# Resonating with the ghost of a hand: a TMS experiment

Laila Craighero[a*], Marco Jacono[b] and Sonia Mele[a]

[a] Department of Biomedical and Specialty Surgical Sciences, School of Medicine, University of Ferrara, via Fossato di Mortara 19, 44121 Ferrara, Italy

[b] Department of Robotics, Brain and Cognitive Sciences, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova, Italy

Laila Craighero: laila.craighero@unife.it
Sonia Mele: sonia.mele@unife.it
Marco Jacono: Marco.Jacono@iit.it

**\*Corresponding author:**
Dipartimento di Scienze Biomediche e Chirurgico Specialistiche
Scuola di Medicina
Università di Ferrara
via Fossato di Mortara 19
44121 Ferrara
Italy
E-mail address: laila.craighero@unife.it (L. Craighero).

ABSTRACT

An impressive body of literature in the past 20 years has revealed a possible role played by cortical motor areas in action perception. One question that has been of interest is whether these areas are selectively tuned to process the actions of biological agents. However, no experiments directly testing the effects of the main characteristics identifying a biological agent (physical appearance and movement kinematics) on corticospinal excitability (CS) are present in literature. To fill this gap, we delivered single-pulse transcranial magnetic stimulation to the primary motor cortex and we recorded motor evoked potentials from contralateral hand muscles during observation of point-light-displays stimuli representing a hand having lost its physical appearance (Experiment 1) and kinematics characteristics (Experiment 2). Results showed that physical appearance, natural kinematics, and the possibility to identify the action behind the stimulus are not necessary conditions to modulate CS excitability during stimuli observation. We propose that the involvement of the motor system can be mandatory whenever the perceived stimulus is recognized as reproducible in its final outcome (e.g., position in space, direction of movement, posture of a body part, to-be-produced sound, specific interaction with an object, etc.), and that the peculiar relationship existing between others' actions and the actions executed by the observer could just represent the extreme in which the motor system is able to almost perfectly reproduce the observed stimulus as it unfolds and, consequently, contribute to stimulus perception in the most efficient way.

## 1.    Introduction

The presence of motor activity during action observation (Buccino et al., 2004; Fadiga et al., 2005) represents the clearest proof that this perceptive task automatically recruits the motor system. The privileged electrophysiological technique to study this effect is transcranial magnetic stimulation (TMS). The recording of motor evoked potentials (MEPs) from a given muscle in response to primary motor cortex (M1) stimulation is used to monitor changes in corticospinal (CS) excitability induced by the activity of various brain regions connected with M1 and involved in the concomitant task. Furthermore, the high temporal resolution of TMS is a necessary requirement to verify the dynamic (online) effects that the task has on the motor system of the participant. The fast circulation of a strong electrical current in the coil positioned on the skull induces an electric current in the brain. Consequently, when the underlying M1 cortical neurons are brought over threshold, the descending volley reaches the spinal motoneurons, evoking a MEP detectable by standard electromyography techniques. The presence of a modulation in MEPs amplitude during the execution of perceptive or cognitive tasks indicates a variation in CS excitability induced by the task. However, given the large number of non-primary motor areas establishing excitatory connections with M1, any change in CS excitability does not tell us much about the actual brain structures underlying the facilitation. Nevertheless, a MEPs modulation that is specific to the muscles involved in the task is a clear sign of an involvement of the motor system during task execution in situations in which no overt movements are required to the participants. Many experiments have been devoted to explore the characteristics of this motor involvement, showing that perception of others' actions is constantly accompanied by motor facilitation of the observer's CS system. With the term motor resonance we specifically refer to this motor facilitation characterized by the fact that the pattern of muscle activation of the observer is very similar to the pattern of muscle contraction present during the execution of the observed action (somatotopic specificity) and that muscles activation is temporally strictly coupled with the dynamics of the observed action (high temporal fidelity). This indicates that the perceived action is subliminally reenacted (Fadiga et al., 1995; Borroni and Baldissera, 2008; Brighina et al., 2000; Clark et al., 2004; Gangitano et al., 2001; Montagna et al., 2005). Furthermore, studies indicated that the reenactment is automatic since somatotopic specificity is present even when the individual is not aware of the use of muscles used to perform the action (see Fadiga et al., 1995). Moreover, in TMS experiments a necessary requirement to allow for the delivery of the pulse is the absence of any muscle preactivation which is considered an indication of a possible voluntary movement

preparation. An impressive body of functional magnetic resonance imaging (for a meta-analysis see Caspers et al., 2010), magnetoencephalography (for a review see Hari, 2006) and electroencephalography (for a review see Vanderwert et al., 2013) studies in the past 20 years has demonstrated the presence of overlapping neural networks associated with action perception and execution. However, the presence of brain networks involved in both perception and execution does not guarantee that the perceived action is subliminally replicated with the modalities characterizing motor resonance. For this reason, we will focus the presentation of the state of the art on studies more specifically devoted to investigate, in a direct or indirect way, the effects that the perceived action has on the peripheral motor system, considering these as an index of the reenactment of that action.

Given the evidence of the exact replica of the observed action, the motor resonance effect is commonly considered the result of the encoding of action kinematic aspects (Leonetti et al., 2015). It has been proposed that this effect can be useful during imitation for motor learning (Iacoboni, 1999; Mattar and Gribble, 2005; Vogt et al., 2007) during which the acquisition of precise kinematic information about the single movements to be learned is fundamental. However, it is debated if other action features contribute to determine motor resonance.

One of the main features able to influence the observer's motor response seems to be the nature of the observed agent: the agent may be a human or an artificial device. However, agent's nature may be just inferred: it is known that motor resonance is clearly present even when the agent's hand is covered with a glove and a sleeve (Alaerts et al., 2012), or it is shown as its shadow (Alaerts et al., 2009). In these conditions, it is still recognizable as human hand. Even if no TMS experiments have directly investigated whether humanlike appearance is sufficient to evoke motor resonance, a series of behavioral experiments verified the influence that an observed action has on the execution of the same or a different action when the agent is not a human but a robot. When the agent is a human, the execution of the action is facilitated when the observed action is the same, and interfered when it is a different one (Brass et al., 2000; 2001; Craighero et al., 2002). This interference effect may be considered an indirect evidence of motor resonance: if the motor system is geared up to execute the observed movement, this should result in an interference when the observed movement is qualitatively different from the simultaneously and voluntarily executed one. When the agent is a robot, however, the interference effect is absent. This findings were firstly observed in a study in which subjects made arm movements while observing arm movements made either by another human or by a robot, which were either in the same direction (congruent) or tangential (incongruent) to the subject's own arm movements. The analysis of observers' arm movements revealed that movement variance (considered a measure of interference to the

movement) differed significantly from the baseline movement condition only when subjects watched the human and not the robot making incongruent movements (Kilner et al., 2003). In an another study (Press et al., 2005) participants were required to perform a movement (e.g. opening their hand) following the presentation of a human or robotic hand in the terminal posture of a compatible movement (opened) or an incompatible movement (closed). The action was initiated faster when it was cued by the compatible movement executed by both the human and the robot, however, even when the human and robotic stimuli were of comparable size, color and brightness, the human hand had a stronger effect on performance. Other psychophysical results can be considered an indirect evidence of the influence that the observed action has on its execution, implying the presence of motor resonance only when the actor is a human. Castiello et al. (2002) found that components of manual grasping movements, such as maximum grip aperture and time to reach peak velocity, are affected by prior observation of a human model grasping an object of the same or different size and are not influenced by prior observation of a robotic hand/arm performing the same tasks. Saygin and Stadler (2012) showed participants human and robot actions, during each trial, actions were briefly occluded from view and participants were required to decide whether or not the action's timing continued naturally and coherently (i.e., in time) after occlusion. The authors suggested that to perform the task, participants were necessarily required to mentally continue the action. Results showed that the visual form of the actor affects action prediction and, possibly, the ability to reenact the observed action.

However, what differentiated humans and robots in these experiments was not only the visual form but also movement kinematics, such as the velocity profile and the trajectory of the limb. The typical velocity profile of human goal-directed hand actions is characterized by a fast-velocity initial phase and a low-velocity final phase (Jeannerod, 1984) while, generally, robot velocity profile is characterized by constant velocity or, anyway, by a velocity profile different from the human one. Regarding the trajectory of the limb, human actions are characterized by smooth, curved shapes, while robotic motion is typically jerky and squared. Even if, as previously suggested, the motor resonance effect is commonly considered the result of the encoding of action kinematic aspects, very few studies specifically investigated the role of movement kinematics on motor resonance. In a study a paradigm similar to the one used by Saygin and Stadler (2012) was employed, people were required to infer the final position of a simple dot moving on a screen upwards or downwards. The last part of the trajectory of the dot was masked. The stimulus could either move replicating the kinematics corresponding to the upward and downward velocity profiles recorded during vertical arm pointing movements or with velocity profiles different from those recorded during a natural movement. Results showed that estimation of the final position decreased

in precision and increased in variability for movements that violated the human kinematic laws (Pozzo et al., 2006). Pozzo and his colleagues (Bisio et al., 2010) have utilized the same type of stimuli to investigate the influence that natural and not natural (artificial) kinematics has on the execution of vertical arm pointing movements. Results showed that participants' movements were automatically contaminated by stimulus velocity but only when it moved according to natural kinematics (see also Bouquet et al., 2007 for a very similar experiment and analogous results). Recently, the same lab (Bisio et al., 2014) deepened the study of this problem by substituting the dot with a humanoid robot. Participants observed a humanoid robot and a human agent move their hands into a pre-specified final position or put an object into a container at various velocities. The robot could either move replicating the velocity profile of a human demonstrator previously recorded or with an artificially altered velocity profile. After action observation, participants were requested to either reach the indicated final position or to transport a similar object into another container. Results showed that participants' velocities varied consistently with stimulus velocities except when the humanoid robot violated the natural laws of motion.

Summarizing, all these data suggest that motor resonance, mainly demonstrated by interference on motor execution, seems to be independent from the physical appearance of the agent but restricted to when the agent moves with natural kinematics. However, to the best of our knowledge, no study has directly investigated the role of the physical appearance of the hand, and the role of the kinematics of the movement, on the modulation of MEPs amplitude usually recorded in hand intrinsic muscles during grasping observation. Consequently, we planned a TMS study in which we recorded MEPs from *Opponens Pollicis* (OP) muscle while participants were required to observe stimuli achieved following graphic manipulations of a video representing a hand that grasps an object. In the first experiment, the video was transformed into a Point Light Display (PLD) (see Johansson, 1973) in which the hand and the to-be-grasped object were no more present. We, therefore, exclusively maintained the trajectory and the kinematics of the original movement and canceled every pictorial cue suggesting the presence of an acting hand. In the second experiment, we modified the kinematics of each single dot of the PLD, obtaining a PLD moving with the same trajectory and duration of the original one but at constant velocity, and we compared the effects on MEPs amplitude determined by the observation of the two different stimuli. Our stimuli, therefore, may be conceived as a progressive transformation of a hand into its "ghost", gradually losing its pictorial and kinematics characteristics. The purpose of our study was, then, to directly test what is suggested by literature, and verify if motor resonance is independent from the physical appearance of the agent but dependent from agent's kinematics.

## 2. Experiment 1

Experiment 1 was devoted to test whether the observation of a PLD stimulus, obtained following a graphic manipulation of a video showing a hand grasping a ball, influences CS excitability. From the seminal study performed by Fadiga and his colleagues (Fadiga et al., 1995) it is known that MEPs recorded from hand intrinsic muscles are modulated during observation of a hand executing both transitive and intransitive actions, and that this effect is inconsistent when participants are involved in attentional control conditions. In the present experiment, participants were involved in an attentional task in which, when required, they have to report the final configuration assumed by the dots in the last trial (i.e., "open" or "closed"). The transformation of the videos into PLD stimuli canceled every pictorial cue indicating the hand as well as the presence the to-be-grasped object. Therefore, present experiment directly tested whether motor resonance is independent from the physical appearance of the agent, as indirectly suggested by several behavioral experiments (Pozzo et al., 2006; Bouquet et al., 2007; Bisio et al., 2010, 2014). It must be emphasized that people who participated to this study have never seen the original video and that experimenters were careful in never making reference to the presence of a moving hand.

### 2.1. Materials and methods

#### 2.1.1. Participants

Sixteen students (8 men) of the University of Ferrara (mean age = 21.8, standard deviation = 2.11) participated in the experiment and gave their written informed consent. All subjects were right-handed according to a standard handedness inventory (Briggs and Nebes, 1975) and reported having normal or corrected-to-normal visual acuity. Participants were unaware of the purposes of the study and were debriefed at the end of the experimental session. The procedures were approved by the local Ethics Committee and were in accordance with the guidelines of the Declaration of Helsinki. None of the participants had neurological, psychiatric, or other medical problems or any contraindication to TMS (Rossi et al., 2009, 2011). No discomfort or adverse effects during TMS were reported or noticed.

#### 2.1.2. Electromyography (EMG) recordings and TMS stimulation.

Motor-evoked potentials (MEPs) were recorded from the *Opponens Pollicis* (OP) muscle (see Fadiga et al., 1995) of the right hand. EMG recordings were performed through surface Ag/AgCl disposable electrodes (1 cm diameter) placed in a belly-tendon montage and connected to Aurion ZeroWire system (Aurion S.r.l, Milano, Italy) for amplification, digitization of the EMG signal (sampling rate: 2 kHz) and stored on a PC for off-line analysis. Signal Software (2.02 Version, Cambridge Electronic Design, UK) was used for TMS triggering and EMG recordings.

Focal TMS was performed by means of a 70-mm figure-of-8 stimulation coil (standard Magstim plastic-covered coil), connected to a Magstim Bistim (The Magstim Company, Carmarthenshire, Wales), producing a maximum output of 2 T at the coil surface (rise time of ∼100 μs, decaying back to zero over ∼0.8 ms). The coil was placed over the left motor cortex tangentially to the scalp, with the handle pointing backward and laterally 45° away from the midline, approximately perpendicular to the line of the central sulcus. This orientation induced a posterior-anterior current in the brain, which tends to activate corticospinal neurons indirectly via excitatory synaptic inputs (Di Lazzaro et al., 1998). We chose the above coil orientation based on the finding that the lowest motor threshold is achieved when the induced electric current in the brain is flowing approximately perpendicular to the central sulcus (Brasil-Neto et al., 1992).

First the hand motor area was localized and the optimal scalp position (OSP) for the right OP, defined as the position from which MEPs with maximal amplitude were recorded, was individuated. The resting motor threshold (rMT), defined as the lowest stimulus intensity able to evoke 5 of 10 MEPs with an amplitude of at least 50 μV, was determined by holding the stimulation coil over the OSP. Coil position was marked on the participants' scalp. The coil was held on the scalp by a coil holder with an articulated arm (Manfrotto, Italy), and the experimenter continuously checked the position of the coil with respect to the marks and compensated for any small movements of the participant's head during data collection. Stimulation intensity during the recording sessions was set at 120% of the rMT and ranged from 42% to 71% (mean = 55.06%, SD = 8.40%) of maximum stimulator output. During MEP recordings, the background EMG signal was continuously monitored, and when voluntary contractions of the recorded muscle were detected, participants were encouraged to fully relax their muscles. A pre-stimulus recording of 150 ms was used to check for the presence of EMG activity before the TMS pulse. The peak-to-peak MEP amplitudes (in millivolts) were collected and stored on a computer for offline analysis.

### 2.1.3.   Stimuli and procedure

Each participant sat in a comfortable armchair in a dimly lit room in front of a 19 inch monitor (resolution 1024×768 pixels; refresh frequency 60 Hz) at a distance of 100 cm. They were instructed to keep their right hand on their leg and to fully relax their muscles.

Stimuli consisted in point-light displays (PLD) (Johansson, 1973) of two videos of the same hand approaching the same ball (diameter 10 cm) placed at the center of the screen. In one video the hand started in a pinch shape and subsequently grasped the ball (without lifting it) with a natural hand shaping during the reaching phase, in the other video the hand reached the ball by maintaining the initial pinch shape both during the reaching phase and during the landing onto the superior part of the object. The two videos were segmented into frames and each frame was inserted as a single slide in Microsoft Office PowerPoint (Microsoft Corporation). In each slide, the outline of the hand was drawn by hand using 44 white dots. Subsequently the original video was removed, leaving only the dots. The final slides were used as frames to obtain the two videos again (90 frames, 25 FPS. Duration of the videos: 3600 ms) now consisting in PLD. It is to note that PLD used in biological motion perception normally represent the main joints of an acting individual, and their number is typically quite limited. We decided to use a higher number of PLD covering the entire outline of the hand, and not only representing the main joints, in order to easily implement the modification of velocity of each dot necessary in Experiment 2 and maintaining as much as possible the original shape of the hand. Only PLD videos were used as stimuli. From now on we make use of the following names for the two PLD stimuli: Open, PLD obtained from the video in which the hand changed from a pinch shape to a grasp shape, and Closed, PLD obtained from the video in which the hand maintained its pinch shape (Fig. 1).


Insert Figure 1 approximately here


Before starting the recording session, participants were presented with examples of the stimuli and the experimenter never referred to them as hand-related stimuli. At the end of the experiment, we asked each participant to estimate what the PLD stimuli depicted in order to investigate how they perceived them. None of them reported that the PLD stimuli included a human hand. Almost the totality of them made no suggestion; one participant reported that the PL display represented the shadow of a human hand representing a dog. Participants were instructed to pay attention to the stimuli and were informed that, when requested during the experiment (Vigilance trials), they had to report the general dots configuration at the end of the movement presented in the preceding trial by saying aloud "open" ("aperto" in Italian) for Open stimuli and "closed" ("chiuso" in Italian) for Closed stimuli. The request consisted in the written sentence "Open or closed?" ("Aperto o

chiuso?" in Italian) presented on the screen at the end of the selected trial and was shown in 20 trials (10 after Open stimulus presentation and 10 after Closed stimulus presentation). TMS stimulation was delivered only during Open stimuli presentation at two time intervals: i) in correspondence with Frame 58, which related with the instant of maximum aperture (opening) of the dots (early delay; 2320 ms from the beginning of the video), and ii) in correspondence with Frame 75, which related with the end of the movement (late delay; 3000 ms from the beginning of the video). Closed trials were introduced exclusively to ensure a constant focusing of attention on the videos, given the request to occasionally perform the discrimination task during Vigilance trials. Each participant was submitted to a total of 96 trials divided into: 48 Open trials (16 TMS at early delay, 16 TMS at late delay, 6 no-TMS, 10 Vigilance trials), 48 Closed trials (38 no-TMS, 10 Vigilance trials). Trials were randomized and subdivided into 2 experimental sessions of 48 trials each.

We recorded 4 series of 8 MEPs each, one before and one after each experimental session, while participants observed a white-colored fixation cross presented on a black background. Comparisons of MEPs amplitudes in the 4 series allowed us to check for any CS excitability change related to TMS per se. For each participant the mean amplitude of MEPs recorded in the 4 series served as baseline.

Stimulus presentation timing, EMG recording, and TMS triggering, as well as randomization of stimuli in a block, were controlled using E-prime V2.0 software (Psychology Software Tools Inc., Pittsburgh, PA) running on a PC.

During TMS, no-TMS and Vigilance trials each trial started with the presentation of a central white cross, serving as fixation point, for 1100 ms; then the PLD video was shown (duration of both videos: 3600 ms) and was followed by a white "wait" word on black background for 6000 ms during both TMS and no-TMS trials. During Vigilance trials the wait screen duration was set at 1000 ms and then the question "Open or closed?" appeared on the screen for 1000 ms, followed by the wait screen for 4000 ms. In TMS trials a single TMS pulse was delivered at one of two moments during the trial, either 2320 ms (early delay) or 3000 ms (late delay) after the onset of the stimulus. Total duration of each trial was 10700 ms (Fig. 2).


Insert Figure 2 approximately here


### 2.1.4. *Data Analysis*

The peak-to-peak amplitude of each MEP was calculated. Trials with background activities greater than 50 μV, amplitudes less than 50 μV or amplitudes greater or less than ±2.5 SD from the mean were discarded. Following this screening procedure, the number of discarded MEPs was inferior to 5% across the different experimental conditions, in both early and late delays except for one participant for whom the number of discarded MEPs was higher than 40% across the different experimental conditions. This participant was excluded from the analysis.

For each participant we considered as baseline the mean amplitude of MEPs recorded in the 4 series of TMS stimulations collected one before and one after each experimental session, and we computed the percent change from baseline as follows: % change = (condition - baseline)/ (baseline) x 100. This procedure allowed us to obtain a normalized MEP index of motor facilitation, hereafter referred to as the normalized MEPs which takes into account inter individual differences in baseline corticospinal excitability and allowed improving normal distribution of the variables as checked with the Kolmogorov–Smirnov test for normality. Normalized MEPs at the two TMS delays (early, late) were compared to each other with paired-sample t-test (two tailed) and against 0 with two separate one-sample t-tests (two-tailed) in order to verify facilitation with respect to baseline. A significance threshold of $p<0.05$ was set for all statistical analyses. The data are reported as the *mean ± standard error of the mean (sem)*.

### 2.3.1. Results

MEPs recorded during each baseline series were entered into a one-way ANOVA with series (before first session, after first session, before second session, after second session) as within-subject variables. The main effect of series was not significant ($F_{3,42} = 0.999$, $p= 0.402$, $\eta_p^2 = 0.066$), excluding the presence of CS excitability change related to TMS per se.

Normalized MEPs values for the two TMS delays were compared against 0 with two separate one-sample t-tests to verify whether CS excitability was modulated by the observed video at different time points. For both TMS delays the t-test was significant (early: $t_{15} = 3.841$, $p = 0.0017$; late: $t_{15} = 4.010$, $p = 0.0012$), showing that normalized MEP values were significantly different from 0 (early: 66% ± 17.18; late: 47.05% ± 11.73), indicating an enhancement of CS excitability induced by observation of PLD videos. We did not obtain any difference between the two conditions as revealed by paired-sample t-test analysis ($t_{15} = 1.445$, $p = 0.170$). Figure 3 illustrates in light grey the MEPs of one subject recorded during the baseline and the two delays, and in black the mean of the traces. Figure 4 shows the mean values of the normalized MEPs for the two TMS delays.

Insert Figure 3 and 4 approximately here

## 3.      Experiment 2

The aim of Experiment 1 was to directly investigate if the pictorial characteristics of the hand are necessary to determine motor resonance. Results of Experiment 1 excluded this possibility: PLD stimuli moving with the same velocity, trajectory and duration of a hand when grasping a ball induced a modulation of CS excitability in the observer. These results are congruent with several behavioral studies showing that motor resonance is independent from the physical appearance of the agent when it moves according to natural kinematic laws (Pozzo et al., 2006; Bouquet et al., 2007; Bisio et al., 2010, 2014). In Experiment 2, to directly verify if motor resonance is restricted to when the agent moves with natural kinematics, we recorded MEPs from OP muscle during observation of PLD stimuli moving with the typical velocity profile of goal-directed hand action, characterized by a fast-velocity initial phase and a low-velocity final phase, and during observation of PLD stimuli moving with the same trajectory and duration but at constant velocity.

### 3.1.      Materials and methods

#### 3.1.1.    *Participants*

Fifteen students (9 men) of the University of Ferrara (mean age = 21.46, standard deviation = 1.59) participated in the experiment and gave their written informed consent. All were right-handed according to a standard handedness inventory (Briggs and Nebes, 1975) and reported having normal or corrected-to-normal visual acuity. Participants were unaware of the purposes of the study and were debriefed at the end of the experimental session. The procedures were approved by the local Ethics Committee and were in accordance with the guidelines of the Declaration of Helsinki. None of the participants had neurological, psychiatric, or other medical problems or any contraindication to TMS (Rossi et al., 2009, 2011). No discomfort or adverse effects during TMS were reported or noticed.

#### 3.1.2.    *Electromyography (EMG) recordings and TMS stimulation*

EMG recordings, TMS stimulation and data collection were performed as in Experiment 1. Stimulation intensity during the recording sessions ranged from 41% to 71% (mean = 51.8%, SD = 7.53%) of maximum stimulator output.

### 3.1.3.    *Stimuli and procedure*

Procedure and task were the same as in Experiment 1; the only difference consisted in the type of stimuli presented.

The PLD stimuli used in Experiment 1 (original velocity PLD stimuli) have been manipulated in order to transform the typical goal-directed hand actions movement, characterized by a fast-velocity initial phase and a low-velocity final phase, into a constant velocity movement (constant velocity PLD stimuli), maintaining, however, the same trajectory and the same duration of the original videos. This manipulation required 2D space interpolation and hereafter we provide a simplified description of the algorithm at its bases. Given that the video frame rate was constant, to solve our velocity requirement and to guarantee that the new trajectory replicated the original one, we changed in an appropriate way the position of each single dot, frame after frame. Considering two consecutive frames (t1 and t2), one single dot is described by its X and Y coordinates relative to each frame. Therefore, we call P1 the dot [X,Y] at t1, and P2 the dot [X,Y] at t2. P1 and P2 are separated by a certain distance (dS) (i.e., the displacement of the dot in time). The time between t1 and t2 corresponds to the frame rate of the video (dt). Consequently, the velocity of the dot (dV) is computed by the formula: dV = dS/dt. Therefore, to transform dV into a constant velocity, given a fixed dt, we computed a new dS in order to maintain constant the ratio dS/dt, frame after frame. Having the new dS, we calculated the new X and Y coordinates of P2. The new P2 was placed along the line connecting P1 with old P2 and having the new dS as distance from P1 (see Fig. 5).

Insert Figure 5 approximately here

As in Experiment 1, in Experiment 2, when requested at a given point during the experiment, participants had to report the general dots configuration at the end of the movement presented in the preceding trial (Vigilance trials).

TMS stimulation was delivered during 16 Open original velocity PLD stimuli trials and during 16 Open constant velocity PLD stimuli trials, in correspondence with Frame 58, which related with the instant of maximum aperture of the dots (2320 ms from the beginning of the video).

Each participant was exposed to a total of 96 trials divided into 48 original velocity PLD stimuli and 48 constant velocity PLD stimuli. For each type of velocity, trials were divided into: 24 Open trials (16 TMS, 3 no-TMS, 5 Vigilance trials), 24 Closed trials (19 no-TMS, 5 Vigilance trials). Trials were randomized and subdivided into 2 experimental sessions of 48 trials each.

Before starting the recording session, participants were presented with examples of the stimuli and the experimenter never referred to them as hand-related stimuli or that they differed for some characteristics. At the end of the experiment, we asked each participant to estimate the PLD stimuli depicted and none of them reported that the PLD stimuli included a human hand. Almost the totality of them made no suggestion; one participant reported that the PL display represented a mouth. None of the participants have perceived any difference between the two types of PLD stimuli used in Experiment 2.

As in Experiment 1, to control for any CS excitability change related to TMS per se, and to have a baseline, in the present experiment we recorded 4 series of 8 MEPs each, one before and one after each experimental session, while participants observed a white-colored fixation cross presented on a black background.

Stimulus-presentation timing, EMG recording, and TMS triggering, as well as randomization of stimuli in a block, were controlled using E-prime V2.0 software (Psychology Software Tools Inc., Pittsburgh, PA) running on a PC.

The general procedure was exactly the same used in Experiment 1 (see Fig. 2), except for time of TMS delivery which was always in correspondence of frame 58.

### 3.1.4    Data Analysis.

The peak-to-peak amplitude of each MEP was calculated, and trials with background activities greater than 50 μV, amplitudes less than 50 μV or amplitudes greater or less than ±2.5 SD from the mean were discarded. After this procedure, the number of discarded MEPs was inferior of 5% across the different experimental conditions between the original and constant velocity PLD stimuli.

Data were normalized as in Experiment 1 and percent change from baseline of mean raw amplitudes of MEPs recorded during TMS trials was computed separately for each velocity.

Normalized MEP amplitudes at the two conditions (original velocity, constant velocity) were compared to each other with paired-sample t-test (two-tailed), and against 0 with two separate one-sample t-tests (two-tailed) in order to verify facilitation with respect to baseline. A significance

threshold of $p<0.05$ was set for all statistical analyses. The data are reported as the *mean ± standard error of the mean* (*sem*).

## 3.2.    Results

MEPs recorded during each baseline series were entered into a one-way ANOVA with series (before first session, after first session, before second session, after second session) as within-subject variables. The main effect of series was not significant ($F_{3,42} = 1.40$, $p = 0.255$, $\eta_p^2 = 0.091$). The normalized MEP values for the two conditions were compared against 0 with two separate one-sample t-tests to verify whether CS excitability was modulated by the observation of the PLD stimuli characterized by the different velocity profile. For both conditions the significance of the sample t-test (original velocity: $t_{15} = 4.368$, $p = 0.0006$; constant velocity: $t_{15} = 3.533$, $p = 0.003$) indicates that the normalized MEP values were significantly different from 0 (original velocity: 65.27% ± 14.94 constant velocity: 76.60% ± 21.68). We did not obtain any difference between the two conditions as revealed by paired-sample t-test analysis ($t_{15} = 0.87$, $p = 0.394$). Figure 6 illustrates in light grey the MEPs of one subject recorded during the baseline and the two velocity conditions, whereas  in black the mean of the traces. Figure 7 shows the mean values of the normalized MEPs for the two velocity conditions.

Insert Figure 6 and 7 approximately here

## 4.    Discussion

The purpose of this study was to fill a gap in the literature that explores the characteristics of the observed action necessary to determine the involvement of the observer's motor system. Several neurophysiological, neuroimaging and behavioral studies (Brass,et al., 2000, 2001; Craighero et al., 2002; Cross et al., 2012; Fadiga et al., 2006; Gazzola et al., 2007; Kilner et al., 2003; Oberman et al., 2007; Perani et al., 2001; Shimada, 2010; Tai et al., 2004) investigated the role of physical appearance and of movement kinematics of the agent in activating the observer's neural networks associated with action perception and execution, often with conflicting results. However, the activation in motor areas alone is not yet evidence of the fact that during action observation sensory-motor processes are activated that are also involved in bringing the observed behavior about, as all processing is still accommodated in the central nervous system. The only direct evidence for actual recruitment of the body by action observation is given by results showing that

when TMS is delivered over primary motor cortex, increased motor evoked potentials (MEPs) can be measured during action observation. This increased corticospinal (CS) excitability may depend on an increased excitability in primary motor cortex which, in turn, may depend on an increased excitability in premotor cortex. Premotor cortex is one of the core areas activating during action perception and execution (Cattaneo and Rizzolatti, 2009). Therefore, enlarged MEPs are one of the privileged proofs indicating that action execution and action observation share sensory-motor processes. Although this topic is of great interest, no experiments directly testing the effects of physical appearance and of movement kinematics of the agent on CS excitability are present in literature. To fill this gap, we recorded MEPs from hand intrinsic muscles during observation of Point Light Displays (PLD) stimuli representing a hand having lost its physical appearance and kinematics characteristics.

In Experiment 1 we recorded MEPs from *Opponens Pollicis* (OP) muscle while participants were required to observe PLD stimuli moving with the same velocity, trajectory and duration of a hand when grasping a ball. From the pioneer study by Johansson (Johansson, 1973) it is known that image sequences constructed from point-lights attached to the limbs of a human actor can readily be identified as depicting actions, despite totally lacking visual cues such as color, shading and contours. Several studies have used PLD stimuli to verify the role of physical appearance of the agent in inducing the effects typically determined by human action observation in the central nervous system. Neuroimaging studies of PLD perception have reported activation in the superior temporal gyrus and superior temporal sulcus (STS), in the motion-sensitive region MT and surrounding areas, in the parietal cortex, in other regions in visual cortex (Grèzes et al., 2001; Vaina et al., 2001) and, interestingly, in premotor cortex (Saygin et al., 2004). Another technique used to individuate the neural networks associated with action perception and execution is electroencephalography (EEG), in particular mu frequency band oscillations recorded with scalp electrodes over sensorimotor cortex. The mu rhythm is an 8-13 Hz oscillation generated in sensorimotor cortex that reaches maximal amplitude when individuals are at rest. When subjects move, imagine movement, or observe movements, neurons in this area fire asynchronously, thus reducing mu amplitudes (Cochin et al., 1998; Pineda et al., 2000). It is assumed that mu rhythms reflect the downstream modulation of motor neurons by cells in the premotor cortex (Pineda, 2005). The same stimuli used by Saygin and colleagues (Saygin et al., 2004) have been used to test the effects in an EEG study, and results showed that point-light motion suppresses mu rhythm (Ulloa and Pineda, 2007). Results of Experiment 1, demonstrating a modulation in CS excitability during observation of PLD stimuli, are in line with these findings and provide the first direct demonstration

that the activation induced by these stimuli is not confined to the premotor cortex but extends into primary motor area as well.

In sum, these findings indicate that PLD stimuli, despite totally lacking visual cues, seem to convey sufficient information about movements of the human body to activate sensory-motor processes similar to those typically involved by human action observation, and are in favor of the interpretation claiming that motor resonance effect is the result of the encoding of action kinematic aspects and not of the physical appearance of the agent (Bisio et al., 2010, 2014; Bouquet et al., 2007; Leonetti et al., 2015; Pozzo et al., 2006). Almost the totality of the studies investigating the effects induced by PLD stimuli used matched scrambled animations as control stimuli (Grossman et al., 2000; Saygin et al., 2004; Servos et al., 2002; Ulloa and Pineda, 2007). These animations were created by randomizing the initial starting position of the dots and leaving the motion paths intact. These scrambled movies resembled a cluster of dots moving at different speeds in various directions, with an overall motion "flow" in common. In these movies the original movement was no more detectable. Therefore, these control stimuli differed from the experimental ones for two characteristics: the kinematics of the dots and the possibility to still recognize the original movement. It is important to note that none of our participants reported that the PLD stimuli included a moving human hand and, consequently, our data suggest that CS excitability is modulated by PLD stimuli conserving the original kinematics of the movement even if not identified as depicting actions.

In Experiment 2 we wanted to verify if natural kinematics is not only a sufficient condition, as suggested by results of Experiment 1, but also a necessary condition to activate the same sensory-motor processes activated during action observation. To this purpose, we contrasted the effects on CS excitability determined by PLD stimuli in which the velocity profile of the PL markers were consistent with a real movement, including acceleration and deceleration, with those determined by PLD stimuli in which every PL marker maintained its trajectory and movement time but changed its velocity profile from accelerated/decelerated to constant. The results indicated that natural kinematics is a sufficient condition but not a necessary one, since constant velocity PLD stimuli determined a modulation of CS excitability not different from that determined by accelerated/decelerated PLD stimuli.

Thus, present results indicate that physical appearance, natural kinematics, and the possibility to identify the action behind the stimulus are not necessary conditions to modulate CS excitability during stimuli observation. The obvious question that arises therefore is: Which are the characteristics of the stimulus necessary to activate the observer's sensory-motor system? Only few studies helped us in responding to this question, given the nature of the stimuli used so far in

experiments investigating the presence of common processes between action execution and perception. In general, these stimuli consisted in real agents executing actions in front of participants, in videos of real agents executing actions, or in PLD stimuli readily identified as depicting specific human actions. All these stimuli maintain at least two out of the three not necessary conditions reported above and, moreover, are immediately recognized as humans (or human body parts) performing actions.

We need, therefore, to find suggestions in the available literature investigating the effects induced by the perception of stimuli not evidently visually depicting human actions. The first possible suggestion is given by an fMRI study showing that the observation of hand shadows resembling moving animals activates the premotor cortex, demonstrating that the brain "sees" the performing hand also behind its appearance and typical kinematics (Fadiga et al., 2006). Another suggestion is offered by experiments investigating the effects induced by the listening of sounds produced by human body parts (e.g., two hands clapping). These sounds activate the premotor cortex with a somatotopic organization: the left dorsal premotor cortex is more responsive to the execution and hearing of hand movements than to mouth actions, and the more ventral region is more involved in processing sounds performed by the mouth (Aglioti and Pazzaglia, 2010). Not only listening the sound of actions is able to activate the sensory-motor system, but also listening of words representing actions. A series of experiments clearly demonstrated that listening to action-related sentences modulates CS excitability in a specific way (Buccino et al., 2005; Innocenti et al., 2014) and activates those sectors of the premotor cortex where the actions described are motorically coded (Tettamanti et al., 2005).

All the stimuli used in these experiments possess a common feature: They represent motorically replicable stimulus. Everybody knows that animal hand shadows require hand movements to be performed, and participants know that they may replicate, at least partially, those movements. Also, when we listen to two hands clapping, or we hear footsteps in the hall, we know immediately which part of our body we should move to reproduce that sound, and it is undoubtful that we know how to replicate the action described by a verb. A crucial further suggestion towards this possibility is given by a TMS experiment (Alaerts et al., 2009) in which participants observed shadow animations of abduction/adduction movements of the right index finger, presented from a lateral view. From this perspective, the amount of motion was very small and the hand was absolutely unrecognizable, as well as the different parts of the "moving object" and their potential dynamic relationships (cfr. Figure 1C, in Alaerts et al., 2009). Hand motor resonant responses were comparable to baseline for this observation condition. However, when the subjects were familiarized with the formerly unrecognized shadow animation, by revealing the actual index

motion that it depicted, and the same shadow animation was observed post-familiarization, motor resonant responses suddenly became apparent. Then, it is sufficient to know that the tiny observed movement reflects a clear and consistent dynamic relationship between fingers to determine a motor resonance effect in the recorded hand muscles. It is, therefore, conceivable that the sensory-motor system activates every time the stimulus cues to the perceivers the possibility for them to reproduce it. The only way to produce a perceivable stimulus is by moving, and the sensory consequences of the movement can be the most various: the vision of animal hand shadows on the wall, the listening of applause, or the vision of a hand grasping a cup. However, how much detail does the visual stimulus need to contain in order to be recognised as a replicable movement? The results of Experiment 2, showing a CS modulation in presence of dots moving at constant velocity, but maintaining a clear trajectory, opens the possibility that even more abstract forms of actions, those that have lost almost all the hallmarks of action, may be perceived as motorically replicable. Also stimuli that merely suggest in a dynamic way the presence of a trajectory seem to be able to activate the sensory-motor system, as reported by those studies using single dots to verify the effect of kinematics on the ability to estimate the final position of a masked dot (Bisio et al., 2010; Bouquet et al., 2007), or on the presence of a motor contagion induced by dot velocity (Pozzo et al., 2006). Going further, we can find experimental data that indicate that even static stimuli can suggest the presence of a replicable movement. In a series of TMS experiments, the effects on CS excitability of static snapshots of hands suggesting actions were investigated. Results showed that the observation of static snapshots representing a mimicked grasping action induced an increase in excitability as compared with observation of resting or relaxed hands (Urgesi et al., 2006, 2010). Going even further, the mere presence of a handled object near enough to be actually reachable for the participants, and also when it was out of reach for them, provided that it was ready to an avatar's hand, is able to modulate CS excitability (Cardellicchio et al., 2013). Furthermore, premotor cortex activates in the same way both when participants observed a human model grasping an object and when the agent was simply gazing at the same object (Pierno et al., 2006). All these data definitely indicate that the sensory-motor system seems to be involved whenever the presence of a replicable movement is inferred. This inference is realized when the movement is taking place, when it is known that will be carried out, and when it is probable to occur.

A huge amount of papers have been published in the last decades suggesting the possible role of sensory-motor system involvement during action observation, and the common interpretation is that it is fundamental for inferring others' motor intentions, for predicting the consequences of their actions to the purpose of collaborating or contrasting them, or merely understanding what others are doing (Iacoboni, 2009; Rizzolatti and Craighero, 2004; Rizzolatti and Fogassi, 2014; Rizzolatti and

Sinigaglia, 2007). Present data and discussion of literature add a further possibility: the involvement of the motor system may be mandatory whenever the individual assumes to be able to replicate a real or hypothesized movement, executed by a human, by an animal (Buccino et al., 2004a) or by an object, provided that its final goal (the most various: position in space, direction of movement, posture of a body part, to-be-produced sound, specific interaction with an object, shadow on the wall, etc.) is recognized and reproducible. The involvement of the motor system may be mandatory in all these cases since we need to predict and therefore to promptly react to all external events, and the only way we have to anticipate a future state of something or somebody is to simulate it with our body by recalling the sensory consequences of actions that we started to collect already during our intrauterine life (Zoia et al., 2007) and we begin to use from the first days of life (Craighero et al., 2011). This recall in humans is not limited to transitive actions as in monkeys (di Pellegrino et al., 1992) but extends to intransitive movements (Fadiga et al., 1995) and possibly, as suggested by present results, to moving dots. Our body may tend to replicate a perceived movement even when this is not kinematically similar, albeit, obviously, the replica is better performed when the observed movement satisfies all the features typical of a human movement. This is why, for example, the opening of the hand is initiated faster when cued by a compatible movement executed by both the human and the robot, though the human hand had a stronger effect on performance (Press et al., 2005). And this is why participants were able to infer the final position of a simple dot even when the dot didn't replicated the velocity profiles of a human arm moving, though with less precision and greater variability than when the dot moved with natural kinematics (Pozzo et al., 2006). From this point of view, it's a limit of our body to be less able to simulate a kinematically different movement than a more similar one and, consequently, to be less able to recognize the consequences of the former movement than of the latter one.

As it clearly appears, this interpretation of the results needs further investigation to be maintained, given that objects' movements, until now, have been used exclusively as control stimuli to support the difference between the effects induced on the observer by them and by human actions. In these experimental designs, however, it can't be excluded that when one experimental condition is recognized as representing humans (or human body parts) performing actions, the system can immediately tune to specifically elaborate human actions, inducing a sort of selective attention for human actions. Present work is the first one in which, to investigate the effects on the motor system, only stimuli not recognized as human actions were presented. It is necessary to deepen the investigation, and discover which are the minimum requirements of the stimulus necessary to activate the motor system.

In conclusion, this interpretation of the findings, claiming an involvement of the sensory-motor processes during perception of unspecific but reproducible external events, suggests that literature investigating the role of the motor system in perception underestimated the importance of the effects induced by control stimuli. This attitude was probably prompted by the amazing discovery of mirror neurons in monkeys. From the seminal work made by the Rizzolatti's lab (di Pellegrino et al., 1992), an incredible amount of studies have been devoted to demonstrate the peculiar relationship existing between others actions and the actions executed by the observer. It is undoubtful that this relation is present and that it is special. However, it could just represent the situation in which the compliance of the sensory-motor system is the greatest and, consequently, the effects of its involvement are the most effective.

**References**

Aglioti, S. M., & Pazzaglia, M. (2010). Representing actions through their sound. *Experimental Brain Research*, *206*(2), 141–151. http://doi.org/10.1007/s00221-010-2344-x.

Alaerts, K., Van Aggelpoel, T., Swinnen, S. P., & Wenderoth, N. (2009). Observing shadow motions: resonant activity within the observer's motor system? *Neuroscience Letters 461*(3), 240-244. doi: 10.1016/j.neulet.2009.06.055.

Alaerts, K., de Beukelaar, T. T., Swinnen SP, & Wenderoth, N. (2012). Observing how others lift light or heavy objects: time-dependent encoding of grip force in the primary motor cortex. *Psychological Research 76*(4), 503-513. doi: 10.1007/s00426-011-0380-1.

Bisio, A., Sciutti, A., Nori, F., Metta, G., Fadiga, L., Sandini, G., & Pozzo, T. (2014). Motor contagion during human-human and human-robot interaction. *PloS One*, *9*(8), e106172. http://doi.org/10.1371/journal.pone.0106172

Bisio, A., Stucchi, N., Jacono, M., Fadiga, L., & Pozzo, T. (2010). Automatic versus voluntary motor imitation: Effect of visual context and stimulus velocity. *PLoS ONE*, *5*(10). http://doi.org/10.1371/journal.pone.0013506

Borroni, P., & Baldissera, F. (2008). Activation of motor pathways during observation and execution of hand movements. *Social Neuroscience*, *3*(3-4), 276–288. http://doi.org/10.1080/17470910701515269

Bouquet, C. A., Gaurier, V., Shipley, T., Toussaint, L., & Blandin, Y. (2007). Influence of the perception of biological or non-biological motion on movement execution. *Journal of Sports Sciences*, *25*(5), 519–530. http://doi.org/10.1080/02640410600946803

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J., & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society*, *9*(1), 132–136. http://doi.org/10.1097/00004691-199201000-00014

Brass, M., Bekkering, H., & Prinz, W. (2001). Movement observation affects movement execution in a simple response task. *Acta Psychologica*, *106*(1-2), 3–22. http://doi.org/10.1016/S0001-6918(00)00024-X

Brass, M., Bekkering, H., Wohlschläger, A., & Prinz, W. (2000). Compatibility between observed and executed finger movements: comparing symbolic, spatial, and imitative cues. *Brain and Cognition*, *44*(2), 124–143. http://doi.org/10.1006/brcg.2000.1225

Briggs, G. G., & Nebes, R. D. (1975). Patterns of hand preference in a student population. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *11*(3), 230–238. http://doi.org/10.1016/S0010-9452(75)80005-0

Brighina, F., La Bua, V., Oliveri, M., Piazza, A., & Fierro, B. (2000). Magnetic stimulation study during observation of motor tasks. *Journal of the Neurological Sciences*, *174*(2), 122–126. http://doi.org/S0022-510X(00)00271-9 [pii]

Buccino, G., Binkofski, F., & Riggio, L. (2004). The mirror neuron system and action recognition. *Brain and Language*. http://doi.org/10.1016/S0093-934X(03)00356-0

Buccino, G., Lui, F., Canessa, N., Patteri, I., Lagravinese, G., Benuzzi, F., … Rizzolatti, G. (2004a). Neural circuits involved in the recognition of actions performed by nonconspecifics: an FMRI study. *Journal of Cognitive Neuroscience*, *16*(1), 114–126. http://doi.org/10.1162/089892904322755601

Buccino, G., Riggio, L., Melli, G., Binkofski, F., Gallese, V., & Rizzolatti, G. (2005). Listening to action-related sentences modulates the activity of the motor system: A combined TMS and behavioral study. *Cognitive Brain Research*, *24*(3), 355–363. http://doi.org/10.1016/j.cogbrainres.2005.02.020

Cardellicchio, P., Sinigaglia, C., & Costantini, M. (2013). Grasping affordances with the others hand: A tms study. *Social Cognitive and Affective Neuroscience*, *8*(4), 455–459. http://doi.org/10.1093/scan/nss017

Caspers, S., Zilles, K., Laird, A. R., & Eickhoff, S. B. (2010). ALE meta-analysis of action observation and imitation in the human brain. *NeuroImage*, *50*(3), 1148–1167. http://doi.org/10.1016/j.neuroimage.2009.12.112

Castiello, U., Lusher, D., Mari, M., Edwards, M., & Humphreys, G. W. (2002). Observing a human or a robotic hand grasping an object: differential motor priming effects. *Common Mechanisms in Perception and Action*, *19*, 315–333. Retrieved from <Go to ISI>://000189493700016

Cattaneo, L., & Rizzolatti, G. (2009). The mirror neuron system. *Archives of Neurology*. http://doi.org/10.1001/archneurol.2009.41

Clark, S., Tremblay, F., & Ste-Marie, D. (2004). Differential modulation of corticospinal excitability during observation, mental imagery and imitation of hand actions. *Neuropsychologia*, *42*(1), 105–112. http://doi.org/10.1016/S0028-3932(03)00144-1

Cochin, S., Barthelemy, C., Lejeune, B., Roux, S., & Martineau, J. (1998). Perception of motion and qEEG activity in human adults. *Electroencephalography and Clinical Neurophysiology*, *107*(4), 287–295. http://doi.org/10.1016/S0013-4694(98)00071-6

Craighero, L., Bello, A., Fadiga, L., & Rizzolatti, G. (2002). Hand action preparation influences the responses to hand pictures. *Neuropsychologia*, *40*(5), 492–502. http://doi.org/10.1016/S0028-3932(01)00134-8

Craighero, L., Leo, I., Umiltà, C., & Simion, F. (2011). Newborns' preference for goal-directed actions. *Cognition*, *120*, 26–32. http://doi.org/10.1016/j.cognition.2011.02.011

Cross, E. S., Liepelt, R., Antonia, A. F., Parkinson, J., Ramsey, R., Stadler, W., & Prinz, W. (2012). Robotic movement preferentially engages the action observation network. *Human Brain Mapping*, *33*(9), 2238–2254. http://doi.org/10.1002/hbm.21361

Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., … Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroencephalography and Clinical Neurophysiology - Electromyography and Motor Control*, *109*(5), 397–401. http://doi.org/10.1016/S0924-980X(98)00038-1

Di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., & Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. *Experimental Brain Research*, *91*(1), 176–180. http://doi.org/10.1007/BF00230027

Fadiga, L., Craighero, L., Destro, M. F., Finos, L., Cotillon-Williams, N., Smith, A. T., & Castiello, U. (2006). Language in shadow. *Social Neuroscience*, *1*(2), 77–89. http://doi.org/10.1080/17470910600976430

Fadiga, L., Craighero, L., & Olivier, E. (2005). Human motor cortex excitability during the perception of others' action. *Current Opinion in Neurobiology*. http://doi.org/10.1016/j.conb.2005.03.013

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

Gangitano, M., Mottaghy, F. M., & Pascual-Leone, A. (2001). Phase-specific modulation of cortical motor output during movement observation. *Neuroreport*, *12*(7), 1489–1492. http://doi.org/10.1097/00001756-200105250-00038

Gazzola, V., Rizzolatti, G., Wicker, B., & Keysers, C. (2007). The anthropomorphic brain: The mirror neuron system responds to human and robotic actions. *NeuroImage*, *35*(4), 1674–1684. http://doi.org/10.1016/j.neuroimage.2007.02.003

Grèzes, J., Fonlupt, P., Bertenthal, B., Delon-Martin, C., Segebarth, C., & Decety, J. (2001). Does perception of biological motion rely on specific brain regions? *NeuroImage*, *13*(5), 775–85. http://doi.org/10.1006/nimg.2000.0740

Grossman, E., Donnelly, M., Price, R., Pickens, D., Morgan, V., Neighbor, G., & Blake, R. (2000). Brain areas involved in perception of biological motion. *Journal of Cognitive Neuroscience*, *12*(5), 711–720. http://doi.org/10.1162/089892900562417

Hari, R. (2006). Chapter 17 Action-perception connection and the cortical mu rhythm. *Progress in Brain Research*. http://doi.org/10.1016/S0079-6123(06)59017-X

Iacoboni, M. (1999). Cortical Mechanisms of Human Imitation. *Science*. http://doi.org/10.1126/science.286.5449.2526

Iacoboni, M. (2009). Imitation, empathy, and mirror neurons. *Annual Review of Psychology*, *60*, 653–670. http://doi.org/10.1146/annurev.psych.60.110707.163604

Innocenti, A., De Stefani, E., Sestito, M., & Gentilucci, M. (2014). Understanding of action-related and abstract verbs in comparison: a behavioral and TMS study. *Cognitive Processing*, *15*(1), 85–92. http://doi.org/10.1007/s10339-013-0583-z

Jeannerod, M. (1984). The timing of natural prehensile movements. *Journal of Motor Behaviour*, *16*, 235–254.

Johansson, G. (1973). Visual perception of biological motion and a model for its analysis. *Perception Psychophysics*, *14*(2), 201–211. http://doi.org/10.3758/BF03212378

Kilner, J. M., Paulignan, Y., & Blakemore, S. J. (2003). An interference effect of observed biological movement on action. *Current Biology*, *13*(6), 522–525. http://doi.org/10.1016/S0960-9822(03)00165-9

Leonetti, A., Puglisi, G., Siugzdaite, R., Ferrari, C., Cerri, G., Borroni, P. (2015). What you see is what you get: motor resonance in peripheral vision. *Experimental Brain Research* Jul 14. [Epub ahead of print] PubMed PMID: 26169105

Mattar, A. A. G., & Gribble, P. L. (2005). Motor learning by observing. *Neuron*, *46*(1), 153–160. http://doi.org/10.1016/j.neuron.2005.02.009

Montagna, M., Cerri, G., Borroni, P., & Baldissera, F. (2005). Excitability changes in human corticospinal projections to muscles moving hand and fingers while viewing a reaching and grasping action. *European Journal of Neuroscience*, *22*(6), 1513–1520. http://doi.org/10.1111/j.1460-9568.2005.04336.x

Oberman, L. M., McCleery, J. P., Ramachandran, V. S., & Pineda, J. A. (2007). EEG evidence for mirror neuron activity during the observation of human and robot actions: Toward an analysis of the human qualities of interactive robots. *Neurocomputing*, *70*(13-15), 2194–2203. http://doi.org/10.1016/j.neucom.2006.02.024

Perani, D., Fazio, F., Borghese, N. A., Tettamanti, M., Ferrari, S., Decety, J., & Gilardi, M. C. (2001). Different brain correlates for watching real and virtual hand actions. *NeuroImage*, *14*(3), 749–58. http://doi.org/10.1006/nimg.2001.0872

Pierno, A. C., Becchio, C., Wall, M. B., Smith, A. T., Turella, L., & Castiello, U. (2006). When gaze turns into grasp. *Journal of Cognitive Neuroscience*, *18*(12), 2130–2137. http://doi.org/10.1162/jocn.2006.18.12.2130

Pineda, J. a. (2005). The functional significance of mu rhythms: translating "seeing" and "hearing" into "doing". *Brain Research. Brain Research Reviews*, *50*(1), 57–68. http://doi.org/10.1016/j.brainresrev.2005.04.005

Pineda, J. a., Allison, B. Z., & Vankov, a. (2000). The effects of self-movement, observation, and imagination on ?? rhythms and readiness potentials (RP'S): Toward a brain-computer interface (BCI). *IEEE Transactions on Rehabilitation Engineering*, *8*(2), 219–222. http://doi.org/10.1109/86.847822

Pozzo, T., Papaxanthis, C., Petit, J. L., Schweighofer, N., & Stucchi, N. (2006). Kinematic features of movement tunes perception and action coupling. *Behavioural Brain Research*, *169*(1), 75–82. http://doi.org/10.1016/j.bbr.2005.12.005

Press, C., Bird, G., Flach, R., & Heyes, C. (2005). Robotic movement elicits automatic imitation. *Cognitive Brain Research*, *25*(3), 632–640. http://doi.org/10.1016/j.cogbrainres.2005.08.020

Rizzolatti, G., & Craighero, L. (2004). The mirror-neuron system. *Annual Review of Neuroscience*, *27*, 169–192. http://doi.org/10.1146/annurev.neuro.27.070203.144230

Rizzolatti, G., & Fogassi, L. (2014). The mirror mechanism: recent findings and perspectives. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1644), 20130420. http://doi.org/10.1098/rstb.2013.0420

Rizzolatti, G., & Sinigaglia, C. (2007). Mirror neurons and motor intentionality. *Functional Neurology*, *22*(4), 205–210. http://doi.org/2622 [pii]

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. In *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* (Vol. 120, pp. 2008–2039). http://doi.org/10.1016/j.clinph.2009.08.016

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clinical Neurophysiology*, *122*(8), 1686. http://doi.org/10.1016/j.clinph.2010.12.037

Saygin, A. P., & Stadler, W. (2012). The role of appearance and motion in action prediction. *Psychological Research*, *76*(4), 388–394. http://doi.org/10.1007/s00426-012-0426-z

Saygin, A. P., Wilson, S. M., Hagler, D. J., Bates, E., & Sereno, M. I. (2004). Point-light biological motion perception activates human premotor cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(27), 6181–6188. http://doi.org/10.1523/JNEUROSCI.0504-04.2004

Servos, P., Osu, R., Santi, A., & Kawato, M. (2002). The neural substrates of biological motion perception: an fMRI study. *Cerebral Cortex (New York, N.Y. : 1991)*, *12*(7), 772–782. http://doi.org/10.1093/cercor/12.7.772

Shimada, S. (2010). Deactivation in the sensorimotor area during observation of a human agent performing robotic actions. *Brain and Cognition*, *72*(3), 394–399. http://doi.org/10.1016/j.bandc.2009.11.005

Tai, Y. F., Scherfler, C., Brooks, D. J., Sawamoto, N., & Castiello, U. (2004). The Human Premotor Cortex Is "Mirror" only for Biological Actions. *Current Biology*, *14*(2), 117–120. http://doi.org/10.1016/j.cub.2004.01.005

Tettamanti, M., Buccino, G., Saccuman, M. C., Gallese, V., Danna, M., Scifo, P., … Perani, D. (2005). Listening to action-related sentences activates fronto-parietal motor circuits. *Journal of Cognitive Neuroscience*, *17*(2), 273–281. http://doi.org/10.1162/0898929053124965

Ulloa, E. R., & Pineda, J. a. (2007). Recognition of point-light biological motion: mu rhythms and mirror neuron activity. *Behavioural Brain Research*, *183*(2), 188–94. http://doi.org/10.1016/j.bbr.2007.06.007

Urgesi, C., Maieron, M., Avenanti, A., Tidoni, E., Fabbro, F., & Aglioti, S. M. (2010). Simulating the future of actions in the human corticospinal system. *Cerebral Cortex*, *20*(11), 2511–2521. http://doi.org/10.1093/cercor/bhp292

Urgesi, C., Moro, V., Candidi, M., & Aglioti, S. M. (2006). Mapping implied body actions in the human motor system. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(30), 7942–9. http://doi.org/10.1523/JNEUROSCI.1289-06.2006

Vaina, L. M., Solomon, J., Chowdhury, S., Sinha, P., & Belliveau, J. W. (2001). Functional neuroanatomy of biological motion perception in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(20), 11656–61. http://doi.org/10.1073/pnas.191374198

Vanderwert, R. E., Fox, N. A., & Ferrari, P. F. (2013). The mirror mechanism and mu rhythm in social development. *Neuroscience Letters*. http://doi.org/10.1016/j.neulet.2012.10.006

Vogt, S., Buccino, G., Wohlschläger, A. M., Canessa, N., Shah, N. J., Zilles, K., … Fink, G. R. (2007). Prefrontal involvement in imitation learning of hand actions: Effects of practice and expertise. *NeuroImage*, *37*(4), 1371–1383. http://doi.org/10.1016/j.neuroimage.2007.07.005

Zoia, S., Blason, L., D'Ottavio, G., Bulgheroni, M., Pezzetta, E., Scabar, A., & Castiello, U. (2007). Evidence of early development of action planning in the human foetus: A kinematic study. *Experimental Brain Research*, *176*(2), 217–226. http://doi.org/10.1007/s00221-006-0607-3

**Captions**

Figure 1

PLD: Stimuli used in Experiment 1. Upper panel: Open; PLD obtained from the video in which the hand changed from a pinch shape to a grasp shape. Lower panel: Closed; PLD obtained from the video in which the hand maintained its pinch shape. For each panel three frames are shown. Leftmost: first frame. Center: frame number 58, which related with the instant of maximum aperture of the dots for the Open stimuli (early delay; 2320 ms from the beginning of the video). Rightmost: frame number 75, which related with the end of the movement (late delay; 3000 ms from the beginning of the video). Video: original videos.

Figure 2

Experimental trials sequence for TMS trials, no-TMS trials and Vigilance trials.

Figure 3

The motor evoked potentials (MEPs) of one subject are presented. Superimposed responses (n=16) evoked in Baseline, Early delay, and Late delay conditions are shown in light grey. For the Baseline condition 16 responses were randomly selected from the 32 recorded. Traces are aligned with the instant of TMS delivery. Mean traces for each condition are shown in black. Ordinates are in millivolts (mV).

Figure 4

Mean values of the normalized motor evoked potentials (MEPs) for all subjects relative to the two TMS delays. Abscissa: instant of TMS delivery. Early delay: 2320 ms after the onset of the video (instant of maximum aperture of the dots). Late delay: 3000 ms after the onset of the video (end of the movement). Duration of the video: 3600 ms. Ordinate: percent change from baseline. Thin lines above histograms indicate standard error of the mean.

Figure 5

Panel 1: An example of 2D space interpolation applied to the X coordinate (1A) and to the Y coordinate (1B) of a single dot (i.e., dot number 12) in each of the 90 frames of the video. Red lines represent the original values of X (1A) and Y (1B) coordinates. Blue lines represent the interpolated values of X (1A) and Y (1B) coordinates. Ordinate: X (1A) and Y (1B) values. Abscissa: frame number.

Panel 2: Examples of X, Y coordinates of each of the 44 dots present in each frame, shown in 10 frame steps, and relative to closed videos. 2A: original values. 2B: interpolated values. Ordinate: Y values. Abscissa: X values.

Panel 3: Velocity profile of the original (red line) and of the interpolated (blue line) dots. Note the constant velocity profile of the interpolated dots starting from the movement frames. Ordinate: pixels/frame. Abscissa: frame number.

Figure 6

The motor evoked potentials (MEPs) of one subject are presented. Superimposed responses (n=16) evoked in Baseline, Original velocity and Constant velocity conditions are shown in light grey. For the Baseline condition 16 responses were randomly selected from the 32 recorded. Traces are aligned with the instant of TMS delivery. Mean traces for each condition are shown in black. Ordinates are in millivolts (mV).

Figure 7

Mean values of the normalized motor evoked potentials (MEPs) for all subjects relative to the two experimental conditions. Abscissa: type of velocity profile of the stimuli. Original velocity: typical goal-directed hand actions velocity profile, characterized by a fast-velocity initial phase and a low-velocity final phase. Constant velocity: constant velocity movement. Ordinate: percent change from baseline. Thin lines above histograms indicate standard error of the mean.