

UNIVERSITY OF CALGARY

The Mirror Neuron System and Observational Learning

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

FACULTY OF KINESIOLOGY

CALGARY, ALBERTA

January, 2009

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UNIVERSITY OF CALGARY
FACULTY OF GRADUATE STUDIES

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Abstract

It has been proposed that the mirror neuron system (MNS) is the neural basis for observational learning. The aim of Experiment 1 was to determine if the immediate influence of action observation on the primary motor cortex (M1) was associated with the degree of observational learning induced by repeated action observation. Consistent with this hypothesis, there was a strong positive relationship between the immediate influence of action observation on the M1 and the degree of observational learning. The aim of Experiment 2 was to determine if action content would modulate the immediate effects of action observation and therefore lead to a greater degree of observational learning. In contrast to this hypothesis, it was found that action content did not modulate the immediate effects of action observation and therefore did not lead to a greater degree of observational learning. It is argued that the overall results of the studies provide evidence that the MNS is the neural basis of observational learning.

Acknowledgements

First and foremost I would like to acknowledge Dr. Tim Welsh for all the time, effort and advice that he has given. This has been a great experience and I want to thank you for everything that you have done. I would also like to acknowledge Drs. Deborah Dewey and Libbe Kooistra for all their help and great advice during this process. I would also like to acknowledge Laura Higgins, Laura McDougall, Sanjay Chandrasekharan and Michael Villiger for all their help.

Dedication

I would like to dedicate this thesis to my parents Donald Ray and Rosemary Brown for all their support.

Table of Contents

Approval Page.....	ii
Abstract.....	iii
Acknowledgements.....	iv
Dedication.....	v
Table of Contents.....	vi
List of Tables.....	vii
List of Figures and Illustrations.....	viii
 GENERAL INTRODUCTION.....	 1
Purpose.....	23
References.....	25
 EXPERIMENT 1.....	 31
Abstract.....	33
Introduction.....	34
Methods.....	37
Results.....	41
Discussion.....	42
References.....	46
 EXPERIMENT 2.....	 53
Abstract.....	55
Introduction.....	56
Methods.....	62
Results.....	67
Discussion.....	70
References.....	76
 GENERAL DISCUSSION.....	 85
Conclusions.....	94
References.....	96
 APPENDIX A: ETHICS.....	 99

List of Tables

EXPERIMENT 1	
Table 1	52
EXPERIMENT 2	
Table 1	84

List of Figures and Illustrations

EXPERIMENT 1

Figure 1	49
Figure 2	50
Figure 3	51

EXPERIMENT 1

Figure 1	79
Figure 2	80
Figure 3	81
Figure 4	82
Figure 5	83

General Introduction

Humans are social beings who have to interact and work with others in a variety of contexts. To efficiently work with others, we have to somehow generate an understanding of what the other individual is doing and then coordinate our actions with them. In addition to working with others to get tasks done more efficiently than we could do on our own, our social nature allows us to learn how to perform new movements from watching others. When learning a new movement or behaviour, we can replicate the actions of others through imitation and observational learning. While it is clear that we are able to engage in social cognitive tasks, the question remains as to how is it possible that humans are capable of social behaviours such as imitation, observational learning, empathy and a general understanding of others behaviours. The discovery of mirror neurons may provide a critical first step in developing an understanding of the neural basis for these social behaviours.

The mirror neurons are a unique group of neurons which were discovered in the cortex of macaque monkeys (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). These neurons, first detected in area F5 of the macaque, became active during the observation and execution of similar actions. Since their discovery, a considerable amount of research has been undertaken to identify the specific characteristics of these neurons as well as their possible functions. It has been proposed that these neurons could be the basis for the development of language, imitation, theory of mind, and, of key relevance to the present thesis, observational learning.

Neurophysiological Evidence for Mirror Neurons and Their Firing Properties

The mirror neurons were discovered while Rizzolatti et al. (1996) were investigating how actions are represented in area F5 in the macaque monkeys. Area F5 had previously been shown to code for specific goal-directed mouth and hand actions (Rizzolatti et al., 1988). For instance, some neurons only became active during the execution of grasping movements while others became active during the manipulation of an object. These neurons were also selectively activated depending on the type of grip being used by the monkey (e.g., precision grip vs. whole hand grasping). In this same cortical region, there are neurons which become active when the monkey views an object that affords a certain motor response (i.e., neurons that respond to objects that afford a precision grip versus a whole hand grasp).

During a subsequent investigation of these neurons, another subset of neurons were discovered which became active during the execution and observation of specific goal-directed movements (Rizzolatti, Fadiga, Gallese et al., 1996). The authors called these neurons “mirror neurons” because the neurons seem to form an observation/execution matching system where observed actions of other people are “mirrored” in the brain of the observer. Approximately twenty percent of the F5 neurons which were recorded from demonstrated this mirroring property. Importantly, the presentation of objects alone and action without objects did not activate any of the mirror neurons (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996).

Mirror neurons were shown to be very selective in the motor acts they coded for and only become active during the execution and observation of specific movements with specific grips. Mirror neurons can be classified based on the type of movements that they code. There are grasping, placing, holding and manipulation mirror neurons (Gallese et al., 1996). Some of these neurons code for and will only fire during the execution and observation of specific grips. Therefore, a grasp with a precision grip will activate different neurons than the observation of a whole hand grasp (Gallese et al., 1996). Some neurons are selectively activated depending on the hand being used and the direction of the observed movements (Gallese et al., 1996).

In an effort to further understand how actions are represented in the monkey brain and which visual features are necessary to elicit mirror neuron activation, Umiltà et al. (2001) performed a single neuron recording study in area F5 of the macaque brain while they observed partially hidden hand actions. The monkeys observed trials in which full vision of the action (transport, grasp and lift phases) was available and trials in which the last part of the movement, where the object interaction occurred, was occluded. The mirror neurons which fired during the full vision condition were activated to same degree during the partial vision trials. Importantly, however, this effect was dependent on the monkey knowing that there was an object behind the occluder. During conditions in which there was no object behind the occluder, there was no neuronal activation. These findings indicate that, even when a hand object interaction is not visible, if the monkey knows what action should be occurring, then the mirror neurons will fire (Umiltà et al., 2001).

Mirror neurons can be subdivided into groups which are broadly congruent and strictly congruent (Gallese et al., 1996). Strictly congruent mirror neurons only fire when there is a direct match between the movement that is executed and observed (Gallese et al., 1996). For example, a strictly congruent mirror neuron which codes for a grasp with a precision grip will only become active during the observation of a grasp with a precision grip. Broadly congruent mirror neurons will fire during the execution of a specific movement and the observation of a larger group of similar movements (Gallese et al., 1996). Hence, a particular type of broadly congruent mirror neuron might fire during the execution of a specific action with a specific grip (placing with a precision grip) and during the observation of placing actions using a variety of grips (whole hand grip and precision grip). Another type of broadly congruent neuron fires during execution of a specific action (grasp) and during the observation of multiple actions (grasping, holding, placing) (Gallese et al., 1996). These findings indicate that these neurons can code the specific goal of the action and that, through the generation of an internal representation during action observation, we may be able to understand the meaning of others' actions (Gallese et al., 1996).

Another group of mirror neurons have been found in the inferior parietal lobule of the macaque monkey (Fogassi et al., 2005). Further research into the PF mirror neurons revealed that these particular neurons seemed to code not only for specific action types, but also for the context of a particular movement (Fogassi et al., 2005). Single neuron recordings were taken from the inferior parietal lobule during the execution and observation of similar motor acts in different contexts such as grasping to eat versus

grasping to place an object. The results showed that some mirror neurons fired for both actions, but that the majority of the neurons were activated only during the observation of specific actions with specific goals (Fogassi et al., 2005).

Another class of mirror neurons that have been discovered is the audiovisual mirror neurons (Kohler et al., 2002). This group of mirror neurons fires not only during the observation and execution of action, but also during the hearing of action-related sounds. Single neuron recordings were taken during the execution, the visual observation, and the auditory presentation of the sounds of hand actions. It was discovered that the sound of paper ripping, a peanut breaking, or a stick dropping activated neurons which also fired during the execution, and sometimes observation, of these same actions. These neurons demonstrated the same selective behaviour in their firing that other mirror neurons do indicating that these neurons code specific noises which are associated with specific actions (Kohler et al., 2002). These results provide evidence that actions are coded and represented in the brain with a common representation which contains information related to all the sensory information involved in the movement.

Another group of mirror neurons, named tool-responding mirror neurons, were discovered while investigating how actions not currently in a monkey's repertoire would be coded (Ferrari, Rozzi, & Fogassi, 2005). Single neuron recordings were taken from area F5 during the execution of hand actions as well as during the observation of hand actions and actions with tools. It was found that tools which were unfamiliar to the monkeys did not elicit mirror neuron activity whereas tools that the monkeys were

exposed to, but had not used themselves, elicited mirror neuron activity. The specific characteristics of these tool-responding mirror neurons shows that they fire during the execution of a hand action and during the observation of the experimenter performing a tool action with a similar goal to the movement that the monkey could actually execute (grasp and hold food and observed the experimenter stick and hold food) (Ferrari et al., 2005).

In sum, the discovery of mirror neurons may have important implications for understanding human social behaviour. Rizzolatti et al. (1996) has suggested that the mirror neurons could facilitate the understanding of others via the simulation of observed actions in our own motor system. This conclusion is based on the findings that the mirror neurons become active during the observation and execution of actions and that the firing of these neurons is modulated by specific actions, intentions, action sounds, and expected actions. The activation of an internal representation which is coded with our past experiences gives us insight into the behaviour of those acting around us. It also makes sense that as humans we have a similar, if not more complex, mirror neuron system (MNS) which is likely involved in imitation, observational learning, and understanding others mental states.

Mirror Neuron System Activation in Humans

After the discovery of mirror neurons in macaque monkeys, various neurophysiological techniques have been used to explore whether this action observation/execution system existed in humans. Although there have not been any single neuron recordings in humans to uncover the specific attributes of a particular

mirror neuron, there has been a large amount of neuroimaging and neurophysiological studies to uncover the cortical components and functional properties of a possible human MNS. In sum, evidence accumulated through fMRI (Iacoboni et al., 1999), TMS (L. Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), PET (Rizzolatti et al., 1996), and MEG (Hari et al., 1998) studies suggests that a MNS is present in humans. The putative human MNS is composed of the inferior parietal lobule, lower part of the precentral gyrus, and the posterior part of the inferior frontal gyrus (Rizzolatti & Craighero, 2004). The following is a review of the literature leading to this conclusion. Findings specific to the activation of the MNS and motor system will be reported.

The early imaging work provided information regarding the specific structures in the human brain which would comprise the human MNS. Rizzolatti et al. (1996) used PET to determine brain regions which would become active during the observation of grasping actions. Participants were involved in three conditions: a grasping observation, object grasping, and an object observation condition. During the grasping observation condition, participants observed the experimenter performing grasping movements on objects with various grips. During the object grasping condition, the participants reached out and grasped the same objects that the experimenter manipulated during the viewing condition. Finally, during the object observation condition, participants viewed the objects alone without any action. The results of the study showed that during the observation of various grasping movements there was a significant activation of the middle temporal gyrus BA21, the superior temporal sulcus, and the caudal part of the inferior frontal gyrus BA45, when compared to the activation patterns during the

observation of various objects (Rizzolatti et al., 1996). There was no contrast between the grasping observation condition and the grasping condition. The authors concluded that BA45 in humans corresponds to the area F5 in monkeys and codes for the observation of hand actions.

Decety et al. (1997) used PET to investigate how the content (meaningful and meaningless) and the context (observation for recognition and observation for imitation) of the observed action affected MNS activation in humans. Participants viewed meaningful actions which were pantomimes of actions on objects, whereas the meaningless movements were derived from the American Sign Language (ASL). Participants viewed both types of action with the intent to imitate or with the intent to recognize later. The main findings of the study were that the observation of actions with the intent to imitate activated structures that were involved in action planning, such as the dorsolateral prefrontal cortex and the pre-supplementary motor area. The observation of meaningful actions caused general left hemisphere activation in the frontal and temporal regions regardless of the strategy of observation, whereas the observation of meaningless movements activated right hemisphere structures in the occipitoparietal region. During the observation of meaningful actions with the intent to recognize, the left inferior frontal gyrus BA45 was significantly activated when contrasted with the observation of meaningless movements with the intent to recognize. In summary, the authors found that cortical activation patterns were altered based on action content and that the activation of the inferior frontal gyrus, which is considered part of the MNS, was differentially activated based on action content (Decety et al., 1997).

Grezes and Costes (1998) expanded on the earlier Decety et al. (1997) study by investigating how our perception of meaningful and meaningless movements is altered depending on the goal of observation (imitate vs. passive observation). The methodology was similar to the Decety et al. (1997) study; however the participants did not have to observe the movements with the intent to recognize them later. The meaningful actions consisted of pantomimes of actions on objects and the meaningless actions consisted of actions derived from the ASL - subjects did not have any knowledge of the ASL. When contrasting the cortical activations found during the passive observation of meaningful actions with the observation of a static hand the inferior frontal gyrus, inferior parietal lobule and the precentral gyrus were all active. When contrasting the cortical activations found during the passive observation of meaningless actions with the observation of a static hand, the inferior parietal lobule and the precentral gyrus were active. In this study, the left primary motor cortex was activated during the passive observation of meaningful and meaningless movements, however the inferior frontal gyrus was not activated during the observation of meaningless movements therefore indicating a modulation of MNS activity based on the intent of observation and action content (Grezes & Costes, 1998). No contrast was performed between the meaningful and meaningless conditions therefore it is unknown if the activation found in the primary motor cortex was similar between conditions.

Buccino et al. (2001) used fMRI to investigate how action observation activates premotor and parietal areas in a somatotopic manner. Essentially, it was predicted that the MNS should have a specific pattern of activation based on the contents of the

observed action. A second aim of this study was to determine if the presentation of an object in the action had an effect on MNS activation. Participants observed mouth, arm/hand, and foot actions with or without objects. The observation of non-object related mouth actions caused activation in Brodmann's area 6 and 44 on both sides and in area 45 in the right hemisphere. Right hemisphere activation was stronger and larger than in the left hemisphere. During observation of object related mouth actions, the premotor activation was similar, but actually weaker than during non object related mouth actions.

However, there was additional activation in the parietal lobe during object actions. During the observation of hand actions (non object related) there was bilateral activation of area 6 dorsally to the mouth representation. During the observation of object related hand movements, there was bilateral activation of the premotor cortex plus an activation site in area 44. There was also additional activation in the parietal lobe just dorsal to the mouth actions. During foot activations (non object and object), there was dorsal area 6 activations and during the object condition there was also activation in the posterior part of the parietal lobe. In summary, this study revealed that the MNS has a somatotopic organization and the addition of an object to an action leads to greater parietal lobe activation (Buccino et al., 2001).

Although it has been demonstrated that the MNS is implicated in action observation and imitation, MNS activation might not be automatic. Jonas et al. (2007) is one the first to show that MNS activation is not automatic and that the type of finger movement may play a role on MNS activation. During an fMRI study, they discovered that the MNS did not become active during the viewing of a meaningless finger

movement. When the finger moved with a dot, however, there was significant activation in the inferior frontal gyrus and the inferior parietal lobule (Jonas et al., 2007). In summary, this study shows that MNS activation is not automatic and that contextual information plays a role in MNS activation.

Hari et al. (1998) demonstrated that action observation can lead to activation in the primary motor cortex using MEG. MEG signals were taken when subjects were at rest, when they manipulated a small object with their right hand, and when they observed an individual performing the same action. The observation of an action also altered activation patterns in the primary motor cortex (Hari et al., 1998). The authors state that these changes in MEG recording do not necessarily reflect an increase in motor output, but instead represent changes in the synchrony of motor neurons. The important aspect of this study is that it demonstrates how action observation affects the primary cortex via MNS activation.

Nishitani and Hari (2000) have also shown the timing pattern of how different brain structures are activated during an action observation sequence. This research provides insight into the possible role of different brain regions during the different aspects of action observation. The study consisted of an execution task, that required participants to reach and pinch a manipulandum, an imitation task, which required participants to make a similar movement to the experimenter, and an observation condition, where participants viewed the experimenter performing pinching movements. The execution, imitation, and observation conditions all led to activation in the primary motor cortex. The left BA44 was activated earlier than the left BA4 in all conditions.

Both BA44 and BA4 were activated twice as strongly during the imitation as opposed to during execution and observation (Nishitani & Hari, 2000). This pattern of effects suggests that there is an additive effect of cortical activation during imitation because of the activation associated with the observed movement and the to-be-executed movement. What all these studies demonstrate is that there is a network of cortical centers in humans which become active during the execution and observation of action. These areas include the inferior frontal gyrus, inferior parietal lobule and the precentral gyrus. These neuronal centres have been shown to become active during imitation, action observation, and imagery. MNS activation was also shown to be dependent on the meaning of the movement and the context of action observation. What was important to note from the more recent work was that motor cortex was also activated during action observation. These findings suggest that MNS activation has a direct influence on the activity in the motor system. More direct evidence for this influence comes from transcranial magnetic stimulation (TMS) work.

Transcranial Magnetic Stimulation: Principles, Methods, and Considerations

TMS was described by Barker, Jalinous and Freeston (1985) as a non-invasive way of stimulating the cerebral cortex. Barker, Jalinous, and Freeston (1985) found that magnetically stimulating the primary motor cortex could elicit movements of the body parts which corresponded to the area of the motor homunculus being stimulated. Since that time, magnetic stimulation has, for the most part, replaced electrical stimulation because it is painless (Rossini et al., 1994).

TMS uses the principle of electromagnetic induction to stimulate the cortex of the brain (Maeda & Pascual-Leone, 2003). The magnetic stimulator consists of a coil of wire connected to a large electrical capacitor. This capacitor stores a large amount of electrical current which, upon being discharged, creates a large current that flows through the coil (Rothwell, 1997). A TMS unit normally consists of a capacitor discharge system with voltages of up to 4000v. Once the current is discharged and circulates through the coil, a magnetic field of up to 2.5 Tesla is created with a rise time of 50 to 200 microseconds (Rossini et al., 1994). The magnetic field produces an electrical current parallel and in the opposite direction to the original electrical current in any nearby electro-conductive medium (Maeda & Pascual-Leone, 2003). When the current is of sufficient magnitude, approximately 2000 amps, and the coil is placed tangentially over the cortex, then a depolarization occurs in the neural tissues (Maeda & Pascual-Leone, 2003). The induced current is proportional to the rate of change of the magnetic field (Rothwell, 1997). The peak strength of the magnetic field is related to the magnitude of the current and the amount of turns of wire in the coil (Wasserman, 1998). The electrical current induced in the tissues is weaker than the current in the coil (Wassermann, 1998). The skull does not provide much impedance to the magnetic field, which means that the field is able to penetrate the skull and create an electrical current in the underlying neural tissues (Rothwell, 1997). Because the electrical current is created directly in the neural tissues and there are not any pain receptors in the cortex, TMS is a painless form of stimulation (Barker, Garnham & Freeston, 1991; as seen in review by Rossini et al., 1994). TMS is effective at directly stimulating in the layers of the cortex, however at 4-5

cm from the coil the strength of the magnetic field decreases by half which makes it difficult to stimulate subcortical areas at the level of the basal ganglia or thalamus (Rothwell, 1997).

Work done by Patton and Amassian (1954) using electrical brain stimulation of the motor cortex on cats and monkeys showed that when the brain was stimulated there were two responses, an I wave and a D wave, with the D wave having a shorter latency than the I wave. They proposed that the D wave represents a direct activation of the pyramidal neurons, whereas the I waves represent stimulation of the pyramidal tract via other synapses in the cortex, specifically cortical interneurons. The electrical current from TMS which develops when the magnetic coil is tangential to the skull preferentially activates the cortical interneurons which are oriented in a horizontal fashion, thus creating I waves by trans-synaptically activating the descending pyramidal neurons. Due to the vertical orientation of the pyramidal tract neurons it is unlikely that a D wave could be produced with clockwise magnetic stimulation over the motor cortex (Day et al., 1989). However, Amassian et al (1994) found that by vertically orienting a circular magnetic coil and increasing the stimulus intensity that a direct stimulation of the pyramidal neurons was possible as D waves with the same latency as those done with electrical stimulation were seen. Berardelli, Inghilleri, Cruccu and Manfredi (1990) also found that the D waves could be stimulated with magnetic stimulation with a 10 cm diameter flat coil placed at the vertex of the skull using 80% to 100% of the output available. However, when using TMS, the latency for the D wave was a little bit longer than electrical stimulation, and the amplitude was lower and the threshold was higher

(Berardelli, Inghilleri, Cruccu, & Manfredi, 1990)(Berardelli et al., 1990). Thus, TMS at the levels used in the present study preferentially activate cortical interneurons. This preferential activation is important to note because it demonstrates that the motor-evoked potentials (MEPs) recorded reflect the activity of a network of neurons and not just single pyramidal neurons being activated.

Some minor problems with TMS include how focal the stimulation is on particular brain regions and the variability in locating hot spots (area of maximal representation) in different individuals (Pascual-Leone, Bartres-Faz, & Keenan, 1999). There will be variability in locating structures between different participants, due to interindividual variability in the shape and size of the skull and brain, and in the manner in which it is functionally organized. Therefore, one cannot simply use standard measures to locate structures (Pascual-Leone et al., 1999). However, individual reconstructed head maps using MRI can be digitized and used to guide the correct location of coil placement (for a review see Pascual-Leone et al., 1999). Although it appears as though TMS may not target discrete areas, it has been shown that it generally activates a certain volume of tissue directly below the stimulator. PET and fMRI experiments suggest that TMS is comparable to other brain mapping methods in terms of focality, spatial resolution and the specificity of effects (Pascual-Leone et al., 1999). In addition, because it induces electrical currents tangential to the skull, and the magnitude of the induced currents declines with increasing distance from the coils, it preferentially activates tissue on the peaks of gyri, and has less of an ability to activate tissue on the walls or in the deep pits of sulci.

When looking at the effects of TMS on the central motor pathways, the silent period, which is time when the muscle is unable to contract following the delivery of a TMS pulse, is used to look at inhibitory effects of TMS and MEPs are used for its excitatory effects to study cortical excitability (Rossini et al., 1994). In most TMS experiments of the motor system, the primary motor cortex is stimulated and MEPs for a specific group of muscles are recorded from surface EMG electrodes (Wasserman, 1997). A MEP, the recorded change of electrical potential in a muscle following stimulation of the motor cortex with TMS, is a measure of corticospinal excitability (Maeda & Pascual-Leone, 2003). That is, it provides an index of the sum of the excitatory and inhibitory influences at the level of the cerebral cortex and the spinal cord. MEP amplitude should be measured from the two largest peaks in opposite polarity. The intensity of TMS during a study is typically reported as a percentage of the threshold intensity for evoking an MEP. Because the threshold varies greatly between individuals, one should not report the percentage of the machines output (Wasserman, 1997).

To find the excitability threshold to evoke a MEP, the first step is to locate the region on the skull which corresponds to the muscle being examined. The “hot spot” is considered to be the location where the stimulator intensity is the lowest and MEP latency is the shortest (Rossini et al., 1994). When this point is located, the researcher increases the stimulus intensity in 5% intervals until MEPs are seen in 50% of 10-20 consecutive stimuli (Rossini et al., 1994). Thresholds are generally lower in muscles which have larger neural representations on the motor cortex; these muscles also have smaller latencies (Meyer, Britton, Kloten, Steinmetz, & Benecke, 1991; as seen in review

by Rossini et al., 1994). Variability in the excitability threshold can be effected by wakefulness, drowsiness and mental activity, therefore subjects should have their eyes open and perform simple calculations to reduce variability (for a review see Rossini et al., 1994). The excitability threshold can be facilitated by many factors such as voluntary muscle contraction, continuous vibration of the muscle, conditioning stimuli and limb ischemia (for a review see: Rossini et al., 1994). Measurements of corticospinal and corticocortical excitability are affected by many factors such as different methodologies, age, handedness, fatigue, hyperventilation, attention, imagery, action observation, stimulation of other neurons and drugs. (For a review see Maeda & Pascual-Leone, 2003).

Transcranial Magnetic Stimulation studies of the Mirror Neuron System

Fadiga, Craighero and Olivier (2005) have reviewed the use of TMS as a tool to infer MNS activation and its influence on motor cortices during action observation. Although imaging techniques can provide us with a snapshot of the areas involved in action observation, they do not provide insight into dynamic changes that can emerge due to action observation. The magnitude of TMS-evoked MEPs provides an index of corticospinal excitability. Therefore, changes in the magnitude of MEPs provide information about how the activation of the MNS affects our motor system.

Fadiga, Fogassi, Pavesi, and Rizzolatti (1995) were some of the first researchers to use TMS study the effects of action observation on the excitability of the nervous system. In this experiment, the left motor cortex was stimulated. MEPs were recorded using surface electrodes from various hand muscles. The four experimental conditions

consisted of a grasping observation, object observation, arm movement observation, and a dimming detection. The results showed that the excitability of the nervous system increased when observing the actions of another individual. The increase in MEP was specific to the effectors viewed during action observation. Fadiga et al. (1995) proposed that the MEP facilitation could occur via excitatory cortico-cortical connections from the premotor cortex which would activate the primary motor cortex. These findings indicate that, in humans, action observation has the ability to facilitate the motor system. It was suggested that this effect is likely caused by the MNS (Fadiga et al., 1995).

Gangitano, Mottaghy and Pascual-Leone (2001) demonstrated that facilitation of cortical motor output by action observation is dependent of the specific phase of the observed action. TMS was applied to the motor cortex, during the observation of a hand moving towards a ball and grasping it, at different phases of the movement. Depending on the trial, TMS was delivered with the hand at the starting position (500 ms after onset of the video), 1000 ms, 2000 ms, as the hand opened up to a maximal grip aperture (3000 ms) and as the hand was closing around the object (3500 ms). The largest amplitude MEPs were observed at 3000 ms as maximum grip aperture was reached. The muscles which were recorded from, the first dorsal interosseous and abductor pollicis brevis, are involved in this specific action and therefore it makes sense that at the completion of the movement which is driven by these muscles that the largest MEP facilitation was observed.

Maeda, Chang, Mazziotta and Iacoboni (2001) have shown that frequently observed actions caused a greater facilitation of MEPs than movements which were

viewed from an orientation which was not frequently observed by the participants. Participants viewed a recording of their own movements from an orientation which either mapped away from the body or towards their body. Participants also viewed movements of other individuals from an away or towards the body orientation. The results indicated that corticospinal excitability was increased to a greater degree when participants observed their own action from a self-away orientation and other people's actions from an other-towards orientation.

Aziz-Zadeh, Iacoboni, Zaidel, Wilson and Mazziotta (2004) have shown that action-related sounds in humans can activate the MNS. Participants listened to sounds of paper tearing and typing (hand actions), walking sounds (feet actions) and control sounds such as thunder to see if the action sounds would cause MNS activation and subsequent motor system activation. The sounds of tearing and typing caused a facilitation in MEPs recorded from the FDI indicating a specific motor facilitation caused by a sound associated with a hand action. The foot and thunder sounds did not cause a facilitation of MEPs therefore indicating that there was not a general motor facilitation based on the arousal caused by sound. These data are consistent with the findings of Kohler et al. (2002) in the monkey in which sounds activated mirror neurons. Interestingly, the motor facilitation caused by the hand action sounds was only present in the left hemisphere. The authors concluded that in the left hemisphere actions can be coded via sound, visual and motor-related information whereas the right hemisphere codes actions using vision and motor information.

TMS has also been used by Brighina, La Bua, Oliveri, Piazza and Fierro (2000) to investigate whether single or more complex movements caused differential action in the MNS. Stimulation was applied to both the right and left motor cortices. Motor responses were measured in the contralateral abductor pollicis brevis. Participants had three different conditions: a single movement observation, a motor sequence observation, and a fixed target condition. The single movement observation consisted of viewing an arm elevation or thumb abduction. The motor sequence observation consisted of a simple sequence and a complex sequence of finger and thumb movements. During the fixed target observation participants observed the palmar surface of the examiner with his hand at rest. The results showed that there was a right to left asymmetry present and that during the observation of isolated muscle movements the right cortex had an increased excitability. The authors also found that more complex movements caused a greater facilitation in the left motor cortex.

In summary, TMS research has shown that action observation can cause a facilitation of the motor system. This facilitation, however, is modulated by the complexity, familiarity, orientation, time, and action sounds.

Observational Learning and Motor Memory

During the acquisition of motor skills, it is quite common to learn from observing another individual perform an action. The MNS is a good candidate for supporting this type of learning process due to the fact that the observation of another's actions activates our own motor representations (Vogt & Thomaschke, 2007). The online simulation of the observed movement in the MNS and the task-specific excitatory influence it has on the

motor system could help to build the motor representation which could then be accessed when performing the skill.

TMS has been used in a number of studies to look at learning. The first study relevant to the present discussion investigated the plasticity of a movement representation induced by physical practice (Classen, Liepert, Wise, Hallett & Cohen, 1998). In this study, the authors examined whether the direction of TMS-evoked thumb movements would change from a baseline direction following physical practice. After 1800 trials of thumb movements in the opposite direction to that determined at baseline, the TMS-evoked movements changed towards the direction of training. These results showed that the practice of simple unidirectional thumb movements caused a reorganization of the movement representation initially displayed via TMS (Classen, Liepert, Wise, Hallett, & Cohen, 1998)

The unique methodology that has been developed to use TMS to monitor changes in the representation of a movement in the motor cortex following action observation followed very closely from the research on physical practice (Stefan et al., 2005). This type of research provides insight into the possible role of the MNS in motor learning and to study the effects of physical practice on motor representations. EMG recordings were taken from surface electrodes placed over the extensor pollicis brevis and flexor pollicis brevis. Kinematic measures were taken using an accelerometer to determine the direction of thumb movements based on first peak acceleration vectors in the horizontal and vertical axes of the principal movement plane. A figure-8 magnetic coil was used and was firmly fixed to a frame so that movement could be avoided. The experiment lasted

three sessions; each session involved a different condition of either physical practice or observational practice. The methodology used to study this phenomenon involved determining a baseline direction for TMS-evoked thumb movements, this involved applying 60 TMS stimuli at 0.1 Hz to the optimal scalp site to elicit thumb movements. Once the baseline direction of TMS evoked thumb movements was determined, the participants either watched a video of thumb movements in the same direction or in the opposite direction to the baseline, or they physically practiced thumb movements in the opposite direction to baseline. TMS was used to monitor changes in the direction of TMS evoked thumb movements following each session. Stefan et al. (2005) were able to show that observation alone could change the representation of a movement in the primary motor cortex because the direction of thumb movements changed after the observation of movements in the opposite direction to the baseline. They proposed that the MNS is the likely candidate for the changes that were observed. Essentially, they suggested that each time an action was observed, the MNS would activate the specific parts of primary motor cortex which corresponded to the observed movements. Therefore, the effects of the repetitive action observation are seen in the primary motor cortex.

In a review by Krakauer and Shadmehr (2006), the authors suggested that M1 is important in the early consolidation of a motor memory. In support of this conclusion, he described a study by Muellbacher et al. (2002) where the application of rTMS to the primary motor cortex during the initial learning phase of a pinch grip movement disrupted the consolidation of the newly learned movement. If the rTMS was applied 6

hrs later, on the other hand, the disruption did not occur. Krakauer and Shadmehr (2006) also described a study by Li et al. (2001) where single unit recording in M1 were recorded while monkeys adapted to changes in force fields. Some of the neurons which coded for a particular direction changed the direction which they coded for during or following, the training. During a washout period, the neurons re-established their coding for their original preferred direction. Together these studies indicate that the primary motor cortex is involved in the early consolidation of a learned movement and that directionally coded neurons can temporarily change the direction they code for with training.

In sum, what these studies show is that the primary motor cortex is important in the development of short-term memory traces that form as the results of training. These cortical representations can be changed by physical practice as well as observational practice. The MNS is the likely candidate for facilitating this process because the observation of the actions would activate cortical representations which would alter neuron activity in the primary motor cortex.

Purpose of the Present Studies

The purpose of the present studies was to look at the relationship between the MNS and observational learning. Specifically, Experiment 1 examined the relationship between the immediate effects of MNS activation during action observation on the motor system (change in the amplitude of TMS evoked thumb movements during action observation) and the short term effects of repeated action observation on the changes in cortical representation (change in the direction of TMS evoked thumb movements). The

purpose of Experiment 2 was to investigate how action content might affect MNS activation on the motor system and hence modulate the effectiveness of repeated action observation in changing the cortical representation of movement.

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EXPERIMENT 1

The Neurophysiological Basis for Observational Learning

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Abstract

It has been proposed that the mirror neuron system (MNS), a system of neurons which increases in activity when we perform or observe movement, is the neural basis for observational learning. The aim of the present study was to test this hypothesis by using transcranial magnetic stimulation to determine if the immediate influence of action observation on the excitability of primary motor cortex (M1) is associated with the degree of observational learning. Consistent with our hypothesis, the change in the excitability of the M1 during action observation was correlated with the amount of change in the representation of action in M1 following repeated action observation. We argue that these results demonstrate that the MNS is the neural basis of observational learning.

Introduction

Observational learning is the process by which we translate the actions that we watch another person perform into our own motor system so that we can recreate the observed behaviour. It has been proposed that the neural substrate for observational learning is the mirror neuron system (MNS) (Stefan et al., 2005; Vogt & Thomaschke, 2007). The MNS was first discovered in the cerebral cortex of the macaque monkey and consists of specialized neurons that become active when the monkey physically executed actions and, importantly, when the monkey observed the execution of these same actions (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). These neurons can be further divided into multiple subgroups that are specialized for specific characteristics of the movements being performed or observed (i.e., types of actions, action goals, action effects) (Iacoboni & Mazziotta, 2007). It is believed that humans also have a MNS. The main evidence in favour of a human MNS comes from brain imaging studies (Buccino et al., 2001; Iacoboni et al., 1999; Rizzolatti et al., 1996; Hari et al., 1998). These studies have shown that cortical areas that become active in humans, such as the rostral part of the inferior parietal lobule, inferior section of the precentral gyrus, and the posterior part of the inferior frontal gyrus, are homologues to the areas in the cortex of the macaques in which mirror neuron activity has been (Rizzolatti & Craighero, 2004).

It has been suggested that the MNS generate a representation of the observed action in the brain of the observer and that this representation is then accessed by motor and other cognitive systems for the purpose of imitation, communication, empathy, and

understanding others mental states (Iacoboni & Mazziotta, 2007). Neurophysiological support for the immediate influence of action observation and the MNS on the representation of action in the human motor system has been provided by studies using transcranial magnetic stimulation (TMS). For example, Fadiga et al. (1995) found that the observation of an action caused an increase in the amplitude of motor evoked potentials (MEPs) measured from the muscles that would be active if the observed action had been performed. They suggested that the task-specific increase in cortical excitability develops because the observation of action activates a representation of that action in the motor system of the observer via the MNS.

Although observation-evoked response representations may be immediately used for imitation and communication, these representations may also form the basis of observational learning. There is ample evidence from behavioural research that demonstrates that observational learning enhances the development of a motor skill (Vogt & Thomaschke, 2007). It is only recently, however, that researchers have started to uncover neurophysiological evidence regarding changes in the brain that are associated with more long-term effects of action observation (observational learning). Stefan et al. (2005) suggested that the repetition of observation-evoked response representations (via MNS activation) could form the basis for observational learning. Specifically, the response codes evoked through observation could simulate the response codes activated in the motor system during actual performance and, through repeated response code activation; the brain could generate and refine the neural representation of that specific behaviour.

Using TMS, they investigated how action observation changed the cortical representation of an action. Specifically, they determined a baseline (prior to training) representation for a specific action (i.e., thumb movement in a specific direction) in the primary motor cortex (M1) by measuring the average direction for TMS-evoked thumb movements while the participant rested. Participants then took part in a training session that required them to generate thumb movements or to observe a video of thumb movements that were in the opposite direction to the pre-training direction. At the end of each training session, a measure of the direction of TMS-evoked thumb movements was recorded. Results indicated that physical practice generated the largest change in the direction of TMS-evoked thumb movements; however, observation of the thumb movements alone also resulted in a significant change in the direction of the TMS-evoked thumb movements. These data suggest that the repeated observation of action was able to alter the cortical representation of action in the M1 in a manner consistent with physical practice. The authors proposed that the MNS was involved in generating the changes in cortical representation that occurred during observational learning because the mirror neurons became active during repeated observation of actions and altered the activity of M1 in a task-specific manner (L. Fadiga et al., 1995).

In sum, one line of research has shown that the immediate effect of action observation is a task-specific increase in the excitability of M1 (L. Fadiga et al., 1995) and another has demonstrated that repeated action observation can change the cortical representation of an action (Stefan et al., 2005). It has been suggested that the same neural system, the MNS, is responsible for both of these influences. The purpose of the

present study was to test this hypothesis by examining the relationship between the immediate and long-term effects of action observation on the representation of action in M1. It was hypothesized that if the same system (i.e., the MNS) contributes to both effects, the immediate and long-term effects of action observation would be highly correlated.

Materials and Methods

Participants

Twelve subjects (3 women, 9 men), ages 20 – 39 ($M = 26.4$, $SD = 5.31$), were recruited from the University of Calgary population. Each participant read, completed and signed an informed consent form, medical history questionnaire, and questions for self-report prior to testing. Once the participants filled and signed the forms, they were familiarized with the experimental set-up and the task. The participants were given written and verbal instructions about the task. The study consisted of one experimental session that lasted approximately 2 hrs. The participants were financially compensated. The procedures of the study complied with the ethical standards of the 1964 Declaration of Helsinki regarding the treatment of human participants in research and were approved by the University of Calgary Research Ethics Board.

Recording and stimulation procedures

This study employed similar recording and stimulation procedures used in the study by Stefan et al. (2005). At the beginning of each session, participants sat in a chair in front of a table and a computer screen. Once the participant was seated, an infrared light-emitting diode (IRED) from an active marker system (Optotrak Certus) was

attached to the tip of the right thumb. The position of the IRED marker was recorded at 500 Hz. Surface EMG electrodes were placed on the extensor pollicis brevis (EPB) and the flexor pollicis brevis (FPB). In order to minimize electrical impedance and noise, the skin over these muscle bellies was shaved with a razor and cleaned with 70% alcohol pads.

Subjects sat in a chair with their right arm immobilized in a semipronated position with their fingers supported and the thumb free to move (Figure 1). EMG recordings were made of the EPB and FPB at a sampling rate of 4000 Hz and signal amplification of 1000 (bandwidth 20-450 Hz). EMG was recorded using a Delsys Bangoli-8 system and custom LabView software for a window that began 100 ms prior to and 1500 ms after TMS stimulation. TMS (Magstim 200) was delivered through a figure eight coil (70 mm diameter wings) to elicit contractions of the FPB and the EPB. The optimal scalp position was identified using suprathreshold stimulus intensity and marked on the scalp. The resting motor threshold was quantified as the minimum stimulator output which elicited a motor evoked potential greater than 50 mV (peak-to-peak) from the FPB on 5 out of 10 consecutive trials (Rossini et al, 1994). All videos were displayed on a 19" LCD screen approximately 70 cm in front of the participants.

Experimental Design

Sixty TMS pulses at 115% of the resting MEP threshold were delivered over the region of M1 to elicit thumb movements and MEPs in FPB while the participants stared at a blank screen. Thumb movements (defined as the change in the position of the IRED) were analysed using custom MatLab software. The analysis of the initial 60 thumb

movements was used to determine the baseline cortical representation of the thumb. MEPs from FPB were also recorded at this point to determine the resting excitability of the motor pathways.

Following these baseline measures, the excitability of M1 during action observation was assessed. Participants observed a video of a randomized set of flexion and extension movements of the thumb. TMS at 115% of resting threshold was delivered over the cortical representation for the thumb 500ms after the thumb movement in the video was initiated. This protocol provided a measure of the immediate changes in excitation following action observation.

The observational learning training session consisted of 9 blocks of 200 trials (lasting approximately 3.5 mins). Each trial contained one thumb movement at a rate of 1 Hz. Participants were told to relax their hand muscles and to watch a video which consisted mostly of thumb movements in the opposite direction to that determined at baseline (i.e., in the flexion or extension direction). To ensure that the participants were focusing their attention on the movement on the screen, 6% of the trials were in the opposite direction to the other thumb movements and the participants were required to count these oddball trials (Stefan et al., 2005). The average error in recognizing the oddball movements was 1.9% across all participants with the maximum individual amount of error at 3.7%. The hand in the video was in the same apparatus that the participants were tested in. Hence, the thumb of the right hand was seen from a side view with the hand in a semipronated position so that the participants could clearly see the thumb moving into flexion or extension through the sagittal plane (see Figure 1). To

ensure that the participants were not physically practicing the thumb movements, EMG of the FBP and EPB were constantly monitored and participants were reminded to relax on the rare occasion that an increase in EMG was detected. Fifteen TMS pulses were applied to the cortical representation of the thumb in M1, in between each block to monitor the time course of the changes in cortical representation induced by action observation (data not reported). Following the 9 training blocks, a post-training measure of the cortical representation of thumb movements was taken by applying 60 TMS pulses to M1 at rest while participants viewed a blank screen.. The direction of these TMS-evoked thumb movements were recorded and analysed.

Data Reduction

Because of recording difficulties (TMS stimulus artefact), we were unable to analyze MEPs recorded from EPB. As such, all MEP analysis is based on MEPs evoked from FBP. The excitability of the cortical representation in M1 was determined by examining the mean peak-to-peak amplitudes of the MEPs. Peak-to-peak amplitude was defined as the difference between the highest and lowest voltage for each MEP from approximately 19-60 ms after TMS delivery (exact time range differed for each participant). Prior to the calculation of mean peak-to-peak amplitude, two outlier procedures were conducted. First, because of the known relationship between voluntary muscle activation and MEP amplitude (Rossini et al., 1994), individual MEP amplitudes were eliminated from the data set if the mean background EMG in the epoch 5-85 ms prior to TMS delivery was greater than 3 standard deviations above the mean background EMG for all trials during that time. Second, to eliminate the influence of outliers due to

random influences, individual MEP amplitudes were eliminated from the data set if that specific amplitude was 4 standard deviations greater than the mean MEP amplitude.

None of the data were eliminated for these reasons.

The direction of the TMS-evoked thumb movement was determined by examining the acceleration vectors in the vertical axis (Stefan et al. 2005). Movements which had a positive acceleration in the vertical axis were considered extension movements.

Movements with a negative acceleration in the vertical axis were considered flexion movements. The total amount of thumb movements in each direction was divided by the total number of thumb movements elicited to determine the percentage of thumb movements in each direction.

Results

A two-tailed paired sample *t*-test was used to analyze the difference in peak-to-peak MEP amplitude of the FPB at rest and during the observation of flexion movements. The data from one participant was not available for this analysis due to a recording error. The initial analysis of the MEP amplitude data did not reveal a significant increase in the size of the MEPs during the observation of flexion actions ($M = 527.03$ mV, $SD = 512.80$) compared to rest ($M = 348.34$ mV, $SD = 256.80$), $t(10) = 1.28$, $p > 0.22$ (Table 1). Detailed inspection of the data, however, revealed extensive inter-participant variability in the responsiveness of M1 during action observation; about half of the participants showed increases in MEP amplitude, whereas the remaining participants did not. A subsequent analysis was done with the participants separated into “high” and “low” responder groups. The analysis of the high-responders showed that there was a

significant increase in the size of the MEPs during action observation ($M = 802.24$ mV, $SD = 558.08$) compared to rest ($M = 310.43$ mV, $SD = 258.22$), $t(5) = 3.28$, $p < 0.05$.

There was no significant difference in the size of the MEPs during action observation ($M = 196.8$ mV, $SD = 134.83$) compared to rest ($M = 393.83$ mV, $SD = 277.10$) for the low-responders, $t(4) = 2.24$, $p > 0.05$.

A paired sample t -test was used to analyze the before and after percentage of TMS-evoked thumb movements in the direction of the training video. There was a significant increase in the percentage of TMS-evoked thumb movements in the training direction from pre- ($M = 13.86\%$, $SD = 12.50$) to post- training ($M = 28.45\%$, $SD = 22.96$), $t(11) = 2.26$, $p < 0.05$ (Figure 2).

To address the specific aim of the present study, a Pearson R correlation was used to determine the relationship between MEP facilitation during action observation (AO) ($MEP_{AO} - MEP_{REST}$) and the effectiveness of action observation in changing the direction of TMS-evoked thumb movements ($\%_{POST} - \%_{PRE}$). Results revealed a high, positive, and significant correlation between the amount of change in the direction of TMS-evoked thumb movements and the change in the amplitude of the MEP during action observation, $r = 0.79$, $p < 0.005$ (Figure 3). Thus, the participants who demonstrated the greatest immediate cortical responsiveness had the greatest change in the cortical representation of action following the training session.

Discussion

We predicted that if the MNS contributes to observational learning, then there should be a relationship between immediate changes in M1 activity during action

observation and the changes that occur after repeated exposure to the action stimulus.

The present study revealed that alterations in the excitability of M1 during action observation correlated with the degree of change in the cortical representation of action induced by repeated action observation. This correlation supports the hypothesis that the MNS is responsible for both the immediate and long-term effects associated with action observation.

Specifically, what has been illustrated in the current study is that participants who demonstrated a facilitation in the size of a MEP during action observation were more likely to have a change in cortical representation of movement following repeated action observation. This relationship suggests that the two effects are linked and have a common neural substrate. We argue that the MNS is the common neural substrate because brain imaging studies have shown that action observation activates neurons in the MNS (premotor and posterior parietal cortices) and primary motor cortex (Rizzolatti & Craighero, 2004). Thus, the neural codes for a specific response are evoked in the motor system when that response is observed. If one considers learning to be a product of changes in synaptic activity and/or cortical reorganization caused by the repeated activation of specific neural networks (Bi & Poo, 2001), then it follows that larger neural activations would lead to a larger reorganization than smaller activations when the number of activations are equivalent. Here, we have established this relationship for observational learning.

An intriguing finding was that, as a group, there was no significant difference in the size of MEP amplitude during action observation. However, our more detailed

analyses revealed that we had high and low responders during action observation. This finding is consistent with our expectations that there would be individual variability in the effects of action observation on MNS activation and, hence, the changes in cortical representation induced via action observation. Individual differences in the effectiveness of observational learning have been noted in behavioural studies (Braaksma, Rijlaarsdam, van den Bergh, & van Hout-Wolters, 2004).

Our finding of individual differences is interesting because it suggests that the sight of the thumb movements did not activate their MNS and/or influence M1 in some participants. These individual differences cannot be explained by a lack of attention to the videos as all participants were successful in counting the number of “oddball” thumb movements in the videos. Individual differences, however, could be related to two factors. First, there may be individual differences in the responsiveness of the MNS during action observation. Second, the type of thumb movements and/or the context in which they were viewed could explain these differences. It has been documented that mirror neuron activation can be modulated by instruction (Zentgraf et al., 2005), intent and meaning (Decety et al., 1997; Grezes & Costes, 1998), and familiarity with the movement (Maeda, Chang, Mazziotta, & Iacoboni, 2001). For example, there has been mixed results in the effectiveness of non-meaningful movements, such as those used in the present study, in activating the MNS. Whereas (Iacoboni et al., 1999) showed MNS activation during the observation of meaningless finger movements, (Jonas et al., 2007) found that meaningless finger movements did not lead to MNS activation in their study.

Future research has been designed to further our understanding of these individual differences.

In sum, the critical finding of the present study was the correlation between the immediate and longer-term effects of action observation. These results suggest that a common neural substrate, the MNS, is responsible for both short- and long-term changes in cortical activity. The MNS has been suggested to be involved in a number of cognitive processes including imitation, communication, and observational learning (Iacoboni & Mazziotta, 2007). The present study provides evidence for a clear link between some of the processes that have been ascribed to the MNS and suggests that action observation and the MNS has a critical role in shaping human behaviour.

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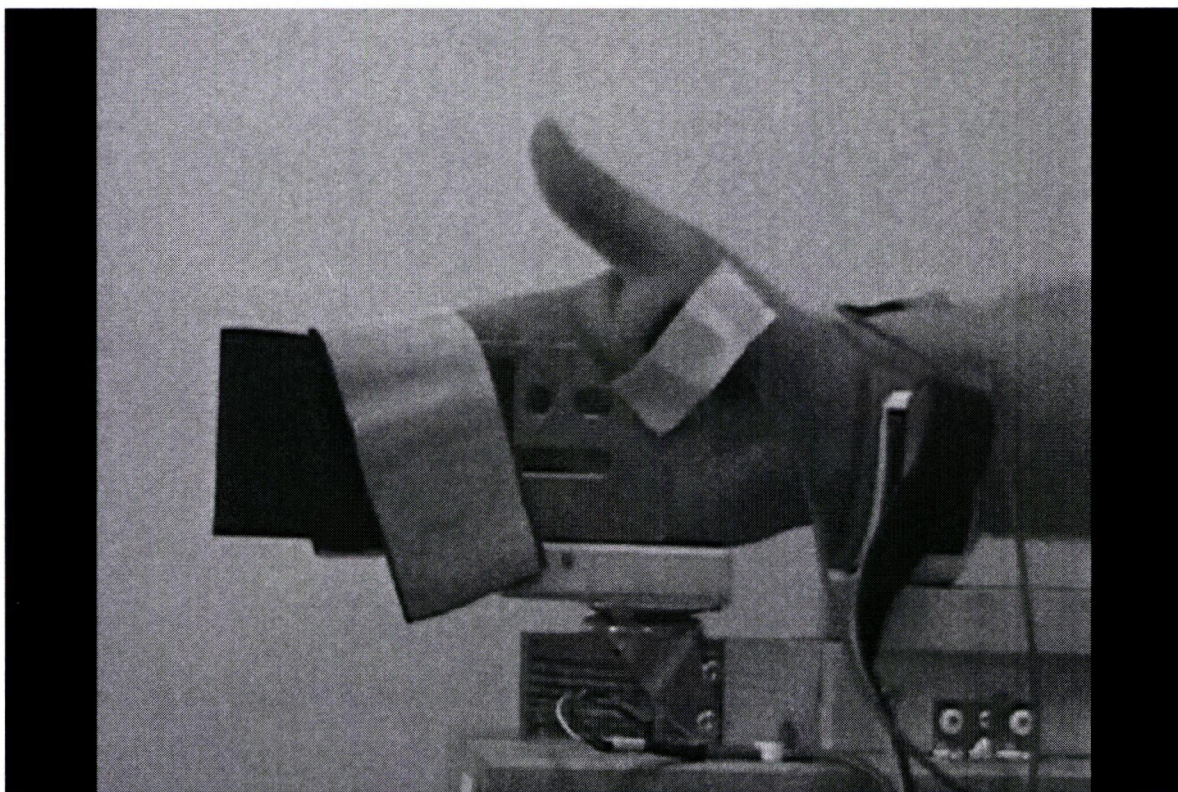


Figure 1. Picture of apparatus.

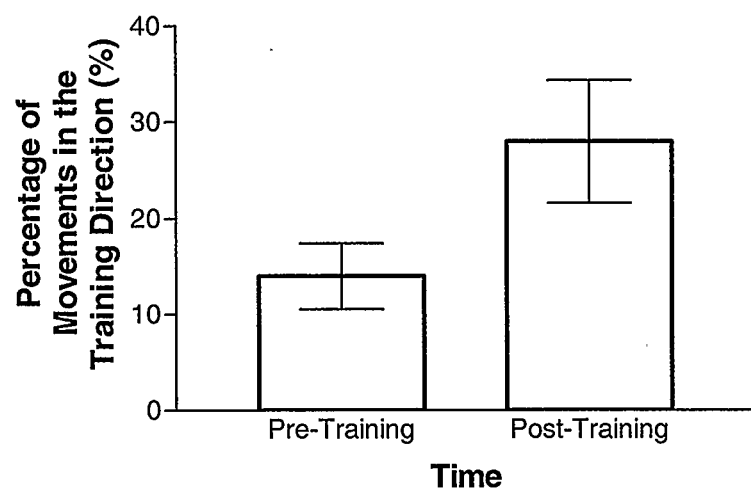


Figure 2. Means of the percentage of TMS evoked thumb movements landing in the training direction pre- and post-training.

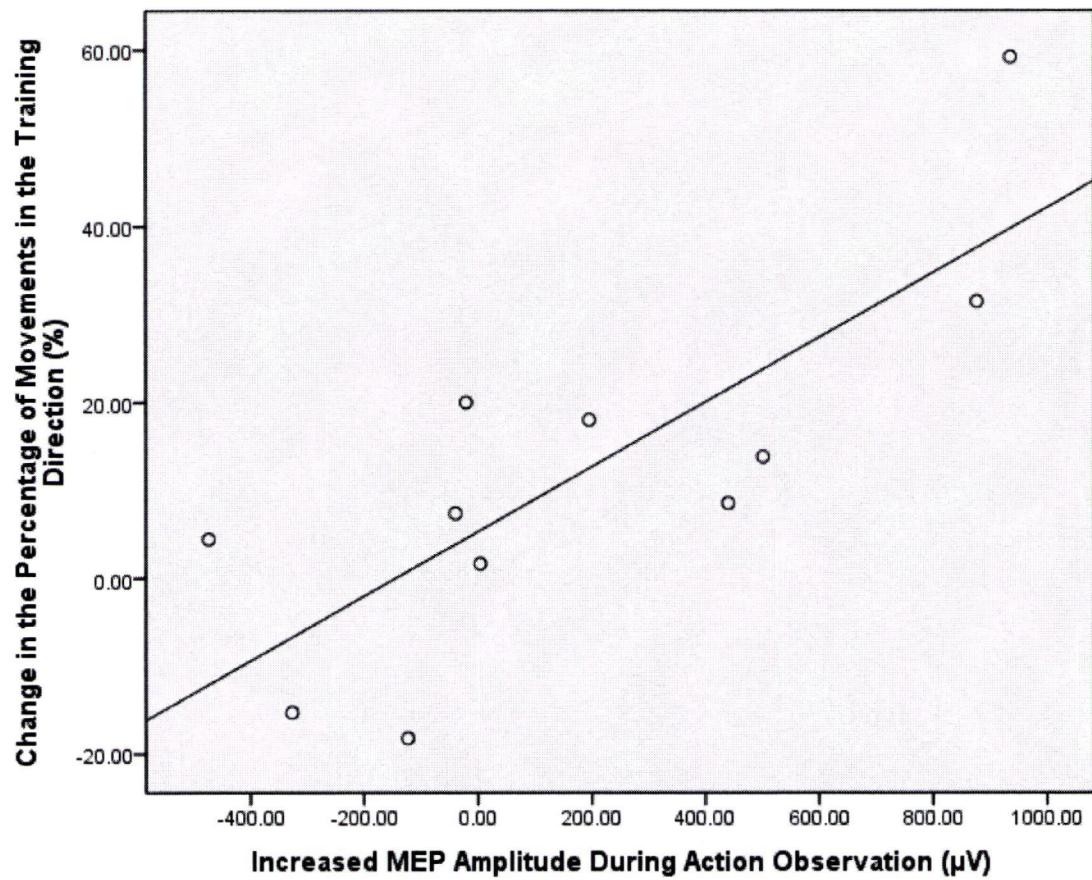


Figure 3. Relationship between the immediate MNS activation during action observation and the effectiveness of action observation in changing the cortical representation of movement.

Table 1

Motor Evoked Potential (MEP) Amplitude at Rest and during
Action Observation

	Rest		Observation	
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
MEP Amplitude	348.34	256.80	527.03	512.80

EXPERIMENT 2

Action Content during Action Observation and its Effect on Observational Learning

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Abstract

Previous research has shown that there is a relationship between the immediate effects of action observation on the primary motor cortex (increased MEPs during action observation) and the effectiveness of repeated action observation in changing the cortical representation of movement (observational learning). The aim of the present study was to use TMS to determine if the action content of an observed action would increase the amplitude of motor evoked potentials and likewise increase the effectiveness of observational learning. The findings of the present study demonstrate that the observation of simple thumb movements on objects did not lead to an increase in the amplitude of MEPs or the effectiveness of observational learning.

Introduction

The mirror neuron system (MNS), a system of neurons that become active during the observation and execution of actions, has been suggested to be the main neural system which facilitates observational learning (Stefan et al., 2005; Vogt & Thomaschke, 2007). Specifically, it is thought that, when an individual observes an action, the MNS generates a representation of the observed action and activates the motor system of the observer. Through repeated observations and associated motor system activations, it is thought that the neural coding of that response is enhanced in the observer in the same way that physical practice enhances that motor representation. Because of this hypothesized role of the MNS in observational learning, an understanding of the factors which affect MNS activation could have implications in maximizing the effectiveness of observational learning.

Investigations of the MNS in humans conducted to understand the factors that modulate MNS activity have used primarily neuroimaging and neurophysiological techniques. The neuroimaging research involving fMRI and PET has shown that the MNS (which consists of the inferior frontal gyrus and inferior parietal lobule) is activated during the observation of actions, the imagery of actions, and the execution of actions (see Rizzolatti & Craighero, 2004 for a review). Of greater relevance to the present paper, neurophysiological studies have typically employed stimulation techniques, such as transcranial magnetic stimulation (TMS), to assess the influence of action observation on the excitability of the motor system. For example, Fadiga et al. (1995) were the first to directly show that action observation alters the activity of motor cortex. In their study,

they demonstrated that action observation can increase the amplitude of motor-evoked potentials (MEPs) recorded from the muscles that would be active during the actual performance of the observed action. This finding indicates that action observation causes an activation of the motor system, which is likely mediated via the MNS. It has been suggested that these immediate modulations in activity provide the basis for social cognitive processes communication, imitation, and the coordination of actions (Rizzolatti & Craighero, 2004).

While the studies reviewed thus far have revealed the immediate effects of action observation on the MNS and motor system, Stefan et al. (2005) was the first to show that repeated action observation can alter the cortical representation of movement in the primary motor cortex in a manner consistent with physical practice. The critical measure in their study was the change in the direction of TMS-evoked thumb movements from pre-training to post-training. Specifically, a baseline direction of TMS-evoked thumb movements was taken at the beginning of the experimental session to determine the initial cortical representation of the thumb muscles in the primary motor cortex. A given participant would, for example, have 80% of the TMS-evoked thumb movements going in the flexion direction and 20% into extension. Such a pattern of thumb movements was taken to indicate that the flexion action dominated the representation of thumb movements in motor cortex prior to training.

Following the assessment of the baseline representation, participants engaged in three different training sessions. In the key training condition, participants watched a video of thumb movements in the opposite direction to their baseline direction. The

direction of TMS-evoked thumb movements was then recorded after training to determine if the cortical representation had changed. A change in cortical representation would be indicated by higher percentage of thumb movements in the training direction (i.e., in the opposite direction to the dominant baseline direction) in the post training assessment than in the baseline assessment. In our exemplar participant, such a change would be indicated by an increase from 20% extension (80% flexion) movements prior to training to 40% extension (60% flexion) movements after training. The key finding of their study was that the direction of TMS-evoked thumb movements changed after this observational training session with more of the movements going into the training direction after training than before training. The authors suggested that the MNS was responsible for inducing this “learning” effect because the learning effect occurred following action observation alone. While this conclusion is consistent with neuroimaging (Grezes et al., 1998) and neurophysiological (Fadiga et al., 1995) work suggesting a relationship between MNS and motor system activation, there was no specific neurophysiological evidence to support their conclusion.

Ray et al. (submitted; see Experiment 1 of the thesis) have recently tested Stefan et al.’s (2005) hypothesis, that the MNS is involved in observation learning, by investigating the relationship between the immediate (e.g., Fadiga et al., 1995) and short-term (Stefan et al., 2005) effects of action-observation on the motor system. Ray et al. hypothesized that, if the MNS is responsible for facilitating both the immediate changes in cortical excitability and the representation in the primary motor cortex, then there

should be a relationship between the immediate (facilitation of MEPs) and short-term (changes in cortical representation) effects of action observation.

Similar to the Stefan et al. (2005) study, Ray et al. recorded a baseline direction of TMS-evoked thumb movements to determine the initial representation of the thumb muscles in motor cortex. To determine the immediate effects of action observation on the primary motor cortex (e.g., Fadiga et al., 1995), MEPs were recorded from the extensor pollicis brevis and the flexor pollicis brevis during the observation of thumb flexion and extension movements. Following the baseline and excitability measurements, participants engaged in a training session in which they observed thumb movements in the opposite direction to the dominant direction determined at baseline. The direction of TMS-evoked thumb movements was recorded again after training to determine if there was a change in cortical representation (e.g., Stefan et al., 2005).

The key finding of Ray et al. was that the immediate effects of action observation (change in MEP amplitude during action observation) were strongly correlated with the short term effects of action observation (change in cortical representation). That is, participants who showed small increases or decreases in MEP amplitude during action observation demonstrated small or no learning effects, while the participants that showed the large increase in MEPs during action observation showed the large learning effects. Ray et al. concluded that this relationship indicates that the effectiveness of action observation in activating the primary motor cortex on a single observation is related to the amount of change in cortical representation following repeated observations (observational learning). The observed relationship is consistent with the hypothesis that

a common neural mechanism, likely the MNS, is responsible for both the immediate and short-term effects of action observation on motor cortex.

What was somewhat puzzling about the Ray et al. results, however, was that there was a group of the participants that showed no increase in MEPs during action observation and no change in the direction of TMS-evoked thumb movements following training. Although some intersubject variability was expected, the imaging and TMS literature had not identified a similar subgroup in the average population that was not responsive during action observation (see, however, the research on people with autism spectrum disorder; (Theoret et al., 2005; Williams, Whiten, Suddendorf, & Perrett, 2001).

A clue to understanding why a subgroup emerged might be found through careful examination of the neuroimaging literature. Although the neuroimaging literature consistently reports MNS activation during action observation, action observation does not always lead to activation in the primary motor cortex (Rizzolatti et al, 1996; Buccino et al. 2001; Jonas et al., 2007). Studies in which action observation caused activation in the primary motor cortex (Grezes et al., 1998; Hari et al., 1998) have used actions on objects and pantomimes of actions on objects as the stimuli. In addition, Decety et al. (1997) found that movements that they classified as having more meaning (pantomimes of actions on objects) caused greater left hemisphere MNS activation. This suggests that action content may modulate MNS activity and/or its effects on the primary motor cortex. Therefore, if TMS is applied to the left hemisphere to elicit movement of the right limb to study changes in MNS activation on the primary motor cortex, increasing action content could potentially lead to greater changes in motor system excitability. Brighina et al.

(2000) reported some data consistent with this conclusion. They showed that increased movement complexity caused a greater facilitation of MEPs when the left hemisphere was stimulated. Thus, it is possible that some individuals in the Ray et al. (submitted) study were not responsive to the videos because the content of the observed action was not sufficiently meaningful to evoke MNS and/or motor cortex activation.

The present study was designed to follow-up on the work of Ray et al. (submitted) by attempting to determine if action content (actions on object versus action without objects) influences the immediate and short term effects of action observation. To this end, assessments of cortical excitability and the changes in the cortical representation of movement were made in different groups of participants who saw videos of thumb movements with and without objects. The “no object” group was essentially a control group and completed the protocol used in the previous Ray et al. study. This group viewed videos of thumb movements without objects during both the immediate phase and the training phase of the study. The “object” group was the experimental group. They differed from the “no object” group in two important ways. First, during the immediate phase of the study, TMS was applied to motor cortex while they watched videos of thumb movements without objects and thumb movements on objects. A comparison between the magnitude of MEPs (the index of motor cortex excitability) during the videos with and without objects provided an index of the immediate affect that action content had on motor cortex excitability. It was predicted that, if action content (simple actions on objects vs. simple actions without objects) affects the activation of the MNS and/or its effect on the motor system, then the observation of actions with objects should elicit

larger MEPs than the observation of actions without objects. The second major difference between the two groups was that the “object” group watched videos of actions on objects during the training sessions, whereas the “no object” group watched videos of thumb movements without objects. A comparison of the amount of change in the cortical representation of thumb movements between the two groups provided an index of the influence of action content on observational learning. It was predicted that if the observation of simple actions on objects leads to a greater MNS and motor system activation, then the observation of simple actions on objects should also lead to greater changes in cortical representation. If this is the case, the “object” group should demonstrate a greater amount of change in cortical representation (i.e., learning) than the “no object” group.

Methods

Participants

Twenty-four subjects (10 women, 14 men), ages 20 – 33 ($M = 24.75$, $SD = 3.32$), were recruited from the University of Calgary population via email and personal communication. Each participant read, completed and signed an informed consent form, a medical history questionnaire, and questions for self-report prior to testing. Twenty-one participants were right-handed and 3 were left-handed. Once the participants filled and signed the forms, they were familiarized with the experimental set-up and the task. The participants were given written and verbal instructions about the task. The study consisted of one experimental session that lasted approximately 2 hrs. The participants

were compensated with a \$20 CAD honorarium. The procedures of the study complied with the ethical standards of the 1964 Declaration of Helsinki regarding the treatment of human participants in research and were approved by the University of Calgary Research Ethics Board.

Recording and stimulation procedures

This study employed similar recording and simulation procedures to those used in the study by Stefan et al. (2005) and Ray et al (see Experiment 1). At the beginning of each session, participants sat in a chair in front of a table and a computer screen. Once the participant was seated, surface EMG electrodes were placed on the extensor pollicis brevis (EPB) and the flexor pollicis brevis (FPB). After the electrodes were in place an infrared light-emitting diode (IRED) from an active marker system (Optotrak Certus) was attached to the tip of the right thumb. The position of the IRED marker was recorded at 500 Hz.

Subjects sat with their right arm resting in the apparatus in a semipronated position with their fingers immobilized and the thumb free to move (Figure 1). EMG recordings were made of the EPB and FPB at a sampling rate of 4000 Hz and signal amplification of 1000 (bandwidth filter of 20-450 Hz). EMG was recorded using a Delsys Bangoli-8 system and custom LabView software for a window that began 100 ms prior to and 1500 ms after TMS stimulation. TMS (Magstim 200) was delivered through a figure-eight coil (70 mm diameter wings) to elicit contractions of the FPB and the EPB. The optimal scalp position was identified using suprathreshold stimulus intensity and marked on the scalp. The resting motor threshold was then quantified as the minimum

stimulator output which elicited a motor evoked potential greater than 50 μ V (peak-to-peak) from the FPB on 5 out of 10 consecutive trials (Rossini et al., 1994). All videos were displayed on a 19" LCD screen approximately 70 cm in front of the participants.

Experimental Design

Participants were divided into two experimental groups. The “no object group” observed thumb flexions and thumb extensions without an object. The “object group” observed thumb flexions on an object (squish a sponge ball) and thumb extensions on an object (flipping a coin). Participants in each group followed a nearly identical experimental design. The key difference between groups was the content of the videos observed during training.

Following the identification of the optimal scalp location and the resting motor threshold, sixty TMS pulses at 115% of the resting MEP threshold were delivered over the region of M1 that elicited thumb movements and MEPs in FPB and EPB while the participants stared at a blank screen. Thumb movements (defined as the change in the position of the IRED) were analysed using custom MatLab software. The initial 60 thumb movements were analyzed to determine the baseline cortical representation of the muscles that controlled the thumb. MEPs from FPB and EPB were also recorded at this point to determine the resting excitability of the motor pathways.

Following these baseline measures, the excitability of M1 during action observation was assessed. Participants in the “no object” group observed a video that consisted of a random order of flexion (Figure 2A) and extension (Figure 2B) movements with no object present. TMS at 115% of resting threshold was delivered over the cortical

representation for the thumb 500 ms after the thumb movement in the video was initiated. The TMS pulse coincided with the completion (maximal vertical displacement) of the thumb flexion or extension. This protocol provided a measure of the immediate changes in excitation following action observation. Participants from the “object” group observed two different sets of videos during the assessment of the influence of action observation on primary motor cortex excitability. The first set of videos consisted of a random order of flexion (Figure 2A) and extension (Figure 2B) movements with no object present. The second set consisted showed a ball being squished (Figure 2C) by the thumb (thumb flexion) and a coin being flipped (Figure 2D) (thumb extension). The video of thumb movements without an object was always presented first so that the observation of actions on objects did not provide any content or meaning to these meaningless movements.

The observational learning training session consisted of 9 blocks of 200 trials (lasting approximately 3.5 min. each). The “no object” group observed a video of a simple thumb flexion or extension, whereas the “object” group observed a video of the thumb flipping a coin or squishing a foam ball. Each trial contained one thumb movement and trials were presented at a rate of 1 Hz. Participants were told to relax their hand muscles and to watch a video, which consisted mostly of thumb movements in the opposite direction to that determined at baseline (i.e., in the flexion or extension direction). To ensure that the participants were focusing their attention on the movement on the screen, 6% of the trials showed a video of a thumb movement in the opposite direction to the other thumb movements and the participants were required to count these

oddball trials. The average error in recognizing the oddball movements was 0.01% across all participants with the maximum individual amount of error at 0.05%. The hand, in both videos that the “object group” observed and the one video that the “no object group” observed, was placed on top of a table with a blank wall in the background. The thumb in the video was the model’s thumb of the right hand. The thumb was seen from a side view with the hand in a semipronated position so that the participants could clearly see the thumb moving into flexion or extension through the sagittal plane (see Figure 2A, 2B, 2C, 2D). To ensure that the participants were not physically practicing the thumb movements, EMG of the FBP and EPB were constantly monitored and participants were reminded to relax on the rare occasion that an increase in EMG was detected. Following the 9 training blocks, a post-training measure of the cortical representation of thumb movements was taken by applying 60 TMS pulses to M1 at rest while participants viewed a blank screen. The direction of these TMS-evoked thumb movements were recorded and analysed.

Data Reduction

Because of recording difficulties (TMS stimulus artefact), we were unable to analyze MEPs recorded from EPB for the “no object” group. As such, all MEP analysis for this group is based on MEPs evoked from FPB. The MEP analysis for the “object” group was based on MEPs evoked from FPB and EPB. The excitability of M1 was determined by examining the mean peak-to-peak amplitudes of the MEPs. Peak-to-peak amplitude was defined as the difference between the highest and lowest voltage for each MEP from approximately 19-60 ms after TMS delivery (exact time range differed for

each participant). Prior to the calculation of mean peak-to-peak amplitude, two outlier procedures were conducted. First, because of the known relationship between voluntary muscle activation and MEP amplitude), individual MEP amplitudes were eliminated from the data set if the mean background EMG in the epoch 5-85 ms prior to TMS delivery was greater than 3 standard deviations above the mean background EMG for all trials during that time. Second, to eliminate the influence of outliers due to random influences, individual MEP amplitudes were eliminated from the data set if that specific amplitude was 3 standard deviations greater than the mean MEP amplitude. 5 % of the data were eliminated for these reasons.

The direction of the TMS-evoked thumb movement was determined by examining the acceleration vectors in the vertical axis. Movements which had a positive acceleration in the vertical axis were considered extension movements. Movements with a negative acceleration in the vertical axis were considered flexion movements. The total amount of thumb movements in each direction was divided by the total number of thumb movements elicited to determine the percentage of thumb movements in each direction.

Results

Influence of Action Observation on Cortical Excitability

Object Observation Group.

To address the influence of action content on the amplitude of MEPs recorded during action observation, a within-subjects one-way (rest, object observation, no object observation) ANOVA was used to analyze the difference in peak-to-peak MEP amplitude of the FPB at rest, during the observation of flexion movements on an object, and during

the observation of flexion movements without an object. The main effect for the action content on the MEP amplitude data did not reveal a significant increase in the size of the MEPs during the observation of flexion actions, $F(2, 11) = 1.88, p > 0.05$ (Table 1). Likewise, a within subjects one-way (rest, object observation, no object observation) ANOVA was used to analyze the difference in peak-to-peak MEP amplitude of the EPB at rest, during the observation of no object extension movements and object extension movements. The main effect for the action content on the MEP amplitude data did not reveal a significant increase in the size of the MEPs during the observation of extension actions, $F(2, 11) = 2.53, p > 0.05$.

No Object Observation group.

A two-tailed paired sample t -test was used to analyze the difference in peak-to-peak MEP amplitude of the FPB at rest and during the observation of flexion movements. Curiously, the analysis of the MEP amplitude revealed a significant decrease in the size of the MEPs during the observation of flexion actions ($M = 336.43 \mu V$, $SD = 309.03$) compared to rest ($M = 466.68 \mu V$, $SD = 384.39$), $t(11) = 3.06, p < 0.05$ (Table 1).

Influence of Repeated Action Observation on the Change in Cortical Representation

A 2 (Time: before, after) by 2 (Group: object group, no object group) mixed ANOVA, with Time as the repeated measures factor and Group as the between-subjects factor, was used to analyse differences in the before and after percentage of TMS evoked thumb movements from baseline to post training (change in cortical representation). Interestingly, there was no significant main effect for Time, indicating that there was no significant change in the direction of TMS evoked thumb movements from baseline to

post training, $F(1, 22) = 3.51, p > 0.05$ (Figure 3). There was no main effect for Group, indicating that there was no significant difference between groups, $F(1, 22) = 0.57, p > 0.05$. Finally, there was no significant interaction between Group and Time, $F(1, 22) = 0.92, p > 0.05$. The non-significant interaction indicates that there was no significant difference between groups in the change of direction of TMS evoked thumb movements from baseline to post training.

Relationship Between the Immediate and Short Term Effects of Action Observation on M1

Object Observation Group.

A Pearson R correlation was used to determine the relationship between MEP facilitation during the object action observation condition (OAO) ($MEP_{OAO} - MEP_{REST}$) and the effectiveness of action observation in changing the direction of TMS-evoked thumb movements ($\%_{POST} - \%_{PRE}$). Results revealed a positive, non-significant correlation between the amount of change in the direction of TMS-evoked thumb movements and the change in the amplitude of the MEP during action observation, $r = 0.27, p > 0.05$ (Figure 4).

No Object Observation Group.

A Pearson R correlation was used to determine the relationship between MEP facilitation during action observation (AO) ($MEP_{AO} - MEP_{REST}$) and the effectiveness of action observation in changing the direction of TMS-evoked thumb movements ($\%_{POST} - \%_{PRE}$). Results revealed a negative, non significant correlation between the amount of

change in the direction of TMS-evoked thumb movements and the change in the amplitude of the MEP during action observation, $r = 0.12$, $p > .05$ (Figure 5).

Discussion

The purpose of the present study was to investigate the influence of action content on the immediate and short term effects of action observation on the motor system. Previous neuroimaging research has shown that action on objects or pantomimes of action on objects causes activation in the primary motor cortex (Hari et al., 2000) and a stronger left hemisphere MNS activation (Decety et al., 1997). It has been suggested that the increases in activity occurred because actions on objects have more meaning than actions without objects. Based on this research, it was hypothesized that the addition of an object to a simple thumb movement would lead to an increase in the amplitude of TMS MEPs, and to greater changes in cortical representation following repeated action observation. The results of the present study indicate that action content (the addition of an object to simple thumb movements) did not cause a significant increase in MEPs during action observation, or induce a significantly greater change in cortical representation. Therefore, it is concluded that, while the presence of an object or pantomimes of actions on objects may modulate the activity of the MNS (Hari et al., 2000; Decety et al., 1997), it did not differentially affect the activation of motor cortex, and the changes in the cortical representation of action associated with observational learning in the present study. The following discussion will highlight some of the potential reasons why action content may not have influenced the immediate and short term effects of action observation on motor cortex.

Action Content, Complexity, and Context

The addition of an object to a simple thumb movement was hypothesized to increase the meaning of the movement for the observer and increase activation of the MNS and the motor system. It is possible, however, that the addition of an object to the movements used in the present study did not convey more meaning than the movements without objects. The movements used in the Decety et al. (1997) study, which found differential effects of action content, were pantomimes of actions on objects, which conveyed a clear meaning and purpose, such as sewing a button. In the present study, however, the actions on objects were the flipping of a coin and the squishing of a sponge ball and there was no additional contextual information provided to the participants as to why these objects were being acted upon. Therefore, although the present results indicate that modulating action content (adding an object to a movement) does not cause a greater facilitation of MEPs during action observation, or learning following repeated action observation, it is possible that increasing the information conveyed by these actions, or using other movements which convey more meaning might have a more beneficial effect.

Other factors that could have lead to the absence of object-based modulation of the motor system in the present study are the complexity of the observed movements and/or the hemisphere that was stimulated. Brighina et al. (2000) have shown that the observation of complex movements leads to a greater facilitation of MEPs when the left primary motor cortex is stimulated, whereas the observation of simple movements caused a greater facilitation of MEPs when the right hemisphere is stimulated. In the present

study, the left motor cortex was stimulated and the observed movements were simple flexion/extension movements of the thumb. Because the left hemisphere is more responsive during the observation of complex actions, it is possible that the movements in the present study were too simple to elicit a significant difference in left hemisphere activation. Therefore, an increase in motor system activation might have been detected if the observed movements in the present study were more complex or if the right hemisphere was stimulated.

A final possibility is that contextual differences may also play a role in the influence of action observation on the motor system. Ray et al. (submitted) have previously investigated the effects of observing a thumb flexion or extension on facilitating the amplitude of MEPs. Their initial inspection of the data did not reveal a significant increase in MEPs during action observation. When the participants were divided into subgroups of high and low responders, however, there was a significant difference in the size of MEPs at rest and during the observation of action for the high responders only. In contrast, the “no object” group in the present study demonstrated a significant decrease in MEPs whereas the “object group” showed neither a significant increase nor decrease in MEPs. One key difference between these two studies was the context of the thumb movements in the videos. In the previous study, the video showed an individual moving the thumb with the hand positioned in the actual apparatus the participants were in during the study. In contrast, in the video used in the present study the hand was placed on a table and no cues related to the experimental set up were provided. It is possible that the absence of these contextual cues in the video in the

present study negatively affected the ability of the individual to simulate the observed action in the video. Thus, contextual difference could explain why significant changes in the amplitude of MEPs during action observation were found in one study and not the other.

Individual Differences

In the Ray et al. (submitted) study, although there was a significant positive correlation between increases in MEPs and the change in cortical representation, some individuals showed decreases in cortical excitability during action observation and little or no learning and other individuals showed increases in cortical excitability during action observation and positive changes in the cortical representation of movement. Thus, there were large individual differences in the responsiveness of motor cortex during action observation. Interestingly, this correlation was not replicated in the present study. Although in the “object” group there was a trend for participants who had shown a facilitation of MEPs to have a larger change in the cortical representation, the relationship was not as strong as that seen in Ray et al. (submitted).

Another possible explanation for the absence of learning effects and a breakdown in the relationship between the immediate and short term effects of action observation on the motor system is individual differences in the efficiency of transforming the effects of immediate action observation into the longer term effects of action observation. Previous research has shown that although the MNS is activated during action observation, MNS activation may not always influence the primary motor cortex (Rizzolatti et al., 1996; Buccino et al. 2001; Jonas et al., 2007). Thus, for the individuals in the present study, the

motor cortex may not have been activated during action observation. Alternatively, it could be that the excitatory influence from MNS may be inhibited by another system. The possibility that the MNS was active, but that its influence was inhibited in primary motor cortex is particularly relevant for the “no object” group who showed a significant decrease in the size of MEPs during action observation in comparison to baseline. Interestingly, this was not the case for the participants in the other group who watched the same video. This difference in the patterns of neural responses across groups demonstrates that there is marked variability in the effects of action observation on different individuals and it is still unclear why these differences exist. However, it is quite possible that these individual differences lead to the failure to replicate the clear relationship between the immediate and short-term effects of action observation reported in the previous Ray et al. (submitted) study.

Another way in which individual differences could influence the immediate and short term effects of action observation on the motor system, is through changes in the responsiveness of an individual’s MNS and/or motor cortex during action observation throughout the course of the study. For example, some participants might not have been responsive to the video when they initially viewed the video, but after numerous repetitions, the observation of the videos could have begun to activate the MNS and therefore affect the motor system. Such individuals would demonstrate small increases or decreases in the magnitude of MEPs during the initial phase of the study, but would demonstrate a significant degree of learning by the end of the training phase.

Alternatively, individuals who were initially responsive to the video might have

habituated to the video over the extended training period. These individuals would demonstrate large increases in MEPs during initial phase of the study, but little or no learning at the end of the training period. While there is no clear answer to this question at present, it is possible that the relationship between the immediate effects of action observation and the short term effects could be modulated by individual differences in the efficiency of transforming activated representations into a new cortical representations and potential changes in this process after repeated exposure.

Conclusion

In conclusion, the present study revealed that the addition of an object to a simple thumb movement did not significantly increase the amplitude of MEPs during action observation or the degree of learning following an extended period of action observation. While it remains an open question, it is possible that contextual differences, simplicity of the observed movement, and a possible lack of meaning might have contributed to the null effects. Additionally, the present study indicates that there may be additional factors that can modulate the relationship between the immediate and short term effects of action observation and changes in the cortical representation of the thumb. Factors influencing this relationship could be the individual's efficiency in developing new cortical representations and changes in the responsiveness of the individual to the observed action over time. Future research will be conducted to explore these possibilities.

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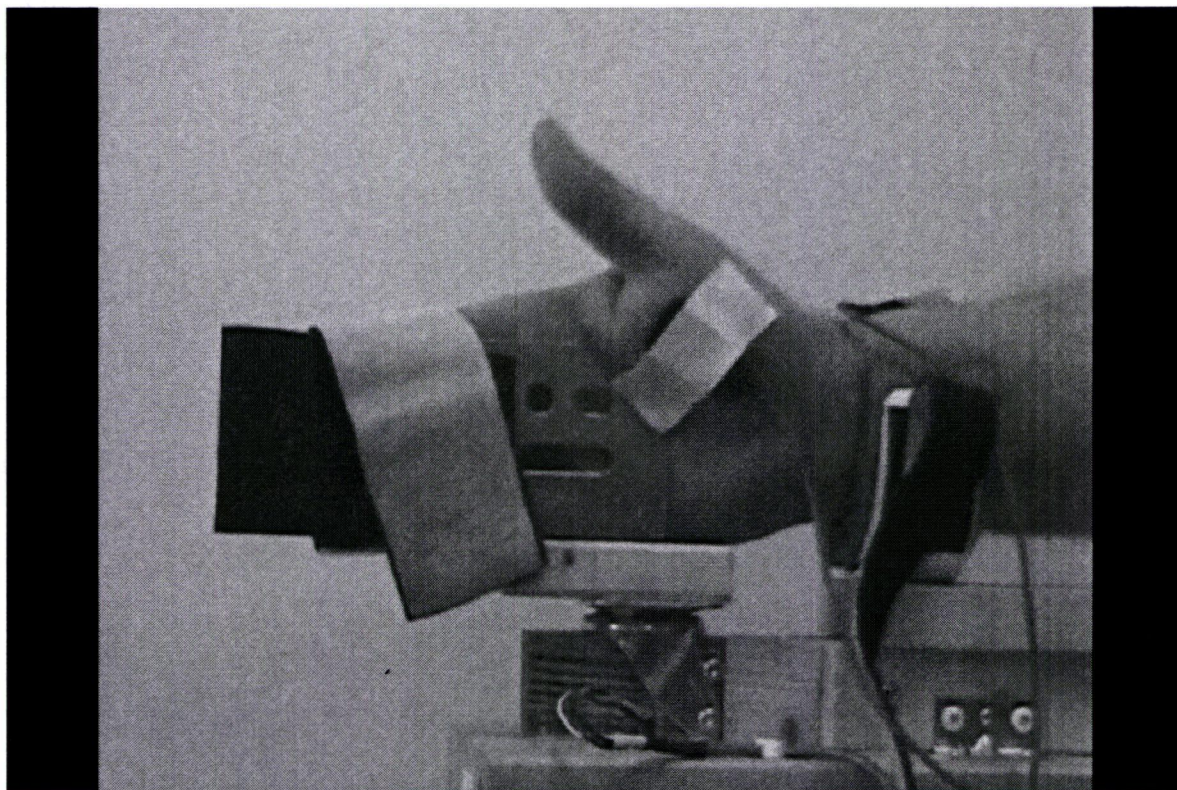


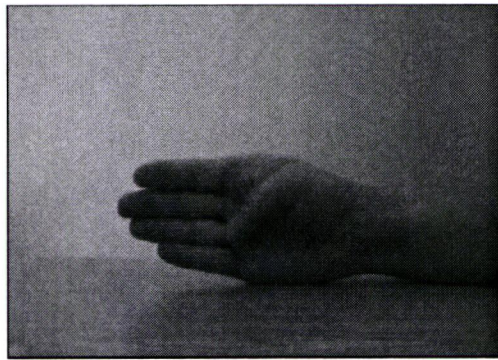
Figure 1. Picture of apparatus.



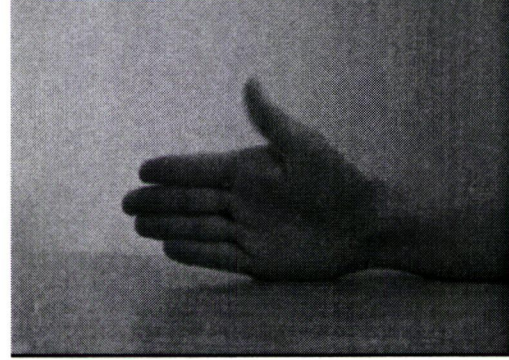
A)



B)



C)



D)

Figure 2. Pictures of thumb movements viewed during action observation: A) ball squish, B) coin flip, C) thumb flexion, D) thumb extension.

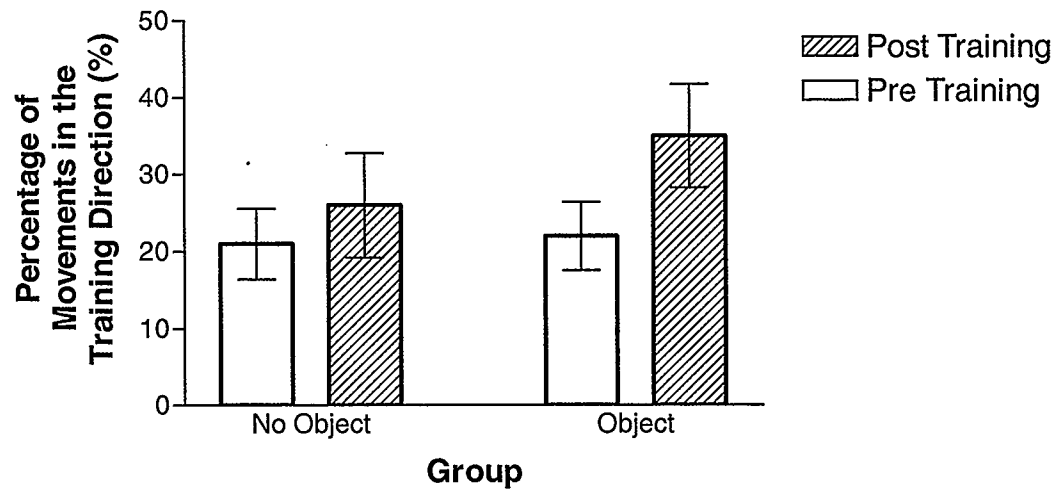


Figure 3. Influence of Repeated Action Observation on the Change in Cortical Representation.

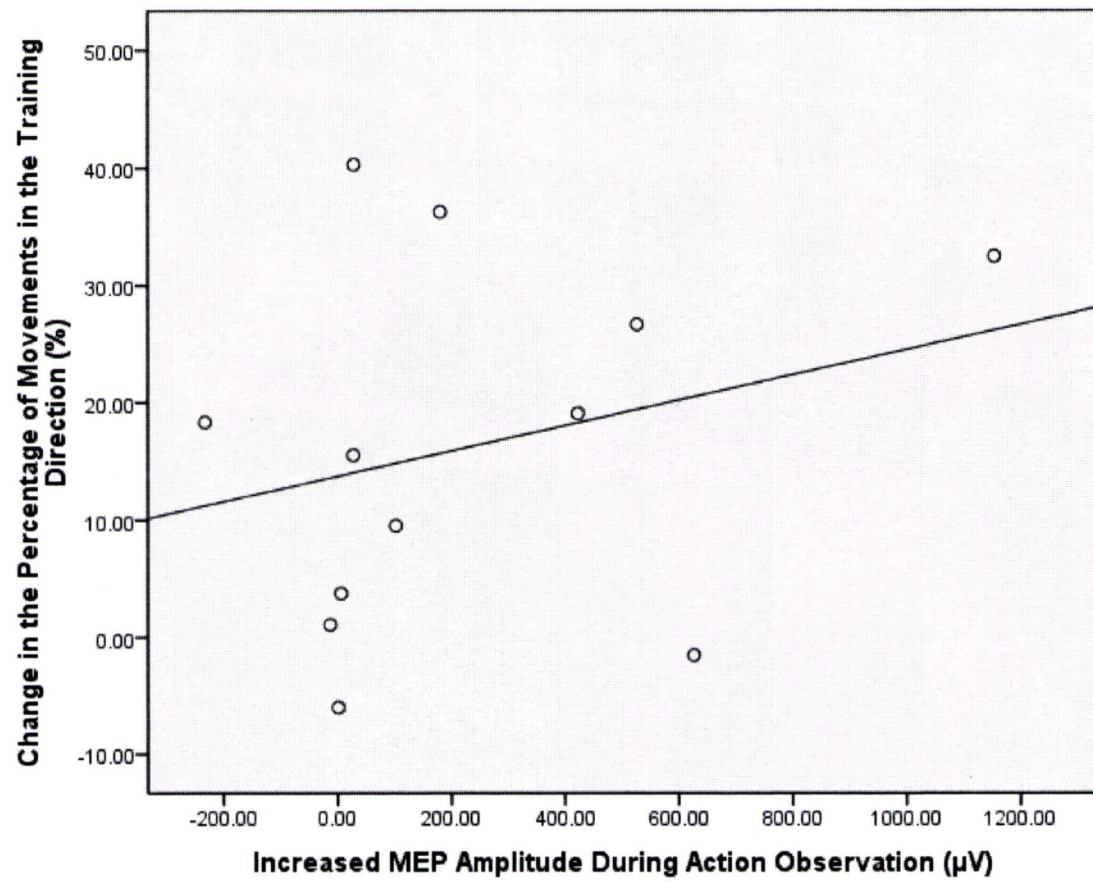


Figure 4. Relationship between the immediate and short term effects of action observation on the primary motor cortex during the observation of actions with objects.

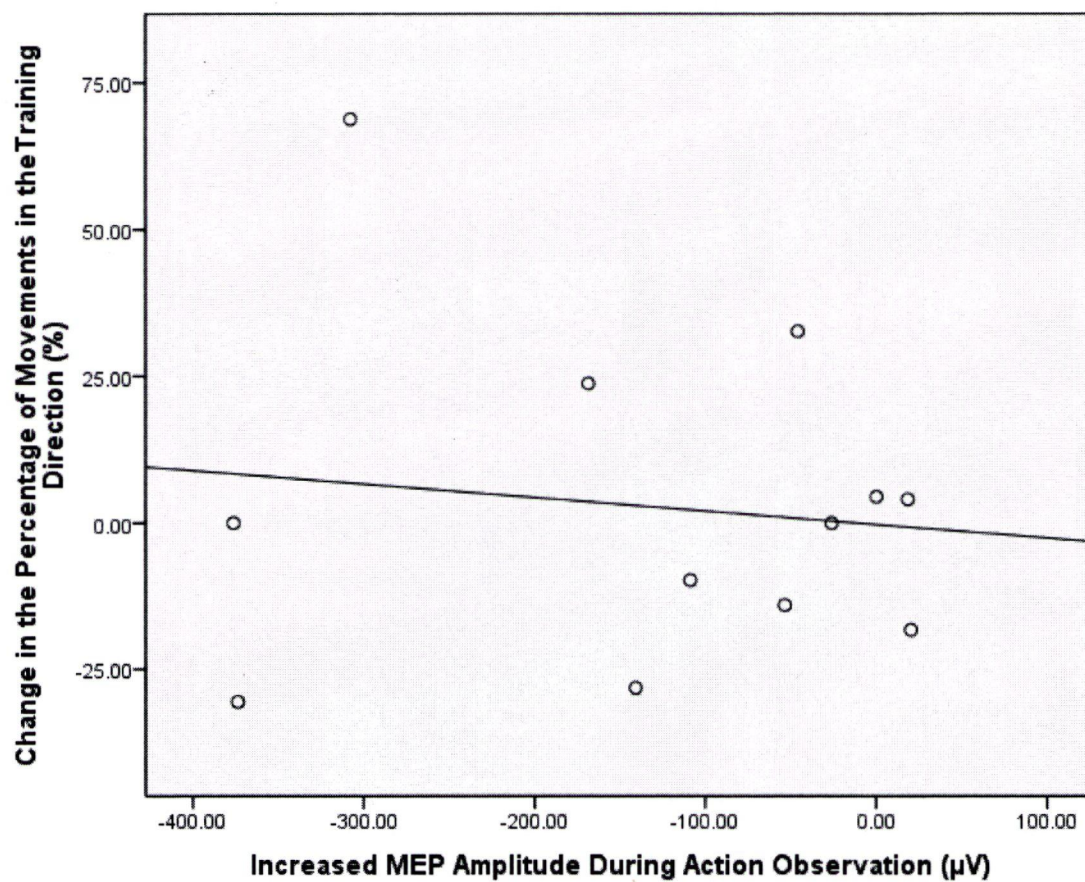


Figure 5. Relationship between the immediate and short term effects of action observation on the primary motor cortex during the observation of actions without objects.

Table 1

Motor Evoked Potential (MEP)Amplitude at Rest and during
the Observation of Thumb Movements

	Rest		No Object Observation		Object Observation	
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
MEP Amplitude Object Group	439.67	487.31	556.56	419.17	675.11	848.77
MEP Amplitude No Object Group	460.35	384.39	334.32	309.32	NA	NA

General Discussion

The general purpose of the experiments reported in the present thesis was to investigate the role the mirror neuron system (MNS) may play in observational learning. Observational learning is an effective method of skill acquisition and development. In observational learning, the observer uses information from an actor they are watching perform a task to develop or improve their own actions. The MNS has been put forward as a candidate for facilitating this process based on the findings that the observation of an action activates the same network of neurons that are involved in the execution of the observed action (Stefan et al., 2005; Vogt & Thomaschke, 2007). Thus, the MNS may provide a direct interface between the perception and execution of action. In the case of observational learning, the activation of response codes through observation may replace the voluntary activation of these response codes during physical practice and induce a learning effect.

In the present thesis, the potential role of the MNS in observational learning was examined by using transcranial magnetic stimulation (TMS) to assess the influence of action observation on the excitability and coding of movements in motor cortex. The main hypothesis was that if the MNS is responsible for activating the motor system during action observation and observational learning that occurs through repeated activations, then there should be a relationship between the magnitudes of the immediate and short-term effects of action observation on motor cortex as assessed through TMS. While the results of Experiment 1 supported this hypothesis, the results of Experiment 2

were not as clear. The following is a discussion of the results of the present studies and how they contribute to the existing literature on MNS activation.

The Relationship between the Immediate and Short Term Effects of Action Observation

The specific purpose of Experiment 1 was to investigate the potential involvement of the MNS in observational learning by looking at the relationship between the immediate (facilitation of MEPs) and short term effects (change in cortical representation) of action observation. Because both the immediate and short-term effects of action observation have been attributed to MNS influence on the motor system, it was hypothesized that the immediate and short term effects of action observation should be strongly related. In support of this hypothesis, the results of Experiment 1 revealed that there was a strong and positive correlation between the amplitude of MEPs recorded during action observation and the magnitude of change in the direction of TMS-evoked thumb movements following repeated observations. Thus, the data suggest that the observation of movements which consistently activate strong internal representations in primary motor cortex are able to elicit greater temporary changes in the cortical representation of movement. This is the first study to demonstrate that there is a strong positive correlation between the immediate and short term effects of action observation on the motor system. This finding supports the hypothesis that the MNS is involved in observational learning.

Experiment 2 was conducted to build on the results of Experiment 1 by determining if increases in action content, a factor known to modulate MNS activation (Decety et al., 1997), improves observational learning. In contrast to predictions,

increased action content did not result in increased motor cortex activation or learning. In addition, the strong positive correlation between the immediate and short term effects of action observation was not replicated. Initially the results regarding the relationship between the immediate and short term effects of action observation on the motor system appear to be inconsistent with Experiment 1. The differences in the findings of these two studies have suggested some new questions such as: How is it possible to have a change in cortical representation if the observation of action did not lead to the activation of the movement representation in the primary cortex? Why did some individuals who showed responsiveness to the videos not demonstrate a significant change in the direction of TMS evoked thumb movements? While there is no clear answer to these questions at this point, it is likely that individual differences, in part, could account for the different pattern of results in Experiments 1 and 2.

One possible explanation for the lack of a correlation between the immediate and short term effects of action observation on the motor system in Experiment 2 is that participants had different patterns of short and long-term responsiveness to the observed actions. For example, some participants might not have been initially responsive to the video, but the video started to activate the observer's internal representations as the number of viewings increased. Such an individual would have low levels of MEP modulation during the initial phase of the study, but demonstrate a fair degree of learning by the end of the study. On the other hand, other participants might have had the opposite pattern of responsiveness in that they were initially highly responsive, but habituated over the course of the study. These individuals might be the participants who

showed an increase in the amplitude of MEPs during action observation, but no changes in the direction of TMS evoked thumb movements. Because no studies thus far have explored the time course of activation in MNS and the motor system over extended periods of time, a future area of research should focus on understanding how the effects of action observation on the primary cortex might change over time. Such research would provide a clearer picture of the relationship between the MNS and observational learning.

Another factor that might have influenced the relationship between the immediate and short term effects of action observation could be individual differences in the rate of learning. For example, it might be that the amount of time and the total number of trials used in the present studies were insufficient to alter the cortical representations in some participants. For these individuals, although action observation might have activated a movement representation, it is possible that the activated representation was unable to create a change in cortical representation over the period of time of the training. The different time course of learning might account for participants who might have been responsive during the immediate action observation phase, but demonstrated different levels of learning by the end of the training period.

Taken together, the results of Experiments 1 and 2 suggest that there is a relationship between the immediate and short term effects of action observation. These data also suggest that the MNS is likely involved in observational learning. It is important to note, however, that the results of Experiment 2 suggest that the effectiveness of action observation in changing the cortical representation of movement may also be modulated

by changes in an individual's responsiveness to the observed actions and their rate of learning.

The Influence of Action Content on the Immediate and Short Term Effects of Action Observation

If the MNS is responsible for both the immediate and short term changes in motor cortex (as suggested by the relationship between the effects of action observation on the motor system and changes in cortical representation seen in Experiment 1), then understanding the various factors that could influence the activation of the MNS is critical. The purpose of Experiment 2 was to investigate whether action content, specifically the addition of an object to a thumb flexion and extension, would influence the immediate (facilitation of MEPs) and short term (change in cortical representation) effects of action observation. It was hypothesized that if the action content of an observed action modulates the influence of the MNS on the primary motor cortex, then a larger facilitation of MEPs would be observed during the observation of actions on objects and, subsequently, a greater change in cortical representation would occur during the repeated observation of actions on objects. The findings from Experiment 2 were not consistent with this hypothesis in that the addition of an object to thumb flexion and extension movements did not cause a significantly greater facilitation of MEPs when compared to rest and during the observation of thumb movements without objects. In addition, the repeated observation of thumb movements with objects did not cause a significantly greater change in cortical representation when compared with the repeated observation of thumb movements without the object. While it is likely that individual

differences played a role in absence of effects in Experiment 2 (see above), a number of methodological issues may also have play a role.

The Influence of Action Content on Hemispheric Activation

The finding that action content did not lead to significantly greater MEPs during action observation was quite surprising. Decety et al. (1997) have previously investigated the influence of action content on MNS activation using PET. They reported that the observation of meaningless movements (derived from the ASL) led to greater right hemisphere activation of the MNS, whereas the observation of meaningful movements (pantomimes of actions on objects such as sewing a button) led to greater changes in the left hemisphere MNS activation. This pattern of activation suggests that when investigating the effects of action observation on the amplitude of MEPs, observing actions on objects, would convey more meaning and should lead to a greater facilitation in MEPs when the left primary cortex is stimulated. In the present study, even though the left hemisphere was stimulated, the addition of an object did not significantly increase MEP amplitude. It is possible that the absence of an object effect occurred because the action on the object was still fairly simple. It might not have conveyed more meaning than the movements without objects. Thus, without additional contextual information, the addition of an object did not increase the meaning of the observed movements sufficiently. For instance, if observing the coin flip actually conveyed some information regarding the observer's situation (i.e., heads they win and tails they lose), then the addition of the coin would certainly convey more meaning. With additional contextual information, it is possible that the effect of object would emerge. Future research that

includes the observation of movements that convey more meaning based on contextual differences could provide a more thorough understanding of the influence of action content on the immediate effects of action observation on the motor system

Another explanation for the discrepancy between the Decety et al. (1997) study and those of Experiment 2 is that the effects from their study might actually be explained by differences in complexity and not meaning. Brighina et al. (2000) have previously shown that the observation of complex actions causes greater left hemisphere MNS activation, whereas the observation simpler movements causes a greater right hemisphere MNS activation. The thumb movements with and without objects observed in Experiment 2 were simple single digit actions and, hence could be modulating right hemispheric activity. TMS was applied to the left hemisphere in the present study. Therefore, it is possible that action content might have had a different influence on the immediate effects of action observation on the motor system if the right hemisphere was being stimulated with TMS in the Experiment 2.

While action content was not associated with the predicted effect on the magnitude of MEPs, it should be noted that action content likewise did not affect the short-term changes in motor cortex. Although the absence of an influence of action content on learning is not consistent with the predicted increase in learning, it is consistent with the finding that action content did not significantly influence the immediate effects of action observation. That is, if action content is not affecting the primary motor cortex during a single observation, then it is unlikely that repeated action observation would cause a greater change in cortical representation. Thus, overall, the

findings Experiment 1 and 2 regarding the relationship between the immediate and short term effects of action observation on the motor system indicate that both processes share a common neural mechanism.

Intersubject Variability in the Immediate Effects of Action Observation on the Motor System

In Experiment 1, the observation of thumb movements had a remarkably different effect among participants. It was found that there was a high responding group who had a significant facilitation of MEPs during action observation and there was a low responding group who did not display significant facilitation of MEPs during action observation. In Experiment 2, the observation of thumb movements without an object did not cause a facilitation of MEPs for participants from the “object group”, whereas participants from the “no object group” displayed a significant decrease in the amplitude of MEPs. Taken together, the findings from Experiment 1 and 2 demonstrate that the observation of an action influences an individual’s motor system in different ways. The following discussion will highlight some of the potential reasons why this variability in responses might occur.

Salience of the Observed Movement

It has previously been shown that observing movements, which we practice and are familiar with, cause the greatest activation in the MNS (Calvo-Merino et al., 2005). Although the thumb is used every day, either on its own or in conjunction with other digits, it is typically not used in the manner demonstrated during the videos. For instance, when typing, the thumb is actually abducting to press the space bar. Individuals

who play a lot of video games or do a lot of text messaging could be more responsive to the thumb flexion and extension movements than those who do not. Therefore, the observation of a thumb movement, which has no saliency to a particular individual, might not activate the MNS, whereas for those individuals who find the movement salient the MNS could become active. This could explain some of the intersubject variability seen in Experiments 1 and 2. This suggests that it may be beneficial to take inventory of an individual's movement history to determine the salience of particular movements used in action observation paradigms.

Inhibition of the Influence of Action Observation on the Motor System

For the individuals in Experiment 2 who demonstrated a decrease in the amplitude of MEPs during action observation, it is also possible that the MNS was activated but, as opposed to sending a facilitatory response to the motor system, it might be that a strong inhibitory response was sent. Alternatively, the inhibitory signal might have been sent from another area to motor cortex to prevent an overt motor response. As previously mentioned, PET and fMRI studies do not always show a significant activation of the primary motor cortex during action observation (Buccino et al., 2001; Rizzolatti et al., 1996). Thus, it could be that the motor cortex had not been activated because the participants were inhibiting activation that might be evoked by the MNS to prevent this excitation from eliciting unwanted movements. Therefore, it is possible that the decrease in MEPs during action observation was caused an inhibition placed on the MNS activity.

Effects of the Orientation of the Observed Actions

Another factor that might affect an individual's responsiveness to the presentation of the thumb movements is the orientation of the observed movement relative to the observer. Maeda et al. (2001) have shown that when observing our own movements, there is a greater facilitation in the size of MEPs when the movements are viewed from a self-away orientation and that when observing other individuals movements a towards-self orientation leads to a greater facilitation of MEPs. In the present studies, neither a self-away nor a towards-away orientation was used. Instead, a 90 degree from observer orientation was employed to remain consistent with the Stefan et al. (2005) protocol. Therefore, it could be that some participants had problems mentally rotating the movements onto a mapping of themselves or someone else. This difficulty could then prevent MNS activation due to the observer's inability perceive the movements in orientations with which they are familiar.

Conclusions

In summary, the present thesis demonstrates that there is a relationship between MNS activity and the change in cortical representation induced via action observation. However, the data also suggest that this relationship may be modulated by individual differences in the participant's ability to form or alter cortical representations, and the individual's interpretation of the meaning, complexity, orientation and the saliency of the observed movement. Future research should explore these individual differences and the influence of action content on the immediate and short term effects of action observation. For example, the influence of action content could be investigated by including an

additional video which has more meaning based on contextual information (observing a coin flip which conveys winning or losing a particular situation). One limitation of the present research is that the strong positive correlation between the immediate and short term effects of action observation on the motor system was not replicated in Experiment 2. To further understand the relationship between the immediate and short term effects of action observation on the motor system, a recording of the MEPs during action observation could be taken in between blocks during the observation training. This would provide information about changes in the individuals' responsiveness to the observed movement over time. Another limitation in the current study was the variability in the responsiveness of individuals to the same video. To reduce this problem, future research studies should consider developing inclusion criteria based on movement history.

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Appendix A



FACULTY OF UNIVERSITY OF
MEDICINE CALGARY

2007-02-15

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Dear Dr. Welsh:

RE: Does activation of the mirror neuron system predict the effectiveness of observational learning.

Ethics ID: E-20701

The above-named research, including the Questionnaire (Medical History Questionnaire and Questions for Self Report), Consent Form (Version 2 dated January 04, 2007), and Protocol has been granted ethical approval by the Conjoint Health Research Ethics Board of the Faculties of Medicine, Nursing and Kinesiology, University of Calgary, and the Affiliated Teaching Institutions. The Board conforms to the Tri-Council Guidelines, ICH Guidelines and amendments to regulations of the Food and Drugs Act re clinical trials, including membership and requirements for a quorum.

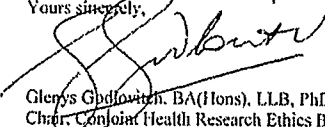
You and your co-investigators are not members of the CHREB and did not participate in review or voting on this study.

Please note that this approval is subject to the following conditions:

- (1) access to personal identifiable health information was not requested in this submission;
- (2) a copy of the informed consent form must have been given to each research subject, if required for this study;
- (3) a Progress Report must be submitted by February 15, 2008, containing the following information:
 - i) the number of subjects recruited;
 - ii) a description of any protocol modification;
 - iii) any unusual and/or severe complications, adverse events or unanticipated problems involving risks to subjects or others, withdrawal of subjects from the research, or complaints about the research;
 - iv) a summary of any recent literature, finding, or other relevant information, especially information about risks associated with the research;
 - v) a copy of the current informed consent form;
 - vi) the expected date of termination of this project.
- 4) a Final Report must be submitted at the termination of the project.

Please accept the Board's best wishes for success in your research.

Yours sincerely,


Glenys Goddard, BA(Hons), LLB, PhD
Chair, Conjoint Health Research Ethics Board

GG/emcg

c.c. Dr. Wayne Giles (information) Research Services
Office of Information & Privacy

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