Action Complexity Modulates Corticospinal Excitability During Action Observation

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary educational institution

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Abstract

Observing an action engages the same brain regions as executing an action. Activity in the motor areas of the brain can be measured indirectly using non-invasive techniques, such as single-pulse transcranial magnetic stimulation (TMS). The current study aimed to investigate the time-course of motor cortex activity during action observation of a simple and complex action. In Experiment 1 single-pulse TMS was used to investigate whether the time-course, and magnitude of change in motor cortex excitability was mediated by complexity of an observed action. Measures of motor cortex excitability acting on two intrinsic hand muscles and two forearm muscles were obtained during observation of a simple and a complex action. Experiment 1 found excitability in an intrinsic hand muscle was greater during observation of a complex action compared to a simple action, at the time-point at which the actions were kinematically different. Paired-pulse TMS can be used to measure excitability of motor cortex inhibitory processes. In Experiment 2, single and paired-pulse TMS was used to determine whether greater excitability during observation of the complex action compared to the simple action was associated with reduced motor cortical inhibition. Experiment 2 showed no significant difference in motor cortical inhibition between the complex and simple action. Findings suggest action complexity modulates changes in motor cortex excitability during action observation.

Action Complexity Modulates Corticospinal Excitability During Action Observation

Successful interaction with our environment requires a capacity to execute actions, and a capacity to understand others actions. During action observation we make sense of, and understand the intentions of others' actions (Rizzolatti & Craighero, 2004). From an evolutionary perspective, mirror neurons have an important function in recognising and understanding the actions of members of our own species (Rizzolatti & Craighero, 2004; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). The ability to understand actions, and their goals, allows for an action to be imitated, which in turn facilitates learning of motor skills (Rizzolatti & Craighero, 2004), and re-learning motor skills after a stroke (Ertelt et al., 2007). Due to brain injury after a stroke, it can become difficult to perform actions associated with daily tasks, which were once automatic and unconscious processes. This difficulty in re-learning motor skills is due to the complex nature of many actions, for example, drinking from a cup involves using the whole hand, reaching and grasping the cup, lifting the cup, and bringing the cup to the mouth to drink (Lee, Roh, Park, Lee, & Han, 2013). Action understanding involves engagement of the motor system, particularly the motor cortex which is the part of the brain that controls movement of the muscles in the body.

The mirror neuron system is the neural substrate which supports action understanding. (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). The mirror neuron system is engaged throughout action observation as it encodes contextual information for goal-directed actions (Rizzolatti & Craighero, 2004). Mirror neurons are a class of neurons which were originally discovered in the motor areas of the macaque monkey brain (di Pellegrino et al., 1992). This discovery of mirror neurons was made by recording from electrodes measuring single cell activity in the macaque monkey brain during action observation (di Pellegrino et al., 1992). Neurons were activated

when the monkey observed an action and when the monkey performed a similar action (di Pellegrino et al., 1992). The engagement of the mirror neuron system represents the activation of a motor representation that corresponds to the observed action – this forms the basis for understanding motor actions (Rizzolatti et al., 1996). Growing evidence suggests that this mirror neuron system in the macaque monkey also exists in humans. Functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) have been used to investigate the mirror neuron system in humans.

Using Functional Magnetic Resonance Imaging During Action Observation

FMRI shows changes in oxygenated blood flow during brain activity. The increase in blood flow is thought to reflect an increase in neural activity, therefore brain regions which show an increase in blood flow during action observation are thought to be involved in the task (de la Rosa, Schillinger, Bulthoff, Schultz, & Uludag, 2016). Studies using fMRI in humans have reliably shown the same brain regions are active during action observation, namely the inferior frontal gyrus, premotor cortex, and the inferior parietal lobe (Filimon, Nelson, Hagley & Sereno, 2007; Gatti et al., 2016). These brain regions form the fronto-parietal mirror neuron system, which is primarily associated with hand actions (Filimon et al., 2007). The brain regions active in fMRI studies during action observation are the human correlates of the brain areas active in the macaque monkey during single cell recordings; this is taken as evidence of a similar mirror neuron system in humans as the one found in monkeys (Chong, Cunnington, Williams, Kanwisher, & Mattingley, 2008).

Using fMRI, Filimon et al. (2007) identified cortical areas associated with observation of reaching and execution of reaching. During these two conditions the same brain regions became active, and were part of the fronto-parietal mirror neuron system (Filimon et al., 2007). As the same brain regions were active during different

hand movements, this suggest activation of the fronto-parietal mirror neuron system is specific to hand actions (Filimon et al., 2007). Additionally, Gatti et al. (2016) examined the existence and location of mirror neuron activity during observation and execution of motor tasks which differed in complexity. This included a simple action involving opening and closing of the four fingers to the hand, and a complex action involving opening of the four fingers and abducting/adducting the fingers (Gatti et al., 2016). Findings showed more brain regions were active during the performance of a complex task compared to the simple task (Gatti et al., 2016). Thus, recruitment of brain regions in the mirror neuron system is differentially modulated by action complexity. The fMRI literature is valuable in characterising the brain regions engaged during action observation, however this technique is limited as it cannot fully characterise the role of the motor cortex during action observation. As such, TMS can be used to target mirror neuron activity in the motor cortex.

Using Transcranial Magnetic Stimulation During Action Observation

TMS is a non-invasive, non-painful, brain stimulation technique used to stimulate the brain (Hallett, 2007). TMS stimulation is delivered through a wound coil held over the scalp; TMS emits an electric pulse which creates a magnetic field, and when applied to the scalp, creates electrical current flow in the underlying brain region (Hallett, 2007). When a single TMS pulse is applied to the motor cortex the underlying cells depolarise, creating action potentials (Hallett, 2007). These action potentials create a pathway of activity which causes a response in the contralateral target muscle. The muscle responses are recorded by electromyography (EMG) and are known as the motor evoked potential (MEP) (Hallett, 2007). The MEP is interpreted as a measure of corticospinal excitability (Rizzolatti & Craighero, 2004). The increase in MEP amplitude during observation of actions compared to an alternative condition is taken as

evidence of a mirror neuron response (Naish, Houston-Price, Bremner, & Holmes, 2014).

TMS has been used to investigate the engagement of the motor system in action observation. Fadiga, Fogassi, Pavesi, and Rizzolatti (1995) used TMS to investigate whether the mirror neuron system found in the macaque monkey was present in humans. The study recorded responses from hand and forearm muscles. TMS was applied during observation in the later phases of a goal-directed action and a non-goal directed action. Fadiga et al. (1995) found MEP amplitude was larger during observation of the goal-directed action compared to the non-goal directed action, and this response was specific to the hand muscle used to perform the action. The larger MEP amplitude is taken as an increase in corticospinal excitability, which indicates the engagement of the motor cortex during action observation. Fadiga et al. (1995) conclude that the increase in excitability of the motor cortex during action observation is evidence for the existence of the human mirror neuron system. The increase in corticospinal excitability during action observation has been replicated extensively (see review by Naish, Houston-Price, Bremner, and Holmes, 2014) with numerous hand action types (e.g., reaching and grasping, finger movement sequences, pinching). On this basis, TMS is a useful technique to investigate the engagement of the motor system in action observation.

A Model for Investigating Action Observation

A systematic review by Naish et al. (2014) formalises some of the gaps in the action observation literature by identifying these areas in which further research is required: timing of corticospinal excitability, muscle-specific responses during action observation, and the direction of activity (i.e., excitatory and inhibitory). Based on the research to date, Naish et al. (2014) proposed a model outlining how action observation

modulates corticospinal excitability. The model outlines an early general increase in corticospinal excitability, which occurs between 60-90ms after action onset, and then returns to baseline (Lepage, Tremblay, & Theoret, 2010). It is suggested that this early increase in corticospinal excitability is due to the motor system detecting biological movement (Naish et al., 2014). While this early increase in corticospinal excitability is general, there is a later increase from 200ms which is muscle-specific (Cavallo, Heyes, Becchio, Bird, & Catmur, 2014; Naish & Obhi, 2015). Muscle-specificity is the second component of the model. The interaction between timing and muscle-specificity is important, as a mirror neuron response is determined when the increase in corticospinal excitability is specific to the muscle being used to perform the action (Cavallo et al., 2014). As such, these first two components, timing and muscle-specificity, are tightly coupled.

The third core component of Naish et al.'s (2014) model refers to the direction of the MEP, that is, whether cortical excitatory or inhibitory processes are mediating changes in corticospinal excitability during action observation. Naish et al. (2014) suggest inhibitory processes may be active during action observation to prevent the overt imitation of the action. Although it is not well defined what role inhibitory processes play in action observation, Naish et al. (2014) propose the increase in corticospinal excitability could be mediated by a decrease in inhibition or an increase in excitation, or a combination of both.

Timing and Muscle-Specificity

It is important to investigate the timing of mirror neuron responses as it indicates the length of time it takes for an observed action to be understood (Cavallo et al., 2014). Furthermore, it is proposed that a 'mirror' response can only be determined when an increase in corticospinal excitability is specific to the muscle used in the observed

action (Cavallo et al., 2014). Timing and muscle-specific changes in corticospinal excitability have been investigated in both simple and complex actions.

Simple actions.

The earliest increase in corticospinal excitability has been found at 60-90ms after action onset (Lepage et al., 2010). Lepage et al. (2010) examined the timing and muscle-specificity of corticospinal excitability during observation of an index finger abduction/adduction and a static hand, while recording muscle activity from the first dorsal interosseous (FDI) and the abductor digiti minimi (ADM). At 60-90ms after action onset – when the index finger is abducted – the MEP increased in FDI and ADM. This is considered a general increase in corticospinal excitability as FDI is used to perform the action not ADM, yet excitability occurred in both muscles. Although the study examined time-points up to 270ms after action onset, no other significant time-points were found. This suggests there is a general non-muscle specific increase in corticospinal excitability between 60-90ms after action onset.

In contrast, other studies did not find this early general increase in corticospinal excitability. Cavallo et al. (2014) examined the timing of MEPs recorded from FDI and ADM while individuals observed an index finger and little finger abduction. Findings showed MEPs increased from 200ms after action onset, and this was specific to the muscle used to perform the action; MEPs increased in FDI during index finger abduction but not in ADM, which abducts the little finger (Cavallo et al., 2013). Although the study measured MEPs from 100ms after action onset there was no early modulation, which is in contrast to the findings from Lepage et al. (2010). Naish and Obhi (2015) replicated the study by Cavallo et al (2014) with an inclusion of a static hand as a baseline measure. This study found a muscle-specific increase in MEP

amplitude at 200ms and no increase in MEP amplitude at earlier time-points (i.e., 100ms), supporting the results from Cavallo et al. (2014).

Although these studies show the motor cortex is engaged during action observation of a simple action (i.e., finger abduction), the results are somewhat limited in terms of generalisability to complex actions due to the variation in length of the action. As action observation is important for successful interaction with our environment it is important to investigate changes in timing and muscle-specificity related to action complexity, in order to fully understand the neural mechanisms underlying action observation.

Complex actions.

One of the key pieces of evidence for the human mirror neuron system is the action-specific modulation of corticospinal excitability. Gangitano, Mottaghy, and Pascual-Leone (2001) tested whether corticospinal excitability was selective to the phases of the movement and the muscles used to perform the action, during observation of a hand reaching and grasping a ball. Findings showed MEP amplitude increased in FDI during observation of the action compared to baseline, at the time-point at which the thumb and index finger were at maximal aperture forming a grasp, thus when FDI is most engaged in performing the action (Gangitano et al., 2001). This increase in MEP amplitude was not found in the little finger muscle. Findings suggest corticospinal excitability is greater during key movement phases in the action, and is specific to the muscle used to perform the action. These findings were replicated in a later study (Gangitano, Mottaghy, & Pascual-Leone, 2004).

Additionally, Montagna, Cerri, Borroni, and Baldissera (2005) investigated timing of corticospinal excitability in the forearm and hand muscles during observation of a hand reaching and grasping a ball. Findings showed larger MEPs in the forearm

muscle – which is engaged in flexing the fingers - during the opening phase of the hand.

Also, MEPs were larger in FDI during the hand closing phase, when FDI is used to

close the index finger and thumb (Montagna et al., 2005). Although this research is

valuable in demonstrating the timing of corticospinal excitability is movement and

muscle-specific, there is a gap in the literature as the differences between simple and

complex actions in terms of timing, muscle-specificity and inhibition is not well

characterised.

Direction of Cortical Processes

Paired-pulse TMS can be used to measure inhibition within the motor cortex,

that is, intracortical inhibition (Kuijrai et al., 1993). The MEP elicited by paired-pulse

TMS is smaller than the MEP elicited by single-pulse TMS. The MEP suppression is

due to the activation of intracortical inhibitory circuits by the conditioning stimulus

(CS) (Hallett, 2007). In paired-pulse TMS the first pulse is a CS and the second pulse is

the test stimulus (TS). The CS is delivered at a subthreshold level which is not sufficient

to elicit an MEP. After a short interval between 1-3ms, the TS is delivered at a

suprathreshold level, which is sufficient to elicit an MEP (Hallet, 2007; Kuijrai et al.,

1993). This is termed short-interval intracortical inhibition (SICI). Paired-pulse TMS

has been used to investigate the role of SICI in action observation.

Strafella and Paus (2000) investigated changes in corticospinal excitability in the

motor cortex during action observation, and the inhibitory processes contributing to

motor cortex excitability. The study recorded MEPs from FDI and the bicep muscle

during observation of handwriting, and were compared to MEPs taken while

participants were at rest (baseline) (Strafella & Paus, 2000). The findings show that

corticospinal excitability increased in FDI and the bicep during observation of

handwriting, and SICI decreased in FDI and the bicep during observation of

handwriting compared to rest. The findings showed motor cortex excitability is specific to the muscle used to perform the action, inhibitory processes modulated during action observation contribute to motor cortex excitability, and during action observation there are both excitatory and inhibitory processes active (Strafella & Paus, 2000). Similary, Patuzzo, Fiaschi, and Manganotti (2003) found that during observation of hand movements there was a decrease in inhibition, and both excitatory and inhibitory circuits were active in the motor cortex. Due to the limited studies investigating SICI during observation of hand movements, the timing and role of SICI during action observation is not well characterised.

Current Study

The current study aimed to compare the difference in corticospinal excitability and inhibition, between a simple and a complex action, at specific time-points throughout action observation. Action complexity was determined by the number of muscles involved in the action, and the number of movements needed to execute the action. On this basis, a hand pointing at a ball was considered a simple action, and a hand grasping a ball was considered a complex action. By investigating the influence of action complexity on corticospinal excitability and SICI, the current study builds on the action observation model outlined previously (Naish et al., 2014). The current study aligns with Naish et al.'s (2014) recommendations that muscle activity should be recorded from more than one muscle, and more than one action should be investigated. The current study meets these recommendations by recording from four muscles (two intrinsic hand muscles and two forearm muscles) and including a simple and complex action in the experimental design.

The current study comprised two experiments. Experiment 1 investigated the timing of corticospinal excitability during observation of a simple action and complex

action. Hypothesis 1 states that MEP amplitude will be larger during observation of simple and complex actions compared to observation of a static hand. Hypothesis 2 states that MEP amplitude will be larger during observation of a complex action at the time-point in which the kinematics of the action are clearly differentiated from the simple action. In this context, kinematic refers to the motion of body parts used to perform the action (Magill, 2014). The variation in kinematics between the actions is referred to as kinematically different henceforth.

Based on the results from Experiment 1, Experiment 2 investigated whether the difference in the timing of corticospinal excitability between a simple and complex action was modulated by a decrease in SICI. It was hypothesised that a significant increase in corticospinal excitability during action observation in Experiment 1 would be associated with a decrease in SICI.

Method

Experiment 1

Participants

Twenty-seven participants were recruited via flyers posted at Murdoch University and in the community. Murdoch University psychology students received course credit for participating in the study, all non-psychology students received a $10 ColesMyer voucher for participating. The sample size was deemed appropriate based on the number of participants used in previous research of a similar nature (Cavallo et al., 2014; Gangitano et al, 2001). All participants were right hand dominant as measured by the Edinburgh Handedness Inventory (Oldfield, 1971) ($M = 83.20$, $SD = 17.83$), in which scores above 40 indicate right-hand dominance. All participants were free from any neurological condition and were not taking medication influencing brain activity or the central nervous system; this is in line with the international safety guidelines for the
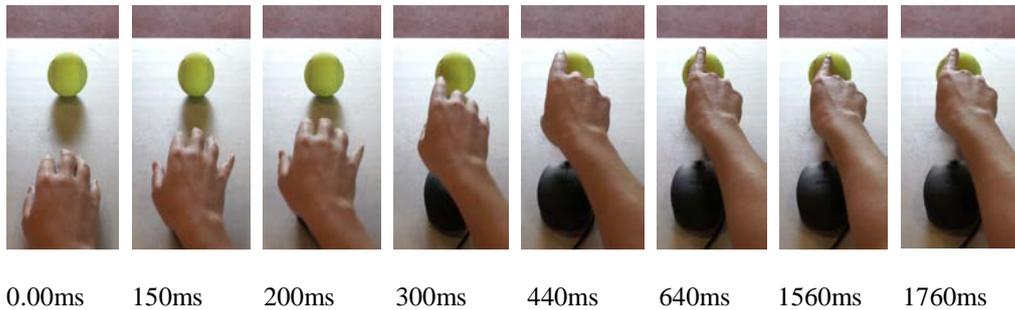
use of TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). Participants were screened for suitability to receive TMS by completing the TMS safety screen form (Appendix A). Data was collected in a single 2-hour experimental session in the Action and Cognition laboratory at Murdoch University. All participants were given an information letter (Appendix B), provided informed written consent (Appendix C), and completed the Edinburgh Handedness Inventory (Oldfield, 1971) (Appendix D). The study was approved by Murdoch University Ethics Committee 2014/247; a project summary is provided as an ethics requirement (Appendix E).

Materials

Action videos.

Participants watched three custom-made video clips – static hand, simple action and complex action - filmed from the observers' perspective (see Figure 1). Each video lasted 3000ms. The static hand video shows an image of the hand at rest in the same 'starting position' as the action videos ("static:"), the simple action video shows a hand pointing to a tennis ball ("point"), and the complex action video shows a hand grasping a tennis ball ("grasp"). These videos will be referred to as static, point, and grasp henceforth. All video clips were filmed in the Performance laboratory at Murdoch University at the same time of day to ensure the background of all videos was consistent. The timing of the point and grasp action videos were aligned in two ways. Firstly, in both videos the hand started to move at 360ms after the video started, this is referred to as action onset. Secondly, the kinematic movements for both actions occurred at the same time-points. This was a crucial step in the study design as it allowed the MEPs between both conditions to be compared. The static hand video was used as a baseline measure.

Point (simple action)

| 0.00ms | 150ms | 200ms | 300ms | 440ms | 640ms | 1560ms | 1760ms |

Grasp (complex action)

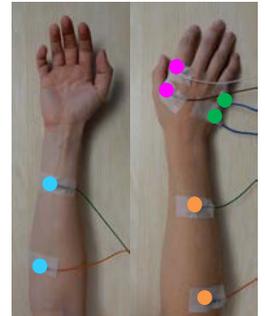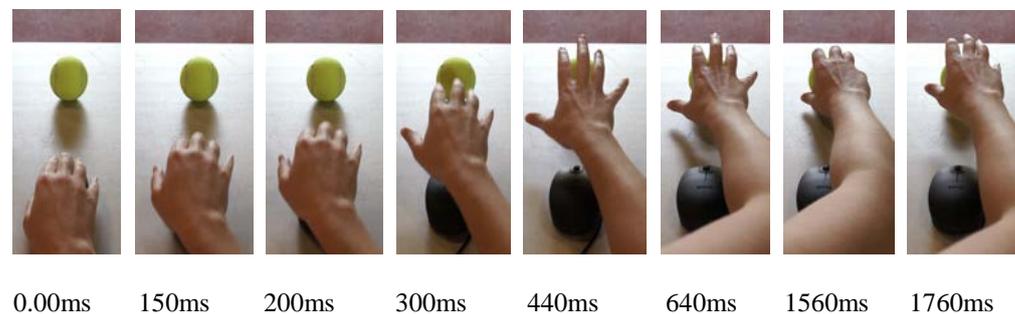| 0.00ms | 150ms | 200ms | 300ms | 440ms | 640ms | 1560ms | 1760ms |

Figure 1: The images show the action phases when TMS was delivered (except for 0.00ms) for the point (top panel) and the grasp (bottom panel). The time noted under the picture of the action reflects milliseconds (ms) after action onset. The image at 0.00ms shows the beginning of the video, this image was used as the "static" condition. The pictures on the far right side show where the electrodes were placed for the four target muscles, with one electrode on the muscle belly and one reference electrode; FDI (pink), ADM (green), FDS (blue), and EDC (orange).

Electromyography activity.

Participants were seated in a comfortable arm chair with their arms and hands resting on a pillow, enabling relaxation of the target muscles. MEPs were recorded from surface Ag-AgCI electrodes placed on the first dorsal interosseous (FDI), abductor digiti minimi (ADM), flexor digitorum superficialis (FDS), and extensor digitorum communis (EDC) of the right hand and forearm (see Figure 1 for electrode placement). The active electrodes were placed over the belly of each muscle (approximately the central part of the muscle), the reference electrode was placed over the tendon insertion point for the hand muscles and off the muscle for the forearm muscle, and a ground electrode placed on the bone (Zipp, 1982). Raw EMG was amplified (1000x), bandpass

filtered (10-1000Hz), digitised with 14-bit resolution sampling rate of 4kHz (Cambridge

Electronic Design, UK), and displayed on a computer screen through Signal software

program and stored for offline analysis.

Transcranial magnetic stimulation.

Single-pulse TMS was administered via two Magstim 200 stimulators (Magstim

Co., Whitland, Dyfed, UK) connected through a BiStim module to a figure-of-eight

coil. The coil was placed over the left hemisphere at a 45° angle tangentially to the mid-

line of the skull with the handle pointing backwards. The optimal site ("hot spot") for

TMS was identified as the position on the scalp that consistently elicited large MEPs.

The hot spot was identified by applying suprathreshold stimuli starting at the cortical

representation area for the hand and systematically moving the coil across the scalp in

an anterior-posterior, and dorsal-ventral direction. The hot spot for FDI was marked on

a secure cap on the participants' head enabling reliable re-placement of the coil. FDI

was the primary target muscle as this muscle is important for index finger movement,

and is the main muscle used to perform both actions. The resting motor threshold

(RMT) was defined as the minimum TMS intensity (% maximum stimulator output)

required to elicit an MEP with a peak-to-peak amplitude of at least 50μV in at least 5

out of 10 consecutive trials (Rossini et al., 1994). Single-pulse TMS stimuli were

delivered at an intensity of 120% of RMT. The two earliest time-points (150, 200ms)

were based on previous research examining simple actions, which found corticospinal

excitability increased from 200ms after action onset (Cavallo et al., 2014; Naish &

Obhi, 2015). The next five time-points (300, 440, 640, 1560, 1760ms) relate to

movement phases, in line with previous research investigating corticospinal excitability

in complex actions (i.e., reaching, grasping, pinching). At these time-points the actions

are kinematically different.

Procedure

All participants were seated 70cm from a computer monitor displaying the videos. Participants were instructed at the beginning of the experiment to relax the muscles in their right hand and forearm, and to pay attention to the videos. Each trial was monitored online for visible background muscle activity; in trials where the muscle activity was excessive, an instruction to relax the hand was given to the participant. The videos and TMS pulse were presented using PsychoPy (Pierce, 2007). The timing of TMS pulses was set relative to action onset, 360ms after video onset. The TMS pulse was delivered at one of these time-points from action onset: 150, 200, 300, 440, 640, 1560 and 1760ms (see Figure 1 for the movement phase at each time-point). The same time-points were used in the static hand. Each trial consisted of a fixation cross for 1000ms, video presentation for 3000ms, and a black screen for 4500ms (inter-trial interval (ITI)). The fixation cross triggered the EMG recording. The order of videos and TMS pulses was pseudo-randomised. Trials were delivered in blocks, with one block consisting of 42 trials – 14 trials for each static, point and grasp. In each block, the video plus specific time-point occurred twice. There were eight blocks of trials, and each block finished with a black screen displaying "End of block". There were 336 trials in total (112 trials each for static, point and grasp), equalling 16 trials for each video and time-point combination. In each block of trials, at three random points during an ITI, participants were asked: "what was the last action?". In this attention task responses were recorded to gauge participant's attention level.

Experiment 2

Participants

Thirteen participants were recruited for Experiment 2. Recruitment of participants, compensation for participating in the study, assessment of handedness, and

assessment of participants' suitability to receive TMS was consistent with Experiment 1. All participants were right hand dominant as measured by the Edinburgh Handedness Inventory (Oldfield, 1971) ($M = 89$, $SD = 13.79$). Data was collected in a single 2.5-hour experimental session in the Action and Cognition laboratory at Murdoch University. All participants were given an information letter (Appendix F), provided informed written consent, completed the Edinburgh Handedness Inventory (Oldfield, 1971), and completed the TMS safety screen from as per Experiment 1.

Materials

Electromyography activity and action videos.

EMG recording protocols were consistent with Experiment 1, with the exception that EMG was only recorded from FDI. Participants watched the same three custom-made videos used in Experiment 1.

Transcranial magnetic stimulation.

Single-pulse and paired-pulse TMS was delivered by the same machine used in Experiment 1. Determination and marking of the hot spot, and defining RMT, followed the same procedure as Experiment 1. Consistent with Experiment 1, single-pulse trials were delivered at 120% of RMT. As this experiment was investigating the modulation of SICI during action observation, a paired-pulse protocol was used. In the paired-pulse trials, the first TMS pulse was the CS, which was set at these sub-threshold intensities of RMT: 50%, 60%, 70% and 80%. The second TMS pulse was the TS, which was set at a suprathreshold intensity of 120% of RMT. The inter-stimulus interval (ISI) between the paired-pulses was 2ms.

Procedure

All participants were seated 70cm from the computer monitor displaying the videos. The instructions for relaxation of the hand muscle and online monitoring of

background muscle activity was consistent with Experiment 1. During the static video, a single or paired-pulse TMS stimuli was delivered at either 300ms or 640ms after action onset; these are the time-points in which the MEP was differentially modulated in the two action conditions (point, grasp) in Experiment 1. Each trial consisted of a fixation cross for 1000ms, video presentation for 3000ms, and a black screen for 9000ms (as the ITI). The ITI was longer in Experiment 2 due to the Magstim needing more time to charge between paired-pulse trials. The fixation cross triggered the EMG recording. The order of videos and TMS pulses was pseudo-randomised. Trials were delivered in blocks, with one block consisting of 30 trials - 10 trials for each static, point and grasp. In each block, the video plus time-point occurred once. Six blocks of trials were delivered twice, totalling 12 blocks of trials. Each block finished with a black screen displaying "End of block". There were 360 trials in total (120 trials each for static, point and grasp), equalling 10 trials for each video, time-point, and CS intensity combination. In each block of trials, at two random points during an ITI, participants were asked: "what was the last action?". In this attention task responses were recorded to gauge participant's attention level.

Data analysis: General

Data were analysed offline, EMG activity from each trial was visually inspected for background voluntary muscle activity. Trials were excluded if EMG activity in the 300ms prior to the TMS pulse exceeded 10 microvolts. Peak-to-peak MEP amplitude in millivolts (mV) was calculated from the EMG recording in the 10-50ms period following the TMS pulse. The Statistical Package for Social Sciences (SPSS) (version 23) was used to analyse the data. All data was analysed using a within-subjects design, and an alpha level of .05 was applied to determine significance. If Mauchly's test of sphericity assumption was violated, the Greenhouse-Geisser statistic was interpreted.

Although there were moderate violations of the Shapiro-Wilks statistic in a number of measures, the ANOVA is robust against normality violations (Field, 2012), thus allowing the interpretation of these parametric statistics. All graphs were created using GraphPad Prism 6.

Data analysis: Experiment 1

Sixteen MEP's were collected for each time-point per participant, with a minimum of 10 MEPs included in the analysis after excluding trials. Due to excessive background muscle activity, some trials were excluded. Overall, this equated to 5% of trials excluded in FDI, 7% in ADM, 7.8% in FDS, and 9.3% in EDC.

Hypothesis 1 aimed to determine whether MEP amplitude was increased in the action conditions compared to the static condition. The raw MEPs were used in a two-way repeated measures (RM) analysis of variance (ANOVA), with a factor of condition with three levels (static, point, grasp) and a factor of time with seven levels (150, 200, 300, 440, 640, 1560, 1760ms). Separate RM ANOVAs were conducted for FDI, ADM, FDS, and EDC.

In order to normalise the data for statistical analysis in hypothesis 2, it was important to ensure there was no significant differences between time-points in the static condition. As the static image does not change, it is not expected that there will be a difference across time. An RM ANOVA was conducted using the raw static condition data, with time as a single factor with seven levels. There was no difference between time-points for FDI ($F(6, 120) = 1.31$, $p = .258$, partial $\eta^2 = .06$), ADM ($F(6, 120) = .85$, $p = .535$, partial $\eta^2 = .04$), and FDS ($F(1.85, 37.09) = 1.24$, $p = .300$, partial $\eta^2 = .06$). As there was no difference in MEP amplitude in the static conditions across time, the average static MEP for each muscle was calculated. The average static MEP was used in normalising the data from the action condition for each participant; mean MEP

amplitude from each time-point in the action conditions was expressed as a ratio of the average MEP amplitude from the static condition. When conducting this analysis with the static EDC data, the RM ANOVA revealed a main effect of time ($F(6, 120) = 2.23$, $p = .044$, partial $\eta^2 = .10$). Therefore, it is not appropriate to take the average of all time-points in the static condition. As a result of this, the RM ANOVA was conducted again using the four time-points in which the actions were kinematically different; this was deemed appropriate on the basis that hypothesis 2 only relates to these time-points. The RM ANOVA conducted with time as a single factor with four levels (300, 440, 640, 1560, 1760ms) showed no main effect of time ($F(3, 60) = .57$, $p = .637$, partial $\eta^2 = .03$). As there was no difference in MEP amplitude in the static condition across these four time-points, the MEPs in the action conditions were normalised to the average of the static MEP across the four time-points.

   To determine whether the normalised MEP amplitude was different between the point and grasp actions at time-points in which the actions were kinematically different, separate two-way RM ANOVAs were conducted with the normalised data. The two-way RM ANOVA was conducted with action as a factor with two levels (point, grasp), and time as a factor with seven levels (150, 200, 300, 440, 640, 1560, 1760ms) for FDI, ADM and FDS. If there were any significant main effects or interactions, a two-way RM ANOVA was conducted with action as a factor with two levels (point, grasp) and time as a factor with four levels (300, 440, 640, 1560ms). As this was previously conducted for EDC there was no need to run this analysis again. These four time-points were selected for further analysis as they are directly related to the time-points in which the actions are kinematically different. The final time-point, 1760ms, was deemed similar to 1560ms therefore was not included in this analysis. Paired-samples t-tests

were conducted for post-hoc analysis following significant main effects and interactions.

Results: Experiment 1

Participants were recruited between June-August 2016. Of the 27 participants recruited for the study, two were excluded due to unforeseen technical errors with the TMS machine, and four were excluded due to excessive background EMG activity. Therefore, data from 21 participants (13 females, age range 18-37 years old, $M = 25.95$, $SD = 6.10$) were analysed. Participants achieved 98.4% accuracy on the attention task, consistent with previous research (Gangitano et al., 2004).

First dorsal interosseous (FDI)

A RM ANOVA was conducted to test whether raw MEP amplitude was greater in the action conditions compared to the static condition. This ANOVA showed no main effect of condition ($F(1.57, 31.40) = 1.23$, $p = .297$, partial $\eta^2 = .06$), no main effect of time ($F(3.60, 72.00) = 2.37$, $p = .067$, partial $\eta^2 = .11$), and no condition * time interaction ($F(5.90, 118.06) = 1.74$, $p = .119$, partial $\eta^2 = .08$).

Figure 2 shows normalised MEP amplitude for the point and grasp conditions across the seven time-points. A RM ANOVA was performed to test whether normalised MEP amplitude (MEP action/average MEP static) was greater during the grasp than the point. This ANOVA showed no main effect of action type ($F(1, 20) = .09$, $p = .770$, partial $\eta^2 = .004$) and no action * time interaction ($F(3.70, 73.97) = 2.10$, $p = .094$, partial $\eta^2 = .095$). However, there was a significant main effect of time ($F(3.71, 74.20) = 2.74$, $p = .038$, partial $\eta^2 = .12$). To further investigate the main effect of time, a second two-way RM ANOVA was conducted with the normalised MEPs from the four time-points in which the actions are kinematically different. This ANOVA showed no main effect of action ($F(1, 20) = .02$, $p = .88$, partial $\eta^2 = .001$) and no main

effect of time ($F(3, 60) = 1.21$, $p = .313$, partial $\eta^2 = .06$). However, there was an action * time interaction, ($F(3, 60) = 4.18$, $p = .009$, partial $\eta^2 = .17$).

To further investigate the interaction, paired samples t-test were conducted to determine whether normalised MEP amplitude was different between the two actions at the four key time-points (300, 440, 640, 1560ms). There was no significant difference in normalised MEP amplitude between the point and grasp at 440ms ($t(20) = 1.16$, $p = .257$, $d = .22$) and at 1560ms ($t(20) = .40$, $p = .694$, $d = .13$). However, at 300ms, the normalised MEP amplitude in FDI was significantly larger in the point than the grasp, ($t(20) = 2.27$, $p = .034$, $d = .35$). At 640ms the normalised MEP amplitude was significantly higher in the grasp than the point, ($t(20) = 2.68$, $p = .014$, $d = .66$).
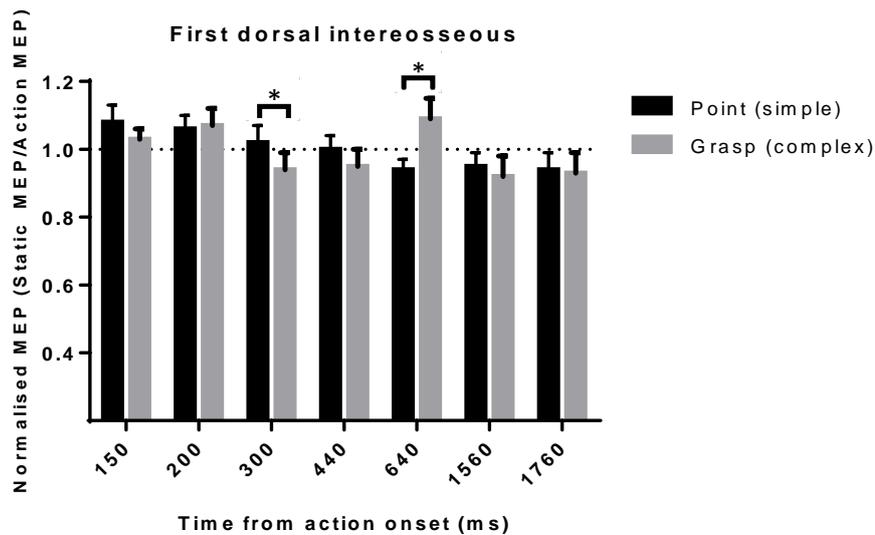

Figure 2: Normalised MEP amplitude for the point and grasp conditions across the seven time-points in FDI. The y-axis shows the action MEP amplitude normalised to the mean static MEP amplitude for all participants. The x-axis shows the time-points (from action onset) TMS pulses were delivered during observation of the videos. Error bars represent standard error of the mean (SEM). The dotted line at 1.0 represents the static condition; normalised MEPs less than 1.0 were lower in amplitude than the static (baseline) condition. * denotes significant difference between the action types at $p < 0.05$.

Abductor digiti minimi (ADM)

A RM ANOVA was conducted to test whether raw MEP amplitude was greater in the action conditions compared to the static condition. This ANOVA showed no main

effect of condition ($F(1.22, 24.32) = .67$, $p = .449$, partial $\eta^2 = .03$), no main effect of time ($F(6, 120) = 2.17$, $p = .05$, partial $\eta^2 = .10$), and no condition * time interaction ($F(12, 240) = 1.04$, $p = .417$, partial $\eta^2 = .05$).

Figure 3 shows normalised MEP amplitude for the point and grasp conditions across the seven time-points. A RM ANOVA was performed to test whether normalised MEP amplitude (MEP action/average MEP static) was greater during the grasp than the point. This ANOVA showed no main effect of action type ($F(1, 20) = .10$, $p = .754$, partial $\eta^2 = .005$) and no action * time interaction ($F(6, 120) = .78$, $p = .588$, partial $\eta^2 = .04$). However, there was a significant main effect of time ($F(6, 120) = 3.72$, $p = .002$, partial $\eta^2 = .16$). To further investigate the main effect of time, a second two-way RM ANOVA was conducted with the normalised MEPs for the four time-points in which the actions are kinematically different. This ANOVA showed no main effect of action ($F(1, 20) = .36$, $p = .553$, partial $\eta^2 = .02$), no main effect of time ($F(3, 60) = 2.70$, $p = .054$, partial $\eta^2 = .12$), and no action * time interaction ($F(3, 60) = .70$, $p = .556$, partial $\eta^2 = .03$).
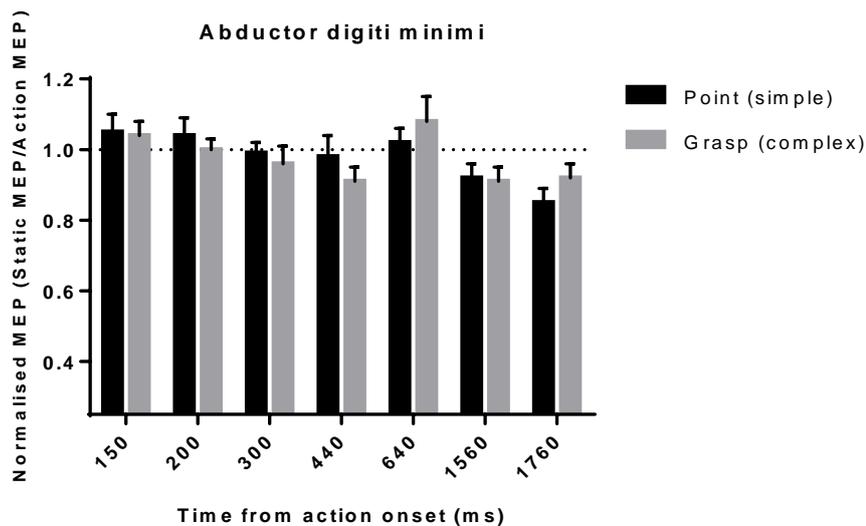

Figure 3: Normalised MEP amplitude for the point and grasp conditions across the seven time-points in ADM. The y-axis shows the action MEP amplitude normalised to the mean static MEP amplitude for all participants. The x-axis shows the time-points (from action onset) TMS pulses

were delivered during observation of the videos. Error bars represent standard error of the mean (SEM). The dotted line at 1.0 represents the static condition; normalised MEPs less than 1.0 were lower in amplitude than the static (baseline) condition.

Flexor digitorum superficialis (FDS)

A RM ANOVA was conducted to test whether raw MEP amplitude was greater in the action conditions than the static condition. This ANOVA showed no main effect of time ($F(1.75, 34.91) = 2.77$, $p = .083$, partial $\eta^2 = .12$) and no condition * time interaction ($F(3.75, 74.98) = .95$, $p = .438$, partial $\eta^2 = .05$). However, there was a main effect of condition ($F(1.39, 27.77) = 5.78$, $p = .015$, partial $\eta^2 = .22$). The main effect of condition was further investigated using pairwise comparisons, which show that the mean MEP in the static condition is significantly larger than the mean MEP in the grasp condition ($p < 0 .05$).

Figure 4 shows normalised MEP amplitude for the point and grasp conditions across the seven time-points. A RM ANOVA was performed to test whether normalised MEP amplitude (MEP action/average MEP static) was greater during the grasp than the point. This ANOVA showed no main effect of action type ($F(1, 20) = 3.82$, $p = .065$, partial $\eta^2 = .16$) and no action * time interaction ($F(3.88, 77.65) = .45$, $p = .770$, partial $\eta^2 = .02$). However, there was a significant main effect of time ($F(6, 120) = 3.72$, $p = .002$, partial $\eta^2 = .16$). To further investigate the main effect of time, a second two-way RM ANOVA was conducted with the normalised MEPs for the four time-points in which the actions are kinematically different. This ANOVA showed no main effect of action ($F(1, 20) = .28$, $p = .600$, partial $\eta^2 = .01$), no main effect of time ($F(3, 60) = 1.50$, $p = .234$, partial $\eta^2 = .07$), and no action * time interaction ($F(3, 60) = .38$, $p = .77$, partial $\eta^2 = .02$).
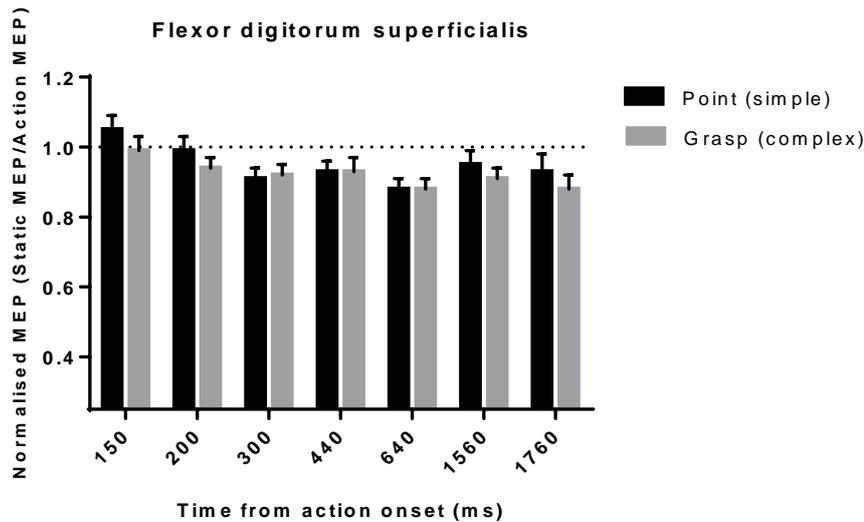
Figure 4: Normalised MEP amplitude for the point and grasp conditions across the seven time-points in FDS. The y-axis shows the action MEP amplitude normalised to the mean static MEP amplitude for all participants. The x-axis shows the time-points (from action onset) TMS pulses were delivered during observation of the videos. Error bars represent standard error of the mean (SEM). The dotted line at 1.0 represents the static condition; normalised MEPs less than 1.0 were lower in amplitude than the static (baseline) condition.

Extensor digitorum communis (EDC)

A RM ANOVA was conducted to test whether raw MEP amplitude was greater in the action conditions compared to the static condition. This ANOVA showed no main effect of condition ($F(1.53, 30.62) = 1.01$, $p = .358$, partial $\eta^2 = .05$) and no condition * time interaction ($F(4.63, 92.51) = 1.70$, $p = .149$, partial $\eta^2 = .08$). However, there was a main effect of time ($F(2.88, 57.53) = 5.31$, $p = .003$, partial $\eta^2 = .21$).

Figure 5 shows normalised MEP amplitude for the point and grasp conditions across the four time-points related to when the actions were kinematically different. A RM ANOVA was performed to test whether normalised MEP amplitude (MEP action/average MEP static across four time-points) was greater during the grasp than the point. This ANOVA showed no main effect of action type ($F(1, 20) = .58$, $p = .455$, partial $\eta^2 = .03$) and no action * time interaction ($F(3, 60) = 2.37$, $p = .080$, partial $\eta^2 = .11$). However, there was a significant main effect of time ($F(3, 60) = 2.98$, $p = .038$,

partial η^2 = .13). To further investigate the main effect of time, paired samples t-test

were conducted to determine whether normalised MEP amplitude was different between

the two actions at the four key time-points (300, 440, 640, 1560ms). There was no

significant difference in normalised MEP amplitude between the point and grasp at

300ms ($t(20)$ = -.87, p = .395, d = .30), 640ms ($t(20)$ = -.09, p = .93, d = 0), or 1560ms

($t(20)$ = .19, p = .849, d = .07). However, at 440ms normalised MEP amplitude was

significantly higher in the point than the grasp ($t(20)$ = 2.48, p = .022, d = .69).
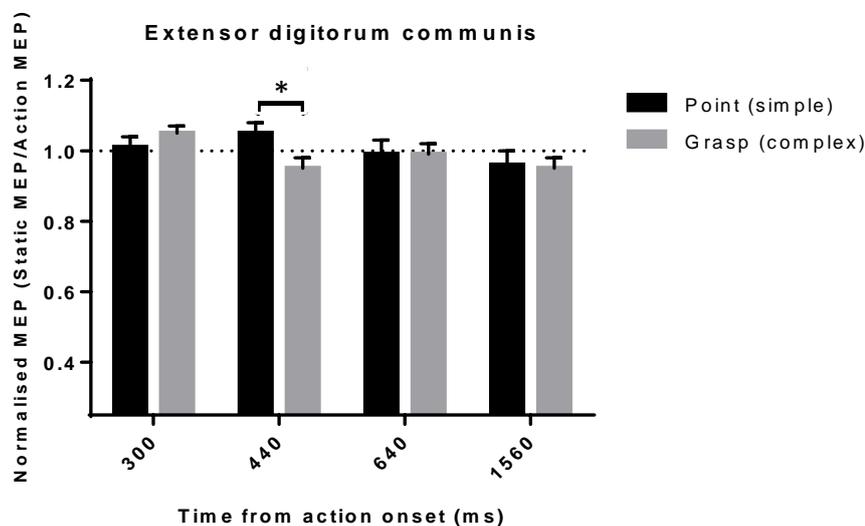

Figure 5: Normalised MEP amplitude for the point and grasp conditions across four time-points in EDC. The y-axis shows the action MEP amplitude normalised to the mean static MEP amplitude for all participants. The x-axis shows 4 of the time-points (from action onset) TMS pulses was delivered during observation of the videos. Error bars represent standard error of the mean (SEM). The dotted line at 1.0 represents static condition; normalised MEPs less than 1.0 were lower in amplitude than the static (baseline) condition. * denotes significant difference between the action types at p <0.05.

Data analysis: Experiment 2

Twelve MEPs were collected for each time-point per participant, with a

minimum of 10 MEPs included in the analysis after excluding trials. Due to excessive

background muscle activity, some trials were excluded. Overall, this equated to 4% of

trials being excluded.

Single-pulse MEP amplitude

In order to normalise the data for statistical analysis, the single-pulse MEP amplitude in the action conditions (point, grasp) were normalised to single-pulse MEP amplitude in the static condition for both time-points. Using the normalised data, paired samples t-tests were conducted separately for the two time-points of interest (300, 640ms) to examine whether there was a difference in MEP amplitude between actions at each time-point.

Short-Interval Intracortical Inhibition

SICI was quantified by expressing the mean MEP amplitude from the paired-pulse trials as a ratio of the mean MEP amplitude of the single-pulse trials. The ratio was calculated for each CS intensity. The SICI ratios were used in separate two-way RM ANOVAs to examine whether a decrease in SICI was mediating the change in MEP amplitude at 300ms and 640ms found in Experiment 1. Separate two-way RM ANOVAs were conducted for each time-point (300, 640ms), with a factor of condition with three levels (static, point, grasp) and a factor of CS intensity with four levels (50%, 60%, 70%, 80%).

Results: Experiment 2

Participants were recruited between June-August 2016. Of the 13 participants recruited for the study, one was excluded due to unforeseen technical errors with the TMS machine. Therefore, data from 12 participants (8 females, age range 20-39 years old, $M = 25.25$, $SD = 5.15$) were analysed. Participants achieved 100% accuracy on the attention task.

Single-pulse MEP amplitude

The descriptive statistics in Table 1 (see below) show the normalised mean MEP amplitude of the single-pulse trials in the point and grasp in Experiment 1 and

Experiment 2, at 300ms and 640ms. In Experiment 2, at 640ms the normalised mean

MEP amplitude in the grasp was 15% larger than the point. Paired-samples t-tests

showed no significant difference between the point and grasp at 640ms ($t(11) = -1.04$, p

$= .322$, $d = .42$). Although the difference in normalised mean MEP amplitude between

the grasp and the point at 640ms was not statistically significant, the numerical

difference was comparable to the results of Experiment 1, in which normalised mean

MEP amplitude in the grasp was 16% larger than the point.

In Experiment 2, at 300ms the normalised mean MEP amplitude in the point was

1% larger than the grasp. Paired-samples t-tests showed no significant difference

between the point and grasp at 300ms, ($t(11) = .09$, $p = .932$, $d = .03$). Unlike the results

at 640ms, at 300ms the difference in normalised mean MEP amplitude was not

comparable to the results of Experiment 1. The results at 300ms in Experiment 2 may

not have been replicated due to the smaller effect size ($d = .35$) found at this time-point

in Experiment 1.

Table 1

Normalised mean MEP amplitude of the single-pulse trials recorded from FDI in

Experiment 1 and Experiment 2

	300ms		640ms	
	Exp 1	Exp 2	Exp 1	Exp 2
Action	M (SEM)	M (SEM)	M (SEM)	M (SEM)
Point	1.02 (.05)	.89 (.08)	.94 (.03)	.93 (.08)
Grasp	.94 (.05)	.88 (.07)	1.09 (.06)	1.07 (.11)

Note: Exp = Experiment; M = Mean; SEM = Standard Error of the Mean

Short-Interval Intracortical Inhibition

Experiment 1 results showed greater MEP amplitude in the point compared to the grasp at 300ms, and greater MEP amplitude in the grasp compared to the point at 640ms. In Experiment 2, MEP amplitude and SICI was measured at 300ms and 640ms.

Figure 6 shows SICI ratios at each CS intensity for the static, point and grasp at 300ms. The figure shows SICI increases as the CS intensity increases, as expected. A RM ANOVA was performed to test whether the differences in MEP amplitude between the point and the grasp at 300ms in Experiment 1 was mediated by a change in SICI. This ANOVA showed that at 300ms, there was no main effect of condition ($F(2, 22) = .511$, $p = .607$, partial $\eta^2 = .04$) and no condition * CS intensity interaction ($F(3.40, 37.34) = .72$, $p = .563$, partial $\eta^2 = .06$). However, there was a main effect of CS intensity ($F(1.81, 19.89) = 21.86$, $p = <.001$, partial $\eta^2 = .67$). The linear trend for CS intensity showed no main effect of condition ($F(1, 11) = .66$, $p = .434$, partial $\eta^2 = .06$) and no condition * CS intensity interaction ($F(1, 11) = .05$, $p = .824$, partial $\eta^2 = .005$). However, there was a main effect for CS intensity ($F(1, 11) = 28.64$, $p = <.001$, partial $\eta^2 = .72$).
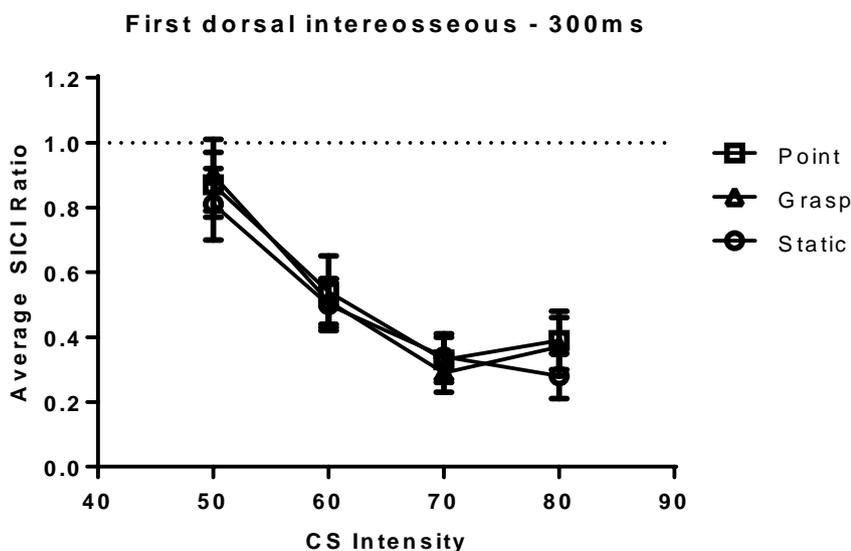
Figure 6: Modulation of SICI present in the point, grasp, and static conditions at 300ms after action onset. The y-axis shows the static and action (point and grasp) conditions MEP amplitude normalised to the mean MEP amplitude of the single-pulse TMS trials. The x-axis shows the conditioning stimulus (CS) intensity of the TMS pulse delivered prior to the test stimulus (TS) (120% of RMT). Values below 1.0 represent inhibition. Error bars represent Standard Error of the Mean (SEM).

Figure 7 shows SICI ratios at each CS intensity for the static, point and grasp at 640ms. The figure shows SICI increases as the CS intensity increases, as expected. A RM ANOVA was performed to test whether differences in MEP amplitude between the point and the grasp at 640ms in Experiment 1 was mediated by a change in SICI. This ANOVA showed that at 640ms there was no main effect of condition ($F(2, 22) = 3.12$, $p = .064$, partial $\eta^2 = .22$) and no condition * CS intensity interaction ($F(2.22, 24.44) = .92$, $p = .421$, partial $\eta^2 = .08$). However, there was a main effect of CS intensity ($F(1.53, 16.81) = 21.41$, $p = <.001$, partial $\eta^2 = .66$). The linear trend for CS intensity showed no main effect of condition ($F(1, 11) = 3.20$, $p = .101$, partial $\eta^2 = .23$) and no condition * CS intensity interaction ($F(1, 11) = 2.35$, $p = .154$, partial $\eta^2 = .18$). However, there was a main effect of CS intensity ($F(1, 11) = 25.06$, $p = <.001$, partial $\eta^2 = .70$). Although SICI was numerically greater in the grasp than the point, this difference was not statistically significant.
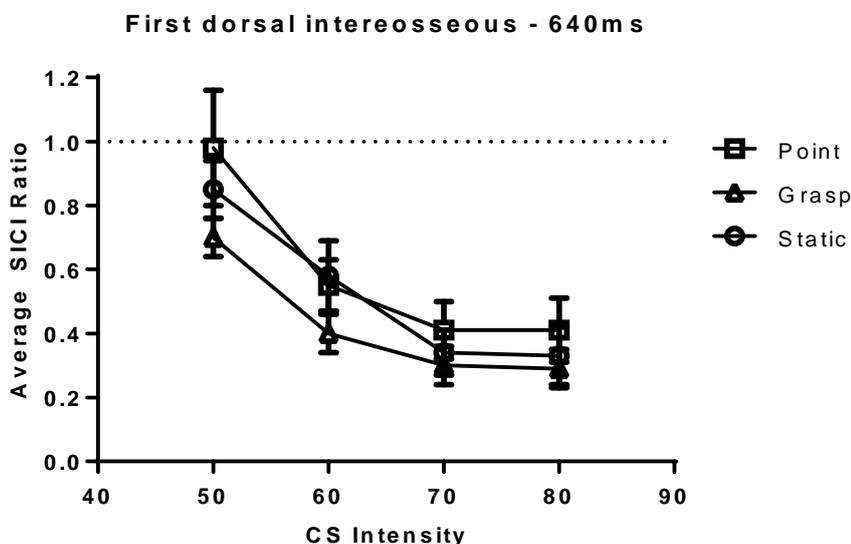
Figure 7: Modulation of SICI present in the point, grasp, and static conditions at 640ms after action onset. The y-axis shows the static and action (point and grasp) conditions MEP amplitude normalised to the mean MEP amplitude of the single-pulse TMS trials. The x-axis shows the conditioning stimulus (CS) intensity of the TMS pulse delivered prior to the test stimulus (TS) (120% of RMT). Values below 1.0 represent inhibition. Error bars represent Standard Error of the Mean (SEM).

Discussion

The primary aim of the current study was to systematically examine the effects of action complexity on corticospinal excitability and SICI during action observation. The key findings from of this study are; corticospinal excitability is differentially modulated by the action observed and corticospinal excitability is specific to the muscles used to perform the action at particular movement phases. Together, corticospinal excitability is affected by action complexity in a time-specific, and muscle-specific, manner.

Corticospinal Excitability Changes During Action Observation

The first hypothesis in Experiment 1 predicted MEP amplitude would be greater in the action conditions compared to a static image of a hand. Results from Experiment 1 showed no difference in MEP amplitude in any of the four target muscles during observation of the actions compared to the static condition. This suggests that corticospinal excitability was not significantly modulated during observation of a moving hand compared to a static hand. Therefore, hypothesis 1 was not supported. Although this finding is in contrast to previous research, which showed MEP amplitude increased during observation of a reach and grasp action compared to baseline (Gangitano et al., 2001, 2004; Hardwick et al., 2012; Strafella & Paus, 2000), it is important to note that in these studies the baseline measures were conducted while participants observed a fixation cross, observed a black screen, and were at rest. This is a key difference in the methodological design of the current study as the baseline measure was an image of a static hand, which looked the same as the beginning of the

action videos. It has been suggested that baseline measures during action observation should be a biological image (i.e., hand at rest), as it allows the findings to be interpreted as a direct result of the action observed rather than due to general observation of a hand (Naish & Obhi, 2015). In this way, the similarity in excitability between static and action conditions in the current results may be due to the static image used as a baseline measure. This supports findings from Loporto, McAllister, Edwards, Wright, and Holmes (2012) who found that facilitation of MEPs was similar across observation of the pinching action and observation of the static hand image. Although in the current study there was no significant difference between MEP amplitude in the action conditions compared to the static condition, the MEP amplitude was systematically modulated by action kinematics.

Timing and Muscle-Specific Changes in Corticospinal Excitability

In Experiment 1, hypothesis 2 predicted MEP amplitude would be larger during observation of the complex action at the time-point in which the action was kinematically different to the simple action. Results from Experiment 1 show that the modulation of corticospinal excitability during action observation is time-dependent and related to the complexity of the action.

At 640ms, MEP amplitude in FDI was greater during the grasp than the point. At this time-point the actions are distinctly kinematically different and the goal of the action is clearly identifiable, thus hypothesis 2 is supported. As this finding had a medium-large effect size (Cohen's $d = .66$) (Cohen, 1992) and was replicated in Experiment 2, it is likely that this is a robust finding. The medium-large effect size indicates the grasp action moderately influenced corticospinal excitability. This finding is considered muscle-specific as FDI is most engaged in the action at this time-point. This finding supports previous research in complex actions (e.g., reach and grasp)

which found corticospinal excitability increased in FDI when the hand was open to grasp a ball (Gangitano et al., 2004; Montagna et al., 2005).

In addition to supporting hypothesis 2, the current study found interesting results related to muscle-specific increases in corticospinal excitability. At 300ms, MEP amplitude in FDI was greater during the point than the grasp. This time-point is when the index finger starts to extend and the goal of the point action is first detected. This result suggests an increase in corticospinal excitability in FDI at the time-point at which it first appears the muscle is engaged in the action. This finding supports previous research which found corticospinal excitability increased from 200ms after action onset in simple actions (Cavallo et al., 2014; Naish & Obhi, 2015). However, it is important to note that this finding was not replicated in Experiment 2. This could be due to the small sample (N = 12); however, the effect size in Experiment 1 was small-medium (Cohen's d = .35) (Cohen, 1992) so requires further testing.

At 440ms, MEP amplitude in EDC was greater during the point than the grasp. At this time-point it is clear that the index finger is extending, which requires the use of the EDC muscle, and the goal of the action is more easily identifiable. As this finding had a medium-large effect size, (Cohen's d = .69) (Cohen, 1992) it indicates that EDC is moderately engaged in the point action at this time-point. This result is considered muscle-specific as the primary role of the EDC muscle is to extend the fingers. This finding is supported by Romani, Cesari, Urgesi, Facchini, and Aglioti (2005) who found MEPs increased in FDI, but not ADM, during observation of an index finger adduction/abduction; as FDI is used to perform the action and ADM is not used to perform the action. Similarly, Montagna et al. (2005) found MEPs were larger in FDI than FDS when the hand was starting to close when nearing a ball; this is when FDI would be most engaged as the index finger and thumb start to form a grasp. This study

also found larger MEPs in FDS during the opening of the hand to form a grasp

(Montagna et al., 2005).

As the results in the point action at 300ms in ADM and 440ms in EDC occur

when the actions are increasingly becoming kinematically different, it could be

interpreted that the simple action, rather than the complex action, created more

corticospinal excitability during kinematically different time-points. However, the

current study was primarily concerned with the time-point when the actions are

distinctly different and the goal of the action is clear, which occurs at 640ms.

The lack of change in FDS across all time-points in the current study is not too

surprising given that this muscle is not really seen to be involved in the observed

actions. However, this is in contrast to previous research which found MEPs in FDS

increased during opening of a hand in a grasp action (Montagna et al., 2005). The lack

of change in MEP amplitude in ADM in the current results is somewhat surprising

considering ADM is involved in opening the hand for grasping. However, previous

studies report inconsistent results for ADM during index and little finger abduction,

reaching and grasping, and pinching (Gangitano et al., 2004; Hardwick, McAllister,

Holmes, & Edwards, 2012: Loporto et al., 2012). Therefore, further testing of ADM in

action observation is required. Future research should consider recording muscle

activity while participants perform the action, to determine how much ADM is involved

in performing the action.

Changes in Short-Interval Intracorical Inhibition

In Experiment 2 it was hypothesised that at 300ms there would be a decrease in

SICI in the point compared to the grasp. At 640ms it was hypothesised there would be a

decrease in SICI in the grasp compared to the point. This hypothesis was not supported

at 300ms or 640ms. At 300ms there were similar levels of inhibition in all conditions

(static, point, grasp), and at 640ms there was numerically more SICI present in the grasp compared to the point and static; however, these results were not statistically significant. As such, the following interpretation of the pattern of SICI should be treated with caution.

The increase in SICI found in the current study is inconsistent with previous research, which found SICI significantly decreased during observation of a handwriting task compared to MEPs collected while participants were at rest (baseline) (Stafella & Paus, 2000). Although inconsistent with the current results, it is important to consider the decrease in SICI reported by Strafella and Paus (2000) in the context of three important methodological considerations: handwriting is a complex task involving multiple movement phases (i.e., there is variation in the muscles used to perform the task); the timing of the TMS pulse was not reported, this is important as Experiment 1 demonstrated that corticospinal excitability is differentially modulated during various time-points in an action; and, the baseline measure was taken while participants were at rest. The current study design does not have the same methodological constraints as the timing of TMS pulses was specific to the early time-points after action onset and the kinematic movements of the action, and the design included a static hand as a baseline measure as recommended by previous research (Fadiga et al., 1995; Loporto et al., 2010: Naish et al., 2014). As the current results are incongruent with the literature, future research is required to fully characterise the role of SICI in action observation.

It is worth noting that if the trend for an increase in SICI is a robust finding, this would suggest the increase in corticospinal excitability found in Experiment 1 was not mediated by a reduction in SICI as hypothesised. Instead, the increase in MEP amplitude found in Experiment 1 could be mediated by different cortical processes. The MEP amplitude reflects net corticospinal excitability, which is influenced by the

activity of intracortical inhibitory and excitatory circuits (Ziemann, Rothwell, & Ridding, 1996). As such, the increase in MEP amplitude during action observation could be the result of an increase in the excitability of excitatory circuits, or a decrease in the excitability of inhibitory circuits (other than SICI), or a combination of both (Naish et al., 2014).

What do the Results of this Study Contribute to the Action Observation Model?

The current study supports Naish et al.'s (2014) model as there was a muscle-specific increase in corticospinal excitability in FDI and EDC from 300ms after action onset. The results extend the model by finding that corticospinal excitability is differentially modulated by action complexity. Although the results for SICI were non-significant, Figure 7 shows that SICI numerically increased in the grasp compared to the point and static at 640ms. As Naish et al. (2014) suggest that inhibitory and excitatory processes may occur together during action observation, the findings indicate that action complexity might influence the amount of excitation and inhibition at different time-points in the action. To our knowledge, this study was the first to measure corticospinal excitability and SICI across different time-points with actions of different complexity. This is the first step in comparing the time-course of corticospinal excitability and inhibitory processes between actions based on their complexity, hence more research is needed to further understand the neural mechanisms active during action observation.

Limitations and Future Research

In the current study, TMS parameters were optimised for FDI as this muscle is engaged in performing the point and grasp actions. Although it could be suggested that setting the hot spot and resting motor threshold for FDI, rather than targeting each muscle individually is a limitation, it is appropriate to use the stimulation parameters for FDI when recording from intrinsic hand muscles and forearm muscles as the cortical

representation area of these muscles overlap. This is evident from Experiment 1 results, which show MEPs were evoked for the target muscles when using TMS parameters optimised for FDI (average static MEP amplitude (mV) in: ADM = .84, FDS = .49, EDC = .62). Hence, TMS-evoked responses occur in these muscles despite that the hot spot is not specific to each muscle. It is worth noting that to test each muscle individually would have required four separate sessions with each participant, which is beyond the scope of the current study, and not necessary to answer the primary hypothesis.

Additionally, as the baseline measure was incorporated into the blocks of trials with the action conditions, and all blocks were pseudo-randomised, it may be likely that participants were expecting the hand to move, thus increasing the excitability of the motor system. In previous research, when baseline measures were completed prior to action observation trials, larger MEPs were found during observation compared to baseline (Hardwick et al., 2012). However, other studies which did pseudo-randomised trials with a baseline measure have found an increase in corticospinal excitability compared to baseline (Fadiga et al., 1995; Lepage et al., 2010; Loporto et al., 2012; Naish & Obhi 2015). As these results are inconsistent, future research should consider comparing differences between collecting baseline data prior to observation trials to including the baseline measures within each block in pseudo-randomised order. Another consideration for future research examining timing, is to include a cue prior to the video to provide contextual information. In everyday life contextual information helps determine others actions; since this is missing in a laboratory setting, providing a cue may assist in creating a more naturalistic environment.

Furthermore, while the actions used in the current study are important to investigate as they are performed automatically, often daily, it is worth noting that there

is a spectrum of task complexity. Although the study differentiated action complexity based on movement kinematics and muscle involvement, the two actions used are towards the simple end of the complexity spectrum. Future research should consider using actions with greater complexity, such as fine motor control requiring object manipulation.

Generalisation of Findings

The results of the current study contribute to our knowledge about mirror neuron activity in two ways: first, the magnitude of change in mirror neuron activity is affected by timing and action complexity – that is, there was an increase in mirror neuron activity when the hand was closer to grasping the ball and looked like the hand was going to pick-up the ball. Secondly, mirror neurons respond to specific muscles, which are active during execution of the action. This second assertion comes from the findings in FDI when the index finger is starting to extend in the point action, and then later when the whole hand is outstretched about to grasp the ball, and in the EDC muscle when the index finger is further extended.

As action observation interventions are currently used in rehabilitation settings to improve upper limb function (Ertelt, 2007; Franceschini et al., 2012; Lee, Roh, Park, Lee, & Han, 2013), the current study makes a valuable contribution to our understanding of the neural mechanisms of action observation. Since this study was conducted in healthy adults, the next step would be to replicate the study in a neurologically impaired population. It would be beneficial to use the same reach and grasp actions in this population as commonly people with neurological impairment lose function in their hands, which makes grasping very difficult. Therefore, improvement in these action can provide meaningful change in tasks associated with activities of daily living. The purpose of conducting this study in a neurologically impaired population

would be to investigate whether the neural mechanisms supporting action observation

behave in the same manner as found in healthy adults. The findings from a study such

as this may be important for improving the efficacy of action observation training which

is used in rehabilitation to improve motor function.

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