity in the motor regions of the brain during action observation (see Loporto et al., 2011; Naish et al., 2014; Chapter 2.4 for reviews). Specifically, this technique uses a suprathreshold test stimulation (TS) to generate a MEP, the amplitude of which provides a marker CSE (Bestmann and Krakauer, 2015; Ruffino et al., 2017). This technique provides a useful indicator of overall activity in the extended motor system, although specific neural networks can be overlooked. Paired-pulse TMS (ppTMS) utilises a subthreshold conditioning stimulation (CS) immediately prior to the TS used in spTMS, providing a measure of corticocortical excitability through separate networks: ICI at an interstimulus interval (ISI) of 1-5ms prior to the TS, and ICF at an ISI of 6-20ms prior to the TS.

When applied to the motor regions of the brain, the TS evokes either D- (direct) or I- (indirect) waves (Day et al., 1989; Chapter 2.3). If the induced current flow in the motor cortex following a TS is in a posteroanterior direction (see Chapter 3.3.1), and the TS intensity is not too high (Loporto et al., 2011; Chapter 3.3.3), I-waves are evoked. This is due to the descending volleys being of a trans-synaptic origin and results in the reflection of motor cortex excitability in the recorded MEP amplitude (Sakai et al., 1997; Opitz et al., 2013). It is difficult, however, to identify the nature

of any CSE modulation, as CSE reflects both inhibition and facilitation mechanisms (Nakamura et al., 1997; see Chapter 2.3).

A subthreshold CS paired with a suprathreshold TS allows for exploration of the individual ICI and ICF networks in the brain, which provides a better indicator of corticocortical excitability at the point of stimulation. The first evidence of ICI and ICF in humans was provided using ppTMS (Kujirai et al., 1993). When a CS was delivered 1-6ms prior to the TS, the resulting MEP was inhibited. A facilitatory effect on the MEP was demonstrated when the CS was delivered 10 and 15ms prior to the TS. This finding has since been replicated consistently (e.g., Nakamura et al., 1997; Abbruzzese et al., 1999; Strafella and Paus, 2001), with later evidence narrowing down the ISI ranges to 1-5ms for ICI (Rothwell et al., 2009) and 6-20ms for ICF (Ziemann et al., 1996).

At a low sub-threshold stimulation intensity, approximately 80% RMT (Ziemann, 2001), the CS avoids evoking significant descending volleys. This is evidenced by epidural spinal cord recordings (e.g., Nakamura et al., 1997; Di Lazzaro et al., 1998), and a lack of change in the H-reflex following both ICI (Kujirai et al., 1993) and ICF (Ziemann et al., 1996). Such findings indicate that any modulation in MEP amplitudes occurs at a corticocortical level. Further evidence exploring individual I-waves indicate that modulation occurs upstream of corticospinal neurons (Di Lazzaro et al., 1998; Hanajima et al., 1998; Trompetto et al., 1999) and that ICI and ICF networks have separate mechanisms (e.g., Kujirai et al., 1993; Ziemann et al., 1996; Strafella and Paus, 2001). This is in contrast to spTMS which explores inhibition and facilitation networks simultaneously as a measure of overall cortical

output (Ni and Chen, 2008; Rothwell et al., 2009). Using ppTMS to explore the individual inhibition and facilitation networks separately during action observation allows for a better understanding of the effects of a variable at a corticocortical, rather than corticospinal, level.

Even though ppTMS offers the potential to explore individual mechanisms, it is rarely used in action observation research, where the use of spTMS is more common. Facilitation of CSE during action observation in comparison to control conditions is commonly demonstrated using spTMS (e.g., Fadiga et al., 1995; see Naish et al., 2014). This may not indicate, however, the nature of any modulation of the individual inhibitory and facilitatory circuits (Ni and Chen, 2008; Rothwell et al., 2009). In one of the few ppTMS experiments to test this effect, Strafella and Paus (2000) demonstrated a reduction in ICI (3ms ISI) and ICF (12ms ISI) during action observation compared to control conditions. Similarly, Patuzzo et al. (2003) demonstrated facilitated CSE using spTMS and reduced ICI using ppTMS during action observation in comparison to the control conditions. In addition, in contrast to Strafella and Paus (2000), Patuzzo et al. (2003) found no modulation in ICF during action observation. Such corticocortical excitability findings enable an in-depth mechanistic exploration of the observation-execution matching mechanism, with Patuzzo et al. (2003) demonstrating that CSE modulation shown with spTMS may be a result of inhibitory, rather than facilitatory mechanisms.

With the ability to explore individual neural inhibitory and facilitatory circuits, ppTMS appears to be ideal in testing the effects of action observation by providing an indicator of corticocortical activity, rather than the corticospinal activity

indicated by spTMS. However, there are limited findings on these effects to date. As such, the present experiment aimed to explore single- and paired-pulse paradigms during action observation to develop the skills and knowledge to perform TMS action observation experiments. In order to gain a full understanding of TMS, a pilot comparison of single- and paired-pulse techniques was performed. It was hypothesised that, in comparison to spTMS, ppTMS MEP amplitudes would be (i) inhibited at 3ms ISI and (ii) facilitated at 12ms ISI. In addition, it was hypothesised that, in comparison to a static control image, action observation would result in (iii) a facilitation of CSE during spTMS, and (iv) a reduction of ICI during ppTMS with a 3ms ISI. Due to the mixed findings in previous research, no hypothesis was made regarding the effect of action observation on ICF during ppTMS with a 12ms ISI.

5.2. Methods

5.2.1. Participants

Five right-handed individuals (3 male, 2 female, mean LQ 87.54 ± 5.14) aged 22-31 years (mean age 26.80 ± 3.49 years) participated in this experiment. A small sample size was used to pilot the ppTMS procedure, and to learn how to use TMS and become familiar with the TMS procedure.

5.2.2. Procedure

The EMG and TMS procedures used, including the OSP procedure, were as outlined in Chapters 4.2.2 and 4.2.3, with the exception of the stimulation type and experimental stimulation intensity. This experiment involved both spTMS and ppTMS. The RMT procedure was identical (as per Chapter 4.2.3.2), with the addition

of the ppTMS experimental stimulation intensities being set at 80% (Ziemann, 2001) and 110% (Loporto et al., 2013) of RMT for the conditioning pulse, and test pulse respectively. Paired-pulse interstimulus intervals were set to 3ms for ICI and 12ms for ICF (Strafella and Paus, 2000). The modal OSP was 4cm lateral, 1.5cm anterior to Cz, and the mean RMT was 50.2% ± 10.61.

The general experimental procedure was as outlined in Chapter 4.5.1. Participants observed two 10-second videos: 1) an experimental video containing four index finger-thumb pinches of a blue foam ball at a rate of 0.4Hz; and 2) a control video of a static hand holding the same ball between the index finger and thumb (see Figure 5.1). The videos were presented across six blocks, with three blocks containing the experimental video and three blocks containing the control video. One stimulation type was delivered per video, and, in total, all three stimulation types were used for each video: 1) spTMS; 2) 3ms ISI ppTMS; and 3) 12ms ISI ppTMS. Five trials of each stimulation type were randomised within each 15 trial block. This resulted in 30 trials of each stimulation type and 45 trials of each video, for a total of 90 experimental trials. The stimulations were applied at the point of maximal FDI contraction (Gangitano et al., 2001) during either the third (6300ms) or fourth (8800ms) pinch of each video. This was to prevent the predictability of the stimulation (Loporto et al., 2012). Prior to each block, five stimulations were delivered whilst participants observed a black fixation cross on a white screen. These control MEPs were used to normalise the data in this experiment.

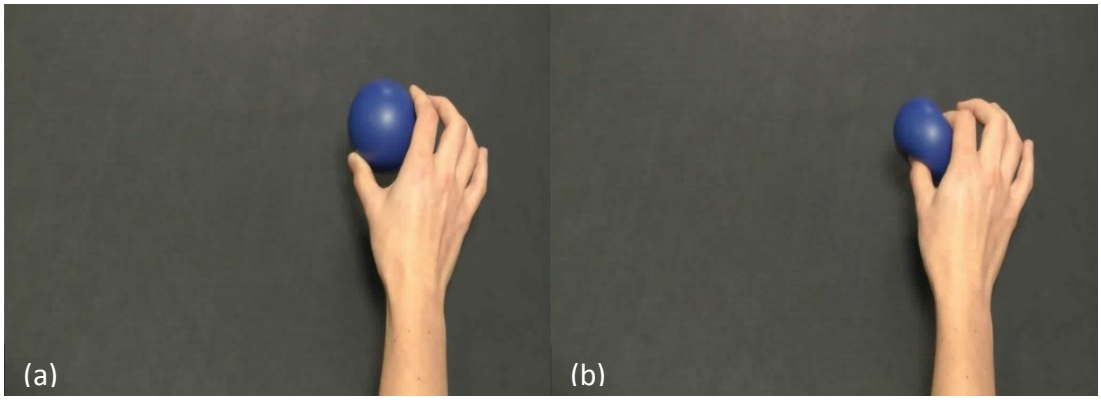


Figure 5.1. Still images from the control condition (a) and experimental condition (b)

5.2.3. Data analysis

Statistical analysis on five participants' data would not have sufficient power to detect significant results, so no such analysis was performed. Instead, descriptive comparisons of the data were implemented using percentage change values. The data for this experiment was normalised as a percentage of the unconditioned spTMS MEPs produced during the fixation cross condition. This was to allow for better comparisons with the results from previous literature, where data was also normalised as a percentage of a control MEP (e.g., Strafella and Paus, 2000).

5.3. Results

5.3.1. FDI data

The comparisons in the FDI muscle showed the smallest MEP amplitudes were recorded during the 3ms ISI ppTMS, followed by the spTMS, and the largest MEP amplitudes were recorded during the 12ms ISI ppTMS (see Table 5.1; Figure 5.2). Greater MEP amplitudes were recorded in the FDI during action observation compared to the static observation during the spTMS, 3ms ISI ppTMS and 12ms ISI ppTMS stimulation types (see Table 5.2; Figure 5.3).

5.3.2. ADM data

In the ADM muscle, the spTMS produced the smallest MEP amplitudes, followed by the 3ms ISI ppTMS, and the 12ms ISI ppTMS produced the largest (see Table 5.1; Figure 5.2). Greater MEP amplitudes were produced during action observation compared to the static observation during the 3ms ISI ppTMS and 12ms ISI ppTMS stimulation types. MEP amplitudes recorded from the ADM during the spTMS stimulation type were of similar size during both the action and static observation (see Table 5.2; Figure 5.4).

Table 5.1. Percentage change from the fixation cross values of the MEP amplitudes produced by each stimulation type compared to the other stimulation types for the right FDI and ADM muscles

	spTMS		3ms ISI		12ms ISI	
	3ms ISI	12ms ISI	spTMS	12ms ISI	spTMS	3ms ISI
FDI	15.47	-44.61	-15.47	-52.03	44.61	52.03
ADM	-39.77	-66.48	39.77	-44.35	66.48	44.35

Table 5.2. Percentage change from the fixation cross values of the MEP amplitudes produced by each stimulation type during the action compared to static conditions for the right FDI and ADM muscles

	spTMS	3ms ISI	12ms ISI
FDI	17.33	11.18	30.25
ADM	4.24	15.12	43.25

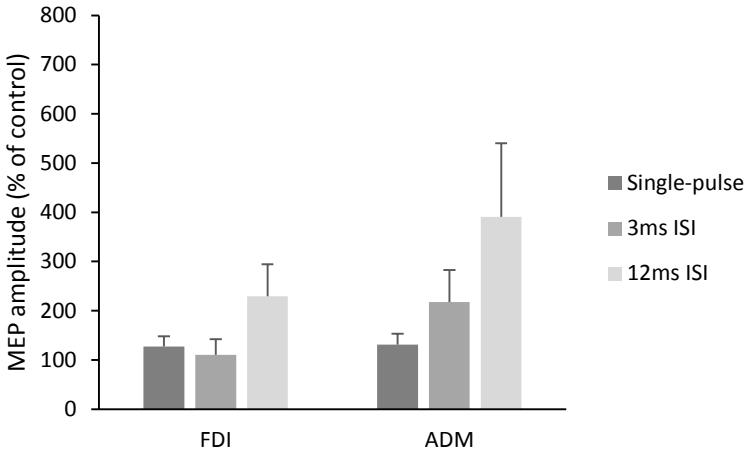


Figure 5.2. Mean MEP amplitudes, displayed as percentage of control, recorded during each stimulation type from both muscles

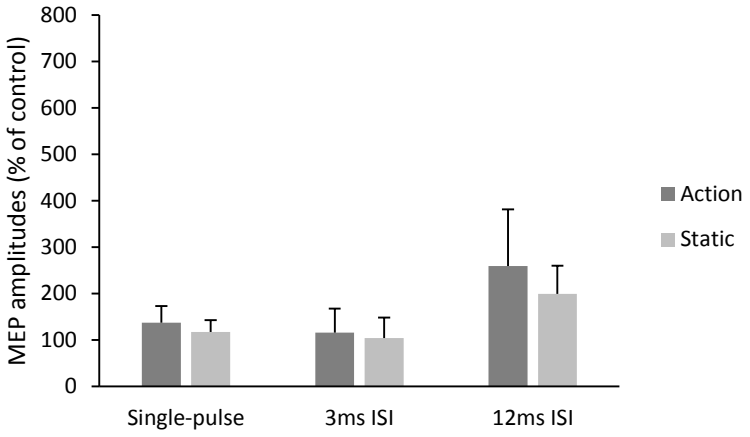


Figure 5.3. Mean MEP amplitudes, displayed as percentage of control, recorded during both conditions from the FDI muscle

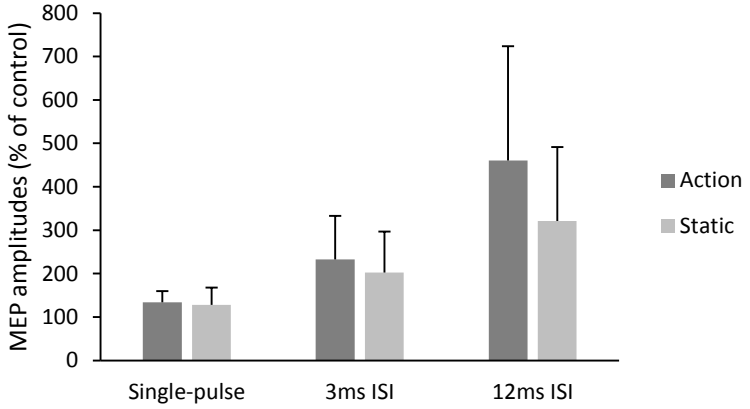


Figure 5.4. Mean MEP amplitudes, displayed as percentage of control, recorded during both conditions from the ADM muscle

5.4. Discussion

This experiment aimed to test the ppTMS protocol in comparison to the more commonly used spTMS protocol during action observation. The results would then be used to inform the TMS protocol utilised in the subsequent studies presented in this thesis.

5.4.1. 3ms ISI ppTMS inhibition

The first hypothesis stated that MEP amplitudes would be inhibited using ppTMS with an ISI of 3ms in comparison to spTMS. In partial support of the first hypothesis, percentage change comparisons demonstrated that ppTMS with a 3ms ISI resulted in a 15.47% inhibition of MEP amplitudes in the FDI muscle compared to spTMS. This supports previous findings that a subthreshold CS 3ms prior to a suprathreshold TS inhibits MEP amplitudes (Kujirai et al., 1993; Nakamura et al., 1997; Strafella and Paus, 2001). However, in contrast to previous findings, MEP amplitudes were facilitated in the ADM muscle at the same ISI by 39.77% compared to spTMS. This may reflect methodological issues within this experiment.

Using a single scalp position to stimulate two individually is a commonly used method in spTMS (Fadiga et al., 1995; Gangitano et al., 2004; Naish and Obhi, 2015), though it may result in a less-than-optimal stimulating site for one or both of the muscles. As the FDI was the target experimental muscle, it is possible that the control muscle (i.e., the ADM) was being stimulated outside of its OSP. Historically, this has not presented an issue within spTMS research, as this technique has been used consistently to good effect (e.g., Fadiga et al., 1995; see Loporto et al., 2013). This may be as spTMS generates an overall indication of CSE, reflecting both

inhibitory and facilitatory responses (Nakamura et al., 1997). With ppTMS exploring separate inhibitory and facilitatory mechanisms, however, it may pose a greater problem. This is apparent in the experimental stimulation intensity used. The intensity for the TS used was 110%, based on the spTMS recommendations by Loporto et al. (2013). However, the optimal stimulation intensity for the TS in ppTMS to elicit ICI is suggested to be 110-120% (Garry and Thomson, 2009). A TS lower than 110% has the potential to reduce ICI (Garry and Thomson, 2009), and so, with a less-than-optimal OSP for the ADM muscle, it is possible that the TS was less than 110% of the actual RMT to stimulate this muscle. This would result in the inhibitory effect not being shown in the ADM, as in the present experiment.

5.4.2. 12ms ISI ppTMS facilitation

The second hypothesis stated that MEP amplitudes would be facilitated using ppTMS with an ISI of 12ms in comparison to spTMS. With a 12ms ISI, the CS in ppTMS facilitated the TS MEP amplitudes in comparison to spTMS in both the FDI and ADM muscles by 44.61% and 66.48% respectively. This finding supports both the second hypothesis and previous research (Kujirai et al., 1993; Ziemann et al., 1996; Strafella and Paus, 2001). The issue of coil position present within ICI, discussed above, appears not to have as much of an effect on ICF as the facilitation effect was present for both muscles. It is possible that the facilitatory mechanism may not be as sensitive to changes in the stimulation intensity, and, therefore, may not require as accurate coil positioning as the inhibitory mechanism does. Previous research has only explored this issue within ICI (Garry and Thomson, 2009), however, so this postulate requires further exploration.

5.4.3. spTMS comparisons

The third hypothesis stated that spTMS MEP amplitudes would be facilitated during action observation compared to the static hand condition. The percentage change comparison of the spTMS data indicated a 17.33% facilitation of CSE during the observation of an index finger-thumb ball pinch compared to the static hand observation in the FDI muscle. This facilitation was muscle specific, as only 4.24% was present in the ADM muscle. The percentage change comparisons compare similarly to previous research indicating muscle specific CSE facilitation during action observation compared to a control condition (Fadiga et al., 1995; Gangitano et al., 2004; Loporto et al., 2012). This finding simultaneously reflects both inhibitory and facilitatory responses (Nakamura et al., 1997) through an overall measure of CSE.

5.4.4. 3ms ISI ppTMS comparisons

The fourth hypothesis stated that 3ms ISI ppTMS MEP amplitudes would be facilitated, indicating a reduction in ICI, during action observation compared to the static hand condition. Using ppTMS with a 3ms ISI, an 11.18% and 15.12% reduction in ICI was visible in both the FDI and ADM muscles respectively during action observation compared to static observation. This effect in the FDI supports the fourth hypothesis and previous findings (Strafella and Paus, 2000; Patuzzo et al., 2003), but the reduction in ICI in the ADM muscle was unexpected. ICI has shown muscle-specific effects, similar to those demonstrated in spTMS research, whereby MEP amplitude modulation is limited to the active muscle in the observed action (Fadiga et al., 1995; Strafella and Paus, 2000). This unexpected finding, however,

could be an extension of the OSP and stimulation intensity methodological limitations discussed above.

5.4.5. 12ms ISI ppTMS comparisons

Due to the mixed results regarding ICF during action observation found in previous research, no hypothesis was made for the 12ms ISI ppTMS MEP amplitudes during action and static observation conditions. Strafella and Paus (2000) reported a reduction in facilitation during action observation, and Patuzzo et al. (2003) demonstrated no significant modulation in ICF during action observation compared to controls. In the current experiment, 30.25% and 43.25% increases in ICF are present in both the FDI and ADM muscles respectively during action observation compared to static observation. If the ICF network is involved in an observation-execution matching mechanism as seen in spTMS (e.g., Fadiga et al., 1995; Montagna et al., 2005), then it could be hypothesised that ICF should be reduced, as it is during action execution (Ridding et al., 1995). The findings of (Strafella and Paus, 2000) support this, although the overall mixed findings regarding the ICF network raises questions whether or not it is involved in this mechanism. It is clear that further research is required to explore these individual neural networks during action observation.

5.4.6. Additional limitations and considerations

As this was a pilot experiment, the limitation of having a small sample size of five participants was accepted during the experiment's design process. This number of participants was chosen as a pilot sample to test the initial feasibility of replicating previous ppTMS research. The ppTMS experiments that have been discussed in this

chapter, however, used eight (Strafella and Paus, 2000) and ten (Patuzzo et al., 2003) participants. More recently, it has become common practice to test approximately 20 participants in TMS action observation experiments (e.g., Uithol et al., 2015; Wright et al., 2016; D'Innocenzo et al., 2017), as an insufficient sample size reduces the likelihood of finding an effect (Button et al., 2013). In addition, each of the six conditions only contained 15 trials. Due to the intraindividual variability of MEP amplitudes (Kiers et al., 1993; Choudhury et al., 2011), thirty trials are suggested to be sufficient to provide a reliable measure of CSE (Cuypers et al., 2014; Goldsworthy et al., 2016). This was not possible in this experiment, however, due to the large number of conditions. Six conditions containing 30 trials each would have doubled the number of trials and duration of the experiment, increasing the likelihood of participants becoming fatigued and altering their goal-directed attention as a result (Boksem et al., 2005). This would have raised ethical issues within the method, and so is an accepted methodological limitation.

The secondary aim of this experiment was to inform the use of either spTMS or ppTMS in future action observation experiments included in this thesis. The use of ppTMS allows a more detailed mechanistic understanding of activity at a corticocortical level but is not a perfect method. For example, it is postulated that ICI modulation is likely the summation of both ICI and ICF, and so cannot be interpreted as a modulation in inhibition or facilitation if it has only been tested at one CS intensity (Peurala et al., 2008). Also, ICI and ICF are affected differently at different CS intensities (Wagle-Shukla et al., 2009), so testing multiple CS intensities (Peurala et al., 2008; Wagle-Shukla et al., 2009) and measuring both ICI and ICF (Peurala et al., 2008) is required to obtain a rigorous measure of inhibition and

facilitation. In addition, there is evidence of high inhibition and facilitation variability between individuals (Orth et al., 2003; Du et al., 2014). Using one ISI and one CS will not sufficiently capture the unique inhibition and facilitation profiles of each participant (Orth et al., 2003; Du et al., 2014). Longer and more testing sessions would be required to maintain scientific rigour whilst accommodating for the multiple CS intensities and ISIs. By not utilising a CS, spTMS overcomes a lot of these problems by removing additional interindividual variability. This does prevent the exploration of inhibitory and facilitatory mechanisms, but still provides a useful marker of CSE: an overall indication of cortical and spinal excitability. Also, within action observation research, spTMS is widely used, whereas the use of ppTMS is lacking and, as a result, the role of ICI and ICF mechanisms during action observation are not fully understood.

Due to the limited sample size and lack of statistical analysis, conclusions cannot be drawn on the use of either spTMS or ppTMS in the subsequent studies based on the data presented in this experiment. Based on the literature discussed regarding the use of spTMS and ppTMS, however, it is clear that more research is required to understand the individual ICI and ICF networks and the role they have on cortical activity during action observation. As the primary aim of this thesis was to inform action observation interventions for motor (re)learning, the additional exploration required to understand inhibitory and facilitatory mechanisms fully would detract from the focus of the thesis. Therefore, the following experiments in this thesis all used spTMS to explore CSE during action observation.

Chapter 6. Experiment 1

Screen position preference offers a new direction for action observation research: preliminary findings using TMS.

[published as Riach, M., Wright, D. J., Franklin, Z. and Holmes, P. S. (2018) 'Screen position preference offers a new direction for action observation research: preliminary findings using TMS.' Frontiers in Human Neuroscience, 12(26)]

6.1. Introduction

As discussed in Chapter 2.4, TMS has been used extensively to explore CSE in the during action observation. This research has shown consistently that MEP amplitude, a marker of CSE (Ruffino et al., 2017; Chapter 2.3), is facilitated during action observation compared to control conditions (Fadiga et al., 1995; Naish et al., 2014; Chapter 2.4). The visual perspective from which the action is observed further modulates CSE (Chapter 2.4.1). Specifically, observing an action from a first-person visual perspective facilitates CSE compared to observing the same action from a third-person visual perspective (e.g., Maeda et al., 2002; Alaerts et al., 2009). In addition, greater neural activity is generated when the spatial reference frame of the observed action matches that of the observer (Vogeley and Fink, 2003), which can be achieved by presenting stimuli on a horizontal screen positioned over the observer's own limb. Despite the evidence supporting the use of a combined first-person visual perspective and egocentric reference frame for action observation, a vertical screen position has been the most frequently employed position for presenting videos in action observation experiments using TMS.

There are some instances of researchers utilising a horizontal screen position during action observation experiments (e.g., Kaneko et al., 2007; Wright et al., 2016). The

more common use of a vertical screen position, however, is understandable. Both television and computer screens are generally viewed at this orientation, and we view others' actions from a similar reference frame. When using vertically positioned screens to present first-person visual perspective self-agency actions, however, the observed action is viewed from an allocentric reference frame that is incongruent to the observer's viewing position. This may not be optimal for observation interventions attempting to create an egocentric, first-person visual perspective using a vertical screen. It would present a less embodied, more 'detached' action relative to the observer's own body, reducing a sense of ownership. In the case of viewing a forearm and hand aiming to mimic the viewer's own limb and hand, the presented action becomes an upward rotation (90°) in the sagittal plane away from the more anatomically accurate transverse plane in which self-executed hand and arm actions are normally viewed and executed. The vertical reference frame could make the movement seem biomechanically impossible due to the limb rotation and displacement of the observed action from the observer's body. This may modulate CSE during action observation as a result (Romani et al., 2005; Borroni et al., 2011). Observing actions through vertically orientated screens, therefore, mimics an other-based movement, even if the action is filmed from a first-person visual perspective.

Given these concerns, it would seem paradoxical that research continues to use vertical screen presentations for action observation. In order to represent the action with greater anatomical accuracy, the action observation stimuli should be presented on a horizontal screen in the observer's peripersonal space. This would provide an egocentric reference frame, and, therefore, retain a greater perception

of self-agency and ownership with the presented action. Such ownership and self-agency mechanisms are required to access the motor representation optimally (Jeannerod, 2001). A first-person visual perspective video observed on a horizontally angled screen located in the observer's peripersonal space, therefore, provides a more accurate egocentric reference frame for observation of hand and arm movements for both research and applied interventions.

Additional considerations should be made based on the affect relating to each participant's screen position viewing preferences (see Chapter 2.4.4.2). Perspective preference is an accepted methodological variable within motor imagery research (Hall, 1997; Calmels et al., 2006). With the shared partial neural substrate between motor imagery and action observation, individuals may also possess viewing preferences during action observation. Action observation viewing preference is evident within a third-person visual perspective (Ustinova et al., 2010), and, based on shared partial neural substrates of motor imagery and action observation, may modulate CSE based on this preference (see Chapter 2.4.4.2).

Taken together, it is important to investigate the corticospinal response of individuals viewing the same action on vertically and horizontally orientated screens, whilst also considering the influence of each participant's affect on action observation viewing preference. The first aim of this experiment was to determine whether different screen positions modulate CSE during observation of hand movements filmed from a first-person visual perspective and aiming to present self-agency. The second aim was to establish whether CSE was modulated when accounting for participants' viewing preference for screen position. It was hypothesised that a first-person visual perspective video viewed on a horizontal

screen would facilitate CSE to a greater extent than the same video observed on a vertical screen. Given the complexity of the inter-relationships, no directional hypotheses were made for viewing preference and CSE.

6.2. Methods

6.2.1. Participants

Twenty-four individuals (16 male, 8 female) aged 19-37 years (mean age 23.96 ± 4.41 years) participated in the experiment. Eighteen participants were right-handed (mean LQ 87.34 ± 9.98), and six were left-handed (mean LQ -29.98 ± 31.12).

6.2.2. Procedure

The general TMS and interview procedures were identical to those outlined in Chapters 4.2 and 4.4. The modal OSP was 4cm lateral, 1.5cm anterior to Cz, and the mean RMT was 46.71% ± 6.05.

The general experimental protocol was as outlined in Chapter 4.5.1. Each participant took part in four conditions. The two experimental conditions involved observation of an index finger-thumb pinch of a blue foam ball (Figure 6.1(b)) on either a horizontally positioned (15^{o} to the table, distance of 45cm, centre of the stimulus 25^{o} from eye-level, Figure 6.2(a)) or a vertically positioned (90^{o} to the table, distance of 90cm, centre of the stimulus 3.5^{o} from eye-level, Figure 6.2(b)) screen. The two control conditions required observation of a static hand holding the same ball between the index finger and thumb on either the horizontally or vertically positioned screen (Figure 6.1(a)). Conditions were split into four blocks of 30 trials, with each block containing 15 action observation and 15 static control trials presented in a random order, resulting in a total of 30 trials per condition. Two blocks were presented for the horizontally positioned screen and two for the

vertically positioned screen. The screen position presentation order was randomised. Due to the horizontal screen position requiring participants to look down, clear imaging of the eyes was difficult due to lowering of the upper eyelids. As such, eye-tracking was not used in this experiment.

The videos showed a Caucasian female's right hand and forearm filmed from a first-person visual perspective, with the hand positioned to the right of the screen to enhance the first-person visual perspective. A post-experiment manipulation check confirmed that the majority of participants perceived the observed hand to be of their own sex (71%). The action and static videos were of 10 seconds duration, with the action observation video containing four pinches at a rate of 0.4Hz. One stimulation was delivered per trial at the point of maximal FDI contraction during the second or third ball pinch, and at the same time points during the static videos (either 3650ms or 6150ms after video onset). There were 64 stimulations for each time point.

On completion of the TMS protocol, one-to-one deductive semi-structured interviews were conducted with each participant to explore their experiences of each screen position. Questions targeted action observation experiences such as visual perspective, movement agency, movement kinaesthesis and peripersonal space to analyse commonly recurring themes in action observation and motor imagery research (see Holmes and Calmels, 2008 for a review of these themes). Example questions included: "What were your opinions of the two different screen positions that you saw in the experiment?" and "What physical and emotional sensations were you aware of whilst watching the ball pinches?" Probes were used to ensure a thorough consideration and response from each participant. These

included “Can you describe these differences?” and “Was that present during one screen position more than the other, or about the same?” (see Appendix 6 for full interview guide).

Following this, all participants completed a bespoke questionnaire focusing on their affect and experiences during each screen condition. Example questions included: “How strongly did you feel that the hand you were watching was your own?” and “How strong was the feeling that you were performing the movement?” A 6-point Likert scale recorded responses ranging from 0 “*not at all like my own*” to 5 “*strongly like my own*”, and 0 “*no feeling at all*” to 5 “*very strong feeling*” respectively. Each question was answered once for each screen position, to allow for a comparison between the horizontal and vertical screen position. For the question, “On which screen did you prefer watching the ball pinch?”, a single 7-point Likert scale was used to allow for a middle, “*no preference*”, response (see Appendix 7 for full questionnaire).

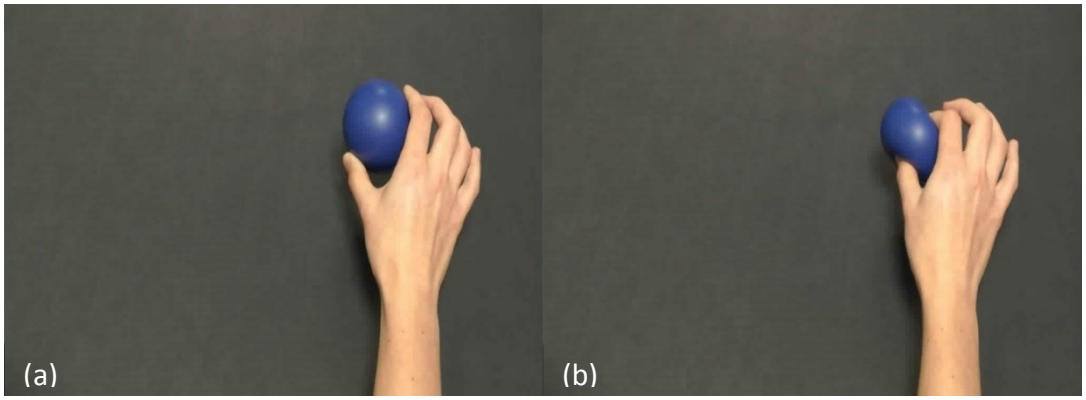


Figure 6.1. Still images from the control condition (a) and experimental condition (b)

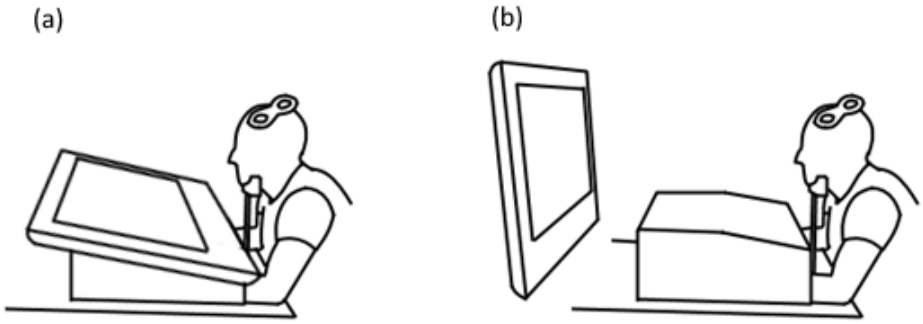


Figure 6.2. Experimental setup demonstrating the horizontal (a) and vertical (b) screen positions

6.2.3. Data Analysis

6.2.3.1. Overall TMS data

Data analysis for the MEP amplitudes was as outlined in Chapters 4.5.2.1 and 4.5.2.3. A 2 (screen position: horizontal, vertical) x 2 (video: action, static) repeated measures ANOVA was performed on all participants' *z*-score MEP data. Significant effects were explored further using post-hob pairwise comparisons with Bonferroni corrections.

6.2.3.2. Questionnaire and interview data

Data analysis for the interview data was as outlined in Chapter 4.5.2.4. Also, strategies to enhance analytic rigour included comparisons of themes between the questionnaire and interview responses. The themes and questionnaire response comparisons were verified further following discussion with the wider research team to ensure they were comprehensive and inclusive in relation to the themes relating to screen position preference. Due to the non-parametric nature of the data, Wilcoxon signed-rank tests were used on the questionnaire data to compare

the responses for the horizontal and vertical screen positions. These were then compared to the interview data where appropriate.

6.2.3.3. Screen position preference TMS data

Based on the analyses of the qualitative data, the *z*-score MEP data were grouped by screen position preference. Data analysis for the screen position preference group *z*-score MEP data was as outlined in Chapters 4.5.2.1 and 4.5.2.3. A 2 (screen position: horizontal, vertical) x 2 (video: action, static) repeated measures ANOVA was performed on the horizontal preference (*n* = 16, 5 female, 3 left-handed) group data. Descriptive data only is presented for the vertical (*n* = 7, 2 female, 3 left-handed) group data due to the small number of participants reporting a preference for this screen position. No analysis was performed on the no preference group data (*n* = 1, female, right-handed). In addition, descriptive data is provided plotting the difference in MEP amplitude between action observation on the horizontal and vertical screens against screen position preference.

6.3. Results

6.3.1. Preliminary TMS data

The 2 (screen position) x 2 (video) repeated measures ANOVA on the number of rejected trials showed no significant main effect for screen position, $F_{(1,23)} = 2.63$, $p = 0.12$, $\eta_p^2 = 0.10$, no significant main effect for video, $F_{(1,23)} = 0.55$, $p = 0.47$, $\eta_p^2 = 0.02$, and no significant screen position x video interaction effect, $F_{(1,23)} = 1.23$, $p = 0.28$, $\eta_p^2 = 0.05$.

The 2 (screen position) x 4 (video) repeated measures ANOVA on the pre-stimulation baseline EMG data of the remaining trials showed a significant main

effect for screen position, $F_{(1,23)} = 7.30$, $p = 0.01$, $\eta_p^2 = 0.24$, with a significantly greater baseline EMG signal during the vertical screen position (12.55 ± 1.22) compared to the horizontal screen position (11.27 ± 0.91). There was no significant main effect for video, $F_{(1,23)} = 1.07$, $p = 0.31$, $\eta_p^2 = 0.05$, and no significant screen position x video interaction effect, $F_{(1,23)} = 1.13$, $p = 0.30$, $\eta_p^2 = 0.05$.

6.3.2. Overall TMS data

Raw MEP amplitudes recorded during each of the conditions are reported in Table 6.1. A 2 (screen position) x 2 (video) repeated measures ANOVA on the *z*-score data from all participants showed no significant main effects for screen position, $F_{(1,23)} = 3.11$, $p = 0.09$, $\eta_p^2 = 0.12$, or video, $F_{(1,23)} = 1.40$, $p = 0.25$, $\eta_p^2 = 0.06$, and no significant screen position x video interaction, $F_{(1,23)} = 0.47$, $p = 0.50$, $\eta_p^2 = 0.02$ (see Figure 6.3).

Table 6.1. Raw MEP amplitudes recorded from the FDI muscle during each condition

	Raw MEP amplitude (μV)	
	Horizontal screen position	Vertical screen position
Static hand	1504.54 (± 204.27)	1366.4 (± 196.86)
Action observation	1602.71 (± 217.91)	1428.44 (± 217.28)

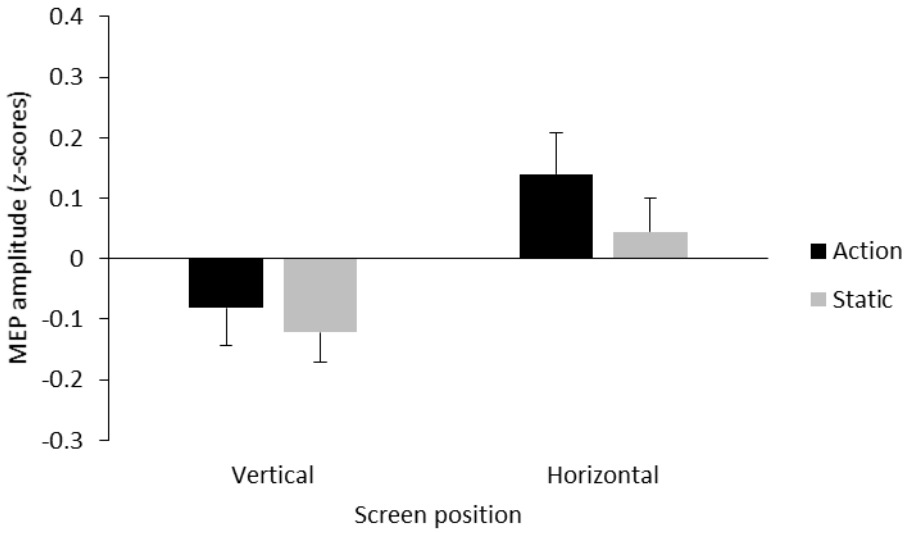


Figure 6.3. Mean MEP amplitudes, displayed as *z*-scores, recorded during the action and static conditions on the vertical and horizontal screen position from the right FDI muscle

6.3.3. Questionnaire and interview data

The horizontal and vertical screen position conditions and elements related to participants' viewing experiences of these conditions (e.g., visual perspective, movement agency, movement kinaesthesis, peripersonal space) provided the structure for the thematic analysis. Analysis of the interviews suggested a primary theme of self-agency, relating to the participants' kinaesthetic experience and self-agency realism was associated with both screen positions but to a greater or lesser extent depending on screen preference. Data from the interview and questionnaire are presented under the deductive themes of realism and movement ownership, and imagery emerging from the interview.

6.3.3.1. Realism and movement ownership

Videos presented on the horizontal screen position were generally perceived to be more "realistic" (e.g., participant (P)1.6; P1.18; P1.21) than those presented on the vertical screen ("Because it doesn't look like my hand. Especially because my hand

was not in the same position" (P1.24)) and this was associated with perceived ownership of the observed limb: "it [the hand/arm] did seem like mine, more on the [horizontal screen]" (P1.22). Participants reported that this was, in part, aided by the congruent positioning of their own hand/arm with the model's hand/arm. Participants reported that the positional congruency enhanced the ownership of the hand/arm ("I felt it was where my hand was, I actually felt like I was looking at my hand" (P1.8); "I felt like when it was flat [horizontal screen], it was easier to identify as my own [hand]" (P1.23)), promoted perceived interaction with the video ("it felt like my hand was going into the screen as it was under the screen" (P1.3)), and provided a greater sense of movement ownership ("When it was on the horizontal screen… and my hand was underneath the screen, it made me feel like it was my hand that was moving" (P1.21)).

In support of the qualitative data suggesting screen position differences in affect and preference, a Wilcoxon signed-rank test on the questionnaire responses to the question "How strongly did you feel that the hand you were watching was your own?" supported the contention that participants experienced a significantly greater ownership of the observed hand in the horizontal screen condition (median = 4, IQR = 2) compared to the vertical screen condition (median = 2, IQR = 1), $Z = -3.09$, $p < 0.01$. An increased sense of embodiment with the observed hand also gave some participants a desire to perform, or a perception that they were actually performing, the observed action. For example, when observing the videos on the horizontal screen, some participants reported that they "felt as though [he/she] wanted to act that same movement" (P1.6), or that he/she wanted to interact with

the movement actively and “grab it [the ball] when it [the hand] was squeezing it [the ball]” (P1.4).

6.3.3.2. Imagery

The interviews indicated that some participants employed concurrent imagery behaviour whilst observing the action. During the interview, 21 participants reported the spontaneous use of imagery in some form, with nine discussing a range of imagery modalities in detail. These were raised during varying questions, including “What were your opinions of the two different screen positions that you saw in the experiment?” and “What physical and emotional sensations were you aware of whilst watching the ball pinches?” For example, participants reported they felt like they were “doing [the movement] with [their] brain” (P1.14), and even generated kinaesthetic and haptic imagery (“the feeling of the, what seemed to be like a stress ball kind of material” (P1.18)), and auditory (“I could kind of hear it going, like a noise to it” (P1.23)) elements to the image. Participants who reported experiencing these multimodal images also reported that they were generated to a greater extent, but not exclusively, during the observation of the ball pinch on the horizontal, compared to the vertical, screen position, and during the action, compared to static, videos. If they did not raise the differences themselves, participants were prompted to compare the different conditions.

Interview data suggested that the horizontal screen position, with its associated imagery, gave participants the perception that they were actively involved in performing the movement. Participants reported feeling “as though [they were] grasping the ball” and that this may have been due to action observation on the horizontal screen generating tactile sensations, such as feeling “… the resistance of

the ball in the ball squeezing" (P1.23). From the questionnaire data, a Wilcoxon signed-rank test on the responses to the question "How strong was the feeling that you were performing the movement?" confirmed that participants had a greater feeling that they were performing the movement when observing videos on the horizontal screen (median = 3.5, IQR = 2.5) compared to the vertical screen (median = 2, IQR = 1), $Z = -3.39$, $p < 0.01$.

6.3.4. Screen position preference data

In response to the question "On which screen did you prefer watching the ball pinch?", seven (29%) participants reported a vertical screen position preference, 16 (67%) reported a horizontal screen position preference, and one (4%) indicated no preference for either screen position (Figure 6.4).

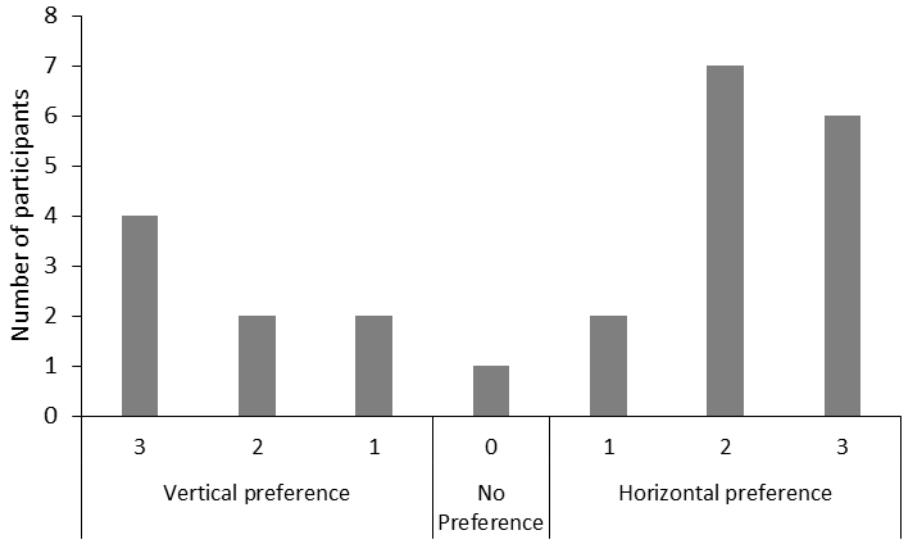


Figure 6.4. Frequency of responses to the question "On which screen did you prefer watching the ball pinch?"

6.3.5. Screen position preference TMS data

A 2 (screen position) x 2 (video) repeated measures ANOVA on the *z*-score data from participants who reported a horizontal screen position preference ($n = 16$) revealed significant main effects for screen position $F_{(1, 15)} = 6.05$, $p = 0.03$, η_p^2 =

0.29, and video $F_{(1, 15)}$ = 8.38, p = 0.01, η_p^2 = 0.36. MEPs were significantly greater during observation of the action videos compared to the static videos irrespective of screen position and significantly greater during trials on the horizontal screen position compared to the vertical screen position irrespective of video type. No significant screen position x video interaction effect was found $F_{(1, 15)}$ = 2.29, p = 0.15, η_p^2 = 0.13 (see Figure 6.5). Due to the small number of participants who reported a preference for the vertical screen position (n = 7), only descriptive data is presented for this group (see Table 6.2). In addition, descriptive data plotting the difference in individual participants' MEP amplitudes obtained during action observation on the horizontal and vertical screens is presented against their subjective screen position preference scores for all participants (see Figure 6.6).

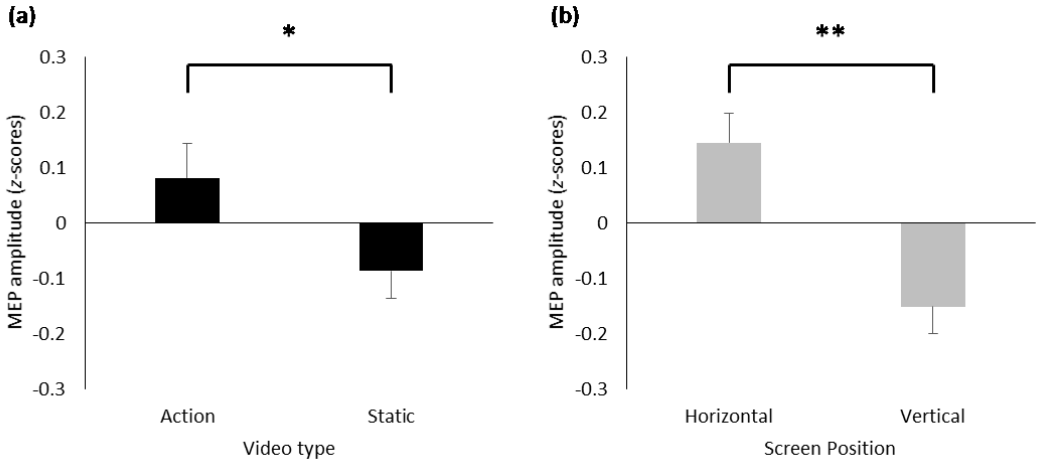


Figure 6.5. Mean MEP amplitudes, displayed as z-scores, from the horizontal preference group, for (a) the action and static conditions (*p = 0.01) and (b) the horizontal and vertical screen positions (**p = 0.03) from the right FDI muscle

Table 6.2. Mean MEP amplitudes displayed as *z*-scores, for the action and static conditions on the vertical screen position for the right FDI muscle

	Mean MEP amplitude (*z*-scores)	
	Horizontal screen position	Vertical screen position
Static hand	-0.002 (± 0.12)	0.10 (± 0.03)
Action observation	-0.12 (± 0.10)	0.03 (± 0.12)

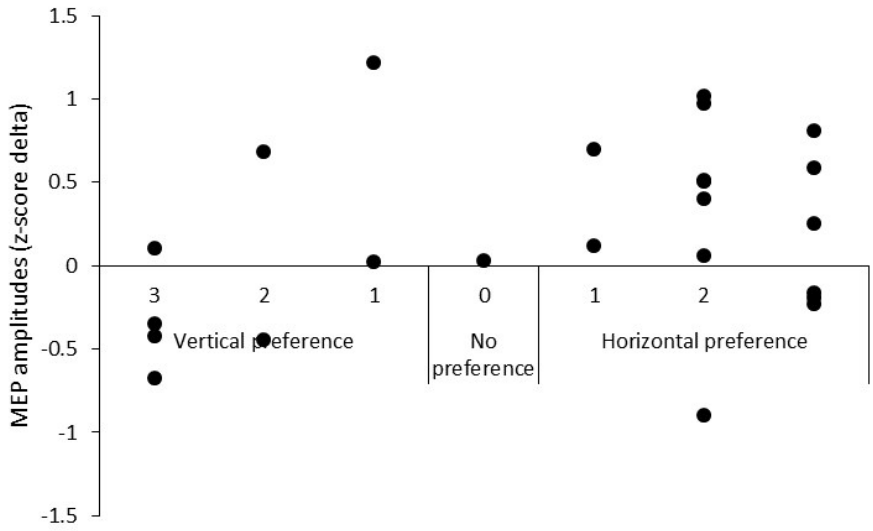


Figure 6.6. The difference in MEP amplitude, displayed as *z*-score delta, between action observation on the horizontal and vertical screen positions plotted against screen position preference

6.4. Discussion

The first aim of this experiment was to determine whether different screen positions modulate CSE during the observation of a hand movement filmed from a first-person visual perspective. In contrast to the hypothesis, there were no significant differences in MEP amplitude between the two different screen positions or the type of video presented. The lack of significant difference in MEP amplitude when viewing action compared to static hand videos was surprising as, since the seminal experiment of Fadiga et al. (1995), research has generally

demonstrated that action observation produces an increased CSE in the muscle(s) used to perform the action when compared to a static control (Loporto et al., 2011; Naish et al., 2014). Despite this well-established facilitation effect during action observation, some studies have, however, reported that only certain types of action observation facilitate CSE in comparison to control conditions (e.g., Donne et al., 2011; Enticott et al., 2010). Collectively, the results from these studies indicate that facilitation of CSE is more likely when the action is perceived as particularly meaningful to the individual. In part, the extent to which the action was considered meaningful by each participant may vary because of their preference for the screen positions and may explain the lack of significance. The qualitative data suggested that 67% of individuals preferred viewing the action on the horizontal screen. Participants with the horizontal screen viewing preference may have attributed less meaning to the videos on the vertical screen due to the different reference frame, thereby introducing a confound to MEP results when screen position viewing preference was not considered. When the TMS data control for screen preference as a marker of attributed meaning, the experimental versus control effect becomes evident within the participants, who indicated a preference for the horizontal screen condition. This will be discussed in more detail later.

A second aim was to establish whether CSE was modulated when accounting for participants' viewing preference for screen position. Follow-up analysis of TMS data for just those participants who preferred the horizontal screen position demonstrated significantly greater MEP amplitudes for this sub-group when they observed videos displayed on the horizontal screen compared to the vertical screen. Furthermore, and consistent with the literature (see Loporto et al., 2011;

Naish et al., 2014), MEP amplitudes were also significantly greater during the observation of the ball pinch action compared to the observation of the static hand. These data suggest that screen position and its induced viewing preference can highlight differences in MEP amplitude and could explain the lack of significance between conditions in the initial analysis. As the preliminary analysis indicated significantly greater baseline EMG activity during the vertical screen compared to the horizontal screen, the results cannot be completely attributed to the manipulation of the screen position. As the difference was minimal and beneath the 50μV threshold that is recognised as the minimum electrical potential of an MEP (Rossini et al., 1994), however, it is unlikely that this difference influenced the results.

71% of participants reported that they perceived the observed hand as their own sex. This may have contributed to participants' sense of ownership and self-agency of the observed hand. In addition, during the horizontal screen position, anatomical and perceptual congruency with the physical task is emphasised. Combined, this may have resulted in the participants reporting a greater sense of ownership and self-agency during the horizontal screen position compared to the vertical screen position. These qualitative data suggest that the horizontal screen position optimally presented participants with visual and affective cues to reinforce self-attribution for the movement of the action.

The greater CSE during the videos presented on the horizontal screen position in participants with a preference for the horizontal screen position suggests that the sense of agency was increased for these participants. It is proposed that, in part, this may be a function of the dorsal visual stream for these participants during the

videos on the horizontal screen. Amongst participants with a horizontal screen position preference, the vertical screen position may require an imagined rotation of the observed image to provide an ego-relative remapping (Filimon, 2015) of the hand in an attempt to experience the richer motor simulation only present in their preferred environment. When the reference frame requires the remapping of the action, there is predominantly activation of ventral, rather than dorsal, visual stream processing (van Polanen and Davare, 2015; Filimon, 2015). It is possible, therefore, that greater activation of the dorsal posterior-parietal pathway is present during the horizontal screen position when the observer holds a preference for it. This may explain some of the contribution to the greater MEP amplitudes during the preferred horizontal condition when compared to the inferotemporal pathway of the ventral stream associated with the non-preferred vertical screen condition, although further research is required to test this postulate.

Further mechanistic explanation for the screen position effect on CSE can be found in the work of (Jeannerod, 2001). He proposed that activation of the motor cortex and descending motor pathways during action observation generates signals that propagate upstream to parietal and premotor cortex which allow monitoring of the simulation and a realisation that the participant is the agent of the covert activity, even though there is no overt behaviour. Therefore, for the horizontal screen preference group, the greater CSE suggests that the screen position generates cortical activity that is associated with a greater feeling of self-identification and, therefore, ownership and self-agency of the observed action, even though they are viewing a model's arm and hand producing the action. The qualitative data also highlighted that not only did the horizontal screen position give a significantly

greater sense of self-ownership but that it also gave a more realistic kinaesthetic feeling about performing the movement compared to the vertical screen position. The viewer can only use visual, imagined kinaesthetic, and predicted proprioceptive information to make a judgement about the sense of ownership of the hand and limb in the two conditions. However, in action observation conditions, the latter is significantly compromised, placing greater emphasis on the visual and kineasthetic cues. On the horizontal screen, the congruence of the visual perception and kinaesthetic imagery with the predicted proprioceptive information from the viewer's own arm seemed to have provided greater ownership and agency of the movement in contrast to the vertical screen position where the visual perceptions are incongruent to the observer's kinaesthesis and expected proprioceptive feedback. These findings concur with studies using the rubber hand illusion (Schütz-Bosbach et al., 2009). The importance of vision's contribution to the sense of ownership and movement agency has been shown extensively in these studies with the authors concluding, in line with the present findings, that motor facilitation depends strongly on the agent to whom the observed action is attributed.

In this experiment, the illusion of a sense of ownership and realism extended to the perception of haptic afference of the ball's texture and kinaesthetic sensations associated with finger flexion but, for most participants, only whilst viewing the action on the horizontal screen during the action condition. This supports similar findings from Farnè et al. (2000) who showed that the brain could form visual representations of a non-owned body part. The authors identified that the rubber hand illusion was only evident when participants saw the rubber hand as congruent to the positioning of their own hand. In contrast, and in support of why CSE was

significantly lower during the vertical screen in the horizontal preference group, the illusion was significantly reduced when the position of the rubber hand was incongruent to the observer's own hand. It was suggested that this phenomenon is due to the dominance of vision over proprioception in the perception of limb ownership. More specifically, provided that they look plausible with respect to the subject's own body, the visual cues of a 'fake' arm and hand become self-attributed, and a sense of ownership may arise. In the case of this experiment, the horizontal screen does this whereas the vertical screen presents a misaligned posture that requires imagined rotation to regain positional plausibility. This effect may have been influenced, however, by the different viewing angle and distance of the two screen positions. The centre of the stimuli presented on the vertical screen position was close to eye-level (3.5^{o} from eye-level) and positioned 90cm away from the participants, whilst the centre of the stimuli presented on the horizontal screen position required a greater deviation from eye-level (25^{o} from eye-level) and was positioned only 45cm away from participants. Although these were necessary methodological inclusions, this may have resulted in the stimulus appearing to be presented at a different angle and size on the two screen positions. This could have influenced participant comfort and preference, and possibly CSE if the stimulus size appeared closer to anatomical congruency on one screen position compared to another.

In an experiment with some similarities to the present experiment, (Kaneko et al., 2007) reported a facilitation of CSE and kinaesthetic experience when participants observed abduction movements of an index finger from an egocentric viewpoint on a horizontal screen. A vertical screen presentation, however, resulted in reduced

kinaesthesis and lower CSE. Based on the present findings and interpretation of the data, the vertical screen position provided participants with a different spatial reference frame and reduced visual cues for self-agency and ownership compared to the horizontal screen. This could be further explained by what Jeannerod and Pacherie (2004) have described as an error in self-predication because the vertical screen presents the viewer's arm/hand as their own, but in a visually incongruent position to their own that contributes to proprioceptive error. The proprioceptive error can be seen through lower levels of CSE in the vertical screen position compared to the horizontal screen position for the horizontal preference group. In order to maintain a sense of limb and movement ownership, a recalibration of the position is required, similar to the ego-relative remapping process described by Filimon (2015). Participants may experience an enhanced kinaesthesis during action observation when the feeling of where their hand is meant to be is congruent with where they see the modelled hand. This supports the notion that it is important to ensure action observation tasks that aim to mimic self-actions are delivered from an egocentric reference frame in peripersonal space and filmed from a plausible anatomical viewpoint.

The evidence from the qualitative data suggests that participants used concurrent coordinative imagery during the horizontal screen position, but possibly not during the vertical screen position. In line with the arguments presented above, the differences in visual cues between the two screen positions and the associated kinaesthesis may be contributory to the difference in MEP amplitude. Vogt et al. (2013) proposed stronger activations in motor execution-related areas when the observed and imagined tasks are fully congruent, as would be proposed here for

the horizontal screen condition. In contrast, the lower MEPs for the vertical screen position could be associated with the less congruent observation-imagery behaviour, with the observed action only being coordinative with the imagery, which was not reported to have been employed, in order to rotate the image of the hand and retain the perception of self. The horizontal screen condition seems to have given participants the perception that they were performing the observed action and experiencing greater kinaesthetic imagery, shown through facilitated corticospinal activity (Stinear et al., 2006). Due to the more congruent visual perspective participants may have found it easier to use appropriate imagery in the horizontal screen position and this, in turn, may have contributed to the greater MEPs.

Taken together, these results demonstrate that anatomical and perceptual congruency with the physical task, alongside the consideration of participants' screen position viewing preferences, have the potential to modulate CSE during action observation. These findings, therefore, have important implications for the design and delivery of action observation interventions in motor (re)learning settings. Specifically, structured action observation interventions have been shown to contribute significantly to improvements in motor function in situations where an individual's movement capability has been compromised, for example following a stroke or in individual's with Parkinson's disease (Buccino, 2014). Despite the apparent efficacy of action observation as an adjunct to physical therapy for motor rehabilitation, these interventions continue to present first-person visual perspective action observation on vertically-orientated screens in the observer's extrapersonal space and screen position viewing preference is rarely considered.

Although not completely supported by the MEP data, the results within the horizontal screen position preference group alongside the qualitative data indicate that these variables may be important in order to optimise action observation interventions. Importantly, advances in mobile information technology now allow for the relatively easy creation and delivery of action observation interventions via tablet and smartphone devices (McCormick and Holmes, 2016). The portability of such devices makes it considerably easier to manipulate the positioning of the screen, where appropriate for the task being viewed, to achieve perceptual and anatomical congruency with the observed action, and match the individual's screen position viewing preference. As such, future research should seek to expand on these TMS findings, and establish the efficacy of integrating screen position preference into action observation interventions for motor (re)learning within clinical populations.

Chapter 7. Experiment 2

Action observation with a congruent contextual background facilitates corticospinal excitability: A combined TMS and eye-tracking experiment.

[published as Riach, M., Holmes, P. S., Franklin, Z. C. and Wright, D. J. (2018) 'Observation of an action with a congruent contextual background facilitates corticospinal excitability: A combined TMS and eye-tracking experiment.' Neuropsychologia, 119 pp. 157-164.]

7.1. Introduction

As reviewed in Chapter 2.4.2, activity in the motor regions of the brain has been shown to be modulated by different visual contexts in which an action is presented for observation. For example, in a fMRI experiment, Iacoboni et al. (2005) reported a significant increase in activity in the premotor cortex and the inferior frontal gyrus when the observed action occurred within a contextually relevant background scene compared to a blank background. Utilising TMS, Enticott et al. (2010), Donne et al. (2011), and Amoruso and Urgesi (2016) supported the findings of Iacoboni et al. (2005) by demonstrating that action observation with meaningful visual context facilitated CSE to a greater extent than action observation without meaningful visual context. Taken together, this research indicates that visual contextual information about the goal and intention of the observed action facilitates CSE, and may inform the design and delivery of action observation interventions for motor (re)learning.

Evidence indicates the effect of providing context on modulating activity in the motor regions of the brain, but further research is needed to clarify some of the findings. For example, as Iacoboni et al. (2005) only explored background scenes that were related to the observed action to provide context compared to a blank background, it is possible that any modulation in neurophysiological activity was due to the presence of additional visual information, rather than the congruence of such information to the observed action. It makes it difficult, therefore, to claim that the modulation of neural activity was a result of a meaningful visual context. Initial findings from Amoruso and Urgesi (2016) have indicated that the congruency of the context modulates CSE, but did not address the issue of Iacoboni et al. (2005) experiment as additional background information was not provided. It would be beneficial, therefore, to explore this effect further to expand the understanding of how the manipulation of context influences CSE.

One area of visual context that would be beneficial to explore further would be visual attention. The manipulation of background contexts in action observation experiments may modulate participants' visual attention, which may be associated with changes in CSE (Conte et al., 2007; see Chapter 2.4.4.1). The inclusion of eye-tracking techniques, therefore, would benefit the interpretation of any CSE modulation in the present experiment.

This experiment aimed to determine the effect of background context on CSE and visual attention during action observation. It was hypothesised that: (i) CSE would be facilitated during action observation conditions compared to a control condition; (ii) the facilitation effect would be specific to the muscles involved in the execution

of the observed action; (iii) the facilitation of CSE would be greater when an action is observed against a congruent contextual background compared to either a plain black background or an incongruent contextual background; and (iv) both the number of fixations and the percentage fixation duration on the background scene would be greater during action observation conditions with a congruent or incongruent contextual background compared to action observation with a plain black background.

7.2. Methods

7.2.1. Participants

Twenty-four volunteers (16 male, 8 female) aged 19-32 years (mean age 22.42 ± 3.23 years) participated in the experiment. Twenty-one participants were right-handed (mean LQ 82.27 ± 16.07), and three were left-handed (mean LQ -35.08 ± 42.61).

7.2.2. Procedure

The general TMS and eye-tracking procedures were identical to those outlined in Chapters 4.2 and 4.3. The modal OSP was 4cm lateral, 1.5cm anterior to Cz, and the mean RMT was 47.46% ± 8.19. A vertical screen position was used to enable accurate eye-tracking (see Chapter 3.4.3).

The general experimental procedure was as outlined in Chapter 4.5.1. Each participant observed four conditions (Figure 7.1): a static right hand holding a sponge between the index finger and thumb against a plain black background (Figure 7.1(a)); and a right hand performing two index finger-thumb pinches of a

sponge against either a plain black background (Figure 7.1(b)), a background containing objects that were incongruent with the activity of daily living of pinching a sponge to wash dishes, such as a cardboard box, tools, and pens (Figure 7.1(c)), or a background containing objects that were congruent with the activity of daily living of pinching a sponge to wash dishes, such as a sink, plates, and cutlery (Figure 7.1(d)). The location of the background objects was kept similar, though the object features differed to ensure no overlap of the background context between conditions. In addition, an index finger-thumb pinch was used over a possibly more ecologically-valid whole hand squeeze to explore the muscle-specificity effect between visible experimental and control muscles. Kinematic equivalence across conditions was not measured, but the recorded actions were matched visually to keep the movement consistent across all conditions. The experiment was split into four blocks of 32 trials, with each block containing eight trials of each condition presented in a random order.

The videos showed a Caucasian male's right hand and forearm filmed from a first-person visual perspective, with the hand positioned to the right of the screen to enhance anatomical congruence and perception of ownership (Chapter 6). All videos were seven seconds in duration, and the action observation videos contained two pinches at a rate of approximately 0.3Hz. For the three experimental conditions, one stimulation was delivered per trial at the point of maximal contraction of the FDI muscle during either the first or second sponge pinch (either 1700ms or 4900ms after video onset). Two stimulation timings were used to reduce the predictability of the stimulation (Loporto et al., 2012). Participants were given a break of approximately 2 minutes between each block.

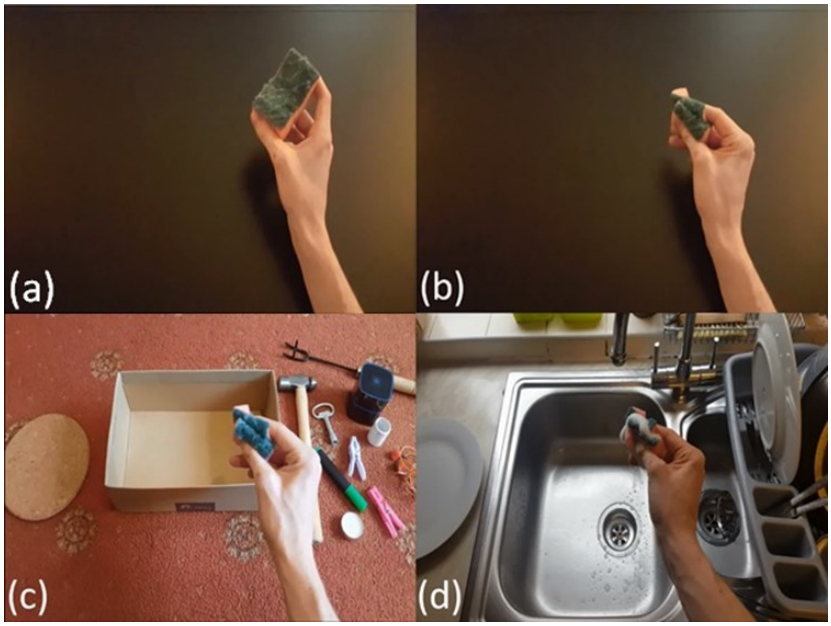


Figure 7.1. Still images from (a) the control condition, and three experimental conditions: (b) a plain black background, (c) a contextually incongruent background, and (d) a contextually congruent background

7.2.3. Data Analysis

7.2.3.1. TMS data

Data analysis for the MEP amplitudes was as outlined in Chapters 4.5.2.1 and 4.5.2.3. A 2 (muscle: FDI, ADM) x 4 (condition: static hand, no context background, incongruent context, congruent context) repeated measures ANOVA was performed on the *z*-score MEP data. Significant effects were explored further using post-hoc pairwise comparisons with Bonferroni corrections.

7.2.3.2. Eye-tracking data

Data analysis for the eye-tracking data was as outlined in Chapters 4.5.2.2 and 4.5.2.3. Individual trials were analysed using two separate dynamic AOI for the sponge and hand. A third dynamic AOI was defined as the remaining background scene to cover all elements relating to the congruency of the background context

(i.e., the backdrop and all background objects; Figure 7.2). The fixation count data and percentage fixation duration data were analysed using separate 3 (AOI: sponge, hand, background) x 4 (condition) repeated measures ANOVAs.

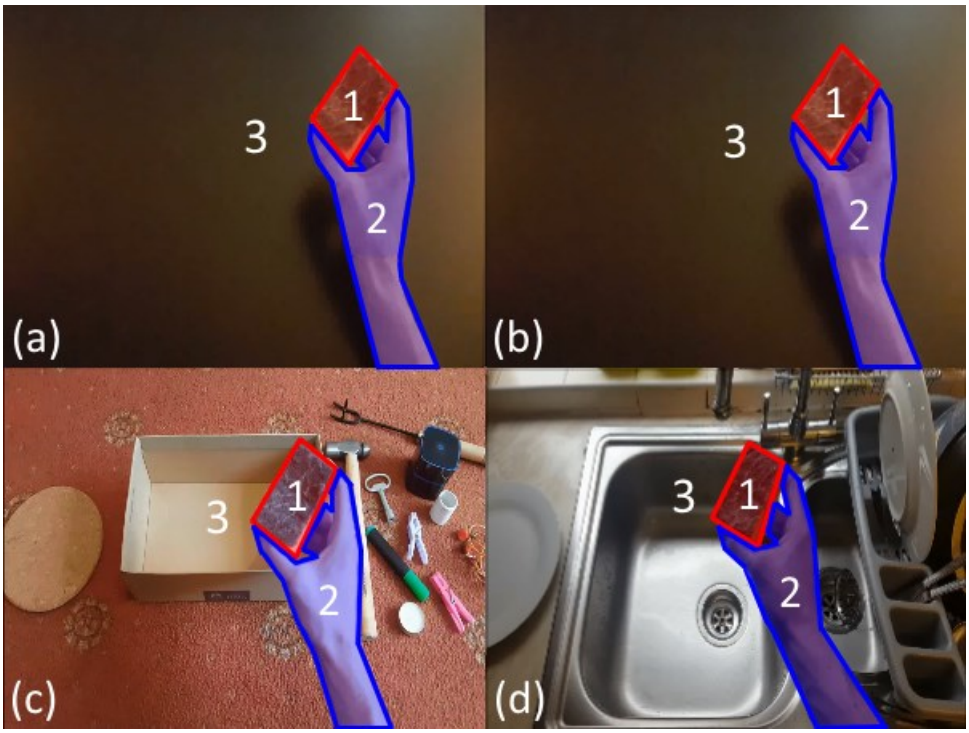


Figure 7.2. Dynamic AOI were used to cover the (1) sponge, (2) hand, and (3) remaining background scene on the (a) static hand, (b) plain black background, (c) incongruent context background, and (d) congruent context background conditions

7.3. Results

7.3.1. Preliminary TMS data

The 2 (muscle) x 4 (condition) repeated measures ANOVA on the number of rejected trials showed a significant main effect for muscle, $F_{(1,23)} = 7.09$, $p = 0.01$, $\eta_p^2 = 0.24$, with significantly more trials rejected from the ADM muscle (2.52 ± 0.65) compared to the FDI muscle (1.03 ± 0.24). Mauchly's test indicated that the assumption of sphericity had been violated for the main effect for condition ($\chi^2_{(5)} = 13.57$, $p = 0.02$). Following a Greenhouse-Geisser correction ($\varepsilon = 0.73$), no

significant main effect for condition was found, $F_{(2.19,50.35)} = 2.18$, $p = 0.50$, $\eta_p^2 = 0.03$. In addition, no significant muscle x condition interaction effect was found, $F_{(3,69)} = 1.60$, $p = 0.26$, $\eta_p^2 = 0.06$.

The 2 (muscle) x 4 (condition) repeated measures ANOVA on the pre-stimulation baseline EMG data of the remaining trials showed no significant main effect for muscle, $F_{(1,23)} = 0.09$, $p = 0.77$, $\eta_p^2 = 0.004$. Mauchly's test indicated that the assumption of sphericity had been violated for the main effect for condition ($\chi^2_{(5)} = 26.35$, $p < 0.001$) and the muscle x condition interaction effect ($\chi^2_{(5)} = 27.46$, $p < 0.001$). Following Greenhouse-Geisser corrections ($\varepsilon = 0.56$ and $\varepsilon = 0.57$ respectively) there was no main effect for condition, $F_{(1.67,38.29)} = 0.13$, $p = 0.84$, $\eta_p^2 = 0.01$, and no significant muscle x condition interaction effect, $F_{(1.70,39.10)} = 2.05$, $p = 0.15$, $\eta_p^2 = 0.08$.

7.3.2. TMS data

Raw MEP amplitudes recorded from the FDI and ADM muscles for each of the conditions are reported in Table 7.1. The 2 (muscle) x 4 (condition) repeated measures ANOVA on the *z*-score data showed a significant muscle x condition interaction effect, $F_{(3,69)} = 4.80$, $p < 0.001$, $\eta_p^2 = 0.24$. Pairwise comparisons showed that MEP amplitudes recorded from the FDI muscle were significantly greater during the congruent context condition than MEPs recorded from both the static hand ($p = 0.02$, $d = 0.65$) and plain black background ($p = 0.02$, $d = 0.65$) conditions (Figure 7.3). In addition, MEPs recorded from the ADM muscle were significantly

greater during the static hand condition compared to the plain black background condition (p = 0.01, d = 0.74; Figure 7.3).

Table 7.1. Raw MEP amplitudes recorded from the FDI and ADM muscles for each condition

	Raw MEP amplitude (μV)	
	FDI	ADM
Static hand	1423.60 (± 221.9)	914.85 (± 174.95)
Plain black background	1470.17 (± 229.2)	769.42 (± 143.3)
Incongruent context	1548.85 (± 261.05)	805.41 (± 161)
Congruent context	1604.49 (± 255.28)	801.23 (± 145.19)

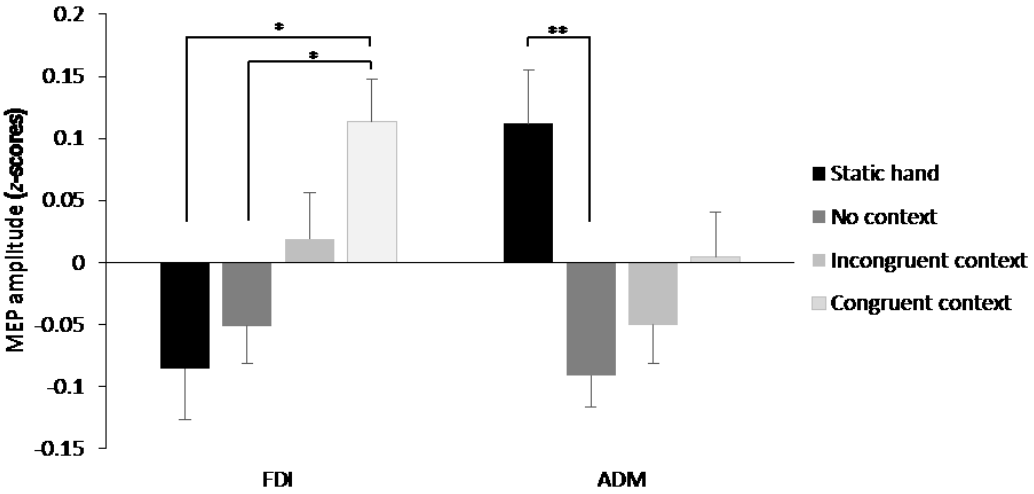


Figure 7.3. Mean MEP amplitudes, displayed as z-scores, recorded during each condition from the FDI and ADM muscles, *p = 0.02, **p = 0.01

7.3.3. Eye-tracking data

For the number of fixations data, Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2_{(20)}$ = 34.35, p = 0.03). Following a Greenhouse-Geisser correction (ε = 0.65), the 3 (AOI) x 4 (condition) repeated measures ANOVA

on the number of fixations data showed a significant AOI x condition interaction, $F_{(3.92,90.04)} = 25.12$, $p < 0.001$, $\eta_p^2 = 0.52$. Pairwise comparisons revealed that more fixations were made on the sponge compared to both the hand and background AOI during all conditions (all $p \leq 0.004$, all d from 0.76 to 2.28; see Figure 7.4). In addition, more fixations were made on the background during both the congruent and incongruent context conditions compared to the static hand ($p < 0.001$, d = 1.02 and 1.23 respectively) and plain black background ($p < 0.001$, d = 1.03 and 1.19 respectively) conditions (see Figure 7.4). The difference between the number of fixations on the background during the incongruent and congruent context conditions approached significance ($p = 0.08$, $d = 0.54$). Significantly more fixations were made on both the static hand and incongruent context conditions compared to both the plain black background and congruent context conditions (all $p \leq 0.02$, all d from 0.27 to 0.53; see Figure 7.4).

For the percentage fixation duration data, Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2_{(20)} = 47.06$, $p = 0.001$). Following a Greenhouse-Geisser correction ($\varepsilon = 0.69$), the 3 (AOI) x 4 (condition) repeated measures ANOVA on the percentage fixation duration data showed a significant AOI x condition interaction, $F_{(4.14,95.13)} = 8.69$, $p < 0.001$, $\eta_p^2 = 0.28$. Pairwise comparisons revealed that participants spent a greater percentage of time fixating on the sponge compared to both the hand and background AOI during all conditions (all $p < 0.001$, all d from 1.33 to 3.14; see Figure 7.5). In addition, participants spent a greater percentage of time fixating on the background during both the congruent and incongruent context conditions compared to the static hand (both $p \leq 0.001$, d =

0.94 and 0.98 respectively) and plain black background (both $p \leq 0.001$, $d = 0.92$ and 1.03 respectively) conditions (see Figure 7.5). No significant difference was found in the percentage fixation duration on the background during the incongruent and congruent context conditions ($p = 1.00$, $d = 0.12$). A greater percentage of time was spent fixating on the hand during the congruent context compared to the incongruent context condition ($p = 0.004$, $d = 0.81$; see Figure 7.5).

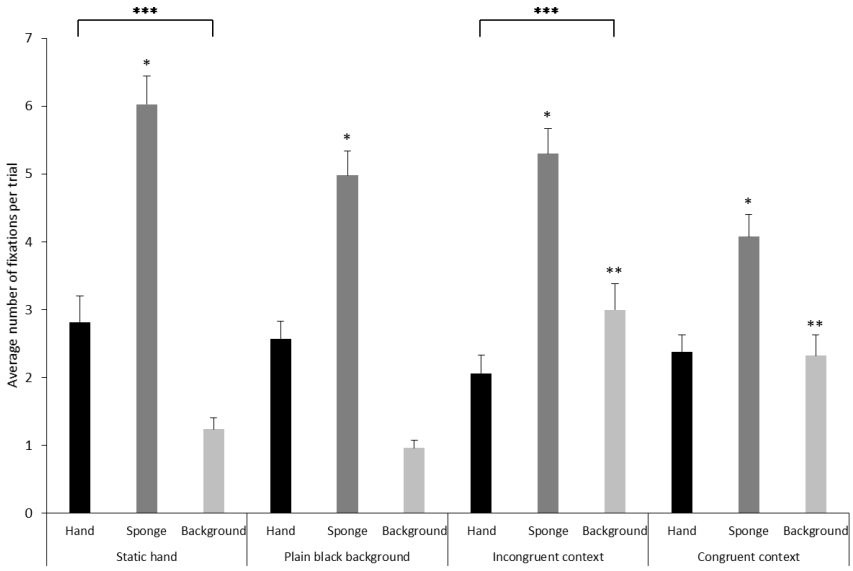


Figure 7.4. The average number of fixations per trial recorded in each AOI during each condition. * indicates significantly more fixations on the sponge AOI compared to the hand or background AOI in each condition ($p \leq 0.004$). ** indicates significantly more fixations on the background AOI in the incongruent and congruent conditions compared to the static hand and plain black background conditions ($p < 0.001$). *** indicates significantly greater total number of fixations during both the static hand and incongruent context conditions compared to both the plain black background and congruent context conditions (all $p \leq 0.02$)

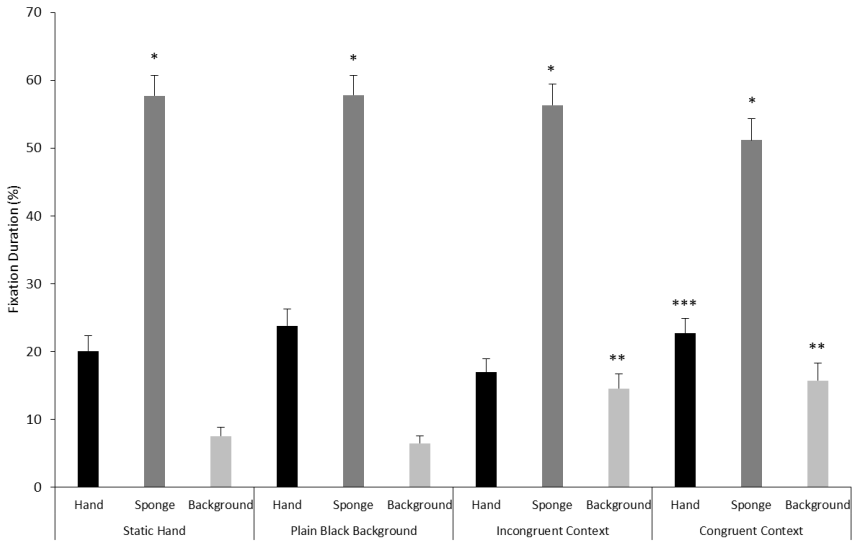


Figure 7.5. The percentage fixation duration recorded on each AOI during each condition. * indicates significantly greater fixation percentage duration on the sponge AOI compared to the hand or background AOI in each condition ($p < 0.001$). ** indicates significantly greater percentage fixation duration on the background AOI in the incongruent and congruent conditions compared to the static hand and plain black background conditions ($p \leq 0.001$). *** indicates significantly greater percentage fixation duration on the hand AOI in the congruent condition compared to the incongruent condition ($p = 0.004$)

7.4. Discussion

This experiment aimed to determine whether the visual context in which an action is observed modulates CSE and visual attention during action observation. The findings indicate that action observation presented with a congruent contextual background produces a facilitation of CSE compared to the control condition. This effect was only present in the FDI muscle that would be involved in the execution of the observed action, providing support for the well-established muscle-specificity effect during action observation (Naish et al., 2014). In contrast, action observation presented with an incongruent contextual background or a background devoid of contextual information did not significantly facilitate CSE relative to the control condition. Furthermore, the presence of the congruent context facilitated CSE to a greater extent than action observation with a plain black background, indicating

that the presence of contextually relevant information enhances the facilitation of CSE during action observation. The preliminary analyses confirm that these differences between conditions cannot be accounted for by increased muscle activity prior to stimulation and, therefore, can be attributed to the manipulation of the visual contextual information.

These findings indicate that CSE was only facilitated during action observation with a congruent context and not for action observation with a plain black background or with an incongruent context. Previous studies have reported that observation of actions perceived to be meaningful by the individual are more likely to produce a facilitation of CSE, compared to actions perceived to be less meaningful (Enticott et al., 2010; Donne et al., 2011). In the present experiment, it can be suggested that only the congruent context condition provided contextually meaningful information to the participants to allow them to infer the goal and intention of the observed action. The presence of this information may enable the observer to utilise attentional and comparator cognitive mechanisms underlying motor simulation, allowing for the accurate selection of appropriate motor representations required for action execution (Jeannerod, 2004; O'Shea and Moran, 2017) which would be expected to reflect as a facilitated MEP profile. This may explain why only the congruent context condition resulted in a facilitation of CSE in comparison to the control condition. These findings support Iacoboni et al. (2005) results by demonstrating that the facilitation of CSE only occurred when the action was observed with a congruent contextual background. The lack of facilitation effect in the incongruent context condition also extends the findings of Iacoboni et al. (2005) by confirming that it is the congruency of the context to the observed action that

produces the facilitation effect, not just the presence of additional visual information in the background scene. In addition, the findings of the present experiment support and extend the findings of Amoruso and Urgesi (2016) by demonstrating that action observation with a congruent, but not an incongruent, context facilitates CSE. These findings highlight the need to consider contextual meaning in both research and applied settings.

The eye-tracking data in this experiment provides further support for the explanation that the congruence of the background scene facilitated CSE. The data revealed that, across all conditions, participants made more fixations and spent a greater percentage of time fixating on the sponge compared to the hand or the background scene. This indicates that visual attention may have been drawn towards understanding the interaction element of the observed action (Wright et al., 2018). To generate a motor response to an observed action, individuals use observable information such as objects and movements to understand the observed stimuli, though the sponge alone may not have been sufficient (Manthey et al., 2003). Additional information that may have provided further understanding of the observed action was present in the congruent and incongruent context conditions. This resulted in participants making more fixations and spending more time fixating on the background scene during both the congruent and incongruent context conditions compared to the static hand and plain black background conditions. This indicates that the presence of objects in the background scene altered participants' visual attention during action observation, diverting visual attention towards elements of the stimuli that could provide higher understanding of the observed action. As the videos were presented in a random order, it is

conceivable that participants fixated more and for longer on the background scene in the congruent and incongruent conditions in an attempt to identify and extract this additional information about the goal and intention of the observed action for each individual video (Kelly and Wheaton, 2013). One explanation, therefore, for why CSE may have been facilitated in the congruent context condition and not the incongruent context condition could be that participants were able to understand the action better when fixating on the congruent background, but not when fixating on the incongruent background (Wurm and Schubotz, 2017). The congruent context condition provided the additional information relating to the action goal (i.e., washing dishes) that the incongruent context condition did not provide. Such information is required for motor planning and provides complete access to motor representations similarly to action execution (Jeannerod, 2004), and to high-level contextual representations (Amoruso et al., 2016), which may have contributed to the facilitation of CSE in this condition.

Participants spent similar lengths of time fixating on the background during the congruent and incongruent context conditions, but there was a trend (p = 0.08) for more fixations on the background during the incongruent, compared to the congruent, condition. This enabled participants to fixate longer on the movement itself, with significantly greater lengths of time spent fixating on the hand AOI during the congruent context compared to the incongruent context condition. This could indicate that participants had to search actively for visual information regarding the goal and intention of the observed action during the incongruent context condition. As they may not have been able to extract this information from the visual scene, the additional fixations distracted visual attention away from the

movement itself. As this information was not directly identifiable in the incongruent or plain black background conditions, the visual features of the observed actions may have been analysed and interpreted inferentially (Molenberghs et al., 2012). Inferring the goal and intention of an action to access appropriate motor representations is possible when context congruent with the goal and intention is provided, even when an incomplete action is shown (Amoruso and Urgesi, 2016). With such important task-relevant information being inaccessible to the observer during action observation in the incongruent or plain black background conditions, however, incomplete, or even incorrect, selection of motor representations may have occurred, even though the complete action was observed. This may be due to the lack of congruency between the observed action and the objects displayed in the background scene (Kelly and Wheaton, 2013; Ocampo and Kritikos, 2010; Wurm and Schubotz, 2017). In addition, a greater total number of fixations were made during the static hand and incongruent context conditions compared to both the plain black background and congruent context conditions. Increased eye movement has been shown to result in a reduction of CSE (D'Innocenzo et al., 2017). The behavioural response of increased fixations on the incongruent background context may explain why CSE was not facilitated when actions were observed with an incongruent contextual background.

Kelly and Wheaton (2013) postulated that both contextual and physical knowledge is required to understand hand-object interactions. As the observed task was a common activity of daily living, it is likely that the physical knowledge of the observed action was available to participants in all three action observation conditions of this experiment (e.g., how the object is typically used, the weight and

texture of the sponge). The importance of this knowledge is demonstrated in the eye-tracking data, as participants fixated on the sponge AOI a greater number of times than the hand and background AOI across all conditions. Contextual knowledge, however, is still required for a better understanding of the observed action. In the present experiment, only the congruent context condition would have provided sufficient and appropriate contextual information for each participant to understand the action and infer the goal of the observed action (Kelly and Wheaton, 2013).

A mechanistic explanation for the findings in this experiment is that distinct, but connected, neural substrates are involved in processing observed objects, actions and context (Epstein, 2005; Schubotz et al., 2014). The strength of the association between the three sources of information affects the strength and likelihood of co-occurring activation within the substrates (Wurm and Schubotz, 2017). The congruent context condition in the present experiment presented each participant with strong visual associations between the object, the action, and the context. This may have allowed for more efficient processing of the observed action in the ventral processing stream by encouraging pre-activation of action information, including the goal of the action (Ganis and Kutas, 2003; Bar, 2004; Wurm et al., 2012; Wurm and Schubotz, 2017). This initial expedited understanding of the observed action through the ventral stream may then have manifested as enhanced dorsal stream processing during observation of the action, and a facilitation of CSE as this stream terminates in a key area of the human action observation matching system. When one of the three sources of information are missing, it is still possible that information regarding the absent source can be predicted from two remaining

sources (Wurm and Schubotz, 2017). Consequently, an increased prediction error becomes apparent throughout the ventral processing stream; the extent to which is dependent on the type of action information (Kilner et al., 2007). The prediction error could explain why CSE was not facilitated during the incongruent and plain black background conditions, as incomplete information (i.e., missing valuable contextual information) resulted in reduced goal and intention-related cognition. Specifically for the incongruent context condition, it is possible that the incongruence of the context misled the observers' expectations of the action (Wurm and Schubotz, 2012), resulting in slower action recognition through the ventral stream and, therefore, diminished utilisation of the dorsal processing stream and interference in the motor response. This would be expected to reflect in the lack of facilitation of CSE found in the present experiment.

The finding that CSE was facilitated in the ADM muscle during the static hand condition compared to the plain black background condition was unexpected. A possible explanation for this could relate to the spontaneous use of motor imagery in this condition for some participants. It is possible that the image of a static hand holding the sponge may have contained implied movement information (Kourtzi and Kanwisher, 2000; Proverbio et al., 2009; Pavan et al., 2011) and encouraged participants to spontaneously imagine performing a sponge squeeze action that included flexion of all the fingers. Due to the randomisation of the conditions, only 25% of the videos observed within each block showed no movement. Although this randomisation was included to control for coil movement across conditions, a consequence may have been that participants expected to observe a movement during static hand trials. This may have resulted in participants unintentionally

engaging in motor imagery of a whole-hand squeeze whilst observing the static hand. Such spontaneous use of motor imagery in this condition may have resulted in the facilitation of CSE in the ADM muscle during the static hand condition (Fadiga et al., 1999; Stinear et al., 2006). This phenomenon would not be expected during the other three action observation conditions, where the limited involvement of the ADM muscle is more obvious and when, due to the observation of an action, motor resonance becomes stronger. Further research is required to test this speculation.

It is likely that the reported findings are due to the visual contextual information provided and the congruency of the context to the action. It is possible, however, that other issues could have influenced the results to some extent. For example, as each video was filmed independently, kinematic equivalence could not be matched exactly. Differences in the kinematic profiles of an observed action (McCabe et al., 2014; Aihara et al., 2015), including the force used to perform an observed action (Alaerts et al., 2010), can modulate CSE. Careful attempts were made to match the movement visually for each video as closely as possible to minimise the possible confounding effect that this could have had on the results. In addition, due to the different backgrounds, each video may have generated different levels of interest for the participant. This may have altered attention from observing the goal of the action to the background based on the participant's interest, resulting in modulated CSE. This appears unlikely, however, as a greater number of fixations and a greater percentage of time spent fixating on the sponge AOI compared to the hand and background AOI was observed across all conditions.

Action observation interventions have been shown to contribute to improvements in motor (re)learning (see Buccino, 2014). Holmes and Wright (2017) suggested that interventions that elicit increased activity in the extended motor system may indicate more optimal intervention techniques. The findings of the current experiment indicate that including congruent contextual information in action observation interventions can promote increased activity in the motor system. Provision of such information may, therefore, contribute to more effective action observation interventions for motor (re)learning, and future research should establish the veracity of this claim.

Chapter 8. Experiment 3

Emotional valence modulates corticospinal excitability during observation of a reach and grasp of food items: A TMS, eye-tracking and interview experiment.

8.1. Introduction

As discussed in Chapter 2.4.3, the emotional valence of an object has shown to modulate CSE. Most research demonstrates that observation of unpleasant and pleasant images facilitate CSE compared to neutral stimuli (Hajcak et al., 2007; Van Loon et al., 2010), or that observation of unpleasant images facilitates CSE compared to pleasant images (Coelho et al., 2010; Van Loon et al., 2010; Enticott et al., 2012).

Enticott et al. (2012) and Nogueira-Campos et al. (2014) utilised unpleasant or pleasant items to prime participants' emotional valence immediately prior to action observation and movement preparation respectively. This resulted in facilitated CSE when primed with unpleasant compared to pleasant items, potentially indicating an adaptive avoidance of perceived threat through the activation of withdrawal networks. Taken together, these findings suggest that modulation of activity in the motor regions of the brain may be caused by preparing to withdraw or avoid unpleasant observed stimuli (see Chapter 2.4.3).

Nogueira-Campos et al. (2016) performed the only experiment to explore the effect of emotional valence of objects on CSE during action observation. They reported CSE facilitation during observation of a reach and grasp of unpleasant compared to pleasant objects. These findings provide similar CSE profiles to those of previous

emotional valence research (Enticott et al., 2012; Nogueira-Campos et al., 2014), indicating that such a modulation of CSE is maintained throughout movement preparation and action observation. Nogueira-Campos et al. (2016) propose that the facilitation of CSE during observation of interactions with unpleasant stimuli compared to pleasant stimuli could be a result of the need to overcome aversive-like circuits in order to execute the observed action with unpleasant stimuli, and/or the need to prevent overt execution of the observed action whilst observing the pleasant stimuli.

Previous research has provided useful contributions to understanding the effect of emotional valence on CSE during action observation. There are, however, further issues that need to be addressed. Nogueira-Campos et al. (2016) provide the only research exploring the emotional valence of an object during action observation. This research used a pre-determined list of objects and did not contain a static control condition. Nogueira-Campos et al. (2016) tested a large array of emotionally valent objects, ultimately using 10 objects that participants had rated as unpleasant (e.g., a spider), and 10 objects rated as pleasant (e.g., chocolate). This method ensures that items generally considered to be unpleasant or pleasant are used, but it does not allow consideration of each individual participant's preference. As preference has shown to be a necessary consideration during motor simulation (Hall, 1997; Calmels et al., 2006; Chapter 6), this should be controlled. In addition, not including a static control condition makes it impossible to determine whether the results are due to observing an action, objects, or additional factors such as the individual meaning attributed to the observation. As well as utilising a static control, additional measures such as eye-tracking (see Chapter 2.4.4.1) and interviews (see

Chapter 2.4.3; Chapter 2.4.4.2) would be useful inclusions to determine the nature of CSE modulation whilst observing actions involving emotionally valent objects. Eye-tracking would indicate any changes in visual attention due to the emotional valence of the observed object (Chapter 2.4.4.1), and interviews would enable an understanding of the observer's experiences during action observation (Chapter 2.4.4.2). Measuring visual attention and exploring each participant's experiences whilst observing emotionally valent stimuli, therefore, would be beneficial to understanding the applications of emotional stimuli to action observation interventions.

The aim of this experiment was to determine the effect of the emotional valence of objects on CSE and visual attention during action observation. It was hypothesised that: (i) CSE would be facilitated during action observation to a control condition; (ii) CSE facilitation would be specific to the muscle involved in the execution of the observed action; (iii) the facilitation of CSE would be greater when the observed action involves reaching for a food item with a negative emotional valence compared to a positive or neutral emotional valence; and (iv) the number of fixations and percentage fixation duration on the food item would be greater when that food item had a negative emotional valence compared to a positive or neutral emotional valence.

8.2. Methods

8.2.1. Participants

Twenty-four volunteers (19 male, 5 female) aged 20-33 years (mean age 24.63 ± 3.85 years) participated in the experiment. All participants were right-handed (mean LQ 79.97 ± 16.20).

8.2.2. Procedure

The general TMS, eye-tracking and interview procedures were identical to those outlined in Chapters 4.2, 4.3, and 4.4. The modal OSP was 4cm lateral, 0cm anterior to Cz, and the mean RMT was 45.38% ± 6.81. A vertical screen position was used to enable accurate eye-tracking (see Chapter 3.4.3).

Due to the nature of exploring the emotional valence of food items, the decision was made to individualise the videos observed by each participant based on their preferences. To achieve this, prior to the experiment, each participant completed a questionnaire to determine individual food items that they most or least preferred, or for which they had a neutral preference. This questionnaire required each participant to list three food items for each preference and to rate them on a seven-point Self-Assessment Manikin valence scale (Appendix 8). The portrait version was employed; adapted from the Self-Assessment Manikin scales developed by Lang (1980) and first utilised by Suk (2006). The food items that were indicated to be least preferred, neutral in preference, and most preferred were selected for the videos where appropriate. However, participants occasionally specified food items where properties such as the size and ease with which it could

be picked up and eaten using a reach and grasp action were implausible. If the food items were of different sizes, the grasp could not be matched closely across conditions. In such cases, the item with the next strongest preference was selected. This process resulted in each participant seeing a different variety of food items, with some participants seeing the same item as another but in a different condition (e.g., a reach and grasp of an egg was watched as a neutral preference video by one participant, and as a most preferred video by another; see Appendix 9 for complete information of the food items used).

The general experimental protocol was as outlined in Chapter 4.5.1. Each participant took part in four conditions. A control condition involved observing a static right hand against a black background with an empty plate (Figure 8.1(a)). Three experimental conditions involved the observation of a right hand performing a reach and grasp of a food item from a plate and brought towards the "mouth" (off the bottom of the screen). The food items used for each condition were items that individual participants indicated they most or least preferred, or for which they had a neutral preference (see Figure 8.1(b) for an example). Kinematic equivalence across conditions was not measured, but the recorded actions were matched visually to keep the movement consistent across all conditions. Each condition was split into four blocks of 32 trials, with each block containing eight trials of each condition presented in a random order.

The videos showed a Caucasian male's right hand and forearm filmed from a first-person visual perspective, with the hand positioned to the right of the screen to enhance anatomical congruence and ownership. All videos were 5.5 seconds in

duration, with the action observation videos containing one reach and grasp. One stimulation was delivered per trial at the point of maximal FDI contraction during the grasp action, and at the same time points during the static videos (2400ms after video onset).

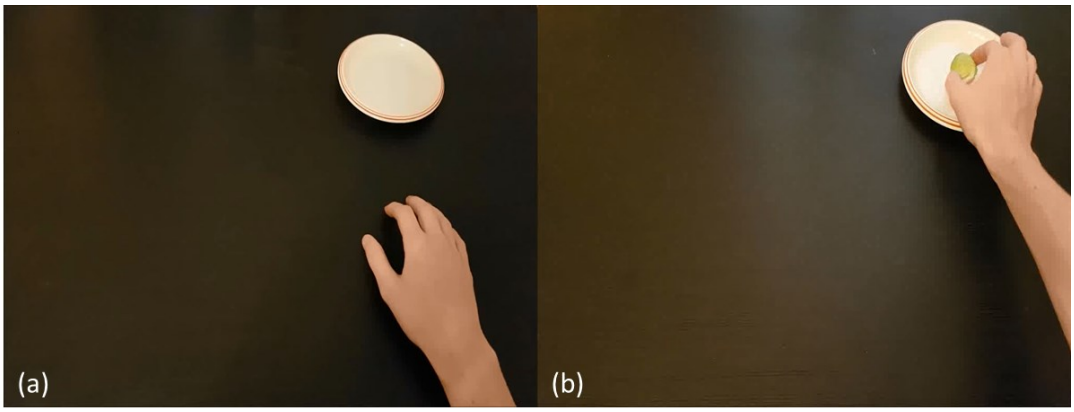


Figure 8.1. Still images from (a) the control condition and (b) an example action observation condition

On completion of the TMS protocol, a one-to-one deductive semi-structured interview was conducted with each participant to explore their experiences of each condition. Questions targeted action observation experiences such as previous experiences with each food item and associated emotional responses. Example questions included: “What were your opinions of the four different videos that you saw in the experiment?” and “Why did you select the [food item] as your [most/least] preferred item?” Probes were used to ensure a thorough consideration and response from each participant. These included “Can you describe these differences?” and “Was that present during one food item video more than the others, or about the same?” (see Appendix 10 for full interview guide).

8.2.3. Data analysis

8.2.3.1. Emotional valence data

Following food item selection, a one-way repeated measures ANOVA was performed on the emotional valence scores. Significant effects were explored further using post-hoc comparisons with Bonferroni corrections.

8.2.3.2. TMS data

Data analysis for the MEP amplitudes was as outlined in Chapters 4.5.2.1 and 4.5.2.3. A 2 (muscle: FDI, ADM) x 4 (condition: static hand, least preferred food item, neutral preference food item, most preferred food item) repeated measures ANOVA was performed on all participants' *z*-score MEP data. Significant effects were explored further using post-hoc pairwise comparisons with Bonferroni corrections.

8.2.3.3. Eye-tracking data

Data analysis for the eye-tracking data was as outlined in Chapters 4.5.2.2 and 4.5.2.3. Individual trials were analysed using two separate dynamic AOI for the food item and hand. A third AOI was defined as the plate on which the food item was placed (Figure 8.2). The fixation count data and percentage fixation duration data were analysed using separate 3 (AOI: hand, food, plate) x 4 (condition) repeated measures ANOVAs.

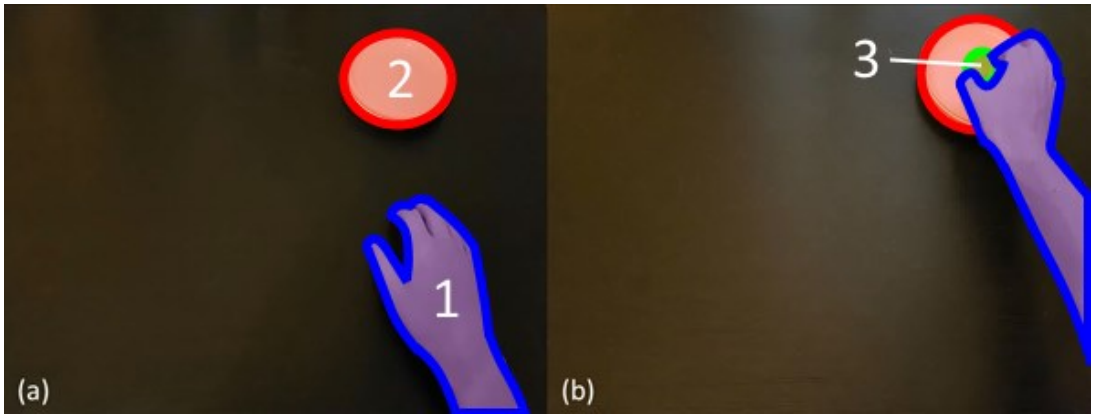


Figure 8.2. Dynamic AOI were used to cover the (1) hand, (2) plate, and (3) food item on the (a) static hand and (b) reach and grasp observation conditions

8.2.3.4. Interview data

Data analysis for the interview data was as outlined in Chapter 4.5.2.4.

8.3. Results

8.3.1. Emotional valence data

Following food item selection, the average valence scores were as follows: least preferred (1.58 ± 0.15); neutral (5.46 ± 0.12); and most preferred (8.42 ± 0.15). A one-way ANOVA revealed a significant main effect for valence scores $F_{(2,46)}$ = 533.42, $p < 0.001$, $\eta_p^2 = 0.96$ with the valence scores for the most preferred food items significantly greater than the valence scores for the neutral preference ($p < 0.001$, $d = 3.10$) and least preferred food ($p < 0.001$, $d = 5.85$) items. In addition, valence scores were significantly smaller for the least preferred food items compared to the neutral preference ($p < 0.001$, $d = 4.09$).

8.3.2. Preliminary TMS data

The 2 (muscle) x 4 (condition) repeated measures ANOVA on the number of rejected trials showed a significant main effect for muscle, $F_{(1,23)} = 7.06$, $p = 0.01$, η_p^2

= 0.24, with significantly more trials rejected from the ADM muscle (2.07 ± 0.57) compared to the FDI muscle (0.45 ± 0.18). No significant main effect for condition was found, $F_{(3,69)} = 1.80$, $p = 0.16$, $\eta_p^2 = 0.07$. In addition, no significant muscle x condition interaction effect was found, $F_{(3,69)} = 0.32$, $p = 0.81$, $\eta_p^2 = 0.01$.

The 2 (muscle) x 4 (condition) repeated measures ANOVA on the pre-stimulation baseline EMG data of the remaining trials showed no significant main effect for muscle, $F_{(1,23)} = 3.28$, $p = 0.08$, $\eta_p^2 = 0.13$, and no significant muscle x condition interaction effect, $F_{(3,69)} = 0.72$, $p = 0.54$, $\eta_p^2 = 0.03$. Mauchly's test indicated that the assumption of sphericity had been violated for the main effect for condition ($\chi^2_{(5)} = 11.56$, $p = 0.04$). Following a Huynh-Feldt correction ($\varepsilon = 0.88$) there was no main effect for condition, $F_{(2.63,60.58)} = 0.98$, $p = 0.40$, $\eta_p^2 = 0.04$.

8.3.3. TMS data

Raw MEP amplitudes recorded from the FDI and ADM muscles for each of the conditions are reported in Table 8.1. The 2 (muscle) x 4 (condition) repeated measures ANOVA on the *z*-score data showed a significant main effect for condition, $F_{(3,69)} = 4.94$, $p = 0.004$, $\eta_p^2 = 0.18$. Pairwise comparisons revealed that MEP amplitudes were significantly greater during the static hand condition compared to the most preferred food item condition ($p = 0.02$, $d = 0.55$), and during the least preferred food item condition compared to the most preferred food item condition ($p = 0.04$, $d = 0.44$) conditions (Figure 8.3).

Table 8.1. Raw MEP amplitudes recorded from the FDI and ADM muscles for each condition

	Raw MEP amplitude (μV)	
	FDI	ADM
Static hand	845.66 (± 103.24)	639.97 (± 96.76)
Least preferred	854.82 (± 115.47)	595.38 (± 98.10)
Neutral preference	800.36 (± 105.63)	547.75 (± 90.82)
Most preferred	792.60 (± 106.01)	556.84 (± 89.68)

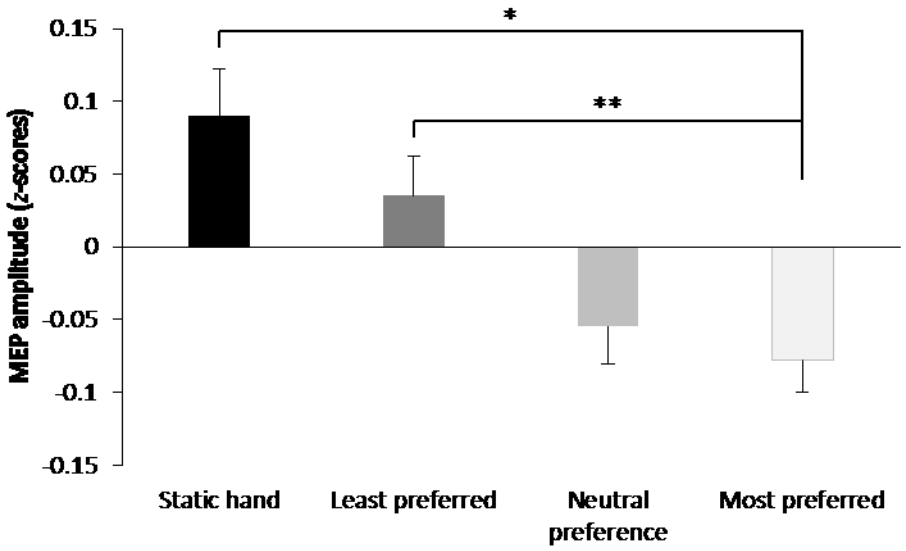


Figure 8.3. Mean MEP amplitudes, displayed as *z*-scores, recorded during each condition, *p = 0.02, **p = 0.01

8.3.4. Eye-tracking data

For the number of fixations data, Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2_{(20)} = 109.63$, $p < 0.001$). Following a Greenhouse-Geisser correction ($\varepsilon = 0.33$), the 3 (AOI) x 4 (condition) repeated measures ANOVA

on the number of fixations data showed a significant AOI x condition interaction, $F_{(2.00,46.05)} = 33.12$, $p < 0.001$, $\eta_p^2 = 0.59$. Pairwise comparisons revealed that more fixations were made on the food compared to both the hand ($p = 0.03$, $d = 0.56$) and the plate ($p = 0.04$, $d = 0.54$) AOI during the least preferred food item condition (see Figure 8.4).

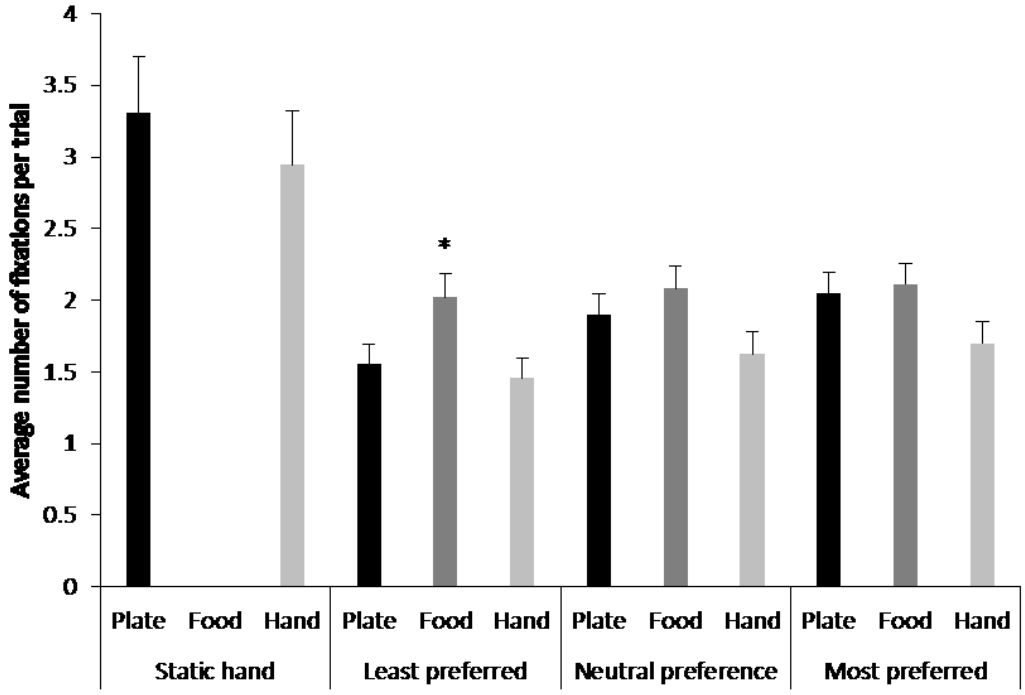


Figure 8.4. The average number of fixations per trial recorded in each AOI during each condition. * indicates significantly more fixations on the food AOI compared to the plate and hand AOI in the least preferred food item condition ($p \leq 0.04$)

For the percentage fixation duration data, Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2_{(20)} = 106.87$, $p < 0.001$). Following a Greenhouse-Geisser correction ($\varepsilon = 0.33$), the 3 (AOI) x 4 (condition) repeated measures ANOVA on the percentage fixation duration data showed a significant AOI x condition interaction, $F_{(1.96,45.11)} = 33.90$, $p < 0.001$, $\eta_p^2 = 0.60$. Pairwise comparisons revealed that participants spent a greater percentage of time fixating

on the food compared to both the hand (all $p \leq 0.01$, all d from 0.67 to 1.07) and the plate (all $p \leq 0.02$, all d from 0.61 to 1.11) AOI during all action observation conditions (see Figure 8.5).

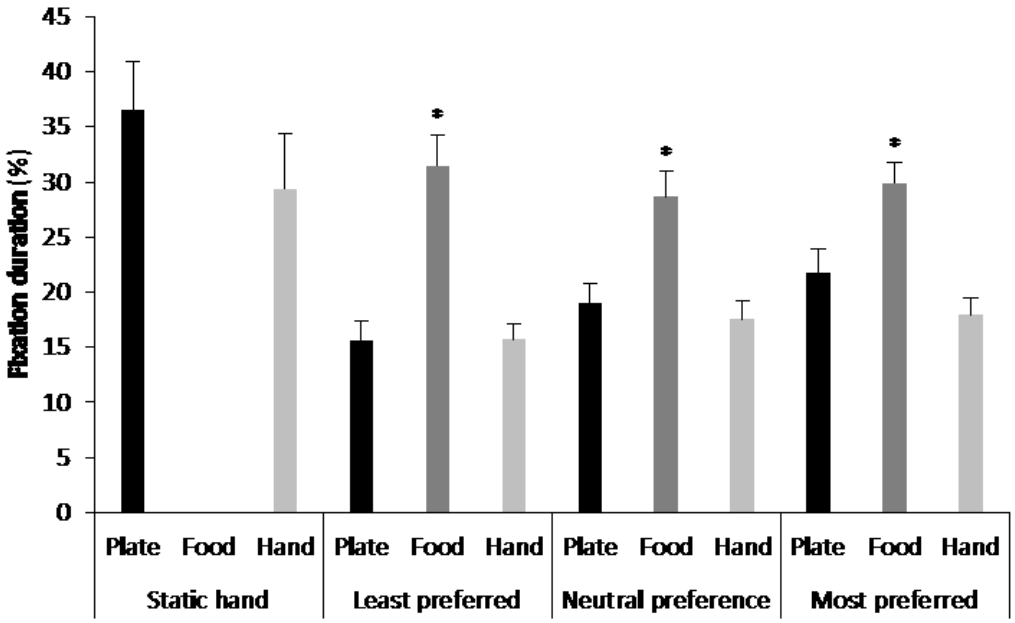


Figure 8.5. The percentage fixation duration recorded on each AOI during each condition. * indicates significantly greater percentage fixation duration on the food AOI compared to the plate and hand AOI in each action observation condition ($p \leq 0.02$)

8.3.5. Interview data

Elements related to participants' viewing experiences of the action and food items, and the participants' preference for the food items, provided the structure for the thematic analysis. Analysis of the interviews suggested primary themes of viewing experience and preference. The viewing experience theme related to participants' engagement with the observation and spontaneous use of motor imagery. The preference theme related to the extent to which participants discussed their feelings towards each food item and their previous experiences with the food, with greater or lesser detail provided depending on preference.

8.3.5.1. Viewing experience

Videos containing an action were reported to be more engaging than the videos containing a static hand, regardless of the preference for the food item involved (e.g., participant (P)3.12; P3.18; P3.24). Participants reported that "all three of the movement [videos]" (P3.4) were engaging to the observer, possibly due to both the movement and the presence of food items, as participants were "following the hand movement" (P3.12) and curious "to see what food it was" (P3.14). In addition, it was reported that they "weren't as engaged" (P3.15) in the control condition containing the static hand. This led to participants "not concentrating as much" (P3.9) and feeling "switched off" (P3.20) during the static hand trials.

Out of the 24 participants, 18 reported the spontaneous use of motor imagery modalities across the different conditions (e.g., P3.1; P3.11; P3.23). Details regarding motor imagery were raised during various questions and prompts, such as "Did you experience any differences as a consequence of viewing the action with different objects?" and "Can you describe what you thought about when you observed the hand grasping the [most/least preferred food item]?" Participants reported that they felt like they were thinking about "grabbing it and eating [the food item]" (P3.16). Kinaesthetic imagery modalities relating to reaching ("I could feel myself almost reaching for [the food item]" (P3.3)), grasping ("I was imagining myself grabbing a piece" (P3.1)), and eating ("biting it, chewing it" (P3.18)) were all reported. In addition, wider sensory experiences were reported in relation to the touch ("you do imagine what it feels like even if that's not part of the task" (P3.5)),

taste (“it made you imagine eating it and tasting it” (P3.22)) and smell (“awareness of the smell” (P3.11)) of the food items.

8.3.5.2. Preference

Participants discussed their previous experiences with the food that influenced their preference, and the emotional response to observing the food. Participants more frequently discussed their previous experiences with the least preferred, compared to the most preferred food items. The first time a participant tried the least preferred food item was a common discussion point, with one participant reporting that observing the food item led them to go “back to that moment when [they] found out [they] didn’t like them” (P3.9). Multiple participants reported previous experiences of feeling “ill after eating [the food item]” (P3.7), including actually “throwing up” (P3.8). Some participants felt like they were “made to eat it” (P3.3) when they were younger, with one reporting that their mother “still puts sprouts on [their] Sunday dinner” (P3.4). Such levels of detail and recall were not present when discussing the most preferred food item (e.g., “I guess it’s something that I suppose I’ve consumed from when I was a child” (P3.10); and “I quite like chocolate cake” (P.3.7)).

Similar patterns were found in the discussions regarding their emotional response, with more detailed accounts being provided for the least preferred to the most preferred food items. Participants discussed the sensations and experiences that they relate to the least preferred food item, such as stating that they “don’t like the texture of [the food item] or the smell” (P3.4), or that “it smells bad and it doesn’t taste very good” (P3.11). In contrast, participants reported that the most preferred

food item was simply “a bit more desirable” (P3.10), with some even indicating uncertainty as to why they held it in greater preference (e.g., “I don’t know, I just like eating it” (P3.19)). From the discussions relating to preference, it appears that participants developed and maintained stronger emotions related to the least preferred food item compared to the most preferred food item.

8.4. Discussion

This experiment aimed to determine the effect of the emotional valence of objects on CSE and visual attention during action observation. In contrast to a well-established body of research (e.g., Fadiga et al., 1995; see Loporto et al., 2011; Naish et al., 2014 for reviews), the results of this experiment did not demonstrate a facilitation of CSE across any experimental conditions in comparison to the control condition. Specifically, no differences were found between observation of a reach and grasp of an unpleasant and neutral food item compared to the control condition. In addition, the results indicate that CSE was inhibited during observation of a reach and grasp of a food item judged to be pleasant compared to the control condition. Inhibition of CSE during action observation is not a common finding, but some variables, such as observing both with the intention to imitate (Hardwick et al., 2012) and painful events (Avenanti et al., 2005) have demonstrated an inhibition effect. Finally, the results also indicate that the emotional valence of a grasped food item in an observed reach and grasp action does modulate CSE, with a facilitation of CSE during observation of the unpleasant food item condition compared to the pleasant food item condition. The observed effects, however, were not specific to the muscle involved in the execution of the observed action

(i.e., the FDI muscle), with only a significant main effect for condition found. This is again in contrast to well-established muscle specificity research (see Fadiga et al., 1995; Naish et al., 2014). The preliminary analyses confirm that the findings cannot be accounted for by increased muscle activity prior to stimulation and that the emotional valence scores were significantly different across all conditions. The findings, therefore, can be attributed to the manipulation of the emotional valence of the objects.

The least preferred and neutral preference food item conditions did not differ significantly from the static control condition. This is in contrast to the consistent finding of facilitated CSE during action observation compared to static observation (see Fadiga et al., 1995; Naish et al., 2014). Although unexpected, this finding may be an indicator of avoidance behaviours being selected during the unpleasant food item condition (Coelho and Purkis, 2009). When considering the muscle-specific facilitation of CSE (Naish et al., 2014), it is possible that movement preparations were made to withdraw the arm away from the stimuli whilst observing the unpleasant food item. Given the mechanics of this action, this would likely be reflected in muscles such as the bicep as the arm is retracted and the elbow flexed. This could explain why no CSE modulation was found in the FDI and ADM muscles, as they would not be expected to be involved in such a withdrawal action. This postulate, however, requires testing empirically in future research. In addition, research has indicated that observation of actions perceived to be meaningful, such as the unpleasant and pleasant food items in the present experiment, are more likely to facilitate CSE (Donne et al., 2011; Enticott et al., 2012; see Chapter 7.4).

This may explain the neutral preference food item not producing a facilitation of CSE, as it was not perceived as meaningful in comparison to the other conditions.

The MEP data revealed an inhibition of CSE during observation of the reach and grasp for a pleasant food item compared to the static hand condition. This is in contrast to the common finding of CSE facilitation during action observation, (see Fadiga et al., 1995; Naish et al., 2014). However, this finding does support a smaller body of research that shows some variables may result in inhibition effects during action observation (e.g., Villiger et al., 2011; Hardwick et al., 2012). For example, whilst Enticott et al. (2010) demonstrated facilitated CSE during observation of a reach and grasp of a non-food-related item, Villiger et al. (2011) showed inhibited CSE during observation of a reach and grasp of a food item. Villiger et al. (2011) argue that the inhibition effect whilst observing a reach and grasp for a food item may reflect the observer refraining to execute the observed action. There may be inhibitory mechanisms acting to prevent overt execution during action observation involving non-food-related items, but such mechanisms may be activated to a greater extent during action observation involving food items. This is thought to be a result of the emotional valence associated with observing food items, and may have induced the CSE inhibition seen in the present experiment.

In support of the postulate that increased emotional valence generated the inhibition effect reported in this experiment, Nogueira-Campos et al. (2014) only found corticospinal inhibition whilst participants prepared to reach and grasp for a pleasant object compared to when a pleasant object was presented and no action preparation was required. This difference was not seen during the same tasks with

unpleasant objects. In their experiment, the stimulation was delivered whilst preparing to perform an action. This is comparable to the present experiment, where the participant's task was to observe, but not to perform, an action. The selected 'motor programmes' at any moment are influenced by the goal of the action (Manthey et al., 2003; Donne et al., 2011; Kelly and Wheaton, 2013), so it appears that, due to the inhibition of CSE, participants were refraining from executing the observed action during the pleasant stimulus condition (Villiger et al., 2011). It may be a strong resistance of executing an action towards a desirable object, therefore, that resulted in inhibited CSE (Cross and Iacoboni, 2014; Nogueira-Campos et al., 2014).

Within the action observation conditions, greater CSE was demonstrated during observation of a reach and grasp of an unpleasant compared to a pleasant food item. This is in support of previous research involving movement preparation tasks (Nogueira-Campos et al., 2014), and the observation of both images (Coelho et al., 2010; Van Loon et al., 2010) and actual movement (Nogueira-Campos et al., 2016). It is thought to be a result of connections between emotion and motor responses (Coelho et al., 2010; Lang et al., 2000). Specifically, the superior temporal sulcus and inferior frontal gyrus, thought to be part of an extended action observation-execution matching mechanism (Eaves et al., 2016), are sensitive to affect whilst processing action-related information (Ferri et al., 2013). The superior temporal sulcus combines both positive and negative affect with action-related information, whilst the inferior frontal gyrus only combines negative affect with such information (Ferri et al., 2013). In addition, the premotor cortex has also been shown to respond to negative affect (Pichon et al., 2009). Increased activation of

the motor regions because of negative affect compared to positive affect could explain the facilitation of CSE during the unpleasant food item condition compared to the pleasant food item condition. During the interview, participants indicated stronger affect during the unpleasant food item condition, as they discussed greater detailed accounts of the emotional valence of the unpleasant food item compared to the other food items.

The eye-tracking data indicated that the unpleasant object may have been perceived as a source of negative affect or a threat, as a greater number of fixations were made on the unpleasant object compared to the hand and plate in the same condition (Alshehri and Alghowinem, 2013; Strauss et al., 2016). Such differences were not found, however, whilst observing the pleasant or neutral object conditions. This becomes more evident when considering the percentage fixation data. This shows that participants spent longer fixating on the food items compared to the hand and plate across all conditions, indicating that gaze was drawn towards the target and goal of the action (D'Innocenzo et al., 2017; Wright et al., 2018), but it was the nature of the threatening unpleasant object that modulated visual attention. The interview data support the postulate that the unpleasant food item was perceived as a threat. Participants discussed emotive responses relating to the unpleasant food item in detail, but with considerably reduced detail when discussing those related to the pleasant food item. Participants recalled detailed accounts of previous experiences with the unpleasant food item and generated detailed imagery regarding the taste, smell and texture. Such recall of negative experiences is common when discussing the origins of negative associations, such as phobias (Merckelbach et al., 1991).

Taken together, the TMS, eye-tracking, and interview findings all indicate important survival responses to unpleasant food items. These physiological and behavioural responses to the unpleasant food item indicate a potential avoidance of exposure to harm. The negative affect associated with observing unpleasant stimuli appears to stimulate 'flight-or-fight' mechanisms (Grecucci et al., 2011:312), reflecting in subsequent selection of appropriate motor response, eye movement and cognitive recall to alter the behaviour of the observer (Coelho and Purkis, 2009; Grecucci et al., 2011). These innate responses are automatic, and cannot be consciously controlled (Mineka and Öhman, 2002).

Aside from the possible confounds discussed already, it is possible that the results may also have been influenced by the kinematic profiles of each video. As each participant observed three individualised action videos including their selected food items, kinematic equivalence could not be matched exactly. Manipulating the kinematic profile of an observed action, such as the force used (Alaerts et al., 2010) and the grasp aperture (McCabe et al., 2014) of the observed action, modulates CSE. Careful attempts were made to match the movement visually for each video as closely as possible to minimise the possible confounding effect that this could have had on the results, though this can be considered a limitation of the present experiment.

The results of the present experiment indicate that CSE is facilitated during observation of a reach and grasp of an unpleasant, compared to a pleasant, food item. This facilitation would appear to have important behaviour modulation implications as appropriate responses to identify and react to a threat are selected

(Coelho and Purkis, 2009). In addition, observation of the same action with the pleasant food item actually inhibits CSE in comparison to a static hand condition. Based on these results, observing a reach and grasp of food stimuli would be recommended for exclusion from motor (re)learning interventions (*viz.* Holmes and Wright, 2017). The lack of CSE facilitation, and even a significant inhibition, during the food item conditions compared to the static hand condition, may be a result of participants actively refraining from performing the movement due to the experimental paradigm and the necessity for their hand to be stationary. Alongside research indicating the importance of personalising interventions based on participant preferences (Ewan et al., 2010; Buccino, 2014), it appears important that future research explores the emotional valence of other ecologically valid objects. This would determine whether the emotional valence or observing food items produced the present results. In addition, before completely ruling out the use of food items, future research should explore the use of food stimuli during motor (re)learning interventions to ascertain whether or not they should be included.

Chapter 9. General discussion and conclusions

The results from each of the experiments included in this thesis have indicated the importance of the meaning allocated to an observed action. This meaning has been reflected in the modulation of CSE and visual attention, and differences in each individual's reported experiences. In this final chapter, the implications and applications of these results are explored. It is argued that the consideration of individual meaning and the use of additional measures to compliment TMS data are necessary inclusions to further the understanding of both this area of research and interventions for motor (re)learning.

9.1. Thesis summary

The overall aim of the thesis was to identify action observation variables that facilitate CSE in order to provide recommendations for improving the efficacy of action observation interventions for motor (re)learning. Specifically, variables relating to the meaning of the observed action were explored. Aims 1 and 2 (see Chapter 2.4.5) were met in full. Aim 3 was only met in part, as further research is required following Experiment 3 (Chapter 8) to determine the nature of the results. TMS was used to provide a measure of CSE, and interviews and eye-tracking were used to assist in determining the nature of CSE modulation. Aims 4-6 were met in full, as the appropriate TMS technique was able to be determined (Chapter 5), and the additional interview and eye-tracking techniques facilitated the interpretation of the CSE findings (Chapters 6-8).

The pilot experiment explored the use of paired-pulse TMS compared to single-pulse TMS, to explore mechanisms underlying the modulation of CSE during action

observation. As hypothesised, 3ms ISI and 12ms ISI inhibited and facilitated MEP amplitudes respectively in the FDI muscle in comparison to single-pulse TMS. The predicted inhibition effect of the 3ms ISI was not demonstrated in the ADM muscle, however, as both the 3ms ISI and 12ms ISI facilitated MEP amplitudes in comparison to single-pulse TMS. Only MEP amplitudes recorded using single-pulse TMS were as expected in relation to previous research, with an increase in CSE during action observation compared to the control condition, specific to the muscle involved in executing the action (Fadiga et al., 1995; Naish et al., 2014). Neither the 3ms ISI nor the 12ms ISI demonstrated the expected muscle-specific effect, with both the FDI and ADM demonstrating greater MEP amplitudes during the action, compared to control, observation. The facilitation of MEP amplitudes during action observation compared to the static control during 12ms ISI was also unexpected, as previous research indicates either an inhibition effect (Strafella and Paus, 2000) or no effect at all (Patuzzo et al., 2003). With the lack of consistency between the findings of the previous paired-pulse TMS research, and the necessity for methodological research before its use within action observation experiments, single-pulse TMS was determined to be the TMS protocol for the subsequent experiments in this thesis.

Experiment 1 utilised single-pulse TMS to explore the effect of presenting a first-person perspective action on two different screen positions on CSE, with a post-experiment interview to explore each participant's individual experiences. Most research utilises vertical screen positions, even when presenting a first-person perspective. This may create a view of the action that is detached from the self, subsequently detracting from the first-person perspective. A novel aspect of this

experiment was that CSE was explored during action observation presented on a horizontal screen position, with the observed action situated for anatomical congruency with the observer, compared to a vertical screen position, where anatomical congruency could not be matched. No differences were shown in CSE between the two screen positions. Following this analysis, in a further novel contribution to the action observation literature, the data were split based on participants' screen position preference. Although consideration of participants' action observation preferences is not usually taken into account in action observation research, the adoption of this innovative approach resulted in some noteworthy findings. Specifically, participants who reported a horizontal screen position preference in the interview demonstrated greater CSE during action observation compared to the static control irrespective of screen position, and during the horizontal screen position compared to the vertical screen position irrespective of video type. This demonstrates that ensuring anatomical and perceptual congruency with the observed task, combined with consideration of participant viewing preferences, should be important inclusions for action observation interventions for motor (re)learning. As such, individualisation during both action observation experiments and interventions should be considered when conducting future research in this area.

Experiment 2 is amongst the first action observation experiments to record eye movements and CSE simultaneously. This experiment explored the effect of meaning provided by visual context during action observation on visual attention and CSE. Previous research had indicated increased neural activity during action observation embedded in a congruent context (Iacoboni et al., 2005).

Methodological issues associated with the previous research, however, meant that it was unclear whether these findings were due to the effect of the context, or simply the addition of background objects in the video. This experiment explored this further by presenting contextual information that was either congruent or incongruent to the observed action. Results indicated CSE was facilitated when the observed action contained contextual information that was congruent to the action, compared to both a static control condition and action observation devoid of contextual information. This CSE facilitation was not present when the observed action contained contextual information incongruent to the action. In addition, the eye-tracking data indicated a greater number of fixations and percentage fixation duration on the background scene during both the congruent and incongruent conditions. Such eye movement behaviour indicates that participants examined the background scene in an attempt to obtain information regarding the goal and intention of the observed action. It was interpreted that the ability to infer the goal and intention of an observed action from information in the background context may contribute to a facilitation of CSE during action observation. This supports previous research that indicates only observed actions perceived to be meaningful generates a facilitation of CSE (Enticott et al., 2010). Together, these results indicate that the efficacy of action observation interventions for motor (re)learning may be enhanced by including visual information that is congruent to the observed action.

The final experiment, Experiment 3, explored the effect that individual emotional valence attached to observed stimuli had on CSE. Only one previous experiment had examined the effect action observation involving interactions with emotionally valent objects has on CSE. Nogueira-Campos et al. (2016) explored this issue, but

their experiment was limited through a lack of individualisation of the objects presented to each participant based on their preference. To address this, Experiment 3 utilised a novel action observation protocol by presenting participants with a reach and grasp of food items that they had personally indicated they most or least preferred, or for which they had a neutral preference. In contrast to the hypothesised outcome, the results indicated that CSE was not facilitated during any condition compared to a static control. Action observation of an interaction with the most preferred food item, however, did demonstrate a significant inhibition of CSE compared to the control condition. The lack of facilitation during the unpleasant stimuli compared to the control condition appears to be a result of the activation of withdrawal mechanisms, which would not necessarily facilitate CSE in the muscles involved in a reach and grasp (Coelho and Purkis, 2009; Naish et al., 2014). This withdrawal effect would not have been present during the pleasant food item condition, but the inhibition effect may have occurred due to refraining from performing the observed action (Cross and Iacoboni, 2014; Nogueira-Campos et al., 2014). In addition, a greater number of fixations were shown on the unpleasant food item compared to the hand and the plate. This effect was only shown during the unpleasant food item condition, indicating that attention may have been drawn to the unpleasant food item as it was perceived as a threat to the observer. Interview data supports the postulate that the unpleasant food item was perceived as a threat, as participants indicated more detailed emotive responses to this stimuli compared to the pleasant food item. Together, the results of Experiment 3 indicate that observing interactions with food items may not be appropriate stimuli for action observation interventions aimed at motor

(re)learning. The objects selected for action observation research and interventions, however, should be selected based on the individual observer, though further research is required.

9.2. Contribution to knowledge

The experiments included within this thesis are among the first to utilise interview and eye-tracking data alongside TMS data to benefit the understanding of the nature of CSE modulation during action observation. Experiment 1 (Riach, Wright, et al., 2018) and Experiment 2 (Riach, Holmes, et al., 2018) have been published, providing researchers with the information necessary to understand the benefit of utilising such techniques alongside TMS, and to continue their use within future research. As such, a combination of methods alongside TMS during action observation should become more common in the future. The use of additional techniques in the experiments included within thesis provided important insights into the design of action observation interventions. Specifically, demonstrating how the variables manipulated in this thesis modulate CSE provides an indication of how these variables could be used to optimise the design and delivery of action observation interventions for motor (re)learning. Additionally, Experiment 3 was the first experiment to fully individualise action observation stimuli for each participant based on their individual preferences. The results were inconclusive (see Chapter 8), though it does demonstrate that such individualisation is possible, and emphasises the importance of individualisation within research and future applied interventions.

The variables explored in the experiments included within this thesis demonstrate the importance of considering both the meaning of the presented stimuli to each participant, and the preferences of each participant. This can be utilised within research exploring action observation interventions for motor (re)learning. For example, the results of Experiment 1 indicate that presenting action observation stimuli in a manner that matches a participant's screen position viewing preference might enhance the efficacy of action observation interventions. Similarly, the results of Experiment 2 would suggest that presenting action observation interventions with contextually congruent background scenes might also provide more optimal action observation interventions. Technological advances in computer tablet and other mobile devices now enable action observation videos to be easily presented in a manner that matches the observer's viewing preference. Providing a congruent context with the observed action appears not only beneficial, but sensible, as patients are often presented with activities of daily living (e.g., Ewan et al., 2010; McCormick and Holmes, 2016), such as washing dishes. This requires testing, however, before it can be applied to actual interventions for motor (re)learning. Based on the results of Experiment 3, the application of food items and other emotionally valent objects are not, at this stage, recommended for application to motor (re)learning settings, as more research is required to understand the nature of the CSE findings.

The interpretation of the TMS findings of each experiment included in this thesis benefitted directly from the inclusion of eye-tracking and interview techniques. Few experiments have utilised eye-tracking alongside TMS during action observation prior to the experiments included in this thesis (see Chapter 2.4.4.1). The use of

eye-tracking allowed changes in CSE between experimental conditions to be interpreted in relation to differences in visual attention. In addition, no previous experiments have appeared to have utilised in-depth interview techniques to explore individual experiences during action observation to further the understanding of the TMS findings. The interviews enabled an in-depth look at each participant's individual experience of the observation stimuli. This provided an understanding of each participant's affect during different action observation conditions, and was therefore invaluable in the interpretation of significant results. It also appears that Experiment 1 is the first to utilise the qualitative data to provide additional exploration of the TMS data. Where appropriate, such methods should be applied to future action observation research, especially research utilising TMS, to enhance understanding of the whole participant experience during action observation.

Taken together, this series of studies made key contributions to inform the design and delivery of action observation research and interventions. Practitioners utilising action observation interventions for motor (re)learning should consider individualising stimuli based on individual meaning and preference, rather than utilising a battery of generic stimuli that is presented to all patients. This is in line with current NHS goals of working towards personalised care (NHS England, 2018). The NHS aims to provide choice and control to the patient over the planning and delivery of their care. This is in line with the conclusions drawn from the experiments in this thesis, that the patient's individual preferences should be accounting for during this process. Considering individual preferences and context for action observation interventions should enable the patient to experience stimuli

that are particularly meaningful and relevant. This has the potential to result in more effective action observation interventions for motor (re)learning, though this still requires testing in applied settings.

9.3. Future research

The experiments included in this thesis provide useful information regarding the potential importance of multiple variables relating to meaning for action observation interventions for motor (re)learning. However, further research is still required to provide a full understanding of action observation in relation to both CSE and interventions for motor (re)learning.

Experiment 1 demonstrated that, when accounting for participant preference, observing an action on a horizontal screen may facilitate CSE by enhancing anatomical congruency. This is particularly relevant considering the prevalence of mobile devices with video capabilities such as tablets and phones. These devices can be easily oriented to match the observer's preference during action observation intervention videos for motor (re)learning. More research is required, however, prior to this applied stage. For example, the observed hand performing the action was presented to be lifelike in size. This would not be possible on tablet and mobile phone devices due to limitations in the screen size. Research, therefore, should look to examine the effect of different hand and/or screen sizes whilst presenting action observation videos. In addition, measures to explore visual attention may be possible on a horizontal screen position. This could involve using a chin rest that is angled down toward the screen, preventing the participants from having to look down with their eyes only which results in the upper eyelid lowering. Differences in

participant preference may be reflected in their visual attention throughout different screen positions, and would assist in understanding CSE modulation.

A combination of the ideas explored in Experiment 1 and 2 may be an interesting area for future research to examine. It may be beneficial to explore the complexity of the observed action whilst anatomical congruency is matched. Experiment 1 presented a simple index finger-thumb ball pinch, devoid of context, and Experiment 2 could not maintain anatomical congruency due to the use of a vertical screen position. Providing a more complex action to the observer, or providing a meaningful context alongside anatomical congruency should be explored to provide an in-depth understanding of the observer's experiences during each screen position. Likewise, a combination of the protocols utilised in Experiment 2 and 3 would benefit action observation research to inform interventions for motor (re)learning. Patients undertaking action observation interventions for motor (re)learning indicate a preference for observing actions and/or activities that they have an interest in (Ewan et al., 2010). Therefore, research exploring observation of detailed videos involving actions individualised to the participant's interests and containing visual context should be performed.

The results of Experiment 3 require further research to provide clarification on the interpretation of the data. For example, it is unclear whether the CSE findings were due to the emotional valence of the food items, or survival implications of observing interactions with food items. To expand this area of research, alternative items indicated to relate to strong observer preferences should be utilised to provide further understanding of the emotional valence aspect. In addition, CSE

relating to alternative muscles could be explored. Testing the bicep muscle, for example, could possibly indicate motor preparation for withdrawal behaviour whilst observing a hand grasp an unpleasant object through a facilitation in CSE. The bicep would not be expected to demonstrate CSE facilitation due to its lack of involvement in a hand grasp, so this would indicate a desire to remove the hand away from the stimulus, as it would be involved in elbow flexion to bring the hand away from the object and closer to the body.

To understand the precise mechanisms underlying CSE modulation during action observation, further research is required. Research involving paired-pulse TMS would be beneficial as this technique offers a useful insight into individual inhibition and facilitation mechanisms, and so provides an indication of changes in excitability at a corticocortical, rather than a corticospinal, level. Before this research can be performed, however, preliminary research is required to determine the optimal paired-pulse TMS method. For example, the inconsistent findings reported during action observation research utilising paired-pulse TMS may be a reflection of high variability between individuals. Such variability is not yet able to be taken into account without sacrificing either the number of trials required to account for intraindividual variability, measuring multiple conditioning stimulus intensities, or measuring both ICI and ICF (Peurala et al., 2008; Wagle-Shukla et al., 2009; Du et al., 2014; Goldsworthy et al., 2016). Future research should explore such issues in an attempt to determine optimal paired-pulse TMS protocols. Following a prescribed protocol that is able to overcome such issues would enable more comprehensive explorations of the ICI and ICF mechanisms during action

observation, expanding the current understanding of the nature of corticospinal modulation.

All of the variables explored in the experiments included in this thesis could be applied to research exploring action observation interventions for motor (re)learning. The screen position may improve such interventions by presenting the observer with a visual representation that best suits their preference. Furthermore, adding visual context that is congruent to the observed action may help to optimise observation interventions by providing information regarding the action goal and intention. These additions may enhance the motor function improvements beyond those already reported in previous action observation interventions for motor (re)learning (Buccino, 2014). Applied research within patient and healthy populations is, therefore, needed to determine the efficacy of these additions within observation interventions for motor (re)learning. With the findings of Experiment 3 not demonstrating a facilitation in CSE, more research is required to understand the mechanisms underlying the findings before applying to motor (re)learning settings. Even with the inhibition of CSE, however, presenting most preferred food items during action observation interventions may also benefit motor (re)learning in an applied setting where the participant may be able, and even encouraged, to emulate the observed action (Ewan et al., 2010). This may prevent the CSE inhibition demonstrated in Experiment 3 that may be a result of having to refrain from movement execution due to methodological constraints (Cross and Iacoboni, 2014). This requires further testing in both experimental and applied settings, however, before such conclusions can be drawn.

9.4. Conclusions

The experiments included in this thesis explored variables relating to the meaning of an observed action using TMS. By combining the TMS and interview data, Experiment 1 provided evidence that individual experiences relating to the observed conditions are important considerations. Specifically, future research and interventions should account for the observer's viewing preference relating to the screen position. Experiment 2 expanded on previous research, indicating that providing context that is congruent to an observed action may benefit interventions for motor (re)learning. The eye-tracking data revealed that this might have been due to participants seeking additional relevant information relating to the observed action, that only a congruent context provides. The final experiment, Experiment 3, utilised a novel approach to action observation and TMS research by completely individualising the videos that each participant observed. Participants observed a reach and grasp of food items that they indicated they most or least preferred, or for which they indicated a neutral preference. The TMS findings expanded on a single previous experiment that explored emotionally-valent objects involved in action observation. Experiment 3 appears to be the first to include TMS, eye-tracking, and interview techniques within a single experiment. The additional techniques provided novel insight into the TMS data, demonstrating that the least preferred food item was perceived as a threat. Taken together, these data provide a greater knowledge of the effect of the meaning attached to an observed action by both groups and individuals.

Future research should consider the use of additional measures to further explore and understand CSE modulation during action observation. Eye-tracking and interview techniques have enhanced the understanding of each variable during action observation, though further research is still required in both experimental and applied settings. They do demonstrate, however, potential avenues for optimising observation interventions. This especially applies to the use of context that is congruent to the observed action and individualising each intervention based on the patient's preferences. As our understanding of CSE during action observation is enhanced, the number of variables that can be explored in applied settings is increased. Such research, therefore, is pivotal in the future optimisation of the design and delivery of action observation interventions for motor (re)learning.

References

Abbruzzese, G., Assini, A., Buccolieri, A., Marchese, R. and Trompetto, C. (1999) 'Changes of intracortical inhibition during motor imagery in human subjects.' *Neuroscience letters*, 263(2) pp. 113-116.

Adhikari, S. P., Tretriluxana, J., Chaiyawat, P. and Jalayondeja, C. (2018) 'Enhanced Upper Extremity Functions with a Single Session of Action-Observation-Execution and Accelerated Skill Acquisition Program in Subacute Stroke.' *Stroke Research and Treatment*, 2018

Aglioti, S. M., Cesari, P., Romani, M. and Urgesi, C. (2008) 'Action anticipation and motor resonance in elite basketball players.' *Nature neuroscience*, 11(9) pp. 1109-1116.

Aihara, T., Yamamoto, S., Mori, H., Kushiro, K. and Uehara, S. (2015) 'Observation of interactive behavior increases corticospinal excitability in humans: A transcranial magnetic stimulation study.' *Brain and cognition*, 100 pp. 1-6.

Alaerts, K., Heremans, E., Swinnen, S. P. and Wenderoth, N. (2009) 'How are observed actions mapped to the observer's motor system? Influence of posture and perspective.' *Neuropsychologia*, 47(2) pp. 415-422.

Alaerts, K., Senot, P., Swinnen, S. P., Craighero, L., Wenderoth, N. and Fadiga, L. (2010) 'Force requirements of observed object lifting are encoded by the observer's motor system: a TMS study.' *European Journal of Neuroscience*, 31(6) pp. 1144-1153.

Allan, G. (2003) 'A critique of using grounded theory as a research method.' *Electronic journal of business research methods*, 2(1) pp. 1-10.

Allison, T., Puce, A. and McCarthy, G. (2000) 'Social perception from visual cues: role of the STS region.' *Trends in cognitive sciences*, 4(7) pp. 267-278.

Alshehri, M. and Alghowinem, S. (2013) *An exploratory study of detecting emotion states using eye-tracking technology.* IEEE.

Altschuler, E., Vankov, A., Hubbard, E., Roberts, E., Ramachandran, V. and Pineda, J. (2000) *Mu wave blocking by observation of movement and its possible use as a tool to study theory of other minds.* Vol. 68:

Amoruso, L. and Urgesi, C. (2016) 'Contextual modulation of motor resonance during the observation of everyday actions.' *NeuroImage*, 134 pp. 74-84.

Amoruso, L., Finisguerra, A. and Urgesi, C. (2016) 'Tracking the time course of top-down contextual effects on motor responses during action comprehension.' *Journal of neuroscience*, 36(46) pp. 11590-11600.

Anand, S. and Hotson, J. (2002) 'Transcranial magnetic stimulation: neurophysiological applications and safety.' *Brain and cognition*, 50(3) pp. 366-386.

Avenanti, A., Bueti, D., Galati, G. and Aglioti, S. M. (2005) 'Transcranial magnetic stimulation highlights the sensorimotor side of empathy for pain.' *Nature neuroscience*, 8(7) p. 955.

Aziz-Zadeh, L., Maeda, F., Zaidel, E., Mazziotta, J. and Iacoboni, M. (2002) 'Lateralization in motor facilitation during action observation: a TMS study.' *Experimental brain research*, 144(1) pp. 127-131.

Bähr, F., Ritter, A., Seidel, G., Puta, C., Gabriel, H. H. and Hamzei, F. (2018) 'Boosting the Motor Outcome of the Untrained Hand by Action Observation: Mirror Visual Feedback, Video Therapy, or Both Combined—What Is More Effective?' *Neural plasticity*, 2018

Balslev, D., Braet, W., McAllister, C. and Miall, R. C. (2007) 'Inter-individual variability in optimal current direction for transcranial magnetic stimulation of the motor cortex.' *Journal of neuroscience methods*, 162(1) pp. 309-313.

Bar, M. (2004) 'Visual objects in context.' *Nature reviews. Neuroscience*, 5(8) p. 617.

Barker, A. T., Jalinous, R. and Freeston, I. L. (1985) 'Non-invasive magnetic stimulation of human motor cortex.' *The Lancet*, 325(8437) pp. 1106-1107.

Bestmann, S. and Krakauer, J. W. (2015) 'The uses and interpretations of the motor-evoked potential for understanding behaviour.' *Experimental brain research*, 233(3) pp. 679-689.

Bestmann, S. and Duque, J. (2015) 'Transcranial magnetic stimulation decomposing the processes underlying action preparation.' *The Neuroscientist*, p. 1073858415592594.

Bestmann, S., Harrison, L. M., Blankenburg, F., Mars, R. B., Haggard, P., Friston, K. J. and Rothwell, J. C. (2008) 'Influence of uncertainty and surprise on human corticospinal excitability during preparation for action.' *Current Biology*, 18(10) pp. 775-780.

Biagi, L., Cioni, G., Fogassi, L., Guzzetta, A., Sgandurra, G. and Tosetti, M. (2016) 'Action observation network in childhood: a comparative fMRI study with adults.' *Developmental science*, 19(6) pp. 1075-1086.

Blascheck, T., Kurzhals, K., Raschke, M., Burch, M., Weiskopf, D. and Ertl, T. (2014) *State-of-the-art of visualization for eye tracking data.* Vol. 2014:

Block, H., Bastian, A. and Celnik, P. (2013) 'Virtual lesion of angular gyrus disrupts the relationship between visuoproprioceptive weighting and realignment.' *Journal of cognitive neuroscience*, 25(4) pp. 636-648.

Boksem, M. A., Meijman, T. F. and Lorist, M. M. (2005) 'Effects of mental fatigue on attention: an ERP study.' *Cognitive brain research*, 25(1) pp. 107-116.

Boniface, S., Mills, K. and Schubert, M. (1990) 'The optimum direction and orientation of the maximal inducing current for magnetic human brain stimulation with a double coil.' *Journal of physiology*, 426

Borroni, P., Montagna, M., Cerri, G. and Baldissera, F. (2008) 'Bilateral motor resonance evoked by observation of a one-hand movement: role of the primary motor cortex.' *European Journal of Neuroscience*, 28(7) pp. 1427-1435.

Borroni, P., Gorini, A., Riva, G., Bouchard, S. and Cerri, G. (2011) 'Mirroring avatars: dissociation of action and intention in human motor resonance.' *European Journal of Neuroscience*, 34(4) pp. 662-669.

Both, S., Everaerd, W. and Laan, E. (2003) 'Modulation of spinal reflexes by aversive and sexually appetitive stimuli.' *Psychophysiology*, 40(2) pp. 174-183.

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J. and Hallett, M. (1992) 'Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity.' *Journal of clinical neurophysiology*, 9(1) pp. 132-136.

Brass, M., Bekkering, H., Wohlschläger, A. and Prinz, W. (2000) 'Compatibility between observed and executed finger movements: comparing symbolic, spatial, and imitative cues.' *Brain and cognition*, 44(2) pp. 124-143.

Braun, V. and Clarke, V. (2006) 'Using thematic analysis in psychology.' *Qualitative Research in Psychology*, 3(2) pp. 77-101.

Brinkmann, S. and Kvale, S. (2015) *Interviews: Learning the craft of qualitative research interviewing.* 3 ed.: Sage Publications.

Buccino, G. (2014) 'Action observation treatment: a novel tool in neurorehabilitation.' *Phil. Trans. R. Soc. B*, 369(1644) p. 20130185.

Buccino, G., Gatti, R., Giusti, M. C., Negrotti, A., Rossi, A., Calzetti, S. and Cappa, S. F. (2011) 'Action observation treatment improves autonomy in daily activities in Parkinson's disease patients: results from a pilot study.' *Movement Disorders*, 26(10) pp. 1963-1964.

Buccino, G., Lui, F., Canessa, N., Patteri, I., Lagravinese, G., Benuzzi, F., Porro, C. A. and Rizzolatti, G. (2004) 'Neural circuits involved in the recognition of actions performed by nonconspecifics: An fMRI study.' *Journal of cognitive neuroscience*, 16(1) pp. 114-126.

Buccino, G., Molinaro, A., Ambrosi, C., Arisi, D., Mascaro, L., Pinardi, C., Rossi, A., Gasparotti, R., Fazzi, E. and Galli, J. (2018) 'Action Observation Treatment Improves Upper Limb Motor Functions in Children with Cerebral Palsy: A Combined Clinical and Brain Imaging Study.' *Neural Plasticity*, 2018

Burden, A. (2017) 'Surface electromyography.' *In* Payton, C. J. and Burden, A. (eds.) *Biomechanical Evaluation of Movement in Sport and Exercise: The British Association of Sport and Exercise Sciences Guide*. Routledge,

Button, K. S., Ioannidis, J. P., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. and Munafò, M. R. (2013) 'Power failure: why small sample size undermines the reliability of neuroscience.' *Nature Reviews Neuroscience*, 14(5) pp. 365-376.

Callow, N. and Roberts, R. (2010) 'Imagery research: An investigation of three issues.' *Psychology of Sport and Exercise*, 11(4) pp. 325-329.

Calmels, C., Holmes, P., Lopez, E. and Naman, V. (2006) 'Chronometric comparison of actual and imaged complex movement patterns.' *Journal of Motor Behavior*, 38(5) pp. 339-348.

Carrasco, M. (2011) 'Visual attention: The past 25 years.' *Vision research*, 51(13) pp. 1484-1525.

Catmur, C. (2013) 'Sensorimotor learning and the ontogeny of the mirror neuron system.' *Neuroscience letters*, 540 pp. 21-27.

Catmur, C., Walsh, V. and Heyes, C. (2007) 'Sensorimotor learning configures the human mirror system.' *Current biology*, 17(17) pp. 1527-1531.

Causer, J., Janelle, C., Vickers, J. and Williams, A. (2012) 'Perceptual training: What can be trained.' *In* Hodges, N. and Williams, A. (eds.) *Skill acquisition in sport: Research, theory and practice*. Oxford, UK: Routledge, pp. 306-324.

Center for the Study of Emotion and Attention. (1999) The international affective picture system: Digitized photographs. The Center for Research in Psychophysiology, University of Florida Gainesville, FL.

Chaminade, T., Meltzoff, A. N. and Decety, J. (2005) 'An fMRI study of imitation: action representation and body schema.' *Neuropsychologia*, 43(1) pp. 115-127.

Chapman, P. R. and Underwood, G. (1998) 'Visual search of driving situations: Danger and experience.' *Perception*, 27(8) pp. 951-964.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.-P., Magistris, M. R., Mills, K., Rösler, K. M., Triggs, W. J. and Ugawa, Y. (2008) 'The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee.' *Clinical Neurophysiology*, 119(3) pp. 504-532.

Cheng, Y., Meltzoff, A. N. and Decety, J. (2006) 'Motivation modulates the activity of the human mirror-neuron system.' *Cerebral Cortex*, 17(8) pp. 1979-1986.

Choudhury, K. R., Boyle, L., Burke, M., Lombard, W., Ryan, S. and McNamara, B. (2011) 'Intra subject variation and correlation of motor potentials evoked by transcranial magnetic stimulation.' *Irish journal of medical science*, 180(4) pp. 873-880.

Cochin, S., Barthelemy, C., Roux, S. and Martineau, J. (1999) 'Observation and execution of movement: similarities demonstrated by quantified electroencephalography.' *European Journal of Neuroscience*, 11(5) pp. 1839-1842.

Coelho, C. M. and Purkis, H. (2009) 'The origins of specific phobias: Influential theories and current perspectives.' *Review of General Psychology*, 13(4) p. 335.

Coelho, C. M., Lipp, O. V., Marinovic, W., Wallis, G. and Riek, S. (2010) 'Increased corticospinal excitability induced by unpleasant visual stimuli.' *Neuroscience letters*, 481(3) pp. 135-138.

Cohen, J. (1988) *Statistical power analysis for the behavioral science.* Hillsdale, NJ: Lawrence Earlbaum Associates.

Cohen, L. G., Roth, B. J., Nilsson, J., Dang, N., Panizza, M., Bandinelli, S., Friauf, W. and Hallett, M. (1990) 'Effects of coil design on delivery of focal magnetic stimulation. Technical considerations.' *Electroencephalography and clinical neurophysiology*, 75(4) pp. 350-357.

Collet, C. and Guillot, A. (2012) 'The rehabilitation function of motor imagery after peripheral injury or central stroke.' *In* Lagana, R. and Esposito, S. M. (eds.) *Rehabilitation: Practices, Psychology and Health*. Nova Science Publishers, pp. 53-77.

Conte, A., Gilio, F., Iezzi, E., Frasca, V., Inghilleri, M. and Berardelli, A. (2007) 'Attention influences the excitability of cortical motor areas in healthy humans.' *Experimental brain research*, 182(1) pp. 109-117.

Coombes, S. A., Cauraugh, J. H. and Janelle, C. M. (2007) 'Emotional state and initiating cue alter central and peripheral motor processes.' *Emotion*, 7(2) p. 275.

Corey, D. M., Hurley, M. M. and Foundas, A. L. (2001) 'Right and left handedness defined: a multivariate approach using hand preference and hand performance measures.' *Cognitive and Behavioral Neurology*, 14(3) pp. 144-152.

Cornell, E. D., Macdougall, H. G., Predebon, J. and Curthoys, I. S. (2003) 'Errors of binocular fixation are common in normal subjects during natural conditions.' *Optometry and Vision Science*, 80(11) pp. 764-771.

Covington Jr., W. O. (no date) *Homunculus (Topographic) Diagram.* [Online image] [Accessed on 28th July 2018] http://willcov.com/bio-consciousness/diagrams/Homunculus%20(Topographic)%20Diagram.htm

Craighero, L., Zorzi, V., Canto, R. and Franca, M. (2014) 'Same kinematics but different objects during action observation: Detection times and motor evoked potentials.' *Visual Cognition*, 22(5) pp. 653-671.

Creswell, J. W. (2013) *Qualitative inquiry and reearch design: choosing among five approaches.* Los Angeles: Sage Publications.

Creswell, J. W. and Plano Clark, V. L. (2017) *Designing and conducting mixed methods research.* Los Angeles, USA: Sage Publications.

Cross, E. S., Kraemer, D. J., Hamilton, A. F. d. C., Kelley, W. M. and Grafton, S. T. (2009) 'Sensitivity of the action observation network to physical and observational learning.' *Cerebral cortex*, 19(2) pp. 315-326.

Cross, K. A. and Iacoboni, M. (2014) 'To imitate or not: avoiding imitation involves preparatory inhibition of motor resonance.' *Neuroimage*, 91 pp. 228-236.

Cui, Y. and Hondzinski, J. M. (2006) 'Gaze tracking accuracy in humans: Two eyes are better than one.' *Neuroscience letters*, 396(3) pp. 257-262.

Cushion, C. and Jones, R. L. (2006) 'Power, discourse, and symbolic violence in professional youth soccer: The case of Albion Football Club.' *Sociology of sport journal*, 23(2) pp. 142-161.

Cuypers, K., Thijs, H. and Meesen, R. L. (2014) 'Optimization of the transcranial magnetic stimulation protocol by defining a reliable estimate for corticospinal excitability.' *PloS One*, 9(1) p. e86380.

D'Innocenzo, G., Gonzalez, C. C., Nowicky, A. V., Williams, A. M. and Bishop, D. T. (2017) 'Motor resonance during action observation is gaze-contingent: A TMS study.' *Neuropsychologia*, 103 pp. 77-86.

Dambacher, M. and Kliegl, R. (2007) 'Synchronizing timelines: Relations between fixation durations and N400 amplitudes during sentence reading.' *Brain research*, 1155 pp. 147-162.

Darling, W. G., Wolf, S. L. and Butler, A. J. (2006) 'Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation.' *Experimental brain research*, 174(2) pp. 376-385.

Darwin, C. R. (1872) *The expression of the eotions in man and animals.* London: John Murray.

Day, B., Dressler, D., Maertens de Noordhout, A., Marsden, C., Nakashima, K., Rothwell, J. and Thompson, P. (1989) 'Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses.' *The Journal of physiology*, 412(1) pp. 449-473.

Day, S. (2002) 'Important factors in surface EMG measurement.' *Bortec Biomedical Ltd publishers*, pp. 1-17.

De Souza, L., Hewer, R. L. and Miller, S. (1980) 'Assessment of recovery of arm control in hemiplegic stroke patients. 1. Arm function tests.' *International rehabilitation medicine*, 2(1) pp. 3-9.

Decety, J., Jeannerod, M. and Prablanc, C. (1989) 'The timing of mentally represented actions.' *Behavioural brain research*, 34(1-2) pp. 35-42.

Deubel, H. (2008) 'The time course of presaccadic attention shifts.' *Psychological research*, 72(6) p. 630.

Devanne, H., Lavoie, B. and Capaday, C. (1997) 'Input-output properties and gain changes in the human corticospinal pathway.' *Experimental brain research*, 114(2) pp. 329-338.

Di Lazzaro, V., Restuccia, D., Oliviero, A., Profice, P., Ferrara, L., Insola, A., Mazzone, P., Tonali, P. and Rothwell, J. (1998) 'Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits.' *Experimental Brain Research*, 119(2) pp. 265-268.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., Insola, A., Tonali, P. and Rothwell, J. (2004) 'The physiological basis of transcranial motor cortex stimulation in conscious humans.' *Clinical neurophysiology*, 115(2) pp. 255-266.

Di Lazzaro, V., Capone, F., Di Pino, G., Pellegrino, G., Florio, L., Zollo, L., Simonetti, D., Ranieri, F., Brunelli, N. and Corbetto, M. (2016) 'Combining Robotic Training and Non-Invasive Brain Stimulation in Severe Upper Limb-Impaired Chronic Stroke Patients.' *Frontiers in neuroscience*, 10

Di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V. and Rizzolatti, G. (1992) 'Understanding motor events: a neurophysiological study.' *Experimental brain research*, 91(1) pp. 176-180.

Dobek, C. E., Blumberger, D. M., Downar, J., Daskalakis, Z. J. and Vila-Rodriguez, F. (2015) 'Risk of seizures in transcranial magnetic stimulation: a clinical review to inform consent process focused on bupropion.' *Neuropsychiatric disease and treatment*, 11 p. 2975.

Dombovy, M. L. (2004) 'Understanding stroke recovery and rehabilitation: current and emerging approaches.' *Current neurology and neuroscience reports*, 4(1) pp. 31-35.

Donaldson, P. H., Gurvich, C., Fielding, J. and Enticott, P. G. (2015) 'Exploring associations between gaze patterns and putative human mirror neuron system activity.' *Frontiers in human neuroscience*, 9

Donne, C. M., Enticott, P. G., Rinehart, N. J. and Fitzgerald, P. B. (2011) 'A transcranial magnetic stimulation study of corticospinal excitability during the observation of meaningless, goal-directed, and social behaviour.' *Neuroscience Letters*, 489(1) pp. 57-61.

Du, X., Summerfelt, A., Chiappelli, J., Holcomb, H. H. and Hong, L. E. (2014) 'Individualized brain inhibition and excitation profile in response to paired-pulse TMS.' *Journal of motor behavior*, 46(1) pp. 39-48.

Duchowski, A. (2007) *Eye Tracking Methodology: Theory and Practice.* London, UK: Springer.

Eaves, D., Riach, M., Holmes, P. and Wright, D. (2016) 'Motor imagery during action observation: a brief review of evidence, theory and future research opportunities.' *Frontiers in Neuroscience*, 10(514)

Edwards, M. G., Humphreys, G. W. and Castiello, U. (2003) 'Motor facilitation following action observation: A behavioural study in prehensile action.' *Brain and cognition*, 53(3) pp. 495-502.

Ehmke, C. and Wilson, S. (2007) *Identifying web usability problems from eye-tracking data.* British Computer Society.

Enticott, P. G., Kennedy, H. A., Bradshaw, J. L., Rinehart, N. J. and Fitzgerald, P. B. (2010) 'Understanding mirror neurons: evidence for enhanced corticospinal excitability during the observation of transitive but not intransitive hand gestures.' *Neuropsychologia*, 48(9) pp. 2675-2680.

Enticott, P. G., Harrison, B. A., Arnold, S. L., Nibaldi, K., Segrave, R. A., Fitzgibbon, B. M., Kennedy, H. A., Lau, K. and Fitzgerald, P. B. (2012) 'Emotional valence modulates putative mirror neuron activity.' *Neuroscience letters*, 508(1) pp. 56-59.

Epstein, C. M. (2006) 'Seizure or convulsive syncope during 1-Hz rTMS?' *Clinical Neurophysiology*, 117(11) pp. 2566-2567.

Epstein, R. (2005) 'The cortical basis of visual scene processing.' *Visual Cognition*, 12(6) pp. 954-978.

Ertelt, D., Small, S., Solodkin, A., Dettmers, C., McNamara, A., Binkofski, F. and Buccino, G. (2007) 'Action observation has a positive impact on rehabilitation of motor deficits after stroke.' *Neuroimage*, 36 pp. T164-T173.

Evans, A. (2007) *A review of the safety of transcranial magnetic stimulation*. [Online] [Accessed on 5th March 2018] http://www.gcrc.uci.edu/neuroimaging/TMSsafetydocument2008.pdf

Ewan, L. M., Kinmond, K. and Holmes, P. S. (2010) 'An observation-based intervention for stroke rehabilitation: experiences of eight individuals affected by stroke.' *Disability and rehabilitation*, 32(25) pp. 2097-2106.

Fadiga, L., Craighero, L. and Olivier, E. (2005) 'Human motor cortex excitability during the perception of others' action.' *Current opinion in neurobiology*, 15(2) pp. 213-218.

Fadiga, L., Fogassi, L., Pavesi, G. and Rizzolatti, G. (1995) 'Motor facilitation during action observation: a magnetic stimulation study.' *Journal of Neurophysiology*, 73(6) pp. 2608-2611.

Fadiga, L., Buccino, G., Craighero, L., Fogassi, L., Gallese, V. and Pavesi, G. (1999) 'Corticospinal excitability is specifically modulated by motor imagery: a magnetic stimulation study.' *Neuropsychologia*, 37(2) pp. 147-158.

Farnè, A., Pavani, F., Meneghello, F. and Làdavas, E. (2000) 'Left tactile extinction following visual stimulation of a rubber hand.' *Brain*, 123(11) pp. 2350-2360.

Ferri, F., Stoianov, I. P., Gianelli, C., D'Amico, L., Borghi, A. M. and Gallese, V. (2010) 'When action meets emotions: how facial displays of emotion influence goal-related behavior.' *PloS one*, 5(10) p. e13126.

Ferri, F., Ebisch, S. J., Costantini, M., Salone, A., Arciero, G., Mazzola, V., Ferro, F. M., Romani, G. L. and Gallese, V. (2013) 'Binding action and emotion in social understanding.' *PloS one*, 8(1) p. e54091.

Ferri, S., Rizzolatti, G. and Orban, G. A. (2015) 'The organization of the posterior parietal cortex devoted to upper limb actions: An fMRI study.' *Human brain mapping*, 36(10) pp. 3845-3866.

Filimon, F. (2015) 'Are all spatial reference frames egocentric? Reinterpreting evidence for allocentric, object-centered, or world-centered reference frames.' *Frontiers in Human Neuroscience*, 9

Fogassi, L., Ferrari, P. F., Gesierich, B., Rozzi, S., Chersi, F. and Rizzolatti, G. (2005) 'Parietal lobe: from action organization to intention understanding.' *Science*, 308(5722) pp. 662-667.

Ford, B. Q., Tamir, M., Brunyé, T. T., Shirer, W. R., Mahoney, C. R. and Taylor, H. A. (2010) 'Keeping your eyes on the prize: Anger and visual attention to threats and rewards.' *Psychological Science*, 21(8) pp. 1098-1105.

Fourkas, A. D., Avenanti, A., Urgesi, C. and Aglioti, S. M. (2006) 'Corticospinal facilitation during first and third person imagery.' *Experimental Brain Research*, 168(1-2) pp. 143-151.

Franceschini, M., Ceravolo, M. G., Agosti, M., Cavallini, P., Bonassi, S., Dall'Armi, V., Massucci, M., Schifini, F. and Sale, P. (2012) 'Clinical relevance of action observation in upper-limb stroke rehabilitation a possible role in recovery of functional dexterity. A randomized clinical trial.' *Neurorehabilitation and Neural Repair*, 26(5) pp. 456-462.

Fu, J., Zeng, M., Shen, F., Cui, Y., Zhu, M., Gu, X. and Sun, Y. (2017) 'Effects of action observation therapy on upper extremity function, daily activities and motion evoked potential in cerebral infarction patients.' *Medicine*, 96(42)

Gaggioli, A., Morganti, F., Walker, R., Meneghini, A., Alcaniz, M., Lozano, J. A., Montesa, J., Gil, J. A. and Riva, G. (2004) 'Training with computer-supported motor imagery in post-stroke rehabilitation.' *CyberPsychology & Behavior*, 7(3) pp. 327-332.

Gangitano, M., Mottaghy, F. M. and Pascual-Leone, A. (2001) 'Phase-specific modulation of cortical motor output during movement observation.' *Neuroreport*, 12(7) pp. 1489-1492.

Gangitano, M., Mottaghy, F. M. and Pascual-Leone, A. (2004) 'Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements.' *European Journal of Neuroscience*, 20(8) pp. 2193-2202.

Ganis, G. and Kutas, M. (2003) 'An electrophysiological study of scene effects on object identification.' *Cognitive Brain Research*, 16(2) pp. 123-144.

Garry, M. and Thomson, R. (2009) 'The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states.' *Experimental brain research*, 193(2) p. 267.

Gastaut, H. J. and Bert, J. (1954) 'EEG changes during cinematographic presentation (Moving picture activation of the EEG).' *Electroencephalography and clinical neurophysiology*, 6 pp. 433-444.

George, M. S., Lisanby, S. H., Avery, D., McDonald, W. M., Durkalski, V., Pavlicova, M., Anderson, B., Nahas, Z., Bulow, P. and Zarkowski, P. (2010) 'Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial.' *Archives of general psychiatry*, 67(5) pp. 507-516.

Gillick, B. T., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., Menk, J., Cassidy, J., Kimberley, T. and Carey, J. R. (2015) 'Safety of primed repetitive transcranial magnetic stimulation and modified constraint-induced movement therapy in a randomized controlled trial in pediatric hemiparesis.' *Archives of physical medicine and rehabilitation*, 96(4) pp. S104-S113.

Girden, E. R. (1992) *ANOVA: Repeated measures.* Sage.

Goldberg, J. H. and Kotval, X. P. (1999) 'Computer interface evaluation using eye movements: methods and constructs.' *International Journal of Industrial Ergonomics*, 24(6) pp. 631-645.

Goldsworthy, M., Hordacre, B. and Ridding, M. (2016) 'Minimum number of trials required for within-and between-session reliability of TMS measures of corticospinal excitability.' *Neuroscience*, 320 pp. 205-209.

Grecucci, A., Koch, I. and Rumiati, R. I. (2011) 'The role of emotional context in facilitating imitative actions.' *Acta Psychologica*, 138(2) pp. 311-315.

Grèzes, J. and Decety, J. (2001) 'Functional anatomy of execution, mental simulation, observation, and verb generation of actions: a meta-analysis.' *Human Brain Mapping*, 12(1) pp. 1-19.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S. and Thickbroom, G. (2012) 'A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee.' *Clinical Neurophysiology*, 123(5) pp. 858-882.

Grosprêtre, S., Ruffino, C. and Lebon, F. (2016) 'Motor imagery and cortico-spinal excitability: a review.' *European journal of sport science*, 16(3) pp. 317-324.

Guillot, A., Collet, C., Nguyen, V. A., Malouin, F., Richards, C. and Doyon, J. (2008) 'Functional neuroanatomical networks associated with expertise in motor imagery.' *Neuroimage*, 41(4) pp. 1471-1483.

Hadar, A. A., Makris, S. and Yarrow, K. (2012) 'Single-pulse TMS related syncopal spell in a healthy subject.' *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 5(4) pp. 652-653.

Hajcak, G., Molnar, C., George, M. S., Bolger, K., Koola, J. and Nahas, Z. (2007) 'Emotion facilitates action: a transcranial magnetic stimulation study of motor cortex excitability during picture viewing.' *Psychophysiology*, 44(1) pp. 91-97.

Hall, C. R. (1997) 'Lew Hardy's third myth: A matter of perspective.' *Journal of Applied Sport Psychology*, 9(2) pp. 310-313.

Hallett, M. and Chokroverty, S. (2005) *Magnetic stimulation in clinical neurophysiology.* Elsevier Health Sciences.

Hanajima, R., Ugawa, Y., Terao, Y., Sakai, K., Furubayashi, T., Machii, K. and Kanazawa, I. (1998) 'Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves.' *The Journal of Physiology*, 509(2) pp. 607-618.

Hardwick, R. M. and Edwards, M. G. (2011) 'Observed reach trajectory influences executed reach kinematics in prehension.' *The Quarterly Journal of Experimental Psychology*, 64(6) pp. 1082-1093.

Hardwick, R. M., McAllister, C. J., Holmes, P. S. and Edwards, M. G. (2012) 'Transcranial magnetic stimulation reveals modulation of corticospinal excitability when observing actions with the intention to imitate.' *European journal of neuroscience*, 35(9) pp. 1475-1480.

Hardwick, R. M., Caspers, S., Eickhoff, S. B. and Swinnen, S. P. (2018) 'Neural Correlates of Action: Comparing Meta-Analyses of Imagery, Observation, and Execution.' *Neuroscience & Biobehavioral Reviews*,

Harris Sr, R. L. and Christhilf, D. M. (1980) *What do pilots see in displays?* Vol. 24: Sage Publications Sage CA: Los Angeles, CA.

Hartman, K. and Altschuler, E. L. (2016) 'Mirror Therapy for Hemiparesis Following Stroke: A Review.' *Current Physical Medicine and Rehabilitation Reports*, 4(4) pp. 237-248.

Hashemirad, F., Zoghi, M., Fitzgerald, P. B. and Jaberzadeh, S. (2017) 'Reliability of motor evoked potentials induced by transcranial magnetic stimulation: the effects of initial motor evoked potentials removal.' *Basic and clinical neuroscience*, 8(1) p. 43.

Henderson, J. M., Weeks Jr, P. A. and Hollingworth, A. (1999) 'The effects of semantic consistency on eye movements during complex scene viewing.' *Journal of experimental psychology: Human perception and performance*, 25(1) p. 210.

Hess, C. W., Mills, K. and Murray, N. (1987) 'Responses in small hand muscles from magnetic stimulation of the human brain.' *The Journal of physiology*, 388(1) pp. 397-419.

Hessels, R. S., Kemner, C., van den Boomen, C. and Hooge, I. T. (2016) 'The area-of-interest problem in eyetracking research: A noise-robust solution for face and sparse stimuli.' *Behavior research methods*, 48(4) pp. 1694-1712.

Hétu, S., Grégoire, M., Saimpont, A., Coll, M.-P., Eugène, F., Michon, P.-E. and Jackson, P. L. (2013) 'The neural network of motor imagery: an ALE meta-analysis.' *Neuroscience & Biobehavioral Reviews*, 37(5) pp. 930-949.

Hickok, G. (2009) 'Eight problems for the mirror neuron theory of action understanding in monkeys and humans.' *Journal of cognitive neuroscience*, 21(7) pp. 1229-1243.

Holmes, P. (2011) 'Evidence from cognitive neuroscience supports action observation as part of an integrated approach to stroke rehabilitation.' *Manual therapy*, 16(1) pp. 40-41.

Holmes, P. and Calmels, C. (2008) 'A neuroscientific review of imagery and observation use in sport.' *Journal of Motor Behavior*, 40(5) pp. 433-445.

Holmes, P. S. (2007) 'Theoretical and practical problems for imagery in stroke rehabilitation: An observation solution.' *Rehabilitation Psychology*, 52(1) p. 1.

Holmes, P. S. and Wright, D. J. (2017) 'Motor Cognition and Neuroscience in Sport Psychology.' *Current Opinion in Psychology*,

Holmqvist, K., Nyström, M., Andersson, R., Dewhurst, R., Jarodzka, H. and Van de Weijer, J. (2011) *Eye tracking: A comprehensive guide to methods and measures.* Oxford, UK: Oxford University Press.

Hopstaken, J. F., van der Linden, D., Bakker, A. B. and Kompier, M. A. (2015) 'The window of my eyes: Task disengagement and mental fatigue covary with pupil dynamics.' *Biological psychology*, 110 pp. 100-106.

Horvath, J. C., Perez, J. M., Forrow, L., Fregni, F. and Pascual-Leone, A. (2010) 'Transcranial magnetic stimulation: a historical evaluation and future prognosis of therapeutically relevant ethical concerns.' *Journal of medical ethics*, 37(3) pp. 137-143.

Hoshi, E. and Tanji, J. (2007) 'Distinctions between dorsal and ventral premotor areas: anatomical connectivity and functional properties.' *Current opinion in neurobiology*, 17(2) pp. 234-242.

Hunnius, S. and Bekkering, H. (2010) 'The early development of object knowledge: A study of infants' visual anticipations during action observation.' *Developmental psychology*, 46(2) p. 446.

Hyde, C., Fuelscher, I., Lum, J. A., Williams, J., He, J. and Enticott, P. G. (2017) 'Primary Motor Cortex Excitability Is Modulated During the Mental Simulation of Hand Movement.' *Journal of the International Neuropsychological Society*, 23(2) pp. 185-193.

Iacoboni, M., Woods, R. P., Brass, M., Bekkering, H., Mazziotta, J. C. and Rizzolatti, G. (1999) 'Cortical mechanisms of human imitation.' *Science*, 286(5449) pp. 2526-2528.

Iacoboni, M., Molnar-Szakacs, I., Gallese, V., Buccino, G., Mazziotta, J. C. and Rizzolatti, G. (2005) 'Grasping the intentions of others with one's own mirror neuron system.' *PLoS Biol*, 3(3) p. e79.

Isaac, A. R. and Marks, D. F. (1994) 'Individual differences in mental imagery experience: developmental changes and specialization.' *British Journal of Psychology*, 85(4) pp. 479-500.

Jacob, R. J. and Karn, K. S. (2003) 'Eye tracking in human-computer interaction and usability research: Ready to deliver the promises.' *In The mind's eye*. Elsevier, pp. 573-605.

Jasper, H. H. (1958) 'Report of the committee on methods of clinical examination in electroencephalography: 1957.' *Electroencephalography and Clinical Neurophysiology*, 10(2) pp. 370-375.

Jeannerod, M. (2001) 'Neural simulation of action: a unifying mechanism for motor cognition.' *Neuroimage*, 14(1) pp. S103-S109.

Jeannerod, M. (2004) 'Actions from within.' *International Journal of Sport and Exercise Psychology*, 2(4) pp. 376-402.

Jeannerod, M. and Pacherie, E. (2004) 'Agency, simulation and self-identification.' *Mind & Language*, 19(2) pp. 113-146.

Julkunen, P., Säisänen, L., Danner, N., Niskanen, E., Hukkanen, T., Mervaala, E. and Könönen, M. (2009) 'Comparison of navigated and non-navigated transcranial magnetic stimulation for motor cortex mapping, motor threshold and motor evoked potentials.' *Neuroimage*, 44(3) pp. 790-795.

Kaneko, F., Yasojima, T. and Kizuka, T. (2007) 'Kinesthetic illusory feeling induced by a finger movement movie effects on corticomotor excitability.' *Neuroscience*, 149(4) pp. 976-984.

Kang, M. J., Hsu, M., Krajbich, I. M., Loewenstein, G., McClure, S. M., Wang, J. T.-y. and Camerer, C. F. (2009) 'The wick in the candle of learning: Epistemic curiosity activates reward circuitry and enhances memory.' *Psychological Science*, 20(8) pp. 963-973.

Kang, Z., Mandal, S., Crutchfield, J., Millan, A. and McClung, S. N. (2016) 'Designs and algorithms to map eye tracking data with dynamic multielement moving objects.' *Computational intelligence and neuroscience*, 2016

Keel, J. C., Smith, M. J. and Wassermann, E. M. (2001) 'A safety screening questionnaire for transcranial magnetic stimulation.' *Clinical Neurophysiology*, 112(4) p. 720.

Kelly, R. L. and Wheaton, L. (2013) 'Differential mechanisms of action understanding in left and right handed subjects: the role of perspective and handedness.' *Frontiers in psychology*, 4 p. 957.

Kiers, L., Cros, D., Chiappa, K. and Fang, J. (1993) 'Variability of motor potentials evoked by transcranial magnetic stimulation.' *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 89(6) pp. 415-423.

Kilner, J. M., Friston, K. J. and Frith, C. D. (2007) 'Predictive coding: an account of the mirror neuron system.' *Cognitive processing*, 8(3) pp. 159-166.

King, N. and Horrocks, C. (2010) *Interviews in qualitative research.* Sage.

Kojima, S., Onishi, H., Sugawara, K., Kirimoto, H., Suzuki, M. and Tamaki, H. (2013) 'Modulation of the cortical silent period elicited by single-and paired-pulse transcranial magnetic stimulation.' *BMC neuroscience*, 14(1) p. 43.

Kolbinger, H. M., Höflich, G., Hufnagel, A., Müller, H. J. and Kasper, S. (1995) 'Transcranial magnetic stimulation (TMS) in the treatment of major depression—a pilot study.' *Human Psychopharmacology: Clinical and Experimental*, 10(4) pp. 305-310.

Kourtzi, Z. and Kanwisher, N. (2000) 'Activation in human MT/MST by static images with implied motion.' *Journal of cognitive neuroscience*, 12(1) pp. 48-55.

Kujirai, T., Caramia, M., Rothwell, J. C., Day, B., Thompson, P., Ferbert, A., Wroe, S., Asselman, P. and Marsden, C. D. (1993) 'Corticocortical inhibition in human motor cortex.' *The Journal of physiology*, 471 p. 501.

Lang, P. J. (1980) 'Behavioral treatment and bio-behavioral assessment: Computer applications.' *In* Sidowski, J. B., Johnson, J. H. and Williams, T. A. (eds.) *Technology in mental health care delivery systems*. pp. 119-137.

Lang, P. J., Davis, M. and Öhman, A. (2000) 'Fear and anxiety: animal models and human cognitive psychophysiology.' *Journal of affective disorders*, 61(3) pp. 137-159.

Leek, E. C. and Johnston, S. J. (2009) 'Functional specialization in the supplementary motor complex.' *Nature Reviews Neuroscience*, 10(1) p. 78.

Leonetti, A., Puglisi, G., Siugzdaite, R., Ferrari, C., Cerri, G. and Borroni, P. (2015) 'What you see is what you get: motor resonance in peripheral vision.' *Experimental brain research*, 233(10) pp. 3013-3022.

Leube, A., Rifai, K. and Wahl, S. (2017) 'Sampling rate influences saccade detection in mobile eye tracking of a reading task.' *J. Eye Mov. Res.*, 10 p. 3.

Li, S., Stevens, J. A. and Rymer, W. Z. (2009) 'Interactions between imagined movement and the initiation of voluntary movement: a TMS study.' *Clinical Neurophysiology*, 120(6) pp. 1154-1160.

Loo, C. K., McFarquhar, T. F. and Mitchell, P. B. (2008) 'A review of the safety of repetitive transcranial magnetic stimulation as a clinical treatment for depression.' *International Journal of Neuropsychopharmacology*, 11(1) pp. 131-147.

Loporto, M., Holmes, P. S., Wright, D. J. and McAllister, C. J. (2013) 'Reflecting on mirror mechanisms: motor resonance effects during action observation only present with low-intensity transcranial magnetic stimulation.' *PLoS One*, 8(5) p. e64911.

Loporto, M., McAllister, C. J., Williams, J., Hardwick, R. M. and Holmes, P. S. (2011) 'Investigating central mechanisms underlying the effects of action observation and imagery through transcranial magnetic stimulation.' *Journal of Motor Behavior*, 43(5) pp. 361-373.

Loporto, M., McAllister, C. J., Edwards, M. G., Wright, D. J. and Holmes, P. S. (2012) 'Prior action execution has no effect on corticospinal facilitation during action observation.' *Behavioural Brain Research*, 231(1) pp. 124-129.

Lu, M. and Ueno, S. (2017) 'Comparison of the induced fields using different coil configurations during deep transcranial magnetic stimulation.' *PloS one*, 12(6) p. e0178422.

Maeda, F., Kleiner-Fisman, G. and Pascual-Leone, A. (2002) 'Motor facilitation while observing hand actions: specificity of the effect and role of observer's orientation.' *Journal of Neurophysiology*, 87(3) pp. 1329-1335.

Magstim. (2018) *Coils*. [Online] [Accessed on 7th March 2018] https://www.magstim.com/products/coils

Maizey, L., Allen, C. P., Dervinis, M., Verbruggen, F., Varnava, A., Kozlov, M., Adams, R. C., Stokes, M., Klemen, J. and Bungert, A. (2013) 'Comparative incidence rates of mild adverse effects to transcranial magnetic stimulation.' *Clinical neurophysiology*, 124(3) pp. 536-544.

Mallet, L., Schüpbach, M., N'Diaye, K., Remy, P., Bardinet, E., Czernecki, V., Welter, M.-L., Pelissolo, A., Ruberg, M. and Agid, Y. (2007) 'Stimulation of subterritories of the

subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of behavior.' *Proceedings of the National Academy of Sciences*, 104(25) pp. 10661-10666.

Manthey, S., Schubotz, R. I. and von Cramon, D. Y. (2003) 'Premotor cortex in observing erroneous action: an fMRI study.' *Cognitive Brain Research*, 15(3) pp. 296-307.

McCabe, S. I., Villalta, J. I., Saunier, G., Grafton, S. T. and Della-Maggiore, V. (2014) 'The relative influence of goal and kinematics on corticospinal excitability depends on the information provided to the observer.' *Cerebral Cortex*, 25(8) pp. 2229-2237.

McConnell, K. A., Nahas, Z., Shastri, A., Lorberbaum, J. P., Kozel, F. A., Bohning, D. E. and George, M. S. (2001) 'The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: a replication in healthy adults comparing two methods of assessing the distance to cortex.' *Biological psychiatry*, 49(5) pp. 454-459.

McCormick, S. A. and Holmes, P. S. (2016) 'Abstract TP157: See, Imagine, Move - Upper Limb Action Therapy (SIMULATe): iPad-based Mental and Physical Motor (re)Learning for Stroke Recovery.' *Stroke*, 47(Suppl 1) pp. ATP157-ATP157.

McLeod, J. (2011) *Qualitative research in counselling and psychotherapy.* Sage.

Merckelbach, H., Arntz, A. and de Jong, P. (1991) 'Conditioning experiences in spider phobics.' *Behaviour Research and Therapy*, 29(4) pp. 333-335.

Mineka, S. and Öhman, A. (2002) 'Phobias and preparedness: The selective, automatic, and encapsulated nature of fear.' *Biological psychiatry*, 52(10) pp. 927-937.

Molenberghs, P., Hayward, L., Mattingley, J. B. and Cunnington, R. (2012) 'Activation patterns during action observation are modulated by context in mirror system areas.' *Neuroimage*, 59(1) pp. 608-615.

Montagna, M., Cerri, G., Borroni, P. and Baldissera, F. (2005) 'Excitability changes in human corticospinal projections to muscles moving hand and fingers while viewing a reaching and grasping action.' *European Journal of Neuroscience*, 22(6) pp. 1513-1520.

Morris, T. and Miller, J. C. (1996) 'Electrooculographic and performance indices of fatigue during simulated flight.' *Biological psychology*, 42(3) pp. 343-360.

Moser, C. and Kalton, G. (2017) 'Question wording.' *In* Bynner, J. and Stribley, K. M. (eds.) *Research Design: The Logic of Social Inquiry*. London: Routledge, pp. 140-155.

Mottura, S., Fontana, L., Arlati, S., Zangiacomi, A., Redaelli, C. and Sacco, M. (2015) 'A virtual reality system for strengthening awareness and participation in rehabilitation for post-stroke patients.' *Journal on Multimodal User Interfaces*, 9(4) pp. 341-351.

Mouthon, A., Ruffieux, J., Wälchli, M., Keller, M. and Taube, W. (2015) 'Task-dependent changes of corticospinal excitability during observation and motor imagery of balance tasks.' *Neuroscience*, 303 pp. 535-543.

Mukamel, R., Ekstrom, A. D., Kaplan, J., Iacoboni, M. and Fried, I. (2010) 'Single-neuron responses in humans during execution and observation of actions.' *Current biology*, 20(8) pp. 750-756.

Nachev, P., Kennard, C. and Husain, M. (2008) 'Functional role of the supplementary and pre-supplementary motor areas.' *Nature Reviews Neuroscience*, 9(11) p. 856.

Naish, K. R. and Obhi, S. S. (2015) 'Timing and specificity of early changes in motor excitability during movement observation.' *Experimental brain research*, 233(6) pp. 1867-1874.

Naish, K. R., Barnes, B. and Obhi, S. S. (2016) 'Stimulation over primary motor cortex during action observation impairs effector recognition.' *Cognition*, 149 pp. 84-94.

Naish, K. R., Houston-Price, C., Bremner, A. J. and Holmes, N. P. (2014) 'Effects of action observation on corticospinal excitability: muscle specificity, direction, and timing of the mirror response.' *Neuropsychologia*, 64 pp. 331-348.

Nakamura, H., Kitagawa, H., Kawaguchi, Y. and Tsuji, H. (1997) 'Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans.' *The Journal of Physiology*, 498(3) pp. 817-823.

Nakayama, M., Takahashi, K. and Shimizu, Y. (2002) *The act of task difficulty and eye-movement frequency for the'Oculo-motor indices'.* ACM.

NHS England. (2018) *Personalised care*. [Online] [Accessed on 11th December 2018] https://www.england.nhs.uk/personalisedcare/

Ni, Z. and Chen, R. (2008) Short-interval intracortical inhibition: a complex measure. Elsevier.

Niehorster, D. C., Cornelissen, T. H., Holmqvist, K., Hooge, I. T. and Hessels, R. S. (2018) 'What to expect from your remote eye-tracker when participants are unrestrained.' *Behavior research methods*, 50(1) pp. 213-227.

Nogueira-Campos, A. A., de Oliveira, L. A. S., Della-Maggiore, V., Esteves, P. O., de Carvalho Rodrigues, E. and Vargas, C. D. (2014) 'Corticospinal excitability preceding the grasping of emotion-laden stimuli.' *PloS one*, 9(4) p. e94824.

Nogueira-Campos, A. A., Saunier, G., Della-Maggiore, V., De Oliveira, L. A., Rodrigues, E. C. and Vargas, C. D. (2016) 'Observing grasping actions directed to emotion-laden objects: effects upon corticospinal excitability.' *Frontiers in Human Neuroscience*, 10

Noohi, S. and Amirsalari, S. (2016) 'History, studies and specific uses of repetitive transcranial magnetic stimulation (rTMS) in treating epilepsy.' *Iranian journal of child neurology*, 10(1) p. 1.

O'Reardon, J. P., Solvason, H. B., Janicak, P. G., Sampson, S., Isenberg, K. E., Nahas, Z., McDonald, W. M., Avery, D., Fitzgerald, P. B. and Loo, C. (2007) 'Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial.' *Biological psychiatry*, 62(11) pp. 1208-1216.

O'Shea, H. and Moran, A. (2017) 'Does motor simulation theory explain the cognitive mechanisms underlying motor imagery? A critical review.' *Frontiers in human neuroscience*, 11

Ocampo, B. and Kritikos, A. (2010) 'Placing actions in context: motor facilitation following observation of identical and non-identical manual acts.' *Experimental brain research*, 201(4) pp. 743-751.

Ohno, K., Higashi, T., Sugawara, K., Ogahara, K., Funase, K. and Kasai, T. (2011) 'Excitability changes in the human primary motor cortex during observation with motor imagery of chopstick use.' *Journal of Physical Therapy Science*, 23(5) pp. 703-706.

Oldfield, R. C. (1971) 'The assessment and analysis of handedness: the Edinburgh inventory.' *Neuropsychologia*, 9(1) pp. 97-113.

Opitz, A., Legon, W., Rowlands, A., Bickel, W. K., Paulus, W. and Tyler, W. J. (2013) 'Physiological observations validate finite element models for estimating subject-specific electric field distributions induced by transcranial magnetic stimulation of the human motor cortex.' *Neuroimage*, 81 pp. 253-264.

Orban, G. A. (2016) 'Functional definitions of parietal areas in human and non-human primates.' *Proc. R. Soc. B*, 283(1828) p. 20160118.

Orth, M., Snijders, A. and Rothwell, J. (2003) 'The variability of intracortical inhibition and facilitation.' *Clinical Neurophysiology*, 114(12) pp. 2362-2369.

Partala, T. and Surakka, V. (2003) 'Pupil size variation as an indication of affective processing.' *International journal of human-computer studies*, 59(1-2) pp. 185-198.

Pascual-Leone, A., Valls-Solé, J., Wassermann, E. M. and Hallett, M. (1994) 'Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex.' *Brain*, 117(4) pp. 847-858.

Pascual-Leone, A., Gates, J. R. and Dhuna, A. (1991) 'Induction of speech arrest and counting errors with rapid-rate transcranial magnetic stimulation.' *Neurology*, 41(5) pp. 697-702.

Pasley, B. N., Allen, E. A. and Freeman, R. D. (2009) 'State-dependent variability of neuronal responses to transcranial magnetic stimulation of the visual cortex.' *Neuron*, 62(2) pp. 291-303.

Patuzzo, S., Fiaschi, A. and Manganotti, P. (2003) 'Modulation of motor cortex excitability in the left hemisphere during action observation: a single-and paired-pulse transcranial magnetic stimulation study of self-and non-self-action observation.' *Neuropsychologia*, 41(9) pp. 1272-1278.

Paus, T., Sipila, P. and Strafella, A. (2001) 'Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study.' *Journal of Neurophysiology*, 86(4) pp. 1983-1990.

Pavan, A., Cuturi, L. F., Maniglia, M., Casco, C. and Campana, G. (2011) 'Implied motion from static photographs influences the perceived position of stationary objects.' *Vision research*, 51(1) pp. 187-194.

Pelosin, E., Avanzino, L., Bove, M., Stramesi, P., Nieuwboer, A. and Abbruzzese, G. (2010) 'Action observation improves freezing of gait in patients with Parkinson's disease.' *Neurorehabilitation and neural repair*, 24(8) pp. 746-752.

Pereira, M. G., Volchan, E., de Souza, G. G. L., Oliveira, L., Campagnoli, R. R., Pinheiro, W. M. and Pessoa, L. (2006) 'Sustained and transient modulation of performance induced by emotional picture viewing.' *Emotion*, 6(4) p. 622.

Peurala, S. H., Müller-Dahlhaus, J. F. M., Arai, N. and Ziemann, U. (2008) 'Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF).' *Clinical Neurophysiology*, 119(10) pp. 2291-2297.

Pichon, S., de Gelder, B. and Grèzes, J. (2009) 'Two different faces of threat. Comparing the neural systems for recognizing fear and anger in dynamic body expressions.' *Neuroimage*, 47(4) pp. 1873-1883.

Pieters, R. G., Rosbergen, E. and Hartog, M. (1996) 'Visual attention to advertising: The impact of motivation and repetition.' *ACR North American Advances*,

Pitcher, J. B., Ogston, K. M. and Miles, T. S. (2003) 'Age and sex differences in human motor cortex input–output characteristics.' *The Journal of physiology*, 546(2) pp. 605-613.

Pomeroy, V. M., Clark, C. A., Miller, J. S. G., Baron, J.-C., Markus, H. S. and Tallis, R. C. (2005) 'The potential for utilizing the “mirror neurone system” to enhance recovery of the severely affected upper limb early after stroke: a review and hypothesis.' *Neurorehabilitation and Neural Repair*, 19(1) pp. 4-13.

Poole, A., Ball, L. J. and Phillips, P. (2004) 'In search of salience: A response-time and eye-movement analysis of bookmark recognition.' *In People and computers XVIII—Design for life*. Springer, pp. 363-378.

Pope, C. and Mays, N. (2006) *Qualitative Research in Health Care.* Blackwell Publishing.

Press, C., Heyes, C. and Kilner, J. M. (2011) 'Learning to understand others' actions.' *Biology Letters*, 7(3) pp. 457-460.

Proverbio, A. M., Riva, F. and Zani, A. (2009) 'Observation of static pictures of dynamic actions enhances the activity of movement-related brain areas.' *PLoS One*, 4(5) p. e5389.

Purdy, L. (2014) 'Interviews.' *In* Nelson, L., Groom, R. and Potrac, P. (eds.) *Research methods in sports coaching*. London, UK: Routledge, pp. 161-170.

Purdy, L. G. and Jones, R. L. (2011) 'Choppy waters: Elite rowers’ perceptions of coaching.' *Sociology of sport journal*, 28(3) pp. 329-346.

Rayner, K., Pollatsek, A., Ashby, J. and Clifton Jr, C. (2012) *Psychology of reading.* Psychology Press.

Riach, M., Holmes, P. S., Franklin, Z. C. and Wright, D. J. (2018) 'Observation of an action with a congruent contextual background facilitates corticospinal excitability: A combined TMS and eye-tracking experiment.' *Neuropsychologia*, 119 pp. 157-164.

Riach, M., Wright, D. J., Franklin, Z. and Holmes, P. S. (2018) 'Screen position preference offers a new direction for action observation research: preliminary findings using TMS.' *Frontiers in Human Neuroscience*, 12(26)

Ridding, M., Taylor, J. L. and Rothwell, J. (1995) 'The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex.' *The Journal of Physiology*, 487(2) pp. 541-548.

Rizzolatti, G. and Craighero, L. (2004) 'The mirror-neuron system.' *Annu. Rev. Neurosci.*, 27 pp. 169-192.

Rocca, M. A., Falini, A., Comi, G., Scotti, G. and Filippi, M. (2008) 'The mirror-neuron system and handedness: A “right” world?' *Human brain mapping*, 29(11) pp. 1243-1254.

Romani, M., Cesari, P., Urgesi, C., Facchini, S. and Aglioti, S. M. (2005) 'Motor facilitation of the human cortico-spinal system during observation of bio-mechanically impossible movements.' *Neuroimage*, 26(3) pp. 755-763.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A. and Group, S. o. T. C. (2009) 'Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research.' *Clinical neurophysiology*, 120(12) pp. 2008-2039.

Rossini, P., Burke, D., Chen, R., Cohen, L., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P. and George, M. (2015) 'Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an IFCN Committee.' *Clinical Neurophysiology*, 126(6) pp. 1071-1107.

Rossini, P. M. (2014) '1994–2014 Twenty years from the first guidelines for electrical and magnetic stimulation of brain, spinal cord and spinal roots.' *Clinical Neurophysiology*, 125(5) pp. 865-866.

Rossini, P. M., Barker, A., Berardelli, A., Caramia, M., Caruso, G., Cracco, R., Dimitrijević, M., Hallett, M., Katayama, Y. and Lücking, C. (1994) 'Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee.' *Electroencephalography and clinical neurophysiology*, 91(2) pp. 79-92.

Rothwell, J., Day, B., Thompson, P. and Kujirai, T. (2009) 'Short latency intracortical inhibition: one of the most popular tools in human motor neurophysiology.' *The Journal of physiology*, 587(1) pp. 11-12.

Ruffino, C., Papaxanthis, C. and Lebon, F. (2017) 'Neural plasticity during motor learning with motor imagery practice: Review and perspectives.' *Neuroscience*, 341 pp. 61-78.

Ryan, F., Coughlan, M. and Cronin, P. (2009) 'Interviewing in qualitative research: The one-to-one interview.' *International Journal of Therapy and Rehabilitation*, 16(6) pp. 309-314.

Sacco, P., Thickbroom, G. W., Thompson, M. L. and Mastaglia, F. L. (1997) 'Changes in corticomotor excitation and inhibition during prolonged submaximal muscle contractions.' *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, 20(9) pp. 1158-1166.

Sakai, K., Ugawa, Y., Terao, Y., Hanajima, R., Furubayashi, T. and Kanazawa, I. (1997) 'Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil.' *Experimental Brain Research*, 113(1) pp. 24-32.

Salvucci, D. D. and Goldberg, J. H. (2000) *Identifying fixations and saccades in eye-tracking protocols.* ACM.

Sandbrink, F. (2008) 'The MEP in clinical neurodiagnosis.' *In* Wassermann, E. M., Epstein, C. M., Ziemenn, U., Walsh, V., Paus, T. and Lisanby, S. (eds.) *The Oxford Handbook of Transcranial Magnetic Stimulation*. New York: Oxford University Press, pp. 237-282.

Sanes, J. N. and Donoghue, J. P. (2000) 'Plasticity and primary motor cortex.' *Annual review of neuroscience*, 23(1) pp. 393-415.

Sartori, L., Begliomini, C. and Castiello, U. (2013) 'Motor resonance in left-and right-handers: evidence for effector-independent motor representations.' *Frontiers in human neuroscience*, 7

Schubotz, R. I., Wurm, M. F., Wittmann, M. K. and von Cramon, D. Y. (2014) 'Objects tell us what action we can expect: dissociating brain areas for retrieval and exploitation of action knowledge during action observation in fMRI.' *Frontiers in psychology*, 5

Schütz-Bosbach, S., Avenanti, A., Aglioti, S. M. and Haggard, P. (2009) 'Don't do it! Cortical inhibition and self-attribution during action observation.' *Journal of Cognitive Neuroscience*, 21(6) pp. 1215-1227.

Sczesny-Kaiser, M., Höffken, O., Tegenthoff, M. and Schwenkreis, P. (2013) 'Convulsive syncope after single-pulse TMS.' *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 6(5) p. 830.

SensoMotoric Instruments. (2016) *iViewETG User Guide.*

SR Research. (2018) *EyeLink 1000 Plus*. [Online] [Accessed on 4th May 2018] https://www.sr-research.com/products/eyelink-1000-plus/

Stewart, D. W. and Shamdasani, P. N. (2014) *Focus groups: Theory and practice.* 3 ed., Vol. 20. Sage publications.

Stinear, C. M., Byblow, W. D., Steyvers, M., Levin, O. and Swinnen, S. P. (2006) 'Kinesthetic, but not visual, motor imagery modulates corticomotor excitability.' *Experimental Brain Research*, 168(1-2) pp. 157-164.

Stokes, M. G., Chambers, C. D., Gould, I. C., Henderson, T. R., Janko, N. E., Allen, N. B. and Mattingley, J. B. (2005) 'Simple metric for scaling motor threshold based on scalp-cortex distance: application to studies using transcranial magnetic stimulation.' *Journal of neurophysiology*, 94(6) pp. 4520-4527.

Strafella, A. and Paus, T. (2001) 'Cerebral blood-flow changes induced by paired-pulse transcranial magnetic stimulation of the primary motor cortex.' *Journal of neurophysiology*, 85(6) pp. 2624-2629.

Strafella, A. P. and Paus, T. (2000) 'Modulation of cortical excitability during action observation: a transcranial magnetic stimulation study.' *Neuroreport*, 11(10) pp. 2289-2292.

Strauss, G. P., Ossenfort, K. L. and Whearty, K. M. (2016) 'Reappraisal and distraction emotion regulation strategies are associated with distinct patterns of visual attention and differing levels of cognitive demand.' *PloS one*, 11(11) p. e0162290.

Sugg, K., Müller, S., Winstein, C., Hathorn, D. and Dempsey, A. (2015) 'Does action observation training with immediate physical practice improve hemiparetic upper-limb function in chronic stroke?' *Neurorehabilitation and Neural Repair*, 29(9) pp. 807-817.

Suk, H.-J. (2006) *Color and Emotion-a study on the affective judgment across media and in relation to visual stimuli.* Universität Mannheim.

Tamir, M. and Robinson, M. D. (2007) 'The happy spotlight: Positive mood and selective attention to rewarding information.' *Personality and Social Psychology Bulletin*, 33(8) pp. 1124-1136.

Taylor, J., Allen, G. M., Butler, J. E. and Gandevia, S. (1997) 'Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimulation.' *Experimental Brain Research*, 117(3) pp. 472-478.

Thomas, G. and James, D. (2006) 'Reinventing grounded theory: some questions about theory, ground and discovery.' *British educational research journal*, 32(6) pp. 767-795.

Trompetto, C., Assini, A., Buccolieri, A., Marchese, R. and Abbruzzese, G. (1999) 'Intracortical inhibition after paired transcranial magnetic stimulation depends on the current flow direction.' *Clinical neurophysiology*, 110(6) pp. 1106-1110.

Tsukazaki, I., Uehara, K., Morishita, T., Ninomiya, M. and Funase, K. (2012) 'Effect of observation combined with motor imagery of a skilled hand-motor task on motor cortical excitability: difference between novice and expert.' *Neuroscience letters*, 518(2) pp. 96-100.

U.S. National Library of Medicine. (2018) *Electromyography*. [Online] [Accessed on 7th March 2018] https://meshb.nlm.nih.gov/record/ui?name=Electromyography

Ueno, S., Tashiro, T. and Harada, K. (1988) 'Localized stimulation of neural tissues in the brain by means of a paired configuration of time-varying magnetic fields.' *Journal of Applied Physics*, 64(10) pp. 5862-5864.

Ueno, S., Matsuda, T. and Fujiki, M. (1990) 'Functional mapping of the human motor cortex obtained by focal and vectorial magnetic stimulation of the brain.' *IEEE Transactions on Magnetics*, 26(5) pp. 1539-1544.

Ugawa, Y., Terao, Y., Hanajima, R., Sakai, K. and Kanazawa, I. (1995) 'Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation.' *Clinical Neurophysiology*, 97(6) pp. 451-454.

Uithol, S., Franca, M., Heimann, K., Marzoli, D., Capotosto, P., Tommasi, L. and Gallese, V. (2015) 'Single-pulse Transcranial Magnetic Stimulation Reveals Contribution of Premotor Cortex to Object Shape Recognition.' *Brain stimulation*, 8(5) pp. 953-956.

Urgesi, C., Candidi, M., Fabbro, F., Romani, M. and Aglioti, S. M. (2006) 'Motor facilitation during action observation: topographic mapping of the target muscle and influence of the onlooker's posture.' *European Journal of Neuroscience*, 23(9) pp. 2522-2530.

Ustinova, K., Perkins, J., Szostakowski, L., Tamkei, L. and Leonard, W. (2010) 'Effect of viewing angle on arm reaching while standing in a virtual environment: potential for virtual rehabilitation.' *Acta Psychologica*, 133(2) pp. 180-190.

van den Bos, M. A., Geevasinga, N., Menon, P., Burke, D., Kiernan, M. C. and Vucic, S. (2016) 'Physiological processes influencing motor-evoked potential duration with voluntary contraction.' *Journal of neurophysiology*, 117(3) pp. 1156-1162.

Van Loon, A. M., van den Wildenberg, W. P., van Stegeren, A. H., Ridderinkhof, K. R. and Hajcak, G. (2010) 'Emotional stimuli modulate readiness for action: a transcranial magnetic stimulation study.' *Cognitive, Affective, & Behavioral Neuroscience*, 10(2) pp. 174-181.

Van Orden, K. F., Jung, T.-P. and Makeig, S. (2000) 'Combined eye activity measures accurately estimate changes in sustained visual task performance.' *Biological psychology*, 52(3) pp. 221-240.

van Polanen, V. and Davare, M. (2015) 'Interactions between dorsal and ventral streams for controlling skilled grasp.' *Neuropsychologia*, 79 pp. 186-191.

Vecchio, M., Gracies, J.-M., Panza, F., Fortunato, F., Vitaliti, G., Malaguarnera, G., Cinone, N., Beatrice, R., Ranieri, M. and Santamato, A. (2017) 'Change in coefficient of fatigability following rapid, repetitive movement training in post-stroke spastic paresis: A prospective open-label observational study.' *Journal of Stroke and Cerebrovascular Diseases*, 26(11) pp. 2536-2540.

Vickers, J. N. (2009) 'Advances in coupling perception and action: the quiet eye as a bidirectional link between gaze, attention, and action.' *Progress in brain research*, 174 pp. 279-288.

Villiger, M., Chandrasekharan, S. and Welsh, T. N. (2011) 'Activity of human motor system during action observation is modulated by object presence.' *Experimental brain research*, 209(1) pp. 85-93.

Vogeley, K. and Fink, G. R. (2003) 'Neural correlates of the first-person-perspective.' *Trends in cognitive sciences*, 7(1) pp. 38-42.

Vogt, S., Di Rienzo, F., Collet, C., Collins, A. and Guillot, A. (2013) 'Multiple roles of motor imagery during action observation.' *Frontiers in Human Neuroscience*, 7 p. 807.

Wagle-Shukla, A., Ni, Z., Gunraj, C. A., Bahl, N. and Chen, R. (2009) 'Effects of short interval intracortical inhibition and intracortical facilitation on short interval intracortical facilitation in human primary motor cortex.' *The Journal of physiology*, 587(23) pp. 5665-5678.

Wassermann, E., Cohen, L., Flitman, S., Chen, R. and Hallett, M. (1996) 'Seizures in healthy people with repeated" safe" trains of transcranial magnetic stimuli.' *The Lancet*, 347(9004) pp. 825-826.

Wassermann, E. M. (1998) 'Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996.' *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 108(1) pp. 1-16.

Watson, J. B. and Rayner, R. (1920) 'Conditioned emotional reactions.' *Journal of experimental psychology*, 3(1) p. 1.

Weiss, P. H., Rahbari, N. N., Hesse, M. D. and Fink, G. R. (2008) 'Deficient sequencing of pantomimes in apraxia.' *Neurology*, 70(11) pp. 834-840.

Werhahn, K., Fong, J., Meyer, B.-U., Priori, A., Rothwell, J., Day, B. and Thompson, P. (1994) 'The effect of magnetic coil orientation on the latency of surface EMG and single motor unit responses in the first dorsal interosseous muscle.' *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section*, 93(2) pp. 138-146.

Westin, G. G., Bassi, B. D., Lisanby, S. H. and Luber, B. (2014) 'Determination of motor threshold using visual observation overestimates transcranial magnetic stimulation dosage: Safety implications.' *Clinical Neurophysiology*, 125(1) pp. 142-147.

Williams, J., Pearce, A. J., Loporto, M., Morris, T. and Holmes, P. S. (2012) 'The relationship between corticospinal excitability during motor imagery and motor imagery ability.' *Behavioural brain research*, 226(2) pp. 369-375.

Wolf, S. L., Lecraw, D. E., Barton, L. A. and Jann, B. B. (1989) 'Forced use of hemiplegic upper extremities to reverse the effect of learned nonuse among chronic stroke and head-injured patients.' *Experimental neurology*, 104(2) pp. 125-132.

World Medical Association (2013) 'World medical association declaration of helsinki: Ethical principles for medical research involving human subjects.' *JAMA*, 310(20) pp. 2191-2194.

Wright, D. J., McCormick, S. A., Williams, J. and Holmes, P. S. (2016) 'Viewing Instructions Accompanying Action Observation Modulate Corticospinal Excitability.' *Frontiers in Human Neuroscience*, 10

Wright, D. J., Wood, G., Franklin, Z. C., Marshall, B., Riach, M. and Holmes, P. S. (2018) 'Directing visual attention during action observation modulates corticospinal excitability.' *PLoS One*, 13(1) p. e0190165.

Wurm, M. F. and Schubotz, R. I. (2012) 'Squeezing lemons in the bathroom: contextual information modulates action recognition.' *Neuroimage*, 59(2) pp. 1551-1559.

Wurm, M. F. and Schubotz, R. I. (2017) 'What's she doing in the kitchen? Context helps when actions are hard to recognize.' *Psychonomic bulletin & review*, 24(2) pp. 503-509.

Wurm, M. F., Cramon, D. Y. and Schubotz, R. I. (2012) 'The Context–Object–Manipulation Triad: Cross Talk during Action Perception Revealed by fMRI.' *Journal of Cognitive Neuroscience*, 24(7) pp. 1548-1559.

Yahagi, S. and Kasai, T. (1999) 'Motor evoked potentials induced by motor imagery reveal a functional asymmetry of cortical motor control in left-and right-handed human subjects.' *Neuroscience Letters*, 276(3) pp. 185-188.

Zaidi, A., Clough, P., Cooper, P., Scheepers, B. and Fitzpatrick, A. P. (2000) 'Misdiagnosis of epilepsy: many seizure-like attacks have a cardiovascular cause.' *Journal of the American College of Cardiology*, 36(1) pp. 181-184.

Ziemann, U. (2001) 'Paired pulse techniques.' *In* Pascual-Leone, A., Wassermann, E., Rothwell, J. and Puri, B. (eds.) *Handbook of transcranial magnetic stimulation*. 1 ed.: London: Arnold, pp. 141-162.

Ziemann, U., Rothwell, J. C. and Ridding, M. C. (1996) 'Interaction between intracortical inhibition and facilitation in human motor cortex.' *The Journal of Physiology*, 496(3) pp. 873-881.

Ziemann, U., Tergau, F., Wassermann, E. M., Wischer, S., Hildebrandt, J. and Paulus, W. (1998) 'Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation.' *The Journal of physiology*, 511(1) pp. 181-190.

Supplementary material 1. Coil orientation pilot demonstration

S1.1. Introduction

In attempts to design a set protocol for TMS research, groups have explored the effect of coil orientation on the subsequent MEP amplitude. Brasil-Neto et al. (1992) first explored this, testing angles in 45° increments ranging from 45° to 315°. Their results demonstrated that a 45° coil orientation inducing current in a postero-anterior direction produced the largest MEPs. Further research indicated the importance of ensuring correct coil orientation throughout an experiment, as inducing the current in a lateral-medial direction stimulated direct waves, whilst a current induced in a poster-anterior direction stimulates indirect waves (Werhahn et al., 1994), and reflects the overall excitability of the motor regions of the brain (Opitz et al., 2013). Incorrect coil orientation, therefore, does not reflect CSE, and can reduce MEP amplitudes (see Chapter 3.3.1).

The aim of this pilot experiment was to demonstrate the effect that manipulating coil orientation has on MEP amplitudes. It was hypothesised that the 45° coil orientation, inducing current flow in a poster-anterior direction would elicit the largest MEP amplitudes.

S1.2. Method

S1.2.1. Participants

One volunteer (female, aged 22 years, right-handed: LQ 88.24) participated in the experiment. Only one participant was required to demonstrate the effect manipulating the coil orientation has on MEP amplitude, as the biological effect is so profound due to neural alignment (see Brasil-Neto et al., 1992).

S1.2.2. Procedure

The general TMS procedure was identical to that outlined in Chapter 4.2. A coil orientation of 45° to the midline between nasion and inion landmarks of the skull was used to determine the OSP and RMT, but was then manipulated in the experiment.

The general experimental procedure was as outline in Chapter 4.5.1, with the exception that the participant observed a video of six seconds in duration containing a fixation cross (Figure A2.1), rather than an action. One stimulation timing was used (3000ms after video onset), as no indicators of the onset of the stimulation were available during the fixation cross observation to influence predictability of the stimulation. The participant observed the fixation cross with the coil orientation at 45°, 135°, 225° and 315° in relation to the midline between nasion and inion landmarks of the skull (Figure A2.1). The experiment was split into four blocks of 10 trials, with each block containing only one coil orientation.

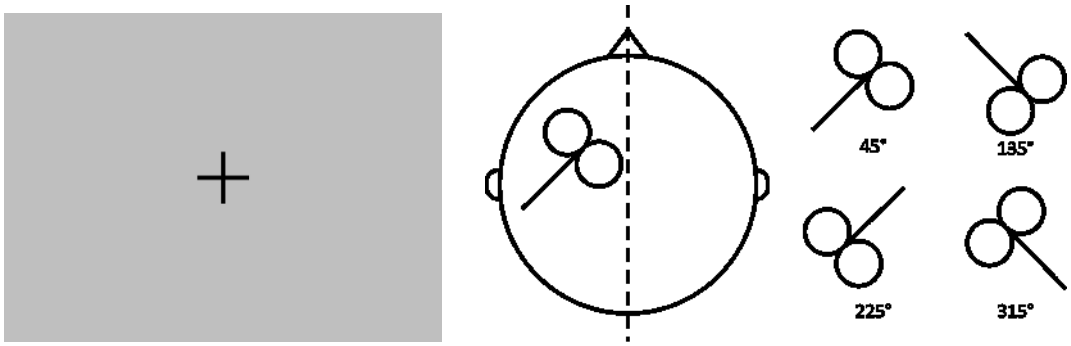


Figure S1.1. The fixation cross stimulus (left), and each coil orientation relative to the midline between nasion and inion landmarks of the skull (right).

S1.2.3. Data analysis

Due to only testing one participant, no statistical analysis was performed on the *z*-score MEP data. Instead, descriptive data is provided.

S1.3. Results

Raw MEP amplitudes recorded from the FDI and ADM muscles for each of the conditions are reported in Table A2.1. The data showed the largest MEP amplitudes during the 45° coil orientation for both the FDI and ADM muscles (Figure A2.2). The MEP amplitudes recorded during the 135° coil orientation from the ADM, and the 225° and 315° coil orientations from both the FDI and ADM did not exceed the 50μV resting motor threshold limit.

Table S1.1. Raw MEP amplitudes recorded from the FDI and ADM muscles for each condition

	Raw MEP amplitude (μV)	
	FDI	ADM
45° coil orientation	3037.77 (± 456.31)	516.62 (± 82.37)
135° coil orientation	166.41 (± 98.99)	15.68 (± 4.89)
225° coil orientation	28.23 (± 7.51)	28.38 (± 8.22)
315° coil orientation	6.04 (± 1.16)	10.43 (± 3.75)

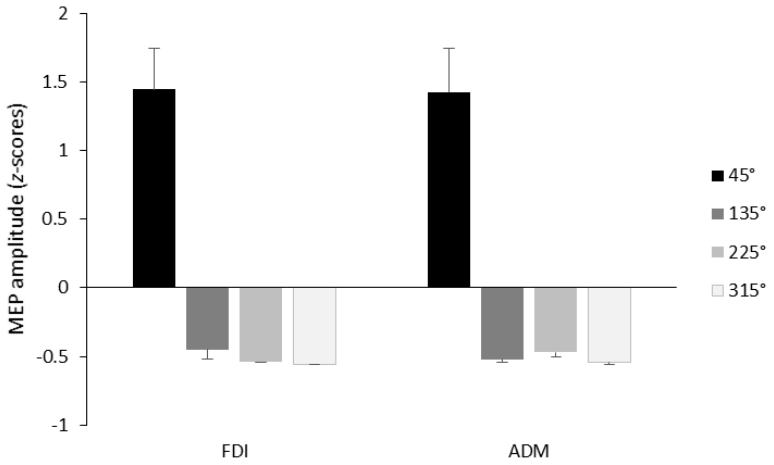


Figure S1.2. Mean MEP amplitudes, displayed as *z*-scores, recorded during each coil orientation from the FDI and ADM muscles.

S1.4. Discussion

This experiment aimed to demonstrate the effect of coil orientation on MEP amplitudes. Greater MEP amplitudes were shown when the coil orientation was at a 45° angle to the midline of the skull in a poster-anterior direction. This supports previous research demonstrating the importance of coil orientation (Brasil-Neto et al., 1992; Werhahn et al., 1994). As such, the experiments included in this thesis all utilised the coil orientation recommended by Brasil-Neto et al. (1992).

Appendix 1. The TMS adult safety screen (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.

Have you ever suffered from any neurological or psychiatric conditions?
YES / NO

If YES please give details (nature of condition, duration, current medication, etc).

Have you ever suffered from epilepsy, febrile convulsions in infancy
or had recurrent fainting spells?
YES / NO

Does anyone in your immediate or distant family suffer from epilepsy?
YES / NO

If YES please state your relationship to the affected family member.

Do you suffer from migraine?
YES/ NO

Have you ever undergone a neurosurgical procedure (including eye surgery)?
YES/ NO

If YES please give details.

Do you currently have any of the following fitted to your body?
YES / NO

Heart pacemaker, Cochlear implant, Medication pump,

Surgical clips, Metal plates, Stent

Are you currently taking any unprescribed or prescribed medication?
YES / NO

If YES please give details.

Are you currently undergoing anti - malarial treatment?
YES / NO

Have you drunk more than 3 units of alcohol in the last 24 hours?
YES / NO

Have you drunk alcohol already today?
YES / NO

Have you had more than one cup of coffee, or other sources of caffeine, in the last hour?
YES / NO

Have you used recreational drugs in the last 24 hours?
YES / NO

Did you have very little sleep last night?

YES / NO

Have you already participated in a TMS experiment today?

YES / NO

Are you left or right handed?

Left / Right

Date of Birth

__/__/__

Name (in CAPITALS)_____

Signature_____ Date_____

Appendix 2. Edinburgh handedness inventory (EHI; Oldfield, 1971)

Edinburgh Handedness Inventory – Oldfield (1971)

Name: Date:

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.

Some of the activities require both hands. In these cases, the part of the task, or object, for which hand preference is wanted is indicated in parentheses.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Right	Left
Writing		
Drawing		
Throwing		
Scissors		
Comb		
Toothbrush		
Knife (without fork)		
Spoon		
Hammer		
Screwdriver		
Tennis racquet		
Knife (with fork)		
Cricket bat (lower hand)		
Golf club (lower hand)		
Broom (upper hand)		
Striking match (match hand)		
Opening box/jar (lid hand)		
Dealing cards (card dealing hand)		
Which foot do you prefer to kick with?		
Which eye do you use when using only one?		

Appendix 3. Information sheets for participants

Appendix 3.1. Information sheet for participants for the pilot experiment

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study:

An exploration of different brain activation mechanisms during action observation: A pilot study.

Ethics Committee Reference Number:

27.11.15(i)

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 explore two methods of measuring activity in areas of the brain related to controlling movement during the observation of an action. The results will identify the optimal method of measuring brain activity during observation, and will therefore inform the design of future studies exploring action observation.

3) Why is the study being performed?

During the observation of an action, specific areas of the brain related to performing that action become active. This study aims to use two methods of measuring this brain activity during the observation of an action compared to observation of a static image. This study seeks to explore both methods and determine the most appropriate technique for future research.

4) Why am I being asked to take part?

You have been asked to take part in this study because you are aged 18-35 years, are right-handed, and are fit to undergo transcranial magnetic stimulation (TMS) based on your responses to a screening questionnaire.

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 study 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, on written request to the Principal Investigator, and this will not be included when the research is reported. If you decide not to take part or withdraw from the study, this will not 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. You will be given a copy of the consent form together with this information sheet to keep.

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

If you agree to take part, you will be required to attend one testing session in the psychophysiology lab in the Exercise and Sport Science building at MMU Cheshire in Crewe. The testing session will last approximately 1.5 to 2 hours. You will first complete a screening questionnaire to determine your eligibility to take part, and will be shown all of the equipment that will be used during the study, including a short video of the procedure. Next, you will sit in front of a computer screen with your head on a chin-rest. Two electrodes will be placed on your hand (this is safe and painless), which will be used to measure muscle activity in the hand. Finally, a plastic-coated coil, which is used to deliver stimulations causing small contractions in your hand muscles, will be positioned over your head. Again, this is safe and painless, but please refer to section 7 of this document for further information.

Once the setup is complete, you will watch a video on the screen. There will be 2 variations of the video that include observing a hand squeezing a sponge ball and a static hand holding a sponge ball. You will observe each video 45 times for a total of 90 observations. You will be given a 5 minute break between blocks of 15 videos where you can move around to prevent any discomfort from prolonged sitting.

During some trials you will receive one stimulation pulse, and others you will receive two stimulation pulses. Both types of stimulation will cause small contractions in your hand muscles. You are unlikely to notice the difference between trials, as the two-pulse stimulation trials occur in such quick succession. These stimulations will activate the area of the brain responsible for controlling hand movements and cause a small muscle contraction in your whole hand, which will be recorded by the electrodes on your hand. You should not feel any discomfort during this procedure, but we will stop testing immediately if you report otherwise.

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

If your responses to the screening questionnaire indicate that you are eligible to take part, then there should be no disadvantages to participating in this study. Occasionally, some participants report mild headaches following TMS, but this can be alleviated with over-the-counter pain medication. If the problems persist for 12 hours or into the next day, please contact your GP.

If you do experience any discomfort during the procedure, please remember that you are free to withdraw from the study at any time.

8) What are the possible benefits of taking part?

There will be no direct benefits to yourself, but you are assisting in improving the methodology of an area of research that will inform interventions used with stroke patients. These interventions can be used to improve skill learning and aid with the rehabilitation of stroke patients to regain movement in affected limbs.

9) Who are the members of the research team?

The Principal Investigator is Mr Martin Riach (m.riach@mmu.ac.uk), who designed the experiment and will be responsible for data collection. He can be contacted via e-mail if any further information is wanted.

The Research Supervisors are Dr David Wright (d.j.wright@mmu.ac.uk) and Prof Paul Holmes (p.s.holmes@mmu.ac.uk) from the Department of Exercise and Sport Science at MMU Cheshire, and can be contacted via e-mail. Dr Wright and Prof Holmes helped to design the experiment and will oversee the project.

10) Who is funding the research?

The research is funded by Manchester Metropolitan University.

11) Who will have access to the data?

All of the information collected during the course of the research will be kept confidential, and used only for the purposes of the study and any associated publications. All electronic data will be stored on a password protected computer in a coded format, and the questionnaire responses will be stored in a locked drawer separate from the rest of the data. This will only be accessible to the Principal Investigator and named Research Supervisors.

The data will only be kept for the duration of the principal investigator's research project and for the purposes of publication. Following this, all data will be securely destroyed. If the results of the study are published, this will be done so in a confidential manner so that you will not be identifiable as a participant. If you wish to receive a copy of any publication that may result from this research, please contact the Principal Investigator (m.riach@mmu.ac.uk).

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

If you feel like your rights have been violated during the course of this research, or if you wish to make a complaint, then please contact the address below:

MMU Ethics Committee
Registrar & Clerk to the Board of Governors
Head of Governance and Secretariat Team
Manchester Metropolitan University
All Saints Building, All Saints
Manchester M15 6BH
Tel: 0161 247 1390

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

13) Finally, a thank you!

Thank you for considering participating in this research. If you have any further questions, please do not hesitate to ask.

Appendix 3.2. Information sheet for participants for Experiment 1

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study:

An investigation into the effects of viewing angle on cortical activity during action observation.

Ethics Committee Reference Number: 30.09.14(i)

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 this experiment is to investigate the activity of certain brain areas when people observe human movements. Specifically, the study aims to discover the effects of viewing angle on activity in the brain. The results are likely to inform how to best to design and deliver observation-based learning interventions.

3) Why is the study being performed?

Research has shown that when we observe human movements, areas of the brain responsible for controlling physical movement become active. This process is thought to be the reason why observation interventions are helpful as a type of movement (re)learning technique. It is important to establish how best such interventions can be designed and delivered. Researchers have suggested that the viewing angle may influence the extent to which areas of the brain are activated during action observation. However, no research has yet been conducted to support these claims. This study is therefore being conducted to address these issues.

4) Why am I being asked to take part?

This study requires participants who are right handed and aged between 18-35 years. You have been asked to take part in the experiment as you fit these criteria. In addition, your responses to the Transcranial Magnetic Stimulation Adult Safety Screen indicated that you are unlikely to suffer adverse side effects to the brain stimulation technique, and you are therefore eligible to participate in this experiment.

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 study 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, on written request to the Principal Investigator, and this will not be included when the research is reported. If

you decide not to take part or withdraw from the study your relationship with any of the staff at the Manchester Metropolitan University will not be affected.

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 given a copy together with this information sheet to keep.

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

Before agreeing to take part in this experiment, you will be asked to complete a screening questionnaire to assess your suitability to participate in the study. If this indicates that you are eligible to take part, you will be asked to attend the Psychophysiology Laboratory at Manchester Metropolitan University (Cheshire campus) for a single testing session that will last about two hours.

You will be asked to sit at a desk whilst electrodes are attached to the skin overlying three muscles on your right hand. These electrodes will be used to record muscular activity in your hand throughout the experiment. Attachment of the electrodes and recording of muscular activity is a safe and harmless procedure. A tight-fitting cap will then be placed on your head and head measurements will be taken to identify the area of the brain that controls hand movements. You will then be asked to place your head onto a head and chin rest, and a polyurethane-coated coil will be placed close to the previously marked areas of the scalp. You will be asked to observe 120 short videos (10 seconds each) showing simple hand movements or objects on screen. The coil will deliver one stimulation during each video and this will activate the area of the brain that controls hand movements. This stimulation procedure is safe and may be experienced as a faint tap to the scalp. It should not cause any pain or discomfort, but it may feel a little strange at first as a small, involuntary contraction of your hand muscles will occur. We will record the size of the muscular contractions through the electrodes attached to your hand to gain an indication of how active your brain was at the time of stimulation. The procedure will be explained to you again verbally when you arrive at the laboratory and you will be given the opportunity to watch a short video demonstrating the procedure and to ask any questions.

At the end of the testing session you will be asked to take part in a brief semi-structured interview, lasting no more than 20 minutes, where you will be asked questions regarding your experiences of observing the videos from two different screen angles. This will complement the data recorded from the experimental stimulation and allow for a better interpretation of the results.

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

Although the Transcranial Magnetic Stimulation procedure being used in this experiment is safe, there is a small risk that it can cause headaches or discomfort. These symptoms are rare but usually only last for a short period of time. If these symptoms develop during the experiment, the session will be terminated immediately.

As a follow up measure, the primary investigator will contact you by telephone in the evening, if the symptoms persist you will be advised to contact your GP. You will be asked to complete a screening questionnaire prior to participating, and if your responses indicate that you may be susceptible to these adverse effects you will not be asked to take part in the experiment.

8) What are the possible benefits of taking part?

Participation in this experiment you will help further our scientific understanding of the brain processes involved in observation of human movement. This may result in a greater understanding of how best to design and implement observation interventions in movement rehabilitation settings.

9) Who are the members of the research team?

The Principal Investigator in this experiment is Mr Martin Riach (m.riach@mmu.ac.uk) from Manchester Metropolitan University. Mr Riach helped to design the experiment and will be responsible for data collection.

Additional members of the research team include Dr David Wright (d.j.wright@mmu.ac.uk) and Professor Paul Holmes (p.s.holmes@mmu.ac.uk) from Manchester Metropolitan University. Dr Wright will supervise the project and may be involved in data collection. Both Dr Wright and Professor Holmes were involved in designing the experiment.

All members of the research team have extensive experience using the techniques and procedures involved in this experiment and will be pleased to answer any questions you may have about your involvement.

10) Who is funding the research?

This research is being funded by the Centre for Health, Exercise and Active Living (HEAL) at Manchester Metropolitan University.

11) Who will have access to the data?

All information and data collected during the course of this research will be kept confidential and will only be used for the purposes of this study. Electronic data from the experiment will be kept on a password protected PC in the Psychophysiology

Laboratory at Manchester Metropolitan University. Data from the questionnaires will be stored in a locked filing cabinet. In both cases the data will be coded to protect your identity. Only the Principal Investigator and other members of the research team will have access to the data, which will be stored for 5 years. After this time, all electronic data (raw and processed) will be deleted and the questionnaire data will be shredded. It is likely that the results from the study will be communicated at academic conferences and published in scientific journals. In this event, data will be presented in a manner that will not allow your identity to be determined. Should you wish to obtain a copy of any publication that results from this research, please email the Principal Investigator (m.riach@mmu.ac.uk).

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

If you wish to make a complaint regarding your involvement in the study please contact :

MMU Ethics Committee

Registrar & Clerk to the Board of Governors

Head of Governance and Secretariat Team

Manchester Metropolitan University

All Saints Building, All Saints

Manchester M15 6BH

Tel: 0161 247 1390

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

Thank you for considering participating in this experiment.

Appendix 3.3. Information sheet for participants for Experiment 2

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study:

The effect of background context on brain activity during action observation.

Ethics Committee Reference Number:

MR-16-01

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 this experiment is to investigate the activity of certain brain areas when people observe human movements. Specifically, the study aims to discover the effects of viewing actions presented against contextually relevant and contextually irrelevant backgrounds. The results are likely to inform how to best to design and deliver observation-based learning interventions.

3) Why is the study being performed?

Research has shown that when we observe human movements, areas of the brain responsible for controlling physical movement become active. This process is thought to be the reason why observation interventions are helpful as a type of movement (re)learning technique. It is important to establish how best such interventions can be designed and delivered. Researchers have suggested that the context in which movements are observed may influence the extent to which areas of the brain are activated during action observation. However, more research is needed to support these claims. This study is therefore being conducted to address these issues.

4) Why am I being asked to take part?

This study requires participants who are right handed and aged between 18-35 years. You have been asked to take part in the experiment as you fit these criteria. In addition, your responses to the screening questionnaire indicated that you are unlikely to suffer adverse side effects to the testing, and you are therefore eligible to participate in this experiment.

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 study 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, on written request to the Principal Investigator, and this will not be included when the research is reported. If you decide not to take part or withdraw from the study, this will not 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. You will be given a copy of the consent form together with this information sheet to keep.

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

Before agreeing to take part in this experiment, you will be asked to complete a screening questionnaire to assess your suitability to participate in the study. If this indicates that you are eligible to take part, you will be asked to attend the Psychophysiology Laboratory at Manchester Metropolitan University (Cheshire campus) for a single testing session that will last about around an hour and a half.

You will be asked to sit at a desk whilst electrodes are attached to the skin overlying two muscles on your right hand. These electrodes will be used to record muscular activity in your hand throughout the experiment. Attachment of the electrodes and recording of muscular activity is a safe and harmless procedure. A tight-fitting cap will then be placed on your head and head measurements will be taken to identify the area of the brain that controls hand movements. You will put on a pair of eye-tracking glasses, similar to a normal pair of spectacles, which will monitor your eye-movements throughout the experiment. Following the setup, your head will be rested on a head and chin rest, and a plastic-coated coil will be placed close to the previously marked areas of the scalp. You will be asked to observe 128 short videos (10 seconds each) showing simple hand movements or objects on screen. The coil will deliver one stimulation during each video and this will activate the area of the brain that controls hand movements. This stimulation procedure is safe and may be experienced as a faint tap to the scalp. It should not cause any pain or discomfort, but it may feel a little strange at first as a small, involuntary contraction of your hand muscles will occur. We will record the size of the muscular contractions through the electrodes attached to your hand to gain an indication of how active your brain was at the time of stimulation. The procedure will be explained to you again verbally when you arrive at the laboratory and you will be given the opportunity to watch a short video demonstrating the procedure and to ask questions regarding the procedure.

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

Although the Transcranial Magnetic Stimulation procedure being used in this experiment is safe, there is a small risk that it can cause headaches or discomfort. These symptoms are rare but usually only last for a short period of time. Should the symptoms persist, please contact your GP. You will be asked to complete a screening questionnaire prior to participating, and if your responses indicate that you may be susceptible to these adverse effects you will not be asked to take part in the experiment. In the unlikely event of you suffering these symptoms the experiment will be stopped immediately and suitable aftercare guidance will be provided.

8) What are the possible benefits of taking part?

Participation in this experiment you will help further our scientific understanding of the brain processes involved in observation of human movement. This may result in a greater understanding of how best to design and implement observation interventions in movement rehabilitation settings.

9) Who are the members of the research team?

The Principal Investigator in this experiment is Mr Martin Riach (m.riach@mmu.ac.uk) from Manchester Metropolitan University. Mr Riach designed the experiment and will be responsible for data collection.

Additional members of the research team include Dr David Wright (d.j.wright@mmu.ac.uk) and Professor Paul Holmes (p.s.holmes@mmu.ac.uk) from Manchester Metropolitan University. Dr Wright and Professor Holmes will supervise the project and were both involved in designing the experiment.

All members of the research team have extensive experience using the techniques and procedures involved in this experiment and will be pleased to answer any questions you may have about your involvement.

10) Who is funding the research?

This research is being funded by the Centre for Health, Exercise and Active Living (HEAL) at Manchester Metropolitan University.

11) Who will have access to the data?

All information and data collected during the course of this research will be kept confidential and will only be used for the purposes of this study. Electronic data from the experiment will be kept on a password protected PC in the Psychophysiology Laboratory at Manchester Metropolitan University. The data will be coded to protect your identity. Only the Principal Investigator and other members of the research team will have access to the data, which will be stored for 5 years. After this time, all electronic data (raw and processed) will be deleted. It is likely that the results from the study will be communicated at academic conferences and published in scientific journals. In this event, data will be presented in a manner that will not allow your identity to be determined. Should you wish to obtain a copy of any publication that results from this research, please email the Principal Investigator (m.riach@mmu.ac.uk).

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

If you wish to make a complaint regarding your involvement in the study please contact :

MMU Ethics Committee
Registrar & Clerk to the Board of Governors
Head of Governance and Secretariat Team
Manchester Metropolitan University
All Saints Building, All Saints
Manchester M15 6BH
Tel: 0161 247 1390

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

Thank you for considering participating in this experiment.

Appendix 3.4. Information sheet for participants for Experiment 3

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Information Sheet for Participants

Title of Study:

The effect of object preference on brain activity 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 study?

The purpose of this experiment is to investigate the activity of certain brain areas when people observe human movements. Specifically, the study aims to discover whether having a preference for certain objects changes brain activity whilst

observing interactions with such objects. The results are likely to inform how to best to design and deliver observation-based learning interventions.

3) Why is the study being performed?

Research has shown that when we observe human movements, areas of the brain responsible for controlling physical movement become active. This process is thought to be the reason why observation interventions are helpful as a movement (re)learning technique. It is important to establish how best these interventions can be designed and delivered. Researchers have suggested that observing actions involving interactions with an object for which the observer has a strong preference for may influence the extent to which areas of the brain are activated during action observation. However, more research is needed to support these claims.

4) Why have I been invited?

We have asked you to take part in the study because you are aged between 18 – 40 years of age and a student at Manchester Metropolitan University. In addition, your responses to the Transcranial Magnetic Stimulation Adult Safety Screening questionnaire indicated that you are unlikely to suffer adverse side effects to the brain stimulation technique, and you are, therefore, eligible to participate in this experiment.

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 study 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, on written request to the Principal Investigator, and this will not be included when the research is reported. If you decide not to take part or withdraw from the study, this will not affect your relationship with any of the staff at the Manchester Metropolitan University.

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

Before agreeing to take part in this experiment you will be asked to complete a screening questionnaire to assess your suitability to participate in the study. Should this questionnaire indicate that you are eligible to take part, you will be asked to attend the Psychophysiology Laboratory at the Cheshire campus of Manchester Metropolitan University for a single testing session that will last about two hours.

Prior to testing, you will be asked to rank a series of objects by preference. You will then be asked to sit at a desk whilst electrodes are attached to the skin overlying two muscles on your right hand to record muscular activity throughout the experiment. This is a safe and harmless procedure. A tight-fitting cap will then be placed on your head and head measurements will be taken to identify the area of the brain that controls hand movements. You will put on a pair of eye-tracking glasses, similar to a normal pair of spectacles, which will monitor your eye-movements throughout the experiment.

Following the setup, your head will be rested on a head and chin rest, and a stimulating coil will be placed over the scalp. You will then be asked to view observe 120 short videos (approximately 10 seconds duration each). The coil will deliver one stimulation during each video, which will activate the area of the brain that controls hand movements and cause a muscular contraction to occur in your right hand. This stimulation procedure is safe and should not cause any pain or discomfort, but it may feel a little strange at first. We will record the amplitude of the muscular contractions through the electrodes attached to your hand to gain an indication of how active your brain was at the time of stimulation. Following the experiment, you will take part in a brief semi-structured interview, lasting approximately 20 minutes, where you will be asked questions regarding your experience of observing the different objects.

The procedure will be explained to you again verbally when you arrive at the laboratory and you will be shown a short video demonstrating the stimulation procedure so that you can see what it involves before deciding to take part. You will also be given the opportunity to ask any questions about the experiment procedure.

7) What will I have to do?

If you agree to take part in this project you will not be required to make any changes to your lifestyle. You will be asked to attend one testing session during the day at Manchester Metropolitan University's Cheshire campus. This testing session will last approximately two hours, during which you will complete the procedure outlined above. The session will be scheduled for the most convenient day and time for you. All testing procedures will be explained to you verbally at each session.

8) What are the possible disadvantages and risks of taking part?

Although the Transcranial Magnetic Stimulation procedure being used in this experiment is safe, there is a small risk that it can cause headaches or discomfort. You will be asked to complete a screening questionnaire prior to participating, and if your responses indicate that you may be susceptible to these adverse effects you will not be asked to take part in the experiment. These symptoms are rare and usually

only last for a short period of time. In the unlikely event that these symptoms develop during the experiment, the session will be terminated immediately. As a follow up measure, the primary investigator will contact you by telephone in the evening to check that you are feeling better, and if the symptoms persist you will be advised to contact your GP.

9) What are the possible benefits of taking part?

We cannot promise the study will help you directly but the information we get from the study will help to improve our knowledge of the brain processes involved in observing interactions with different objects. This will help to inform rehabilitation techniques. By taking part in this project it will give you the opportunity to gain insight into how to conduct a higher level study, which may benefit any future research projects you undertake yourself. You will also be shown how different types of equipment work.

10) What if there is a problem?

If you have a concern about any aspect of this study you should contact the Principal Investigator (email: m.riach@mmu.ac.uk, tel: 0161 247 5086) who will do their best to answer any questions. If you remain unhappy and wish to complain formally, then please contact the researchers university through the address provided below:

Registrar & Clerk to the Board of Governors

Head of Governance and Secretariat Team

Manchester Metropolitan University,

All Saints Building, All Saints,

Manchester, M15 6BH

Tel: 0161 247 1390.

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

11) Will my taking part in the study be kept confidential?

All of the information collected will be kept confidential and used only for the purposes of this study and any associated publications or conference presentations. The data will be stored in a coded format, participant names and codes will not be kept in the same location and the storage system will be password protected. Only the Principal Investigator and named members of the research team will have access to the data. The data will be kept for the duration of the study and for any necessary period associated with resulting publications.

If the results of the study are used in conferences or published in scientific journals at some point in the future, participants will not be identified in any way. As a participant you have the right to request a copy of any publication resulting from the research.

12) What will happen if I don't carry on with the study?

If you withdraw from the study all the information and data collected from you, to date, will be destroyed and your name will be removed from all the study files.

13) What will happen to the results of the research study?

Once the study has ended, we aim to publish and present the results from this study in scientific journals and conferences. Should this happen, you will not be identified in any way. As a participant, you have the right to obtain a copy of any publication resulting from the research.

14) Who is organising or sponsoring the research?

The research is being funded by the Research Centre for Health, Exercise and Active Living at Manchester Metropolitan University.

15) Further information and contact details

If you would like general or specific information about the research project the Principal Investigator is Martin Riach. Please do not hesitate to email him on m.riach@mmu.ac.uk or telephone on 0161 247 5086. If you are unhappy with the principal investigator or would like to make a complaint about the research then please contact the address below:

Registrar & Clerk to the Board of Governors

Head of Governance and Secretariat Team

Manchester Metropolitan University,

All Saints Building, All Saints,

Manchester, M15 6BH

Tel: 0161 247 1390.

The other members of the research team are Dr David Wright (e-mail: d.j.wright@mmu.ac.uk, telephone: 0161 247 5534), Prof Paul Holmes (p.s.holmes@mmu.ac.uk) and Dr Zoe Franklin (z.franklin@mmu.ac.uk) from the Department of Exercise and Sport Science at MMU Cheshire.

16) Finally, a thank you!

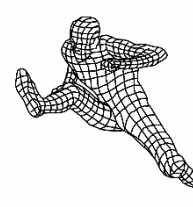
Thank you very much for considering participating in this study. I hope that the information I have provided is in enough detail for you. If you have any questions before you agree to participate, please do not hesitate to ask.

Appendix 4. Informed consent forms

Appendix 4.1. Informed consent form for the pilot experiment

Manchester Metropolitan University — Cheshire —

Department of Exercise and Sport Science



Informed Consent Form

Name of Participant:

Principal Investigator: Mr Martin Riach (m.riach@mmu.ac.uk)

Supervisor: Dr David Wright (d.j.wright@mmu.ac.uk)

Project Title: An exploration of different brain activation mechanisms during action observation: A pilot study.

Ethics Committee Approval Number: 27.11.15(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 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.

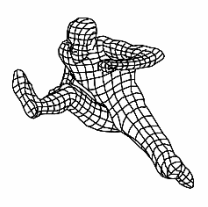
Signed (Participant)		Date	
Signed (Investigator)		Date	

cc

Appendix 4.2. Informed consent form for Experiment 1

Manchester Metropolitan University — Cheshire —

Department of Exercise and Sport Science



Informed Consent Form

Name of Participant:

Principal Investigator: Mr Martin Riach (m.riach@mmu.ac.uk)

Supervisor: Dr David Wright (d.j.wright@mmu.ac.uk)

Project Title: An investigation into the effects of viewing angle on cortical activity during action observation

Ethics Committee Approval Number: 30.09.14(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 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.

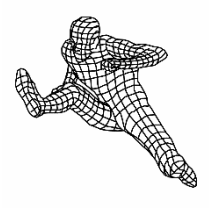
Signed (Participant)		Date	
Signed (Investigator)		Date	

dd

Appendix 4.3. Informed consent form for Experiment 2

Manchester Metropolitan University — Cheshire —

Department of Exercise and Sport Science



Informed Consent Form

Name of Participant:

Principal Investigator: Mr Martin Riach (m.riach@mmu.ac.uk)

Supervisor: Dr David Wright (d.j.wright@mmu.ac.uk)

Project Title: The effect of background context on brain activity during action observation.

Ethics Committee Approval Number: MR-16-01

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 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	

ɛd consent form for Experiment 3

1st September 2017
Martin Riach
Department of Exercise and Sports Science
Seeley 1-14
Manchester Metropolitan University
Tel: 01612475086

Manchester Metropolitan University

:onsent Form

Name of Researcher:

Participant Identification Code for this project:

	Please initial box
1. I confirm that I have read and understood the information sheet dated 1st September 2017 for the above project and have had the opportunity to ask questions about the interview procedure.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason to the named researcher.	☐
3. I understand that my responses will be sound recorded and used for analysis for this research project.	☐
4. I give/do not give permission for my interview recording to be archived as part of this research project, making it available to future researchers.	☐
5. I understand that my responses will remain anonymous.	☐
6. I agree to take part in the above research project.	☐
7. I understand that at my request a transcript of my interview can be made available to me.	☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

To be signed and dated in presence of the participant

Once this has been signed, you will receive a copy of your signed and dated consent form and information sheet by post.

Appendix 5. Discarded MEP data

Appendix 5.1. MEP data discarded from the pilot experiment

	Number of data removed	Percentage of data removed
FDI	15	2.50%
ADM	4	0.67%
Both	19	3.17%

Appendix 5.2. MEP data discarded from Experiment 1

	Number of data removed	Percentage of data removed
FDI	122	4.24%
ADM	167	5.80%
Both	289	5.02%

Appendix 5.3. MEP data discarded from Experiment 2

	Number of data removed	Percentage of data removed
FDI	99	3.22%
ADM	242	7.88%
Both	341	5.55%

Appendix 5.4. MEP data discarded from Experiment 3

	Number of data removed	Percentage of data removed
FDI	43	1.40%
ADM	199	6.48%
Both	242	3.94%

Appendix 5.5. Total MEP data discarded across all experiments

	Number of data removed	Percentage of data removed
Pilot experiment	19	3.17%
Experiment 1	122	4.24%
Experiment 2	341	5.55%
Experiment 3	242	3.94%
Total	724	4.59%

Appendix 6. Interview guide used in Experiment 1

What were your opinions of the two different screen angles that you saw in the experiment?

- Did you experience any differences as a consequence of viewing the ball pinch on differently angled screens?
- Can you describe these differences?
- Did you experience any sensations whilst viewing the ball pinch?
- If so, were these different when viewing on different screens?

Did the hand on two screens seem like your hands or someone else's?

- If it felt like your own hand, did it feel more like your own with one screen angle more than the other?
- If it felt like someone else's hand, why was that? Can you explain what you mean?
- Was this feeling stronger on one of the screens more than the other?
- If their own - Did it feel like it was you performing the movement?
    - Can you describe the feeling?
    - If so, was this feeling different whilst observing videos on each screen angle?

When looking at either screen angle, did you feel that you had to mentally rotate the image?

- If so, did one screen angle require greater or less of the mental rotation to better feel the ball pinch movement?

Were you aware of watching the ball pinch differently on the two screens?

- Can you describe these differences?

What physical and emotional sensations where you aware of whilst watching the ball pinches?

- E.g. if no responses – tingling in hand, urge to move your own hand, feeling that you were performing the movement?
- Present during one angle more than the other, or about the same?

Did you feel like the ball was in your hand on either screen?

- If yes, could you almost "touch and pinch" the ball as if it was in your own hand
- Was this present during one angle more than the other?

Did you notice any differences in the size of the muscle contractions in your hand?

- If different, describe contraction during each screen angle/video type

Appendix 7. Questionnaire used in Experiment 1

How strongly did you feel that the hand you were watching was your own?

Horizontal screen angle:

Not at all like my own					Strongly like my own
1	2	3	4	5	6

Vertical screen angle:

Not at all like my own					Strongly like my own
1	2	3	4	5	6

How strong was the feeling that you were performing the movement?

Horizontal screen angle:

No feeling at all					Very strong feeling
1	2	3	4	5	6

Vertical screen angle:

No feeling at all					Very strong feeling
1	2	3	4	5	6

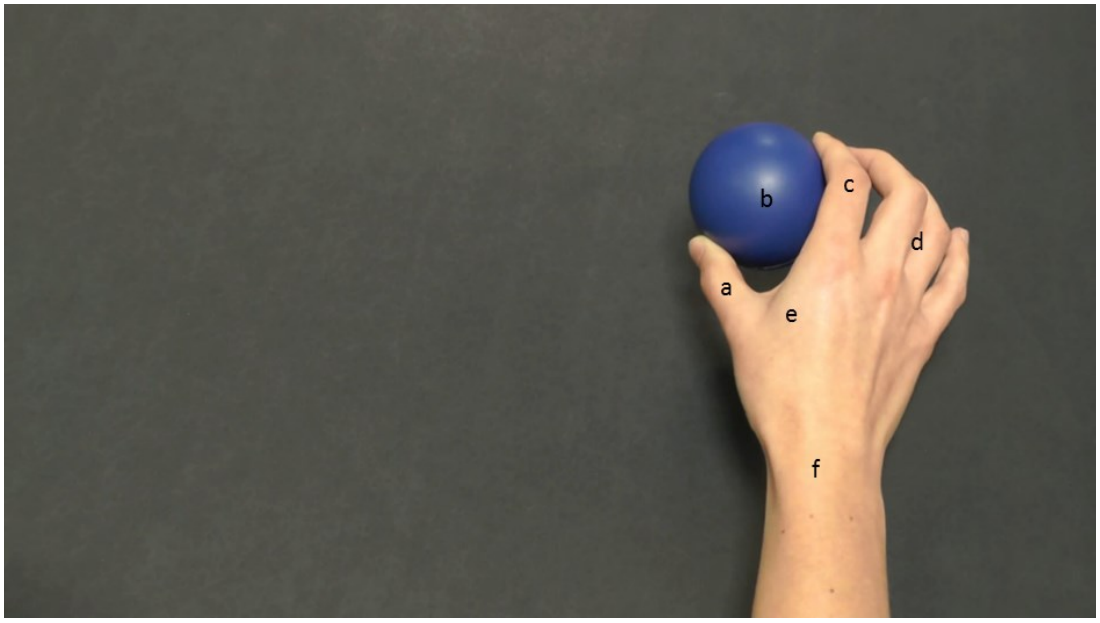
How often did you feel the need to mentally rotate the image on the screen?

Horizontal screen angle:

Not at all					Every video
1	2	3	4	5	6

Vertical screen angle:

Not at all					Every video
1	2	3	4	5	6



Which parts of the video do you think you watched the most during the video?

Horizontal screen angle:

( ) a. Thumb

( ) b. Ball

( ) c. Index finger

( ) d. Other fingers

( ) e. Muscle

( ) f. Wrist

( ) None of the above

Vertical screen angle:

( ) a. Thumb

( ) b. Ball

( ) c. Index finger

( ) d. Other fingers

( ) e. Muscle

( ) f. Wrist

( ) None of the above

Were there any other areas of the video that you watched? (tick more than one if applicable)

Horizontal screen angle:

( ) a. Thumb

( ) b. Ball

( ) c. Index finger

( ) d. Other fingers

( ) e. Muscle

( ) f. Wrist

( ) None of the above

Vertical screen angle:

( ) a. Thumb

( ) b. Ball

( ) c. Index finger

( ) d. Other fingers

( ) e. Muscle

( ) f. Wrist

( ) None of the above

How strong did you feel the muscle contractions were during each screen angle?

Horizontal screen angle:

No feeling at all Very strong feeling

1 2 3 4 5 6

Vertical screen angle:

No feeling at all Very strong feeling

1 2 3 4 5 6

Which screen angle did you prefer watching the ball pinch on?

Vertical No preference Horizontal

3 2 1 0 1 2 3

Did you perceive the hand to be:

( ) Male

( ) Female

( ) Unsure

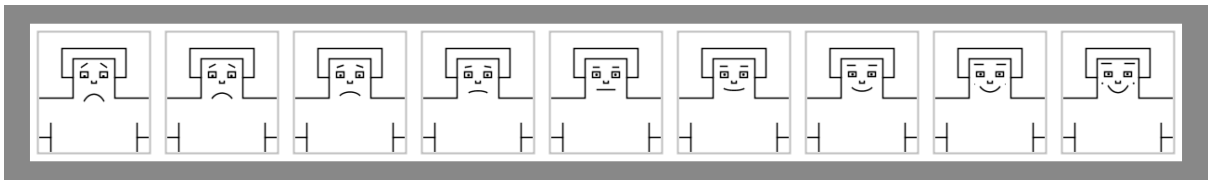
Appendix 8. Self-assessment manikin valence scale (Lang, 1980; Suk, 2006)

Name:_____

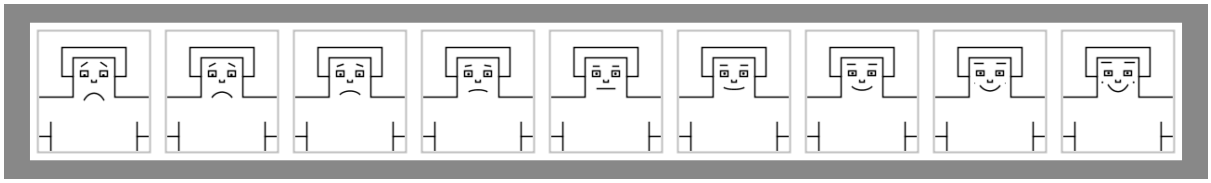
Date:_____

Please write your three most preferred food items in the spaces provided, and indicate the extent of your preference for that food item by putting an X on the scale provided underneath.

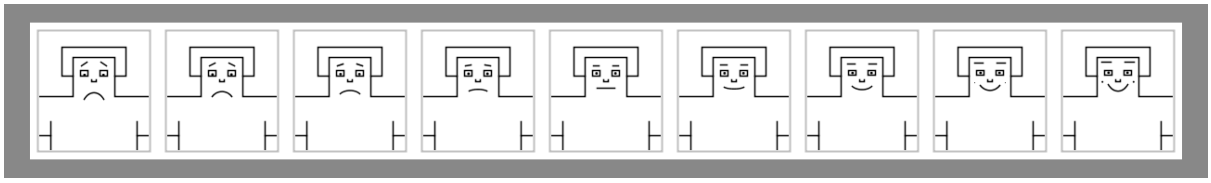
Most preferred food item 1:_____



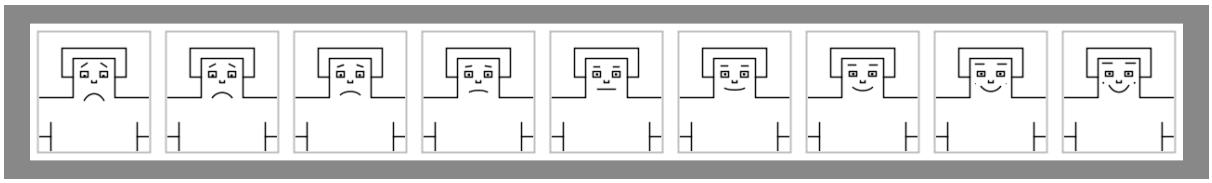
Most preferred food item 2:_____



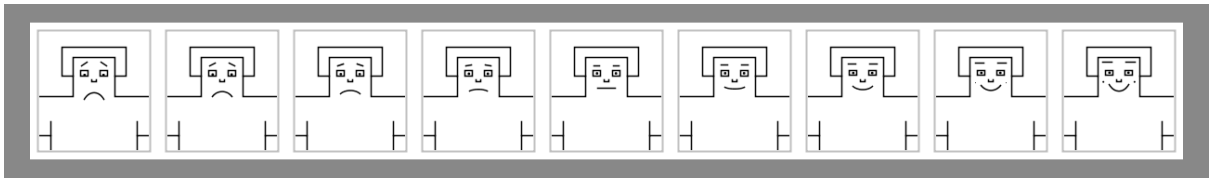
Most preferred food item 3:_____



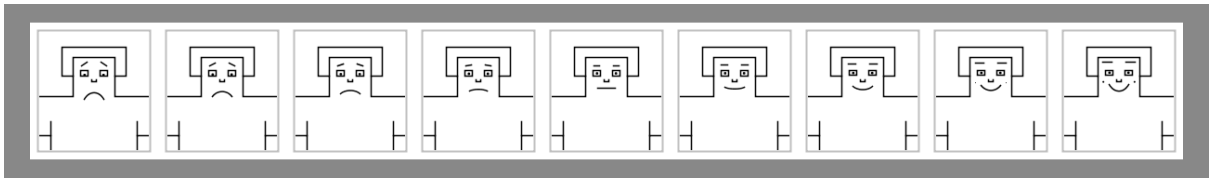
Neutral preference food item 1:_____



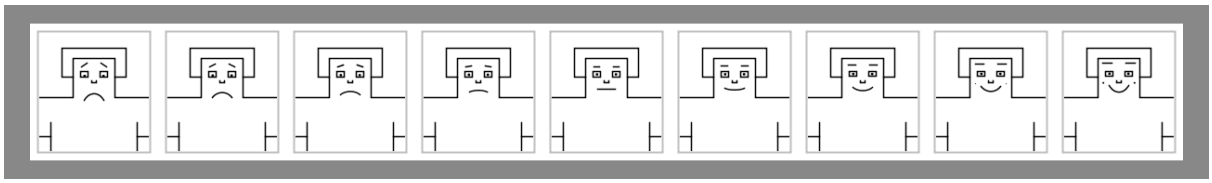
Neutral preference food item 2:_____



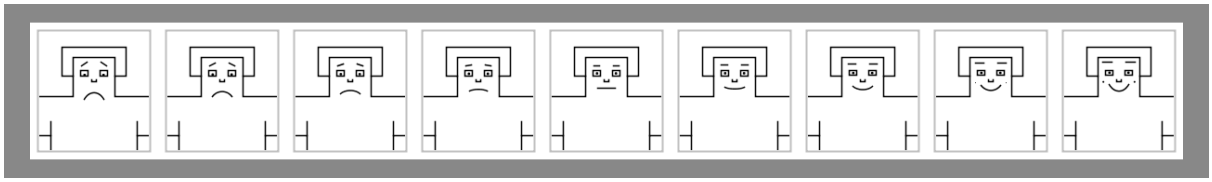
Neutral preference food item 3:_____



Least preferred food item 1:_____



Least preferred food item 2:_____



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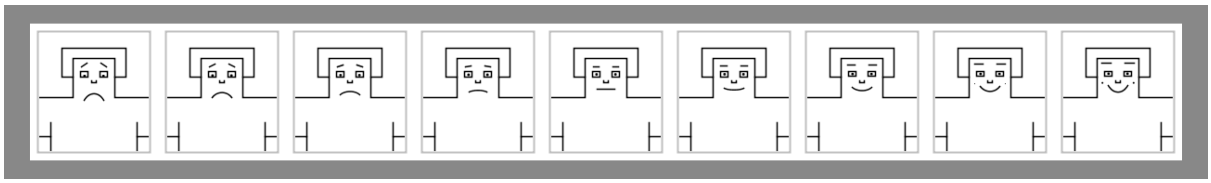
Least preferred food item 3:_____



Appendix 9. Food items used in Experiment 3

Table A9.1. The food items observed by each participant for the least preferred, neutral preference, and most preferred conditions

	Least preferred	Neutral preference	Most preferred
PP1	Kidney	Crisp	Calamari
PP2	Mushroom	Pasta	Chocolate
PP3	Cauliflower	Sausage Roll	Gammon
PP4	Sprout	Bacon	Cheese
PP5	Olive	Chip	Cake
PP6	Sprout	Mushroom	Custard cream
PP7	Mushroom	Pasta	Cake
PP8	Ginger	Egg	Pasta
PP9	Olive	Crisp	Egg
PP10	Pear	Cheese	Chip
PP11	Cabbage	Chip	Sweet
PP12	Sushi	Carrot	Cheese
PP13	Sprout	Quiche	Pizza
PP14	Cauliflower	Carrot	Pizza
PP15	Mushroom	Chip	Pizza
PP16	Cabbage	Chip	Beef jerky
PP17	Cheese	Crisp	Garlic bread
PP18	Kidney	Bread	Chorizo
PP19	Celery	Cabbage	Chocolate
PP20	Sprout	Chocolate	Pizza
PP21	Celery	Carrot	Sweet potato chip
PP22	Celery	Crisp	Chocolate

PP23	Mushroom	Sprout	Steak
PP24	Beetroot	Bread	Chip

Table A9.2. The frequency of food items observed by the participants for the least preferred, neutral preference, and most preferred conditions

	Total	Least preferred	Neutral preference	Most preferred
Bacon	1		1	
Beef jerky	1			1
Beetroot	1	1		
Bread	2		2	
Cabbage	3	2	1	
Cake	2			2
Calamari	1			1
Carrot	3		3	
Cauliflower	2	2		
Celery	3	3		
Cheese	4	1	1	2
Chip	6		4	2
Chocolate	4		1	3
Chorizo	1			1
Crisp	4		4	
Custard cream	1			1
Egg	2		1	1
Gammon	1			1
Garlic bread	1			1
Ginger	1	1		

Kidney	2	2		
Mushroom	5	4	1	
Olive	2	2		
Pasta	3		2	1
Pear	1	1		
Pizza	4			4
Quiche	1		1	
Sausage roll	1		1	
Sprout	5	4	1	
Steak	1			1
Sushi	1	1		
Sweet	1			1
Sweet potato chip	1			1

Appendix 10. Interview guide used in Experiment 3

What were your opinions of the four different videos that you saw in the experiment?

- Did you experience any differences as a consequence of viewing the action with different objects?
- Can you describe the differences?
- Where you more engaged when watching some of the videos more than others? If yes, which videos did you feel more engaged in watching?

Why did you select the XXX as your most preferred item?

- Can you describe what you thought about when you observed the hand grasping this item?
- Did you experience any particular feelings, emotions or sensations when you observed the hand grasping them? If so, can you describe these?

Why did you select the XXX as your least preferred?

- Can you describe what you thought about when you observed the hand grasping this item?
- Did you experience any particular feelings, emotions or sensations when you observed the hand grasping them? If so, can you describe these?

Were you aware of watching the action differently with the different objects and static hand video?

- Can you describe the differences?
- Prompt – looking in the same place during each video?
- Were you more attentive during certain videos? If so, which were you most or least attentive in? Why do you think you were more attentive in some videos compared to others?

What physical and emotional sensations were you aware of whilst watching the different videos?

- E.g. if no responses – tingling in hand, urge to move your own hand, feeling that you were performing the movement, wanting to pull away?
- Present during one video more than others, or about the same?

Did you feel like the hand you were observing was your own?

- If yes, why? Was this more on certain videos?
- If no, why not?

Did you notice any differences in the size of the muscle contractions in your hand?

- If different, describe contraction during each video