Doctoral School in Cognitive and Brain Sciences, XXV cycle

Motor Resonance meets Motor Performance

Neurocognitive investigations with Transcranial Magnetic Stimulation

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1.MIRROR NEURONS: HOW DID WE GET THERE

1.1 Neurons in F5: the discovery of goal-directed motor neurons in the premotor cortex.

Until the 1970's an influential concept formulated by Woolsey and colleagues (Woolsey et al., 1952) on the organization of the cortical motor system was that no premotor areas were present anterior to Brodmann's area 4 and that Brodmann's area 6 portion of the cortex is not functionally segregated from area 4 but it constitutes a unique complex in which proximal and axial movements are represented. A separate representation of body movements would be found, according to that hypothesis, only in the supplementary motor area (SMA) which was named by Woolsey as "M2". Starting from the early 1980's neurophysiological research on the cortical motor system focused on the existence and organization of the multiple premotor areas localized in the frontal lobe, anteriorly to the primary motor cortex. The amount of evidence based on lesions, odology and intracortical recordings and microstimulation studies on monkeys, was showing that several, now well identified, different full representations of bodily movements in BA6 (Graziano & Aflalo, 2007; Dum & Strick, 2002), functionally separated from BA4.

The group of Rizzolatti and coworkers focused its attention on the functional properties of neurons in the ventral sector of the premotor cortex (vPM, or inferior area

6), the cortical portion comprised behind the arcuate sulcus and below the spur (Figure 1). The authors employed a naturalistic experimental paradigm in which the activity of single neurons was recorded during the occurrence of spontaneous movements performed by the animals. In this way they defined different neuronal populations according to their firing properties. In a first work single neuron activity in inferior area 6 was recorded in two exemplars of *Macaca Nemestrina* while the animals were performing active movements (Rizzolatti et al., 1987). The authors observed that the neurons recorded in the portion of the inferior area 6 closest to the arcuate sulcus coded more than movements or specific muscular contractions. Indeed almost one third (91 out of 315) of the total amount of neurons coded goal-directed actions. Among the goal-directed neurons the 73% responded to arm actions only, while the remaining 27% responded both to arm and mouth movements; these neurons were in turn not sensitive to non-transitive movements, that is, moving the arms or opening and closing the mouth didn’t result in an increase of the firing rate if the action was not directed to an object.

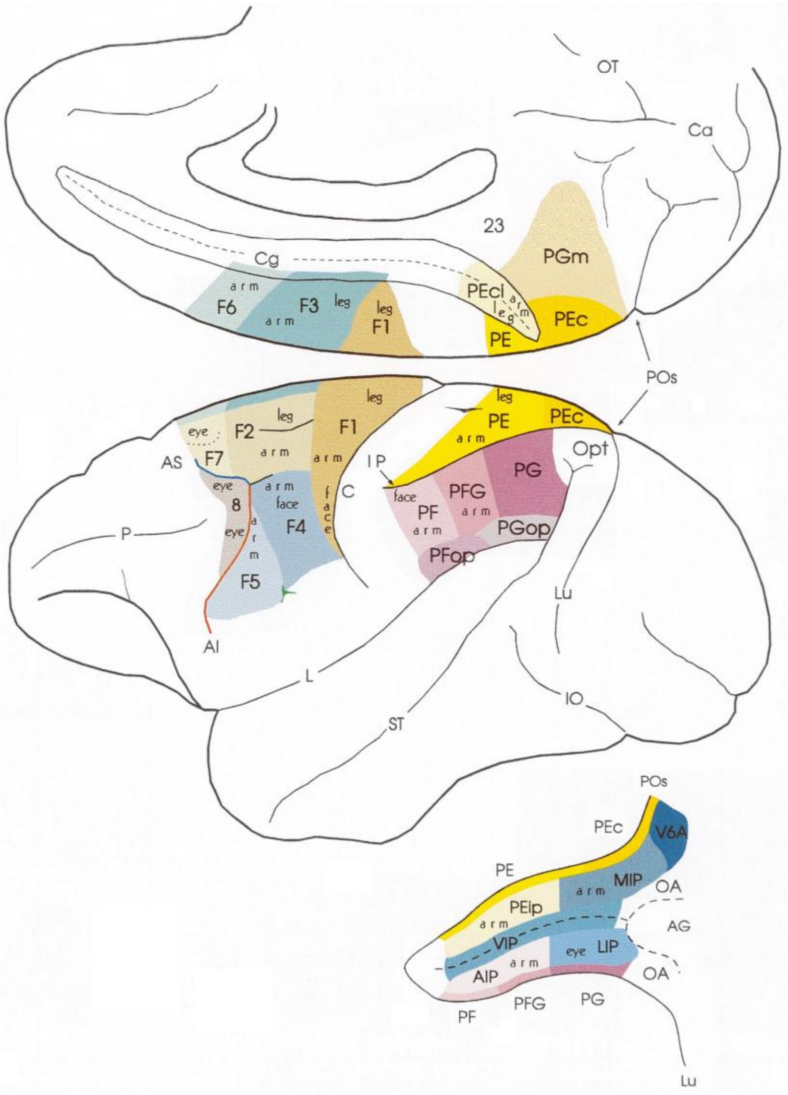


Figure 1:
Lateral and mesial view of the monkey brain.

Although goal-directed neurons were not coding every type of goal-directed actions, the authors were able to classify them into elementary categories according to the action they fired with: *“Preparation for grasping and grasping”, “Grasping”, “Holding”. “Preparation for grasping and grasping”* neurons began to fire before the execution of a distal grasping movement and stopped when the object was grasped, with the maximal discharge reached when the animal started to move its fingers. These neurons also responded to the mere visual presentation of food. *“Grasping”* neurons was the largest class of the recorded neurons (43, almost half of the goal-directed neurons). These neurons have been further differentiated according to their different firing properties in relation to different types of grip: 25 of them fired during precision grip (grasping performed by the index and thumb) while 13 responded to whole hand grasping; 5 responded to both type of grip. These neurons fired before the hand touched the object. *"Holding"* neurons began the discharge when the hand made a contact with the object and continued until the object was released.

In a subsequent work (Rizzolatti et al., 1988) the authors confirmed and enriched the data from Rizzolatti et al. 1987 recording 216 neurons from the F5 sector of the frontal cortex of three monkeys. The data showed that the vast majority of the recorded neurons in F5 (193/216, 89%) responded selectively to motor-acts, that is, they fired in correspondence to the appearance of specific "chunks", or sequences of movements that compose an entire action. Motor acts will be defined later as "movements directed toward an object which eventually allows an effective interaction between the used effector and the target of the movement (Rizzolatti & Luppino, 2001)". Moreover motor-acts categories were increased: those included neurons responding to distal movements as *"Grasping with the hand", "Grasping with the hand and the mouth", "Holding", "Tearing", "Complex"* and neurons responding to proximal acts like *"Reaching"* and *"Bringing to the mouth or the body"*. Most of these neurons were the *"Grasping with the hand"* neurons, followed by the *"Grasping with hand-and mouth"* neurons.

The majority of the *"Grasping with the hand"* neurons (90, 42% of the goal-directed neurons) were selective for the type of grasping. Precision grip was the most represented one (36 neurons, 40%), followed by finger grip (grasping performed with fingers other than the index, 34 neurons, 38%), and by whole-hand grasping (grasping performed with the whole hand, 6 neurons, 7%); the remaining 14 (15%) “Grasping with the hand” neurons were not selective for a specific type of grasp. None of these neurons showed differences in firing rate according to the spatial position of the object to be grasped, and only 5 out of the 41 were selective for the contralateral limb, with the others responding to both hands.

Neurons classified as *“Grasping with the hand and mouth”* (52, 24% of the goal-directed neurons), fired regardless of whether the effector was the hand or the mouth. The feature they seemed to encode was to take possession of the object. In almost half of these neurons (46%) the firing was not selective for the type of grasping used by the monkey, while the remaining 54% were instead selective: 20 neurons were selective for the precision grip, 8 neurons were selective for finger grip, and none for the whole hand grip). All of the *“Grasping-with-hand and mouth”* neurons that were tested with movements of both the ipsilateral and contralateral hand (37 neurons) showed no selectivity for the hand used. The time of onset of spikes varied largely: 35 neurons spiked before appearance of the distal movement, 37 with finger extension, 32 with finger flexion. All stopped firing as soon as the action has been completed.

“Holding” neurons (20, 9% of the goal-directed neurons) were described. These neurons fired when the grasped object was held in the hand and stopped as soon as the monkey ended the grasping. Also this class of neurons showed specificity for the type of grip, 30% was specific for precision grip, 15% for finger grip, 15% for whole grasping while the other 40% doesn’t show specificity for grip. Interestingly some of these neurons started to fire slightly before the object was held, while others began as soon as the hand touched the object. This observation led the authors to consider the distinction between “grasping” and “holding” neurons in a temporal “continuum” within the same action rather than a clear-cut categorization. “Tearing neurons” became active when the monkey broke or tore objects, starting the firing as soon as the hand touched the object. Among F5 neurons discharging to proximal movements the authors recorded neurons firing to reaching movements to any part of the space or to a big sector of it. “Bringing to the mouth neurons” were found firing when the monkey brought a grasped object to the mouth. The activity of these neurons was independent from the initial position of the arm; these neurons did not fire if the monkey grasped the food, moved towards him, with the mouth.

The most striking finding however was that neurons encoding distal motor acts in F5 fired also for somatosensory and visual stimulation. Out of the totality of the recorded neurons, 73 responded to somatosensory stimulation and their receptive field was detected on the hand for those neurons that spiked in association with hands movements, and the same was true for hands-and-mouth neurons, whose receptive field could be located on the hands, on the mouth, or on both the hands and the mouth. Other than neurons responding to somatosensory stimulation, the authors found 30 distal F5 neurons spiking in response to motivational visual stimuli. All these neurons belonged either to the “Grasping with the hand”, or to the “Grasp with the hand and the mouth” classes. Plus, a relationship between the size of the object and the type of grasping was found, that is, small objects triggered precision grip and unspecific neurons were triggered by both small and big objects. No whole-hand neurons were found to be triggered by visual stimuli. Among F5 neurons coding for proximal motor acts, half of the “Reaching” neurons fired in response to visual stimuli alone, with a good correspondence between the selectivity of the space sector to which they fired when the monkey reached and the position of the visual stimulus. Conversely the majority of the "Bringing to the mouth" neurons did not respond neither to visual nor somatosensory stimulation, but the few that did fire to sensory stimulation showed a receptive field in the perioral space or on the mouth.

Altogether these data pointed out a selectivity of the F5 neurons more abstract than the single muscle or single movement representation, indeed the same movement performed in the context of different motor acts corresponded to different firing patterns of the recorded neurons. Grasping-related neurons active during the flexion of the fingers didn't respond when the monkey pushed away the object through finger flexion. Finally, the finding of neurons that respond both to hand and mouth grasping excluded at the origin any possible explanation of these results in terms of coding of simple movements. The authors' interpretation was that PMv contained a "vocabulary of motor acts" that could be accessed via somatosensory and visual routes. Extending this concept, these data indicate that in the motor system, perceptual features of the space and objects around us are coded in terms of potential motor acts.

As a whole, the level of abstraction of the action coding in inferior area 6, was, in a hierarchical level, corresponding to that of "motor acts", i.e. it was clearly superior to that of single joint displacements but was inferior to that of general action meanings or even of intentions. The authors suggest that "… abstract commands without a well defined motor specification are very likely issued in cortical areas further away from the primary motor cortex that area 6. Inferior parietal lobe and prefrontal cortex, both connected to inferior area 6, are the most likely candidates for this more abstract role." (Rizzolatti et al., 1987).

1.2 Canonical Neurons

Visuomotor neurons in F5 responding to the vision of objects and firing also when the monkey is grasping an object with that same geometry have been defined as "canonical neurons". They have been subsequently studied in order to better describe their visual responses in relation to the sight of manipulable objects. Murata and colleagues (Murata et al., 1997) studied the firing properties of these neurons in order to relate their activity to the actual movement and to the observation of the graspable object. They tested a monkey in four different conditions in which the monkey had a) to grasp the object with the lights on, b) with lights off (grasping in the dark), c) had to fixate the object or d) had to fixate a spot of light. The objects presented were a ring, a plate, a cone, a cube a sphere, a cylinder.

Among the 165 neurons studied, 25 were classified as motor neurons and 24 as visuo-motor neurons. The former fired only during the grasping of a visually presented object, and the latter fired also when the object was visually presented but not grasped by the monkey. Among visuo-motor neurons, 16 neurons were selective, that is, their firing rate was higher for the grasping of a small set of objects. Interestingly the visuo-motor neurons classified as selective showed the same selectivity for the visual presentation of objects, in the condition in which the monkey had only to fixate the object without grasping it. These data clearly showed that the specificity of visuo-motor neurons was action-related, meaning that these neurons coded the potential action to be performed on the observed object, ultimately representing a praxic description of the object.

1.3 Mirror neurons

In one of the experiments on the visual properties of neurons in PMv, di Pellegrino and colleagues (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992) focused on disambiguating the neural activity of stimulus-associated responses from the activity related to movement when the monkey was presented with an object to be grasped. However the authors made an intriguing observation that is considered to date one of the most exciting and discussed findings in neuroscience. The researches realized that when they performed goal-directed actions in front of the monkey some neurons from F5 fired even if the monkey was still. The feature distinguishing these neurons from the canonical ones is that the latter did not respond to the observation of an action while the former did not respond to the visual presentation of a manipulable object alone. The first and most extensive investigation of the mirror neurons properties was done by Gallese and colleagues (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996); they recorded 532 neurons from F5 in the monkey's premotor cortex, and 92 of them had mirror properties (17,3% of the recorded neurons). A neuron has been defined as a mirror neuron if "…it discharged both when the monkey made active movements and when it observes specific meaningful action performed by the experimenter" (Gallese et al., 1996). From a motor point of view these neurons were indistinguishable from other F5 neurons, indeed they showed the same motor-act specificity exhibited by other F5 neurons. Analogously to goal-directed neurons described by Rizzolatti et al. in 1988, the majority of mirror neurons were sensitive to the observation of "Grasping with the hand" actions (60, 65% of mirror neurons), followed by "Grasping with the Hand and the mouth" actions.

The presentation of objects (including food), emotional gestures and actions executed with tools, even if very similar to those performed with the hands did not evoke any response. Some neurons were sensitive to the side of the hand that performed the action or to its trajectory. The strongest visual stimuli capable of eliciting a response in these neurons were hand-object interactions or mouth-object interactions, as grasping, placing and manipulating, and holding actions; these responses did not apparently habituate and were consistent across trials. “Grasping neurons” responded to the observation of a hand approaching an object and grasping it. Some of these neurons (18) were also specific for grasping type, and some start firing at different times compared to the onset of the observed grasping. “Placing neurons” fired as soon as the experimenter placed the food on an empty tray and stopped soon after the experimenter moved the hand away from the food. “Manipulating neurons” fired when the experimenter touched and moved an object with his finger in order to take possession of it. Some neurons fired for observed hands interaction like a hand holding an object and bringing it to the other hand. “Holding neurons” started to fire when the experimenter hold an object and stopped when he moved the hand away from it. Interestingly 25 (4.6%) neurons among the 532 recorded, did not show any motor, but only visual properties (called mirror-like neurons). Mirror neurons showed different degrees of visuo-motor congruence, for this reason they were assigned to 3 different categories: strictly congruent, broadly congruent, non-congruent. Strictly congruent neurons (31% of the mirror neurons) matched both the meaning of the action and how the action is performed, so for example they fired when the monkey performed a precision grasp and when the monkey saw a precision grasp.

Broadly congruent (60%) mirror neurons were further divided in 3 classes. A) The first class (7% of mirror neurons) of these neurons were motorically specific for a particular type of action executed in a specific way, for example they spiked when the monkey executed a precision grip, but responded to different kind of observed grips (for example they spiked to the observation of both precision and whole-hand grip). B) The second class (50% of mirror neurons) of broadly congruent mirror neurons were active when the monkey performed one action (independently of how it is executed), but responded to two or three different observed actions. C) The third class of broadly congruent (2% of mirror neurons) discharged when, for example, the monkey grasps with the hand, but also when it observes a grasping performed with the mouth. The last category, non-congruent mirror neurons, comprises neurons for which the visuo-motor relation is not obvious (9%).

In order to exclude the possibility that the activity of the mirror neurons was just a covert execution of the observed action the authors checked whether neurons in F1 discharged after the discharge of mirror neurons: 0/49 tested neurons in F1 discharged in this condition, in accordance with the EMG signal from the monkey’s hand.

1.4 Translating neural activity into cognitive processes: the “classical” interpretation of mirror neurons.

The first attempt to explain the role of mirror neurons ascribed to them the function of creating an internal representation of the action observed (Jeannerod, 1994; Gallese et al., 1996). This internal representation can serve different functions, the most plausible at first glance is imitation. In order to imitate an action one has to observe it, match the different parts of the body with his own body and then relate the action observed with his ones in his own motor system. According to the researchers who discovered them, mirror neurons could indeed match the action observed, and relating it with the F5 “motor vocabulary”, in order to express the correspondent motor behavior. This hypothesis, although interesting and catchy, was initially ruled out (at least for explaining monkeys’ mirror neurons function), because monkeys have poor imitation skills (Rizzolatti & Craighero, 2004), but see (Brass & Heyes, 2005). The interpretation that has become the classical one asserts that mirror neurons serve the understanding of others’ actions. The concept of action understanding has been defined in different ways (Hickok, 2009). The early definitions describe “action understanding” as either: a) “…the capacity to recognize that an individual is performing an action, to differentiate that action from others analogous to it, and to use this information to act appropriately”, (Gallese et al., 1996) or b) ”…the capacity to achieve the internal description of an action and to use it to organize appropriate future behavior” (Rizzolatti, Fogassi, & Gallese, 2001).

How does this process of action understanding take place? In an influential theoretical paper Rizzolatti and colleagues (Rizzolatti et al., 2001) described two possible hypotheses that could explain how actions are understood. The *Visual Hypothesis* for understanding an action is based on the visual analysis of the different elements that form an action. The core of the hypothesis asserts that “a description of motor events in visual terms is sufficient to for action understanding”, but most importantly that “…no motor involvement is required”. According to the authors the main weakness is that the *visual hypothesis* “…does not indicate how the validation of the meaning of the observed action is achieved”.

An alternative hypothesis is called *Direct Matching Hypothesis*: it assumes that there is a direct route that maps “the visual representation of the observed action onto motor representation of the same action. An action is understood when its observation causes the motor system to resonate. So when we observe a hand grasping an object the same population of neurons that control the action execution of grasping movements becomes active in the observer’s motor areas” (Rizzolatti et al., 2001). The *Direct Matching hypothesis* does not exclude the possibility that other, more cognitive processes based on object and movement description could also participate in this function. It stresses however the primacy of the direct matching between the observation and execution of action (Rizzolatti et al., 2001), as there is no need for a visual description of the action for action understanding.

What is the putative role of mirror neurons in these two routes? Basically mirror neurons are supposed to perform the direct match between visual representation of an action and the motor representations that allow the execution of the action observed. Mirror neurons are, from the motor point of view indistinguishable from those found by Rizzolatti et al. (1988), and as those, they code motor acts. The mirror neurons system recognizes and parses the action observed, (defined as “a sequence of motor acts that, at its end, produces a reward for the acting individual”) into the motor acts by which the action is composed. The *direct matching hypothesis* assumes that the action observed can be understood after the simulation in the motor system has taken place, namely, the observers can understand the observed action after the activation of the same motor representations needed to perform that action.

2. MIRROR NEURONS TODAY: CURRENT RESEARCH ON MIRROR NEURONS IN NON-HUMAN PRIMATES

Despite 20 years have passed by since their discovery, research on mirror neurons has increased steadily. In recent years research on mirror neurons revealed new features of these type of neurons. Moreover, neurons that show mirror properties has been found also in other cortical brain areas out of F5 such as the primary motor cortex, the dorsal premotor (Tkach, Reimer, & Hatsopoulos, 2007) and parietal cortex (Fogassi et al., 2005). This widespread distribution of visuomotor neurons with mirror properties has led to the concept of “mirror mechanism” to describe the overall pool of mirror neurons rather than the classical term of “mirror neuron system” (Cattaneo & Rizzolatti, 2009).

2.1 Mirror neurons in the parietal lobe: action-constrained neurons

Mirror neurons have been discovered in the monkey’s parietal lobe. In order to study the properties of the PFG area (see Figure 1). Fogassi and colleagues (Fogassi et al., 2005) trained monkeys in two conditions: in the first one they had to grasp a piece of food and place it in a container located either nearby or on the monkey’s shoulder, or, in a second condition, they had to grasp the food and eat it. Authors found that the majority of the recorded neurons in PFG discharged selectively during the same motor act (i.e. grasping) but only if it was embedded in one of the two chain of motor acts (grasp-to-place chain, or grasp-to-eat chain). Authors also tested some neurons that were active both during grasping execution and grasping observation: among these neurons about half were selective for the chain of actions executed. Among these motor-selective neurons, the great majority showed the same selectivity to the observation of action chains, that is, if a neuron was sensitive selectively for performing a grasp-to-eat action, then it was selectively activated also when the monkey watched a grasping-to-eat action. The coding performed by these neurons is not about the type of motor act that triggers their firing but it is related to the action in which the motor act is embedded, leading to the interpretation that the parietal mirror neurons can indeed extract motor intentions when the monkey observes an action performed by another conspecific (Rozzi, Ferrari, Bonini, Rizzolatti, & Fogassi, 2008).

2.2 Mirror neurons in the primary motor cortex

Also the primary motor cortex of the monkey contains neurons with mirror properties. One of these demonstrations has been provided by Tkach et al. (2007) who trained the monkey to move a cursor in the direction of a visual target appearing on a screen. The cursor was moved by the monkey’s hand but the hand was out of the monkey’s sight. However the cursor was visible so that the monkey can guide its movement based on a visual feedback. The whole session was recorded and then played on a screen while the monkey was still, just observing the moving cursor of its previous performance. Neurons both in primary motor cortex and in dorsal premotor showed a pattern of activity during the observation phase that closely resembled the one detected during the execution phase. This finding has been interpreted as the evidence that the monkey is covertly generating a motor command while observing its own previous performance even when it was not required to move. Similar results have also been found by

Dushanova and Donoghue (Dushanova & Donoghue, 2010). Interestingly pyramidal tract neurons with mirror-like properties have also been found in F5 (Kraskov, Dancause, Quallo, Shepherd, & Lemon, 2009); when the monkey observed a grasping action about half of the pyramidal neurons that were found to be modulated by action observation, showed a complete suppression while the other half exhibited facilitation.

2.3 Variant visual properties of premotor mirror neurons

The first descriptions of mirror neurons properties seemed to exclude their sensitiveness to some visual manipulations, such as the point of view of the observed action, the distance at which the observed action is performed, and the target of the observed action. However recent evidence from the group lead by Martin Giese in Tübingen, shows that some of these features do indeed modulate mirror neurons activity in F5.

(Caggiano, Fogassi, Rizzolatti, Thier, & Casile, 2009) described mirror neurons sensitive to the distance at which the observed action was performed. The experimenter grasped an object in front of the monkey but at different distances, classified either as peri-personal or as extra-personal. The 27% and the 26% of the recorded mirror neurons exhibited a visual preference respectively for peri-personal space and for extra-personal space. The remaining mirror neurons did not show any selectivity. Authors then presented the monkey with the action performed at 5 different distances with the 3rd one considered a half-way between peri-personal and extra-personal space; results showed that the bigger the distance of the observed action the lower the activity of peri-personal selective mirror neurons and vice-versa for extra-personal selective ones. Nevertheless it was not clear whether the tuning was based on a metric space, meaning that the boundaries between peri-personal and extra-personal space was fixed, or on an operational space, meaning that the boundary was dynamic and dependent from the monkey potential range of action. Researchers disambiguate between the two hypotheses by placing a transparent panel between the monkey and the object that would have been grasped by the experimenter. This manipulation changed the tuning of 9 out of 21 recorded making extra-personal mirror neurons less selective and more active when the action was performed in the peri-personal space, while some peri-personal neurons stopped firing. The last demonstration lead the authors to distinguish between two categories of space-selective mirror neurons: the distance selective and the operational selective neurons.

In a second work by Caggiano et al., (2011) the authors showed that among the mirror neurons recorded in F5 the 43% did not distinguish between naturalistic or filmed actions. The authors tested also the sensitiveness of those neurons responding to filmed actions, to the point of view from which the observed action has been performed (180°, 90°, 0° subjective point of view): about 74% of 201 neurons were view dependent, while the remaining 26% was not. Among the view-dependent mirror neurons the 59% were sensitive to more than one point of view, 10% was sensitive to the 180° degree, 12% to the 90° and finally the 18% was sensitive to the 0° point of view. The finding that a 26% of the total mirror neurons recorded did not modulate the firing rate according to the point of view favored, according to the authors, the hypothesis that those mirror neurons mediate action understanding without being influenced by the visual aspects of the action observed.

Finally a more recent work from the same group Caggiano et al. (2012) focused on the role of the object on which the observed action is performed. The authors varied the subjective value of the object grasped by the experimenter in order to understand whether the F5 mirror neurons would have been modulated. In the first experiment they presented the monkeys with food grasping, or with non-food grasping actions while recording from F5: they found a clear modulation of the firing rate of the 68% of the mirror neurons recorded, in particular 92 on 102 neurons recorded responded better when the monkey observed food grasping, while 10 neurons showed the opposite pattern; finally the 32% of the mirror neurons recorded did not show any preference. Furthermore the authors disambiguated the role of the rewarding object that is represented by the food, from the role of the reward itself. They showed the monkey two objects, a red and a grey one. In the 20-30% of the trials, after the experimenter grasped the red one, the monkey was rewarded; authors found that almost half (46%) of the mirror neurons tested exhibited a stronger response when the rewarded object was grasped, while 13% exhibited a stronger response for the non rewarded object and the remaining 36% didn't show a preference.

2.4 Mirror responses in other sensory modalities

Most works on mirror neurons tested the visual modality as the main sensory route to the motor system. There is not a strong rationale from this choice and in fact this could be driven by the empirical difficulties in testing other sensory modalities. In fact, whenever a sensory pathway different from the visual one has been tested, mirror sensorimotor responses have been found in the premotor cortex. Kohler et al., (2002) described neurons in F5 that were sensitive to action-related sounds but not to non action-related sounds. After the auditory selectivity has been proved for particular action-related sounds as tearing a piece of paper or braking peanuts, authors sought for a correspondent visual selectivity for those same actions. This was indeed the case: out of 29 auditory selective neurons, 22 were visually selective for the same actions. The majority of these audio-visual neurons (16) responded with the same intensity to each single modality, that is vision only, sound only or motor only, while the minority (10) responded more vigorously when visual and auditory modalities were presented simultaneously. In the 3 remaining neurons the response was higher for the auditory modality alone.

The presence of neurons that code actions in multimodal terms suggest that mirror neurons are not just visuo-motor neurons able to transform information coded in the visual modality into motor representations of the same action, but seemed to represent actions in an abstract form, without the need of a visual description of the action, as predicted by the direct matching hypothesis. Umilta et al. (2001) tested this hypothesis by showing the monkeys a grasping action and a pantomimed grasping action (i.e. without an actual piece of food to be grasped) in two different conditions: in one the action was fully visible in all its phases, in the other instead the crucial part of the action, the final phase of the grasping, was hidden by an opaque screen. Crucially before placing the screen the experimenter put or took away the piece of food so that the monkey knew whether a piece of food was there to be grasped or not. If the mirror neurons code the meaning of the action then their firing should be comparable in both conditions in which the actual grasping was showed, while it should be absent in the other two conditions in which no goal-directed action was performed. Half of the mirror neurons that spiked in the actual grasping-full view condition spiked even in the actual grasping-hidden condition, but not in the pantomimed condition. This datum has been interpreted as the evidence that mirror neurons mediate action understanding even when there is no access to the visual representation of the action.

Finally mirror neurons have been proved to be sensitive also to the observation of tools use (Ferrari, Rozzi, & Fogassi, 2005). Indeed tool use has been exploited by

Umilta et al., (2008) in order to disentangle whether the F5ab neurons coded the movement or the goal of a motor act. Monkeys have been trained in using normal and inverse pliers in order to grasp a piece of food. The actual movement of the index finger and the thumb is the opposite in the two conditions, so that in order to reach the same goal, the monkey has to perform two opposite movements. Neurons in F5 fired independently of whether the monkey was using the normal or the inverse pliers, but according to the temporal phase of aperture-closure of the pliers. This demonstration showed that is the goal of the action that is coded, not the movement that has to be performed in order to grasp the food. The same criterion has been hypothesized for mirror neurons and the correspondent demonstration has been provided by Rochat et al. (2010).

Other than action understanding some evidence has been found that the mirroring process serves also the attentional sharing (Shepherd, Klein, Deaner, & Platt, 2009). The authors described neurons in the LIP region of the parietal cortex of the monkey that were more active both when the monkey oriented the gaze in a specific direction and when the monkey observed another monkey directing its gaze in the very same direction.

2.5 Summary

The most recent data on monkey show that mirror neurons are sensitive to different visual and non-visual features of the observed action. Mirror neurons, besides being sensitive to the observed motor act, are sensitive to the point of view from which the action is observed, to the peri-personal distance at which the action is showed, and also to the subjective value of the object grasped by an actor. Nevertheless visual input is not necessary in order to trigger mirror neurons as long as the meaning of the action is accessible in another way. Neurons with mirror properties have also been found in primary motor cortex and in the PFG section of the parietal cortex, which along with F5 constitutes a functional unit for action and intention understanding (Bonini et al., 2010).

3. MIRROR NEURONS IN HUMANS

The most important question concerning mirror neurons is whether they exist also in humans and if they accomplish the same function ascribed to the monkeys mirror neurons. The techniques used to test the existence of these neurons in humans are different from those used for monkeys mirror neurons, so the evidence of the mirror neurons existence is indirect.

3.1 Behavioral Data

From a behavioral point of view, the cognitive processes ascribed to mirror neurons, that is, to covertly simulate the observed action in the observer’s motor system, is testable (and it has been tested) in the following way: participants have to react to one of two visual target stimuli (non-action stimuli) that are presented in synchrony with one of two possible motor actions on the background. Typically the possible responses to the target stimuli are the same actions that are presented on the background of the visual stimuli (Heyes, 2011 for a complete review on this topic).

If the motor representation of the actions presented in the background of the visual stimuli triggers the correspondent representation of that action in the observer’s motor system, then the reaction times should be faster when the background stimulus represents the same action that the participant has to perform, compared to a neutral condition in which no action is shown (and so there is no correspondence with the action to be performed); vice-versa, if the action shown does not correspond to the motor response that has to be provided, then reaction times should be slower compared to a neutral condition.

The first data supporting this hypothesis were provided by a series of experiments conducted by Marcel Brass within the theoretical framework of the common coding theory of action and perception (Prinz, 1997). Authors presented participants with two numbers (1 and 2) placed between the index and the middle finger of a left hand showed in frontal perspective. Participants' task was a choice reaction time, they had to lift their own index finger if the number "1" appeared, or to lift their own middle finger if the number "2" appeared. When the numerical stimulus appeared a hand action was shown simultaneously on the background, that could be an index finger lifting, a middle finger lifting or no action (baseline). The result, replicated many times in literature shows that participants' motor response was automatically influenced by the background hand action even if they had to ignore it, yielding slower reaction times when the finger lifting was incongruent with the background action, and vice-versa it yielded faster reaction times when the motor response was congruent with the background action.

This automatic effect is a particular kind of stimulus-response compatibility that in principle can easily result in a spatial compatibility effect if the side of the action observed and the side of the response are the same. In the first experiments carried out by Brass et al. (2000) this confound variable was not controlled; however more recent studies have clearly demonstrated that the automatic imitation effect is a pure one (Bertenthal, Longo, & Kosobud, 2006; Catmur & Heyes; Chong, Cunnington, Williams, & Mattingley, 2009). In particular eleven studies using different action pairs have controlled for spatial compatibility effects and have found an automatic imitation effect anyways (Heyes, 2011). Also the time course of a spatial compatibility effect and the one of an automatic imitation effect differ, with the spatial effect being appearing earlier than the imitative one (Catmur & Heyes, 2011). These effects have been replicated many times in literature and they extend beyond hand actions as shown by (Gillmeister, Catmur, Liepelt, Brass, & Heyes, 2008) with foot or mouth actions (Leighton & Heyes, 2010).

This compatibility effect has been called “automatic imitation” and it is, one the behavioral side, what has to be expected if the simulation is automatically triggered by the vision of an action. Indeed if the same representations needed to perform an action are needed for understanding that action, then when participants are presented with a different action compared to the one they have to perform, the motor representations exploited for understanding the action have to be inhibited and substituted by a voluntary response selected from the same motor representations, while in the congruent trials the inhibitory process does not take place, and this why in the incongruent trials responses are slower than the congruent ones.

Is this effect really automatic? Are the motor representations of the action observed accessed even when participants do not need to condition their motor response to the type of action observed? This hypothesis has been further tested in a series of block designed experiments (Brass, Bekkering, & Prinz, 2001) in which participants had to perform motor responses in simple reaction time to the visual presentation of two possible actions. Participants were presented with either the tap the lift of an index finger: whichever movement observed, participants had to perform, depending on the block, a lifting or a tapping of their own index finger as fast as they could. Results showed that even when no conditioned motor choices has to be performed, motor responses to incongruent trials were slower compared to the responses to congruent trials (the magnitude of the effect is around 20 ms). Also this effect has been replicated with different effectors (Heyes, Bird, Johnson, & Haggard, 2005; Sturmer, Aschersleben, & Prinz, 2000).

Behavioral data turn out to be extremely useful in drawing inferences on the processes that take place in the mind/brain during motor responses to action observation, however a very important question to answer to is whether the areas of the brain homologous to F5 and PF show more activity in action observation tasks. In order to achieve this information techniques as neuroimaging techniques can be exploited.

3.2 Neuroimaging Data

Neuroimaging techniques comprise functional magnetic resonance (fMRI) and positron emission tomography (PET). These techniques exploit respectively the variation of the oxygen consumption and the variation of glucose consumption in different parts of the brain as an index of neural electrical activity. The main advantage of these techniques is their high spatial resolution that allows to mark with extreme precision very defined spots of activity in the brain. Many of the fMRI experiments dealing with action observation basically confirm that part of the motor system that is used in action execution is also recruited when observing actions, imitating actions. Soon after the discovery in monkey, researchers had to focus on two aspects in order to understand whether evidence for mirror neurons could be found also in humans; the first was the common activation of particular brain areas both during action observation and execution, while the second was the localization of that putative area in the frontal lobe. The hypotheses about the localization of the homologue of F5 were restricted to the premotor cortex and the pars opercularis of the inferior frontal gyrus, also known as the Broca’s area.

After the discovery of mirror neurons in macaques brain, the Rizzolatti’s group tested the existence of a putative mirror neurons in the human brain with a PET experiment (Decety et al., 1994). They hoped the frontal cortex, in particular the Broca’s area and the premotor ventral cortex, to be engaged when participants observed actions. Unfortunately they were not able to find any selective activity shared both by observation and execution in theany of the voxels in the frontal lobe that could have corresponded to the homologue of monkey F5. The reason was attributed to the fact that visual stimuli were virtual reality-generated hands (Rizzolatti & Sinigaglia, 2008). However, when Rizzolatti and colleagues (Rizzolatti et al., 1996) presented participants with real hands instead of virtual hands, Broca’s area was found active while participants were observing motor acts. Is Broca’s area to be considered the homologue of monkeys’ F5? Research on humans have shown that Broca’s area, an area considered to be involved only in language related articulatory processing (Fadiga, Craighero, & D'Ausilio, 2009), is, at least in part, involved in object manipulation.

Binkowski and colleagues (Binkofski et al., 1999) asked participants to manipulate either complex objects or a sphere without covertly naming the manipulated object; in another condition subjects were required to covertly name the object they were manipulating. Results showed that area 44 was involved in the manipulation of complex objects, while the naming task activated the pars opercularis that falls within area 45 (Binkofski & Buccino, 2006; Buccino, Binkofski, & Riggio, 2004)

This is an issue not solved yet, because other evidences are not in line with the interpretation that the pars opercularis of Broca's area is the homologue of F5. A recent work (Morin & Grezes, 2008) posed the question performing a meta-analysis of 24 fMRI studies testing action observation and found that the crucial difference between the ventral premotor cortex and area 44 was that the first, differently from the latter, shared with monkey F5 the sensitiveness to goal directed actions; instead area 44 was not sensitive to this fundamental feature of the observed action. Nevertheless other more recent meta-analyses ascribe a role found Broca's area rather than premotor ventral cortex to be more active in action observation (Caspers, Zilles, Laird, & Eickhoff, 2010).

In their review Caspers et al. (2010), analyzed 139 among fMRI and PET studies and found a network of object-related hand action observation that comprised Brodmann area 44, lateral premotor cortex, inferior parietal area PFt, Superior Parietal area 7A, the posterior middle temporal gyrus and V5 bilaterally, as well as the primary somatosensory cortex and the anterior IPS (area hIP3) on the right hemisphere. On the contrary non-object related hand actions did not show any significant cluster of activation in left frontal areas. Observed actions performed with non-hand actions activated Brodmann area 44 more than hand-action. The involvement of Brodmann 44 and parietal areas in action not performed with hands has been tested for the first time by Buccino and colleagues (Buccino et al., 2001). The authors showed object-related and non-object-related actions performed with different effectors: the presentation of object related actions activated the premotor cortex and the posterior parietal cortex in a somatotopic manner with foot actions more dorsal than hand actions and mouth actions. A somatotopic activation in correspondence to action-related sound presentation has been found by Gazzola and colleagues (Gazzola, Aziz-Zadeh, & Keysers, 2006) with a dorsal cluster in premotor areas responding more to hand actions and a more ventral spot responding more to mouth actions. However contrasting results on this topic have been described by the different meta-analyses, with the one of Caspers et al. (2010) showing no somatotopy in parietal cortex, but a different degree of activity in Broca's area and in more dorsal premotor cortex respectively for non-hand action and for hand actions. The meta-analysis by Morin and Grézes (2008) instead showed no clear somatotopy in premotor areas.

Considering the results of many fMRI experiments on action observation, it is clear that the inferior frontal gyrus and the inferior parietal lobe do not make the whole story when participants observe actions. Indeed the superior temporal sulcus (STS) has been considered by few researchers to be part of the mirror neuron system even if its neurons do not show motor properties in monkeys. Neurons contained in STS have been described to be sensitive to biological motion (Allison, Puce, & McCarthy, 2000; Puce & Perrett, 2003) and they are strongly connected to the PF/PFG complex in the inferior parietal lobule. Many researchers believe that this area represents the action concepts in a more abstract way than the premotor areas. In particular STS is thought to recognize and understand actions. Other than classical mirror cortices, posterior temporal areas have been found active during action understanding tasks (Lingnau & Petris, 2012). Other theoretical perspectives however consider the posterior temporal cortex and in particular STS, an important part of the action understanding process, but according to those STS lacks the necessary degree of generalization that would allow it to be a likely underpinning of the action understanding process (Rizzolatti & Sinigaglia, 2010).

Generalization is instead considered the main distinctive feature that makes Broca’s area/ventral premotor cortex the likeliest substrate for action understanding in humans; for example the motor system and in particular the mirror neuron network has been found active even when the action observed is performed by a non-biological effector, as a mechanical arm. Gazzola and colleagues (Gazzola, Rizzolatti, Wicker, & Keysers, 2007) showed a mechanical arm/hand or a human hand grasping objects and found no difference on the brain activations between the two conditions, with both movies activating the mirror neuron network; this results was interpreted as evidence that the mirror neuron system is more sensitive to the meaning of the action compared to the way the action is performed or to the visual features of the action observed. However other neuroimaging results such as Tai and colleagues (Tai, Scherfler, Brooks, Sawamoto, & Castiello, 2004) are at odds with these results, describing activity in mirror areas when participants were shown with human actions, but not when presented with mechanical ones. Another convincing demonstration of the generalization properties of the mirror neurons system comes from aplasics patients, people born without hands (Gazzola, van der Worp et al., 2007). If the mirror system codes the goal of the action, and not the way the action is performed, then the prediction is that mirror areas become active when aplasics watch actions performed by people with hands. Indeed this is exactly what it has been found; mirror areas active when the aplasics perform an action with feet were active when they observed the same action performed with the hands. On the contrary however other evidences point to a less degree of abstraction in the premotor cortex; Oosterhof et al. (2012) showed through an MVPA fMRI experiment that occipito-temporal cortices are activated independently of the view point (1st or 3rd person perspective) from which the observed action was observed, while PMv was only active in the 1st person perspective, the frontal mirror areas would not generalize the viewing point.

According to the classical interpretation on the mirror neurons functions, in order to simulate an action the motor representation of that action has to exist in the observer's motor system. The role of the experience has an influence on the functioning of the mirror neuron system. Consistent results with this idea have been provided by Calvo-Merino and colleagues (2005); the authors showed different videos of capoeira dance moves to classical dancers, capoeira dancers or naïve people. They showed that in capoeira dancers the activations in mirror areas were higher compared to the other two categories of people. Nevertheless this increasing activation in mirror areas could be determined not by the intense visuo-motor experience, but only because capoeira dancers had more visual experience than the others. In order to disentangle the role of the visuo-motor experience from the solely visual experience the authors exploited a characteristic of capoeira dances: since some moves are performed only by one gender, the authors compared the mirror activation of males and females to the presentation of males and females moves. It turned out that the visuo-motor experience was crucial in order to activate the mirror system since males mirror system was more active during the presentation of male moves and the same applied for females watching female moves. Other evidences point to a fundamental role of the experience not just to a modulatory one. Catmur et al. (2008) presented participants with hands and feet actions. Participants were scanned and the activation found in PMv showed a more pronounced activation for the observation of hands actions compared to feet movements. Afterwards participants had to perform a counter-imitative training, that is, they had to respond with a foot movement to the presentation of the hand movement and vice-versa. After the training participants underwent another scan in which the hands and feet actions were presented again. Results showed that observed feet actions produced now a more pronounced activation of the premotor cortex compared to the observation of the hands, suggesting that the mirror neurons are easily reconfigurable as counter-mirror neurons.

The question arises of whether the mirror neuron system is activated even when observing action not in our own motor repertoire. Buccino et al. (2004) showed two kind of movies to the participants: the first type of movies showed either a man, a monkey or a dog biting some food, while in the other type of movie showed a man speaking, a monkey lipsmacking and a dog barking. In the first condition the parieto-premotor cortices were activated by each movie, while in the second condition the ventral premotor-Broca's area complex was active only when the participants saw the man speaking. This was interpreted as the evidence that the mirror neuron system maps the observed action when we know how to perform the action observed, but not as in the case of a barking dog, when the action was not in participants' motor repertoire.

A large number of fMRI studies found that ventral premotor cortex/inferior frontal gyrus and the inferior parietal lobule were active while participants observed actions (for recent meta-analysis studies see Caspers et al. (2010) and Molenberghs, Cunnington, & Mattingley (2009). However this evidence is not sufficient in order to assure that mirror neurons are present also in humans. What is lacking in these studies is the capacity to identify that the same neuronal population (and not only the same area) is actually active both when the participant observes an action and when he performs that very same action.

FMRI experiments can exploit the effect of the repeated presentation of the same stimulus on the BOLD signal. Typically the BOLD signal decreases after the repeated presentation of the same stimulus. The rationale is the following: let's assume that the presentation of the stimulus "A" as well as the stimulus "B" activates the area "X" with no statistical difference between the BOLD activity caused by the stimuli: this is not evidence that the same population of neurons is caused to be activated by the two stimuli, but it may be that two different populations intermixed in the same area (neurons "Xa" and "Xb") are selectively activated respectively by one of the two stimuli. In order to avoid this potential confound a repetition suppression technique has been devised. If the heightening of the BOLD signal is caused by the activity of the neural population "Xa" activated by the first presentation of the stimulus "A", then, if the same neuronal population is caused to be engaged also by the presentation of the stimulus "B", then a reduction in the BOLD signal should be present in correspondence of the presentation of the stimulus "B"; otherwise, if the population "Xa" is not activated by the stimulus "B", but the "Xb" is engaged, then there should be no repetition suppression at the presentation of the stimulus "B" (Grill-Spector, Henson, & Martin, 2006).

If mirror neurons are present in human brain, then the consecutive observation of an action and the performance of that action (but not of a different one), should cause a suppression in the BOLD signal in "mirror areas" (cross-modal repetition suppression). The first experiment using this paradigm has been performed by Dinstein et al. (2007). The authors asked participants to play the rock-paper-scissors game inside the scanner: they had to play their game against a videotaped hand that played as the participant opponent. The authors then considered couples of trials consecutively observed (participants saw two trials consecutively showing a hand rock-shaped for example) and those in which participants performed the same action consecutively. Authors found that BOLD signal was suppressed in some areas both for consecutive observation of the action and for consecutive executions; among the areas suppressed authors found both the premotor ventral cortex and the anterior intraparietal sulcus, suggesting the existence of mirror neurons in the human brain. However this experiment did not show any cross-modal repetition suppression of the BOLD signal leaving the open question of whether, within those areas different populations were coding information about observed and executed actions. In an independent experiment (Chong et al. 2008) participants observed non goal-directed actions, and then executed the same actions (or different ones) cued by verbal labels describing the to-be-executed action. Authors found cross-modal repetition suppression in the right supramarginal gyrus when participants observed actions previously executed, but this suppression was not present within the region of the ventral premotor cortex/inferior frontal gyrus.

Lingnau et al. (2009) performed a similar experiment in which participants had to observe or perform meaningless actions. Their results show no sign of cross-modal adaptation when participants had to observe actions previously performed. Taken together these experiments favor the hypothesis that mirror neurons do not exist in the human brain. However another repetition-suppression experiment (Kilner, Neal, Weiskopf, Friston, & Frith, 2009) provided evidence for repetition suppression effects both within parietal and ventral premotor cortex. The distinctive feature of this experiment in comparison with the others is that observed and performed actions were goal-directed differently from the meaningless and symbolic ones previously used.

Although fascinating, the paradigm employed by the authors has been criticized by Rizzolatti & Sinigaglia (2010), arguing that the neuronal site of the repetition effect is on the synaptic level; this implies that if the neuronal population is excited consecutively by two different synapses, there should not be any repetition effect, and this is why the above cited works show no consistent results. Taken together neuroimaging results do not provide a clear- cut evidence of the existence of mirror neurons. Nevertheless since early experiments, fMRI and PET data clearly showed that the inferior frontal and the intraparietal cortices were almost constantly engaged in action observations tasks. fMRI as well as other neurocognitive techniques, do not provide evidence for causal relationships between activity in brain areas and tasks performance. One way to overcome these limitations is to seek for mirror neurons analyzing the cognitive pattern of patients who exhibit focal brain damages.

3.3 Neuropsychological data

Even if lesion studies on monkeys mirror neurons oddly have not been performed, humans patients have been described whose neuropsychological profile was consistent with the existence of mirror neurons in Broca's area and, more in general, with the idea that motor representations are used to understand an observed action.

Evidence in agreement with the classical interpretation found basically two type of evidence: the first is that, on group studies, the performance on action recognition and action production processes are correlated. This suggests a link between action and perception closer than previously thought. Negri et al. (2007) tested patients with unilateral focal lesions and found, at a group level, a significant correlation between different tasks: in particular pantomime recognition was correlated with object use (r=.581), and pantomime imitation with pantomime recognition (r=.597). Similar results were obtained by Buxbaum et al. (2005); the authors tested 44 ideomotor apraxic and non-apraxic patients in imitation of object related pantomime imitation, non-object related pantomimes, semantic and spatial recognition tasks. The authors found that ideomotor apraxic patients were more impaired not only in imitating pantomimes but also in recognizing pantomimes. Moreover a significant correlation between object-related pantomime imitation and object-related pantomime recognition was found among the whole patient population.

Paralleling the predictions of BOLD activity in fMRI, clues on the existence of the mirror neurons should also come from the performance in action recognition in patients with lesions to the Broca's area/ventral premotor cortex. Fazio et al. (2009) tested 6 patients without apraxia presenting focal lesions with maximal overlapping on the Broca's area. They were more impaired in sequencing pictures representing different phases of actions than in sequencing non action-related events. A similar result comes from Cattaneo et al. (2012) who performed a similar experiment but testing cerebellar patients.

One of the most striking evidences pointing towards the involvement of the inferior frontal gyrus in action understanding comes from Pazzaglia et al. (2008). The authors tested a group of 33 patients, 21 out of them presenting a left focal lesion. Patients were divided in groups according to the results of two tests for testing ideative and ideomotor apraxia. Then participants performed a gesture recognition task involving the judgment of transitive and intransitive symbolic actions performed correctly or in an incorrect way. A lesion mapping subtraction analysis revealed that patients presenting signs of apraxia, compared with non-apraxics, had lesion maximally overlapping in the inferior frontal gyrus and inferior parietal cortex. The described evidence tells however only half of the story. Using different approaches in analyzing patients' cognitive system other authors found that a parallel deficit in action production and recognition is not a necessary condition. A more detailed description of these results will be given in the following chapter.

Finally, even more compelling evidence is available on the existence of an automatic simulation process when watching actions. This evidence comes from patients first described by Lhermitte et al. (1983), who exhibit an automatic, involuntary tendency to imitate the observed actions, even if they are told not to imitate the examiner. This deficit of the voluntary motor process has been called "imitative behavior". Typically these patients exhibit lower anterior frontal lesions in the vast majority of the cases (96%) (Lhermitte, Pillon, & Serdaru, 1986). However a less striking relation has been found by De Renzi et al. (1996) who found that the 39% of the frontal patients they examined showed imitation behavior with lateral and medial frontal lesions. Also lesions outside frontal lobe, mostly in basal ganglia and thalamus, produced imitation behavior in a minority of cases. None of these two experiments found imitative behavior in retrorolandic patients and interestingly, patients suffering from imitative behavior imitate both meaningful and meaningless actions (Archibald, Mateer, & Kerns, 2001). Patients are not the only source of evidence for causal relationship between brain and behavior; available evidence for causal relationship between brain activity and behavior comes from transcranial magnetic stimulation studies. This technique is particularly valuable to study the motor system and it has been exploited massively in studying its role in action observation.

3.4 Transcranial Magnetic Stimulation (TMS)

The first ever demonstration of a motor resonance effect in humans (and of a putative mirror neurons system) has been provided by Fadiga et al. (1995). The paradigm used by the authors has become the classic one for testing the motor system involvement in action observation. The rationale of the demonstration is the following: the authors chose to look for the excitability of the motor system as a measure of action simulation. In particular the assumption was that if the action observed is automatically represented in the brain, then the active representation of the observed action in participants' own motor system should activate in turn neurons representing the muscles involved in the production of that very same action.

Transcranial Magnetic stimulation (TMS) allows testing the simulation hypothesis: the suprathreshold stimulation of the primary motor cortex depolarizes cortical interneurons that in turn excite the corticospinal tract, eventually producing a hyper-synchronized depolarization of the muscular fibers innervated by the alpha motorneurons. The electromyographic signature of this depolarization is a wave called Motor Evoked Potential (MEP). The amplitude (or the area underlying the wave) of the MEPs is considered to be an index of cortical excitability. So if the muscles involved in the production of an action become automatically active in participants' own motor system when those actions are observed, then the stimulation of the portion of the primary motor cortex representing hand movements and muscles, should produce MEPs on the recorded muscles whose amplitude should be higher compared to MEPs amplitudes evoked when participants are watching a dimming light or when participants are watching action whose production would not involve the recorded muscles.

The authors showed the participants 4 different visual stimuli: a grasping action towards an object, an arm movement, an object alone, and finally a dimming light. Four muscles were recorded from participants' arm-hand: Extensor Digitorum Communis (EDC), Flexor Digitorum Superficialis (FDS), First Dorsal Interosseus (1DI), and Opponens Pollicis (OP). Transcranial magnetic stimulation has been delivered on participants' left primary motor cortex (M1), on the hand "hot spot" in order to produce a constant MEP on the recorded muscles. Results showed that MEPs were higher in all recorded muscles during action observation conditions compared to the other ones. Moreover MEPs amplitudes on the recorded muscles differed also between "arm movement" and "grasping actions toward an object" conditions. In particular MEPs from OP muscle were not different from baseline in the arm movement condition. The hypothesis of a neural system that matches observed actions with the motor representations of those same actions would predict that the pattern of MEPs amplitudes on the recorded muscles would reflect the electromyoghraphic activity of those very same muscles when movements shown in the "arm movement" and "grasping towards an object" conditions were actually performed.

If this was the case OP should not have been involved in performing those arm movements shown in the "arm movement" condition, but it would have been only engaged in the "grasping toward an object" condition. This is exactly what the authors found, a differential involvement of the OP muscle in performing grasping action (in which OP was involved) and meaningless arm movements (in which OP it was not). Thus results have been interpreted as the evidence of a specific visuo-motor matching system that automatically translates visual information on the observed action into the motor representation of that action, that is very likely underpinned by human mirror neurons.

Since the seminal work produced by Fadiga and coworkers, many features of the human resonant system have been discovered. What are the characteristics of this visuo-motor matching system? One interesting feature of the human resonant system is that, as described by Fadiga and colleagues, the observer's motor system is modulated also by meaningless actions. Many experiments after the first one have replicated this datum using a range of different meaningless and intransitive movements (Alaerts, Heremans, Swinnen, & Wenderoth, 2009; Alaerts, Swinnen, & Wenderoth, 2009b; Catmur, Mars, Rushworth, & Heyes, 2011; Catmur, Walsh, & Heyes, 2007; Cattaneo, Caruana, Jezzini, & Rizzolatti, 2009; Maeda, Kleiner-Fisman, & Pascual-Leone, 2002; Patuzzo, Fiaschi, & Manganotti, 2003; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006). For example Maeda et al. (2002) showed participants simple finger movements as index finger abduction/adduction, or thumb abduction/adduction. 1DI and Abductor Pollicis Brevis (APB) were the muscles recorded from the participants hand; these muscles are respectively the major actors in index finger and thumb abduction/adduction. When the observed hands were oriented in an egocentric perspective, MEPs from FDI were higher when observing index finger abductions/adductions, and vice-versa MEPs from APB were higher when observing thumb abduction/adductions. Alaerts et al. (2009b) presented participants with right wrist extension and flexion movements while they recorded from participants' Flexor Carpi Radialis muscle (FCR), responsible for wrist flexion, and from the Extensor Carpi Radialis (ECR), responsible for wrist extension. In line with previous data, they found a differential MEP’s amplitude modulation when the hands were presented in an egocentric perspective, with higher amplitude in MEPs from FCR when showing wrist flexions and vice-versa for ECR.

Mirror neurons are supposed to transform the observed directly into its correspondent motor representation. So it is likely that the visuo-motor features of the observed actions modulate the activity of the resonant motor system. One of these features is the spatial compatibility between the observed action and the position of the onlooker hands. The study by Maeda et al. (2002) found a modulation of the motor resonance effect depending on the orientation of the presented hand, that is, if the hand was presented in an ego-centric perspective, meaning, with the fingers pointing away from the observer’s body, the above described motor resonance effect was found, however if the hand was oriented in an allocentric perspective, that is with the fingers pointing towards the observers’ body, the effect decreased.

In Maeda and colleagues’ study spatial-dependent features, the direction of the movement observed and the hand orientation covaried, so that it was not possible distinguishing whether the spatial compatibility played a role in causing the motor resonance effect. Urgesi et al. (2006) tested participants lying their hand palm-up or palm down while they were presented with index and little finger abduction of either a palm-down or a palm-up right hand. The authors recorded MEPs from 1DI and Abductor Digiti Minimi (ADM) muscles. Importantly, hands were shown in an egocentric perspective; in this way, keeping the orientation fixed, they varied the spatial compatibility of the observed action. Results showed motor facilitation of MEPs on those muscles involved in the observed action irrespectively from the posture of the observed hand. Nevertheless, 1DI modulation was obtained only when participants kept their own hand palm-down, while ADM modulation was obtained only when participants kept their own hand palm-up. This result has been interpreted in the light of electromyographic activations of the recorded muscles during the actual execution of index or little finger abductions with the hand palm-up or palm down: it turned out that in a palm-down position 1DI muscle was more involved than in the palm-up position and vice-versa for ADM. Thus spatial features seem to play a minor role in the motor resonance effect; instead what drives the motor system modulation is the topographic matching of the observed hands movements onto the observers' motor system.

In the same vein, the above cited work by Alaerts et al. (2009b), tested whether the motor facilitation observed in the literature were principally muscle-dependent or spatial-dependent driven. They showed participants extending or flexing right wrists in a palm-up or palm down position while recording from ECR and FCR; this manipulation allowed a complete separation of the muscular factor from the spatial factor. Results showed that if participants' right hand was oriented in a compatible way with the one observed, then, as predicted, MEPs from ECR, the muscle responsible for wrist extension, were higher compared to those from FCR, the muscle responsible for wrist flexion, and vice-versa when the presented movement was a wrist flexion. However when placing participants' hand in an incompatible posture compared to the one in the stimuli, a significant difference was only found on ECR muscle but not on the FCR. Although separate analyses for the two muscles showed an effect of the model's movement in both muscles, while no effect of the posture was found leading to the conclusion that the motor resonance is predominantly influenced by muscle-dependent than by spatial-dependent matching. These data point to a minor role of the spatial effects in favor of a muscular-dependent coding.

The laterality of the motor resonance effect has been tested by Aziz-Zadeh and colleagues (Aziz-Zadeh, Maeda, Zaidel, Mazziotta, & Iacoboni, 2002) showing a non-differential motor facilitation both in the left and the right hemispheres when showing participants right or left hands index finger abductions respectively. However the motor facilitation was more pronounced for the left hemisphere for right hands. Given the specificity of the monkey's mirror neurons for goal-directed actions (but not for non goal-directed actions), raises the question of whether the motor facilitations observed during action observation tasks are due to the same mirror neuron system. Is this system involved in goal coding?

Cattaneo et al. (2009) explored this issue using pliers. The experimenter used two kinds of pliers, one regular pliers were opened when the fingers were extended, while the inverse pliers were opened when fingers flexed. Participants had to observe the experimenter performing either goal directed (grasping) or non goal-directed actions using either normal or inverse pliers. In the meanwhile participants' left primary motor cortex was stimulated and MEPs were recorded from OP muscle. If the motor resonance effect is driven exclusively by muscular-dependent matching between the observers' motor system and the observed action, then this would have predicted an opposite modulation of MEPs amplitude from OP when participants observed grasping performed with normal or inverse pliers, otherwise. On the contrary, if the motor resonance effect is influenced by the goal, MEPs amplitude should have not differed between the inverse and the normal pliers usage observation. The authors found an interesting interaction between the type of pliers and the context (goal or non goal-directed actions), that is, when the action performed was a non-goal-directed one then the motor facilitation observed on OP was in accordance with the actual movement performed, instead, if the action was an object grasping, then, motor facilitation was independent from the type of pliers used by the experimenter.

Enticott et al. (2010) even found no effect on cortical excitability when participants were presented with pantomimed grasped actions compared to static hands. They only found a significant motor facilitation for observed grasping actions. Donne et al. (2011) replicated this effect showing that action observation facilitated the motor system only when the action was a goal directed one as a grasping or a social one as handshaking or “thumbs up” gestures. Related to this topic Gangitano, Mottaghy, & Pascual-Leone (2001), recording from 1DI muscle, found that motor facilitation caused by the observation of an observed grasp action followed in time the different phases of the “reaching-finger preshaping-finger closure” performed by the actor. However it was not clear whether the motor program activated by the observed action was fragmented and constantly updated in order to follow the different phases of the actual action, or it was a complete non-fragmented loading of the motor program observed. The authors put some unpredictable movements in the grasping action showed to the participants and tested whether the motor facilitation followed the sudden change. The results are in agreement with the complete loading hypothesis, that is, once the motor resonance takes place it continues independently of what the observer is watching.

Other experiments have shown that the motor resonance effect deals more with the action than with the actual movement (Urgesi, Moro, Candidi, & Aglioti, 2006). The authors presented participants a still hand snapshot, a snapshot of a hand in the middle of a grasping movement, and finally a snapshot of a hand in the end posture of a grasping movement. Motor facilitation on 1DI was detectable only for the implied movement picture, but not for the still hand and for the end-posture picture. Motor resonance does not need an actual movement to take place. The great majority of the TMS studies testing the involvement of the motor system in action observation have been conducted testing the modulation of the cortical excitability of the portion of the primary motor cortex stimulated. However mirror neurons have been found in the premotor ventral region of the monkey brain, not in the primary motor cortex (but see Tkach et al. 2007). So basically these studies do not test directly the mirror neurons activity. In order to test whether mirror neurons are responsible for the motor facilitation observed in many TMS studies on action observation, in one study (Avenanti, Bolognini, Maravita, & Aglioti, 2007) the excitability of the participants' PMv, primary somatosensory cortex (S1) and M1 has been transiently reduced after an off-line low-frequency repetitive TMS (rTMS). Soon after, MEPs were recorded while participants observed still hands, biomechanically possible movements or biomechanically impossible movements. MEPs after rTMS on the PMv reduced the motor excitability selectively for biomechanically possible actions, while the stimulation on the S1 influenced MEPs amplitude only in biomechanically impossible conditions. rTMS on M1 reduced the overall cortical excitability in a non-specific way.

Other experiments from the same group tested the effects of an on-line “virtual lesion” on PMv when performing action judgments and body parts judgments (Urgesi, Candidi, Ionta & Aglioti 2007). The stimulation impaired the performance only on action judgments but not on body parts discrimination (which in turn has been impaired selectively by on-line rTMS on the extrastriate body area). A research from the same group (Romani, Cesari, Urgesi, Facchini, & Aglioti, 2005) demonstrated that a motor facilitation on MEPs recorded from 1DI muscle was triggered both by biomechanically possible actions and by impossible biomechanically actions. However interfering with

PMv impairs selectively the judgment on possible but not on impossible actions (Candidi, Urgesi, Ionta, & Aglioti, 2008).

These results, and those of Avenanti et al. (2007), suggest that the main hub for transforming visual representation of observed actions into correspondent motor representations is the premotor ventral cortex and the motor facilitation observed in the single pulse TMS on M1 reflects its activity. This experiment strongly suggests that PMv contains mirror neurons, but it is possible that the effect of the repetitive stimulation spread also in different brain networks connected to the stimulated area, and thus it does not exclude that the effects showed are not due to the inhibition of PMv, but rather to other inhibited areas, or that the differential effect they found was due to the inhibition of neurons with different functional properties than those of mirror neurons.

The work by Avenanti et al. (2007) does not deal with the cognitive functions ascribed to the mirror neurons. A rather new and promising paradigm has been used (Cattaneo, Sandrini, & Schwarzbach, 2010) in order to clarify which type of information is encoded in different sectors of the cortex. For this purpose the authors used the TMS counterpart of the repetition effects on the BOLD signal in fMRI, that is the “TMS adaptation” (Silvanto, Muggleton, Cowey, & Walsh, 2007). When some property of a stimulus that is encoded by a specific neural population is repeated many times, it causes that particular neural population to adapt to the stimulus. It has been demonstrated that a single pulse stimulation delivered on the neural population adapted, boosts the firing of these neurons. Cattaneo and colleagues showed participants two motor acts (pushing or grasping an object) performed with two different effectors (hand or foot). The combination of these characteristics produced 4 types of movies: grasping with the hand, grasping with the foot, pushing with the hand, pushing with the foot. In each trial these clips were shown for 40 seconds and then participants were presented with 8 consecutive pictures representing all these actions. Participants were required to answer whether the depicted motor acts were same or different from the ones showed in the clips, independently of the effector used by the actor. Single pulse TMS was delivered on the PMv, IPL, STS at the onset of each picture. As predicted by the TMS adaptation paradigm, if the portion of the cortex codes the goal of the action independently of the effector, then the TMS on the test stimuli should lower the reaction times for recognizing the adapted motor act compared to the non-adapted motor act independently of the effector used. Results showed that when stimulations occurred on PMv and IPL participants were faster in recognizing the adapted motor act independently of the effector used to perform it, while the stimulation on STS, decreased the reaction times on the adapted actions only if the effector used was the same of the adapting clips. This results demonstrated that PMv generalizes the goal of the observed action, while the STS does not, leading to the conclusion that STS is not endowed with enough generalization properties in order to accomplish the action understanding process.

In conclusion TMS experiments showed a reliable picture of how the simulation process takes place in the motor system. However in literature the debate has been fueled by arguments and criticism against the mirror neuron (simulatory) theory of action understanding. Even if 20 years have passed by, the debate on how and what mirror neurons accomplish and represent (assuming that they exist in humans) is still a matter of an incandescent debate.

4. CRITICISMS TOWARDS THE CLASSICAL INTERPRETATION

The discovery of mirror neurons has been described as the driving force behind “the great leap forward” in human evolution' and that “…mirror neurons will do for psychology what DNA did for biology”. (Ramachandran, 2000). Despite the interest and the excitement raised by this discovery, many neuroscientists have criticized different aspects of the mirror (simulatory) theory of action understanding. Following, the most common issues about the mirror neurons and the mirror neuron theory of action understanding are briefly described.

4.1 Definitions

According to Rizzolatti et al. (2001) “…when we observe a hand grasping an object the same population of neurons *that control the action execution* of grasping movements becomes active in the observer’s *motor* areas” and “…An action is understood when its observation causes the *motor* system to resonate (Rizzolatti et al. 2001)”. The mechanism described implies that “In our brain there are mechanisms (mirror mechanisms) that allow us to directly understand the meaning of the actions and emotions of others by internally replicating (simulating) them without any explicit reflective mediation (Gallese, Keysers, & Rizzolatti, 2004)”. Thus what turns out from the description of the mirror mechanism (simulation, motor resonance or mirroring) is that understanding and recognizing an action is a *motor process* that involves those structure that code *motor* representations necessary to perform that action: “Action understanding does not depend, according to this view, on the activation of visual representations (an activation obviously present) followed by their interpretation by the central conceptual system, but by the "penetration" of visual information into the experiential ('first person') *motor* knowledge of the observer (Gallese et al. 2004)".

The definition of "action understanding" has been somewhat unstable and changeable, understanding is a very broad concept even if the core of its meaning seems easily graspable. The concept of action understanding could be interpreted in several ways, leading in principle to radically different conclusions when testing hypotheses involving this concept (Hickok, 2009; Uithol, Haselager & Bekkering 2011). The first paper by Gallese et al. (1996) described a potential mechanism ascribed to the mirror neurons, that of understanding motor events, namely defined as "...the capacity to recognize that an individual is performing an action, to differentiate this action from others analogous to it and use this information in order to act appropriately". Rizzolatti et al. (2001) defined it as "…the capacity to achieve the internal description of an action and to use it to organize appropriate future behavior" (Rizzolatti et al. 2001)" leaving however unexplained the meaning of "internal representation".

Nelissen, Luppino, Vanduffel, Rizzolatti, & Orban (2005) made a step further: "A mere visual representation, without involvement of the motor system, provides a description of the visible aspects of the movements of the agent, but does not give information critical for understanding action semantics, i.e., what the action is about, what its goal is, and how it is related to other actions" letting the reader to interpret the motor representations as equivalent to action semantics, or suggesting that the access to action semantics is possible only through the access to the *motor* representation of that action.

4.2 Single-cases in cognitive neuropsychology

As suggested by Gallese et al. (1996), distinguishing actions is a *motor* process since one has to simulate the action and then he will be able to understand it. This hypothesis is testable by looking at the cognitive performance of patients with neurological disease or focal lesions. As described in the preceding chapter, evidence favoring a simulative account of action understanding comes from group studies involving brain-damaged patients. However another possible evidence comes for single-patients studies; if action execution and action understanding/recognition are the same process that is supposed to be a motor one, then it would be impossible finding patients that would show a poor performance on action recognition but an almost or completely spared ability in producing those same actions, that is a strong single dissociation between those two cognitive processes; neither would it be possible to find the reverse pattern, with impaired production and complete spared understanding. This double dissociation has been found before the discovery of mirror neurons. Importantly, testing this hypothesis can be independent from the lesioned area that causes the cognitive deficits. The aim is testing whether the *cognitive* representations involved in action execution are the same involved in action understanding as predicted by a simulative account of action understating.

In cognitive neuropsychology a dual route model for action recognition/production has been developed based on single cases showing dissociations among several cognitive abilities (Rothi, Ochipa, Heilman & Kenneth, 1991). The dual route model was derived by reading and writing neuropsychological models in which patients who could not read irregular or psedo-words, could however read regular words by using a direct orthographical to phonological route that allowed participants to read regular words by applying grammatical rules, let them reading the word as sum of these orthographical chunks. Instead an indirect route accounted for the pattern showed by patients who could read both regular and irregular words, but not pseudo-words. These patients had, according to the model, a deficit on the direct route because they could not apply the grammatical rules in order to read the pseudo-words correctly. Within the indirect route other dissociations can appear. For example, patients that could understand the meaning of written words, could not however read them aloud, demonstrating a selective deficit in the phonological output lexicon. However the opposite dissociation could arise in which patients could not name pictures correctly (demonstrating an deficit in the phonological lexicon) but they understand the meaning of regular and irregular written words. This led to the conclusion that the orthographical input lexicon where the visual representation written words is stored, is independent of the phonological output lexicon in which the phonological patterns of the words are represented.

The same logic was applied to the gesture recognition and production. Indeed it has been demonstrated a dissociation between action production and pantomime/gesture recognition.

Rothi, Mack, & Heilman (1986) reported two non-apraxic patients who were almost flawless in pantomiming on verbal command or on picture but they performed poorly both in pantomime discrimination and in their comprehension. This condition was defined as “Pantomime agnosia” because they were able to imitate and to perform on verbal command pantomimes they could not recognize. Another group (Cubelli, Marchetti, Boscolo, & Della Sala, 2000) tested the model of limb apraxia proposed by

Rothi et al. (1991). Patient number 3 showed pantomime agnosia not being able to discriminate and comprehending observed meaningful gestures that however he could produce on verbal command. It could be argued that pantomime recognition is a more difficult task compared to producing an action to verbal command; however the opposite dissociation, a deficit in producing actions or pantomimes with a spared ability to visually recognizing them, has been described by the same authors. Along with these data, Negri et al. (2007) showed that even if correlations between production and recognition abilities were statistically significant, some patients with spared pantomime recognition abilities presented impaired object use and, vice-versa, patients with spared object use showed impaired pantomime recognition abilities.

For what concerns patients with lesions to the putative mirror neurons in the inferior frontal gyrus, data are puzzling; indeed demonstrating a correlation between lesions to Broca’s area and action recognition abilities without a parallel deficit in action production, should make the observation not sufficient in order to support a mirror neuron theory of action understanding. Pazzaglia et al. (2008) showed that, on a group level, patients presenting apraxia were more impaired in action recognition compared to non-apraxic patients. However at a single-case level one third (7 on 21) of the apraxic patients were spared in recognizing actions and pantomimes/symbolic gestures (Gallese, Gernsbacher, Heyes, Hickok & Iacoboni, 2011).

4.3 Criticism on the simulation hypothesis

Other than the evidence on patients, the mirror neuron theory of action understanding has been criticized based on evidence on monkey. Gergely Csibra

(Csibra, 2005) dealt with the property shown by mirror neurons of spiking when the monkey observes transitive but not meaningless intransitive actions. According to the mirror neuron theory of action understanding, the simulation process is accomplished by mirror neurons, allowing an action to be understood. Given these premises, Csibra argued that mirror neurons should simulate any observed action, including intransitive actions, since is the simulation process they performed that allows the understanding of an action, otherwise the logical conclusion is that the action has been understood before mirror neurons activity. Available data though favor a “first understand then simulate” mechanism rather than a “simulate to understand” one. According to Csibra, mirror neurons pattern of activation is more compatible with the coding of the action goal, not with his simulation. Related to the latter point, the author highlights a paradox within the direct matching hypothesis: in the theoretical papers describing direct matching hypothesis, mirror neurons are considered both to code the goal of an action in a quasi-independent way from how the goal is performed, but they are also suppose to simulate the observed action in order to understand the goal. But the two alternatives are mutually exclusive.

4.4 Criticism on the neurons classification

A second criticized point concerns the fact that if the simulation is for understanding observed actions, than broadly congruent neurons would not allow a very good action understanding since their simulation of the observed act would be incorrect. That is, let’s assume that the neuron recorded fires when a precision grasp is performed. According to the simulation theory when a precision grasping action is observed that neuron should simulate the action, firing in response to the observed action. However the same neuron could spike also to the observation of a whole-grip. If the simulation hypothesis holds true, then this would result in a mis-simulation and the action would not have been completely understood. Related to this criticism Uithol et al. (2011) made some considerations about the strategy for categorizing mirror neurons in terms of representational content, referring with this term to represented feature of the object to be represented (the action performed or observed). In strictly congruent neurons the object to be represented by the neuronal firing is the same in both observation and execution. The representational content abstracts from the performer of the movement (the monkey itself or another monkey/human). So the neuronal vehicle covariates its activity with the execution and the perception of the same representational content (the action performed in a specific way). Differently, neurons defined as broadly congruent fire to the observation of the same actions but performed in different ways, while on the motor side, they respond only to a specific way of producing an action. Once this discrepancy has been realized, a strategy was adopted to move the correspondence a level up, not at the level of “how” a specific action was realized, but on the type of action, independently on how the action was realized. The logic then is the following: the less the compatibility between the execution and observation responses, the more the abstraction of the representational content in order to continue speaking of mirroring. The authors argued that even for those neurons that did not show a clear relationship between execution and observation, a shared representational content can be found; in this case for example, the action was performed on an object in both modalities.

The last mentioned consideration triggers another question about what piece of information is it coded by a single neuron in PMv. The assumption made in the early experiments is that the information on the goal is coded on a single neuron (Rizzolatti et al. 2001); however this assumption excludes the possibility that the representational content is represented by a representational vehicle constituted by the pattern of activity of a number of neurons, in which the single neuron activity is not to be considered the vehicle of the information represented; indeed within this view the sum of the subparts (single neuronal activities) is not equal to the whole (Uithol et al. 2011). Even assuming that the representational content is coded on the single neuron activity, recent works on monkeys arise some speculation on the degree of generalization provided by the mirror neuron activity. In the recent works by Caggiano et al. (2009, 2011, 2012) it is suggested that mirror neurons are sensitive to much more visual features than previously thought. In one of these papers (Caggiano et al. 2011) it has been realized that this empirical evidence is not in line with the goal coding hypothesis. If the goal of an action is coded by single neurons, then their spike should not be affected by the point of view, by the distance, or by the value of the grasped object. Caggiano et al. (2011) showed that some mirror neurons responded only to peculiar orientations of the observed action; for example when the action has been carried out in front of the monkey some neurons increased their firing rate, but the spiking did not change from baseline when the action was performed in an egocentric perspective. The 26% of the neurons recorded in that experiment were view-independent, that is they responded to the grasping action independently from the point of view from which the action was presented. This 26% can be claimed to be coding the goal of the action, because the goal is supposed to be independent from the sensory features of the action (including the orientation). However is that degree of generalization preserved when *that 26% of mirror neurons* is tested *at the same time* on other properties as the distance of the action performed or the subjective value of the object grasped? The degree of generalization should be the same even when the different combinations of these three variables are tested simultaneously. This experiment has not been carried out (yet), but it is worth considering the possible outcomes, since the idea that mirror neurons code the goal of an action in a quite abstract way predicts that the combination of perspective, point of view, sensitiveness to filmed actions and subjective value would not influence the firing in neurons coding specific goals.

4.5 Motor or multimodal neurons?

Another important concern is defining what does the adjective “motor” means, and how should it be used. This issue is crucial in order to understand which type of representation is engaged when observing actions. It is often implicit that mirror neurons are motor neurons endowed with sensory properties. However considering them “motor” neurons only because they are contained into an area that contains motor neurons is somehow misleading. For example Gallese et al. (1996) described neurons in F5 that responded *only* to the sight of actions, but not to the performance of that action. These neurons, called “mirror-like”, roughly match in number the strictly congruent mirror neurons (25); interestingly they were not endowed with motor properties even if they have been found in monkey’s premotor cortex. So assuming that the action goal is coded on single neurons, mirror-like neurons are high-order *visual* neurons specifically tuned to the vision of different specific actions. The same applies for mirror neurons:

these neurons are endowed with visual, motor and auditory properties. In order to describe them correctly a good definition would be “multimodal” (assuming a more embodied perspective), or “amodal” neurons coding the goal of an action independently of the sensory property through which the “goal” of the action has been accessed. These considerations have an effect on the empirical side. For example, if an action recognition deficit is correlated with lesions to the inferior frontal gyrus, this does not say much about the simulatory processes supposed to take place in order to understand an action as long as there are no action production deficits (Fazio et al. 2009); on the contrary, it suggest that action recognition processes can be damaged selectively without a parallel deficit on the action production side (possibly damaging those mirror-like neurons not endowed with motor properties for example).

4.6 Summary

In conclusion it can be noticed that the number of demonstrations and counter-demonstrations for the role of the mirror neurons in action understanding is, as previously shown, large. The criticism towards the mirror neuron theory of action understanding, mainly, is not about the neuronal properties (firing both in execution and observation) of the neurons, but on their role in cognition. The simulation process that is supposed to transform visual representations into motor representations is described in cognitive terms, and in cognitive terms can be faced. Since the exact relationship between neuronal activity and cognitive processes is not known, (especially in rather high-level cortical areas as the premotor cortex can be) the processes that mirror neurons are supposed to compute can be performed even by different physical representational vehicles (from example, as suggested by Uithol et al. 2011, by a network of neurons). Then, in order to demonstrate the classical mirror neurons theory to be true in humans, the theory can be decomposed in at least two components: the first recites that in order to understand the action observed, the observer has to simulate it, meaning that the visual representation should access the motor representation of that action in order to understand it. The other part of the theory asserts that this simulation is performed by mirror neurons. So in principle it would be possible that 1) the simulation process does not exist in the terms described, but mirror neurons do, 2) mirror neurons and simulation both exist but the mirror neurons neuronal properties are not related to the cognitive processes of the simulation, 3) simulation exist but not mirror neurons, and finally as asserted by the classical theory, 4) simulation process is carried out by mirror neurons. Data on mirror neurons are not straightforward for different reasons, going from the clarity of the "action understanding" concept, passing through the single-patient showing production/recognition dissociations, ending with the representational content coded by a single neuron. In next chapters a series of experiments will be described. The focus of the present research is both to determine whether motor structures contribute to action understanding and to find a paradigm or a cognitive set that allows the simulation to be stopped in order to test whether the simulation is necessary for action understanding.

PART 2: EXPERIMENTAL WORK

5. AIM OF THE STUDIES

The classical mirror neuron theory of action understanding asserts that when we observe an action, the representations that are engaged for performing it, are automatically activated (Rizzolatti & Craighero 2004). So a basic step forward would be gaining information about the role of the simulation in action understanding in normal subjects. In order to do so a state dependent TMS experiment has been carried out. The fundamental idea is to adapt a neural population and then testing the effects of this adaptation on the understanding of actions (Chapter 6 and 7).

Subsequently a complementary issue has been investigated in the present work. Is it possible to stop the simulation? Many experiments concerning action observation test the putative mirror activity in the brain when the participants are still, at rest. However participants are able not to move even if their motor cortex is engaged by the task. So at least part of the motor system can be voluntarily fully controlled, so that one can decide when to move and what action to perform. Pushing the argument a step further, we are able to voluntarily react to observed actions (especially in sports or in competitive actions) without imitating the observed action. How can this be reconciled with the classical mirror neuron theory of action understanding? According to the classical theory, the covert simulation in our own motor system happens to be automatic and has to be performed in order to understand an action (Rizzolatti et al. 2001, 2004). However there should be some mechanisms that allow both not to imitate all the time and to react as we decided to react, not to being “slaves” of our mirror neuron system.

One reliable way to test whether action understanding is a motor process is to determine if actions can be understood when the motor representations of that action are not accessed in normal population (Chapter 8,9 and 10). But before being able to test action understanding without the involvement of correspondent motor representations, these motor representations should become inaccessible; in other words, the first hypothesis that has to be tested is whether and how the motor resonance can be stopped.

In monkey this would have been extremely easy, but surprisingly it has been argued that, in the particular case of mirror neurons, it is not possible to disrupt mirror neurons for the following reasons: 1) Mirror neurons have been found bilaterally in the brain, so disrupting them in one hemisphere would leave the other intact leading to a potential conclusion that mirror neurons are not for action understanding, even if they were. Then the logical suggestion would have been to disrupt both sides. However this suggestion has been rejected claiming that 2) the tissue that would have been damaged would have lead to widespread cognitive dysfunctions, not allowing action understanding to be tested, 3) there are other mechanisms that may mediate action (Rizzolatti & Craighero 2004).

As stated at the end of the previous chapter, part of the aim of the present research is to find a paradigm, or a particular cognitive set, that does not allow the simulation of the observed actions. Some evidence that the motor resonance effect can be modulated (and even reversed) in humans, has been provided by the group lead by Cecilia Heyes within the field of the mirror neurons’ development. In chapter 8 a description of the theory supported by this group will be described followed by the testing of their predictions. Chapter 9 tested the motor simulation during a motor performance task in response to action stimuli, and chapter 10 describes the results of an exploratory experiment that aims to test a new hypothesis on the role of action pre-selection during motor performance in response to action stimuli.

6. STUDY 1: CROSS-MODAL ADAPTATION IN THE VENTRAL PREMOTOR CORTEX – A TMS-ADAPTATION STUDY

6.1 Introduction

According to the mirror mechanism hypothesis, the goal of others' actions is at least in part understood by its match to elements of one's own motor repertoire. There are two implications of this hypothesis: (i) vision of another's action modulates one's own motor system and (ii) vice versa motor activity affects action perception (Schutz-Bosbach & Prinz, 2007). Several experiments have now confirmed the finding that the observers' motor system resonates to action observation (Avenanti et al., 2007; Cattaneo et al., 2009; Desy & Theoret, 2007; Fadiga et al., 1995; Koch et al.; Maeda et al., 2002; Strafella & Paus, 2000), however, the bi-directionality of the mirror mechanism is less clear. The experiments dealing with this topic indeed have shown a wide range of effects, from interference to facilitation (Schutz-Bosbach & Prinz, 2007) and the interpretation of such a variable phenomenology remains largely hypothetical. Some studies show an effect of body position in space or of limb posture on the perception of others' bodily movements (Alaerts, Swinnen et al., 2009b). In these works, congruent body posture facilitated motor resonance to observed actions as measured by motor evoked potentials. Other studies showed online effects of action on perception as a contrast or interference effects of action on congruent visual stimuli (Miall et al., 2006; Musseler & Hommel, 1997; Musseler, Steininger, & Wuhr, 2001; Zwickel, Grosjean, & Prinz, 2007, 2010a, 2010b) or as online facilitation of concurrent performed hand postures and observed actions (Blaesi & Wilson, 2010). Evidence in favor of an effect of movement on action perception is still however under-represented and controversial compared to the well-established observation-execution phenomenology and this certainly contributes to the debate as to whether the motor system really mediates action understanding (Rizzolatti & Sinigaglia, 2010).

A recent innovation in the study of the human mirror mechanisms is that of investigating cross-modal effects on action and vision. The rationale for this approach is that the firing of a multimodal neuron is modified by its preceding input in any of the two modalities to which it responds. Then, effects of its firing history driven by history in one modality can be observed in the other modality. This approach has been exploited mainly using the imaging technique of repetition-suppression, which is the decrease in blood oxygen level-dependent signal to repeated stimuli (Grill-Spector et al. 2006). The repetition-suppression paradigm has yielded various results, from no cross-modal adaptation at all (Dinstein et al. 2007), to asymmetric adaptation (Lingnau et al., 2009), up to full two-way cross-modal adaptation (Chong et al. 2008; Kilner et al. 2009). Nonetheless, because imaging studies produce correlational data, the findings cannot provide evidence of the causal role of a visuo-motor matching mechanism in the process of action cognition.

In the first experiment, we sought to elicit the behavioral correlate of cross-modal adaptation. The hypothesis that was tested is that mirror neurons would be adapted by the repetition of motor acts, and that this adaptation would be evident as a loss in function of visual recognition of actions congruent with motor training. Clear cross-modal motor-to-perception adaptation effects have already been demonstrated in the linguistic domain (Glenberg, Sato, & Cattaneo, 2008). In the second, main experiment, we then used a novel transcranial magnetic stimulation (TMS)-adaptation paradigm (Silvanto, Muggleton, & Walsh, 2008) to investigate the causal role of mirror mechanisms in action recognition. This paradigm is based on the notion that the effects of TMS are state-dependent, i.e. they depend on the initial activity state of the stimulated neurons. Thus, manipulating the firing of subsets of neurons by means of perceptual or motor adaptation allows selective stimulation of those neuronal populations with TMS even when the adapted neurons are spatially overlapping with other cells.

6.2 Materials and Methods

6.2.1 Participants

We tested 20 participants (11 male, 9 female, mean age 29 years) in the behavioral experiment and 10 participants (5 male, 5 female, mean age 27 years) in the TMS experiment. According to the standard handedness inventory (Oldfield, 1975) all subjects were right handed. The present study was approved by the local Ethical Committee for human studies and was conducted in compliance with the Helsinki Declaration of 1975, as revised in 1983. All participants gave written informed consent to the experiment.

6.2.2 Visual stimuli

Visual stimuli consisted in 30 pictures of right hands displacing an object (a yellow table-tennis ball). The orientation of the hand in the pictures was 90°, 180° and −90°with respect to the participant’s viewpoint. The contact point with the object (a sphere) was on five different points: one on the upper pole of the sphere (indicated as point 0 in Figure 2). The remaining four were symmetrically placed along a meridian at 20° and 60° from the vertical axis (points 1, 2, −1 and −2 of Figure 2). The hands were all 90° with respect to the horizontal plane on which the object was positioned. In half of the figures, hands touching objects were female hands and in the other half they were male hands balanced across orientation and position. The background of the figures was homogeneous. Stimuli were presented with the E-Prime® software (Psychology Software Tools Inc.) on a 75 Hz computer screen, with a visual angle of 14° vertically and 19° horizontally.

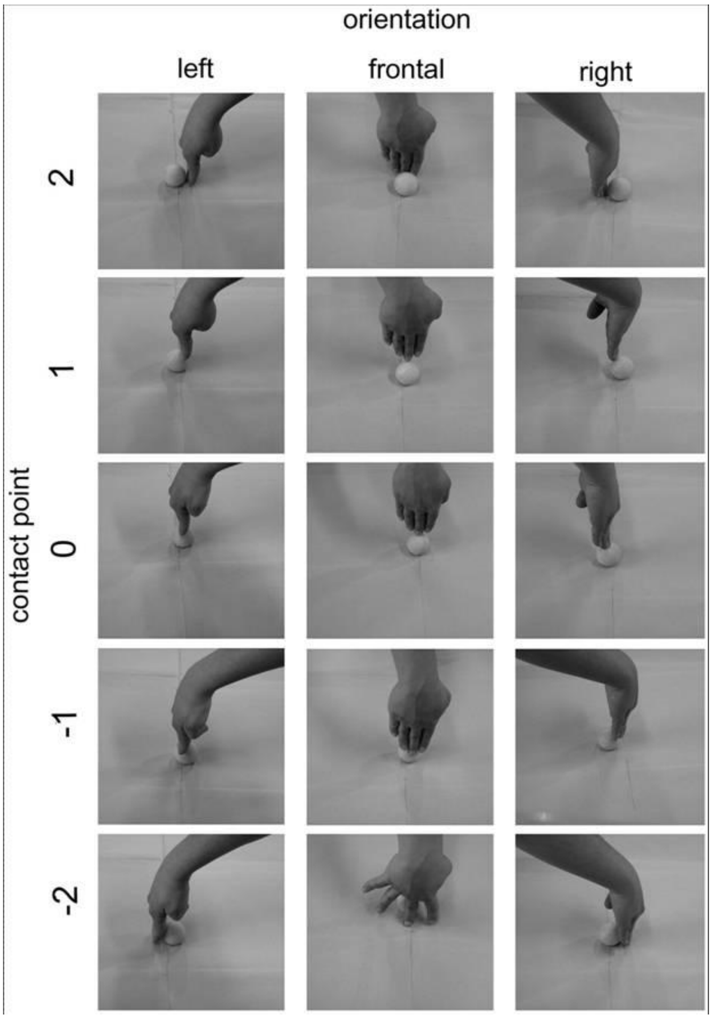


Figure 2
Visual stimuli employed in the two experiments.
The full set of female actor-stimuli in the three different orientations and five different contact points is shown.

6.2.3 Motor adaptation procedure

In both experiments every block started with motor adaptation. On the participant’s right side a bowl full of dried chickpeas was placed. The spherical shape of the container was chosen in order for the chickpeas to fall back in the center of the bowl after each displacement by the subject. In this way adaptation in each direction consisted in purely ‘push’ or ‘pull’ acts without the need of repositioning the objects upon which the participant was acting. Participants performed the action of pushing away or pulling in the chickpeas in the bowl for 60 s. The instruction as to which action to perform was displayed on the screen for 2 s. If the instruction was ‘NEAR’, they had to pull the objects toward themselves; if the instruction was ‘FAR’, subjects had to push the objects away. It should be stressed that in order to avoid any access to visual information concerning their hands’ movements, an opaque screen prevented the participant from seeing his/her own right hand during the whole duration of the motor training.

6.2.4 Behavioral experimental protocol

Subjects sat comfortably on a chair in front of a computer screen wearing earplugs and with the head on a chinrest. The experiment was organized in blocks, each composed of two phases: first the motor adaptation and then the response phase. After the 60 s of motor activity as described above, a beep-sound informed subjects that the training was ended and the test phase was to begin. All the 30 stimuli were presented in every block in random order for 500 ms. A blank screen with a fixation cross preceded the stimulus for 1000 ms and a blank screen with a ‘?’ sign, lasting 1000 ms, followed it. The task consisted in judging if the hand that they were seeing on the computer screen was pushing the objects away or towards him/herself. Subjects were asked to respond as fast and as accurately as possible and they had to respond every time even if in doubt. Responses were given with both feet using a two-pedal response device. The response coding of the left and the right pedals was randomized between subjects. Responses were logged only during presentation of the hand picture and on the ‘?’ sign, but not on the fixation cross. In this way responses given 1500 ms after the onset of the target picture were not recorded. The response terminated the picture display, and switching to the next trial. Four blocks of motor adaptation in each of the two directions were repeated in random order in the behavioral experiment (eight blocks in total). Therefore 240 (30 trials × eight blocks) single responses were collected from every subject. Moreover, according to this structure, single experimental conditions (i.e. same motor adaptation and same contact point) were repeated 24 times in each experiment. Each participant underwent two practice blocks prior to the real session. For the purpose of data analysis, the ‘pull’ responses were coded as −1 and the ‘push’ responses as +1.

6.2.5 Preliminary estimate of the duration of the motor–visual after-effect

After-effects, the perceptual correlates of adaptation, tend to dissipate in time (Thompson & Burr, 2009). Therefore, as a preliminary step, to assess the duration of the adaptation effect, we examined in the behavioral experiment the crude responses (pull = −1; push = 1) to stimuli with contact point $x = 0$ (ambiguous stimuli—third row in Figure 2) as a function of trial position within each block of categorization trials. Ambiguous trials were repeated for every subject overall 24 times after each of the two adaptation trainings in every subject (six times × four blocks). The responses to these 24 trials were ordered from earliest in a block to latest in a block (Figure 3A). Visual inspection showed that a clear adaptation effect was present for over the first half of the trials in the form of a bias of the response in the direction opposite to the one of the motor activity. This was confirmed statistically by an ANOVA using the mean categorization scores as dependent variable and (i) the trial position, 1–12 or 13–24 and (ii) the direction of motor adaptation, as independent variables. A clear interaction was found between the two factors (trial timing and motor adaptation). *Post hoc* analysis made with Bonferroni corrected *t*-tests showed a clear difference between categorization responses after push or pull motor adaptation only for the first 12 responses (all results are shown in Figure 3B). All the subsequent analyses therefore used only the first 12 responses, where the adaptation effect was predominant.

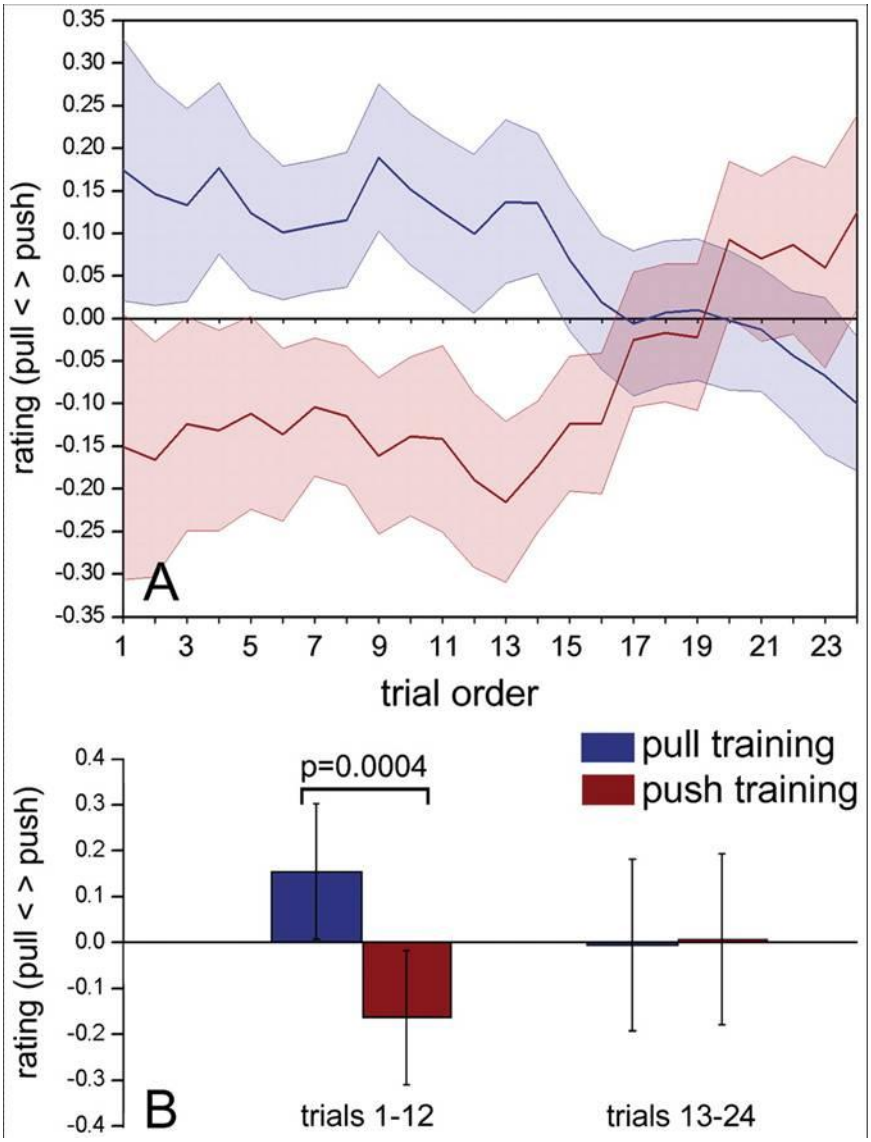


Figure 3
Time course of adaptation effect. **(A)** Mean values of the crude ratings for the ambiguous stimuli (contact point $x = 0$) in all 20 participants to the behavioral experiment sorted by order of appearance in the trials series. The shading represents ± SEM. **(B)** Results of the ANOVA between mean crude ratings of the first half of trials and the second half. The error bars indicate 95% confidence intervals.

6.2.6 Behavioral data analysis

The main analysis on the behavioral data was conducted by fitting individual data sets to independent psychometric functions of the contact point '*x*' (the five contact points are shown in Figure 2). This was done separately for each adaptation direction, taking into account only the first 12 responses in every block. The psychometric function was modeled by means of a normal cumulative distribution function of the contact point *x*, with mean μ and slope σ:

$$\Psi(x; \mu, \sigma) = \frac{1 + \mathrm{erf}[(x - \mu)/\sigma\sqrt{2}]}{2}.$$

Optimal parameters were computed using a maximum likelihood estimation (MLE) procedure (Treutwein & Strasburger, 1999). The maximum of the likelihood function $\Lambda(\mu,\sigma)$ was calculated by performing a grid search on a two dimensional lattice defined by varying μ from −3 to 3 (step 0.01) and σ from 0 to 4 (step 0.01). Rejecting responses given 1500 ms after the onset of the target picture, the average number of data points in each MLE procedure was 58, and was 47 in the worst case.

After each training, the slopes from the two trainings were averaged as $\rho=(\sigma_N+\sigma_F)/2$, where σ_N and σ_F represent the slopes of the psychometric functions estimated in the 'near' and in the 'far' directions of motor adaptation. Then the mean parameter μ was normalized as $\nu = \mu/\rho$. Such normalization allowed us to measure the after-effect in units of participant's psychometric function slope and to plot the grand-average of all the resulting psychometric functions. The normalization procedure was performed assuming that the training would not affect σ, but rather move μ along the 'contact point' *x*-axis, based on the hypothesis that we have adapted a neural population to two opposite and symmetrical tunings of the action observation-execution system. The assumption of σ being training-independent was tested by comparing σ values in the two adaptation conditions by means of a paired-sample *t*-test in the behavioral experiment ($P = 0.29$) and by an ANOVA with two within-subject factors: 'TMS modality' (three levels: sham, PMv and M1) (all $P > 0.56$).

The parameter ν therefore indicates the position of the psychometric functions along a normalized x-axis; the higher it is, the more a subject was biased towards a 'pull' categorization response whereas the lower it is, the more a subject is biased towards a 'push' categorization response. We used therefore the value of ν as dependent variable to characterize the after-effect. The values of ν were compared between the two training directions by using a *t*-test for paired samples.

6.2.7 TMS experimental protocol

In the main experiment, the same experimental paradigm as in the behavioral experiment was used with the addition of a single TMS pulse applied concurrently with the presentation of each target picture. The timing of magnetic stimulation with respect to the target visual presentation was derived from a protocol previously shown to affect adapted populations of neurons in PMv (Cattaneo et al., 2010).

As in the behavioral experiments, subjects underwent blindfolded motor adaptation in one of the two directions for 60 s and were then tested with a series of 30 pictures of hand object interaction. TMS was delivered either as (i) sham stimulation, (ii) real stimulation over left PMv or (iii) real stimulation over left hand-related M1.

Each of the three TMS modalities was delivered in a distinct block. Within each of these blocks the participant performed a total of eight motor adaptations (four in each direction). The order of the three blocks was randomized in each subject. The experiment was therefore organized as a 3 (TMS conditions) × 2 (direction of motor adaptation) design.

6.2.8 Stimulation parameters

Biphasic TMS pulses were applied through a 70-mm-diameter figure-of-eight coil (model MC-B70, MagVenture Denmark) at the onset of each test picture. A MagPro 3100 stimulator (MagVenture Denmark) was used. The coil was attached to a mechanical arm fixed to a tripod and placed tangentially to the skull. Sham stimulation was performed using a sham coil (model MC-P-B70, MagVenture Denmark). Right before the experiment, the individual visible resting motor threshold was assessed, being defined as the lowest stimulation intensity capable of evoking a visible contraction in the relaxed right hand in at least 5 out of 10 consecutive stimuli. The stimulation intensity for the experiment was set to 90% of the individual threshold. The magnetic stimulator was triggered by the e-prime software through the PC’s parallel port. Stimuli were delivered at the onset of each of the 30 test pictures in every block. Coil orientation was parallel to the midline with handle pointing backwards for PMv and sham stimulation and 45° to the midline with handle pointing backwards for M1 stimulation.

6.2.9 Neuronavigation

Prior to the experiment, a high-resolution T1-weighted magnetization-prepared rapid gradient echo sequence scan of the brain of each subject was obtained using a MedSpec 4-T MRI scanner (Bruker BioSpin, Ettlingen, Germany) with an 8-channel array head coil. Before the TMS session, the participant’s head, TMS coil and participant’s 3D reconstruction of brain and scalp from individual MRI images were coregistered in space by means of the BrainVoyager (Brain Innovation BV, The Netherlands) neuronavigation system using the Zebris ultrasound tracker (Zebris, Medical GmbH, Germany). The two target locations, left PMv and left M1, were localized by means of macro-anatomical landmarks, namely the anterior bank of the central sulcus in correspondence with the hand knob of the precentral gyrus for the left M1 and the portion of precentral gyrus below the intersection of the inferior frontal sulcus with the precentral sulcus for the left PMv (Tomassini et al., 2007). An example of coil positioning in the two active TMS conditions is shown in Figure 4. Sham stimulation was applied midway between the M1 and PMv locations.

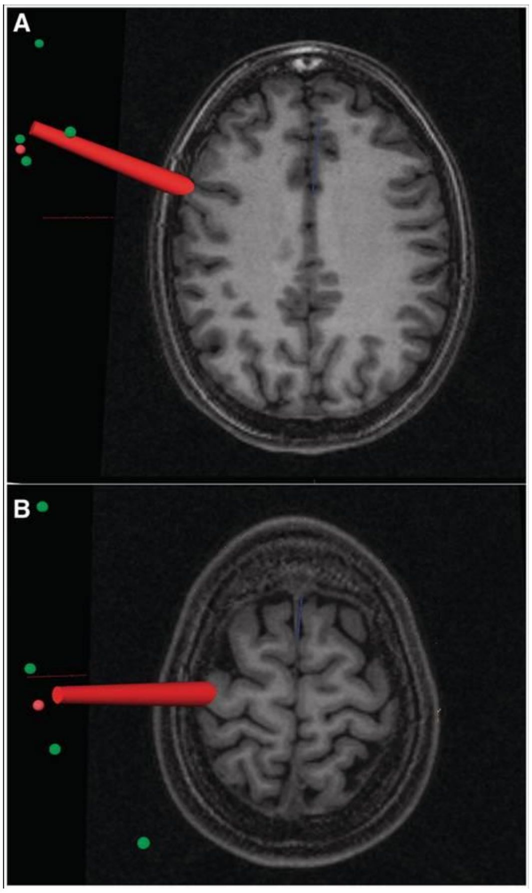


Figure 4
Localization on MRI scans of the left ventral premotor cortex **(A)** and of the left hand-related primary motor cortex (**B**) in one representative subject. The red beam represents the focus of the magnetic pulse.

6.2.10 TMS data analysis

Only the first 12 responses in each series were considered ('Behavioral Experiment' section). All fitting procedures and normalization of the data were performed as described for the experimental experiment. The normalized ν value was then used as the dependent variable in an ANOVA with two within-subjects factors: TMS condition (three levels: Sham, PMv and M1) and adaptation task (two levels: push and pull). *Post hoc* comparisons were made with Bonferroni corrected *t*-tests for paired samples.

6.3 Results

6.3.1 Behavioral experiment

The paired-samples t-test showed a clear difference of ν between the two adaptation directions ($t = -3.50$; DF = 19, $P = 0.0024$). Importantly, the ν values after pull adaptation were smaller than those after push adaptation, being on average −0.106 for the ‘pull’ adaptation and 0.260 for the ‘push’ adaptation (mean psychometric functions and individual data are shown in Figure 5). This difference indicates that, coherently with the adaptation hypothesis, the after-effect following motor adaptation was a strong bias towards the action ‘opposite’ to the one that had been trained with values after pull adaptation being smaller than those after push adaptation. The average number of missed trials (response times > 1500 ms) was overall of 4.8%.

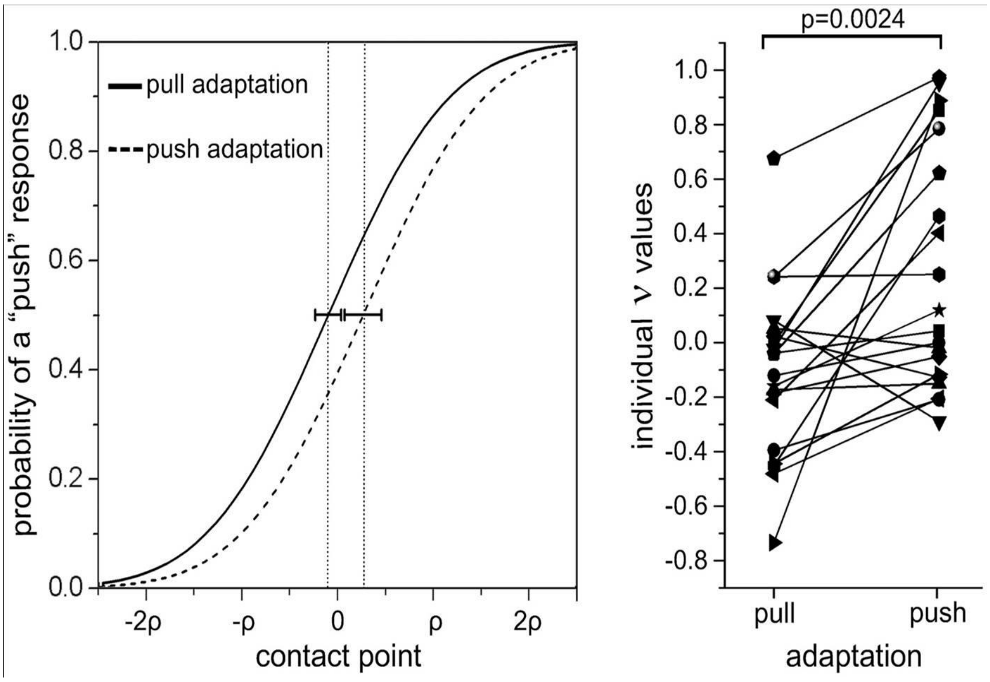


Figure 5
Results of the behavioral experiment. Left: grand-average of all the individual psychometric functions in the two adaptation conditions. The contact point *x* is expressed in units of participant's psychometric function slope ρ. The dotted vertical lines represent ν mean values and horizontal error bars represent 95% confidence intervals. A shift of the psychometric curve towards the left indicates an increased probability of categorizing the stimulus as 'push'. Vice-versa a shift to the right indicates a bias in favor of 'pull' responses. Right: individual values of ν for all 20 participants. Note that ν indicates the stimulus value for which a participant is likely to respond at chance level. A negative value of ν indicates an increased probability of categorizing the stimulus as 'push'. Vice-versa a positive value indicates a bias towards 'pull' responses. The *P*-value refers to pairwise *t*-test.

6.3.2 TMS experiment

No immediate or delayed side-effects of TMS were observed in any participant. None of them reported significant discomfort from stimulation. The results of the ANOVA showed a main effect of the adaptation task [F(1, 9) = 16.807, P = 0.0027] but most importantly we observed a clear interaction of the two factors ‘TMS condition’ and ‘adaptation task’ [F(2, 18) = 6.4205, P= 0.0079]. Post hoc analyses using Bonferroni-corrected t-tests showed that a significant difference was present between ν values in the sham and M1 TMS conditions (corrected P = 0.015 and 0.041, respectively) but no difference was seen in the PMv condition (corrected P = 1.0). The results are schematized in Figure 6. Thus, we confirmed the results of the behavioral experiment in the sham condition, by showing a perceptual bias towards the opposite action. The stimulation over M1 did not change this pattern. In contrast, TMS over PMv strongly perturbed the after-effect.

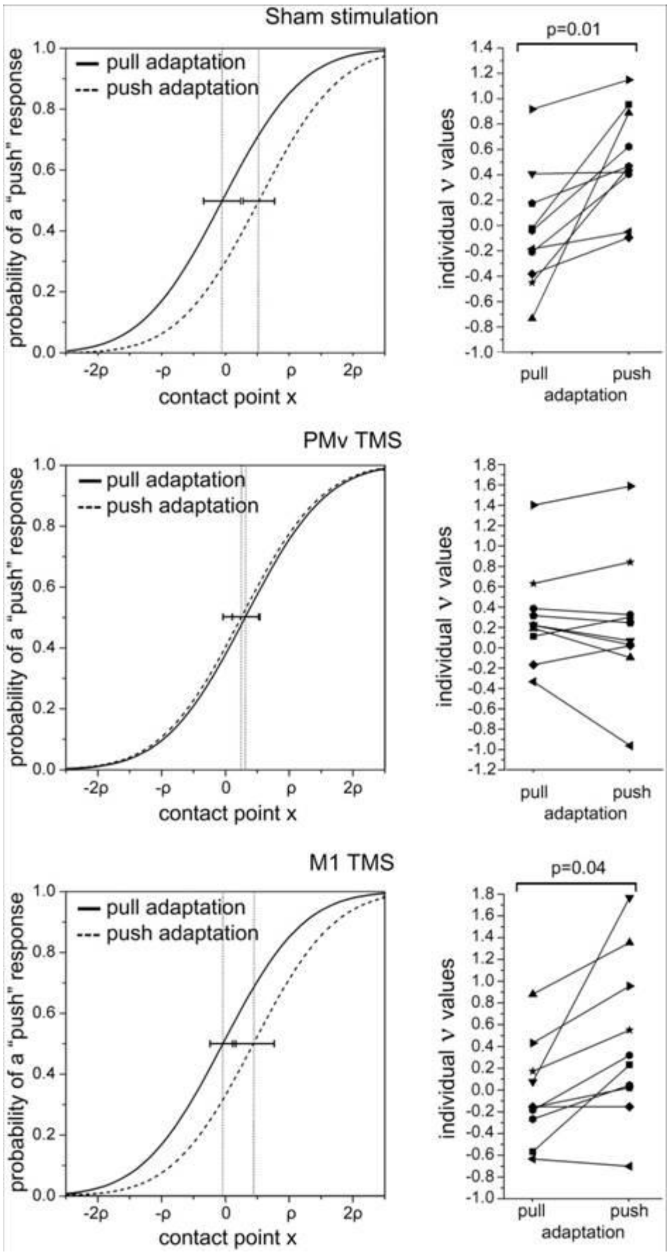


Figure 6
Results of TMS experiment. Left: grand-average of all the individual psychometric functions in the two adaptation conditions. The contact point *x* is expressed in units of participant's psychometric function slope ρ. The dotted vertical lines represent ν average values and horizontal error bars represent 95% confidence intervals. A shift of the psychometric curve towards the left indicates an increased probability of categorizing the stimulus as ‘push’. Vice-versa a shift to the right indicates a bias in favor of ‘pull’ responses. Right: individual values of ν for all 10 participants. The data for the three different TMS conditions are given. Note that ν indicates the stimulus value for which a participant is likely to respond at chance level. A negative value of ν indicates an increased probability of categorizing the stimulus as ‘push’. Vice-versa a positive value indicates a bias towards ‘pull’ responses. The *P*-values refer to significant Bonferroni corrected *P*-values of pairwise *t*-tests.

Mean response times were of 784 ± 76 for Sham stimulation, 797 ± 50 for M1 stimulation and 783 ms ± 46 for PMv stimulation (error values represent within-subject 95% confidence intervals as described in (Loftus & Masson, 1994). A one-way ANOVA did not show any significant difference between these values [$F(2, 18) = 0.43$, $P = 0.65$]. The average number of missed trials (response times > 1500 ms) was of 4.3% in the sham condition, 5.0% in the M1 condition and 4.8% in the PMv condition.

6.4 Discussion

These results demonstrate for the first time in humans that a population of visuo-motor neurons in the left PMv encode congruent observed and executed actions, and that the brain uses their activity in the process of categorizing the motor significance of another's acts. There are multiple aspects of our results that force these conclusions. The first is the clear adaptation effect obtained via a cross-modal motor-to-visual pathway. The finding of behavioral adaptation effects generally identifies the presence in the central nervous system of a population of neurons encoding for the adapted features, as summarized by the aphorism 'If you can adapt it, it's there' (Thompson & Burr, 2009). We show here evidence of complex adaptation crossing over between the two modalities of (i) voluntary object-directed actions and (ii) observed hand–object interactions. This phenomenon therefore identifies a population of neurons firing during both execution and observation of congruent transitive acts, characteristics that satisfy the definition of mirror neurons (Cattaneo & Rizzolatti, 2009).

The anatomical location of this population is indicated by TMS on the basis of the TMS-adaptation paradigm (Silvanto & Pascual-Leone, 2008). According to this paradigm, the effects of TMS are dependent on the activation state of the stimulated neural populations. The phenomenology of the TMS-adaptation paradigm is extremely replicable and consistent: TMS applied over a part of cortex in which the activity of different neuronal populations has been experimentally manipulated by perceptual adaptation produces a behavioral reversal of the perceptual cost of adaptation. In other words, if TMS produces the loss or even the reversal of efficiency differences between responses to adapted stimuli and non-adapted stimuli, it localizes the cortical site where neurons have undergone adaptation. The explanation of the TMS-adaptation paradigm at the neuronal level is still not clear. The most accepted theory is that TMS would be more effective on less active neurons. Leaving aside the possible neural mechanisms, it is undeniable that whenever an interaction between TMS stimulation site and adaptation is found, this localizes the site of adaptation in the stimulated region. Our results therefore localize the polymodal visuo-motor neuronal population in the left PMv. In this experiment we limited our stimulation to the left side on the basis of preceding MRI and TMS data (Kilner et al., 2009; Pobric & Hamilton, 2006). There has been recent debate on whether motor–visual adaptation can actually take place (Rizzolatti & Sinigaglia, 2010). Cortical adaptation is likely to be acting at synaptic level, through synaptic depression (Castellucci & Kandel, 1974; Chung, Li, & Nelson, 2002; Zucker, 1972) even though alternative accounts for the neural mechanisms of adaptation have been proposed (Carandini & Ferster, 1997). Since adaptation is a synaptic phenomenon, it is not surprising that a multimodal neuron receiving afferents from both the visual system and the motor system can undergo memory phenomena related to firing history from both afferences, ultimately leading to cross-modal adaptation which should not affect its firing but rather its synaptic efficiency. In any case, in our work, compared to other controversial studies that investigated cross-modal motor–visual effects (Rizzolatti & Sinigaglia, 2010), we found clear behavioral effects, which make it unequivocal that motor-sensory adaptation has indeed occurred. Also, it should be noted that the waning of the effect in time (Figure 3) is strongly consistent with an adaptation mechanism. It should be stressed here that all participants were blindfolded during the motor adaptation. One possible alternative to the hypothesis of motor-to-visual adaptation is that the adapting stimulus is actually somatosensory feedback. Indeed somatosensation has been demonstrated to play a role in the process of motor simulation that takes place in action observation (Avenanti et al., 2007). However, in our case the two motor adaptations (push and pull) did not differ greatly from the point of view of the kinematics of the repetitive movement, but rather differed radically for their motor meaning. It is therefore unlikely that the effect could be due to proprioceptive afference.

It is known that complex after-effects reflect distributed networks and not single nodes (Mather, Pavan, Campana, & Casco, 2008), and this is probably true also for the present after-effect since adaptation to seen actions has been demonstrated in the temporal cortex and in the PMv (Barraclough, Keith, Xiao, Oram, & Perrett, 2009; Cattaneo et al., 2010). Thus, we do not claim that left PMv is the only site where this perceptual after-effect takes place, but it is certainly one node where cross-modal neurons are present. From this point of view it is remarkable that we did not find any effect when stimulating the primary motor cortex. Although monkey single-cell recordings have clearly found neurons responding to observed motor behavior in M1

(Dushanova & Donoghue, 2010), the data in humans is controversial (Kilner & Frith, 2007). One fMRI study showed observation/execution repetition suppression patterns of Blood Oxygen Level-Dependent (BOLD) signal in the ventral premotor cortex only (Kilner et al., 2009) and not in the primary motor cortex. Another experiment (Gazzola & Keysers, 2009) showed a dissociated response in M1 voxels, consisting in increase in BOLD signal during execution and a decrease during observation. On the contrary many TMS studies have shown modulation of M1 activity to action observation (Alaerts, Swinnen, & Wenderoth, 2009a; Alaerts, Swinnen et al., 2009b; Avenanti et al., 2007; Aziz-Zadeh et al., 2002; Borroni & Baldissera, 2008; Cattaneo et al., 2009; Fadiga et al., 1995; Gangitano et al., 2001; Strafella & Paus, 2000; Urgesi, Moro et al., 2006). However, TMS over M1 is at least in part an index of pre-synaptic signals (Ziemann & Rothwell, 2000) and can reveal information on cortico-cortical input to M1. It is plausible therefore that the motor resonance previously observed with TMS over M1 is a product of mirror activity, but that such resonance is likely to be mediated by cortico-cortical afferents from the ventral premotor cortex (Cattaneo et al., 2005). The present data argue against an active involvement of M1 in action observation. This datum finds support in a recent paper where TMS-induced virtual lesions of M1 did not interfere with motor resonance to observed behavior (Avenanti et al., 2007). The authors concluded that the functional contribution of M1 to the corticospinal resonance to observed actions is not crucial and such resonance probably reflects the functional contribution of other nodes of the action mirror system. In addition to these considerations, it should be stressed again that our experimental adaptation protocol is planned to induce action-related changes rather than movement-related changes. The kinematics of the two adapting trainings of ‘push’ and ‘pull’ are very similar, with rhythmic oscillations of the hand on the wrist joint. Only the action meaning is opposite between the two trainings. It is therefore not surprising that we find stronger effects on PMv neurons rather than M1, considering that most PMv neurons represent actions while only some neurons in M1 represent movements independently from muscles (Kakei, Hoffman, & Strick, 1999; Umilta et al., 2008).

Evidence of mirror neuron activity has been demonstrated mostly uni-directionally, from vision to action. However it has been argued that in the framework of the mirror theory, motor to visual information flow is essential for action perception through visual predictions (Keysers & Perrett, 2004; Miall, 2003). We show here that neurons with mirror properties are capable of adaptation to repeated stimuli in the opposite, motor–visual direction. These findings should not be confused with the numerous sets of data showing a positive correlation between the learning of a new motor skill and its recognition in others (as shown for example in Casile and Giese, (2006); for a review see Schutz-Bosbach & Prinz (2007). We tested here simple, fully-acquired behaviors like pushing or pulling objects, and we observed the dynamic, moment-by-moment changes in the relations between movements in the observer and in the observed, rather than the results of complex motor skill learning.

The function of adaptation in sensory modalities is generally thought to be that of improving perception by changing gain to avoid saturation in ceiling or floor effects (Thompson & Burr, 2009). Accordingly, we show here that the premotor mirror system has indeed perceptual functions and it is embedded in a dynamic and constantly fluctuating framework depending not only on what we see others do, but also, and very strongly, on what we are doing. The fact that action can increase perceptual sensitivity to those events that do not share features with what a person is concurrently doing has been suggested previously (Schutz-Bosbach & Prinz, 2007). The present data add a straightforward hypothesis on the neural mechanisms by which this phenomenon can occur.

This argument leads us to the second most relevant finding in our work: we show that motor adaptation can induce predictable short-lasting changes in how we perceive other's actions and this effect is probably achieved by means of controlled manipulation of the excitability of premotor mirror neurons. This phenomenon can be assimilated to more common visual after-effects. The mirror mechanism is therefore a potential tool for shaping the perception of action. The size of the after-effect is considerable. As shown in Figure 5 and in the upper panel of Figure 6, when subjects respond at chance level after adaptation in one direction, they have a bias in response probability towards 'push' or 'pull' between 14 and 22%. The effects of action on perception have been investigated behaviorally in previous works that showed that perception of visual stimuli was reduced if presented during the planning of an action compatible with the stimulus (Musseler & Hommel, 1997). This effect was originally attributed to brief refractoriness of cognitive codes, which are shared by representations of visual stimuli and motor responses. However following research has showed that the effect is more likely to be attributed to 'perceptual blindness' to stimulus events that share the direction feature with the response (Musseler et al., 2001). In another couple of interesting papers it was shown that concurrent hand movements positively influenced the participants' capacity to detect incongruent hand postures (Miall et al., 2006) or incongruent trajectories (Zwickel et al., 2007). The results of the present experiment are in agreement with the aforementioned findings, in the sense that they all indicate the existence of common modules in the brain shared by perception and action control (Musseler et al., 2001). The present work, however, addresses specifically whether this shared substrate is able to attribute meaning to the observed actions and, most importantly localizes one node of the observation/execution module in the ventral portion of the frontal lobe.

In conclusion, data confirm the long-standing hypothesis that mirror mechanisms exist in the human motor system and that they subserve a process of classifying the meaning of observed actions. Furthermore, the data imply that the system is highly adaptable to increase its sensitivity in quickly evolving social interactions.

7. STUDY 2: THE FRAMES OF REFERENCE OF THE MOTOR-VISUAL AFTER-EFFECT.

7.1 Introduction

The Motor-to-Visual aftereffect (MVA) previously described needs to be better characterized in terms of what features of the adapting task are effective in producing it and in what spatial frame of reference the adaptation occurs. In the present work we try to characterize the MVA according to 3 distinct dimensions. First we assessed the role of the semantic aspects of the task and instructions. Indeed one alternative explanation of the MVA is that the adaptation is not produced by motor training but rather results from lexical-semantic satiation arising from rehearsal of the instruction during the motor training. In this case the phenomena giving rise to the MVA would all be restricted to the conceptual system, involving no cross-talk with the motor system. In order to exclude this possibility we ran the same experiment as in the previous one while reversing the relationship between the instruction and the motor act. In order to do this a puppet was placed on the other side of the bowl, facing the participant: the instructions remained the same as in the previous, but now referred to the puppet, and since the puppet was placed on the other side of the bowl with respect to the participant, the instruction “far” corresponded to the same motor act as the instruction “near” in the previous experiment and vice versa. If adaptation is caused by lexical rehearsal, then the “far from the puppet” training should lead to an MVA centered on the instruction’s object (the agent in the previous experiment but the puppet in the present experiment).

Otherwise if the adaptation is a truly motor one, then the pattern should depend on the direction of movement and therefore, given the same instruction semantics, a direction opposite to that in the previous experiment. The results clearly showed that the instruction semantics are irrelevant to the production of the MVA.

Second we tried to define the spatial frame of reference in which the adaptation phenomenon takes place. The main distinction we tried to capture was that between "intrinsic" and "extrinsic" motor space. This distinction comes from studies in primate neurophysiology of visuomotor neurons in the parietal, premotor and motor cortex. (Kakei et al., 1999; Kakei, Hoffman, & Strick, 2001; Kurata, 2007; Kurata & Hoshi, 2002). Some of these neurons can code the spatial end-position of movement in body-centered coordinates. For example a neuron that fires to produce a clockwise rotation of the wrist independently of the initial wrist position will produce movements with different endpoints. Such visuomotor neurons are located in an "intrinsic" spatial frame of reference. On the contrary, some neurons fire to produce any type of movement that has the same target, irrespective of the initial position of the body part. These neurons can produce a range of different wrist movements as long as the spatial position of the target is the same. The frame of reference of such neurons is said to be "extrinsic". Are the movements generating the MVA mapped in a motor-invariant frame of reference or in an action-invariant (in this case spatial-invariant) frame of reference? To answer this question we performed the second experiment of the present series, in which we manipulated the paradigm so that different movements were used to achieve the same goal (spatial endpoint of the movement). In particular participants were required either A) to use the same movements as in the target pictures (pushing with the dorsum of the hand and pulling with the palmar surface of the hand) to achieve the same goals or B) to use the movements opposite to those in the target pictures in order to achieve the same goals (pushing with the palm of the hand and pulling with the dorsum). Our findings showed that both spatial goal AND movement have an effect in producing the MVA, thus indicating that two distinct neural systems (one operating in intrinsic and the other in an extrinsic motor space) are engaged by the training.

In the last experiment we explored the role of spatial compatibility between the observed action and the participant’s hand posture. We therefore replicated the experiment with the standard instructions but participants were required to orient their hand rightwards or leftwards while acting on the objects and these two conditions were factorially associated with the leftwards or rightwards orientation of the hand in the target pictures. Also in this case we replicated the basic finding of the object displacement direction producing the MVA, with no interaction effects with the spatial congruence or incongruence of the targets.

7.2 Materials and Methods

7.2.1 General Procedure

Each of the present experiments was organized in series of blocks, and each block comprised two distinct phases. In the first phase the participants performed the adaptation motor task for 60 seconds. In the second phase the participants performed the categorization task on a series of static visual stimuli presented serially. Each target picture was presented for 500 ms. In the previous description of the MVA we found that, consistent with an adaptation effect, the MVA appeared to be waning in the first few trials of the categorization phase. Therefore in every experiment we considered for analysis only the first 15 trials of the categorization phase. An illustration of the general procedures is presented in Figure 7.

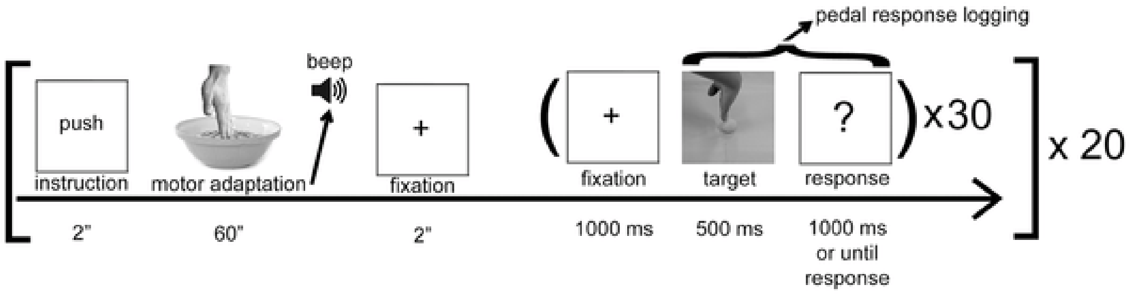


Figure 7
Time-line of the experimental protocol

7.2.2 Participants

We tested 16 participants (7 females, age range: 19–37) in the “Semantic reference” experiment, 20 participants (12 females, age range 22–34) in the “inverted effector” experiment, and 20 participants (14 females, age range 19–33) in the “spatial” experiment. According to the standard handedness inventory (Oldfield 1971) all subjects were right handed except one in the spatial experiment group. The present study was approved by the local Ethical Committee for human studies and was conducted in compliance with the Helsinki Declaration of 1975, as revised in 2008 ("World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects," 2008). All participants gave written informed consent to the experiment.

7.2.3 Visual Stimuli

Visual stimuli presented in the categorization phase of the blocks were the same in all 3 experiments. They consisted of 30 pictures of right hands displacing an object (a yellow table-tennis ball). The full set of images is presented in Figure 2. The orientation of the hand in the pictures was 90°, 180°, and −90° with respect to the participant’s viewpoint. The contact point with the object (the table-tennis ball) was at 5 different points including one on the upper pole of the sphere. The remaining 4 were symmetrically placed along a meridian at 20° and 60° from the vertical axis. The hands were all positioned at 90° with respect to the horizontal plane on which the object was positioned. In half of the figures hands touching objects were female hands and in the other half they were male hands balanced across orientation and position. The background of the figures was homogeneous. Stimuli were presented with the E-prime software (Psychology Software Tools Inc.) on a 60 Hz computer screen, with a visual angle of 14° vertically and 19° horizontally.

7.2.4 Motor Adaptation Procedure

Participants sat comfortably on a chair in front of a computer screen. The objects upon which the participants were asked to act consisted of around 50 dried chickpeas inside a spherical bowl. We used dried vegetables in order to have a series of small, eventually replaceable, objects of uniform size and light weight. Such objects have previously been used in similar use-dependent plasticity induction protocols (Glenberg et al., 2010). The shape of the bowl allowed the chickpeas to fall back naturally in the center of the bowl after the displacement. In this way the direction of chickpeas displacement was univocal for each action. The onset of each block was determined by the participant by pressing the space bar on the keyboard placed in front of them. Every block in each experiment started with an instruction displayed on the computer screen for 2 seconds, describing the action to be performed on the chickpeas during the subsequent adaptation phase. Participants used their right hand to displace the chickpeas according to the different instructions. The bowl was placed in different positions with respect to the participant in each of the experiments. The adaptation phase required continuous acting on the objects for 60 seconds until a beep-sound was emitted by loudspeakers connected to the computer. It should be stressed that in order to avoid any access to visual information concerning their hands' movements, an opaque screen prevented the participant from seeing his/her own right hand during the whole duration of the motor training. In the *"semantic reference"* experiment the bowl was placed in front of the participants. A puppet was placed on the opposite side of the bowl facing the participant (see Figure 8). The experiment consisted in 20 blocks each of which began with an instruction literally translated as "move near to the puppet" ("avvicina al pupazzo" in Italian) or "move away from the puppet" ("allontana dal pupazzo" in Italian). In this way, the externally referenced "move near" instruction corresponded to a self referenced "push" instruction and participants had to continuously move the chickpeas toward the puppet with the back of their hand. Vice-versa a "move away" instruction prompted the act of pushing the chickpeas away from the puppet with the palm of their hand, corresponding to a self-referenced "pull" instruction. The two different instructions were presented in random order across the block series. In the *"inverted effector"* experiment the bowl was placed on the participant's right side at a level lower than the table plane (Figure 9). The experiment consisted of 20 blocks. At the start of each block the instruction indicated both the goal of the action and the hand part with which the action had to be performed, resulting in 4 different instructions: "move away from yourself with the back of the hand", "move away from yourself with the palm of the hand", "move towards yourself with the back of the hand", "move towards yourself with the palm of the hand". Instructions were presented in random order across the blocks. In the *"spatial compatibility"* experiment we tried to make participants perform the push and pull actions using effector positions that were either compatible or incompatible with the target pictures. We therefore presented the target hand pictures with only the right and the left orientations (first and third columns of Figure 2), because the frontal orientation was obviously not reproducible by the participant. Two bowls were simultaneously present in the experimental scene on the computer table, one in front of the subject (from now on defined as Bowl A) and the other one (defined as Bowl B) on his/her right side (Figure 10). In this way the hand acting in the lateral bowl had the same spatial orientation as in the rightward target pictures (in the third column of Figure 2). The participant's hand acting on the frontal bowl was oriented 30–45° leftwards with respect to the midline. A full 90° rotation was attempted at first but this proved to be very fatiguing for participants. The participant's hand when acting on this bowl was oriented as in the leftward directed target pictures (first column of Figure 2). The experiment consisted in 20 blocks, half of which were performed with the anterior bowl and the other half with the lateral one. At the onset of each block the instruction that was presented indicated both the direction of object displacement and the bowl to be used. Therefore the 4 different instructions were “move away from yourself in bowl A”; “move towards yourself in bowl A”; “move away from yourself in bowl B” or “move towards yourself in bowl B”. Instructions were presented randomly.

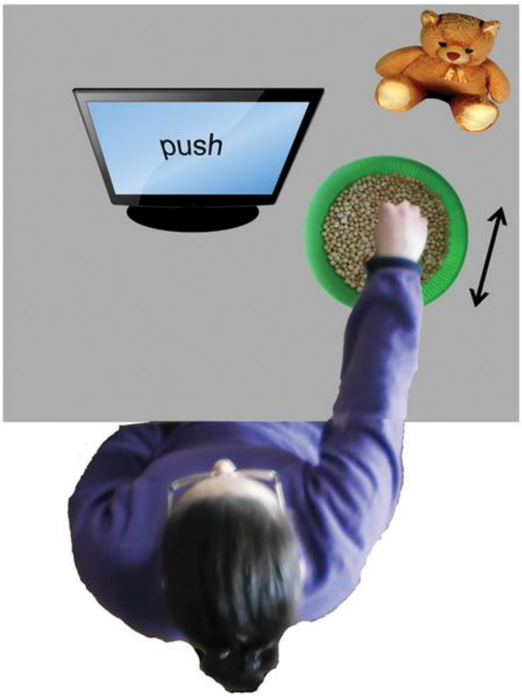


Figure 8
Schematized setup of the “semantic reference” experiment.
The arrow indicates the direction of chickpeas displacement

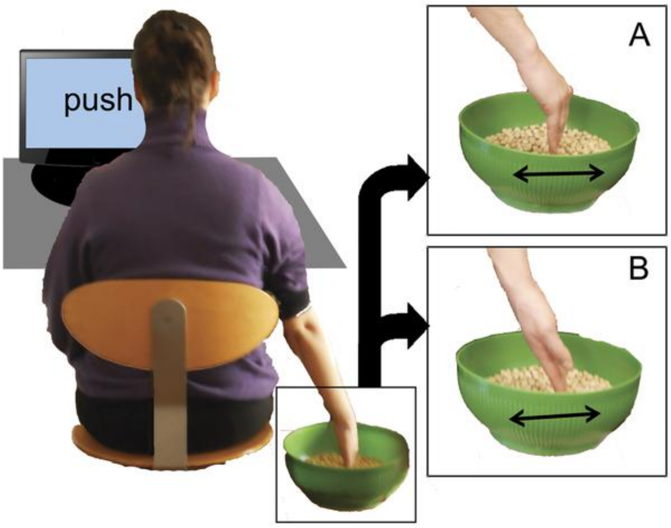


Figure 9
Schematized setup of the “inverted effector” experiment

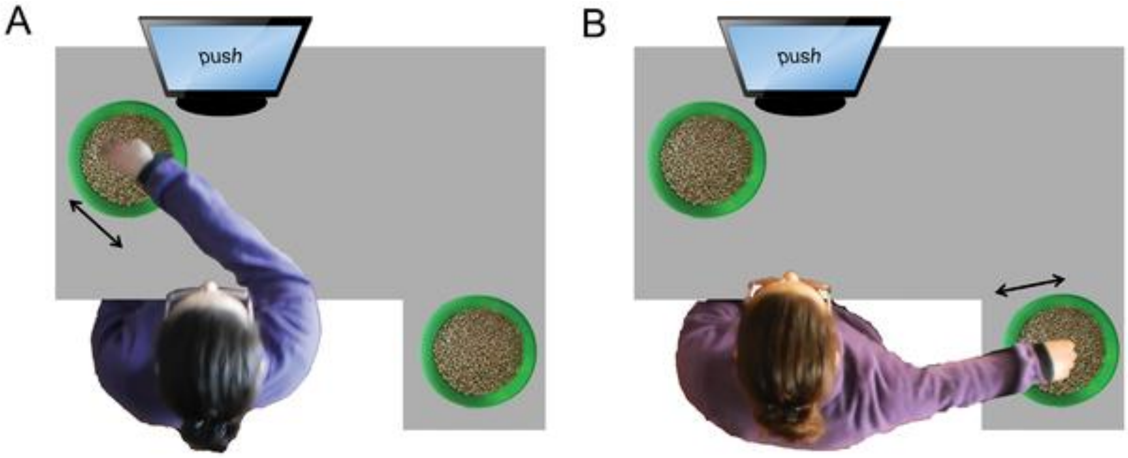


Figure 10
Schematized setup of the “spatial compatibility” experiment.

7.2.5 Categorization Phase

The categorization phase was the same for all 3 experiments. It started 2 seconds after the “beep” sound indicating the end of the motor adaptation phase. Participants were required to quickly take their hand away from the bowl and to put it palm-down on the table. In the first 2 experiments, the presentation comprised 30 target pictures in random order (Figure 7). In the third experiment (“spatial compatibility”) only the hands in the lateral viewpoints were presented (columns 1 and 3 of Figure 2). Participants were required to categorize the action of the depicted agent as “moving the object away from oneself" or "moving the object closer to oneself". Categorization was required to be referred to the depicted agent and not to the observer. For example, the picture in the middle column of Figure 2, at contact point +2 should be correctly categorized as "moving away". Responses were given as fast and accurately as possible by pressing two pedals with the right and left feet. The response mapping on the feet pedals was balanced across participants. Each of the 30 trials began with a red cross in the center of the screen, lasting 1000 ms, preceding the presentation of the visual stimulus (lasting 500 ms). A white screen with a central "?" sign appeared after the target and persisted until the participants' response or until a maximum duration of 1000 ms was reached. This series looped until each of the 30 target pictures were presented (Figure 7). Responses were logged from the onset of the target picture. Responses given after 1500 ms from target onset were not logged.

7.2.6 Data Analysis

The responses to the stimulus set presented in Figure 2 are distributed following a sigmoid psychometric function. This distribution was also verified for the present data by a fitting procedure on the total data set. To simplify the analysis we decided to further consider only the responses to the ambiguous contact position (represented in the middle column of Figure 2). We built a score indicating the average responses to the ambiguous stimuli by attributing a −1 value to single "pull" responses and a +1 value to single "push" responses. Such single-trial values were then averaged within participants and within conditions to obtain a single score ranging between −1 and +1 indicating alternatively, if negative, a prevalence of pull responses and, if positive, a prevalence of “push” responses. This response score was then used in all further analyses as a dependent variable.

In the “semantic reference” experiment the data were analyzed by means of a t-test for paired data comparing individual response scores between the 2 motor adaptation instructions (either “push” or “pull”). Remember that in this experiment the instruction is the opposite as in the other two experiments, that is, the action associated with the “push” instruction in the “semantic reference” experiment corresponds to the one associated with the “pull” instruction in the other two experiments, as well as in the previous description of the MVA. In the “inverted effector” experiment the response scores were averaged within each of the 2 action directions and within each of the two hand postures used in the motor adaptation, in a 2×2 design, resulting in 4 cells per subject. Response scores were then analyzed by means of a 2-way ANOVA with 2 within-subjects factors: ACTION (2 levels: “centrifugal” and “centripetal”) and EFFECTOR (2 levels: “back” and “palm”). In the “spatial compatibility” experiment the response scores were averaged in a 2×2×2 design, within each of the two action directions, within each of the two bowl positions and within each of the 2 orientations of the target picture (see Figure 2), for a total of 12 cells per subject. The data were then analyzed with a 3-way ANOVA with 3 within-subjects factors: ACTION (2 levels: “centrifugal” and “centripetal”), HAND ORIENTATION (2 levels: front or sideways) and TARGET ORIENTATION (2 levels: “left” and “right”).

7.3 Results

The overall rate of responses that were not logged because they were made after the 1500 ms limit was of 3.3% with no significant differences between experiments. None of the participants was excluded from the analysis.

7.3.1 “Semantic Reference” Experiment

The individual and the group results are shown in Figure 11. The t-test showed a significantly different distribution for the paired data ($t(15) = -2.61$, $p = 0.02$). The training with displacement of the object towards the puppet, i.e. away from the agent produced significantly more “moving closer” responses (mean score −0.13; SD: 0.18) than the training with displacement of the object away from the puppet, i.e. towards the agent (mean score 0.08; SD: 0.34).

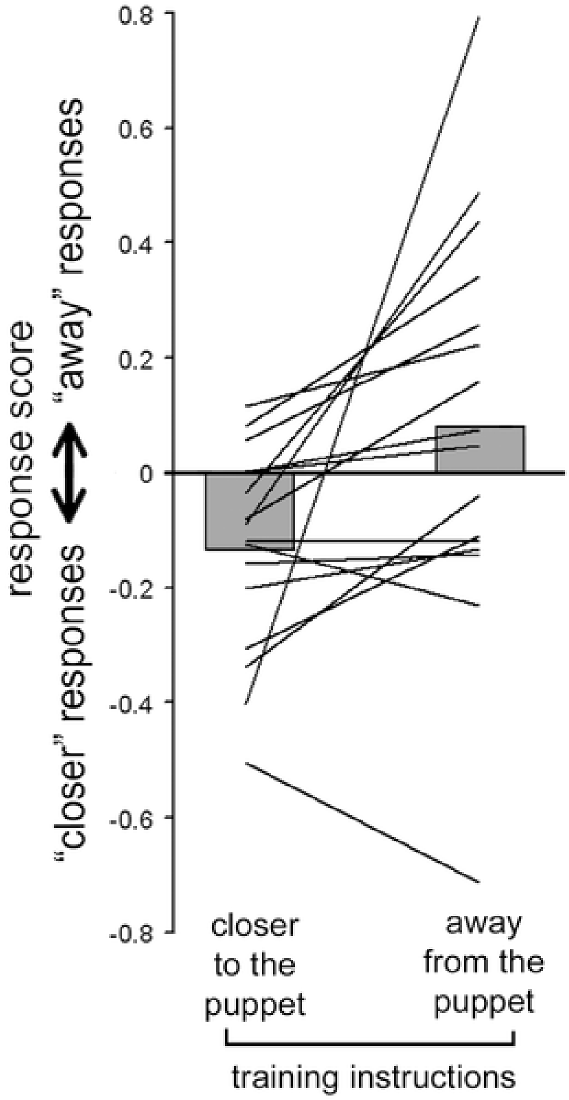


Figure 11
Results of the “semantic reference” experiment

7.3.2 “Inverted Effector” Experiment

The individual and the group results are shown in Figure 12. The ANOVA showed a main effect of both factors: ACTION (F(1, 19) = 6.17, p = 0.02) and EFFECTOR (F(1, 19) = 13.8, p = 0.001). The main effect of ACTION was, again, consistent with our expectations, due to the fact that a repeated displacement of the objects away from the agent produced significantly more “moving closer” responses (mean score: −0.06; SD: 0.42) than the repeated displacement of the objects towards the agent (mean score: 0.15; SD: 0.36). Interestingly, the main effect of EFFECTOR showed that the repeated use of the dorsal surface of the hand produced significantly more “moving closer” responses (i.e. produced a bias in favor of the target picture with the palmar surface of the hand being used; mean score: −0.02; SD: 0.43). By contrast, repeated use of the palmar surface of the hand produced significantly more “moving away” responses (i.e. produced a bias in favor of the target picture with the dorsal surface of the hand being used; mean score: 0.12; SD: 0.37). No interaction between the two factors was present (F(1, 19) = 0.04, p = 0.84).

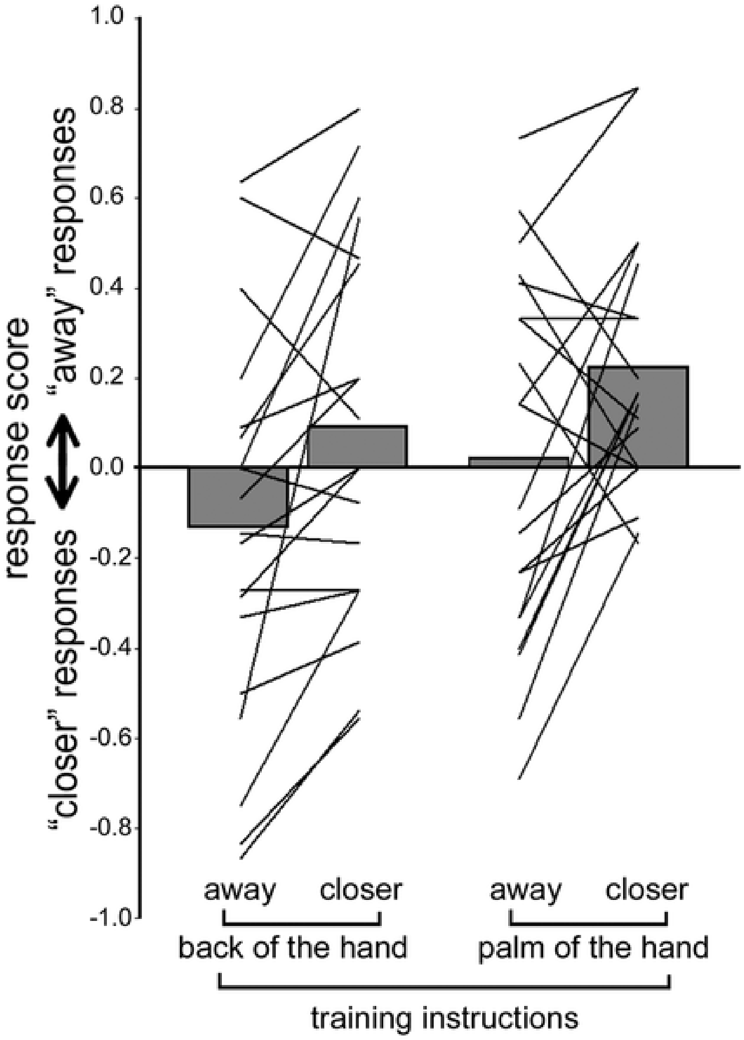


Figure 12
Results of the "inverted effector" experiment

7.3.3 “Spatial Compatibility” Experiment

The individual and the group results are shown in Figure 13. The ANOVA showed a main effect of all 3 factors: ACTION (F(1, 17) = 6.96, p = 0.02), HAND ORIENTATION (F(1, 17) = 5.56, p = 0.03) and TARGET ORIENTATION (F(1, 17) = 9.03, p = 0.008). No interactions between any of the factors were found (all p-values >0.39). The main effect of ACTION consisted, as expected, in a greater rate of “moving closer” responses when the subjects had been pushing the objects away (mean score: −0.16; SD: 0.41) and vice-versa in a greater rate of “moving away” responses when the subjects had been moving closer the objects (mean score: 0.06; SD 0.32). The main effect of HAND ORIENTATION was due to participants responding more frequently “moving closer” to target pictures when they had acted with the hand oriented leftwards, in bowl A (mean score: −0.15; SD: 0.37) and responding more frequently “moving away” when they had acted with the hand oriented rightwards, in bowl B (mean score: 0.05; SD: 0.38). Finally, the main effect of TARGET ORIENTATION consisted in the fact that subjects responded more frequently “moving closer” (mean score: −0.28; SD: 0.42) to pictures showing a hand oriented leftwards (left column of Figure 2) and responded more frequently “moving away” (mean score: 0.18; SD: 0.49) to pictures showing hands directed rightwards (right column of Figure 2).

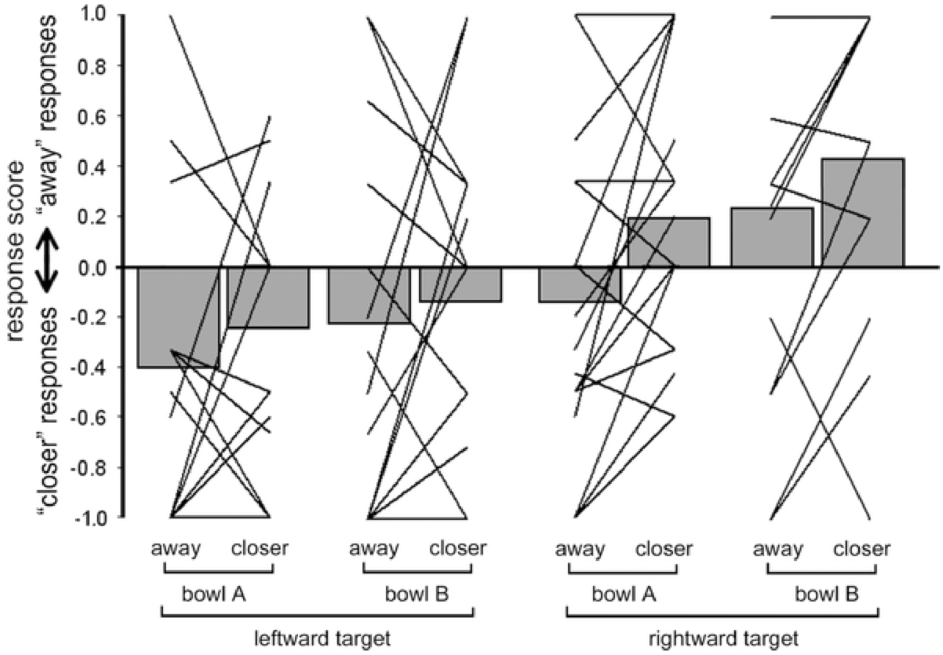


Figure 13
Results of the "spatial compatibility" experiment

7.4 Discussion

The present data indicate that the MVA is a robust and replicable phenomenon. In the present experiments the target visual stimuli were kept constant and identical to our previous description of the MVA. In every experiment we instead manipulated the different components of the adapting actions, so that we could identify the single invariant features between the performed actions and observed target pictures that were responsible for the MVA.

In the first experiment we manipulated the semantics of the verbal instructions for the training actions. This experiment was done to control whether semantic satiation, an adaptation phenomenon in which lexical repetition within a semantic category produces a reduced access to that same category (Balota & Black, 1997; Black, 2001; Kounios, Kotz, & Holcomb, 2000; Smith, 1984) could account for the MVA. Hypothetically, if participants performed a sub-vocal rehearsal of the instructions during the motor adaptation task, this phenomenon could account for the observed MVA. Our results however render this hypothesis unlikely because, although the verbal instructions were inverted compared to the original experiment, the factor that produced the MVA was the direction of the movement referred to the agent rather than to the instructions' semantics. One limitation of the present experiment is that the puppet was not present in the target pictures. In this way it is still possible that an egocentric frame of reference was used also to translate the semantics of the instruction.

Another possible problem is that no internal control condition is present where the instruction "push" actually corresponds to a push movement and this does not allow a direct comparison between congruence-incongruent semantics and action features.

However, an indirect comparison can be made with the results of the second and third experiments, where the instruction "push" actually corresponded to a push movement.

In the second experiment we investigated whether it was the direction of the motor act or the actual movements of the wrist and finger joints that produced the MVA. We therefore applied a well-established paradigm in which the goal of the motor act (moving closer to or away from the objects) was dissociated from the movements required to accomplish it. This paradigm has been successfully applied in non-human (Avenanti et al., 2007; Cantarero et al., 2011) and human (Cattaneo et al., 2009) primates to study the representations of goals and movements in the motor system. Our results show an effect of both movements and action goals, with no interaction between the two. Therefore the MVA is produced by the adaptation of two distinct components of the motor system, one operating in an intrinsic (movement-related) spatial frame of reference and the other operating in an extrinsic (goal-related) frame of reference. We showed that performing centrifugal acts produced a bias in categorizing the target pictures in favor of a centripetal displacement of the object and vice-versa, therefore, the action goals of moving the objects away or towards the agent, irrespective of the movements used to achieve them, are a causal component of the MVA. However, superimposed on this, we also observed that whenever the dorsum of the hand was used to displace the objects, irrespective of the direction of displacement, a bias was produced in the categorization of target pictures towards the pictures with the palm of the hand making contact with the object and vice-versa. In our opinion the most plausible interpretation of this datum is that the actual joint displacement is causally involved in producing the MVA. This finding is supported by results in non-human primates, in the motor systems of which, the double coding of goals and movements is widespread (Schutz-Bosbach & Prinz, 2007). Another interpretation that cannot be ruled out by the present data is that it is the somatosensory rather than the visual information that determines this component of the MVA. According to this hypothesis the adaptation would occur by repeated contact with the dorsal or palmar surface of the fingers during the actual movements and this in turn would produce a bias in the internal representation of the somatosensory consequences of the observed movement.

In the third experiment we explored the role of visual spatial compatibility between the effector performing the adaptation task and the effector shown in the target pictures. The finding of a main effect of action direction replicates once more the finding of an action goal-driven MVA. The most interesting result of this experiment however is a negative finding, i.e. the absence of interactions between the orientation of the acting hand and the orientation of the hand in the target pictures. This datum indicates that the MVA is not generated by matching of purely spatial features between the observers' hand and the observed hand. On the contrary, it is based on the matching of actions or movements, irrespective of the viewpoint. It is also worth reminding the reader here that in all experiments the vision of the subject's own hand was prevented by means of an opaque shield. Finally, in the third experiment, the finding of a main effect of target orientation is of little interest, as it is probably due to an imperfect matching between the visual features of the two oppositely oriented stimuli. Also, the finding of an effect for the position of the bowl, resulting in acts directed to the right or to the left is of little speculative interest since it was not the aim of the experiment.

In conclusion, the present series of experiments shows that the MVA is a phenomenon driven purely by motor behavior and not by non-motor lexical/semantic features or by visual compatibility of the agents' bodies with the observed actions.

Importantly we reinforce the notion that the human motor system contains two distinct coding of self-produced and observed motor behavior, one in an intrinsic frame of reference (Alaerts, Heremans et al., 2009; Alaerts, Swinnen et al., 2009b) and the other in an extrinsic frame of reference (Cattaneo et al., 2009)

8. STUDY 3: TRAINING THE MIRROR

8.1 Introduction

The imitative ability seems to have a very important role in human evolution and social development. What type of mechanism allows to perform an imitative action? Is this capability hard-wired implemented in human mind/brain or is it acquired through experience?

This problem has been raised and described in an influent review by Cecilia Heyes (Heyes, 2001). In this review the author defines "imitation" as the "copying by an observer, a feature of the body movement of a model (Heyes 2001)". "Copying" implies "…a specific causal relationship between the observation of a feature of a model's body movement and the execution by the observer of a body movement with the same feature (Heyes 2001)".

The author argues against a predominant role of putative innate mechanism of imitation, as instead suggested by other influential theories. A few arguments have been typically brought in favor of an innate imitative capacity.

1) Other than humans, only primates and birds are almost the only living beings able to imitate. This argument implies that is not a general visuo-motor matching process that takes place in imitation, but a more specie-specific one.
2) Classical evidence in human neonates suggest that even without any visuo-motor experience, they are able to imitate facial expressions like protruding the tongue or mouth opening (Meltzoff & Moore, 1977).

These arguments however seemed to be flawed by some observations.

According to the author, for what concerns the animal imitation, inferences on the innateness of the imitation capacity are based on studies focused primarily on primates and birds. But it is not known whether other less studied species, are able to imitate. In particular, the fact that some species do not show an imitative behavior in their natural environment, does not mean that they are not “able to” imitate. Indeed some evidences on primates suggest that primates that have been trained or have spent a lot of time with humans are able to imitate (Custance, 1999).

The second and more challenging demonstrations have been flawed by meta-analyses showing that the only constant imitative action performed by neonates is the tongue protrusion (Anisfeld 1996). According to some authors this behavior is more consistent with a releasing mechanism in response either to an observed action or to an event. If this behavior is caused only by a releasing mechanism, then, according to the definition of imitation, it has not to be considered a true imitation yet.

A more viable theory proposed by the author in explaining imitative capabilities is called “Associative Sequence Learning” (ASL). According to the author this theory explains better than others the imitation of both transparent and opaque movements. Transparent movements are defined as those movements whose sensory output is similar to then one executed. Opaque movements are those whose sensory output is different from the movement performed (facial expressions). The ASL theory asserts that imitation is heavily influenced by the experience, and it is constituted by a set of bidirectional links between sensory and motor representations, developed through pavlovian-like associative learning. Mechanisms of imitative behavior are constituted by general mechanisms of visuo-motor associations, and not by dedicated modules.

According to the author two different type of links bind visual and motor experience in imitation: the “direct vertical links” bind motor and sensory representations without any intermediate representation, that is, in a very direct way. The majority of these links are acquired when one is looking at an action he is actually performing: for example grasping a bottle and pouring the liquid in a glass needs the actor to look at his own performance. So the motor representation of an action is linked to the visual representation of that act whenever we are looking at a movement we are actually performing.

Bidirectional links that explain imitation of transparent actions are exploited as well in order to explain opaque actions; indeed, in the case of opaque movements, those links can be developed thanks to the experience with mirrors (that allow to transform an opaque facial expression into a transparent one), through movements performed by others in response to a sensorial stimulus and through the experience of being imitated (Heyes 2001).

Another type of vertical links, the “indirect vertical links”, need an extra intermediate sensorial representation, to be effective. For example when we speak, the motor performance can be link to the visual consequences of the mouth movement by the intermediate auditory representation of that movement.

Finally the ASL hypothesis assumes that movements are constituted by “primitives” that are linked together by “horizontal links” to form an action sequence. Each primitive is linked to its own sensory outcome, so that even the sensory outcomes are linked through other “parallel” horizontal links.

Other then the imitation that is a voluntary visuo-motor matching, ASL can answer questions on the evolution/development of mirror neurons. Usually and implicitly the properties of the mirror neurons have been taken as the result of an evolutionary adaptation, because they are supposed to be evolved *for* action understanding. Within this theoretical framework visuo-motor experience does play a role in modulating the motor resonance when an observer sees an action (Calvo-Merino et al., 2005), however they are supposed not to be entirely a product of a correlated visuo-motor experience. ASL instead asserts that mirror neurons are not evolved for particular evolutionary purposes: the motor neurons (that, according to ASL, will become mirror neurons) are a product of an evolutionary adaptation, and the same applies to the associative learning mechanisms; however "motor neurons and associative learning are not evolved for the purpose of producing mirror neurons (Heyes, 2009)". The mechanisms through which mirror neurons are developed are the same described for imitation; the correlated experience of watching its own action links neurons responsible for performing an action to the ones that represent that action in the visual system. So, assuming that a bidirectional link has been created between these putative visual and motor neurons, then each time an action is observed the visual neurons that code the visual aspects of the actions trigger the activity of the motor ones that code the motor representation of that very same action.

Other inferences can be drawn by assuming that correlated visuo-motor experience forge mirror neurons, with particular attention to the corollary that mirror neurons *are not for* any evolutionary purposes, neither for imitation nor for action understanding, but they can help different cognitive abilities ranging from empathy, to imitation or action understanding.

8.1.1 Catmur et al. 2007

In an interesting experiment performed by the group of Cecilia Heyes (Catmur et al., 2007), participants observed a hand on the screen performing index or little finger abductions while they were receiving single pulse TMS stimulations on their left M1. Authors recorded MEP's amplitude both from the right 1DI and from right ADM muscles. Participants' task was to press the space-bar whenever they saw a flash colored-dot appearing on one out of six possible positions on the screen. Among this group of participants the authors selected the ones who showed the higher motor resonance effect, that is, the ones who showed higher 1DI MEPs compared to ADM MEPs when watching at index finger abductions and vice-versa.

The TMS stimulations times were delivered at 0, 320 and 640 ms from the onset of the movement in the visual stimuli. Importantly, MEPs found at these time points were collapsed, so no time-course was provided.

After this first session (pre-training) participants were assigned to two different conditions, the "compatible" and the "incompatible". Participants assigned to the incompatible condition were presented with the same stimuli used in the pre-training session and their task was to move the opposite finger compared to the one they saw, i.e. if they observed an index finger abduction they had to perform a little finger abduction and vice-versa. The compatible group instead had to react to the stimuli in an imitative fashion. 24 hours later, participants from both groups were tested on the same paradigm used in the pre-training session.

According to the ASL theory if mirror neurons are the product of the life-time correlated experience of seeing its own hands while performing actions, then the correlation experience in abducting its own index finger while seeing a little finger abduction should modify mirror neurons matching properties. The groups assigned to the incompatible condition should have shown counter-mirror properties in the test session, that is, 1DI MEPs should have been higher in amplitude compared to the ADM ones when observing little finger movements and vice-versa.

After the training session the “Incompatible” group showed the counter-mirror effect hypothesized by the ASL theory, differently from the “Compatible” group that showed the same pattern detected on the pre-training session. According to the authors this result showed that just a brief incompatible visuo-motor training can enhance counter-mirror responses.

8.1.2 Catmur et al. 2011

In a second work by Catmur and colleagues (Catmur et al., 2011) two major advances have been carried out compared to the previous one: while the paradigm was the same as the one used in Catmur et al. (2007), a time-course of the cortical excitability on M1 has been tested (200 ms, 250 ms, and 300 ms from the movement onset in the visual stimuli) before the incompatible visuo-motor training. The second major advance is that they used a double coil paradigm, placing the conditioning coil on the right PMv and right dorsal premotor cortex (PMv) and the test coil on the left M1, to test two possible sites responsible for the motor resonance effect. Muscle recording were performed on 1DI and ADM.

Authors found a significant motor resonance effect at all the time points of the time-course when testing cortical excitability on M1 in the pre-training session.

However they also found a facilitatory effect of the conditioning TMS stimulations on PMv and PMd only at 300 ms. Afterwards participants underwent the training session in which they had to perform a counter-imitative reaction time (as in Catmur et al., 2007); 24 hours later the authors tested the effect of the counter-imitative training on the cortical excitability of M1 conditioned on the PMv and PMd pulse, but only at 300 ms. They found that at that time point, the effect of the stimulation on both the two premotor sites facilitated the M1 excitability in the direction of the counter-imitative training.

These experiments show a possible mechanism to reconfigure or inhibit mirror neurons. This method can be exploited in order to test action understanding in front of a reconfigured mirror neurons. However a possible concern arises when considering the time course of the mirror neuron testing after the trainings: an average of 0, 320 and 640 ms (Catmur et al. 2007) or 300 ms (Catmur et al. 2011) is a very late time point. What is the situation before 300 ms?

The chronometry of the mirror effect to action observation is not entirely clear according to the available literature. Many studies used ongoing actions as visual stimuli, though the analyses were time-locked to certain phases of the movement. In such studies the timing of cortical activation is not clear because when watching continuous stimuli, the participants can in principle predict the forthcoming action. In these experiments, imitative responses were between 80 and 200 ms after informative visual cues (Borroni et al., 2005; Cattaneo et al., 2009). One neuromagnetic imaging study recorded activity time-locked to object-hand interaction during an ongoing reach and grasp movement and found M1 to be active around 40 ms from hand-object interaction, but also in this case, the averaging trigger was not the onset of movement but its final phase (Nishitani & Hari, 2000). In that study however some indirect information on the timing of mirror responses can be inferred by comparing the latency of the occipital and motor peaks during imitation, which are separated by around 200 ms. The above papers, regardless of the use of continuous or of event-related paradigms are based on motor events that are predictable by the observer. It has been shown that, during ongoing movements, predictory elements are strongly represented in the observer's motor system (Gangitano, Mottaghy, & Pascual-Leone, 2004; Urgesi et al., 2010).

Only a few studies employed event-related techniques time-locked to the onset of unpredictable movement. These showed motor modulation as early as 90-100 ms after visual or auditory presentation of action stimuli (Lepage, Tremblay, & Theoret, 2010), however this motor modulation was not muscle-specific. Unpredictable movements were also used in the event-related paradigm by Catmur et al. (2011) who found in baseline conditions a muscle-specific mirror effect as early as 200 ms from movement onset. When processing categories of visual information other than upper limb movements, automatic motor responses are known to occur in an early time interval, between 100 and 200 ms from stimulus presentation. This has been shown for a variety of visual stimuli, such as the presentation of manipulable objects (Buccino, Sato, Cattaneo, Roda, & Riggio, 2009; Prabhu et al., 2007), the vision of articulatory lip movements or the visual presentation of Arabic numerals (Sato, Cattaneo, Rizzolatti, & Gallese, 2007; Sato, Catteneo, & Rizzolatti, 2010). Taken together, the data from the literature indicate that when investigating "automatic" visuo-motor imitative responses, the excitability of the motor cortex should be tested in an early time window, roughly between 100 and 300 ms from stimulus onset. A partial time course of the mirror effect in such early interval has been already described (Catmur et al., 2011). However, on that occasion the authors tested the 200, 250 and 300 ms intervals from the onset of observed movement only before the counter-imitative training session, but tested only the 300 ms interval after the training session.

Here the gap has been filled by investigating the chronometry of motor modulation following action observation, before and after associative behavioral trainings in the 100-320 ms interval after visual stimulation. As an instantaneous index of the transient changes in the cortical representation of movements we recorded the kinematics of TMS-evoked movements rather than the muscular electrical activity of MEPs because it better represents the cognitive process we wanted to measure.

Kinematics measure the actual movement, while single muscular contractions may not be univocally associated with a single movement (Brochier, Spinks, Umilta, & Lemon, 2004; Weiss & Flanders, 2004) or with any movement at all, as for example in fixation of an articulation. More importantly, the motor cortex and, even more, the premotor cortex of primates represent motor acts and their endpoint rather than single muscles (for example see Kakei et al., 1999; Kakei et al., 2001; Rizzolatti et al., 1988).

Three different experiments have been performed. In the first (Counter-Imitative) experiment participants were presented with two opposite biological actions, to which they trained with an associative counter-imitative protocol. The second (Imitative) experiment was identical to the first one but the associative training was imitative. In a control experiment (Spatial-Compatibility) participants were presented with spatially oriented non-biological events to which they had to associate spatially non-compatible motor responses. The latter experiment was done to understand whether only spatial features could account for all of the results of the Counter-

Imitative experiment.

8.2 Methods and Materials

8.2.1 Participants

Sixteen volunteers (10 females, age 23-45 years, one left handed) took part in the Counter-Imitative experiment. Ten volunteers (7 female, age 21-28 years) took part in the Imitative experiment and 16 volunteers (9 females, age 21-36 years) participated in the Spatial-Compatibility experiment.

None of the participants took part in more than one experiment. The experiment was approved by the local Ethical Committee and was conducted in compliance with the revised Helsinki declaration (World Medical Association General Assembly, 2008). All participants gave written informed consent to the experiment and were screened for contraindications to TMS (Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

8.2.2 General design

In all 3 experiments we recorded the acceleration of the participants' wrist (W-Acc) evoked by TMS applied to the motor cortex. This measure was used as the dependent variable throughout the experiments. W-Acc was recorded in an event-related design, while participants watched short video clips, consisting in a moving hand in the Counter-Imitative and Imitative experiments and in a moving arrow in the Spatial Compatibility experiment (see below). Importantly, TMS was applied at 4 different time intervals (inter-stimulus intervals – ISIs) from the onset of movement in the movie. Participants underwent a first TMS session in which event-related W-Acc was recorded. They then performed a behavioral associative training session, the rules of which changed in the 3 experiments, and finally underwent another TMS session identical to the first one. In each experiment the within-subject independent factors that were experimentally manipulated were three: 1) being tested before or after the behavioral training, 2) the type of video clip and 3) the ISI.

8.2.3 Video stimuli

Three types of movies were presented in the Counter- Imitative and in the Imitative training, as schematized in Figure 14. They showed a right hand in egocentric perspective turning a round lid in the clockwise direction (CW trials), in the counterclockwise direction (CCW trials) or simply moving down from the lid (Mov trials). The frame rate of the movies was 33 Hz. The first 3

frames were the same in all movies, showing a still hand. The onset of the movement happened in the 4th frame of each video, i.e. at 100 ms from the onset of the movie.

The movements in all trials lasted for 9 frames, i.e. 300 ms. In CW and CCW trials the final wrist rotation was of 18° with respect to the starting position. The timing of TMS was calculated from the onset of the 4th frame, i.e. when the first information on the movement direction was available.

In the Spatial-Compatibility experiment three different types of movies were presented. They showed a white circle with one single black vertical arrow in the middle (resembling the hand of a clock) and are schematized in Figure 15. Three different movie types showed the arrow turning clockwise, counterclockwise or falling down vertically. The movies were designed to match the three conditions (CW, CCW and Mov trials) of the first 2 experiments. Their frame rate was 33 Hz, the first 3 frames showed the still vertical arrow and the onset of the movement was at the 4th frame. Also here the timing of TMS was calculated from the onset of the 4th frame. Also the rotational degree (18°) and movement time (300 ms) were matched to the previous movies. A frame-by frame representation of the single frames in which movement occurred in both the biological and non-biological movies is provided in Figures 14 and 15.

It should be noted that both the hand movies and the arrow movies were designed so that the movement direction could not be guessed from the initial 3 static frames because they were indistinguishable between conditions. All stimuli were presented on a LCD screen, with a visual angle of 14° in width by means of the Cogent 2000, developed by the Cogent 2000 team at the FIL and the ICN and Cogent Graphics developed by John Romaya at the LON at the Wellcome Department of Imaging Neuroscience.

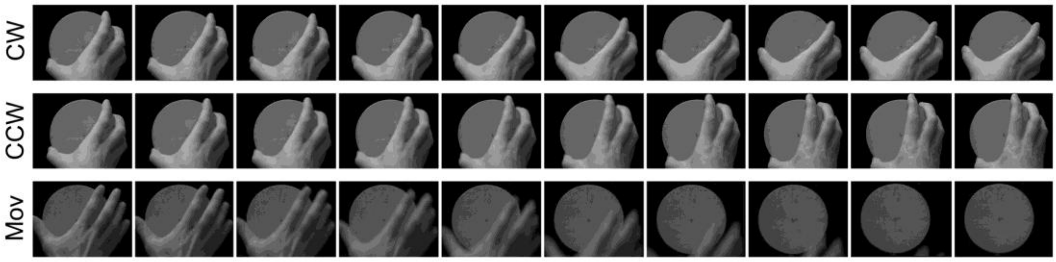


Figure 14
Frame-by-frame representation of the movements presented to the participants in the (A) counter-imitative and imitative experiments. Every clip showed a right hand in egocentric perspective turning a lid clockwise (CW trials), counterclockwise (CCW trials) or simply moving down from the lid (Mov trials). In 9% of trials, a red dot appeared on the screen, to which participants had to respond as fast as possible with a left-hand button press.

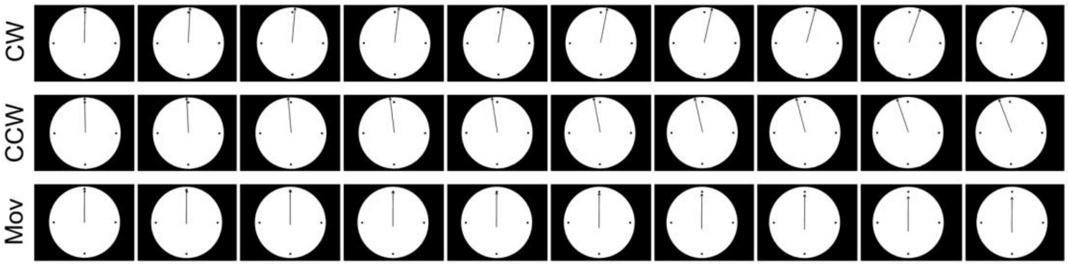


Figure 15
Frame-by-frame representation of the movements presented to the participants in the spatial-compatibility experiment.

8.2.4 TMS sessions

In all 3 experiments, participants were sitting comfortably with their head on a chin-rest, which assured head stability. Figure 16 shows a typical setup. The participants' fingers were fixed on the rim of a round box similar to the one observed in the video. The elbow and the box were placed above two different supports. The forearm was not supported, this in order to reduce friction of a rotational movement of the forearm/wrist. The vision of the own hand was occluded to participants throughout the experiment by means of an opaque shield. Each of the 2 TMS sessions, before and after training, consisted in 740 event-related trials. Each trial started with a fixation cross presented for 2000 ms followed by the movie (1500 ms) and by a blank screen of 1000 ms. TMS was delivered time-locked to clip presentation at different ISIs (see below). Every 74 stimuli participants could take a short pause from TMS, therefore the session could be divided in 10 blocks of 74 trials. The 740 trials consisted of 672 movies (224 CW, 224 CCW and 224 Mov or the equivalent stimuli in the Spatial - Compatibility experiment) plus 68 still frames of a black background with a red dot in the center, all presented in a random order.

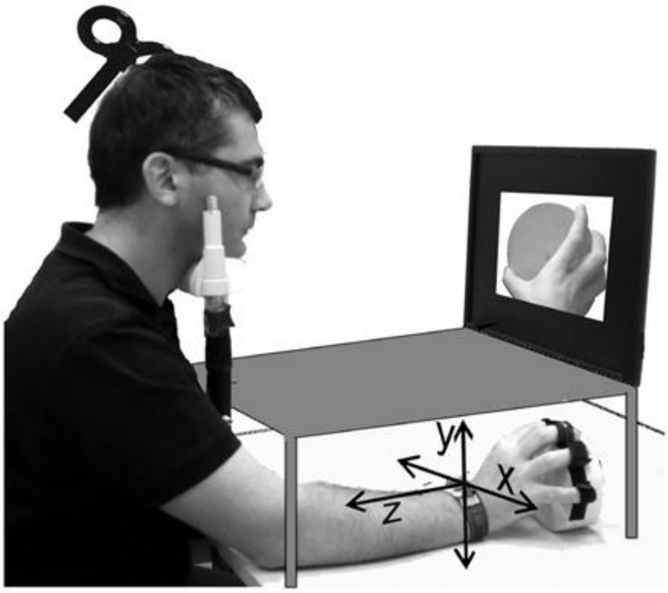


Figure 16
Schematization of the experimental setup showing orientation of the x, y and z axes

The task was to completely relax the right arm and hand, watch carefully the movies and whenever the red dot appeared, to press a key as fast as possible with their left hand. If after 1500 ms from the presentation of the red dot participants did not respond, a “Is anybody out there?” sign was presented in the center of the screen, in order to alert them. Single biphasic stimuli were delivered with a Magpro unit (Magventure, Denmark) connected to an MCB65 figure-of eight coil. Resting motor threshold was visually assessed as the intensity capable of evoking twitches of the thumb or index fingers in 5 out of 10 consecutive trials. This technique is known to over-estimate the threshold assessed with EMG by a factor between 2% (Conforto, Z'Graggen, Kohl, Rosler, & Kaelin-Lang, 2004) and 15% (Hanajima et al., 2007)

Participants were then stimulated with an intensity equal to 130% of threshold.

The TMS site in the actual experiment was detected visually as the site the evoked the most powerful visible wrist turning on the x-axis in the hand at rest. TMS was delivered at four different ISIs corresponding to 100 ms, 150 ms, 250 ms and 320 ms from the onset of movements in the video clips. The Cogent software sent a TTL signal from the PC’s parallel port to an analog/digital input/output device, the CED 1401 (Cambridge Electronic Design, UK) 50 ms before the video onset. It was then the 1401 unit controlled by the Signal software (Cambridge Electronic Design, UK) that, through its digital output triggered the magnetic stimulator at different ISIs in a random order. On the presentation of the red dot no TMS pulse was delivered.

8.2.5 Recording and interpretation of TMS-evoked accelerations

The accelerations were recorded by means of a 2-axis accelerometer (Model DEACCM6G buffered 6G) fixed to the participants' right wrist. The x-axis recorded the horizontal acceleration while the y-axis recorded the acceleration on the vertical axis. Variations over the x-axis were informative of initial clockwise wrist rotations (positive accelerations) and counterclockwise rotations (negative accelerations). The accelerometer output was sampled at 1000 Hz by the CED Micro 1401 analog-to-digital converter, and stored for offline analysis with the Signal software.

Comparing the two-axis system to the movement shown in stimulus movies (compare Figure 14 and Figure 16) it is clear that the x-axis is the main component of the movement that distinguished CW from CCW movements, while CW and CCW trials were undistinguishable on the y-axis component. Therefore the x-axis accelerations were considered to be informative of imitative motor responses while the y-axis was not. The movement shown in Mov stimuli was orthogonal to the x-axis on which wrist rotation occurred and therefore motor responses mimicking it could not be read on the x-axis. The acceleration associated with Mov trials provided therefore a neutral condition serving as baseline that was subtracted from accelerations of CW and CCW trials (see below, ‘data analysis’).

8.2.6 Preliminary evaluation of the TMS-evoked acceleration

The mechanical twitch following synchronous depolarization of muscle fibers such as the one evoked by TMS occurs a few milliseconds after the electrical events spreading in the sarcolemma, which are macroscopically reflected by MEPs. This delay can vary according to muscular and joint properties (Barret, 2009).

The acceleration of body parts in response to TMS of M1 has already been used as an index of motor cortex excitability but limitedly to the thumb (Stefan, Classen, Celnik, & Cohen, 2008; Stefan et al., 2005) and index finger (Ingham, Tucker, Tsao, & Hodges, 2011) movements. TMS-evoked W-Acc has not been described before now in experimental settings. Therefore we evaluated empirically in 6 new participants (3 male, 3 female, mean age 22 years) the timing of appearance of TMS-evoked acceleration. This was done mainly to establish the time interval after TMS in which acceleration was informative of evoked movements but could not yet be contaminated by voluntary reactions to the stimulus. Therefore we asked participants in half of the trials to stay still in the other half react to the TMS pulse as fast as possible with a wrist movement. We recorded the electromyographic (EMG) activity of the right forearm flexor group (including therefore the pronator teres muscle, the main actor of wrist pronation) and the wrist acceleration with the methods described above. The data from a representative subject are shown in Figure 17. We found that significant changes in W-Acc started between 4 and 7 ms from the onset of the MEP (recorded by EMG), and that changes to the W-Acc course related to voluntary activity appeared between 102 and 126 ms after stimulation. We deducted that the time window between 30 ms and 90 ms from stimulation could be safely considered as a genuine expression of TMS-evoked wrist movement.

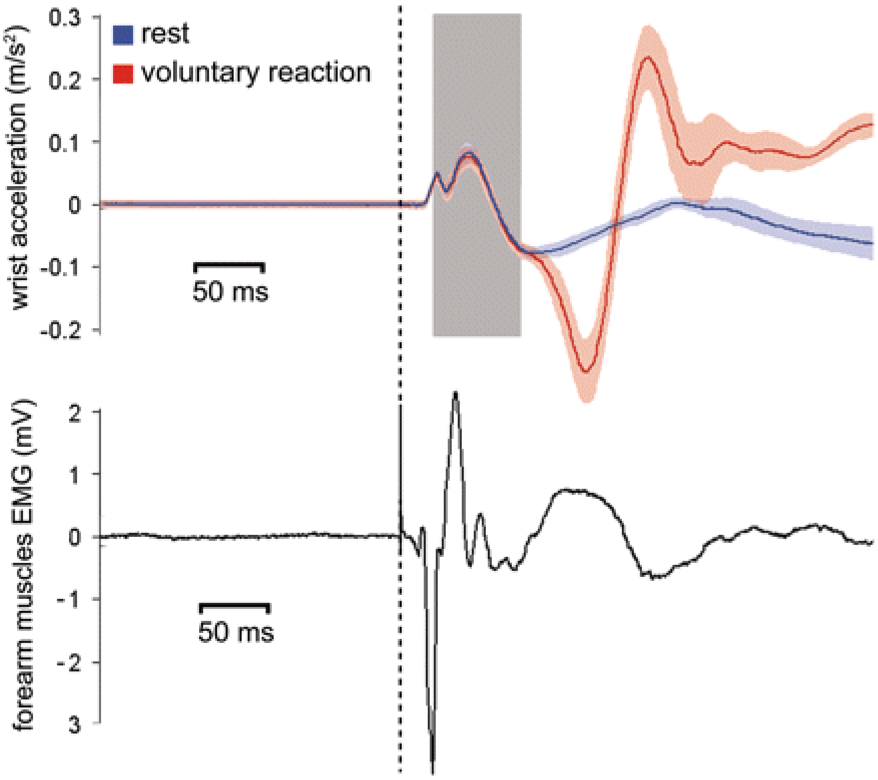


Figure 17
Plot from one representative participant, taken from the pilot evaluation, of the mean x-axis W-Acc (upper panel) and forearm EMG recordings (lower panel) obtained from 25 consecutive 'passive' trials and 25 consecutive 'active' trials. The dashed vertical line represents the time of TMS. The onset latency of the MEP is of 17 ms. Initial deflection of accelerations at 22 ms. Initial deviation of the acceleration trace due to voluntary interference is at 112 ms. The colored shadings represent the s.e.m. of acceleration signals. The gray shading represents the 30–90 ms interval that we subsequently chose to consider for analysis in the main experiment

8.2.7 Data processing

In all 3 experiments the raw W-Acc recordings from each trial were pre-processed separately for each of the two axes by applying the following consecutive steps.

A) baseline correction. It was made in order to adjust the baseline shifts due to the initial position of the hand, because the accelerometer senses also the gravitational acceleration and therefore emits different outputs according to the initial tilt of the still hand. For each trial the mean value of the signal in the 100 ms preceding the magnetic stimulus was subtracted from the subsequent recording.

B) Averaging in the 30-90 ms window. Following the results of the preliminary evaluation of the TMS-evoked acceleration (Figure 17) we averaged the acceleration values between 30 ms and 90 ms from the magnetic stimulus. In this way each single trial of the CW and CCW conditions corresponded finally to one single value for each of the x and y axes. Such value was used as dependent variable in subsequent statistical analyses.

C) Normalization of CW and CCW trials to Mov trials. The W-Acc from Mov trials (or downward arrow movements in the Spatial-Compatibility experiment) was considered as a neutral condition for the purpose of investigating mirror or counter-mirror responses.

We averaged the acceleration values of neutral trials separately for each block of 74 trials, for each of the 4 ISIs and separately for the pre-training and the post-training session in the 30-90 ms time window. These average control data were then subtracted from the individual trials with CW and CCW stimuli (or the equivalent rightward and leftward arrow rotations in the Spatial-Compatibility experiment), thus obtaining a value representing the variation of the acceleration in CW or CCW trials with respect to the neutral ones.

8.2.8 Training session

A wooden manipulandum similar to the lid presented in the Counter-Imitative and Imitative movies was used. The lid of the manipulandum could be turned clockwise or counterclockwise up to 15° from each side of the midline and thanks to a pair of springs it returned in the start position. When the extreme lateral position was reached, a 3.5 V circuit was closed, sending a TTL signal to the computers' parallel port, providing information about the direction in which the lid had been turned, in order to give subjects a feedback on their performance (see below). A potentiometer (10 KOhm Linear) within the manipulandum signaled the angle of the lid. The potentiometer' signal was sampled by the CED Micro 1401 analog to digital converter and recorded with the Spike software (Cambridge Electronic Design, UK). In the training session only CW and CCW movies were presented (or the equivalent stimuli of the Spatial-Compatibility experiment). Participants underwent 864 training trials (432 CW and 432 CCW), divided in 12 consecutive blocks, resulting in 72 randomly presented trials per block. The participants' task was to turn the lid with their right hand, in the same direction (Imitative experiment) or in the opposite direction (Counter-Imitative experiment) to the one shown in the movies. Participants were encouraged to turn the lid as fast as they could during the whole session. For each trial, as soon as the lid was completely turned in the correct direction, feedback was provided by displaying the time of end-contact of the lid, therefore corresponding to the end-of-movement time. If the lid had been turned in the wrong direction, the text “Wrong!” appeared in the center of the screen. If no response was produced, then a “no response” message was displayed. The potentiometer’s signal was used offline to detect the moment of movement onset and subsequently to calculate the subjects’ real reaction time (rather than the displayed end-of-movement time). In the Spatial Compatibility experiment the training task was analogous to that of the Counter- Imitative experiment. Participants had to turn the manipulandum as fast as possible in the direction opposite to the one in which the arrow moved.

8.2.9 Statistical analysis on W-Acc

The single mean acceleration values obtained as described above (see Data Processing section) were averaged within each subject. In this way each participant in each experiment was characterized by two sets (one for each axis) of 16 mean acceleration values corresponding to the two movie types, for each of the 4 ISIs, pre- and post-training. These values were then used as a dependent variable in two (one for each axis) repeated measures ANOVA (2 x 2 x 4) with TRAINING (pre-training, post-training), DIRECTION (Clockwise, Counterclockwise) and ISI (100 ms, 150 ms, 250 ms, 320 ms) as within-subjects factors in each of the 3 experiments. All variables were tested for Normality of the distribution by means of the Kolmogorov-Smirnov test (all variables with $P>0.18$). The ANOVAs were also tested for sphericity in all 3 experiments and the 3-way interactions were found to be spherical for both the x axis (all p-values > 0.22). Three-way interactions were further explored by means of four separate 2-factors ANOVAs (one for each ISI).

One additional unplanned, post-hoc analysis was carried out with the scope of directly comparing the results of the Counter-Imitative experiment with those of the Spatial-Compatibility experiment. We performed 4 separate ANOVAs for each of the 4 ISIs, having one between-subjects factor: EXPERIMENT (2 levels: Counter-Imitative or Spatial-Compatibility) and two within-subjects factors: TRAINING (2 levels: pre-training or post-training) and DIRECTION (2 levels: CW/ or CCW/).

8.2.10 Statistical analysis on the training session

During training the potentiometer signaled instantaneously the angle of the training lid and therefore the participant's responses in each trial. We decided to consider as errors all trials that started in the wrong direction, even if in some trials the trajectory was then corrected in the appropriate direction. To detect these responses the derivative of the potentiometer's output was calculated and the signal was low-pass filtered at 12 Hz. Deviations outside a threshold of rotational velocity of +/- 40°/s were considered as response onset and subsequently categorized in terms of latency and correctness of the response. Responses faster than 90ms were excluded. For each participant and for each of the twelve training blocks we calculated the individual median reaction time (RT) of correct responses and the proportion of errors. Subsequently, two mixed-design ANOVAs were performed separately on RTs and accuracy, with BLOCK as the within-subject variable and EXPERIMENT as the between-subject variable. Possible main effects of BLOCK were subsequently analyzed by means of pairwise t-tests on the data from each pair of consecutive blocks, in order to quantify the improvement in the performance (Figure 18).

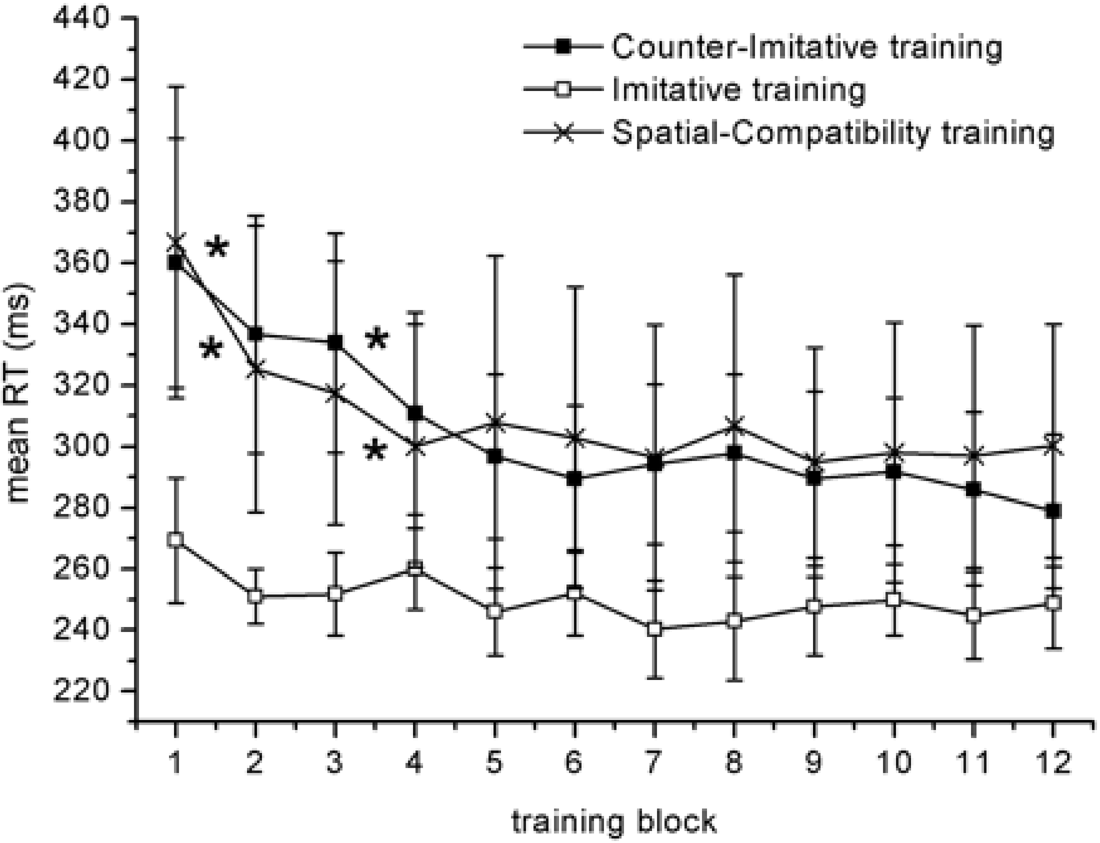


Figure 18
Time course of the mean reaction times recorded during the behavioral trainings of the two main experiments and the control experiment. Asterisks indicate significant differences between consecutive blocks in paired-sample t-tests. Error bars represent 95% CI.

8.3 Results

None of the participants reported undesired effects of TMS. Attendance to the visual stimuli was testified by the low rate of red dot trials in which participants were slower than 1500 ms, consisting in 2.9% for the Counter-Imitative experiment, 2.7% for the Imitative experiment and 3.0% for the Spatial-Compatibility experiment. The average time interval between the end of the training and the beginning of the post-training TMS session was of 232 s (SD: 21s) overall for the 3 experiments.

8.3.1 Counter-Imitative experiment

The most important result on the x-axis data was a significant three way TRAINING*DIRECTION*ISI interaction. As illustrated in Figure 19, in order to analyze this interaction the data were analyzed separately for each of the 4 ISIs, therefore producing 4 distinct ANOVAs with 2 factors, TRAINING and DIRECTION.

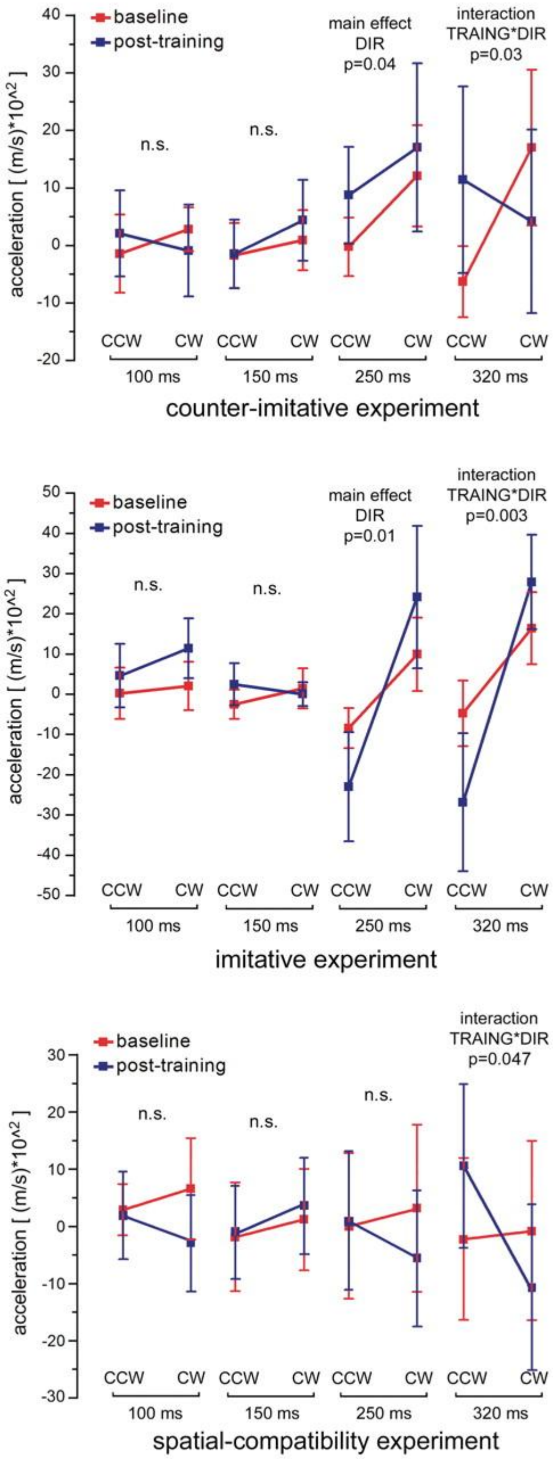


Figure 19
Results of the counter-imitative (upper panel), imitative (middle panel) and spatial-compatibility (lower panel) experiments. The plot represents the mean of TMS-evoked W-Acc values measured on the x-axis of the accelerometer (±95% CI) in CCW and CW trials for all four ISIs, before training and after training. Error bars indicate 95% CI. The results of the two-way ANOVAs on single ISIs made to explore the three-way interaction are shown above each ISI; n.s. = non-significant.

No significant results were observed at 100 ms or 150 ms. At 250 ms we found only a main effect of DIRECTION (F(1, 15)=5.11, p=0.039), showing that at this ISI the training did not influence the subjects' responses which were simulative both before and after training. At 320 ms we did not find main effects but we did find a significant TRAINING*DIRECTION interaction (F(1, 15)=6.10, p=0.026), resulting from a clear mirror effect before the training (paired samples t-test between CW and CCW trials: p=0.01) which was disrupted by training (paired samples t-test: p=0.3). The ANOVA on y axis accelerations did not produce significant results (all p-values >0.21).

8.3.2 Imitative experiment

The ANOVA results on x-axis W-Acc values are shown in Table 1. A significant 3-way interaction was found, is illustrated in Figure 19. The interaction was further investigated by dividing the data into 4 separate ANOVAs for each of the ISIs. At 100 and 150 ms no significant effects were found. At 250 ms a significant main effect of DIRECTION (F(1, 9)=10.41, p=0.01) was found and at 320 both a main effect of DIRECTION (F(1, 9)=9.78, p=0.012) and, more importantly, a significant TRAINING*DIRECTION interaction (F(1, 9)=15.6, p=0.003) were found. No significant effect was found in the analysis on y-axis accelerations.

8.3.3 Spatial-Compatibility experiment

The ANOVA did not show a three way interaction but only a two-way

TRAINING*DIRECTION interaction was present, due to the fact that overall W-Acc values were modulated in a spatially compatible way before training (mean value for leftward trials: -6.5 102*m/s^2. Mean value for rightward trials: 9.5 102*m/s^2. Paired samples t-test, p=0.03) and this difference was not present after training (mean value for leftward trials: 3.1 102*m/s^2. Mean value for rightward trials: -3.7 102*m/s^2. Paired samples t-test, p=0.11). Even in the absence of a significant 3-way interaction, we analyzed the data with four 2-way ANOVAs, in order to compare the results with those of the other two experiments. The values of this analysis are illustrated in the lower panel of Figure 19.

In summary, the ANOVAs produced no significant results at the 100 ms and 150 ms ISIs. At the 250 ms ISI we found significant EXPERIMENT*DIRECTION interaction (F(1,30)=7.51, p=0.01). This interaction was interestingly explained by the fact that, as described above, in the Counter imitative experiment a significant difference (p=0.03) between CW and CCW directions (irrespective of training) was present but in the Spatial-compatibility experiment no such difference was observed (p=0.15). At the 320 ms ISI we found a DIRECTION*TRAINING interaction (F(1,30)=10.77, p=0.003) indicating that, unlike at the 250 ms ISI, at the 320 ms ISI the data from the two experiments were not significantly different.

8.3.4 Training sessions

The RTs in the 12 consecutive blocks of the 3 experiments are shown in Figure 18. The analysis showed a main effect of the factor EXPERIMENT (F(2,39) = 4.13, p = 0.02), with the Imitative training (250.38 +/- 10.4 ms 95% C.I.) faster than the Counter-

Imitative (305,48 +/- 26.38 ms 95% C.I.), (p=0.005) and faster than Spatial-Compatibility (309.52 ms +/- 33.90 ms 95% C.I.), (p=0.01). A main effect of BLOCK (F (11,429) = 13.804, p < 0.0001) and an interaction EXPERIMENT*BLOCK were found (F (22,429) = 1.99, P=0.005).

Figure 18 shows also the pairs of consecutive blocks in which a significant difference was found by means of a pairwise t-test. We showed that a significant improvement was present between the first and second and between the third and fourth blocks in both the Counter-Imitative and the Spatial-Compatibility trainings. On the contrary no significant difference between consecutive blocks was found in the Imitative training. The analysis of the error rates revealed only a main effect of the factor EXPERIMENT with a higher proportion of errors in the Counter–Imitative (0.19 +/- 0.04 95% CI) training than the Spatial-Compatibility training (0.11+/- 0.02 95% CI), (p=0.002). No significant differences were found between the Imitative training (0.14 +/- 0.04 95% CI) and the Spatial-Compatibility training, while a trend towards significance was found between the Imitative and the Counter-Imitative trainings (p=0.071).

8.4 Discussion

The results of the two main experiments (the Imitative and the Counter-Imitative one) show that in non-trained individuals (i.e. in the pre-training session) a clear and repeatable modulation of the motor system is evident at 250ms and 320ms after the first visual information on observed movement is available. This modulation occurs in the same direction as the observed movement, i.e. it is a mirror modulation. In the post-training session of both experiments this mirror effect was unchanged at the 250 ms interval. On the contrary a clear interaction of the training with the evoked responses was observed in the 320 ms interval. This modulation occurred in the direction of the trained visuomotor rule, i.e. subjects' responses were even more mirror-like after the imitative training and were less mirror-like after the counter-imitative training. The present results show that the chronometry of brain activations to action observation distinguishes two different phases. In one early (after 150 ms and before 250 ms) interval the motor cortex contains a mirror-like motor representation that is not modulated in the short term by an associative training. In one late phase (after 250 ms but before 320 ms) the motor cortex contains a motor program that is representative of the recent visuomotor training.

If, as hypothesized by the ASL theory, one single mechanism produces automatic mirror responses and trained counter-mirror responses, we would expect the same effect of training on each ISI. In other words, let's hypothesize that the subjects' responses are described by a function (named *f-mirror*) having as argument the time from movement onset and the features of observed movement.

Observer's response = *f-mirror* (ISI, observed movement)

The ASL theory predicts that the effects of training on the function is that of multiplying it by a constant value, the polarity of which depends on the training rule, that is a negative value with Counter-Imitative training and a positive (or null if a ceiling effect is present) when Imitative training is performed.

Observer's response = K**f-mirror* (ISI, observed movement)

In our specific experimental set we would expect an effect of training at all ISIs where a mirror effect was present before training. The present data however do not fit these predictions. On the contrary, we find a biphasic time-course, with no effect of training on an early ISI (250 ms) irrespective of the direction of training. We propose a different model to explain the present data, assuming the presence of two different mechanisms interacting. The first mechanism is the one producing overlearned automatic mirror responses, which produces fast visuomotor associations, within 250 ms from stimulus onset. The second one mediates the responses compatible with the newly learned visuomotor arbitrary associations and seems to be slower than the first one, given the same visual stimuli, i.e. it becomes apparent only at 320 ms from stimulus onset. Our hypothesis is represented therefore by two distinct functions, named *f-mirror* and *f-executive* with the following temporal constraints: f-mirror produces null results with ISI<250 and f-executive produces null results for ISI<320.

Observer's response = *f-mirror* (ISI, observed response) + K**f-executive* (ISI, observed response)

Before training K=0 and therefore only *f-mirror* produces behavior. After training, K is either a positive or negative constant and therefore the also the *f-executive* function contributes to the observer's response. It is important to stress that this model predicts correctly the data from both main experiments (Imitative and Counter-Imitative). The statistical implications of the model are indeed that a main effect of

DIRECTION is to be expected at 250 ms, irrespective of training, while a TRAINING*DIRECTION interaction is to be found at 320 ms, irrespective of the training direction. As shown in Figure 19, our results fit exactly this prediction.

The present data replicate entirely the ones by Catmur et al. We employ a training of the same type and duration and we obtain data that are compatible with their finding at the late ISI. However testing an early ISI (250 ms) after training produces additional results that are at odds with the interpretation of the data of Catmur et al.. Nevertheless, it should be noted that our data do not argue against an associative origin of the tuning properties of mirror neurons. We clearly demonstrate that a brief visuomotor training is not sufficient to reverse the tuning of mirror neurons but the present data are fully compatible with an ontogenesis of mirror neurons based on visuo-motor associations requiring a much longer history of congruent-visuo-motor experience. In such account, that has been systematically theorized elsewhere (Del Giudice, Manera, & Keysers, 2009; Keysers & Perrett, 2004), Hebbian Learning is at the basis of mirror neuron development and participants train their mirror neurons over the life-span by having great interest in looking at their own actions and such cumulative learning of neurons would be much stronger, and persistent, in the face of a short counter-imitation training.

The ISIs that were chosen in the present experiment represent well defined time points in the course of visual processing along the dorsal stream. The first ISI, 100 ms, represents an ‘early visual’ time, when the visual information is still confined to the early extrastriate visual cortex (see for example Amassian et al. (1989) and Silvanto, Lavie & Walsh (2005). According to our initial hypothesis no modulation of the motor system was expected in this early interval.

The two ISIs of 150 and 250 ms sample the time window in which visual information is likely to be processed in a parieto-frontal system along the dorsal visual stream as shown for different categories of visual information, such as biological movement (Catmur et al., 2011), object geometry (Bernier, Burle, Hasbroucq, & Blouin, 2009) or spatial information (Fierro, Brighina, Piazza, Oliveri, & Bisiach, 2001). It should be noted that neural responses to action observation have been found in two studies as early as 90 ms, albeit in both studies activity was not specific to the type of observed acts (Lepage et al., 2010; van Schie et al., 2008). Interestingly in one of these studies, van Schie and colleagues postulate that the early onset of the lateralized neural activity and the fact that the evoked component was insensitive to the correctness of the observed action suggest the operation of a fast and automatic form of motor resonance that may precede higher levels of action understanding. The last ISI, the 320 ms interval, on the contrary was chosen on the basis of the original description of counter-mirror training (Catmur et al., 2007), in which the intervals between stimulus onset and TMS of 0, 320 and 640 ms were tested. The anatomical structures of the two different neural systems described here can be speculated on the basis of the activation time-course. The most likely substrate of the fast motor resonance process is a temporal-parietal-ventral premotor route (Rizzolatti & Matelli, 2003). The arbitrary association route is probably residing in the prefrontal cortex, in its dorsolateral portion, that is thought to play a role in maintaining representations of stimulus-response associations (Ridderinkhof, Forstmann, Wylie, Burle, & van den Wildenberg, 2010) and in the selection of the responses under conflict (Mansouri, Tanaka, & Buckley, 2009), in the right inferior frontal gyrus which has been found activated during the inhibition of prepotent responses (Aron, Robbins, & Poldrack, 2004) or in the anterior middle prefrontal cortex, that seems to be specifically involved in the inhibition of imitative responses (Brass, Derrfuss, & von Cramon, 2005). It should be noted that, compared to Catmur et al. (2007) we used a continuous movement rather than an implicit one. This means that the 250 ms TMS and the 320 ms TMS occur at different stages of the movement. This datum however accounts only for changes in the time-course of the responses within each training block, but is unlikely to justify the differences that are observed between training blocks at the different ISIs.

The parallel dual route model is essentially contained in the prevailing theories of action control (Ridderinkhof et al., 2010) which postulate that the appearance of a stimulus activates the correct response via a deliberate route and also captivates activation of other responses via a more direct processing route. The two processes necessarily converge at the level of response activation or even of response production. We cannot speculate at this point whether the executive system actively inhibits the fast visuomotor responses or whether a simple competition between the two processes occurs more distally, i.e. at the level of the motor cortex. There are several possible ways to control for a purely spatial compatibility effect in action observation. One is to produce a mismatch between proprioceptive information from one's own hand and the visual information on the observed hand's perspective (Catmur et al., 2007).

Another way, which relies purely on the visual modality, is to test participants with flipped or rotated versions of the observed hand (as for example in Brass et al., 2001). The control we adopted here was to test subjects with non-biological movements matching the biological ones (compare Figures 14 and 15). The data that resulted were considerably variable between individuals and only a two-way interaction (TRAINING*DIRECTION) was found. The training had an effect on the observers' responses in the post-training session but the 3-way ANOVA did not show any specific ISI at which this occurred. We conducted anyway two further post-hoc analyses to better understand the information provided by the Spatial- Compatibility experiment. First we analyzed separately each of the four time points and found significant results only at the 320 ms interval as a 2-way interaction (Figure 19). Second, we compared directly the results of the counter-imitative training and of the spatial compatibility experiment within each of the 4 ISIs.

The significant EXPERIMENT*DIRECTION interaction at the 250 ms ISI showed that the early responses recorded when observing a biological effector unlikely to be due to a spatial compatibility effect. At 320 ms only a DIRECTION*TRAINING was found, corroborating our hypothesis that the responses recorded at the late ISI are the product of short term associative visuomotor learning, independently of whether the visual stimulus is a hand or an arrow. The Spatial-Compatibility experiment would therefore suggest that the results of the Counter-Imitative and imitative experiments would not be due to a spatial compatibility effect between stimulus and response, but that the identity of the stimulus as a hand or a as a non-biological entity does matter in producing the observers’ responses, especially at the relevant early 250 ms interval.

9. STUDY 4: THE TIME COURSE OF COVERT MOTOR RESPONSES TO ACTION OBSERVATION

9.1 Introduction

Evidence from the previous experiment suggests that a short visuo-motor training does not reconfigure mirror neurons in counter-mirror ones.

However, previous TMS data from the accelerometer does not assure that motor resonance cannot be avoided. Indeed it does not push the action selection process enough to tangle with motor resonance. The motor resonance effect has been tested, as in the classical TMS-action observation paradigm, at rest. Although, some evidence that motor resonance is still present comes from the behavioral training session in which the counter-imitative group is slower than the imitative one, it is possible that slowness detected has been determined by the covariant compatible spatial confound, so that the reaction time difference is determined exclusively by the spatial compatibility variable (given the resemblance with the reaction times expressed on the spatial-compatible experiment, this seems to be a likely explanation). In order to test whether the motor resonance effect is detectable even in absence of spatial compatibility a different counter-imitative experiment has been ideated.

The aim of this experiment is to provide a dense time course of the cortical excitability M1 when participants are asked to perform the opposite action compared to the one they observed. In order to measure the cortical excitability at late latencies however a complete relaxation of the muscle recorded is needed. For this reason participants were asked to perform the appropriate action only after a single pulse TMS was delivered. In this way the voluntary input-based action selection has been dissociated from the response release.

9.2 Materials and Methods

9.2.1 Participants

16 healthy participants (11 female, 5 males, mean age 22.4) were enrolled. The experiment has been conducted in compliance with the Helsinki declaration and with the approval of the Ethical local Commettee (World Medical Association General Assembly, 2008). All participants gave written informed consent to the experiment and were screened for contraindications to TMS (Rossi et al., 2009)

9.2.2 Stimuli

Visual stimuli consisted of a single right hand placed between two objects respectively close to the index and the little finger. The hand could abduct either the index or the little finger in order touch one of the objects. Stimuli were not videos but a sequence of two pictures (initial and end-position) presented one after another. The fast presentation of the two pictures induced the sensation of a real movement and allowed a better time control over the onset of the action (see the Procedure and Task section for further details). The hand was presented in a dorsal perspective in the center of the screen; in half of the trials the fingers were pointing leftwards, in half they were pointing rightwards (Figure 20); the orientation was randomized. Usually in most of the TMS experiments presenting index and little finger abductions, the movement observed did not resulted in a goal-directed and/or in a meaningful action (Catmur et al., 2007, 2011).

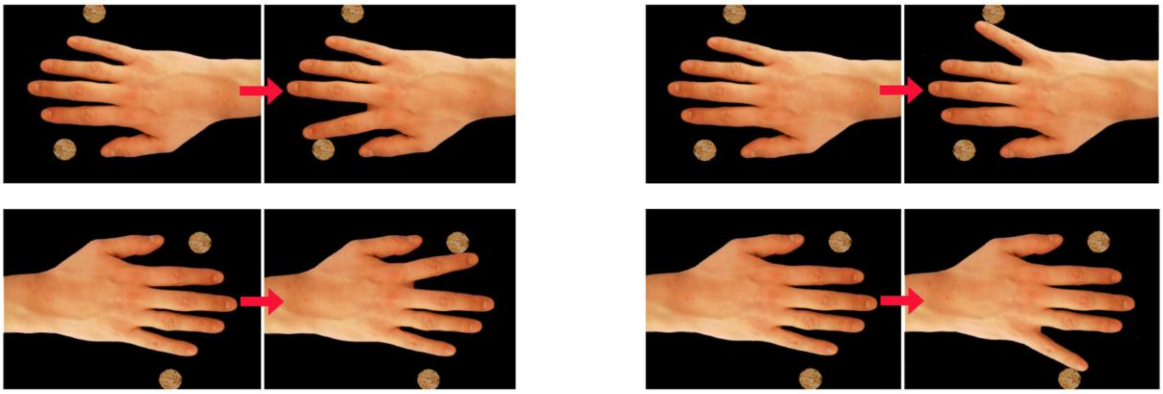


Figure 20

Visual stimuli: hands were shown in two orientations. Two possible motor acts could have been shown: touching the object with the index finger (left panel), or with the little finger (right panel).

9.2.3 TMS

Electromyographic recording (EMG) was performed on the right First Dorsal Interosseus (1DI) and the right Abductor Digiti Minimi (ADM) muscles through Ag-AgCl surface electrodes placed in a belly-tendon montage. The signal recorded has been amplified by means of a 1902 CED amplifier by a 1000x gain and band passed 20 Hz-5000 Hz, and digitized by means of a CED Power 1401 analogical to digital converter, controlled by Signal® software. The EMG signal was sampled 4000 Hz.

Data have been recorded and analyzed off-line.

TMS stimulation was delivered on participants' left primary motor cortex, over the scalp portion on which the biggest MEPs amplitude was recorded using a slightly supra-threshold stimulation. The point was marked on the participants' scalp .

Once the stimulation point was marked, a resting motor threshold (RMT) was determined for each participant. RMT has been defined as the minimum stimulator output intensity capable of producing a Motor Evoked Potentials (MEP) of a peak-to-peak amplitude of at least 50 µV in 5 out of 10 consecutive stimulations, on the 1DI muscle (Rossini et al., 1994). Stimulation intensity across the whole duration of the experiment was set to the 120% of the RMT for each participant). participants in which it was not possible achieving ADM MEPs amplitudes constantly higher than 50 µV were excluded.

Stimulation was performed with a 70 mm figure-of-eight-shaped coil connected to a Magstim Rapid®. The coil was placed tangentially on the scalp with the handle pointing backwards and inclined at 45° with respect to the head midline. Throughout the whole duration of the experiment the coil was held by hand on the participants' scalp.

9.2.4 Procedure & Task

Participants sat on a chair in front of a PC monitor with their chin on a chin-rest. After the surface electrodes were mounted, participants' right hand was placed on a custom made response box constituted by two plates placed respectively nearby to the index and to the little finger. Two adhesive rings were tightened on participants' index and little fingers; when one of these rings touched the nearest plate then a response was detected (Figure 21).

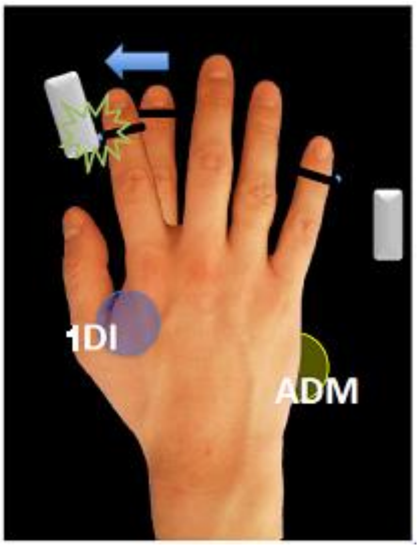


Figure 21

Representation of the participants' hand within the response pad.

Visual stimuli were presented using E-Prime® software on a monitor with a refresh rate of 60Hz. Participants' right hand was oriented orthogonally with respect to the orientation of the hands presented on the screen, with the fingers pointing towards the monitor.

Each trial began with a still hand, then after a random time within 500 and 1000 ms, the target action was presented (index finger or little finger abduction); TMS single pulse was delivered in random order at seven different times from the onset of the end-position picture (0, 100, 133, 150, 200, 250 , 300 ms). Participants were required to react as fast as they could to the observed action with the opposite motor act compared to the one they saw, that is, if they saw an index finger abduction then they had to perform a little finger abduction and vice-versa, but only after having heard the delivering of the TMS pulse. The end-position picture lasted until participants' response was detected for a maximum of 1500 ms; after the response, participants were presented with their reaction time in milliseconds if the response was correct. A "Wrong!" or a "No Response" label was presented respectively either if the response was not correct or not detected. A cross in the center of the screen was presented until the trial had reached a duration of 5000 ms in order to avoid repetitive effects of the TMS stimulations delivered in the preceding trials. (Figure 22). Each time a muscle was visibly contracted participants were required to relax their muscles as much as they could. The whole experiment was constituted by three blocks of 224 trials each, leading to a total number of trials of 672, 48 trials per cell.

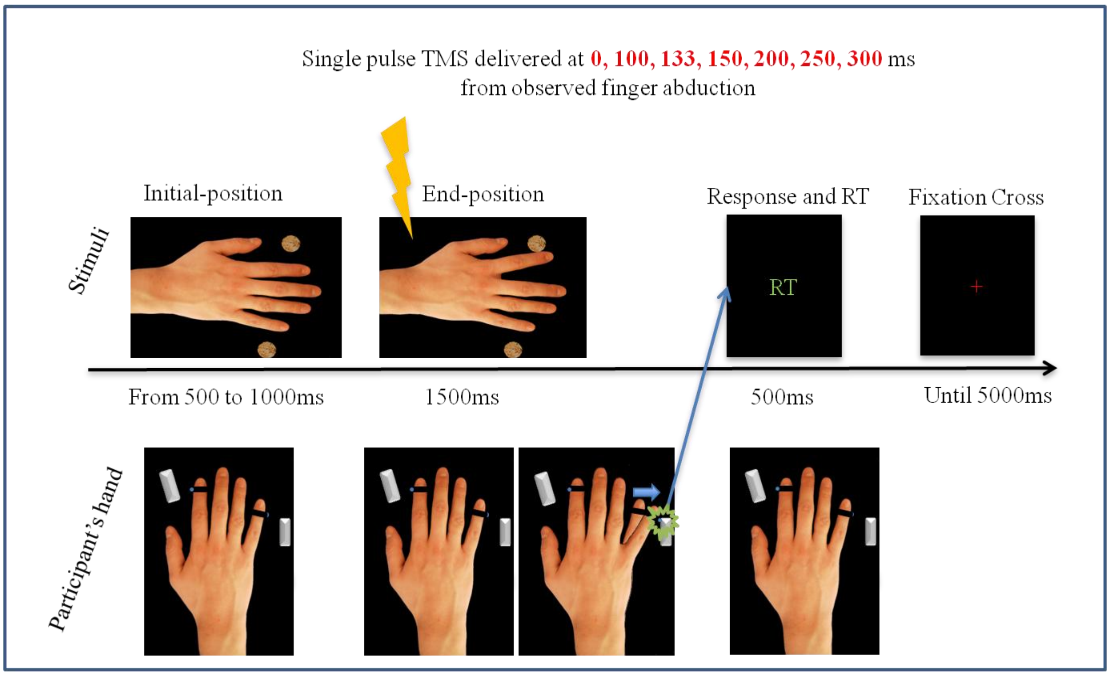


Figure 22

Schematic representation of the task. Presented visual stimuli are depicted above the timeline. Participants' hand is depicted below the time line. Participants' task was to respond with the opposite motor response compared to the one they saw but only after the TMS pulse, that represented the "GO-signal"

9.2.5 Data analysis

For each trial for each participant, peak-to-peak MEPs amplitude was measured on both 1DI and ADM. Trails in which MEPs were smaller than 50 µV on at least one of the two channels were discarded. Trials in which an anticipated response with respect to the TMS go-signal was provided have been discarded. Non-correct responses were excluded from the analysis as well.

1DI and ADM MEPs average at 0 ms time were respectively computed for each participant separately each observed action. The MEPs from all trials of all the other times of stimulation were normalized on 1DI 0 ms average and ADM 0 ms average respectively for 1DI and ADM muscles, separately for each observed action, providing normalized indexes of cortical excitability (1DIn and ADMn).

For each participant (1DIn-ADMn) 20% trimmed mean difference was computed for each time point for each observed action, and used as the dependent variable. In this way, following Catmur et al. (2007), an index of simulation has been created. If this measure is higher when observing index compared to the observation of little finger abductions, than this has to be interpreted as a sign of simulation, while the opposite pattern would reflect a counter-simulative effect.

Analysis performed only on trials in which a correct response was provided. Data were entered in a repeated measures ANOVA (2 x 6) with “OBSERVED FINGER” (Index, Little) and “TIME” (100,133,150,200,250,300) both as within-subject variables.

Pairwise T-test was applied on each of the six times of stimulation (Figure 23).

9.3 Results

Repeated measures ANOVA yielded a significant interaction “OBSERVED FINGER” x “TIME” (F(5,75)=6.1211; P=0.001). Significant pairwise T-tests are shown in Figure 23. A significant effect has been found at two time points of stimulation (P<0.05), namely at 150 ms, in which the normalized (1DI-ADM) difference was higher during the observation of index finger abduction (0.074 mV) than during little finger abduction (-0.044 mV) indicating a mirror effect, and at 300 ms in which the opposite pattern was found, that is, the normalized difference between the two muscles was found higher when observing little finger abductions (0.03 mV) compared to the observation of index finger abductions (-0.166 mV), indicating a counter-mirror effect (Figure 23).

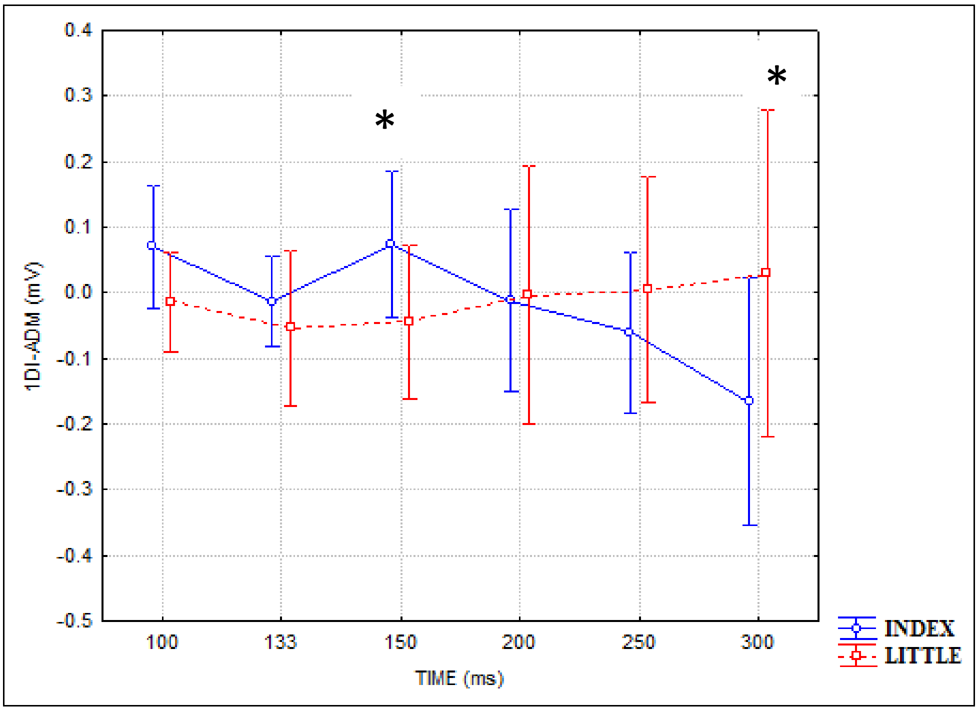


Figure 23
1DI-ADM variation according to the stimulation time.
Blue line represents the 1DI-ADM difference when Index finger abductions are shown, while the red one represents the same difference when little finger abductions are presented. The blue line above the red one indicates a motor resonance effect.
Stars indicate the significant (< 0.05) pairwise T-test. Bars indicates 95% Confidence intervals.

9.4 Discussion

A time course of the cortical excitability has been described showing a biphasic trend in time with an early component determined by a motor resonance effect that is replaced in time by the opposite pattern, a counter-mirror effect induced by the voluntary preparation of the opposite action in response to the one observed. The later pattern is exactly what should be expected when participants are required to performed the opposite action compared to the one they saw. As shown by Tandonnet and colleagues (Tandonnet, Garry, & Summers, 2011) when participants are performing a bimanual choice reaction times have their M1 stimulated close to the reaction time, MEPs from the responding effector increased steadily in amplitude, while in the non-responding effector the MEPs, after an initial increasing, become more and more inhibited.

The presented data are in accordance with the hypothesis that the motor resonance effect is present even when the motor performance apparently does not benefit of a “mirror-like” visuo-motor matching (matching that possibly worsens the performance). Results are compatible with the previously hypothesized dual route model in which a fast activation of the correspondent motor representation of the observed action is automatically activated. According to the dual-route model a second, partially independent route, allows participants to accomplish the task; this route is capable of performing voluntary arbitrary visuo-motor matching. Importantly these results are not ascribable to any potential spatial-compatibility effect since the observed hands are orthogonal to the position of the participants’ hand.

10. STUDY 5: TESTING OUR ACTION PRE-SELECTION HYPOTHESIS

10.1 Introduction

Results from the above described experiments show that motor resonance is present both when participants are required to stay at rest and when they have to produce motor responses. This datum is in agreement with a role of mirror neurons in action understanding as defined by Gallese et al. (1996). However, the mirror neuron theory of action understanding does not explain why we do not constantly imitate others when we are required to produce voluntary reactions in response to observed actions.

If "In our brain there are mechanisms (mirror mechanisms) that allow us to directly understand the meaning of the actions and emotions of others by internally replicating (simulating) them without any explicit reflective mediation (Gallese et al. 2004)" then the motor representation of the observed action should be accessed in order to understand the action. *After* the motor representation is accessed (and thanks to this access), the action is understood because the observer knows the outcome of the observed action *when he does it*.

Reacting with a voluntary action *conditioned* upon an observed one implies though that the action has to be understood before the voluntary action is performed since the latter is conditioned to the understanding of the observed one. Once the action has been understood, the motor representation of the observed action has to be inhibited, or it has to stop itself in order to let the system choose the "response" action that is arbitrarily associated with the “cue” action. This scenario seems to match exactly the picture drawn by the results of the above described experiments: first the simulation in order to understand, then the voluntary reaction in order to react.

However the initial automatic activation of the motor representation of the correspondent observed action should prompt an imitative reaction tendency. One possible explanation of why we do not imitate in response to an observed action could probably rely on the fact that we are not usually required to react as fast as possible to them. This explanation fits with the above presented data but is made unlikely by the fact that in some social conditions people are required to react as fast as they can in a non-imitative way and do not produce imitative errors.

The most highlighting examples come from sports. If the motor representation of an observed action is active as well as other alternative actions, then imitative errors should appear, however it is extremely difficult, even impossible I would say, watching a goalkeeper erroneously imitating the action performed by a player who is kicking the ball (in the context of a penalty kick for example). The same applies when, in the context of a basketball match, the player who is playing in attack shoots the ball to the basket: it is extremely unlikely to see the player in defense reacting with a shooting action in the attempt to perform a blocking action.

A tentative explanation of why imitative errors do not appear in those circumstances could be the following: a goal-keeper has a goal that is catching the ball. In order to maximize the probabilities of a success he may pre-select some acts (from his own “vocabulary”) useful to catch the ball. Plus, he has to inhibit those motor acts that are not useful in catching the ball (for example writing, eating, grasping, scratching etc…). Among these inhibited actions there should be also the action the goalkeeper is observing, that is kicking the ball. Then, once the ball is kicked the goal keeper decision making process is performed on a bunch of pre-selected actions, and not his own whole action repertoire.

If this holds true, then the motor resonance effect observed at early time-points in the condition in which participants had to perform the opposite action with respect to the one they saw, is driven by the sharing of the same action representations by the motor system and the experimental stimuli, meaning that the pre-selected actions in the observers’ motor system are the motor representations of the observed actions.

In the following experiments participants have been required to observe and index finger or a little finger abduction. In one experiment (COUNTER experiment) a group of participants was required to react to the observed action as fast as they could with the opposite action compared to the one they saw, namely, if they observed an index finger abduction they had to perform a little finger abduction and vice-versa. In another experiment (NEUTRAL experiment) participants were required to perform a lifting or a flexion of their middle finger in response both to index or little finger abductions. In half of the trials they received a single pulse TMS on their left M1 at two different times, that is, at 133 ms and at 150 ms from the onset of the movement in the video. Moreover, for both groups, the same actions were performed in response to a non-biological stimulus.

It should be made clear that the main difference between the two conditions is that in the COUNTER condition the response action is the same as one of the cue actions. In the NEUTRAL experiment the response actions are not the same as the cue actions. Therefore, if the action pre-selection hypothesis holds true, then the expectation is to find a faster behavioral performance in the NEUTRAL experiment than in the

COUNTER experiment. On a neurophysiological side the hypothesis is to find a motor resonance effect in the COUNTER experiment, but not on the NEUTRAL experiment.

10.2 Materials and Methods

10.2.1 Participants

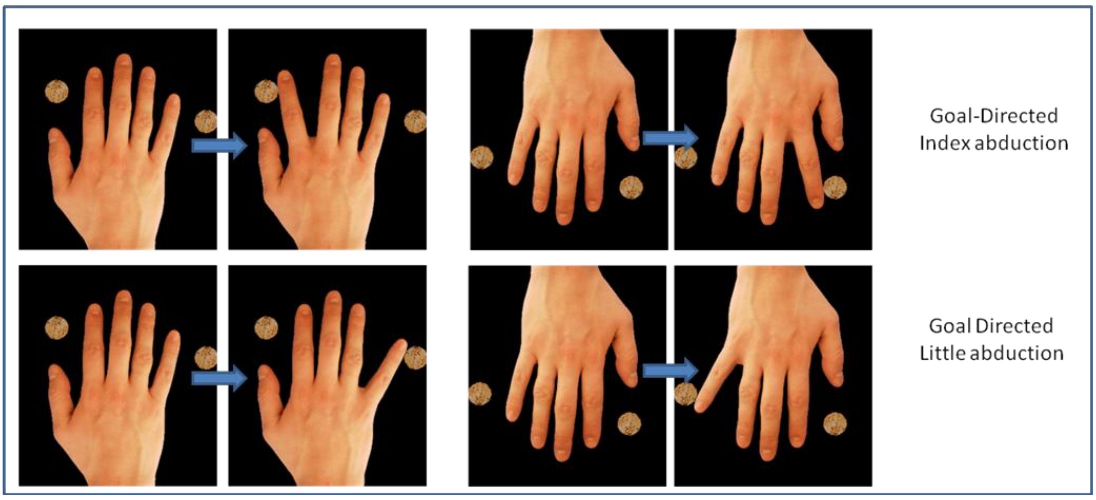
29 healthy participants (23 female, 6 males) were enrolled in the experiment. 16 of them were (14 females and 2 males, mean age =23.3) were assigned to the COUNTER condition, while the remaining 13 (9 females, 4 males, mean age = 23.1) were assigned to the NEUTRAL condition.

The experiment has been conducted in compliance with the Helsinki declaration (World Medical Association General Assembly, 2008) and with the approval of the Ethical local Commettee. All participants gave written informed consent to the experiment and were screened for contraindications to TMS (Rossi et al. 2009)

10.2.2 Stimuli

Differently from the time-course experiment two types of stimuli were presented: the HAND stimuli consisted in 7 right hands (4 female, 3 male hands) placed between two objects respectively close to the index and the little finger. The hand could abduct either the index or the little finger in order touch one of the objects. As for the time-course experiment stimuli were not videos but a sequence of two pictures (initial and end-position) presented one after another. The hand was presented in a dorsal perspective in the center of the screen; differently from the time-course experiment in half of the trials the fingers were pointing upwards (egocentric perspective),while in the other half they were pointing downwards (allocentric perspective) (Figure 24); the orientation of the presented hands was randomized across the whole experiment as well as the action observed.

HAND



SNAKE

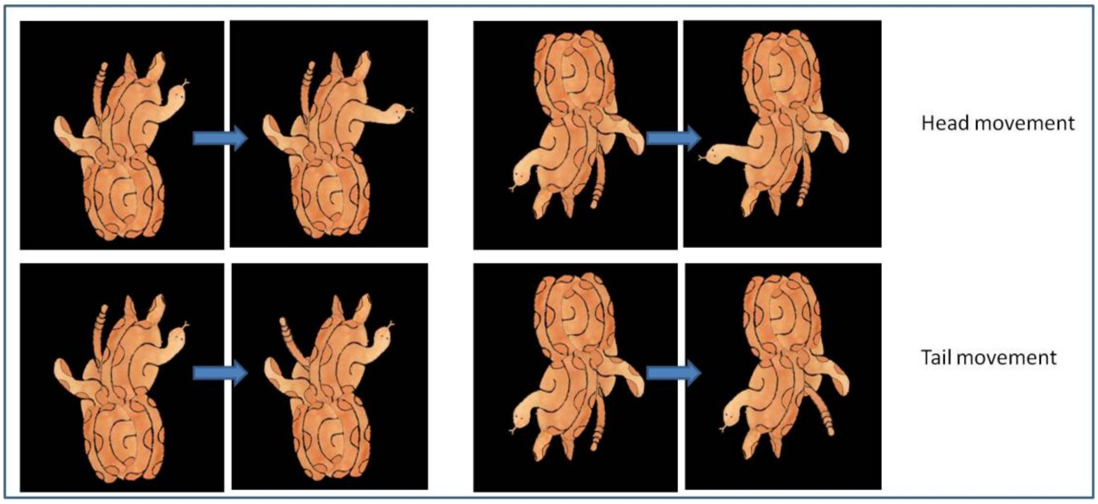


Figure 24
Stimuli observed in both COUNTER and NEUTRAL experiments. In the upper panel an example of both index and little finger abductions are showed both in vertical and up-side down orientation. In the lower panel the SNAKE stimuli are presented, showing tail and head movements both in vertical and up-side down orientations.

The other type of stimulus was a drawing of a bunch of snake bodies, among which only one snake was completely visible. This snake could move either the head or the tail (Figure 24). In order to make the SNAKE stimuli perceptually close to the HAND stimuli, 7 different bunches of snakes were "matched" at the best with one of each of the 7 hands in terms of the total area occupied by the two type of stimuli, and in terms of the initial and the final position of the relevant parts of the stimuli. Three "snake-body parts" were placed approximately in the same position of the finger tips corresponding to the thumb, the middle finger and the ring-finger in the HAND stimuli. The tail of the snake was kept thinner than the head (as the little finger is thinner than the index finger), but it was longer than the head (as the index is longer than the little finger), making in this way a potential resemblance with the hands relevant parts not obvious. The aim of this visual matching between the two type of stimuli was to present very similar stimuli according to the properties that could be relevant to perform a reaction time based on the parts of the stimuli that moved, but on the other hand, the choice of using a quasi-real animal was made in order to make the SNAKE stimuli not as a potential abstract representation of a hand, but as an object that has its own identifiable parts that were neither an index, nor a little finger. In this way HAND stimuli are "simulable" objects, while the SNAKE ones are not.

10.2.3 TMS

Electromyographic recording (EMG) was performed in the COUNTER condition on the Right First Dorsal Interosseus (1DI) and the Right Abductor Digiti

Minimi (ADM) muscles through Ag-AgCl surface electrodes placed in a belly-tendon montage, while in the NEUTRAL condition electrodes were mounted also on the Extensor Digitorum Communis (EDC) and on the Flexor Digitorum Superficialis (FDS). The signal recorded has been amplified by means of a 1902 CED amplifier (Cambridge Electronic Design) by a 1000x gain, band pass at 20-5000 Hz, and digitized by means of a CED Power 1401 analogical to digital converter, controlled by Signal® software. The EMG signal was sampled 4000 Hz. Data have been recorded and analyzed off-line.

TMS stimulation was delivered on participants' left M1, over the scalp portion on which the biggest MEPs amplitude was recorded using a slightly supra-threshold stimulation. The point was marked on the participants' scalp. Stimulation intensity across the whole the experiment was set to the intensity required to evoke MEPs on 1DI of about 0.5 mV at rest for each participant. Stimulation was performed with a 70 mm figure-of-eight-shaped coil connected to a Magstim Rapid. The coil was placed tangentially on the scalp with the handle pointing backwards and inclined at 45° with respect to the head midline. Throughout the experiment the coil was held by a mechanic arm on the participants' scalp.

10.2.4 Procedure & Task: COUNTER experiment

Participants sat on a chair in front of a PC monitor with their chin on a chin-rest. After the surface electrodes were mounted, participants' right hand was placed on a custom-made response box constituted by two plates placed respectively close to the index and to the little finger (the same used in the time-course experiment). Two adhesive rings were tightened on participants' index and little fingers; when one of these rings touched the nearest plate then a response was detected (the apparatus was the same used for the time-course experiment).

Participants' right hand was oriented with the fingers pointing leftwards with respect to the their body; this position kept their right hand orthogonally with respect to the orientation of the hands presented on the screen, in order to exclude potential spatial compatibility effects. Visual stimuli were presented using E-Prime® software on a monitor with a refresh rate of 60Hz.

Throughout the experiment in odd trials a single pulse TMS was delivered, while on the even ones no TMS pulse was given. 896 trials were equally divided in 8 blocks alternatively presenting HAND or SNAKE stimuli. Each block was in turn constituted by 56 TMS plus and 56 no-TMS trials each.

Across the experiment HAND blocks and SNAKE blocks were always alternated, and the order of block presentation counterbalanced; within each block the presentation of the events (index or little finger abductions for HAND stimuli and head or tail movements for SNAKE stimuli) was completely randomized.

Each trial began with a still hand (or a still snake), then, after a random time between 500 and 1000 ms, the target event was presented; in TMS trials a TMS single pulse was delivered at two different times (133, 150 ms) from the onset of the end-position picture. The order of the time of stimulation was randomized. Stimulation times were based on the results of the time-course experiment (150 ms) but, since the task was slightly, but significantly different, it was presumable that a potential mirror effect could have been arisen even before 150 ms, for this reason also stimulations at 133 ms were provided.

Participants were required to react as fast as they could to the observed action with an index finger abduction or a little finger abduction depending on the event they saw. Differently from the time-course experiment, here the go-signal was the event on the screen, not the TMS pulse. The end-position picture lasted until participants' response was detected for a maximum of 1150 ms; after providing the response, participants were presented for 400 ms, with their reaction time in milliseconds if the response was correct. A “Wrong!” or a “No Response” label was presented respectively either if the response was not correct or not detected. A cross in the center of the screen was presented until the trial had reach a duration of 2680 ms. Since the stimulation was delivered in odd trials but not in even ones, the time between the onset of one TMS trial and the following one was of 5360 ms, in order to avoid repetitive effects of consecutive TMS stimulations (Figure 25).

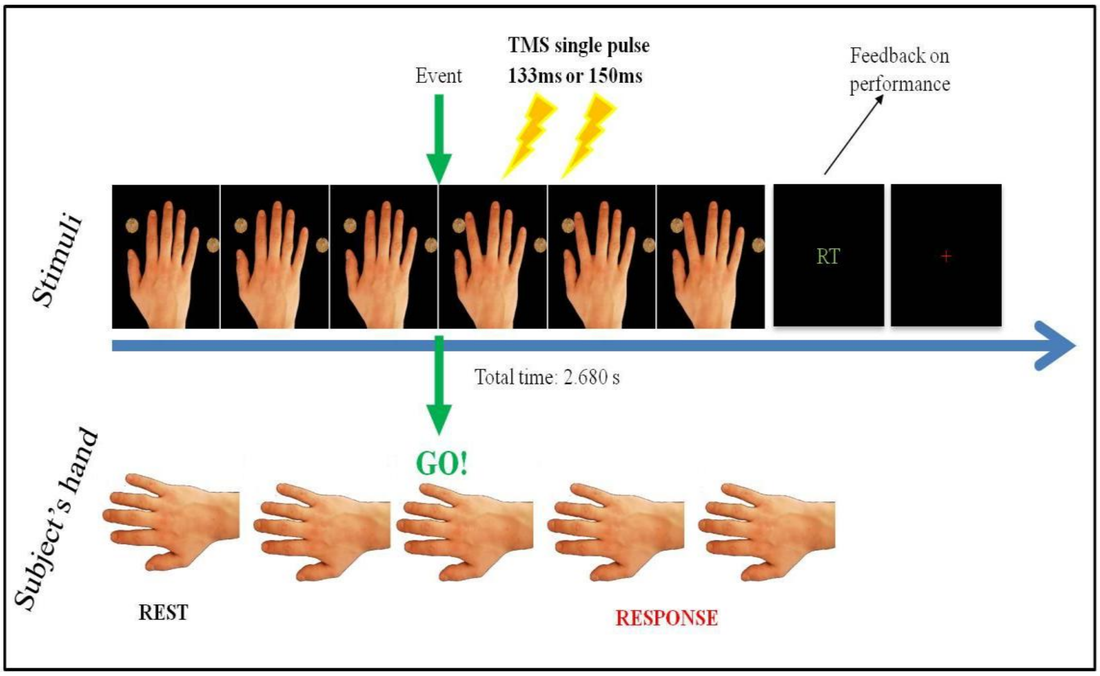


Figure 25

Schematic representation of the procedure and task in TMS-trials. Participants waited for the onset of the action in the observed stimuli. They were required to respond as fast as they could with the visuo-motor matching assigned to them. TMS was delivered at 133 ms and 150 ms from the onset of the observed action in the visual stimuli. After the response participants were provided with their reaction time.

Each time a muscle was visibly contracted, participants were required to relax their muscles as much as they could. At the beginning of the experiment 72 training trials were performed in order to let participants practicing with the response pad. In these 72 trials participants observed a white big dot in the center of the screen and two other smaller dots, a yellow and a blue one, each placed close to one or the other side of the big white dot. Participants were required either to abduct their index if the yellow dot moved or to abduct their little finger if the moving dot was the blue one. Before, participants were required to stay as relaxed as they could between one motor response and following.

Participants were also required to respond as fast as they could but also as accurate as they could in order not to privilege one criterion on the other. Finally they were told not to guess the following event but to respond only after having actually observed an event happening on the screen. This was required in order to prevent a (not successful) strategy in which participants had already decided which action to perform without conditioning their choice upon the visual stimulus event. To strengthen this point an example was made that if the same event appeared three times consecutively, then that event had the same probability to happen in the following trial as the opposite one. If the TMS stimulation produced a hand evoked movement large enough to displace the hand, the hand was tightened on the pad. Since those task requirements were not easily achievable because of the arm position and the muscle relaxation requirements, thin foam pillows were placed under participants' elbow if this helped participants to keep the recorded muscles more relaxed.

Before the beginning of the experiment participants were instructed on the visuo-motor matching they had to perform throughout the experiment. While for the HAND stimuli the participants were always required to abduct the finger opposite to the one that was seen moving, for the SNAKE stimuli half of the participants responded with an abduction of the index finger for a tail movement and with a little finger abduction for a head movement (the other half were required to perform the complementary visuo-motor matching, "see tail→move little finger" and "see head→move index finger"). This was done in order to avoid constant biasing associations between the parts of the snake and those of the hand that shared the same spatial position within each type of stimulus. This means that, according to the position on the screen, the tail would have been considered "as an index finger" and the head "as a little finger" (Figure 24). In this way, this biasing spatial association was avoided across participants. Instructions on the visuo-motor matching, that was always the same throughout the experiment for both HAND and SNAKE stimuli, were repeated at the beginning of each block. Every 2 blocks (2 blocks = 1 session, 4 session in total) the coil has been changed to cool it in order not to let it switching off in the middle of the experiment.

10.2.5 Procedure & Task: NEUTRAL experiment

The procedure for the neutral condition was basically the same as the one described for the COUNTER condition. The most important difference was that participants were required to react to the visual events by lifting their middle finger in order to touch a metal plate placed above it, or by flexing their middle finger, in order to press a button placed underneath it. In order to keep recorded muscles relaxed a little piece of foam was placed above the button; this allowed the middle finger (and the whole hand) to lay relaxed above the button without pressing it. The hand orientation and position were identical to the COUNTER experiment. (Figure 26). As for the COUNTER experiment, 72 training trials were performed before the real experiment. In these practice trials participants had to lift their middle finger and flex respectively when they saw the yellow and the blue dot moving.

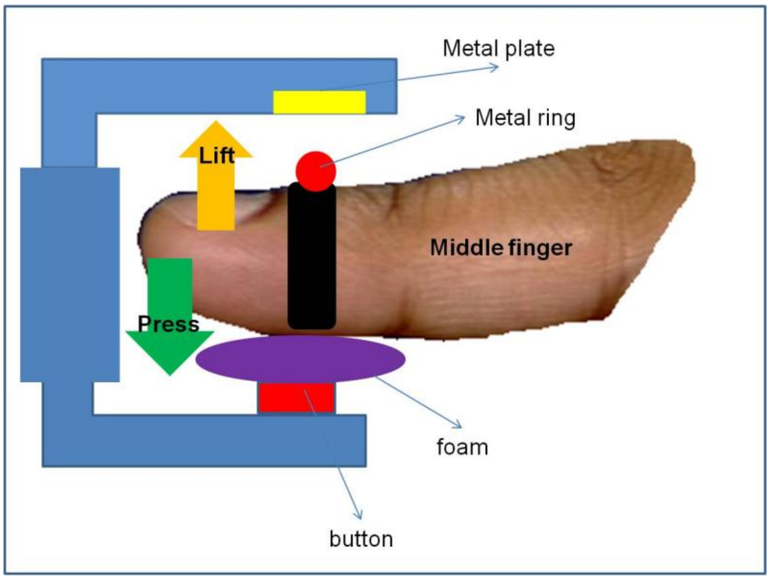


Figure 26
Schematic representation of the position of the middle finger within the response box for the NEUTRAL experiment.

As specified before, the hands were presented both in an allocentric or in an egocentric perspective. The reason is that lifting or flexing the middle finger would have created a spatial compatibility bias when observing events on the screen since the hypothetical spatial plan of both the observed and performed movement was the same. Instead presenting participants with hands in a vertical perspective while they were keeping the arm in a horizontal position diminished the influence of a less likely confounding spatial compatibility effect. Let’s assume that participants were laying their hand with the fingers pointing towards the screen, and the event observed was an index finger abduction when the observed hand was oriented with the fingers pointing rightwards (as in the time-course experiment). This results in a movement in which the observed finger from a (spatial) neutral position, goes up, pointing upwards (Figure 20 left lower panel). Then, if participants were supposed to flex their middle finger in response to the observed event, then this would result in an incompatible spatial movement, since on the vertical axis, the observed finger goes up, while the participants' finger goes down (and vice-versa if the participants were supposed to flex their middle finger in response to a little finger abduction). Now let's consider the present set: the orientation of the hand is, for example, egocentric so in the initial-position picture, both fingers point toward the upper side of the screen, and participants are laying their hand with the fingers pointing leftwards. Then both an observed little or an index finger abduction would result in a lateral movement, but in any case, the finger displaced would continue to point towards the upper side of the screen (Figure 24 HAND left panel). So if participants were required to lift their middle finger in response to an index finger abduction, then the spatial component shared by both the observed and the produced movement would be less compared to the previously described situation.

The visuo-motor matching in the NEUTRAL experiment were balanced both for the HAND and for the SNAKE stimuli. This means that, when watching HAND stimuli, half of the participants were required to lift their middle finger in response to index finger abductions and to flex their middle finger in response to little finger abductions; the other half performed the complementary matching; among these two groups half of the participants were required to lift their middle finger in response to tail movements when observing SNAKE stimuli (and to flex the middle finger in response to the head movements), and half of them were required to flex their middle finger in response to tail movements (and to lift the middle finger in response to the head movements).

10.2.6 General Data Analysis

No-TMS trials were extracted both from the COUNTER and from the NEUTRAL experiment. The analysis has been performed comparing both experiments in the same analysis. For the TMS trials instead two analyses have been performed separately for COUNTER and NEUTRAL experiments.

10.2.7 Data Analysis (no TMS trials)

Reaction Times were collected from participants responses. Non-correct responses or non-detected responses were excluded from the analysis. Reaction times of the two alternative responses were collapsed together. A 20% trimmed mean was computed separately for the reaction times on HAND and on SNAKE stimuli separately for both experiments. 2 x 2 ANOVA has been applied with VIDEO (HAND and SNAKE) as a within-subjects factor and TASK (COUNTER or NEUTRAL) as a between-subjects factor. A post-hoc test Fisher’s LSD was applied to the ANOVA results.

In order to compare the reaction times of the two experiments a normalization ratio between HAND stimuli and SNAKE stimuli was produced in order to account for potential task-specific differences between COUNTER and NEUTRAL tasks. Finally a one-way ANOVA has been performed on the normalized data.

10.2.8 Data Analysis (TMS trials)

MEPs from 1DI and ADM were detected on the EMG traces and the peak-to-peak amplitude was determined. MEPs were first of all inspected visually in order to individuate the time at which the MEPs were produced and when they showed their peaks. The time-window for the algorithm to search the peaks was set at the onset of the MEPs and shortly after the time at which the lowest peak was found. This procedure allowed to search for the MEPs peaks without wrongly considering EMG signal from possible voluntarily responses produced close to the MEPs. Trials showing EMG activity on 1DI or ADM exceeding the amplitude of 50 µV either in the 100 ms before the TMS pulse or between the TMS pulse and the onset of the MEPs, were discarded. Trials in which the TMS produced MEPs lower than 50 µV either on 1DI or ADM were excluded from the analysis as well.

The average amplitude of the MEPs for each of the 4 sessions (two consecutive blocks), was computed separately for each participant for 1DI and ADM. Then all the MEPs were normalized on the MEPs averages corresponding to their session, separately for 1DI and ADM, in order to account for possible differences in the coil position throughout the experiment.

Once the MEPs were normalized on the average of their own session, for each trial a (1DI-ADM) measure was computed. Then for each combination of type of event (index finger, little finger, tail, head) and time of stimulation (133, 150) a 20% trimmed mean of the (1DI-ADM) was computed.

Finally in the COUNTER experiment, considering HAND stimuli, the (1DI-ADM 20% trimmed mean) measure for the observation of a little finger movement was subtracted by the same measure for an index finger movement presentation [1DI-ADM(see index) - 1DI-ADM(see little)]. Importantly, since participants had to perform the opposite movement to the one they observed, the described process for HAND stimuli can be reformulated saying that the measure (1DI-ADM 20% trimmed mean) detected when participants had to respond with the their index finger, was subtracted from the same measure found when participants had to respond with their little finger [1DI-ADM(move little) - 1DI-ADM(move index)]. For the SNAKE stimuli the same process was applied according to the visuo-motor matching assigned to the participants. As for the HAND stimuli, when participants had to respond with the their index finger, the measure (1DI-ADM 20% trimmed mean) was subtracted from the same measure found when participants had to respond with their little finger. So the matching was not performed on the visual components of HAND and SNAKE stimuli, but on the shared motor components between the two stimuli. For example if the instructions for SNAKE stimuli were "see head" → "move the index finger", "see tail → move little finger", then in terms of motor responses the head corresponds, in the HAND stimuli, to the observation of a little finger ("see little finger" → "move index finger") while the tail corresponds to the index finger ("see index finger" → "move little finger"). So the final comparison in terms of observed events would be [1DI-ADM(see index) - 1DI-ADM(see little)] vs. [1DI-ADM(see tail) - 1DI-ADM(see head)]. The comparison can be reformulated equivalently specifying the effector that the participants were required to move [1DI-ADM(move little) - 1DI-ADM(move index)] vs. [1DI-ADM(move little) - 1DI-ADM(move index)].

For what concerns the NEUTRAL experiment the same procedure was applied as well. The dependent variable within the HAND stimuli was always the 1DI-ADM

20% trimmed mean when participants saw index finger abductions minus the same measure when participants observed little finger abductions. Differently from the COUNTER experiment, the visuo-motor matching between the observed event and the motor response changes when considering HAND stimuli. So, in terms of motor responses, the above described difference can be reformulated as [1DI-ADM(lift middle) - 1DI-ADM(flex middle)] if participants were required to lift the middle finger when presented with index finger abductions, while, the same comparison would result in a [1DI-ADM(flex middle) - 1DI-ADM(lift middle)] if participants were required to flex their middle finger when they observed index finger abductions.

Within the SNAKE stimuli the matching was performed based on the effector that was moved in the HAND stimuli: if participants were required to perform a lifting of their middle finger in response to the index finger abduction and a flexion to the little finger abduction [1DI-ADM(lift middle) - 1DI-ADM(flex middle)], then the correspondent situation within the SNAKE stimuli would be considered [1DI-ADM(lift middle) - 1DI-ADM(flex middle)]. Again the comparison between HAND and SNAKE stimuli is performed on the visual stimuli that shared the same motor responses in HAND and SNAKE stimuli, not on the visuo-spatial shared components. In both experiments if the motor resonance takes place the [1DI-ADM(see index) - 1DI-ADM(see little)] measure should be higher when participants watch HAND stimuli compared the same measure in the SNAKE stimuli.

At the end of this procedure participants with less than 20 analyzable trials left on at least one cell were excluded from the subsequent analyses. Two subjects were excluded due to this reason (one female in the COUNTER experiment and one male in the NEUTRAL experiment).

Two separated 2 x 2 ANOVAs for the two experiments have been carried out. For both experiments the variables involved were VIDEO (HAND or SNAKE) and TIME (133 or 150).

10.3 Results

10.3.1 Results: COUNTER experiment (TMS trials)

The ANOVA performed in the COUNTER experiment with VIDEO and TIME as factors resulted in a significant main effect of VIDEO (F(1,13) = 13.13; P = 0.003) with the (1DI-ADM) measure higher when showing HAND stimuli compared to SNAKE stimuli, and with a significant main effect of TIME (F(1,13) =10.61; P=0.006), with the (1DI-ADM) measure higher at the stimulation time of 133 ms than 150 ms. No interaction has been found for VIDEO x TIME (F(1,13) =1.59; P > 0.05) (Figure 27).

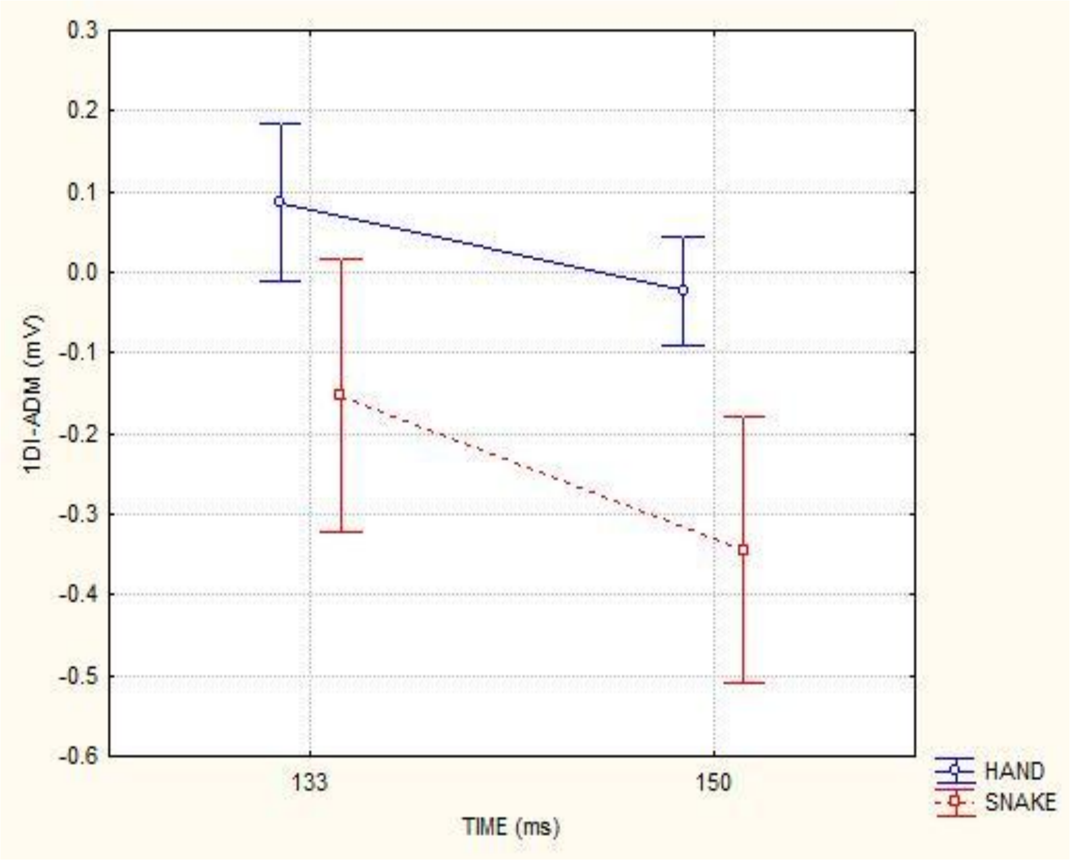


Figure 27
COUNTER experiment.
The Plot shows the [1DI-ADM(see index) - 1DI-ADM(see little)] (equivalent to [1DI-ADM(move little) - 1DI-ADM(move index)]) measure during the observation of HAND stimuli (in blue). The same measure [1DI-ADM(move little) - 1DI-ADM(move index)] for SNAKE stimuli (in red).
If the blue line is above the red one, this indicates that the quantity of motor resonance is higher when participants responded to HAND stimuli compared to SNAKE stimuli. Bars indicates 95% Confidence intervals.

10.3.2 Results: NEUTRAL experiment (TMS trials)

The ANOVA performed on the NEUTRAL experiment with VIDEO and TIME as factors did not yield any significant results.

However a closer inspection of the plots suggests that at 150 ms the (1DI-ADM) measure is indeed significantly higher for the HAND stimuli than for STIMULI. A pairwise T-test was then performed on the stimulation time of 150 ms, comparing the two type of stimuli. A results close but not significant has been found (t(11) = 2.13; P = 0.056) (Figure 28).

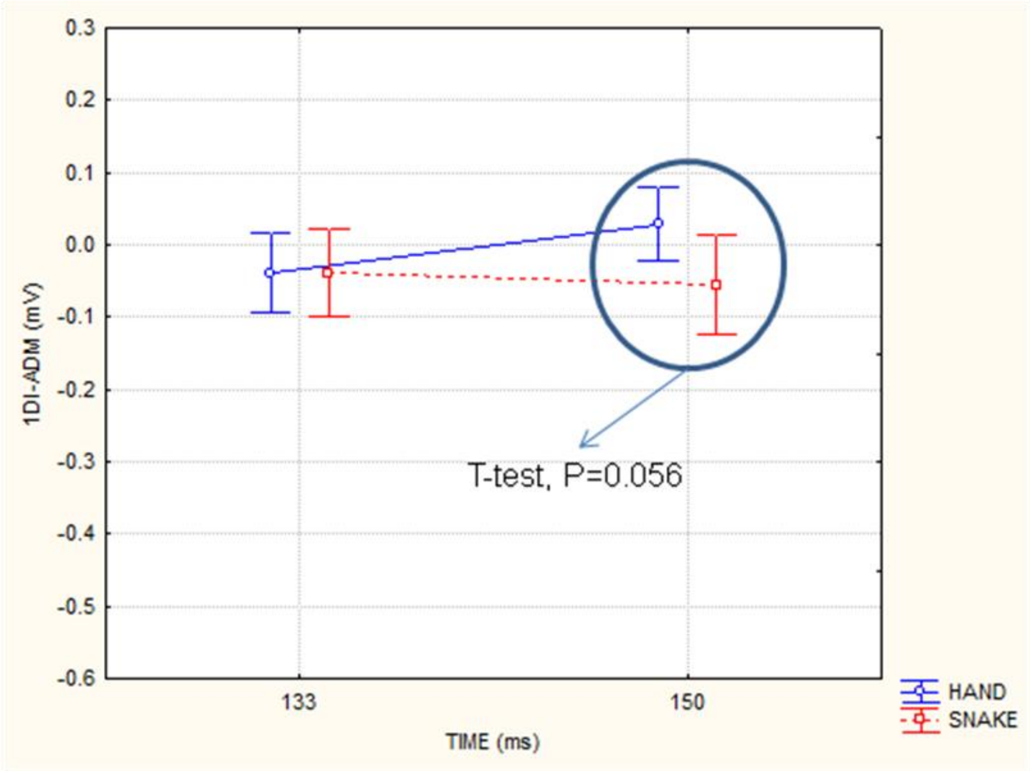


Figure 28
NEUTRAL experiment.
The Plot shows the [1DI-ADM(see index) - 1DI-ADM(see little)] measure when participants performed the required action (lifting or flexing) during the observation of HAND stimuli (in blue) and SNAKE stimuli (in red) (see data analysis for TMS trials for details on the matching). If the blue line is above the red one, this indicates that the quantity of motor resonance is higher when participants responded to HAND stimuli compared to SNAKE stimuli. Bars indicates 95% Confidence intervals.

10.3.3 Results: no-TMS trials

2 x 2 ANOVA on “no-TMS trials” yielded a main effect of the VIDEO with the HAND stimuli slower than the SNAKE stimuli (F(1,24) = 90.46; P < 0.00001). A second main effect of the TASK was found, with the COUNTER task slower than the NEUTRAL task (F(1,24) = 12.65; P=0.002). An interaction has also been found F(1, 24) = 41.7, P < 0 .00001).

Fisher LSD post-hoc analysis revealed a significant difference between HAND (547 ms) and SNAKE (431 ms) in the COUNTER task (P < 0.00001), another significant difference between tasks when responding to HAND stimuli, with RT on HAND stimuli in the COUNTER condition (547 ms) higher than the HAND stimuli in the NEUTRAL condition (408 ms) (P = 0.00076). Finally RT on HAND stimuli in the COUNTER condition (547 ms) are higher than those on SNAKE stimuli in the NEUTRAL condition (388 ms) (P = 0.000002). No other effect has been found significant (Figure 29).

The ratio comparison yielded a significant result with the normalized HAND stimuli in the COUNTER condition slower (1.27 ratio) the normalized HAND stimuli in the NEUTRAL condition (1.05 ratio). F(1,24) = 40.526, P < 0.00001).

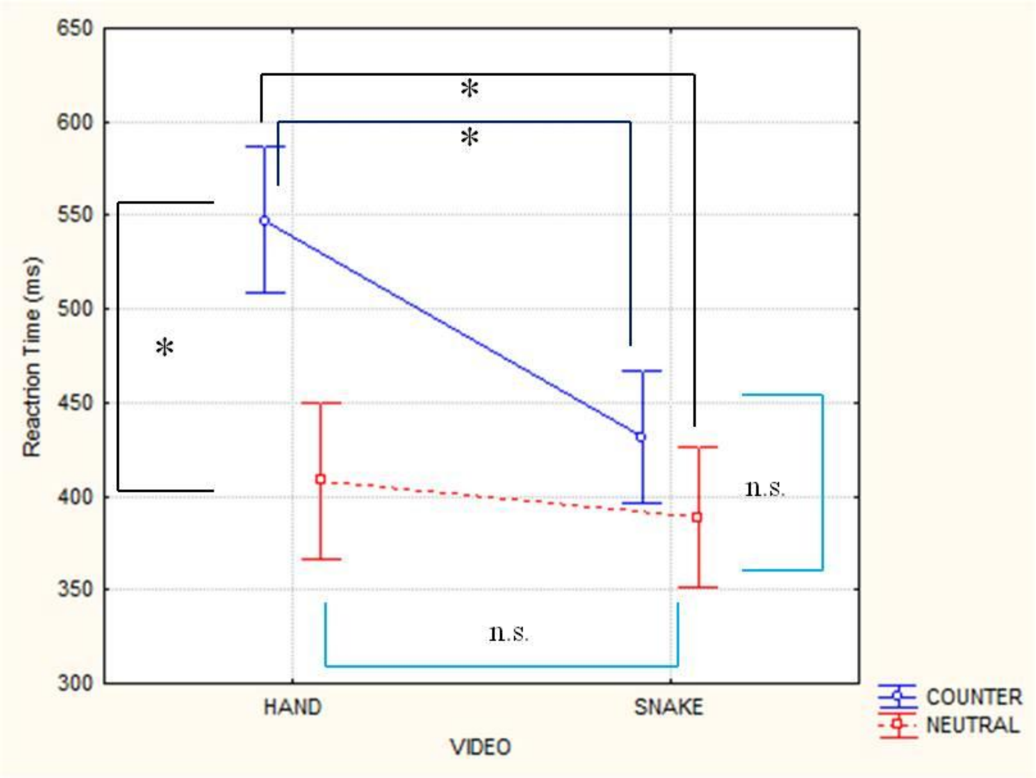


Figure 29
Reaction times in the COUNTER condition (blue) and in the NEUTRAL condition (red) for HAND and SNAKE stimuli. Bars indicates 95% Confidence intervals.

10.4 Interim Discussion

The main effect found in the TMS trials for the VIDEO in the COUNTER experiment suggests that when the task requires to perform the opposite action compared to the one the participants saw, this results in a higher [1DI-ADM(see index) - 1DI-ADM(see little)] measure when a hand is observed, compared to the correspondent comparison on the observation of non-biological stimuli. This result suggests that motor resonance is present when observing a hand, but not when observing a non-biological action. A main effect of the TIME then indicates that the likelihood that participants performed the correct response (the opposite one) increases with time. This scenario confirms in part the results from the time-course experiment since a significant mirror effect has been found at 133 ms other than 150 ms.

For what concerns the results from the NEUTRAL condition, no main effects or interactions have been found. However a pairwise t-test suggests that there is a tendency to the statistical significance at 150 ms meaning that for this particular condition a simulation could arise later in time. If the action pre-selection hypothesis disrupts the simulation process, then there should be no difference between the VIDEO stimuli in this condition. It is possible that the action pre-selection has the effect of delaying the motor resonance but not of disrupting it. The scope of the present work is to find a cognitive set that allows to stop the simulation in order to understand whether the action understanding is possible. Data from the no TMS trials point towards a clear modulation of the motor resonance effect when performing the NEUTRAL task in response to HAND stimuli, in which reaction times are faster compared to the COUNTER task.

Can something be said about action understanding given the present results?
If the putative simulatory process found at 150 ms is used in order to understand the action, the voluntary response provided by participants performing the NEUTRAL condition should be present only *after* the simulation has taken place but not before, since this is the causal relationship hypothesized by a simulative account of action understanding, namely, first the simulation in order to understand, then the correct motor response. In order to test this hypothesis we exploited the recording of the muscles that primarily allow to perform the lifting and flexion of the middle finger.

Secondarily it could be argued that a comparison between the 150 ms time between cannot be performed since the SNAKE is not a good common baseline, since for the COUNTER condition is directly influenced by the voluntary response, but not in the neutral condition. So in order to test at which time the effect differ between the stimuli an ANOVA with TIME (133 ms, 150 ms) as a within factor and TASK (COUNTER, NEUTRAL) as between factor has been carried out.

10.5 Secondary data analysis

MEPs from EDC and FDS have been compared when participants watched HAND stimuli in the NEUTRAL condition in order to understand whether the neurophysiological sign of a correct response was already present in the participants' motor cortex at 150 ms when responding to hand stimuli. Since the muscular relaxation of both muscle has not been controlled, the trials with an EMG trace before the TMS pulse higher than 100 µV were excluded, while a standard (50 µV) criterion has been chosen for EMG contraction between the TMS pulse and the MEP onset. The choice of using this more lenient criterion of exclusion has the advantage of increasing the number of the analyzable trials, but on the other side it could lead to a bigger variability in the data since it is known that the muscle contraction increases the MEPs amplitude.

The analysis process is analogous to the one described for 1DI and ADM. A compound measure constituted by a normalized (EDC-FDS) 20% trimmed mean was performed for each time of stimulation. The final measure is composed by the normalized 20% trimmed mean (EDC-FDS) when participants have to press the button, subtracted from the correspondent measure when participants have to lift of their middle finger [EDC-

FDS(lift middle finger) - EDC-FDS(flex middle finger)]. If the response is present in M1 before the putative motor resonance effect at 150 ms, then the expectation is to find a [EDC-FDS(lift middle finger) - EDC-FDS(flex middle finger)] measure higher than zero and that increases in time (reflecting the higher probability of choosing the correct response).

10.6 Results from the secondary data analysis

A one-way ANOVA on the HAND stimuli with TIME (133 ms, 150 ms) as factor has been performed. The ANOVA yielded a significant effect of the TIME factor (F(1,11) = 6.888; P = 0.024) (Figure 30).

With the [EDC-FDS(lift middle finger) - EDC-FDS(flex middle finger)] measure higher at 150 ms than at 133 ms, single sample two tails t-test has been performed on the two times in order to test whether one (or both) times showed the [EDC-FDS(lift middle finger) - EDC-FDS(flex middle finger)] measure significantly higher than zero when paerticipants were required to respond with a lifting of their middle finger. The t-test performed on both the compound measure at 133 ms and at 150 ms showed a significant difference from zero (respectively t(1,11)=2.73; P= 0.019) and t(1,11)=4.54; P= 0.0008).

For what concerns the direct comparison of the COUNTER and NEUTRAL tasks on HAND stimuli the ANOVA yielded no main effect neither of the TIME nor of the TASK, but only a significant interaction between the two factors (F(1, 24) = 8.21; P = 0.0085). Fisher LSD post-hoc analysis revealed a significant difference of the dependent variable at 133 ms between the two tasks, with the COUNTER (0.086 mV) significantly higher than the NEUTRAL (-0.0384 mV) (P = 0.023). A second significant difference has been found within the COUNTER condition between 133 ms (0.086 mV) and 150 ms (-0.0226 mV). No significant difference between tasks was found at 150 ms (Figure 31).

This result shows that when performing the NEUTRAL task, the correct response is already present at 133 ms. The probability of a correct response is even increased at 150 ms. This increase excludes the possibility that the 133 ms result could be due not to the response selection but to unspecific factors since it does not represent the typical increase of the MEPs when the pulse is delivered before the reaction time (Burle, Bonnet, Vidal, Possamai, & Hasbroucq, 2002; Burle, Vidal, Tandonnet, & Hasbroucq, 2004; Leocani, Cohen, Wassermann, Ikoma, & Hallett, 2000; Tandonnet et al., 2011). If the TMS is delivered close enough to the reaction time, it has been shown that MEPs from the non-responding effector decreases (Tandonnet et al., 2011). Thus, precise predictions can be made: if the increase of cortical excitability is non-selective, then, there should be no such increase in the differential index utilized because both muscles should increase and their difference would be zero. The fact that a sign of a voluntary correct response is present at the appearance of a putative mirror effect or even before, suggests that, at least in the case of a neutral response, there is no need for simulation.

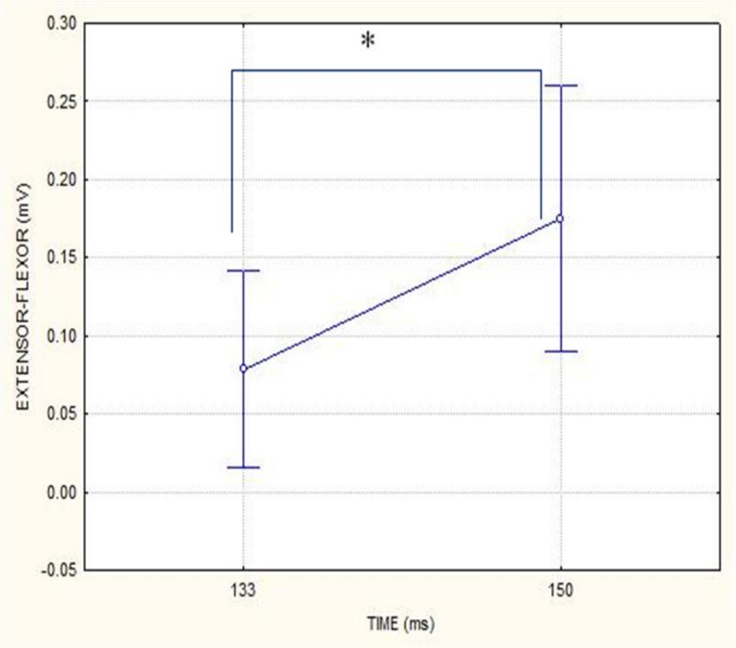


Figure 30
[EDC-FDS(lift middle finger) - EDC-FDS(flex middle finger)] measure in the NEUTRAL condition when participants observed HAND. Bars indicates 95% Confidence intervals.

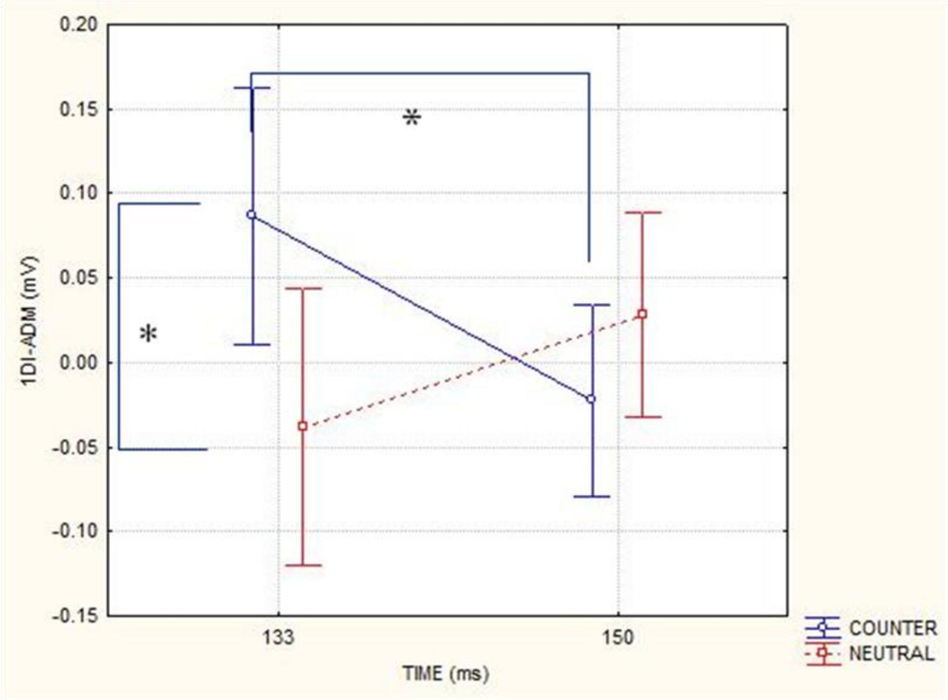


Figure 31
The Plot shows the [1DI-ADM(see index) - 1DI-ADM(see little)] measure when participants performed during the observation of HAND in the COUNTER experiment (in blue) and in the NEUTRAL experiment (in red). Bars indicates 95% Confidence intervals.

10.7 Final Discussion

Taken together these data suggest that action pre-selection effectively modulates the degree of the mirror effect. Higher reaction times have been observed when performing a COUNTER compared to a NEUTRAL task. This difference is in accordance with the idea that motor simulation is not performed (or does not contribute to the task) when the actions to be performed are not the same as the ones observed, but not when they are the same.

The core idea of my interpretation of this behavior is that the subject prepares to react by suppressing all the action representations that are not useful for the task required to perform. In the NEUTRAL condition motor representations that participants have to use are not the ones corresponding to the action they will observe and are therefore actively inhibited, ultimately resulting in the suppression of action mirroring.

On the contrary in the COUNTER condition the observed motor representation cannot be possibly inhibited because they correspond to a possible response. This allows motor mirroring to occur and, in order to react appropriately, participants have to replace the mirror motor pattern with the instructed one on-line. Results from the no-TMS trials point towards this direction. However it could be argued that RT results are not enough to draw the above conclusions and excluding other hypotheses involving motor simulation even in the NEUTRAL task.

TMS data instead provide a neurophysiological account of the simulatory process while observing actions. Results from the COUNTER experiment suggest that the simulation takes place at very early times, specifically at 133 ms with respect to the observation of the snake at the same time. For what concerns the NEUTRAL experiment no significant effect has been found, however a tendency in showing the signature of a simulation can be detected at 150 ms. Then the interim conclusion is that the action pre-selection does modulate the automatic simulation. A direct comparison of the two tasks on the HAND stimuli revealed a significant difference at 133 ms due to the fact that in the COUNTER experiment motor simulation was larger with respect to the same time point in the NEUTRAL task, while at 150 ms no significant difference was found. These results seem to suggest that the action pre-selection can indeed modulate the simulation process. The question that arises at this point is whether it disrupts the effect, delays it, or suppresses it for a brief period.

Simulation is thought to transform visual representations of actions into their correspondent motor representations. Accessing to the motor representation allows action understanding. Assuming that a possible sign of simulation has been detected in the NEUTRAL condition at 150 ms, implies that the correct response can be prepared only after the simulation has taken place, meaning that there should be no signature of the voluntary response until 150 ms, so it has been decided to use the differential amplitude of the two antagonist muscles that allow the lifting or the flexion of the middle finger in the NEUTRAL condition when participants observed HAND stimuli in order to detect whether the correct motor response was already instantiated at 133 or at 150 ms.

Results suggest that a voluntary correct response was already instantiated at 133 ms and it was significantly higher at 150 ms. According to the simulative theory of action understanding no indexes of a correct response should have been found until 150 ms because action mirroring had not happened yet. Taken together these data suggest that the motor simulation is not a necessary condition in order to understand an action.

An alternative explanation of these data, that cannot be disambiguated with the present experiment, is that the TMS stimulation has been delivered on M1, so that it is possible that instead of delaying or disrupting the simulation, the action pre-selection has only interrupted the communication between PMv and M1. So it is possible that the difference detected at the level of M1 between the two tasks is not explainable in terms of delaying or disrupting the simulation effect, but it can be simply interpreted as the action pre-selection made the simulation not detectable from M1.

The comparison between the present experiment and the time-course experiment highlights some differences. A motor resonance effect is present at 133 ms differently from the time-course experiment. One potential explanation is that the present task boosts the visuo-motor system in a more efficient way, so both the voluntary and the simulation systems access more efficiently the motor representations.

11. CONCLUSIONS AND FUTURE DIRECTIONS

Mirror neurons are certainly one of the most interesting discoveries in neuroscience, but most of all in cognitive neuroscience, meaning that the discussions and the debate that they triggered will provide a better view of the relationships between psychological processes and neurons. The core topic of the debate is the claim that representations of higher cognition are based and constituted by low-level sensori-motor representations. In the case of mirror neurons and the simulative theory of action understanding, the idea is that the simulation of the action observed, namely the direct access to the correspondent motor representation of that action, is fundamental in order to understand an action.

Recently a review by Rizzolatti & Sinigaglia (2010) lowered the claim of an essential role of the motor representations in action understanding, meaning that an action can be understood even if the observer does not know how to perform that action. In particular the authors resumed the argument provided at the end of the Hickok’s critical paper (Hickok 2009) against mirror neuron theory of action understanding: the argument recites that action understanding dissociates from the motor capability of knowing how to perform an action. Indeed it is easy to recognize that one is playing the saxophone even if the observer has never played that instrument, or to recognize that a dog is barking even if the observer does not know how to bark. The reformulation of the mirror neuron theory of action understanding states that if a saxophone player and a non-musician look at a musician playing a saxophone, then the two type of understanding are different, namely, the observer who *knows how* to play saxophone understands “from inside” the action, while the non-musician cannot access this type of experiential knowledge. The same theoretical position is shared by other researchers (Gallese et al. 2011).

As suggested by Mahon & Caramazza (2008), it is not necessary to hang on to a theory of motor understanding because the motor areas are active while observing an action: the authors made an example from psycholinguistics in which it has been demonstrated that subjects are faster to decide that two words rhyme when they are orthographically similar (pie - tie) than when they are orthographically dissimilar (rye tie) (Seidenberg & Tanenhaus, 1979). The authors’ argument that “In this case, however, the implication is not that there is a representation in common between phonology and orthography. Instead, it is concluded that the auditory presentation of a word automatically leads to activation of its orthography, and that the decision process promiscuously uses any information that is available for the execution of that task”.

This argument can be redirected to the motor involvement in action understanding: a non-musician can understand that another is playing a saxophone, but if the observer is a saxophone player, then the information that he can achieve through the access to the corresponding motor representations is higher, without linking the understanding concept exclusively to the motor representation access. Then, both these positions (Rizzolatti & Sinigaglia 2010 and Mahon & Caramazza 2008) seem to assert that if one knows how to do an observed action, he/she gains more information about that action, with particular reference to the motor consequences that the action observed implies, so there is more “understanding” in one condition compared to the other. Such understanding is not in terms of action semantics but in terms of experiential knowledge.

It is worth noting that previous models of action understanding, especially those derived from the cognitive analysis of patients with brain lesions, do not posit a role for the motor knowledge in understanding an action. The mirror neuron theory of action understanding, even if it contains some critical problems, has the merit of heaving brought to the scientific community the suggestion that low-level motor systems can contribute to higher cognitive representations. The discovery that areas in the motor system are indeed active when an observer watches an action, was not forecasted by previous models of action comprehension (Rothi et al. 1991; Cubelli et al. 2000). In defense of the mirror neuron theory of action understanding moreover, not many other viable hypotheses have been carried out in order to explain the huge amount of data about the motor involvement in action observation.

The data from the first two experiments described in the present work indeed clearly indicate the involvement of motor representations, in action understanding. In these experiments creating a motor memory of a certain motor act produced a shift in the judgments on the others' actions. On the other side it is interesting to understand the limits of the involvement of the motor representation in action understanding. In our experiments we disrupted the process of automatic simulation and tested whether action understanding persisted under these conditions. We looked for an experimental paradigm in which simulation was abolished and found in the literature a potential method to modulate the mirror neuron characteristics, by reversing their functional properties, i.e. the counter-mirror training. However, testing the time-course of this potential reverse lead us to the conclusion that mirror neuron activity is not abolished in those conditions but rather has to be searched at very early time points after the action onset. The final result is that a short counter-imitative training cannot reverse the motor resonance at early time points, but only at later time points that are however unlikely to represent the time window in which mirror neurons are active (see the dual route model proposal in chapter 8). One potential reason explaining why the motor resonance effect has been found in the counter-imitative experiment is that the test after the counter-imitative training has been performed at rest, as is usually done in other experiments testing action observation with the TMS. So we tested the time course of the cortical excitability in a quasi-ecological condition; participants had to perform a counter-imitative response as soon as they heard the TMS delivering a pulse. Even in this condition an early motor resonance effect was been found followed by the reverse cortical excitability pattern, in line with the task participants had to perform. At this point it seemed that mirroring of observed actions is pervasive, even when the motor resonance does not favor motor performance and, on the contrary it introduces a delay in producing an instructed response.

Motor performance therefore turned out to be a good index to identify situations in which a fully voluntary motor reaction can take place when reacting to an observed action. We therefore decided to abandon the imitative/counter-imitative logic and explore a dimension that was intuitively linked to more efficient motor responses to action observation, that is the production of a response that is neither imitative or counter-imitative but different from all observed motor cues. Behavioral data provided a very strong suggestion on that direction, being more difficult to react in a counter-imitative way compared to reacting with a motor response that is "neutral" with respect to the observed actions. At a neurophysiological level, however, it was observed that in the performance of a "NEUTRAL" task, a tendency toward motor mirroring was found around 150 ms from the onset of the observed action but this occurred later than the earliest signs of production of a correct response. We therefore succeeded in producing an experimental paradigm in which motor simulation was completely separated from motor performance.

A potential mechanism to explain these data is that of the action pre-selection. The hypothesis asserts that if participants are required to react in a conditional fashion to two observed action with other two different motor responses, let's say with a neutral motor response compared to the action observed (i.e., the motor responses do not correspond to the actions observed), then the simulation disappears because the participants had pre-selected only the motor responses needed to perform the task efficiently inhibiting all the others including the actions observed.

The present data indeed show that action pre-selection modulates motor resonance effect, but it does not abolish it completely. The search for a cognitive set, or a paradigm that dissociated action performance form action simulation however was instrumental to reach the final aim of the present thesis, i.e., testing action understanding in absence of simulation. The results in the NEUTRAL condition turned out to be a good situation for testing whether action understanding when simulation has not appeared yet. MEPs from the responding muscles in the NEUTRAL condition showed the sign of the correct response implementation already at 133 ms and significantly more at 150 ms. This last datum however, has to be considered with caution. It could be argued that MEPs increases as the temporal distance between the TMS and the reaction time decreases. So basically the increase of the MEPs reflects the time of a non-specific response implementation, not the implementation of a particular response. More importantly, as stated above, an alternative explanation of these data that cannot be disambiguated with the present experiment is that the TMS stimulation has been delivered on M1, so that it is possible that instead of delaying or disrupting the simulation, the action pre-selection has only interrupted the communication between PMv and M1. So it is possible that the difference detected at the level of M1 between the two tasks is not explainable in terms of delaying or disrupting the simulation effect, but it can be simply interpreted as the action pre-selection made the simulation not detectable from M1.

Although the present evidence is not conclusive, some important points become clear: the time-course is a fundamental feature of motor resonance phenomena, so testing motor resonance at late time points is more risky since voluntary motor processes can overwhelm or increase the signs of simulation, leading to less clear evidence. A second point is that motor performance can be exploited by TMS experiments in order to test the relationship between the fast and involuntary motor resonance. Indeed future research has to test the time-course of the simulation in different contexts and cognitive sets. Finally from these experiments a model relating automatic simulation and voluntary action execution has been provided. The fundamental feature of this dual route model resides in the presence of two routes one that performs automatic visuo-motor matching and the other for arbitrary visuo-motor matching. However the question remains of whether the arbitrary route is fed by the motor representations activated by the simulatory process or by non-motor representations of the action observed, in order to provide motor responses conditioned to the observation of actions.

In conclusion, the role of the sensorimotor systems in cognition is far from being understood. Many questions remain unanswered at present. Some researchers think that the simulation processes are not functionally useful and they consider them to be a by-product or an epiphenomenon of a correlated visuo-motor experience with no or little functional specialization. This explanation is not satisfactory at all. It is possible that the mirror neurons primarily function is not that of action understanding, but it is very unlikely that these neurons have maintained their functional properties throughout evolution, without an evolutionary advantage. Finally, in the physiology of living beings no such thing as epiphenomena exist. If a phenomenon occurs in naturalistic settings it is most likely to have a function. The difficulty in interpreting its function should not be a reason for claiming that a given neuronal property has no function.

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