

Responses of the human motor system to observing actions across species: A transcranial magnetic stimulation study



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ABSTRACT

Ample evidence suggests that the role of the mirror neuron system (MNS) in monkeys is to represent the meaning of actions. The MNS becomes active in monkeys during execution, observation, and auditory experience of meaningful, object-oriented actions, suggesting that these cells represent the same action based on a variety of cues. The present study sought to determine whether the human motor system, part of the putative human MNS, similarly represents and reflects the meaning of actions rather than simply the mechanics of the actions. To this end, transcranial magnetic stimulation (TMS) of primary motor cortex was used to generate motor-evoked potentials (MEPs) from muscles involved in grasping while participants viewed object-oriented grasping actions performed by either a human, an elephant, a rat, or a body-less robotic arm. The analysis of MEP amplitudes suggested that activity in primary motor cortex during action observation was greatest during observation of the grasping actions of the rat and elephant, and smallest for the human and robotic arm. Based on these data, we conclude that the human action observation system can represent actions executed by non-human animals and shows sensitivity to species-specific differences in action mechanics.

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1. Introduction

Over the past twenty years, a wealth of research has provided evidence for the involvement of a specific network of brain structures, referred to collectively as the mirror neuron system (MNS), in recognizing the actions of other individuals. Mirror neurons, first discovered in the brains of non-human primates, are cells in the premotor and posterior parietal cortices that become active during both the execution and observation of specific actions (see Rizzolatti & Craighero, 2004 for review). In the human brain, a similar system is purported to be activated during the observation and execution of actions. Although there is mounting evidence that the putative human MNS is sensitive to an action's end goal, the extent to which MNS responses to goal-directed action rely on the biomechanics and anatomy of the specific effectors involved in action execution has yet to be determined. That is, many goals can be achieved with different effectors. For example, a person may be capable of picking up small objects with their feet as well as their hands. While studies of non-human primates suggest the MNS is

important in representing the *meaning* (e.g., di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996) or *effect* (Umiltà et al., 2001) of a given action, it is unclear whether such representations are reliant on the particular effectors involved in action execution.

There is currently some evidence to suggest that higher-order goals and the context-dependent nature of certain actions can be discerned during the observation of actions with different effectors. For example, Gergely, Bekkering, and Király (2002) have shown that preverbal infants can intuit goals from actions performed with different effectors, even when the effector used to execute an action is unusual. In these examples, the same goal is achieved—lifting an object or turning on a light—but the effector used to achieve the goal is different (e.g., turning on a light with the hand or the head). Preliminary evidence for effector-independent activation of brain regions involved in action observation is also provided by Sartori, Begliomini, and Castiello (2013). The authors used transcranial magnetic stimulation (TMS) to assess activity in the corticospinal tract related to covert motor imitation of observed actions (termed “motor resonance”) in right- and left-handed participants observing a grasping action performed with either the right or left hand. The results showed that observers exhibited motor resonance in their dominant hand regardless of

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the hand with which the action was performed. Such findings indicate that motor resonance may not be a form of direct perceptual-motor matching, but a more complex encoding of the observed movement, involving spatial transformations of the observed action to match the observer's dominant hand. Although [Sartori et al. \(2013\)](#) demonstrated the possibility that the human action observation (i.e., MNS) system may respond during action observation independent from the effector used to execute a particular action, the authors were interested specifically in the effects of handedness, and used only stimuli involving hands as effectors. Further, the nature of higher-level goal representation was not explored. It is thus far unknown whether the human MNS, which becomes active during the observation hand actions, also becomes active when a different effector is used to execute a task with the same outcome goal.

To push this notion of the relationship between goal- and effector-dependency further, it is as of yet unclear how humans recognize, code, and interpret the meaning of other animals' actions when they are executed by effectors humans do not possess. There are many actions that can be performed similarly by both humans and non-human animals. Grasping actions are one such example; non-human animals may execute grasping actions by use of effectors similar to human hands (e.g., the paw of a monkey, raccoon, or rat), or by use of species-specific effectors (e.g., the tail of a monkey or the trunk of an elephant). Regardless of the effector used for action execution, humans are often able to understand the intended goals of non-human actions ([Pinto & Shiffrar, 2009](#); see also [Welsh, McDougall, & Paulson, 2014](#)), but it is presently unclear whether the putative human MNS represents the goals of these actions rather than simply their (bio)mechanics. The present study examined the effector- and species-specific nature of action representations by investigating changes in corticospinal excitation in humans during observation of similar goal-oriented actions across species. Because primary motor cortex may be a component of the putative human MNS (or at minimum reflect the activity of the human MNS, e.g., [Rizzolatti & Craighero, 2004](#); see also [Molenberghs, Cunnington, & Mattingley, 2012](#) for meta-analysis of fMRI data), this exploration of corticospinal activity will shed new light on the coding of action kinematics and action goals during action observation. Prior to outlining the specifics of the present investigation, a brief review of the relevant literature on human and monkey MNS will be provided.

1.1. Coding of goals and effectors in non-human primate MNS

Mirror neurons were first characterized via single-cell recording studies in the monkey brain. [Di Pellegrino et al. \(1992\)](#) first reported that area F5 in the monkey inferior premotor cortex contains cells that became active when the monkey executed a grasping action, and when the monkey observed an experimenter performing the same action. [Gallese et al. \(1996\)](#) subsequently showed that mirror neurons are activated by meaningful actions only. The authors showed monkeys videos of a human performing a hand or mouth action, either on a specific object (e.g., a piece of food) or as a pantomime (non-object-oriented actions). Activity in mirror neurons only increased when the monkey observed a meaningful interaction between the agent and the object; the cells did not become active at the sight of the agent pantomiming or of the object alone. Importantly, a subset of mirror neurons was equally active for hand and mouth movements when the goal of the actions was the same (e.g., to eat). These findings suggest that mirror neurons may be important for representing the meaning of actions based on the agent's intentions, and their activity does not depend on a specific pattern of visual input.

[Umiltà et al. \(2001\)](#) provided further evidence for the representation of meaning and/or intentions in mirror neurons by showing

that some mirror neurons in the monkey brain become equally active during observation of object-oriented grasping actions, even when the end phase of the action (i.e., the actual grasping of an object) is occluded from view. In other words, when the monkey knew an object was present but hidden from direct view by a screen, mirror neurons became active during grasp observation, even when the grasping of the object had to be inferred. When no object was present, mirror neurons did not respond to grasping actions whether in full view or not. [Kohler et al. \(2002\)](#) further showed that some mirror neurons respond to multimodal action cues and do not require visual input at all. The authors found a population of mirror neurons in monkey premotor cortex that were equally responsive when the monkey executed a specific action and when the monkey heard the sound associated with the same action (e.g., ripping a piece of paper). Many of these neurons were also activated when the monkey observed an experimenter performing the same action. Together, these findings provide compelling support that at least some mirror neurons respond to multimodal cues signaling a specific action and thus seem to represent an action's meaning. Studies of the monkey MNS also provide initial evidence for cross-species action representation, as in most research to date, stimuli have comprised human actors.

1.2. The coding of action goals in the human action observation system

Due to the limitations in implementing single-cell recordings in human subjects, research to date on the existence and functional role of the MNS in humans is less conclusive (e.g., [Turella, Pierno, Tubaldi, & Castiello, 2009](#)). Nonetheless there is mounting evidence in favor of a system of brain areas whose collective function is largely homologous to that of monkey MNS (see [Rizzolatti & Craighero, 2004](#) for review). The most compelling evidence to date is provided by [Mukamel et al. \(2010\)](#), who recorded extracellular activity in the brains of patients with intractable epilepsy. The authors found that a significant proportion of the 1177 cells observed demonstrated mirror properties, as indexed by modulation of the cells' firing rate during both action-observation and action-execution.

Of particular relevance to the present study, evidence for the goal-directed nature of the coding in the human action observation system has been garnered from studies in which TMS was used to assess the modulation of the corticospinal tract during the observation of actions. Irrespective of whether or not primary motor cortex is part of the putative human MNS, it seems that the modulation of the activity in the corticospinal tract reflects the coding of observed actions in the central nervous system of the observer. [Fadiga, Fogassi, Pavesi, and Rizzolatti \(1995\)](#) were the first to report that the human primary motor cortex is similarly active during execution and observation of actions. Specifically, they found that motor-evoked potentials (MEPs) in hand muscles were facilitated while subjects either performed or observed grasping actions (compared to a non-motor perceptual task), indicating sub-threshold activation of the motor cortex in both cases. These changes in the corticospinal excitability during action observation are thought to occur because either primary motor cortex is part of the putative human MNS, or because the coding of the response in this action observation system affects the excitability of primary motor cortex.

Regardless of the origins of these changes in corticospinal excitability, it is important to note that there are similarities between the conditions under which these modulations of corticospinal excitability occur in the human primary motor cortex and monkey mirror neurons. For example, [Villiger, Chandrasekharan, and Welsh \(2011\)](#) recently reported that the MEP amplitudes were modulated during the observation of a grasping action on an object both when

the object was in full view or hidden behind screen.¹ These findings mirror those by Umiltà et al. (2001) and support the hypothesis that there is a similarity in the nature of action coding in humans and monkey MNS.

To our knowledge, no TMS studies have explored corticospinal excitability in humans during observation of non-human animal actions. Functional imaging studies of brain activity at the systems level, however, suggest that there are similarities between the human and monkey MNS activity during action observation, especially with respect to the activation of premotor regions during action observation. Buccino et al. (2004) demonstrated using functional magnetic resonance imaging (fMRI) that the human MNS is active during observation of non-human animal actions when the actions are similar to those also typically performed by humans. Participants viewed silent animations of humans, monkeys, or dogs performing actions with the mouth that could be either non-communicative (biting) or communicative (speech/lip movements, barking). The human ventral premotor cortex was equally active for non-communicative mouth movements across all three species. For communicative mouth movements, the premotor cortex responded most strongly to human speech, less strongly to monkey mouth movements, and was not activated for barking. The authors concluded that this region may become activated for any action belonging to the repertoire of human actions, whereas actions not generally performed by humans (e.g., barking) may be recognized based on their visual rather than motor properties. Though the fMRI findings are correlational in nature, these results provide initial evidence that the human MNS may reflect representation of meaningful actions/intentions across species.

2. Purpose and predictions of the present study

The present study sought to determine whether activity in the hand area of the human primary motor system differentiates actions with the same goal when the actions are performed by different species using effectors that are either similar to or different from the human hand. To this end, corticospinal excitation was assessed via TMS while participants viewed videos of grasping actions from three different species (human, rat, and elephant) all with the implied goal of eating (i.e., grasping a piece of food), and a robotic arm control stimulus. The rat was chosen because its grasping front paw is roughly similar in structure to the human hand. The elephant was included in the study to provide an extreme test of the effector-specific nature of activation, because the effector it uses to grasp food (the trunk) is one that humans do not possess.

We elected to use a robotic arm as a control stimulus in this study in line with previous research showing no modulation of the observer's motor system by the presence of a robotic stimulus. Castiello (2003) demonstrated that observing a human model perform a reach-to-grasp action altered the observer's own kinematic trajectory in a later execution of the same action, while observing a robotic arm did not influence subsequent action execution. Further, Sartori, Becchio, Bulgheroni, and Castiello (2009) showed a similar influence on the kinematics of participants' reaching actions by human models, but not by robots. In this study, the authors had participants perform a pre-planned action (a reach), which could be interrupted or not interrupted by a gesture from a confederate (either human or robot). Gestures performed by human confederates changed participants' reach trajectories, while

the same gestures performed by robotic arms did not. Although there is some evidence to suggest that certain types of robots may activate the action observation system in humans, the ability of robotic movements to do so appears to depend on the degree of naturalistic "humanness" of the robot. For instance, Oberman, McCleery, Ramachandran, and Pineda (2007) showed MNS activity in response to robot observation, using a robotic arm designed to appear very humanoid. Their robot (Leonardo) had an articulating arm with a humanoid hand, including a thumb and five fingers. In addition, a behavioral study by Stenzel et al. (2012) reports that a social cognitive action effect (the joint Simon effect; Sebanz, Knoblich, & Prinz, 2003) emerged when participants were interacting with a human-like robot, but not when the robot was more machine-like. In line with these findings, studies which have not found evidence for modulation of the MNS by robotic movement have used more simplistic robotic arms, often with only a rigid plastic hand or two-pronged claw. The robotic arm employed in our study resembles the latter, and thus we expected it to serve as a good control condition.

TMS in the present study was used to target the region of primary motor cortex involved in executing hand movements. Because the hand of the participant remained at rest throughout the task, the degree to which the motor system was activated provides an index of activity elicited by action observation. We hypothesized that if the human motor system represents the end goal of actions regardless of the effector used to complete the action, similar modulations in activity should be elicited during observation of actions with the same goal across species, even when the action is performed by a non-human-like effector, such as an elephant's trunk. It is also possible that primary motor regions in the human brain represent the simple kinetics of human or human-like movement; in this case, we expected to observe increased motor activity for the human condition only.

3. Methods

3.1. Participants

Sixteen University of Toronto students were recruited. Due to technical problems, the data from three participants could not be used in the analysis (TMS artifacts in the electrophysiological data or incomplete data collection). Thirteen participants (5 female; $M_{age} = 25.83$ years, $SD = 3.56$) were included in the final analysis. All participants reported being right-handed and had normal or corrected-to-normal vision. Each participant gave written informed consent prior to participation and received an honorarium of \$15 for their time. Procedures complied with the ethical standards set forth by the 1964 Declaration of Helsinki, and were approved by the Office of Research Ethics at the University of Toronto.

3.2. Stimuli and procedure

The experiment was run on a PC using Experiment Builder software (SR Research Ltd, Mississauga ON). Visual stimuli comprised video clips lasting from 1500 to 4000 ms and were presented on a 19" LCD monitor. Preceding each video, a still image of the video's first frame was presented for an interval of 1000 ms. Stimuli were sized so as to fill most of the screen without appearing distorted. Each trial consisted of a clear resting phase (the static frame), a reach phase (movement toward an object), and a grasping phase (grasping of the object). In the human, elephant, and robot videos, the object that was grasped was a carrot. In the rat video, the rat grasped a food pellet. Examples of stimuli can be seen in Fig. 1.

Prior to viewing the stimuli, participants were fitted with electrodes on the right hand to measure electromyographic (EMG)

¹ In this study the changes in corticospinal activity were inhibitory in nature (see also Gazzola & Keysers, 2009), whereas in similar studies of monkey mirror neurons, facilitation of activity has been largely observed. However, regardless of the directional differences in these effects, that the same manipulation induces modulation in activity in both species provides evidence of cross-species similarity.

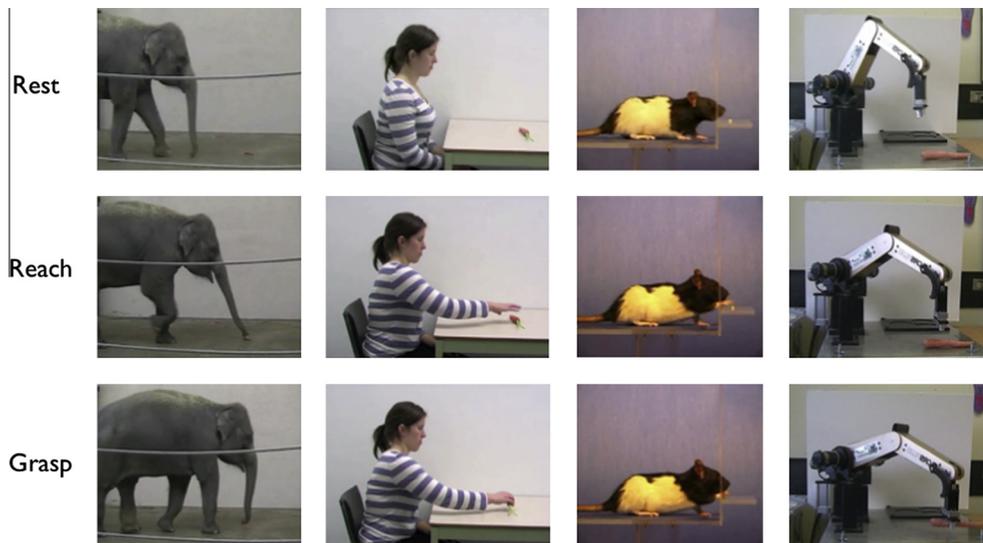


Fig. 1. Depiction of the stimulus set comprising all combinations of Animal Type and TMS Condition (i.e., when TMS stimulation was delivered).

activity in the opponens pollicis (OP), a thumb muscle directly involved in grasping actions via thumb opposition. We also fitted participants with electrodes to record from the first dorsal interosseus (FDI), a finger muscle nominally involved in grasping by stabilizing the index finger during grasping through abduction and flexion of the hand. Because the corticospinal neurons representing the FDI muscle are more active during precision than the power-type grasps (e.g., Hasagawa, Kasai, Tsuji, & Yahagi, 2001) used in the present study, we intended this muscle to serve as a control for comparison with the results from OP.

EMG data were recorded at 3000 Hz for a 200 ms interval. The interval was time-locked to the delivery of magnetic stimulation and began from 50 ms prior to the onset of stimulation and ended 150 ms post-stimulation. TMS and MEP recordings were coordinated using Brainsight software (Rogue Research, Montreal QC). Magnetic stimulation was delivered using a MagStim 200 (The MagStim Company, Carmarthenshire UK) via a figure-eight coil (external wings 7 cm in diameter) to the hemisphere contralateral to the test limb (i.e., the left hemisphere). The optimal scalp location for stimulating the motor cortex for the target muscles was determined via moving the TMS coil over the scalp in 1 cm steps and delivering TMS stimulation until MEP activity was observed in the EMG data. When no MEP was observed at the initial (i.e., 30% of max stimulator output), the output of the stimulator was increased and the exploration procedure was repeated until an MEP was observed. The location eliciting the largest, most consistent MEPs at resting threshold (defined as 6/10 MEPs of at least 50 μ v in peak-to-peak amplitude of both target muscles) was used as the “hot spot” of motor cortex for stimulation during the experiment. The coil was oriented at approximately 45° to the midline and tangential to the scalp in order to induce a posterior-to-anterior flow of current to the primary motor strip. The position and orientation of the coil over the “hotspot” were recorded and loaded into the Brainsight 2.0 neuronavigation system to facilitate accurate (re)positioning of the coil throughout the experiment. During testing, the stimulator was set at an intensity of 120% of the individual’s resting motor threshold.

During each trial, a TMS pulse was delivered at a specific moment during one of the rest, reach, or grasp phases of the movement. The rest TMS pulse was always delivered 1000 ms after the presentation of the initial static picture of the animal or robot. Due to differences in the temporal characteristics of the videos, the absolute timing of the delivery of reach and grasp TMS was

not identical across actor conditions, but was instead tied to the spatial location of the effector during the video. We modeled our reach and grasp stimulation timing after that employed by Gangitano, Mottaghy, and Pascual-Leone (2001). The timing of stimulation for each condition was determined via frame-by-frame visual inspection of each video. The reach TMS pulse was delivered at the point of maximal grasp aperture, when the grasping component of the effector (i.e., the hand, paw, trunk or robotic claw) was maximally extended toward the object (1133–1233 ms after video onset), and the grasp TMS pulse was delivered immediately after closure of the grasping effector, when the object was grasped but prior to its being picked up (1860–3233 ms after video onset²). The order of presentation of videos across species and the TMS pulse conditions during each trial were randomized. Participants viewed stimuli in blocks containing two trials for each combination of Animal Type (Human, Robot, Elephant, Rat) and TMS Condition (Rest, Reach, Grasp), resulting in a block of 24 trials. Participants completed six blocks of trials, taking 1–5 min break between each block, for a total of 144 trials. EMG data was recorded on a trial-by-trial basis, time-locked to the TMS pulse as mentioned above, and stored offline for later analysis. Prior to the onset of each block, the coil position and orientation was checked using the neuronavigation system to ensure accurate placement. As a secondary check of accurate positioning, 2 test TMS pulses were delivered over the hotspot at rMT and the resulting MEPs were inspected to ensure that they were similar to those observed at the end of the mapping.

3.3. Data analysis

EMG data were exported from Brainsight in text format and integrated with the trial information from Experiment Builder so as to match MEPs to the correct trials. Outlier rejection was performed in two phases. First, for each participant, the absolute average EMG amplitude was computed for the window prior to the TMS pulse. Any trials for which the pre-stimulus average exceeded 3 standard deviations of the overall average for that participant were excluded to ensure that MEP amplitudes were not unduly influenced by pre-existing muscle activity. Second, for each

² The timing of grasp stimulation comprised a wider range due to differences in overall video length, with robot motion being generally slower than motion in other conditions. The timing of stimulation during grasping for the elephant, human and rat videos was 1860 ms, 1800 ms, and 1500 ms post-onset, respectively.

participant, mean peak-to-peak MEP values were computed on a by-condition basis. Within each condition, MEPs exceeding 2.5 standard deviations of the average MEP were excluded. Together, these two phases of outlier rejection resulted in excluding 4.9% of the data across participants (rejection rates ranged from 2.8% to 8% of trials per participant). MEP data were positively skewed and therefore transformed using the natural logarithm to better approximate the normal distribution.

Data were analyzed separately for each muscle (EMG channel) using R (R Core Team, 2013), and the R packages *lme4* v0.999999-2 (Bates, Maechler & Bolker, 2013), *languageR* v1.4 (Baayen, 2011) and *LMERConvenienceFunctions* v2.0 (Tremblay & Ransijn, 2013) using multilevel modeling with maximum-likelihood estimation (Baayen, Davidson & Bates, 2008; Faraway, 2006). This technique allows for a classical regression analysis to be performed on repeated measures data by accounting for the interdependence of observations collected within each subject without resorting to data aggregation. Further, rich random effects structures can be modeled in addition to estimating random intercepts for each subject, allowing the researcher to increase the amount of variance explained (e.g., modeling the size of a main effect for each participant) and improve power to detect experimental effects. We subjected data from each muscle to a model fitting procedure to determine a model of optimal fit (i.e., the most parsimonious model accounting for the most explained variance). Importantly, in all analyses, we included individual resting motor thresholds, as well as “Experiment Block,” as covariates in our analyses to account for any variance associated by changes in corticospinal excitability resulting from the TMS procedure over the course of the experiment.

4. Results

4.1. *Opponens pollicis*

An initial model was computed with the simplest random effects structure (by-subjects intercepts only), and data from this model were inspected for normality prior to analysis via quantile–quantile plots of residuals against fitted model values. Random effects were then added to this model to determine the optimal random effects structure, and the optimal fixed effects structure was fitted by starting with a model including all factors of interest (Animal Type: Human, Robot, Elephant, Rat; TMS Condition: Rest, Reach, Grasp) as well as potential covariates (e.g., individual resting motor threshold) and comparing the fit of more complex models with simpler models. Non-significant interaction terms and main effects were removed from the model where appropriate, and models that did not differ significantly in variance explained from the null model were rejected.

Log-transformed MEP amplitude was modeled as a function of individual resting motor threshold and Animal Type. A two-level multilevel model was used to account for the non-independence of observations collected from each participant by estimating a random intercept for each participant using an unstructured covariance matrix and the between-within method of estimating degrees of freedom. Random slopes were also estimated on a by-participant basis for the effect of Block (i.e., to capture any change in MEP amplitudes across blocks). We calculated the proportion reduction in prediction error of this model for each level according to the recommendations of Snijders and Bosker (1994, 1999). At the level of individual trials, the model reduced prediction error by a small amount, $R_1^2 = .117$. At the level of subjects, the model also reduced prediction error by a small amount, $R_2^2 = .116$.

Notably, there was a significant effect of Animal Type (controlling for resting motor threshold), $F(3, 1748) = 3.04$, $p = .0281$ (see also Fig. 2). Post-hoc comparisons revealed that this effect was driven

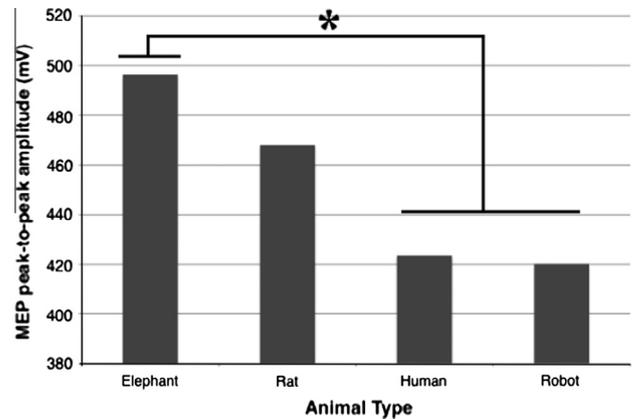


Fig. 2. Main effect of Animal Type. Peak-to-peak MEP amplitude was significantly larger in response to viewing elephant actions than to either human or robot actions. * indicates significantly greater MEP amplitude for the Elephant compared to both the Human and Rat conditions ($ps < .05$).

by larger MEP amplitudes for the elephant stimulus than both the human stimulus, $b = -.158$, $SE = .065$, $t(1748) = 2.45$, $p = .008$, and robot stimulus, $b = -.167$, $SE = .065$, $t(1748) = 2.55$, $p = .005$. MEP amplitudes elicited during the observation of the rat stimulus were marginally larger than those elicited by observing the human stimulus, $b = -.099$, $SE = .065$, $t(1748) = 1.53$, $p = .063$, and significantly larger than those elicited by the robot stimulus, $b = -.107$, $SE = .065$, $t(1748) = 1.65$, $p = .05$. No other post hoc comparisons were significant.

Contrary to predictions, there was no effect of TMS Condition (a term that was removed from the model due to its inability to increase the variance accounted for). In other words, the MEP amplitudes elicited by action observation were largely similar across the rest, reach and grasp phases of each action. The lack of such a modulation by TMS Condition would seem to indicate that MEP amplitudes in the reach and grasp conditions do not differ significantly from MEP amplitudes elicited in the rest condition. This result stands in contrast to previous studies showing alternatively a facilitation of MEPs relative to rest (e.g., Fadiga et al., 1995) or an inhibition of MEPs relative to rest (Villiger et al., 2011) during action observation. Fig. 3 shows the MEP amplitudes elicited across TMS Condition for the different conditions of Animal Type.

Based on a visual inspection of the data presented in Fig. 3, there appeared to be differential effects on MEP amplitudes elicited by grasping relative to rest that were dependent on the animal condition. For the elephant and rat stimuli, MEPs appear larger for the grasp condition relative to the rest condition, while for the human and robot stimuli, MEPs appear smaller in the grasp condition relative to rest. The different directions in these condition effects might be masked in the overall analysis due to MEP amplitude being influenced in opposite directions across the different animal

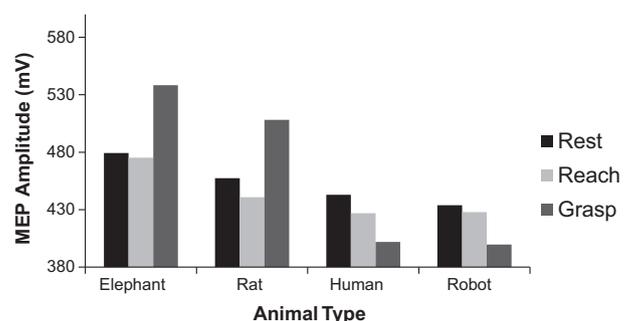


Fig. 3. Model-derived mean MEP amplitudes across phase and animal conditions.

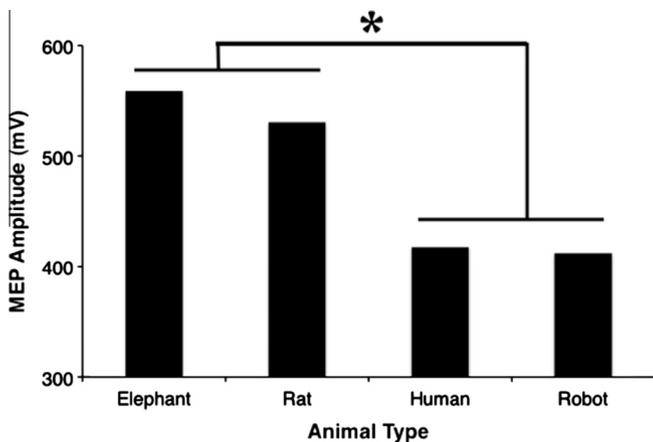


Fig. 4. MEP amplitudes elicited in the grasp condition only. * indicates significantly larger MEPs for both the Elephant and Rat compared to the Human and Robot conditions (all p s < .05).

conditions. Given this possibility, in combination with *a priori* predictions regarding TMS Condition differences, a further exploration of the effect of Animal Type was performed at each phase of TMS stimulation separately to determine if the effect of Animal Type was truly identical in the rest, reach and grasp conditions.

Animal Type effects during rest. A linear mixed effects model was computed for data in the rest condition only, in the same manner described above for the overall analysis. Log-transformed MEP amplitude was modeled as a function of Animal Type, taking into account individual resting motor thresholds as a covariate. Random slopes were also estimated on a by-participant basis for the effect of Block (i.e., to capture any change in MEP amplitudes over time). This analysis revealed no effect of Animal Type, $F(3, 569) < 1$.

Animal Type effects during reach. The same analysis was performed for data from the Reach condition only. This analysis also revealed no effect of Animal Type on MEP amplitudes during observation of reaching, $F(3, 560) < 1$.

Animal Type effects during grasp. The same analysis was performed on data from the Grasp condition only. This analysis revealed a significant effect of Animal Type on MEP amplitude, $F(3, 557) = 3.67, p = .0122$. Post-hoc comparisons revealed that this effect was driven by larger MEP amplitudes elicited by elephants relative to humans, $b = -.29, SE = .127, t(557) = 2.48, p = .007$, and larger MEP amplitudes for elephants than robots, $b = -.30, SE = .12, t(557) = 2.60, p = .005$ (see also Fig. 4). MEPs elicited by rats were also significantly larger than those elicited by both humans, $b = -.24, SE = .12, t(557) = 2.06, p = .02$, and robots, $b = -.25, SE = .12, t(557) = 2.17, p = .015$.

4.2. First dorsal interosseum (FDI)

Modeling of MEP amplitudes for the data from this EMG channel was performed in the same manner described above. The optimal model for estimating MEP amplitude in this case included no fixed effects. Random intercepts and slopes were estimated for the effect of Block as well as the effect of TMS Condition,³ on a by-participant basis. The factors Animal Type and TMS Condition were not included

³ In this case, there was an initially significant effect of TMS Condition. When we included estimates of random slopes for this variable, the main effect of TMS Condition became non-significant, thus it was included in the model as a random effect only. This essentially means that, though there appeared to be some MEP amplitude differences between the Rest, Reach and Grasp conditions, this variance was better captured by accounting for these differences on a by-subject basis (e.g., by capturing individual differences in MEP amplitude changes across conditions rather than capturing an overall effect of condition).

in the final model due to their inability to increase variance accounted for. That is, in this case, the factors of interest do not predict MEP amplitude above and beyond what can be accounted for by individual differences over time. The proportion reduction in prediction error of this model for each level was calculated according to the recommendations of Snijders and Bosker (1994, 1999). At the level of individual trials, the model reduced prediction error by a medium amount, $R_1^2 = .214$. At the level of subjects, the model also reduced prediction error by a medium amount, $R_2^2 = .278$.

5. Discussion

The present study was designed to determine whether the human action observation system, as indexed by corticospinal excitability measured via TMS and EMG, is activated during observation of actions across species when the actions have the same goal. It was predicted that, if the action observation system represents the goals of actions and that representation is reflected in corticospinal activity, then we would observe similar MEP amplitude modulations regardless of species. In contrast, if corticospinal activity elicited during action observation represents the biomechanics of actions executed by a human or human-like effector, then MEP amplitude effects would be species-specific and modulations should only be observed for the human stimuli.

The results showed a difference in MEP amplitudes depending on the species being observed. Viewing elephant and rat grasping elicited larger MEPs than viewing either human or robot grasping. Importantly, these effects were observed only in the OP and not the FDI muscle, indicating that motor resonance was restricted to the specific muscle involved in executing the type of grasp being observed. Though our omnibus analysis for the OP muscle showed no effect of TMS Condition on MEP amplitude, exploratory post hoc analyses suggest that the differences in MEP amplitude across Animal Types observed are limited to the observation of grasping specifically, because no such modulation effects were found in the rest or reaching conditions. Thus, we conclude that, although the action observation system is involved in the coding of actions executed by different species and with different effectors, these regions are differentially activated during action observation across species even when the goal is held constant.

One possible criticism of these findings concerns the variation in timing across our video stimuli. Lepage, Tremblay, and Théoret (2010) have demonstrated that corticospinal excitability during finger movements follows a precise and rapid timecourse, with enhanced, non-specific excitability occurring between sixty and ninety milliseconds post-movement-onset. Though these results were observed during action execution, it is reasonable to expect that such temporal sensitivity would carry over to covert muscular activity during motor resonance. With the temporal onset of our grasp condition having a range between approximately 1500–3200 ms post-stimulus-onset, it may be argued that our results reflect a generalized temporal effect of corticospinal excitability rather than a true species-specific difference. However, there are two aspects of our results that are inconsistent with a simple time-course account. First, we did not find a similar pattern of effects, or indeed any effects at all, in the FDI muscle. If the MEP modulations we observed were an artifact of a generalized excitability, one would expect that any non-specific modulation would be observed in both muscles, while a task-specific modulation would be restricted to the muscle of interest (OP). Secondly, the timing of the grasp phases across the different animal conditions does not accord with a simple temporal explanation. MEP amplitude did not differ between the elephant and rat conditions; both elicited larger MEPs than the human and robot conditions (which did not differ from one another). Any temporal explanation of these find-

ings would require the most similarly-timed conditions to elicit similar patterns of MEPs, while conditions with divergent timing should elicit MEPs of different amplitudes. In the present study, however, the videos that elicited similar patterns of MEP amplitudes (human [1800 ms] and robot [3233 ms], versus rat [1500 ms] and elephant [1860 ms]) had different grasp condition timing. Further, the videos that had the most similar timings (human [1800 ms] and elephant [1860 ms]) elicited different patterns of MEP amplitudes. Therefore, we argue that a simple time-course explanation of the observed MEP modulations does not account for our findings.

Although the data suggest that the human action observation system is activated by the goal-directed actions of other species, there are several novel and informative aspects of the data that need to be addressed. The first notable aspect of the data was that corticospinal activity was similarly affected by the observation of the robot and the human grasping actions. The robot stimulus was originally intended to act as a non-animal control. As such, modulations in corticospinal activation in response to the robot stimulus were not anticipated. Our analysis revealed no differences in MEP amplitudes observed between the robot and human conditions. Oberman et al. (2007) have suggested that the “humanness” of a robot influences its ability to engage the action observation system of an observer. Though we considered our robotic arm to be fairly non-human, given the context of the action (picking up a food item as opposed to an abstract object or a tool) and the life-like features and movement trajectory of the robot arm, it is possible that the robotic arm was interpreted as an animate entity, or as being under human control. A recent study by Cross et al. (2012) supports this view. The authors showed in an fMRI study that the human action observation system is activated during the observation of a robot performing naturalistic dance moves. In this study the authors compared brain responses to observation of a human actor and a Lego-form robot performing both “robotic” and “natural” dance moves. Regions within the putative human MNS became more highly activated by robot observation relative to human observation. However, we argue that in this case it is unclear whether this represents a true MNS response to robotic figures, or whether exactly matching dance moves between human and robot (especially having the human actor perform “the robot”) may have biased participants to interpret the robot as animate. Regardless, the authors demonstrated that life-like movements performed by a robot engage the putative human MNS. The data from the present experiment suggest that the observation of the grasping actions of the human and robotic arm may have had similar effects on corticospinal activation. This finding is consistent with recent data from other studies and suggests that human and robotic arms may be processed similarly when they execute similar goal-directed actions and/or may be visually similar to actions performed by a human model. Further research on the reactivity of the human action observation system to robotic figures within different experimental contexts is needed to address this question.

A second notable aspect of the data is that the human action observation system and the related corticospinal activity appeared to be differentially responsive to the grasping actions of non-human animals. These findings are consistent with the hypothesis that the human action observation system can represent the actions of non-human animals when the action is one a human is capable of performing (i.e., grasping a piece of food; cf. Buccino et al., 2004). Interestingly, the system responds similarly when the action is grasp is performed by the paw of a rat (an effector somewhat resembles a human hand) and the trunk of an elephant (an effector that humans do not possess).

Although there were modulations of the corticospinal activity during the observation of grasping actions by non-human animals, the nature of these modulations depended on whether stimuli

depicted actions executed by human or non-human animals. Specifically, there was a relative decrease in corticospinal excitation during the observation of the grasping actions of the human and robot, whereas there was a relative increase in corticospinal activity during the observation of grasping actions of the elephant and rat. These differences, which were only observed in the grasping phase of the action (see Fig. 3), suggest the presence of MEP facilitation for the elephant and rat and inhibition for the human and the robot.⁴ The reason for the differential activation patterns is not clear. One possibility is that observations of non-conspecific actions may be mediated by different subsets of neuronal populations within the human action observation system. Recording extracellular activity from over 1100 cells in the human brain, Mukamel and colleagues classified four subsets of cells exhibiting mirror properties (that is, a modulation of firing rate during both observation and execution of the same action). The majority of cells increased their firing rate during both observation and execution; however, some neurons decreased firing rates during both conditions, while others increased during observation and decreased during execution and vice versa. The mechanisms of individual neurons with mirror-like properties within the human brain are not well understood. It is possible that different actions or different effectors/agents may differentially engage subsets of neuron populations.

Another possible explanation is that, as with the findings of previous work (Gazzola & Keysers, 2009; Villiger et al., 2011), our results reflect a suppression of MEP amplitude for the human and robot conditions resulting from overcompensation due to the instructions to remain at rest. That is, it is possible that the observation of the human and humanoid robotic arm activated response codes in the observer of sufficient strength to require additional voluntary inhibitory mechanisms to ensure that the system remained at rest and prevent overt action. In contrast, the goal-dominated response codes activated via observation of the rat and elephant were not of sufficient strength to require more active inhibition and, as such, maintained an excitatory influence. A similar set of reactive inhibitory mechanisms has been suggested to underlie patterns of trajectory deviations towards and away from distractors in aiming interference paradigms (see Tipper, Howard, & Houghton, 1999).

A final alternative (and perhaps not mutually-exclusive) explanation of the differential MEP amplitudes for the human and non-human animal conditions could be grounded in the roles primary motor and premotor areas play in coding observed actions. Specifically, this pattern of findings would first appear to contrast with those discussed by Buccino et al. (2004), who showed similar cortical activity for actions across species, as long as those actions were typical of the human motor repertoire (e.g., the human MNS was active for dogs biting, but not barking). However, the regions of activity observed in this fMRI study were not in primary motor cortex, but in ventral premotor regions. It is possible that premotor and parietal regions code the goals and perceptual features of the observed action while primary motor regions code action mechanics more specifically. Thus, if the anatomy of the animal being observed is dramatically different than our own (e.g., an elephant's trunk versus an arm), primary motor regions may be differentially active compared to observing animals with similar

⁴ It should be noted that an additional analysis was performed to determine if there were specific differences between MEPs in the grasping and rest phases. In this analysis difference scores for each of the reach and grasp conditions were calculated relative to the rest condition and submitted to one-sample *t*-tests to determine whether MEP deviations from rest were significantly larger than zero. No significant results were found, possibly due to high intraindividual variability in MEP amplitude within each condition. Additional control conditions will be needed to confirm this hypothesis. Future work should include a non-motor visual control stimulus, such as the perceptual task included in Fadiga et al. (1995), as well as an additional motor stimulus featuring an action with a different goal or no goal.

anatomies. In support of this explanation, Gazzola and Keysers (2009) showed using fMRI that, while primary motor regions were suppressed during action observation, premotor regions increased in activity. The premotor cortex is known to play a role in movement planning and selection based on an integration of visual information with goal information (e.g., Grafton, Fagg, & Arbib, 1998; see also Rushworth, Johansen-Berg, Gobel, & Devlin, 2003 for review). Thus, it might not be surprising that this region might be active during both action execution and action observation since representing the goal of another's action may be viewed as an extension of the ability to coordinate one's own goals and actions. Currently, research supports a role for the premotor cortex in representing the meaning of actions, while the primary motor cortex appears to primarily represent the mechanics of actions. Future work should incorporate measurements of premotor and primary motor activity during action observation across species to better determine the nature of activity in these two regions, and whether they interact with one another. For instance, paired-pulse TMS could be used to modulate premotor activity prior to assessing corticospinal activity in primary motor regions. If the premotor region is involved in representing the meaning of actions across species, while the primary motor regions are involved in representing action mechanics, inhibiting premotor activity during action observation should drastically affect MEP amplitudes measured from primary motor regions during observation of human actions, but not the actions of non-human animals.

In conclusion, the present study provides evidence that the human action observation system represents the actions of non-human animals, even actions executed by effectors that humans do not possess. The changes in corticospinal activity observed here are suggestive of a species-specific effect of action observation on activity in the primary motor cortex, suggesting that this region may not broadly represent the meaning of actions but rather their mechanics. Further work is needed to further explore the nature of this effect and how it may be integrated into the broader domain of knowledge about the human MNS, particularly with respect to action representation in the premotor cortex.

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