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ACTION UNDERSTANDING: THE ROLE OF EXPERTISE IN ADULTHOOD AND ITS DEVELOPMENT IN INFANCY

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Abstract

The ability to understand others' intentions through observation of their action is crucial in social interactions. Several studies involving behavioural and neurophysiological methodologies suggest the existence of mechanisms linking action execution and perception in monkeys and humans that seem to be involved in action understanding. When we observe an action performed by another individual, our motor system internally simulates it, through a matching mechanism which maps the observed action onto the observer's motor repertoire. However the question of how such mechanisms emerge and tune their features during development is still under debate. Developmental studies suggest that these mechanisms may be present in the first year of life, and may be modulated by infants' sensorimotor experience; so far there is no direct evidence in infancy that action observation induces in the observer the recruitment of the same motor program of the observed action. After a review of the current literature in Chapter 1, in Chapter 2 electromyography (EMG) is used for the first time in infancy to assess whether the observed action is directly mapped onto the infant's motor system and internally simulated, and whether the properties of such simulation change during development, in 3-, 6-, 9-month-old infants. This first line of evidence shows that in 3-month-old infants the EMG activity is not modulated by the goal of an observed action which is not yet part of infants' motor repertoire; at 6 months of age, the observed action is simulated on-line, while in 9-month-olds the motor simulation is active at action onset, anticipating the final

goal of the observed action. These results suggest that mirror mechanisms develop gradually, possibly according to infants' greater experience and familiarity with the observed actions.

In adulthood, the human mirror system seems to encode both goal-directed actions and intransitive (i.e. non goal directed) movements, thus suggesting that actions are coded both in terms of their goals and means to achieve them. In Chapter 3, the question whether in action simulation there is a predominance of coding goals or means of the observed action is investigated by showing highly familiar actions (e.g. grasping) executed by unusual effectors (e.g. foot). By means of transcranial magnetic stimulation (TMS) and EMG recordings, it is shown that observing a familiar action performed by an unusual effector activates, in the observer, not only the effector-specific motor program, but also the motor program of the effector usually involved in the observed action, suggesting that the action is remapped with respect to the observer's typical manner of reaching the same goal.

Studies in infancy show that infants' visual and motor familiarity with the observed action may influence their ability to understand its goal. In Chapter 4, by means of eye tracking technique, it is shown that 6-month-olds infants are able to discriminate between a familiar action, such as grasping, and a similar one executed in a biomechanically impossible manner (i.e. violating the constraints of human anatomy). Both biomechanically possible and impossible actions are coded as goal-directed, suggesting that biomechanical plausibility does not impair infants' ability to ascribe goals to the observed actions. However, the familiarity with the possible action, when presented first, exerts an influence in coding the action as goal-directed more in the possible than in the impossible condition, suggesting that information about biomechanical properties of motion is relevant for 6-month-olds' ability to anticipate the goal of the observed action.

Given that infants in the first months of life appear to be sensitive to biological motion and biomechanical constraints of human movements, in Chapter 5 it is assessed whether the ability to discriminate between possible and impossible movements is already present shortly after birth. Two-days-old newborns are able to discriminate between biomechanical possible and impossible intransitive (i.e. non-goal-directed) hand movements, but not between static gestures.

Overall, the present studies suggest that mechanisms linking motor and visual representations of movements are already present at birth, probably thanks to sensorimotor experience in the intrauterine life, and they develops in accordance with the observer's sensorimotor experience with the observed actions or movements.

1. Introduction

1.1 Mechanisms of action understanding

Inferring intentions of others while observing their behavior is crucial in social interactions. When we observe other people acting, we automatically interpret their movements in terms of goals.

Humans show an early inclination to interpret the observed behaviors as goal-directed (e.g. Woodward, 1998; 1999; Woodward and Sommerville, 2000; Csibra et al., 1999; 2003; Csibra, 2003; Gergely and Csibra, 2003; Biro and Leslie, 2007; Hunnius and Bekkering, 2010). A goal-directed action is a sequence of acts or movements performed in order to reach a goal, and it is interpreted as determined by its end state (Csibra et al., 2003). Infants as young as 6 months are sensitive to the end state of an observed action, for instance paying more attention to changes in the actor goal (i.e. the object to be grasped), than to surface aspects of the action, such as changes in the trajectory of the object through space (Woodward, 1998). Infants do not show the same pattern of looking behaviour when the agent simply flops the back of her hand on the target object in a manner that appear accidental, rather than intentional (Woodward, 1999). Furthermore, 12-month-old infants are able to ascribe goals to actions they have not seen completed or to infer from an observed action the presence of an occluded object (Csibra et al., 2003). Moreover, infants as young as 18 months are able to infer goals even from others' unsuccessful attempts (Meltzoff, 1995).

When infants watch unsuccessful acts (i.e. an agent “accidentally” under or overshooting a target), they tend to imitate the inferred actor’s goal rather than the movement actually seen (i.e. the means) (Meltzoff, 1995). Overall, these findings show that from a very young age, when we observe others acting we tend to ascribe goals and intention to them, extracting goals from the observation of their behaviors, even if the goal of the observed actions is not achieved (Meltzoff, 1995).

Different mechanisms have been proposed to explain such abilities. A candidate is the mirror neuron system (MNS). Mirror neurons in monkeys respond to both the execution and the observation of given actions (e.g. Rizzolatti et al., 2001). They have been first discovered in the premotor area F5 of the macaque monkey (di Pellegrino et al. 1992; Gallese et al. 1996), where most of the motor neurons discharge in association with movements that have a specific goal (i.e. motor acts, Rizzolatti et al., 1988). Neurons with similar characteristics were later identified also in the inferior parietal lobule (IPL), particularly in area PF and the anterior intraparietal area (AIP) (Fogassi et al., 1998; 2005; Gallese et al., 2002). These regions receive visual information from the superior temporal sulcus (STS), which has often been considered part of the mirror system (Keysers and Perrett 2004). Although neurons in STS lack motor properties, they respond selectively to the observation of biological movements, and STS is reciprocally connected with PF (Harries and Perrett, 1991), which in turn is reciprocally connected with F5 (Luppino et al. 1999).

Evidence in favor of the hypothesis that mirror neurons may be functional to action understanding comes from neurophysiological studies in monkeys which demonstrate that mirror neurons fire during the execution and the observation of goal-directed actions (e.g. Rizzolatti et al., 2001), but not for the observation of intransitive movements (i.e. body part displacements performed in the absence of a target), such as mimed actions, or during the observation of an object alone (e.g. Gallese et al., 1996).

The information about the goal can be encoded with “different degrees of generality” (Rizzolatti and Sinigaglia, 2010), and mirror neurons can code both the goal and the means of observed actions. Some neurons termed “strictly congruent” mirror neurons fire if the observed action has the same goal (i.e. grasping) and involves the same means for reaching the goal (e.g., precision grip) as the executed action, while “broadly congruent” mirror neurons respond when the observed motor act has the same goal of the executed motor act (i.e. grasping), but is achieved with different means (e.g., whole hand grip) (Gallese et al., 1996). Moreover F5 mirror neurons discharge even when the monkey cannot see the entire action, if the target of the action is occluded (Umiltà et al., 2001), or when the monkey hears the sound of an action (i.e. ripping paper) without seeing it (Kholer et al., 2002), as long as the monkey has sufficient clues to understand its goal. These studies suggest that mirror neurons fire whenever the monkey is able to build a mental representation of an ongoing motor act performed by another agent, even if the monkey does not see it (Cattaneo and Rizzolatti, 2009). Further evidence of the involvement of neurons in F5 in encoding goal-directed actions is provided by a study in which monkeys were trained to grasp objects using normal pliers, that require fingers closing in order to grasp an object, or reverse pliers, that require the opposite hand movement to achieve the same goal (i.e. fingers opening, Umiltà et al., 2008). F5 neurons fire during the grasping phase in both conditions, suggesting that what is coded is the goal of the action, regardless of the specific movement (i.e. closing vs opening of the hand) involved (Umiltà et al., 2008).

Furthermore, mirror neurons in IPL show different patterns of firing during the observation of the same motor act (i.e. grasping), when it is embedded in actions which differ at the level of intention (i.e. grasping to eat or grasping to place), suggesting that mirror neurons in IPL may have a role in understanding the aim of the entire action before the action is concluded (Fogassi et al., 2005).

Evidence based on different neuroimaging and neurophysiological techniques suggests that a cortical network with similar proprieties may also exist in humans (e.g. Fadiga et al., 1995; Rizzolatti et al., 1996a; 1996b; Grafton et al., 1996; Hari et al., 1998; Cochin et al., 1999; Grèzes et al., 2003; Muthukumaraswamy et al., 2004; Pineda, 2005; Gazzola, and Keysers, 2009). In both human and non-human primates, watching or listening to actions performed by others induce a covert motor activation in the observer (e.g. Rizzolatti et al., 1996b; Hari et al., 1998; Buccino et al, 2001; Lewis et al., 2005; Gazzola et al., 2006). The activations of premotor and parietal cortices contingent upon action observation show a somatotopic organization which roughly corresponds to that found when the same body parts are actually moved (Buccino et al., 2001). Similarly, transcranial magnetic stimulation (TMS) studies show that action observation induces a specific covert motor facilitation in the muscles that would be involved in the execution of the observed movements (Fadiga et al., 1995; Strafella and Paus, 2000; Maeda et al., 2002), and that muscular motor facilitation contingent upon action observation couples action execution also in terms of temporal coding (Gangitano et al., 2001; see also Baldissera et al., 2001). Similar to monkeys, in humans the parieto-frontal mirror circuit seems to encode the goal of observed motor acts. For instance, observing either a human or a robot arm grasping an object induces an activation of the mirror system, as detected with functional magnetic resonance (fMRI), despite the difference in shape and kinematics between the human and the robotic effector (Gazzola et al., 2007a). Similarly, listening to sounds related to tools manipulated by hands induces an activation of the parieto-frontal network, while listening to sounds which are not related to actions does not activate the same network (Lewis et al., 2005).

However, unlike in monkeys, the human parieto-frontal mirror circuit is activated also during the observation of intransitive movements (e.g. Lui et al., 2008), which induces an activation of the muscles specifically involved in the execution of the observed movements (e.g. Fadiga et al., 1995). Nevertheless, when mirror motor facilitation is explored in the context of the observation of a goal-directed action, such as grasping, the observation tends to activate the representation of the goal of the observed action, irrespective of the individual movements performed in order to achieve the goal (Cattaneo et al., 2009). For instance, observing an agent grasping an object with normal or reverse pliers (i.e. by closing or opening the hand, respectively) induces a mirror motor facilitation which is modulated by the goal of the action, irrespective of the individual movements actually executed (Cattaneo et al., 2009). These results suggest that the human mirror system encodes both goal-directed actions and intransitive (i.e. not-goal-directed) movements, and probably different brain regions are involved in coding *what* the agent of the observed action is doing and *how* (e.g. Hamilton and Grafton, 2006; Cattaneo et al., 2010, Jastorff et al., 2010). Other behavioral methods using motor priming and interference effects show similar results: A perceived action leads to the recruiting of the corresponding motor command, inducing a facilitation on executing compatible actions, in terms of both type of action shown and effector involved (e.g., Brass et al., 2001; Heyes et al., 2005; Bertenthal et al., 2006; Bach et al., 2007; Longo et al., 2008; Gillmeister et al., 2008; Leighton & Heyes, 2010). Therefore, action observation elicits a covert imitation of the observed action or movement that might allow the observer to have a grasp of both the means (i.e. which body parts are involved and how they move) and the goal or the effect of the observed action, possibly even allowing to predict its outcome (Bertenthal and Longo, 2008). Comparable automatic imitation is induced by the observation of biomechanically possible or impossible hand movements, suggesting that actions are coded more in terms of goals, than of the specific way in which the goal is achieved (Longo et al., 2008). However, when participants' attention is explicitly drawn to the anatomical plausibility of the observed actions, automatic imitation is present only for possible movements, suggesting that coding actions in terms of either goals or means might depend on the situation (Longo et al., 2008). The issue of whether there is a predominance of a goal code or of an effector code in action simulation is the focus of the experiments described in Chapter 3.

It has been suggested that the activation induced by action observation might be functional to action understanding, by enabling the observer to extract and represent the goal of the observed action (Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004; Fogassi et al., 2005). According to the *direct-matching* hypothesis, each time an individual observes an action, that action is mandatorily and automatically duplicated in the observer's motor system (e.g. Rizzolatti et al., 2001). The features of actions done by others are mapped onto the observer's motor representations of the same actions, and a copy of the motor command responsible for the observed action is generated. This immediate internal simulation of the observed action is thought to enable the observer to understand others' actions and intentions without the mediation of cognitive and inferential processes (Rizzolatti et al., 2001).

The direct-matching hypothesis implies that action understanding relies critically on the capacity to produce the same action (e.g. Buccino et al., 2004). However, other studies suggest that actions might be understood even without motor simulation, implying that a direct-matching mechanism might not be the only mechanism by which actions are understood (e.g. Csibra et al, 1999; 2007; Brass et al., 2007). For instance, the *teleological stance* (Csibra et al., 1999; 2003; 2007; Csibra and Gergely, 2007) hypothesizes that agents tend to achieve their goals in the most efficient (i.e. rapid and direct) way available to them in the given situation. Similarly, during action observation, from the first months of life, an early interpretational system established a specific explanatory relation between the action, the goal state and the relevant constraints of the situation (Csibra et al., 1999). The outcome (i.e. the effect) of an action may be interpreted as the goal of an action whenever the outcome is judged to justify the action within the situational constraints given. Such reasoning, that would occur automatically and beyond awareness, can operate even in case the observer lacks the motor program for executing the observed action. For instance, infants are able to understand events that involve movements performed by geometrical shapes (Gergely et al., 1995). In the study from Gergely and colleagues (1995), 12-month-old infants were repeatedly presented with computer animations in which a circle approached another one by jumping over an obstacle. When infants had been habituated to this event, they watched two different events in which the obstacle between the agent and its goal had been removed. One event showed the same "jumping" action as before (i.e. same trajectory), which in this case appeared unnecessary and inefficient in the absence of the obstacle. The second event presented the agent approaching its goal through a direct, more efficient pathway. The infants looked longer at the "jumping" action, even if it was perceptually more similar to the stimulus they were habituated, suggesting that they found it unexpected in this case, and suggesting they have predicted an efficient goal approach (Gergely et al., 1995). Similarly, even if sensorimotor experience seems to play an important role in action perception (e.g. Cannon et al, 2011; Sommerville et al., 2005; Falck-Ytter et al., 2006), infants can attribute goals even to actions they are not able to perform yet, such as a sequence of motor acts involving the mimed action of pouring a liquid from a bottle into a cup (Onishi et al., 2007). Similar results have been found even in monkeys (e.g. Hauser and Wood, 2010). Rhesus monkeys spontaneously tend to infer goals and intentions underlying a human experimenter's action and seem to be able to discriminate between intentional and accidental actions (e.g. Wood et al., 2007a). Moreover, monkeys tend to interpret a human action as goal-directed, understanding its functional consequences, even when it is not part of their species-specific motor repertoire (such as in the case of throwing actions), suggesting that the presence of an exact motor representation of the observed action is not essential in order to comprehend its meaning (Wood et al., 2007b). These results can hardly be explained by motor simulation, since the agent performing the goal-directed actions does not have a body and a motor system similar to the observers' or the action is not part of the observers' motor repertoire. Conversely, they can be interpreted by means of teleological reasoning (Csibra, 2007; Csibra and Gergely, 2007, Hauser and Wood, 2010).

Action understanding may also be based on *action-effect associations* (see ideomotor principle, James, 1890). According to this principle, the representations of actions in the cognitive system are related to the representations of their desired effects (i.e. the goals), through bidirectional associations (Hommel et al., 2001). In action production, if one wants to perform an action, it is sufficient to think of the action's end state (i.e. the goal) and the action necessary to achieve the goal is automatically activated by the motor system (Hommel et al., 2001). These action-effect links would be established through experience by simple associations upon observing the effects that one actions has caused. It has been proposed that the ability to understand others' actions may be based on similar associations between actions and their effects (Elsner, 2007; see also Paulus et al., 2011). These stored action-effect associations are useful to predict the goal of an ongoing observed action. In this case the observer implicitly assumes that the action is directed toward the same goal and in the same manner, as in previous experience.

It is possible that these different mechanisms of goal attribution, which have been suggested as being involved in action understanding, complement each other (e.g. Csibra and Gergely, 2007). Depending on the particular situation (i.e. tasks demands, situational constraints, and available information), one mechanism would be more efficient than another in interpreting the goal of the observed action. For instance, if the observed action is familiar, action-effect associations and motor simulation might allow the observer to understand its goal rapidly, while teleological reasoning might not be equally efficient, given that it requires the recruitment of previous knowledge about the actor, and the physical constraints of the situation. Action-effect associations can help the observer to understand the goal of an action by recalling the effect that is most strongly associated with the observed action, without searching many potentials outcomes. Given that the agent typically presents the same motor features and constraints of the observer, motor simulation usually generates valid predictions. Nevertheless, both action-effect associations and motor simulation might not be efficient in offering solutions if the observed action is novel or highly unfamiliar, as in the case of actions that are not part of the observer's motor repertoire. However, the ability of MNS to generalize the goal of motor acts (see 'broadly congruent mirror neurons') may provide solutions from a type of action to a new one, as long as it has a familiar goal (Rizzolatti and Sinigaglia, 2010). Finally, when the observed action is not part of the observer's motor repertoire and cannot be captured by a motor generalization, as in the case of actions that are not part of the species-specific motor repertoire of the observer (Gergely et al., 1995; Buccino et al., 2004), teleological reasoning can enable the observer to predict the goal of novel actions in any case (Csibra, 2007; Wood et al., 2007a; 2007b).

All these mechanisms rely on previous experience, which is needed in order to create associations between an action and its goal in the case of action-effect associations, or to "resonate" with the observed actions in case of motor simulation, or to build knowledge about the constraints of the situation and the actor in order to interpret it by inferential reasoning (Csibra, 2007; Csibra and Gergely, 2007).

1.2 Role of experience in action perception

The question of how mechanisms linking action execution and perception emerge and possibly modify their activity during development is still under debate. On the basis of studies showing that newborns seem to be able to imitate oro-facial gestures (Meltzoff & Moore, 1977; 1983), it has been suggested that a common coding between perceived and executed actions is innately prewired in humans and already active at birth (Meltzoff and Moore, 1997; Meltzoff and Decety, 2003; Meltzoff, 2005; Lepage and Theoret, 2007). According to this hypothesis, humans possess a supramodal representation of motor acts that couples sensory and motor aspects of human actions and provides the observer with a mechanism that may be the foundation for the later ability to understand others' mind (Meltzoff and Moore, 1997). So far, it is still unclear whether such visuo-motor mechanisms are already present at birth. It has been observed that only one behaviour seems to be reliably imitated by newborns, i.e. tongue protrusion, which may just reflect an increase of arousal, being a common response to many different stimuli, such as flashing lights or music, rather than being an evidence of newborns' imitative ability (Anisfeld, 1991; 1996; Jones, 1996).

A second hypothesis suggests that, rather than being innate, direct links between visual and motor representations of the same action emerge gradually during development, as a product of associative learning, through experience of observing ourselves while performing actions (Keysers and Perret, 2004; Heyes 2010a; 2010b; 2011; Brass and Heyes, 2005; Press et al., 2011). According to this hypothesis, some neurons would acquire mirror properties during development following mechanisms of Hebbian synaptic potentiation (Keysers and Perret, 2004), according to which 'what fires together wires together' (Hebb, 1949). While observing self-generated actions, both visual and motor neurons, in temporal, parietal and premotor regions which are anatomically connected, discharge. Some of the motor neurons would become mirror neurons through the repeated and correlated experience of executing and observing self-generated actions, experience that shape and strengthen links between visual and motor neurons encoding similar actions. Del Giudice and colleagues (2009) argue that active learning of the associations between action execution and perception might be promoted by an innate predisposition to pay attention to self-generated actions and to perform movements with a frequency and a cyclic pattern which could be optimal for enhancing Hebbian learning.

Several studies involving behavioral and neurophysiological methodologies suggest that mechanisms linking action execution and perception might be present in the first year of life, and they might modify their activity during development in accordance with the growing sensorimotor experience of the observer (e.g. Woodward and Guajardo, 2002; Falck-Ytter et al., 2006; Sommerville et al., 2005, Libertus and needham, 2010; Cannon et al, 2011). For instance, infants become sensitive to the goal structure of reaching and grasping action at the same age –around 6 months– in which they start to perform sophisticated and efficient goal-directed reaches (Rochat, 1989; Woodward, 1998). Motor experience and motor trainings seem to affect action perception from a very young age, suggesting that sensorimotor experiences can help infants to learn the consequences of actions which are novel or not much familiar to them yet (e.g. Sommerville et al., 2005, Libertus and Needham, 2010; Cannon et al., 2011; Daum and Gredebäck, 2011; Loucks and Sommerville, 2012). Infants as young as 3 months show an improvement in the ability to comprehend the goal-directedness of an action, even if they are not able to perform it yet, if they are provided with a motor training that allow them to produce it (e.g. Sommerville et al., 2005). Moreover, if infants are allowed to pick up and move objects by means of Velcro attached on both objects and mittens worn by infants, they then show both an increase in reaching-grasping behaviour and changes in their visual exploration of objects, actors and actions in an ecological situation (Libertus and Needham, 2010). If infants are provided with the experience of manipulating objects, they then show more interest in watching people interacting with the same objects, and this effect is not due to the objects or the actors per se (Hauf et al., 2007). Moreover, first-person experience has an important role in making third person inferences: Infants as young as 12-to-18 months turn selectively their head toward an object, looking at it longer when an adult turns his head toward it with the eyes open as compared as when the eyes are closed (Brooks and Meltzoff, 2002), and they follow the adult even if he wears a blindfold (Meltzoff and Brooks, 2004). However, if infants are provided with the first-person experience to wear a blindfold, they subsequently interpret correctly the role of the blindfold, and stop following the blindfolded adult's head movements, providing to be able to use their first-person experience to understand others' perception (Meltzoff and Brooks, 2004).

It has been suggested that the mirror neuron system might have a role in the ability to infer the goal of the observed actions. Indeed, infants tend to manifest anticipatory goal-directed eye movements toward the goal of the action especially when the observed action is already part of their repertoire (Falck-Ytter et al., 2006). The fact that during action observation the eye movement patterns are similar to those produced when the same action is executed suggests that the observed action is mapped onto the observer's motor representation of the same action (see Flanagan and Johansson, 2003). A study involving infrared spectroscopy (NIRS) demonstrated for the first time that infants as young as 6-to-7-months show the same pattern of response then adults in the sensorimotor cortex during action observation (Shimada and Hiraki, 2006). Studies involving electroencephalographic (EEG) technique in infants show '*mu rhythm desynchronization'* for both action execution and observation, suggesting the presence of motor resonance mechanisms during action observation (e.g. Lepage and Theoret, 2006; Nystrom, 2008; Southgate et al., 2009; Stapel et al., 2010; Marshall and Meltzoff, 2011). Moreover such motor resonance appears modulated by the observer's motor experience: For instance, 14- to 16-month-old infants show stronger mu- and beta- desynchronizations during the observation of crawling as compared to walking videos and such desynchronizations are strongly influenced by the observer's own experience with crawling (van Elk et al, 2008). However, a direct evidence for the fact that during action observation the same motor program is recruited by the observer and internally simulated, and whether the properties of such simulation change during development is still lacking in infancy. This particular topic will be the object of study of Chapter 2. Such an evidence is however available in other ages, ranging from 5-to-9-year-old, in which electromyographic (EMG) recordings demonstrate that muscle activity is modulated by the goal of the observed action, as if the action is actually executed by the children (Cattaneo et al., 2007).

Similarly, evidence in adults shows that the activation of the mirror system is related to the observer’s motor experience with the observed actions (e.g. Calvo-Merino et al., 2005; Haslinger et al., 2005; D'Ausilio et al., 2006; Aglioti et al., 2008). Only motor acts belonging to the observer’s motor repertoire seems to activate the mirror neuron system (Buccino et al., 2004); such activations are modulated by the individual motor expertise of the observer (Calvo-Merino et al., 2005), and this effect cannot be ascribed to a mere visual familiarity with the stimuli (Calvo-Merino et al., 2006). Moreover, if people are trained to perform dance steps, the more they practice, the more they show greater activations over time in brain regions classically associated with both action simulation and action observation (Cross et al., 2006). A sensorimotor training can even reverse the typical pattern of response shown by the mirror system (Catmur et al., 2007): For instance, observing an agent performing a movement with a finger typically induces a specific muscular motor facilitation in the same muscle. However, if participant are trained to perform one movement while observing another, the typical mirror effect is reversed (Catmur et al, 2007). These results suggest that, even in adults, the properties of the mirror system are not fixed, once acquired, but they can be dramatically modulated by sensorimotor experience (Catmur et al, 2007, Cattaneo and Rizzolatti, 2009), possibly through sensorimotor associative learning (Heyes, 2010a; 2010b). Similarly, if participants follow a sensorimotor training in which they have to make responses to an observed action using a different effector (i.e. hand responses to foot stimuli and foot responses for hand stimuli), they then show a reverse pattern of activations as measured by fMRI (i.e. voxels typically more activated for hand actions show greater responses to foot actions; Catmur et al., 2008). Moreover, sensorimotor experience can increase automatic imitation even of robotic actions (Press et al., 2007). These results highlight the plastic features of the motor mirror mechanisms, showing that a simple sensorimotor training can possibly result in mirror neurons with counter-mirror properties (Catmur et al., 2007; 2008). It has been suggested that the same mechanisms of sensorimotor learning responsible for the modulation of the MNS responses are involved also in the development of this system, likely through the repeated experience of observing self-generated actions and others' actions during social interactions (e.g., Heyes et al, 2001; 2010; Del Giudice, et al., 2009). According to this hypothesis, it might be the case that observing a familiar action performed by an atypical effector might activate in the observer the motor program of the typical effector used to reach the same goal, by engaging learning-dependent sensorimotor links, and this aspect is assessed in Chapter 3.

1.3 Differentiating between possible and impossible actions and movements

Behavioural and neurophysiological studies in adults demonstrate that observing actions performed by humans usually induces greater premotor and parietal activations and more automatic imitation than similar actions performed by non-biological agents (e.g. Stevens et al, 2000; Kilner et al, 2003; Tai et al., 2004; Press et al., 2005; 2007). Infants from the age of six months tend to attribute mental state to human actions, but not to non-biological agents (e.g. Meltzoff, 1995; Woodward, 1998; Kanakogi and Itakura, 2010; Daum and Gredebäck, 2011), suggesting that the familiarity with human actions, both visual and motor, may play a role in understanding the action goal. Moreover it is also plausible to hypothesize that the mirror neuron system may be biased in favor of human actions (Press et al., 2007). Unlike adults that are able to interpret as goal directed even actions performed by mechanical devices (e.g. Kanakogi and Itakura, 2011), infants in the first months of life may be limited to interpret as goal directed only human actions they are familiar with (e.g. Woodward, 1999). However, it is not clear whether the mere fact that an action is performed by a human agent is enough in order to be coded as goal directed (see Woodward, 1999). For instance, if infants see a familiar action, such as grasping, performed by a human agent in a biomechanically impossible manner (i.e. violating the constraints of human anatomy), will they be able to code that action as goal-directed? In such case the agent is human and the action has a familiar goal, but is performed in a way that is not only unfamiliar to infants, but even impossible to be executed. Whether 6-month-old infants are able to discriminate between a possible goal-directed action and a similar goal-directed action that is biomechanically impossible, and whether such discrimination may affect infants' ability to code the action as goal directed is assessed in Chapter 4.

Studies on adults show that motor simulation can occur even for the observation of biomechanically impossible intransitive movements, and different brain regions seem to be involved in coding the efferent components of movements and their plausibility (e.g. Costantini et al., 2005; Romani et al., 2005, Avenanti et al., 2007; Candidi et al., 2008). Infants in the first months of life are able to discriminate between biological and non-biological motion (e.g. Fox and McDaniel, 1982; Bertenthal et al., 1984;

1987; 1993; Booth et al., 2002; Moore et al., 2007) and there is evidence that such ability may be present even from birth (Simion et al., 2008; Bardi et al., 2011). Moreover infants in their first year of life seem to be sensitive to the violation of biological constraints (Bertenthal et al., 1985; 1987; Reid et al., 2005; Morita et al., 2012), but it is still unclear when the ability to distinguish between biomechanically possible and impossible movements develops during infancy. Given that newborns appear to be also able to discriminate between goal-directed and non-goal-directed actions, suggesting that primitive sensory-motor associations might be already present at birth (Craighero et al., 2011), in Chapter 5 newborns' ability to discriminate between possible and impossible hand movements will be investigated.

2. The early development of human mirror mechanisms: Evidence from electromyographic recordings at 3, 6, and 9 months

2.1 Introduction

Several studies have examined the properties of the mirror neuron system in both monkeys and humans (see Cattaneo and Rizzolatti, 2009 and Rizzolatti and Sinigaglia, 2010 for reviews). Despite the large number of studies in adults, little is known about the origin and early development of the mirror neuron system. On the basis of studies concerning neonatal imitative behaviour (Meltzoff and Moore 1977; 1983), it has been suggested that a rudimentary, largely automatic mechanism linking perception and execution of actions might be present from birth (e.g. Lepage and Theoret, 2007). Given that in adults the mirror neuron system appears to be sensitive to the effect of both experience (e.g. Calvo-Merino et al., 2005) and sensorimotor learning (e.g. Catmur et al., 2007), an alternative hypothesis is that mirror mechanisms may merely represent a byproduct of associative learning that relies on sensorimotor experience (Heyes, 2010a; 2010b). From a neurocostruttivistic framework, Del Giudice and colleagues (2009) argued that an innate predisposition to pay attention to self-generated actions would promote active learning of the association between the perception of actions, and the corresponding motor programs. In this framework, mirror neurons would emerge and possibly adjust their response features through experience, by integrating predispositions with learning during development. In this framework, it is plausible to hypothesize that an early mechanism develops gradually with age and the individual's experience.

Behavioral and neurophysiological evidence, indeed, supports the existence of mechanisms linking action execution and perception in infancy, and suggests that such mechanisms might be modulated by direct sensormotor experience (e.g. Sommerville et al, 2005; Reid et al., 2005; Falck-Ytter et al, 2006; Gredebäck and Melinder, 2010; Libertus and Needham, 2010; Kanakogi and Itakura, 2011; Sanefuji et al., 2008; Cannon et al., 2011).

Behavioral studies show that infants are able to visually anticipate the goal of an observed action which is already part of their own motor repertoire, while this ability seems to be absent in younger infants that cannot already perform that action themselves (Falck-Ytter et al, 2006; Kochukhova and Gredebäck, 2010; Kanakogi and Itakura, 2011).

Studies that have employed electroencephalographic (EEG) techniques reveal in both adults and 8- to-14-month-old infants a decrease in neural synchrony at central sites associated with both action execution and observation (i.e., *mu-rhythm*, Marshall and Meltzoff, 2011; Nyström et al., 2010; van Elk et al., 2008; Southgate et al., 2009; Southgate et al., 2010; Stapel et al., 2010). Noteworthy, such a desynchronization is modulated by the observer's motor experience with the observed action: the larger is the experience, the stronger is the *mu-rhythm* desynchronization (van Elk et al., 2008).

However, all these studies do not provide evidence that the motor system is replicating the observed action, as if actually executed by the infant. A technique which allows to investigate whether the observed action is directly mapped onto the observer’s motor system and internally simulated is electromyography (EMG) (see Fadiga et al., 2005). In adults this technique has shown that action observation triggers specific facilitation of the muscles that would be involved in the actual execution of the observed movement (e.g. Fadiga et al., 1995). Recently, Cattaneo and colleagues (2007) demonstrated in 5-to-9-year-old children that the EMG activity recorded from a muscle involved in mouth opening increases during the observation of an agent grasping an objects and bringing it into the mouth, as compared to when the object is grasped and placed into a container on the agent’s shoulder. The muscle responsible for the final goal of the action increases its activity from action onset, demonstrating that during the grasp-to-bring-to-the-mouth action, the entire grasp-to-eat motor chain is recruited as soon as the action begins (for similar results in adult monkeys, see Fogassi et al, 2005). Such a mechanism can help the observer to immediately anticipate the goal of the observed action (Cattaneo et al., 2007). The demonstration that a specific action chain is activated up to the peripheral muscles provides evidence that, at least from the age of 5 years, the observed action is internally simulated as if it is actually executed by the observer.

In this context, the present study aimed at investigating for the first time in infancy whether the motor system is recruited and selectively modulated by the goal of observed actions already in the first months of life. In particular, we explored if a specific covert activation of the muscles induced by action observation (Cattaneo et al., 2007) is already present in infants and whether it changes during the first months of life.

To this aim, we recorded surface EMG activity from the muscles responsible for mouth opening during the observation of two video-clips showing two actions, which differ with respect to their final goal. The videos displayed an agent either reaching for an object and bringing it to the mouth, or reaching for an object and placing it onto the head. Three experiments were run, involving respectively 3-month-old (Experiment 1), 6-month-old (Experiment 2) and 9-month-old (Experiment 3) infants.

2.2 Experiment 1: 3-month-olds

Methods

Participants. Nineteen full-term 3-month-olds (6 females, mean age=3 months and 10 days, S.D.=5.6 days, range=87-105 days) took part in the study. Twenty-five additional infants were tested, but discarded from the final sample because of fussiness and no completion of the minimum number of trials required for analysis (see below, statistical analysis section). Participants were recruited via a written invitation sent to parents on the basis of birth records provided by the local Register office. The protocol was carried out in accordance with the ethical standards of the Declaration of Helsinki (BMJ 1991; 302: 1194) and approved by the ethical committee of the University of Milano Bicocca. Parents gave their written informed consent.

Stimuli, apparatus and procedure. EMG activity was recorded from mouth-opening muscles during the observation of videos showing an actress reaching for a pacifier, grasping it, and bringing it to the mouth (object-to-mouth action) or reaching for a piece of Lego and placing it onto the head (object-to-head action) (Figure 1). The experiment took place in a sound-proof cabin equipped with a Faraday cage. Participants seated in an infant seat viewing the monitor where videos were displayed at a distance of about 60 cm. Each trial began with a cartoon consisting of an animated image associated with various sounds, and serving as attention grabber. When the infant looked at the animated fixation point, the experimenter started the video. Each video-clip lasted 4 s and consisted of 100 frames, 40 ms each. In particular, for both actions, the 51st frame depicted the exact moment in which the actress's hand touched the object. The actress's actions across the different videos were comparable in terms of kinematics and duration of each action's motor act (i.e., reaching, grasping and bringing). At the end of each trial, a colored circle slowly expanding and contracting was displayed for 3.5 sec, followed by a 500 ms blank screen, thus creating an interval of 4s between trials. A video camera was placed above the monitor and directed to the infant's face. The image of the infant's face was displayed on a screen in front of the experimenter in order to allow constant monitoring of the infant's behavior. The experimenter coded online the duration of the infant's looking toward the stimulus (i.e., looking time). Infant's behavior was also coded offline. The two types of actions were presented in separate and counterbalanced blocks of trials. Each block of trials ended when infants watched 5 trials for at least the 70% of the duration of each video. The computer controlled the sequence and timing of the stimuli, as well as the infants' looking time. The two blocks were alternated each other as long as the infant was focused on the video-clips. The sequence and timing of the stimuli, and the infants' looking time were controlled by the computer (E-prime software, Psychology Software Tools, Pittsburgh, PA).

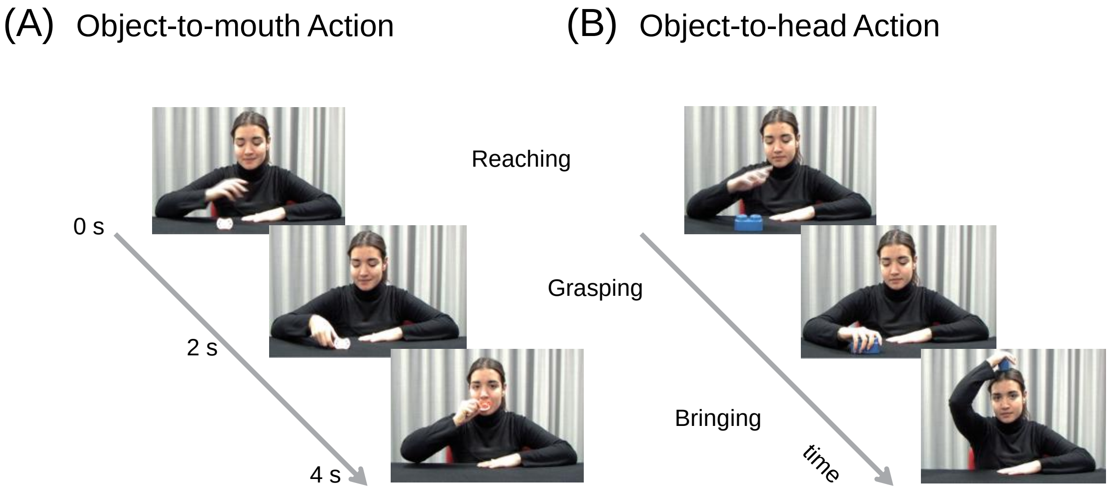

Figure 1. Experimental stimuli. Video-clip frames depicting the two action-stimuli presented: a) Object-to-mouth action. In Experiment 1, the object reached, grasped and brought to the mouth was a pacifier. In Experiment 2 and 3 it could be either a pacifier (group 1) or a piece of Lego (group 2); b) Object-to-head action. The object reached, grasped and brought to the head was a piece of Lego in Experiment 1. In Experiment 2 and 3, it could be either a piece of Lego (group 1) or a pacifier (group 2).

EMG recording. EMG activity was recorded from the infants' suprahyoid muscles (SM), which include digastric, stylohyoid, geniohyoid, and mylohyoid and are mainly involved in mouth opening, swallowing, deglutition, suckling. Two surface electrodes for pediatric use (700 12-K, Neuroline, Ambu) were placed 2 cm apart under the infant's chin symmetrically to the midline. The reference electrode was positioned ~2 cm above the nasion. The EMG signal was recorded by means of a Digitimer electromyogram, amplified (gain 1000), filtered (band-pass: 10Hz-1kHz), sampled at 1kHz, and stored for offline filtering (150 Hz; high-pass: 30 Hz). Impedance was kept between 5 and 10 kOhm. Electrocardiographic signals were filtered out from the EMG signal by means of a high-pass fourth order filter and a 150 Hz notch filter.

Trials in which infants looked less than the 70% of the duration of the video-clip were discarded. Off-line visual inspection of the EMG signal and the videotape of the infants' behavior during the experimental session was conducted on the collected data.

Visual and motor experience with the observed objects. To assess whether individual visual and motor experience with the observed objects could play a role in the modulation of EMG activity during action observation, infants' mothers were presented with a questionnaire at the end of the experimental session. The questionnaire investigated whether the infant was used to utilize the pacifier or not and whether he/she had experience with child's building blocks either as a player or as an observer in case the infant was used to watch older siblings playing with them.

Statistical analysis. Trials were discarded whenever signal noise and motion artifacts contaminated the recordings. Following these criteria, about 40% of the trials were excluded from data processing. Only infants with at least 4 trials per action type were included in the analyses. On average, the analyses were performed on 6 trials for each type of action. For each type of action, the EMG signal recorded during the 4-sec video presentations was functionally segmented into three epochs corresponding to the motor acts of reaching (duration = 1,6 sec), grasping (duration = 0,8 sec), and bringing (duration = 1,6 sec). The area under the curve of the rectified EMG activity was computed on a trial-by-trial basis, normalized (z-scores) and averaged separately for each type of action and epoch. A repeated-measures analysis of variance (ANOVA) was run with Type of Action (object-to-mouth, object-to-head) and Epoch (reaching, grasping, bringing) as within factors. Pairwise comparisons with the LSD test were conducted whenever appropriate. The significance level was set at alpha=0.05.

For each of the three items investigated in the questionnaire (i.e. use of the pacifier, visual, and motor experience with the building blocks toys) an ANOVA was conducted with Type of Action (object-to-mouth, object-to-head) and Epoch (reaching, grasping, bringing) as within factors, and Experience (yes, no) as between factor.

Results

The ANOVA run on SM activation did not show any significant main effect nor interaction (all ps>0.5) (Fig.4). These results showed no modulation of infants' muscular activity contingent upon action observation.

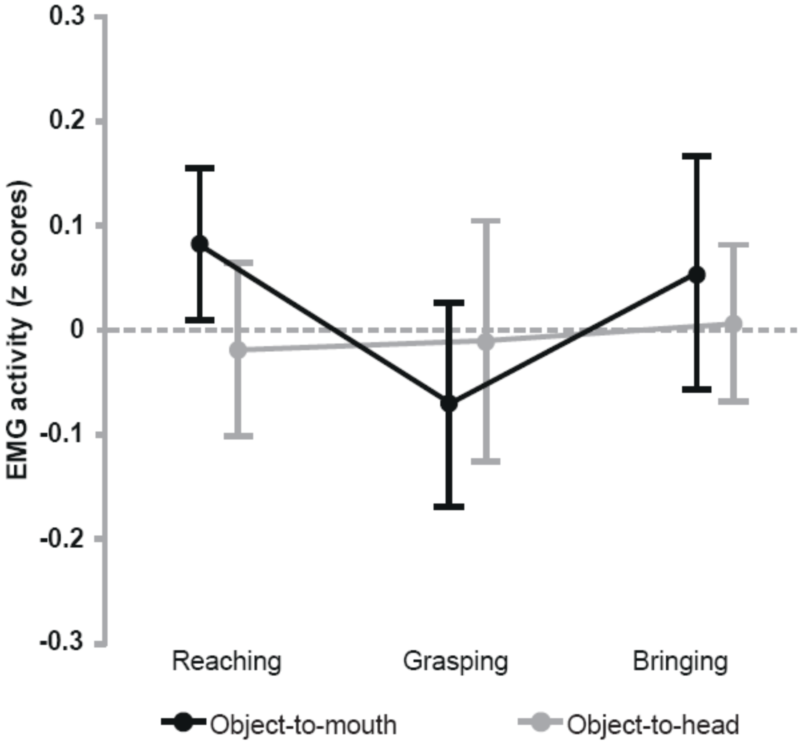

Figure 2. Suprahyoid muscles activation during the reaching, grasping and bringing phases of the Object-to-mouth and Object-to-head actions in 3-month-olds. Error bars indicate SEM.

The analysis for each item of the questionnaire revealed neither significant main effects, nor interactions (all ps>0.1).

2.3 Experiment 2: 6-month-olds

Methods

Participants. Thirty healthy, full-term 6-month-old infants (12 females, mean age=6 months and 9 days, S.D.=9 days, range=167-201 days) were enrolled in the study. Thirty additional infants were tested, but discarded from the final sample because of fussiness and no completion of the minimum number of trials required for analysis (see Experiment 1, statistical analysis section).

Stimuli, apparatus and procedure. In the second and third experiment we chose to control also for the object involved in the observed action. As in the first experiment, EMG activity was recorded from mouth-opening muscles during the observation of videos showing an actress reaching for an object, grasping it, and either bringing it to the mouth (object-to-mouth action) or placing it onto the head (object-to-head action). Participants were assigned to one of two experimental groups: A first group (group 1, 17 participants) observed an agent reaching for a pacifier and bringing it into the mouth, and an agent reaching for a piece of Lego and placing it on the head. A second group (group 2, 13 participants) observed the opposite situation, in which the piece of

Lego was brought to the mouth and the pacifier to the head. Apparatus, procedure and data processing were the same of Experiment 1.

Statistical analysis. Following the same criteria as Experiment 1, on average analyses were performed on 10 trials for each type of action. An analysis of variance (ANOVA) was run with the between factor Group (group 1, group 2) and the within factors Type of Action (object-to-mouth, object-to-head) and Epoch (reaching, grasping, bringing). Pairwise comparisons with the Newman–Keuls test were conducted whenever appropriate. The significance level was set at alpha=0.05.

Results

The ANOVA run on SM activation showed only a significant Type of Action by Epoch interaction ($F_{2,56}$=6.42, p=0.003). Post hoc comparisons revealed a significant increase of the EMG activity in the bringing phase when the object was brought to the mouth as compared to when it was brought to the head ($p<0.000$). For the object-to-mouth action, EMG activity was greater in the bringing phase, as compared to the grasping epoch (p=0.02). On the contrary, in the object-to-head condition, the EMG activity decreased during the bringing phase with respect to the grasping phase ($p<0.02$). These results showed that EMG activity was modulated by the action goal in the bringing phase of observed actions (Fig. 2). Main effects were both non-significant (ps>0.2).

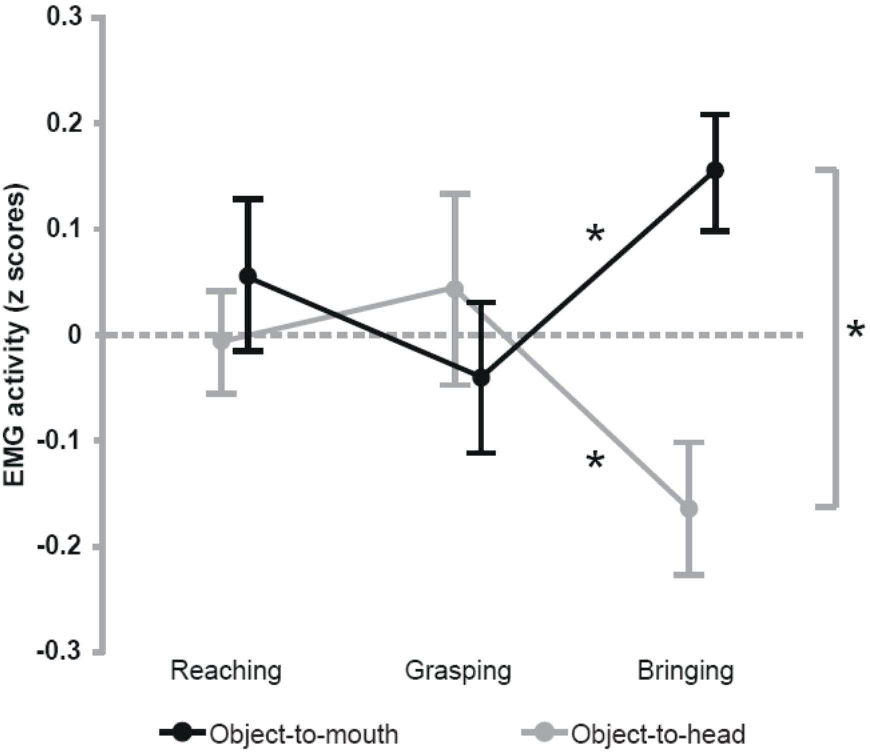

Figure 3. Suprahyoid muscles activation during the reaching, grasping and bringing phases of the Object-to-mouth and Object-to-head actions in 6-month-olds. Error bars indicate SEM.

The analysis run on each item of the questionnaire confirmed only the significant Type of Action by Epoch interaction (all other ps>0.2), suggesting that the results we found were not influenced by the familiarity of the objects involved in the actions.

2.4 Experiment 3: 9-month-olds

Methods

Participants. 30 full-term 9-month-olds (19 females, mean age=9 months and 6 days, S.D.=8.9 days, range=259-290 days) took part in the study. Twenty additional infants were tested, but discarded from the final sample because of fussiness and no completion of the minimum number of trials required for analysis (see Experiment 1, statistical analysis section). Fifteen participants were assigned to group 1 (pacifier to the mouth and piece of Lego to the head) and fifteen to group 2 (piece of Lego to the mouth, pacifier to the head).

Stimuli, apparatus, procedure, EMG recording, and statistical analysis. Stimuli, apparatus, procedure and data analysis were the same of Experiment 2. On average, analyses were performed on 10 trials for each type of action.

Results

The ANOVA carried out on SM activation showed only a significant Type of Action by Epoch interaction ($F_{2,56}$=3.68, p=0.03). Post hoc comparisons revealed a significant increase of the EMG activity in the grasping phase when the object was brought to the mouth as compared to when it was brought to the head (p=0.01). In the object-to-mouth action EMG activity increased in the grasping phase, as compared to the reaching ($p<0.03$) and bringing phases ($p<0.05$) (Fig. 3). Main effects were both non-significant (ps>0.2).

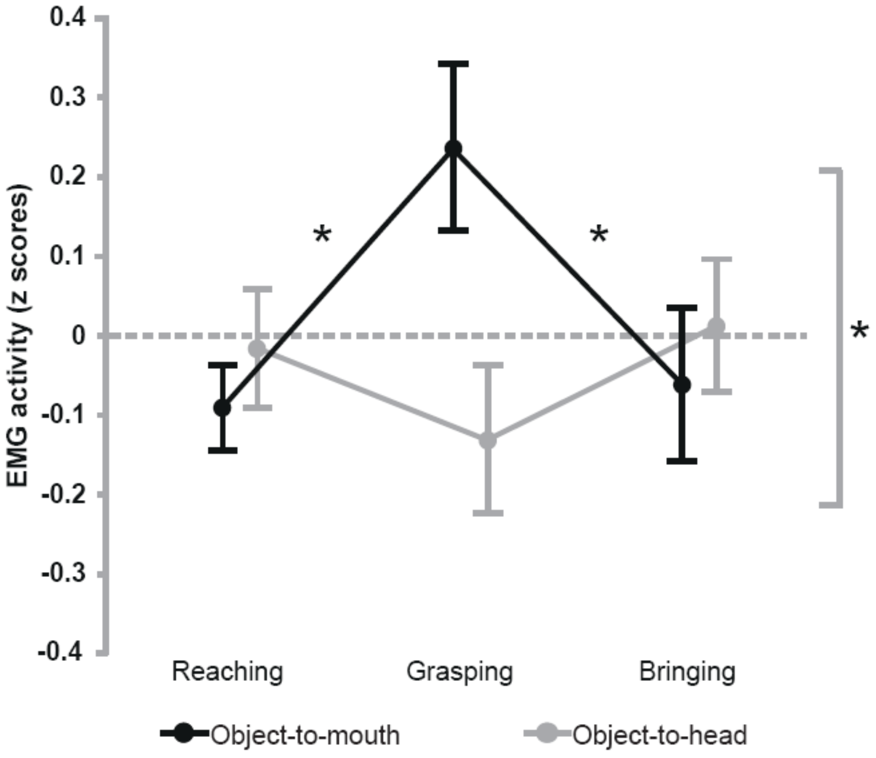

Figure 4. Suprahyoid muscles activation during the reaching, grasping and bringing phases of the Object-to-mouth and Object-to-head actions in 9-month-olds. Error bars indicate SEM.

The analysis run on each item of the questionnaire confirmed only the significant Type of Action by Epoch interaction (all other ps>0.2), suggesting that the EMG modulation contingent upon action observation is not influenced by the familiarity of the objects involved in the actions.

2.5 Discussion

In human adults, EMG recordings (mostly combined with transcranial magnetic stimulation) demonstrated that action observation induces a motor facilitation that is specific for the muscles involved in the observed movements (e.g. Fadiga et al., 1995), thus providing evidence for the presence of an observation/execution matching system assessable also at the muscular level.

In the present study, EMG is employed for the first time to investigate mirror mechanisms in infancy.

EMG activity was recorded from muscles involved in mouth opening while 3, 6, and 9-month-old infants observed an actress reaching for an object and bringing it either to the mouth or to the head. The two displayed actions had different goals, but were similar for biomechanical properties and other low level characteristics.

Results indicated that infants' covert muscle activation was affected by the final goal of the observed action in 6 and in 9-month-olds, but not in 3-month-olds. Moreover such a modulation was specifically induced by the goal of the action, regardless of the nature of the object involved.

Three-month-old infants showed no modulation of their muscular activity due to the observed actions.

In 6-month-old infants, the EMG activity recorded from suprahyoid muscles increased during the observation of an agent reaching for an object, grasping it, and bringing it to the mouth, indicating the presence of a motor-resonance related effect. Conversely, observing an actor bringing an object to the head induced a decrease in the muscular activity. It is possible that this inhibitory effect when the goal did not engage the mouth might be due to infants' tendency to bring to the mouth objects they get in contact with. Observing an object being grasped might favor the selection of the motor program of bring the object to the mouth. When the observed final goal is incongruent with this motor program, motor resonance is inhibited.

In 6 month-old infants, the EMG activity was dynamically and specifically modulated by the action goal during the final stage of the action, i.e. during the motor act of bringing, in which the action goal was achieved.

In 9-month-olds a greater activation of the SM muscle for the object-to-mouth action as compared to object-to-head action was already present during the previous grasping epoch, as in older children (Cattaneo et al., 2007).

Our results suggest that that 6-months-old infants, unlike older children, were not able to anticipate the goal of observed actions. On the contrary, in 9-month-olds and older children, a similar modulation of EMG activity is already present since the onset of the action, and in particular in the grasping phase (Cattaneo et al., 2007).

This may suggest that sensorimotor experience related to action execution plays a role in infants' action understanding. Indeed, 3-month-olds have little or no experience with the observed action of reach-to-grasp: Previous evidence shows that direct grasping begins at about 3 months, and only later (i.e. between 3-6 months) it develops and differentiates in subclasses (e.g. different type of grip; Berthier and Keen, 2006). When the observed action is not part of the observers' motor repertoire yet, infants do not have any corresponding program that can resonate with the observed action (see Buccino et al., 2004). Previous studies suggest that if 3-month-old infants are provided with experience of goal-directed actions like grasping, for instance by using mittens covered with Velcro allowing to pick up objects, they perceived observed grasping action as goal directed significantly more than controls (Sommerville et al., 2005).

Therefore our results are in line with the hypothesis that experience with active execution exerts an influence on action observation, by enhancing infants' perception of action performed by others (Libertus & Needham, 2010; Sommerville et al., 2005;

Del Giudice et al., 2009), and with studies which show that mirror neuron systemis modulated by the specific expertise of the observer (e.g. van Elk et al., 2008; Calvo-Merino et al., 2005, Falck-Ytter et al., 2006).

Overall, our results speak in favor of a gradual development of mirror mechanisms in infancy that may support the ability to understand and anticipate the goal of others' actions. In 3-month-old infants, mirror motor resonance mechanisms for grasping actions are absent. At 6 months of age, the observed action is simulated on-line. In 9 month-olds and children this mechanisms are active already at action onset, probably allowing infants to capture the intention of the agent by anticipating the goal of the action. Therefore, the modulation of the EMG activity due to action goals changes during development, possibly according to the greater experience and familiarity that older children have with the execution of actions (Del Giudice et al., 2009; Calvo-Merino et al., 2005). Indeed, grasping ability continues to develop with age during the first year of life even if infants are already able to grasp an object (Kanakogi and Itakura, 2011).

In monkeys, the activation of mirror neurons is influenced by the action's final goal, as demonstrated by mirror neurons in IPL, which show different activations when the same motor act of grasping is part of actions having different final goals (i.e. grasp-to-eat versus grasp-to-place; Fogassi et al., 2005). These neurons, firing already during the phase of grasping, may allow the observer to understand the agent's intentions by anticipating the subsequent act. It is plausible that this ability can emerge with accumulating experience: the more the infant becomes familiar with an action chain (such as reach-to-grasp-to-bring), the more the infant is able to retrieve the corresponding motor program of the observed action from the very onset.

3. The role of goal and motor expertise during the observation of familiar actions performed by unusual effectors

3.1 Introduction

Several studies in both monkeys and humans suggest the existence of a system, known as the “mirror neuron system” (MNS), which directly maps the features of actions done by others onto the observer’s motor representations of the same actions (e.g. Di Pellegrino et al., 1992; Gallese et al., 1996; Fogassi et al., 1998; Fadiga et al., 1995; Hari et al., 1998; Rizzolatti et al., 1996a; 1996b; Cochin et al., 1999).

A key property of the MNS is the direct matching between observed actions and their internal representations. So far, a key question that still needs to be addressed is how strict the correspondence between the observed action and its internal representation should be in order to trigger the activation of the MNS by action observation.

Neuroimaging studies suggest that only actions belonging to the observer’s motor repertoire are mapped on the observer’s motor system (Buccino et al., 2004), and that mirror motor activations are modulated by the specific expertise of the observer (Calvo-Merino et al., 2005). Moreover, during the observation of actions performed by different body parts (i.e. hand, mouth, foot), the MNS shows a somatotopic pattern of activation which is roughly similar to that found when the same body parts are actually moved (Buccino et al., 2001; Sakreida et al. 2005; Wheaton et al. 2004).

Likewise, Transcranial Magnetic Stimulation (TMS) studies demonstrate that action observation induces a specific motor facilitation, as measured by TMS-induced motor evoked potentials (MEPs), only in those muscles that would be involved in the execution of the observed movements (Fadiga et al., 1995; Strafella and Paus, 2000).

On the other hand, recent evidence shows that mirror activations occur also for the observation of motor acts performed by an effector that is unfamiliar or even not human, as long as the observed action has a familiar goal. For instance, mirror activations were observed during the observation of grasping actions performed by a robotic arm (Gazzola et al, 2007a). Furthermore, individuals with congenital aplasia of the upper limbs show mirror activations during the observation of hand grasping (Gazzola et al., 2007b). Aplasic individuals, although supposedly lacking the motor program to accomplish hand grasping, typically have motor programs to reach the same goal through different effectors (i.e., the foot or the mouth). In this case, the activation of regions involved in the execution of foot and mouth actions could be related to the goal of the action, rather than to the effector used to achieve it. This suggests that, during action observation, the MNS does not merely generate an inner replica of the observed sequence of movements. Rather, the observed action seems to be remapped in accordance with the observers' typical way to perform it.

In humans, unlike monkeys, the MNS also becomes active for the observation of intransitive movements (e.g. Fadiga et al., 1995), indicating that in humans this network encodes both motor acts, which are goal-directed, and simple movements without a goal (Rizzolatti and Craighero, 2004; Rizzolatti and Sinigaglia, 2010). Thus, given that the observed actions can be coded both in terms of goal and means to reach the goal (Rizzolatti and Sinigaglia, 2010), it is critical to assess the effects of viewing actions performed by unusual effectors. If observed actions are translated into the *vocabulary* of actions of the observer (Rizzolatti and Craighero, 2004), and referred to the individual's personal motor repertoire (Calvo-Merino et al., 2005), one could think that a highly familiar action, such as grasping an object, should activate the motor program of the effector typically used to perform that action (i.e. the hand) in a similar way when the effector that performs the observed action is either the typical one, the hand for grasping, or an atypical one, such as the foot for grasping. Indeed, even if the observed action of grasping with the foot is potentially feasible for the observer, it is habitually performed with the hand and may, thus, automatically activate the typical hand grasping motor pattern.

Based on these considerations, we run two experiments in human adults in order to investigate the selectivity of the MNS responses with respect to the habitual correspondence between a given action and the effector that is seen performing that action, in healthy humans. To this aim, mirror motor facilitation was measured by recording MEPs evoked by TMS, contingent upon action observation (Fadiga et al., 1995), by varying across conditions the goal of the action and the effector used to perform such action. In the first experiment, participants were presented with a typical hand action (i.e. grasping an object) performed by either the hand or the foot, and an unspecific action for both effectors (i.e. stepping over an object and then landing on it), performed by either the hand or the foot. In the second experiment, we presented an action which is typically performed with the hand (i.e. grasping an object) and an action which is specific for the foot (i.e. pressing a foot pedal of a car), both executed by either the hand or the foot. If observing a familiar action executed by an unusual effector activates only effector-specific motor programs in the observer, we should find an increase in MEP amplitude only in those muscles involved in the observed action. On the contrary, if a goal-related action is remapped with respect to the observer's typical way of achieving the same goal, regardless of the specific muscles involved, we should find an increase in MEP amplitude only for the effector usually employed by the observer to perform that action. Finally, if observing a common action performed by an uncommon effector activates both kinds of motor mapping, we should find a modulation of MEP amplitude both in the muscles corresponding to the observed effector, and in the muscles corresponding to the effector habitually used to perform that action.

3.2 Experiment 1

Methods

Participants. Ten healthy individuals (8 women; mean age=25 years, range=21–33 years), with normal or corrected-to-normal vision, took part in the study. All participants were right-handed and right-footed, as assessed by the Oldfield handedness questionnaire (Oldfield, 1971) and the laterality preference inventory of Coren (1993). None of the participants had neurological, psychiatric, or other medical problems, nor had any additional contraindication to TMS (Wassermann, 1998). All participants were naïve to the purpose of the study, and gave written informed consent prior to their participation. The experiment was carried out in accordance to the ethical standards of the Declaration of Helsinki, and was approved by the ethical committee of the University of Milano-Bicocca. No discomfort or other adverse effects were reported during TMS.

Experimental task. Participants sat at a distance of 80 cm in front of a PC monitor (Samsung SyncMaster 1200NF) and they watched a series of videos, displaying six situations, two static, namely a right *static hand* and a right *static foot*, and four dynamic situations, namely: 1) a right *hand grasping* and then lifting a pencil, 2) a right *foot grasping* and then lifting a pencil, 3) a right *hand stepping over* a pencil, landing with the wrist over it, and then rising up, and 4) a right *foot stepping over* a pencil, landing with the heel over it and then rising up (see Fig. 1). The actions were chosen as they are all potentially executable by the participants (i.e., they are part of their motor repertoire), as explicitly confirmed by subjective reports at the end of the experimental session.

Each trial started with a central cross presented for 1s, followed by the video-clip presented for 2.1s (70 frames, inter-frame interval of 30.5 msec). The four videos displaying a dynamic situation were carefully matched for the timing of each main component of the action; in all actions, from the onset of the trial, the effector started to move after 250 msec , it was pre-shaped after 1006 msec, got in contact with the object after 1300 msec, and lifted it or raised up after 2000 msec. Luminance, contrast, hue, and saturation were kept constant between frames in each video-clip, and across different video-clips. The inter-trial interval was 8500 msec, during which a blank screen was presented. Each of the four videos displaying dynamic actions was repeated 30 times during each experimental session, thus yielding 120 trials, equally divided in 6 blocks. The four action conditions were presented in a random order within each block. Static conditions were presented in two blocks of 30 trials each, one at the beginning and one at the end of the experimental session, for a total of 60 trials. In each block, 15 trials for each static effector were presented randomly and they served as baseline. Sequence and timing of both the video-clips and the TMS stimuli were controlled by a computer running E-prime software (Psychology Software Tools, Inc, Pittsburgh, PA).

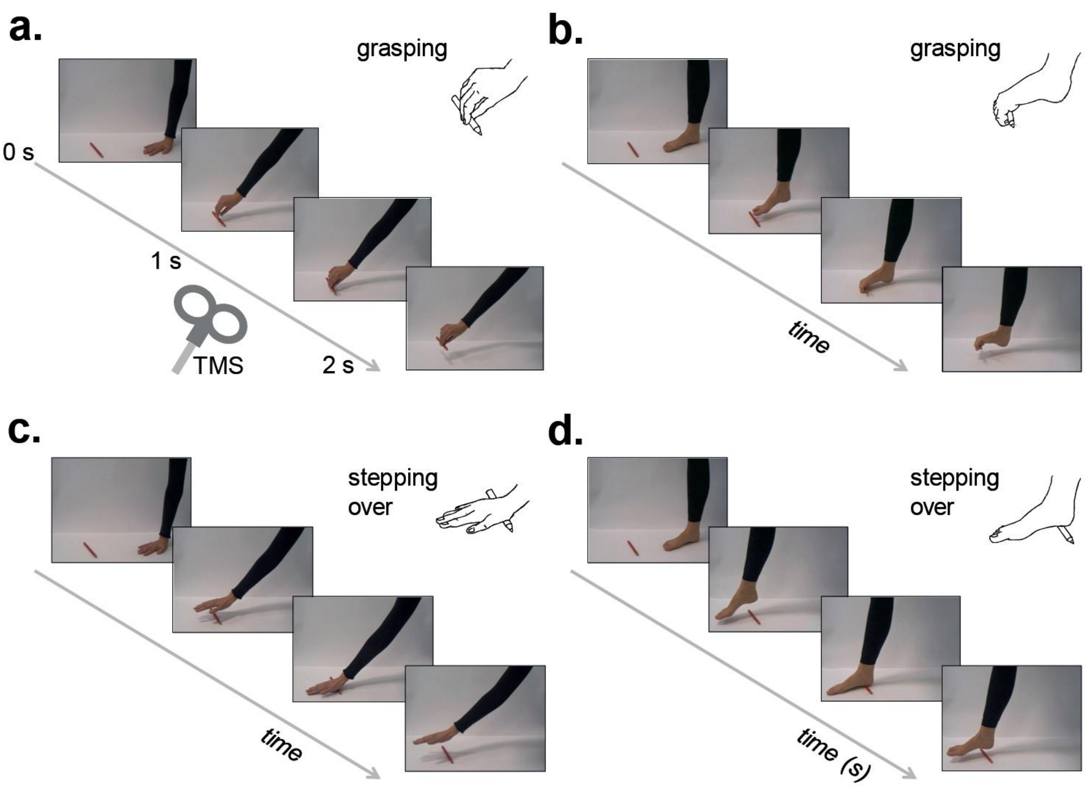

Figure 1. Experimental conditions in Experiment 1. During the experiment, video-clips depicting four actions were presented: a) Grasping with the hand; b) Grasping with the foot; c) Stepping over with the hand; d) Stepping over with the foot. sTMS pulses to the motor hand or foot areas were delivered after a random interval between 1006 and 1982 msec from the beginning of each video-clip.

During the experimental sessions, participants were asked to pay attention to the video-clips, while remaining relaxed. In order to ensure attention to the video-clips, participants were told that, at the end of the experiment, they had to provide a detailed description of both the actions and the objects featured in the videos. Overall, participants provided accurate descriptions. Moreover, at the end of the experiment, they were asked if they were able to perform the observed actions, and in particular how often they used to execute the grasping-with-the foot action. Every subject reported to be able to perform the observed hand and foot actions, but they also unanimously reported that the grasping-with-the foot actions were highly unusual, hence confirming that the grasping-with-the foot action, although feasible, was uncommon for the participants.

TMS protocol. Participants sat comfortably in an armchair, in a dimly lit room. They were instructed to relax and keep their right hand and leg on a pillow. The hand was kept supine on the pillow. The leg rested with the knee slightly bent, and the foot laid with the heel on a comfortable footrest. Two experimental sessions were conducted for each participant, in two different days. In one session, single-pulse TMS (sTMS) was applied over the hand area of the left primary motor cortex (M1), while in the other session sTMS was delivered over the foot area of the left M1. The order of the two sessions was counterbalanced across participants.

In order to stimulate the hand motor region, sTMS was delivered by a 70-mm figure-of-eight coil connected to a Magstim 200 stimulator (Magstim Co., Whitland, UK). For each subject, we first identified the optimal scalp position for inducing reliable MEPs in the contralateral, right first dorsal interosseus (FDI) muscle. After localising the motor area, the target site was marked on a tightly fitting Lycra cap worn by the participant. Throughout the experimental session, the coil was maintained in the optimal position with a mechanical device. The coil was positioned tangentially to the scalp, pointing anteriorly, 45° from the midsagittal axis (Mills et al., 1992).

In order to stimulate the foot motor region, a 110 mm double cone coil was placed over the vertex, oriented perpendicularly to the head. Being designed for inducing a stronger magnetic field, as compared to the figure-of-eight coil, this coil is better suited for stimulating the foot area, which is located medially inside the central sulcus (Hovey and Jalinous, 2008). For each participant, we identified the optimal scalp position for inducing MEPs in the contralateral, right abductor hallucis (AH). The orientation of the coil inducing the strongest AH activation was individually determined for each subject by assessing the amplitude of MEPs by moving the coil relative to the mid-sagittal plane.

For the electromyographic (EMG) recording, two pairs of silver-chloride surface electrodes were placed over the belly of the target muscles: the active electrode was placed over FDI and AH, and the reference electrode over the associated joints, i.e., the metacarpophalangeal joint of the index finger for the FDI, and the first metatarsophalangeal joint for the AH. EMG signals were acquired using a BrainAmp MR recorder and Vision Recorder software (Brainproducts, Munich, Germany) at a sampling rate of 5000 Hz, band-pass filtered (.015-1000 Hz), and stored for offline analysis.

For each area, we determined the resting motor threshold (rMT), corresponding to the lowest stimulation intensity inducing MEPs with peak-to-peak amplitude of at least 50 µV in 5 out of 10 TMS pulses (Rossini et al., 1994). During each experimental session, sTMS intensity was set at 120% of the individual rMT and kept constant throughout the experiment. For the hand area, the mean (± Standard Deviation, S.D.) rMT was 66% (±7.8) of the maximum stimulator output; for the foot area, the mean rMT was 60% (±10.7).

During each experimental session, MEPs were recorded during the observation of the video-clips. Each trial started with the presentation of a video-clip, followed by sTMS delivered after a random interval between 1006 and 1982 msec from the beginning of the video-clip. This temporal window, on the total duration of 2.1 s of each video, corresponded respectively to the moment immediately before the effector got in contact with the object (i.e. in which the fingers were already pre-shaped for the grasping or the stepping over action), and the moment immediately before the effector lifted the object (in the grasping actions) or raised up from the object (in the stepping over actions) after having interacted with it. The interval between sTMS pulses ranged from 10.5 and 11.48 s, in order to rule out any carryover effects of sTMS on cortical excitability (Chen et al., 1997).

The amplitude of the MEPS evoked at an intensity of 120% of the individual resting motor threshold, calculated in terms of areas, was, on average, 3.8 (SD±2) mV*ms in FDI and 3.3 (±2.5) mV*ms in AH.

Statistical analysis. The analysis was conducted on nine out of ten participants. One participant was excluded from the analysis, due to an excess of muscular contraction following unrequested postural adjustments throughout the experiment, that interfered with the pattern of the EMG responses. The EMG trace was rectified and the areas underlying the MEPs were calculated for each trial. The pre-TMS EMG activity, recorded in the 200 msec preceding the TMS pulse, was used as a baseline to check for the presence of EMG activity before the TMS pulse. Trials (=20%) where such activity was present were discarded from further analysis. Mirror facilitation by action observation was calculated by dividing the average MEPs' area in each experimental condition by that of the homologous static condition for each participant (i.e. hand grasping and stepping-over conditions divided by static hand; foot grasping and stepping-over divided by static foot). A repeated-measures analysis of variance (ANOVA) was conducted with three within-subjects factors: Action (grasping, stepping-over), Effector (hand, foot) and TMS-site (hand motor area, foot motor area). For all analyses, the level of significance was set at $p<0.05$. Whenever necessary, post-hoc comparisons were run with the Newman-Keuls Test.

Results and discussion

The ANOVA showed a significant main effect of Action ($F_{1,8}=7.6$, $p<0.02$); the interactions TMS-site by Effector ($F_{1,8}=26.6$, $p<0.001$) and TMS-site by Action ($F_{1,8}=11$, $p<0.01$) were significant. Crucially, the significant Action by Effector by TMS-site interaction ($F_{1,8}=8.3$, $p<0.02$) highlighted the selective effect of the action goal and the motor program used to reach it. Other effects were not significant (all ps>0.5). The Action by Effector by TMS-site interaction was explored via two separate 2-way ANOVAs, one for each TMS-site (hand motor area, foot motor area), with Action and Effector as main factors. With respect to the stimulation of the motor hand area (i.e., MEPs recorded from the FDI), a significant main effect of the Effector ($F_{1,8}=21.3$, $p<0.002$) was found, indicating that the MEP amplitude was greater during the observation of actions performed by the hand (mean ± standard deviation: 1.13 ± 0.4), as compared to actions performed by the foot (0.94 ± 0.3), regardless of the type of action. The main effect of Action ($F_{1,8}=11.5$, $p<0.01$) showed that MEPs amplitude was greater when the subjects observed grasping actions (1.13 ± 0.4), as compared to stepping over actions (0.94 ± 0.3), regardless of the effector. The Effector by Action interaction was not significant ($F_{1,8}=2.8$, $p=0.13$) (see Fig. 2-a and 2-b).

The MEPs recorded from the AH after the stimulation of the foot motor region only showed a significant main effect of the Effector ($F_{1,8}=11.6$, $p<0.01$), with MEPs amplitude greater during the observation of actions performed by the foot (1.11 ± 0.22), as compared to actions performed by the hand (0.92 ± 0.18). Instead, the effects of Action ($F_{1,8}=0.02$, $p=0.87$) and the Effector by Action interaction ($F_{1,8}=0.34$, $p=0.57$) did not reach statistical significance (see Fig. 2-c and 2-d).

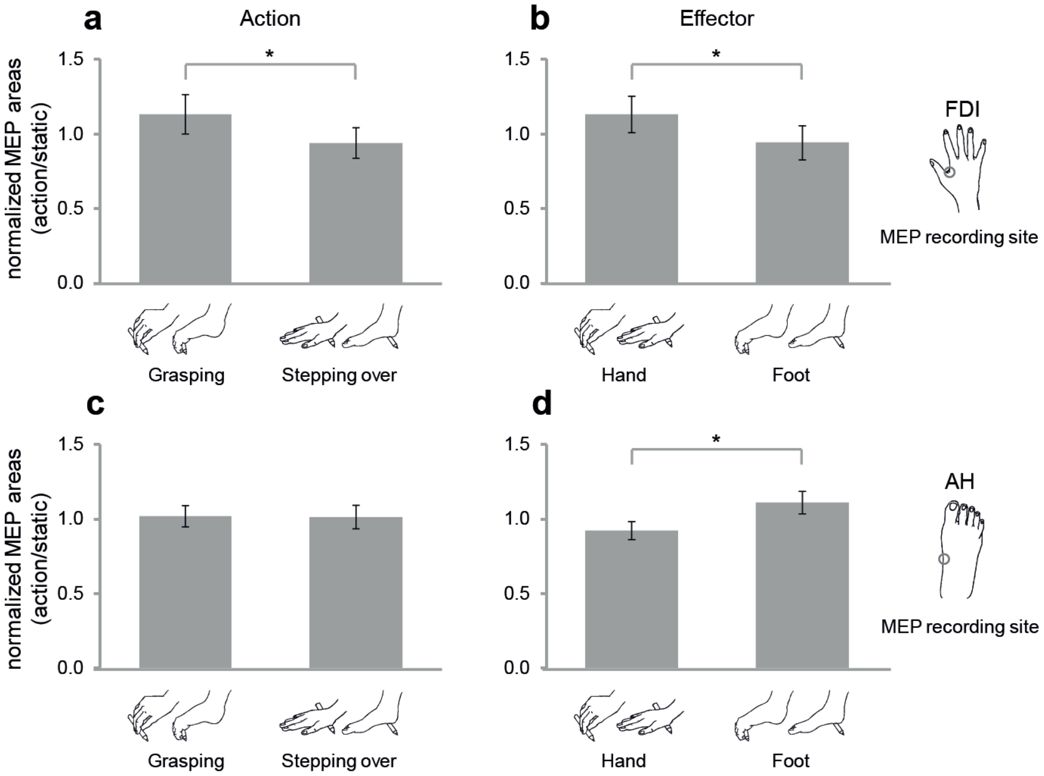

Figure 2. Mirror facilitation by action observation on MEPs in Experiment 1. Figures a-d depict the main effects of action (a-c) and effector (b-d) on MEPs recorded from the muscle FDI (upper panel) and AH (lower panel). Error bars represent the standard error. Asterisks indicate statistical significance at alpha=0.05.

These results show a specific facilitation of corticospinal excitability contingent upon action observation for the muscles that would be involved in the execution of the observed movements. In fact, the observation of actions performed by the hand increased MEP amplitude in FDI, while observing actions executed by the foot increased MEP amplitude in AH, compatibly with a motor resonance of the observed action mapped in somatotopic coordinates. Of interest, MEP amplitude recorded from FDI was enhanced not only by the observation of grasping actions performed with the hand, but also by actions performed with the foot. Conversely, the excitability of the foot motor area was increased only by the observation of grasping actions performed with the foot, but not with the hand. This last result shows that the observation of a familiar action (i.e., grasping) executed by an atypical effector (i.e., foot) induces a motor facilitation also in those muscles that are habitually used to reach the same goal (i.e. hand). The stepping-over action with the foot, being not typical for either the hand and the foot, did not induce any activation of the FDI.

However the control action (i.e., *stepping over*), critically differs from the grasping action, not only for being unspecific for both the hand and the foot, but also for not being a goal-directed action. Therefore, we run a second experiment in which two familiar and common goal-directed actions for either the hand and the foot were shown.

3.3 Experiment 2

In this experiment participants were presented with two familiar, goal-directed actions, one typically performed with the hand (i.e. grasping), and one typically executed with the foot (i.e. pressing a car foot pedal). The actions had both a clear goal, as they were insert in an unambiguous context, and were both familiar to the viewers. By presenting typical and familiar transitive actions for both the hand and the foot, this experiment aimed at investigating whether the foot motor area might be modulated by the observation of a typical foot action performed by an atypical effector (i.e. the hand), in the same way as the hand motor area was modulated by the observation of a grasping action performed by the foot in Experiment 1.

Methods

Participants. Ten healthy participants (all women; mean age=27.7 years, range=23–33 years), with normal or corrected-to-normal vision, all right-handed and right-footed, took part in the study. Two participants took part also in the first experiment. Subjects' selection criteria were the same as in Experiment 1.

Experimental task, TMS protocol, and statistical analysis. Experimental task, TMS protocol and data analysis were the same as in Experiment1, but now participants observed the following video-clips: 1) a right *static hand*; 2) a right *static foot*; 3) a right *hand grasping* and then lifting a pencil; 4) a right *foot grasping* and then lifting a pencil; 5) a right *hand pressing* and then releasing a foot pedal of a car; 6) a right *foot pressing* and then releasing a foot pedal of a car (see Fig. 3). These actions were all potentially executable by the participants, as explicitly confirmed by subjective reports at the end of the experiment, although grasping with the foot or pressing a foot pedals with the hand were rated as totally unfamiliar actions. The temporal pattern of the two dynamic stimuli was matched frame by frame, in the same fashion as Experiment 1, so that the effectors started to move, reached the object and lifted or released it in the same moments in the four videos.

During each experimental session, stimulation intensity was set at 120% of the individual rMT, and was kept constant throughout the experiment. For the hand area, the mean (± Standard Deviation, S.D.) rMT was 63% (±7.6) of the maximum stimulator output; for the foot area, the mean rMT was 60% (±8). Trials in which EMG activity in the 200 milliseconds preceding the TMS stimulus was present (22%) were discarded from the analysis.

The amplitude of the MEPS evoked at an intensity of 120% of the individual resting motor threshold, calculated in terms of areas, was, on average, 2.2 (±1.9) mV*ms in FDI and 1.7 (±1.5) mV*ms in AH.

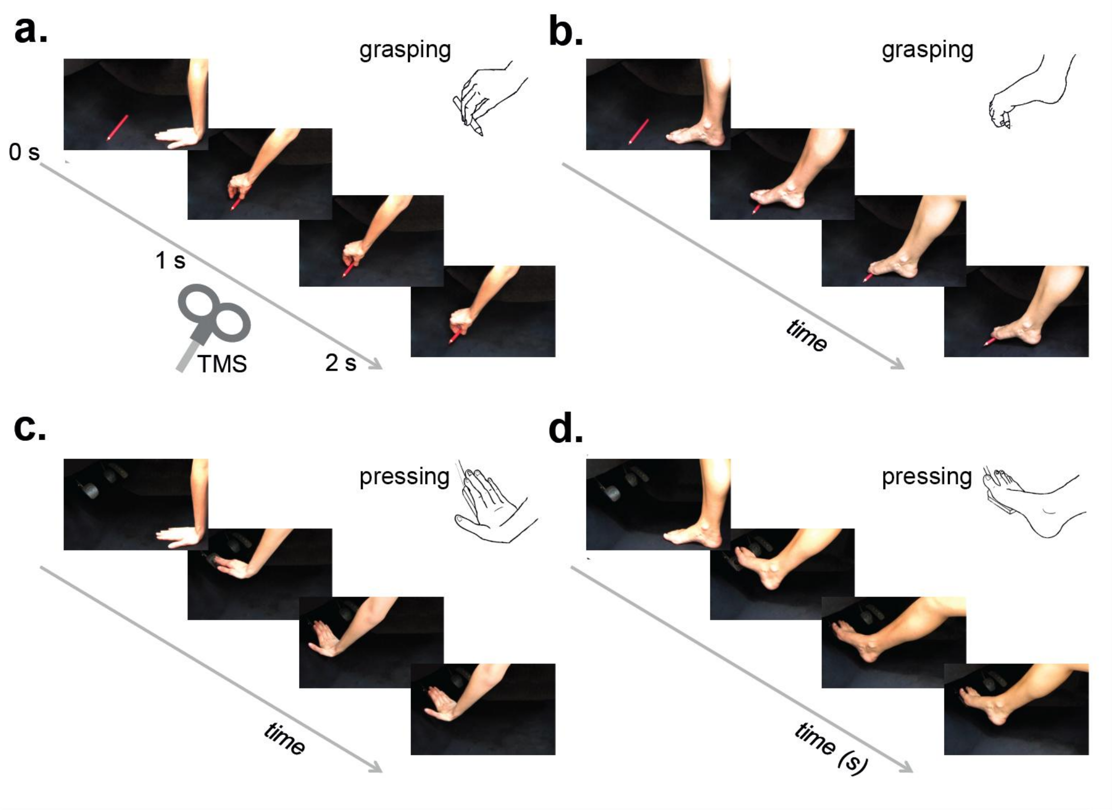

Figure 3. Experimental conditions in Experiment 2. During the experiment, video-clips depicting four actions were presented: a) Grasping with the hand; b) Grasping with the foot; c) Pressing with the hand; d) Pressing with the foot. The sTMS pulse was delivered after a random interval between 1006 and 1982 msec from the beginning of each video-clip.

Results and discussion

A repeated-measures ANOVA was conducted with three within-subjects factors: Action (grasping, pressing), Effector (hand, foot) and TMS-site (hand motor area, foot motor area). The analysis showed a significant TMS-site by Effector interaction ($F_{1,9}$=30, p<0.001), and a significant TMS-site by Action interaction ($F_{1,9}$=10.6,

$p < 0.01$). Other effects were not significant (all ps>0.3). To further explore these interactions, and to test for the specific predictions made in light of the results of the first experiment, we ran a set of planned comparison. On FDI, the MEP amplitude was greater during the observation of actions performed by the hand (mean ± standard deviation: 1.21±0.45), as compared to actions performed by the foot (1±0.43), regardless of the type of action ($F_{1,9}=17$, $p < 0.003$). Moreover, MEP amplitude was greater when the subjects observed grasping (1.18±0.45), as compared to pressing actions (1±0.44), regardless of the effector ($F_{1,9}=11$, $p < 0.01$) (see Fig. 4-a and 4-b).

MEPs recorded from AH after the stimulation of the foot motor region were greater for the observation of actions performed by the foot (1.16±0.32), as compared to actions performed by the hand (1±0.26) ($F_{1,9}=8$, $p < 0.02$). Finally, MEP amplitude increased when participants observed pressing actions (1.16±0.03), as compared to grasping actions (1±0.25), regardless of the effector ($F_{1,9}=6$, $p < 0.03$) (see Fig. 4-c and 4-d).

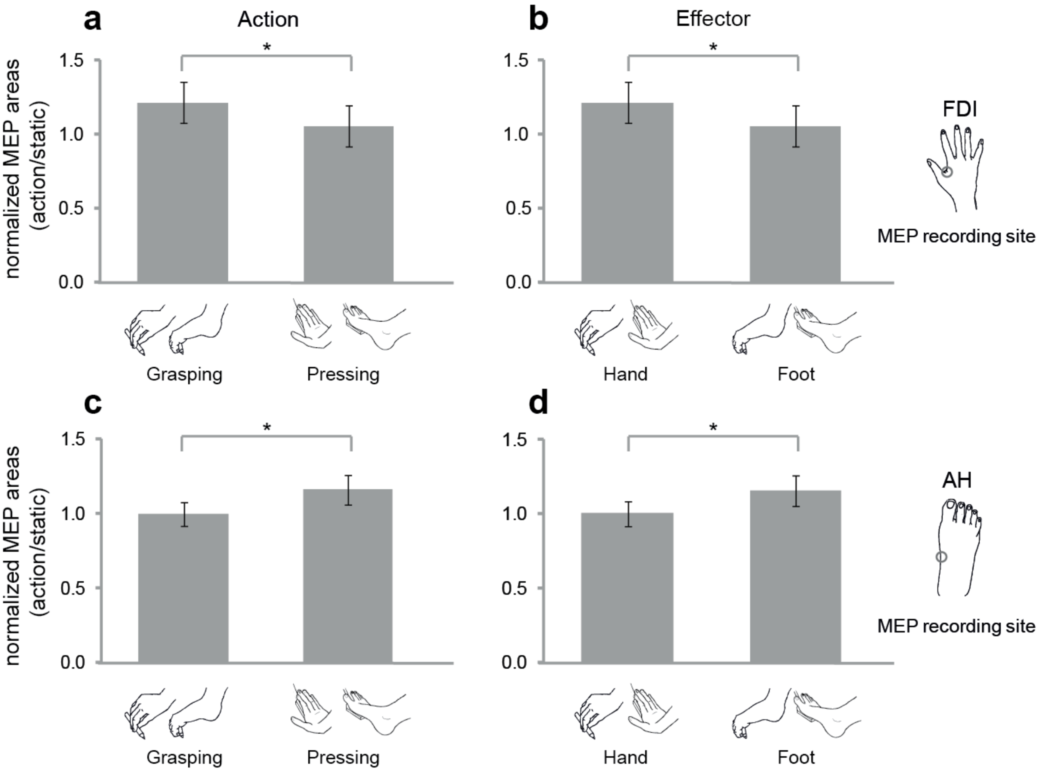

Figure 4. Mirror facilitation by action observation on MEPs in Experiment 2. Figures a-d depict the main effects of action (a-c) and effector (b-d) on MEPs recorded from the muscle FDI (upper panel) and AH (lower panel). Error bars represent the standard error. Asterisks indicate statistical significance at alpha=0.05.

Overall, the results of Experiment 2 confirmed those found in the first experiment, revealing a motor facilitation specific for the muscles that would be involved in the execution of the observed movements, with an increase in the amplitude of MEPs recorded from FDI during the observation of actions performed by the hand, and an increase of MEP amplitude in AH for the observation of actions executed with the foot. Moreover, we found an enhancement in MEP amplitude recorded from FDI also for the observation of a grasping action performed with either the hand or the foot, thus replicating the results of Experiment 1. The novel finding of Experiment 2 is that observing either a foot or a hand pressing a foot pedal, an action typically executed with the foot, induced an increase in MEP amplitude recorded from AH only. These results confirm and extend the results of Experiment 1, showing that the excitability of both hand and foot motor areas is modulated by the observation of a familiar action executed by an unusual effector.

3.4 Discussion

In the present study we recorded MEPs from hand (FDI) and foot (AH) muscles, induced by sTMS delivered to the hand or foot motor areas respectively, during the observation of actions performed by different effectors (hand or foot). In line with previous studies (e.g.: Fadiga et al., 1995; Strafella and Paus, 2000), we have found a facilitation of corticospinal excitability by action observation, which was specific for the effector involved in the actual execution of the observed movements. Indeed, observing an action performed by a hand increased MEP amplitude in FDI, while observing an action executed by the foot increased MEP amplitude in AH.

The crucial finding was that the excitability of the hand motor area was modulated not only by actions performed by a hand, but by grasping actions in general, regardless of the effector used to execute them. Likewise, the foot motor area was modulated not only by actions performed by a foot, but also by the observation of either a foot or a hand pressing a foot pedal. The observation of an action unspecific for both hand and foot (i.e. stepping over) did not modulate specifically neither the hand nor the foot area. This evidence demonstrates that motor facilitation contingent upon action observation is not restricted to the same muscles involved in the observed action, but it occurs even in the muscles typically used to achieve the same action goal. Indeed, when a recognizable action goal (e.g., grasping) is performed through an unusual effector (e.g., the foot), a mirror activation can be observed also in the muscles that are typically used to accomplish the observed action goal (i.e., the hand). If the observed effector did not perform a goal-directed action typically achieved by using a different effector, the mirror motor facilitation is restricted to the observed effector. This is indeed the case of the stepping-over action which is unspecific for both hand and foot and, therefore, induces only an increase of the MEPs recorded from the muscle actually involved in the observed action.

Previous evidence in monkeys showed that mirror neurons in premotor and parietal cortices discharge for both action execution and observation of motor acts (i.e. goal-directed movements), rather than intransitive movements (i.e. displacements of body parts without a specific goal) (Rizzolatti and Craighero, 2004; Umiltà et al., 2001; Kohler et al., 2002; Fogassi et al., 2005; Umiltà et al., 2008). Likewise, neuroimaging and neurophysiological studies in humans suggest the existence of a parieto-frontal network encoding the goals of the observed actions. For instance, frontal and parietal areas are activated by the observation of grasping actions performed by a real arm as well as by a robotic arm, although human and robotic body-parts differ both in terms of kinematics and visual features (Gazzola et al, 2007a). TMS evidence also suggests that when subjects observe a goal-directed action (i.e. grasping an object), what is coded seems to be the action goal rather than the different movements to reach it (Cattaneo et al., 2009). On the other hand, unlike monkeys, the human mirror system is activated not only by goal-directed actions, but also by intransitive actions, such as arm movements or finger flexion (Fadiga et al., 1995; Romani et al., 2005). Given these features of the human MNS, Rizzolatti and Sinigaglia (2010) argued that mirror activations in humans might reflect a double encoding mechanism for action observation. A first mechanism is related to the coding of the motor components of the observed action, namely the displacements of body-parts (i.e., movement mirroring). In this case, there is a mapping of the observed movements onto the observer's inner representation of the same movements. The second mechanism involves a mapping of the observed action goal (i.e., goal mirroring), hence this mechanism is more functional to action understanding. These two mirroring mechanisms appear to be also mediated by different brain regions (Cattaneo et al., 2010, Jastorff et al., 2010). In a recent fMRI study, Jastorff and colleagues (2010) presented participants with various motor acts performed by different effectors (i.e. hand, mouth, foot) and found that the motor acts were coded differently in premotor and parietal cortices according to the effector used or the motor act shown. In particular, in the premotor cortex the motor acts clustered on the basis of the effector, regardless of the type of motor act, while conversely in the parietal cortex the motor acts clustered according to the goal, irrespective of the effector used. The authors suggest that an observed action is encoded firstly in terms of body displacements in the superior temporal sulcus (see also Cattaneo et al., 2010), and then the visual information is sent to the parietal cortex, where the action is abstractly encoded at a higher level in terms of its goal, regardless of the effector involved to achieve it. Finally, the information is sent to the premotor cortex, where the specific program involved in the observed actions, both in terms of goal and effector, is recruited (Jastorff et al., 2010).

A previous TMS study, which investigated the muscle specificity of the motor programs activated during action observation, demonstrated that when participants observed a static effector in front of an object, before grasping it, the cortical excitability was enhanced even in muscles belonging to an effector not involved in that action, as long as it was potentially able to achieve the goal (Lago and Fernandez-del-Olmo, 2011). When participants subsequently saw the effector interacting with the object, the mirror facilitation was muscle-specific. For instance, when the object was grasped with the foot, there was a decrease of MEP amplitude recorded from the hand, as compared to the previous static phase. The authors' account is that only at the beginning of the action the motor program is activated on the basis of the goal of the action, while during effector-object interaction the program is transformed into a muscle-specific program (Lago and Fernandez-del-Olmo, 2011). The different effects found here may be likely due to the different timing of the TMS pulse with respect to the phase of the observed action. Indeed, while in the study by Lago and Fernandez-del-Olmo (2011), TMS pulses were delivered during static phases (i.e. before the effector started to move or after the action was completed), here the TMS pulses were delivered during the effector-object dynamic interaction. An early timing of the TMS pulse with respect to the static effector may have captured affordance-like activations, while our later stimulation may have interacted with the coding of dynamic goal-related aspects of the observed action. This view is further supported by a second main difference between the two studies, related to the overall duration of the observed action: here TMS pulses were delivered during faster actions (2 sec), as compared to those of Lago and Fernandez-del-Olmo (10 s).

Our results support the hypothesis of two parallel coding of actions, i.e., movement mirroring and action mirroring, that are both activated whenever a person observes an action. In daily life, the motor activations expressed by such a double coding of observed actions typically coincide: we generally see people achieving goals using the very same effectors that we would normally use to execute the same actions (see

Gazzola et al., 2007b). In line with the idea of a hierarchical organization of the motor system involving different descriptive levels of the action (i.e. goal, kinematic, muscular levels, see Grafton and Hamilton, 2007), it might be possible that, when the effector involved in the observed action differs from the one that is typically used to execute that action, the brain activates not only the motor program corresponding to the observed effector, but also, at a higher level, the motor program of the effector commonly used to reach that goal, even when it is not actually implicated in the observed action.

Our findings are also in line with recent studies on action priming (see Heyes et al., 2011, for a review), which suggest that automatic imitation is mediated by the MNS through a direct link between perception and action. In automatic imitation paradigms a motor response is facilitated by the observation of a task-irrelevant action that is similar to the required motor response in terms of body part involved and/or type of movement (e.g. Heyes et al., 2005; Bach et al., 2007; Gillmeister et al., 2008; Leighton & Heyes, 2010). For instance, Leighton and Heyes (2010) used a stimulus-response compatibility procedure, showing video-clips of either a hand or a mouth closing or opening, and asking participants to open or close either their hand or their mouth. The authors found a movement compatibility effect, whereby opening responses were faster during the observation of an opening movement (hand or mouth), rather than a closing one, and vice versa. Interestingly, this effect was smaller when the observed effector was incompatible with the one used to give the response, indicating that automatic imitation is influenced by the matching of both the effector and the movement type.

Such imitative processes could be possible through the recruitment of excitatory links between the corresponding sensory and motor representations, emerging from repeated sensory-motor experience of visual and motor representations of familiar action (Heyes et al, 2001; 2010a). In line with the associative sequence learning theory (e.g. Heyes, 2005), these automatic stimulus-response connections can be a product of learning, depending on the repeated experience of observing actions, both self-generated or executed by others, the last observed during social interactions. In this contest, our findings suggest that, during the observation of a familiar action performed by an atypical effector, on one hand the motor program corresponding to the observed effector is recruited by mapping the observed effector's movement on the observer's body schema (Bach et al., 2007). On the other hand, the motor program of the typical effector used to achieve the same goal might be simultaneously recruited by learning-dependent excitatory links, which depend on our typical sensorimotor experience (as in the case of grasping an object which is typically performed by the hand).

In conclusion, while the motor matching of an observed moving body part would allow to simulate the efferent components of the observed action in a strictly congruent fashion, the simultaneous, automatic, matching of the observed action on its prototypical effectors would be of more helpful to understand the action goal based on our experience. This complex mechanism of motor coding would increase our abilities in social interactions.

4. Infants' perception of possible and impossible goal-directed actions: An eye-tracking study in 6-month-olds

4.1 Introduction

When people observe an action, they tend to interpret it in terms of hidden mental states of the actor, such as intentions and desires, which drive that action. Several studies explored how infants code observed actions and which features of the observed action can influence their ability to infer its goal (e.g. Gergely et al. 1995; Meltzoff, 1995; Csibra et al., 1999; 2003).

Previous evidence suggests that infants in their first months of life encode actions performed by others as goal-directed, as long as the observed actions are performed by a human agent (e.g. Woodward et al., 1998; Daum and Gredeback, 2011; Kanagogi and Itakura, 2011). For instance, if infants are visually habituated to an actor who reaches for and grasps one of two toys, then they look longer when the actor grasps a new toy in the same position than when he grasp the old toy in a new position (e.g. Woodward, 1998). This result shows that infants pay more attention to a change in the actor's goal rather than to the spatial location or trajectory of his motion, suggesting that infants tend to encode the observed actions in terms of goals. Infants show this preference only when they observe an action performed by a human actor, but not when they see the same action performed by a rod moving toward and touching the toy (e.g. Woodward, 1998). Infants selectively focus their attention on aspects relevant to the actor's goal over other salient aspects of the action only when the action is performed by a human agent. This line of evidence suggests that biological motion and/or human-related aspects are essential to attribute goals to the observed actions (e.g. Woodward et al., 1998; Daum and Gredeback, 2011). Moreover, studies measuring eye movements demonstrate that infants as young as 6 months show proactive gazes toward the goal of observed actions, thus anticipating it, as long as they have experience in performing those actions, suggesting that goal anticipation during action observation might be mediated by a direct matching mechanism mapping the observed action onto the observer's motor representations of the same action (Falck-Ytter et al., 2006; Gredebäck and Melinder, 2010; Kanagogi and Itakura, 2011). In particular, a study from Falck-Ytter et al. (2006) reveals that anticipatory eye movements for the observation of an actor transporting a series of objects into a bucket are evident in 12-month-old infants and adults, but not in 6-month-olds, the last being not able yet to perform the observed action themselves (Falck-Ytter et al., 2006). Similarly, the ability to anticipate action goal in 12-month-old infants during the observation of feeding actions depends on infants' own experience with similar action sequences of being fed (Gredebäck and Melinder, 2010).

If the presence of a biological agent and infants' sensorimotor experience exerts an influence on the perception of observed actions, what is the role of biomechanical plausibility in infants' coding of goal directed actions? If a human actor performs a familiar action by moving body parts in a biomechanical impossible way, are infants able to notice the violation of biomechanical constraints and does it affect their ability to interpret the action as goal-directed?

In his context, Reid and colleagues (2005) investigated whether 8-month-old infants are able to discriminate between goal directed actions performed in a possible and an impossible manner (i.e. violating biological human constraints). Infants were shown a human arm reaching for and grasping an object in a natural way or in a way that violates the biomechanical constraints of the elbow; only infants with high fine motor skills looked longer at the impossible actions, hence proving to be able to discriminate between the two actions. Therefore, the visual features of the human body and its biomechanical properties seem important for encoding goal directed actions. However, other studies suggest that this is not necessarily the case. For instance, even in case of a non-biological agent such as a rod, 6-month-old infants are able to code the observed actions as goal-directed as long as they are provided with further clues to goal-directedness (Kiraly et al., 2003; Bíró and Leslie, 2007). Moreover, when presented with complex actions, such as reaching for an object by pushing an obstacle away, 6- to 8-month-old infant infer the goal even from biomechanically impossible actions (Southgate et al., 2008). In Southgate and colleagues study (2008), infants were habituated to a video of a human arm moving an obstructing box out of the way and then reaching for and retrieving a target object. In the test phase, a second box blocked a straight reach to the object. The arm could reach for the object either in a biomechanical possible way, by removing the second box, or in a biomechanical impossible manner, i.e. with the arm snaking around the second box and going straight to the object. The possible action reached the goal state in more steps (i.e. less efficiently) than the impossible one did. Infants looked longer at the biomechanical possible but less efficient action, showing that they perceived as novel and more unexpected that action; therefore, the efficiency of the observed action for achieving the goal is more relevant than its plausibility.

The authors interpret this result as an evidence that even impossible actions can be coded as goal directed as they are physically efficient in attaining a goal state. The studies from Southgate and colleagues (2008) and Reid and colleagues (2005) reach different conclusions about how the biomechanical plausibility of motion is coded in the first months of life. It is possible that they found different results due to the different paradigms used in the two studies. Southgate and colleagues (2008) showed a more complex action that involved not only a simple action chain, such as a reach-to-grasp action (Reid et al., 2005), but a goal-directed action composed by more steps, like reaching for and pushing an obstacle away in order to achieve the goal. It is possible that, in the case of a complex action performed in an environment that imposes several constraints, the efficiency in achieving a goal might become more important than the plausibility of the action. However, in both studies the violated biomechanical constraints regarded the reaching phase of the action (i.e. movement of elbow and forearm), but not the grasping phase (i.e. movement of the hand), which represents the action's end state. In other words, the biomechanical properties of the grasping hand, which is the body part relevant to the final goal, were never violated; therefore, in both possible and impossible actions, the final phase of the action (i.e. the object being grasped) was the same. If infants focused their attention on the end state of the action, rather than on the intermediate phases used to achieve the goal, the difference in action plausibility may have remained unnoticed with the use of the above mentioned paradigms.

Moreover, in the study from Southgate and colleagues (2008), the impossible action involves one additional motor component (i.e. the hand pushing an obstacle), and this could have influenced further infant's looking time. Another problematic aspect is that 6- to 8-month-old infants were shown a biomechanical possible action that cannot be performed at that age, namely double-detour reaching action (Diamond, 1990); so neither the possible, nor the impossible actions were part of the infants' motor repertoire, and thus the observed actions are both novel to infants.

To further investigate infants' ability to discriminate possible and impossible actions, in the present study we used eye tracking to investigate whether infants' ability to perceived actions as goal directed is affected by the anatomical plausibility of the end state of an action. In particular, at variance with previous studies, we focus on the ability of 6-month-old infants to discriminate between grasping actions in which the hand grasps an object using a whole hand grip either in a possible (i.e. with the all fingers closing gradually around the object) or impossible manner (i.e. with the fingers moving unnaturally towards the back of the hand, violating the biomechanical constraints of the phalanges). Possible and impossible actions consisted in a simple action chain of reach-to-grasp an object, which was then moved to a final position. We chose to show that the object was moved, after being grasped, in order to present a more salient action-effect, which can provide a facilitatory cue for attribution of goal directedness (Kiraly et al., 2003; Bíró and Leslie, 2007). A grasping action indeed is typically followed by a change in the state of the grasped object. The object is typically moved or picked up when infants grasp it or observe others' grasping actions. Previous studies have shown that 6-month-old infants are facilitated to perceive the action as goal-directed when the agent picks up an object, after having grasped it (e.g. Bíró and Leslie, 2007). We hypothesize that if infants are able to discriminate between possible and impossible hand action, they will look longer at the impossible grasp action, recognizing it as novel and unfamiliar. Moreover, we intend to investigate for the first time whether infants, if capable to discriminate between the two actions, are able to perform predictive eye movements, thus anticipating the action’s goal, in either possible and impossible actions.

In particular, the present study investigated whether 6 month-old infants are able to discriminate between reach-to-grasp actions differing in their anatomical plausibility, and if such discrimination affects infants’ ability to interpret the observed actions as goal-directed. Infants observed grasping actions in which a hand grasped an object using a whole hand grip either in a possible or impossible fashion (i.e. with the fingers bending unnaturally towards the back of the hand). Moreover the study aimed at exploring whether infants interpret the actions as goal directed in both anatomical possible and impossible conditions. The presence of predictive gazes (i.e. gazes which arrive at the target object before the hand reaches for it) is interpreted as infant’s ability to infer the goal of the observed action.

4.2 Methods

Participants. Nineteen healthy, full-term 6-month-old infants (11 females, mean age=6 months and 8 days, S.D.=8 days, range=173-199 days) took part in the study. Nineteen additional infants were tested, but discarded from the final sample because of fussiness (N=10), no completion of the minimum number of trials required for analysis (see below) (N=5), bad calibration and recording (N=4). Participants were recruited via a written invitation sent to parents on the basis of birth records provided by the local Register office. The protocol was carried out in accordance with the ethical standards of the Declaration of Helsinki (BMJ 1991; 302: 1194), and approved by the ethical committee of the University of Milano-Bicocca. Parents gave their written informed consent.

Stimuli apparatus and procedure. Colored computer animations were created using Poser 6 (Smith Micro Software, Inc). Each video was 1500 x 891 pixel (subtending 33.9 x 21.6° of the visual angle) and had a duration of 7.5 s. All videos were matched for luminance, hue, saturation. The videos depicted a female actor seated at a table and facing the viewer, with the right arm resting on the table in front of her, and the hand leaning on the palm, with the fingers aligned. The head of the actress was not visible. In front of the actor, at the opposite side of the table, a cylinder was placed in the middle next to two squares on the table, one on its left and one on its right. At the beginning of each video, to attract infants' attention, a tapping movement of the actress' fingers on the table was shown for 1 s. Then the hand rotated longitudinally on its side, with the thumb up and the fingers aligned (duration: 1s) and started to move toward the cylinder with the fingers preshaping in either a possible or impossible grasp (next 0.3 s), then stopped for 1.14 s, before approaching and getting in contact with the cylinder (next 0.84 s). After grasping the cylinder (0.74 s), the hand moved it onto one of the two squares, which are the object's final location (0.27), and stopped there for the subsequent 2.2 s (Figure 1). The impossible grasp was obtained by bending the fingers in the opposite direction than in the possible grasp (i.e. with the fingers curling backwards and moving towards the back of the hand). The angles of fingers' and phalangeal joints' displacements were matched frame by frame between the possible and impossible conditions.

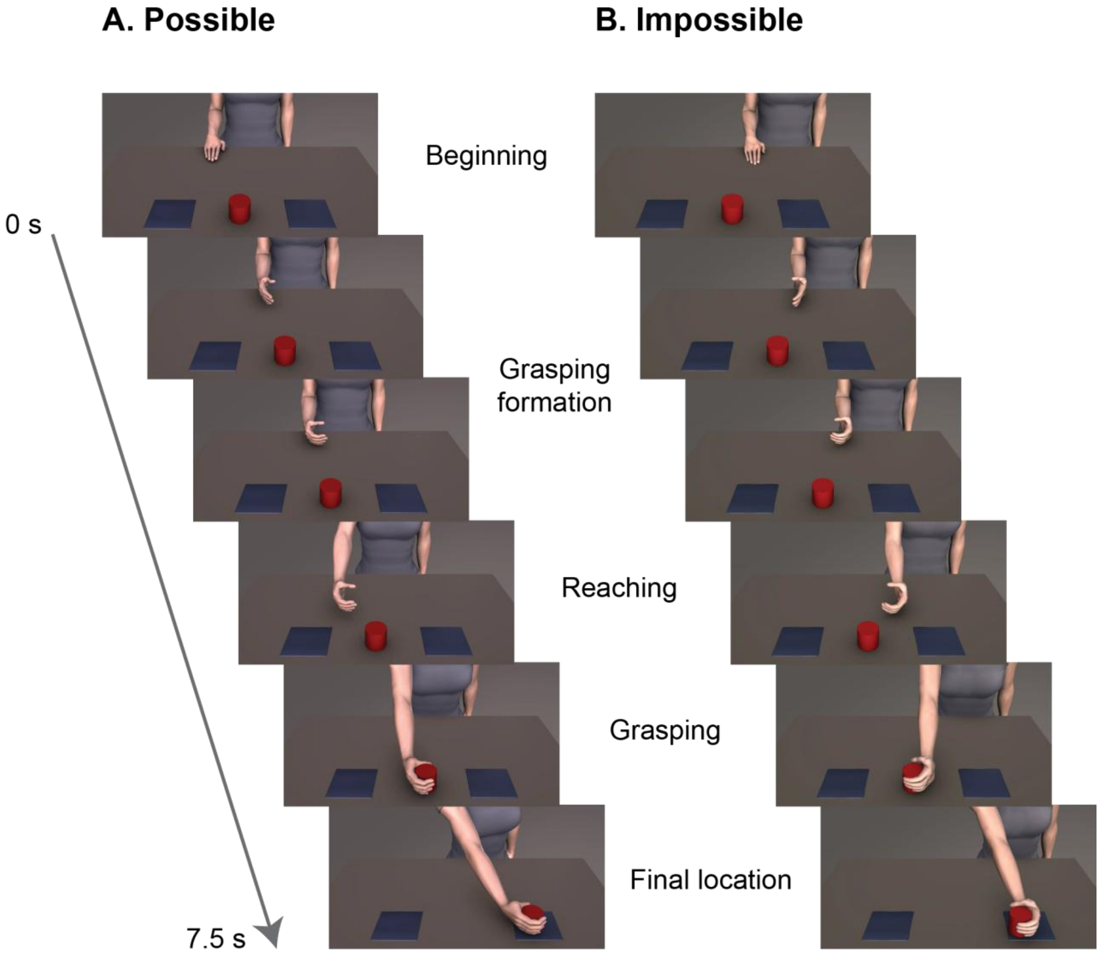

Figure 1. Experimental stimuli. Video-clip frames depicting the two action-stimuli presented: a) Possible and b) Impossible conditions. The animations lasted 7.5 s.

During the observation of the videos, eye movements were recorded by means of an ASL EYE-TRAC 6 eye-tracking system (Applied Science Laboratories, MA). The experiment took place in a dimly illuminated room. Infants were seated on a car safety seat, with the eyes at a distance of approximately 60 cm from a 19'' monitor where the stimuli were presented. After a six-point calibration the experimental session begun. The two stimuli (possible, impossible) were presented in alternated blocks of 6 trials each for a total of 24 trials (12 per condition). Nine out of nineteen participants started with the possible condition (group 1), and the other ten participants started with the impossible condition (group 2). The spatial position of the final location of the object was counterbalanced across participants. Half of the participants were presented with videos in which the object was grasped and then moved onto the square on the right and half with the object moving onto the square on the left. Between trials, an attention-catcher accompanied by sound was presented at the centre of the video until the infant regained his/her attention on the monitor.

Statistical analysis. We defined five Areas Of Interest, i.e. AOI (Figure 2): one covering the position of the hand before the hand moved toward the object (hand AOI), one covering the target object (object AOI), one covering each of the two squares which represent the final location of the object (right square AOI and left square AOI). Together, the object AOI and the square AOI represent the goal area (goal AOI). The last area covered the trajectory of the hand to the object (trajectory AOI).

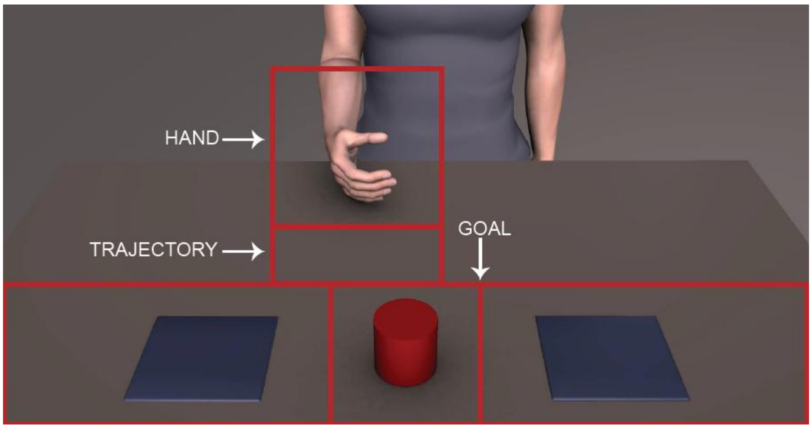

Figure 2. Red rectangles represent AOIs within the scene. The ‘hand AOI’, encompassed the position of the hand before the agent began to move toward the object. The ‘goal AOI’ corresponded to the end target of the action, including both the target object (‘object AOI’) and the object’s final location (either ‘left square’ or ‘right square’ AOI). The middle AOI (‘trajectory AOI’) encompassed the trajectory of the agent’s movement toward the object.

A trial was considered attended, and thus included in the analysis, if the participant looked at the hand AOI during the pre-shaping and formation of the grasp or at the trajectory AOI during the reaching phase before looking at the goal AOI. This was done because we were interested in investigating whether the violation of biomechanical properties of the fingers was noticed by the infants and could influence infants' ability to anticipate the action goal. Participants with less than three attended trials per condition were discarded from the analysis.

To investigate whether infants were able to discriminate between possible and impossible stimuli, we analysed the average looking time to the hand AOI, during the pre-shaping and formation of the grasp, and to the trajectory AOI, during the phase of reaching in the two conditions (possible, impossible), by means of a repeated-measures analysis of variance (ANOVA) with Biomechanical properties (possible, impossible), as within-subjects factor, and Order of presentation (possible-impossible, impossible-possible), as between-subjects factor.

The same analysis was conducted also on the total looking time spent by the infants on the entire duration of the trials.

In order to see whether a gaze shift from the hand AOI to the goal AOI was predictive, thus anticipating the arrival of the hand on the object, or reactive, with the participant's gaze arriving on the object after the hand did, the difference between the timing in which the agent reached the object and the timing in which the gaze arrived on the object was calculated. A positive value indicated the presence of a predictive gaze (i.e. the participant's gaze arrived at the object before the agent), while a negative score indicated a reactive gaze. Latencies of gaze from the hand to the goal, both predictive and reactive, entered a repeated-measured ANOVA with Biomechanical properties (possible, impossible), as within-subjects factor, and Order of presentation (possible-impossible, impossible-possible), as between-subjects, factor.

In order to verify whether biomechanical properties can influence specifically infants' ability to anticipate the action's goal, we conducted an analysis on predictive gazes. For each infants, the proportion of anticipatory gazes on the number of the attended trials was calculated for each condition (possible, impossible), and then analysed via a repeated-measures ANOVA with Biomechanical properties (possible, impossible), as within-subjects factor, and Order of presentation (group 1: possible-impossible, group 2: impossible-possible), as between-subjects factor. Post-hoc comparisons with the Newman-Keuls test were conducted whenever appropriate. The significance level was set at alpha=0.05.

4.3 Results

Infants attended, on average, 7.6 trials out of 12 in the possible condition and 8 in the impossible one.

The analysis conducted on the average time spent looking at the hand revealed a main effect of Biomechanical properties ($F_{1,17}=5$, $p \leq 0.03$). During the formation of the grasp and the reaching phase, infants looked longer at the impossible stimulus (mean=1.48 s, SD=0.5 s) as compared to the possible one (mean=1.3 s, SD=0.5 s), demonstrating that they discriminated between the two stimuli (Figure 3). The preference for the biomechanically impossible stimulus was present in 14 out of 19 participants (binomial test, $p<0.03$). The analysis run on the total time spent looking at the entire trial did not reveal any significant main effect nor interaction (all $ps>0.2$), confirming that the preference for the impossible condition was due to the violation of the biomechanical properties of the hand. In fact average total looking time spent to look at the stimuli in their total duration was similar in the possible (mean=5.3, SD=1.3) and impossible (mean=5.4, SD=1.05) conditions.

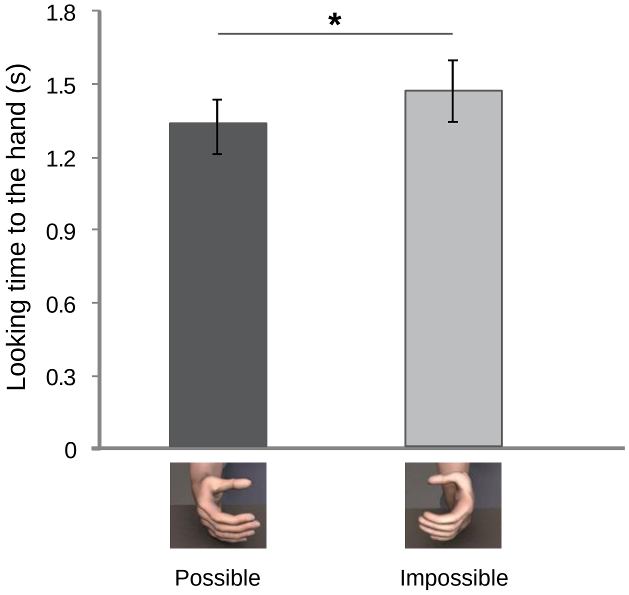

Figure 3. Looking time to the hand during the formation of the grasp and the initial movement in the trajectory toward the object. Error bars indicate SEM.

The analysis on gaze behaviour (i.e. latency of gazes from the hand to the object) showed no significant main effects nor a significant interaction (all ps>0.2). Overall infants made more predictive than reactive gazes, as shown by the fact that latencies of gaze were positive in both possible (0.53 s) and impossible (0.4 s) conditions (Figure 4a). Latencies were significantly different from the chance level, as measured by a one sample t-test against 0, in both possible (t=3.6; p<0.003), and impossible conditions (t=3, p<0.01).

The analysis performed on the proportion of anticipatory gazes showed a significant Biomechanical properties x Order of presentation interaction ($F_{1,17}$=6.3, p<0.03).

Post-hoc comparisons revealed that when infants were presented with the possible condition as first (group 1, possible-impossible), they did more predictive gazes in the possible condition (45% of anticipatory gaze on the total attended trials) than in the impossible condition (23%, p<0.05). When the impossible condition came as first (group 2, impossible-possible), no significant difference was found between possible (37%) and impossible (41%, p=0.4) conditions. The main effect of the factors Biomechanical properties and Order of presentation was not significant (ps>0.2) (Figure 4b).

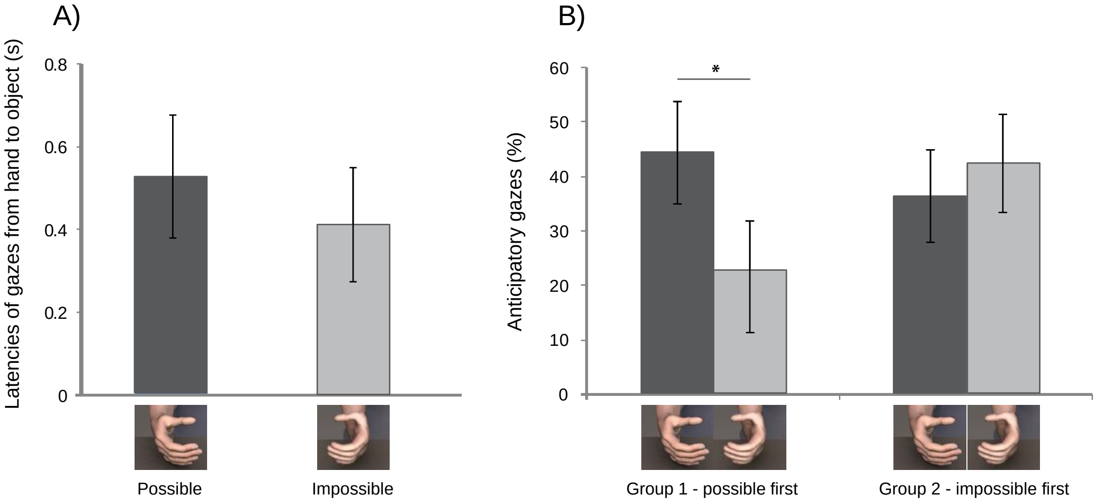

Figure 4. A) Latencies of gaze arrival at goal relative to arrival of hand (difference between the timing in which the agent reached the object and the timing in which the gaze arrived at the object). B) Percentage of anticipatory gazes on the total number of attended trials for each condition in the two groups. Error bars indicate SEM.

4.4 Discussion

The present study showed that 6 month-old infants are able to discriminate between reach-to-grasp actions differing in their anatomical plausibility. Infants interpreted the actions as goal directed in both anatomical possible and impossible conditions.

Finally, infants' ability to discriminate between possible and impossible action exerts an influence on their ability to anticipate the goal of a grasping action.

In particular, the analysis of looking time revealed that infants looked longer at the hand during the formation of the grasp in the impossible condition as compared to the possible one, regardless of the overall time spend to look at the stimuli. This finding indicates that infants were able to discriminate between the two actions and that the preference for the impossible stimulus was probably triggered by the violation of the biomechanical constraints of the hand. Previous studies have demonstrated that, when presented with movements involving a violation of the biomechanical properties of the joint of the elbow, infants are able to discriminate between possible and impossible movements only from the age of 8 months (Reid et al., 2005; Morita et al., 2012). However, in a reach-to-grasp action the elbow is involved in the phase of reaching, but not in grasping which is the goal of the action chain. Since infants tend to focus their attention more on the goal of the action, rather than on the way it is achieved (e.g. Woodward, 1998), it is possible that in our study infants as young as 6-months looked longer at the impossible stimulus because the violation of biomechanical properties concerned the hand, which is the body part interacting with the object, and thus directly relevant in achieving the goal.

Additionally, we showed that 6-month-old infants executed predictive gazes in both possible and impossible conditions, suggesting that they can code as goal directed not only the possible action, but also the impossible one, even if they have recognized the violation of the biomechanical constraints. Previous research indicates that infants tend to code actions as goal-directed only when performed by a biological agent, and not when performed by an unanimated object, unless they are provided with cues helping them to ascribe goal directedness to an action (e.g. Woodward et al., 1998, Daum and Gredeback, 2011, Kanagogi and Itakura, 2011; Bíró and Leslie, 2007). Instead, our findings reveal that observing a human agent performing an action is sufficient to induce an interpretation of the observed action as goal directed, irrespectively of the manner in which the goal is achieve, either possible or impossible. A previous study from Southgate and colleagues (2008) suggest that infants ascribe goals even to biomechanically impossible action, but it is not clear whether infants have noticed the violation of the biomechanical properties of the arm. In our study, by means of an eye tracker, we could demonstrate that infants were able to discriminate between biomechanical possible and impossible actions and that such a discrimination did not impair their ability to code the action as goal directed. The fact that an action is executed by a human may be a sufficient cue to ascribe intentions even to impossible actions.

It should be noted that even in adults, an observed action which implies a violation of the plausibility of human anatomy is coded more at the level of goal, rather than at the level of the movements that constitute it (Longo et al., 2008). For instance, when presented with fingers tapping a surface, both in a biomechanical possible or impossible fashion, comparable automatic imitation is elicited from the two stimuli, suggesting that the actions is coded at the level of the goal, regardless of their biomechanical plausibility (Longo et al., 2008).

It is possible that infants coded as goal directed even biomechanical impossible actions due to their experience with human actions, both executed and observed. Infants could infer others’ goals and intentions through their own experience: Infants tend to interpret humans as intentional agents, and they ascribe intentions even to failed action, probably because infants’ motor and visual self-experience may suggest the presence of a goal beyond the apparent behaviour (Meltzoff, 1995; Csibra and Gergely, 2007).

In our study, infants who were shown the possible condition as first, before the impossible action, did more predictive gazes in the possible condition, than in the following impossible one; instead, when the impossible condition was presented as first, infants did not show any preference. These results suggest that information about the anatomical plausibility of the observed action exerts an influence on the interpretation of that action: even if viewing an impossible action does not impair the interpretation of that action as goal-directed, the view of the possible action as first, which is familiar to infants, may facilitate action understanding.

5. Newborns differentiate between possible and impossible movements

5.1 Introduction

Human adults possess neurophysiological mechanisms that allow to integrate action perception and execution within the observer's motor representation (Rizzolatti and Craighero, 2004). The question of how such mechanisms emerge during development is still under debate. Given that newborns seem to show imitative abilities already at birth (e.g. Melzoff and Moore, 1977; 1983; 1989), it has been hypothesized that a neural system responsible for shared representations that allow to understand the actions of others might be present from birth (e.g. Lepage and Theoret, 2007). However, some authors claim that movements contingent on the observation of the same movement such as tongue protrusion, previously interpreted as evidence of neonatal imitation, might be just reflexive and not a proof of the existence of mirror mechanisms (Jones, 1996; 2009; Anisfeld, 1996; Heyes, 2010a). Moreover, several studies suggest that mirror mechanisms modify their activity during development in accordance with the growing sensorimotor experience of the observer (e.g. Falck-Ytter et al., 2006; Sommerville et al., 2005, Libertus and Needham, 2010; van Elk et al., 2008; Marshall e Meltzoff, 2011). Thus, it is not established whether mirror mechanisms are active at birth, or develop later with age (e.g. Heyes, 2010a; 2010b).

The aim of the present study is to explore the existence of links between the motor representation of a movement and its sensory counterpart in the first days of life . In particular, the study investigates whether newborns are able to visually discriminate between simple possible movements and movements which are impossible to execute because they violate biomechanical human constraints.

When adults observe human body postures or movements that violate biomechanical constraints, such as distorted joint movements, they experience aversive feelings and they judge them as more unpleasant than natural movements (e.g. Costantini et al., 2005; Avenanti et al., 2007; Morita et al., 2012). In adults, action simulation can occur even in response to biologically impossible movements, such as finger movements that are outside the normal range of abduction-adduction movements (Costantini et al., 2005; Romani et al., 2005; Avenanti et al., 2007), triggering a cortico-spinal facilitation of the muscles, as assessed by means of transcranial magnetic stimulation (TMS), that would be involved in the actual execution of the observed movement, as possible movements do (Romani et al., 2005, Avenanti et al., 2007). However, different brain regions appear to be involved in simulating efferent and afferent components of possible and impossible movements (Costantini et al., 2005). Virtual lesion of ventral premotor cortex and primatosensory cortex induced by TMS suppresses mirror motor facilitation during the observation of possible and impossible finger movements, respectively, suggesting that parietal areas might have a role in coding action’s somatic components of observed actions and in coding information about the bio-mechanical plausibility of the observed action (Avenanti et al. 2007). Moreover, in adults motor facilitation contingent upon action observation is induced even by the observation of static body snapshots suggesting motion, either possible or impossible (Urgesi et al., 2006; Candidi et al.; 2008).

Infants as young as 3 to 5 months are able to distinguish between a display depicting a human walking and the same display scrambled, inverted or with dots moving randomly (Fox and McDaniel, 1982; Bertenthal et al., 1984; 1987; 1993). Five-month-old infants are also able to discriminate between three points of light representing the shoulder, elbow, and wrist, moving in a natural or unnatural (i.e. moving out of phase, with the arm appearing disjointed) fashion, suggesting that infants in the first months of life are already sensitive to biological constraints (Bertenthal et al., 1987). Infants from the age of 8-12 months are able to discriminate between movements of the elbow which respect or violate the biomechanical constraints of the joint (Reid et al., 2005; Morita et al., 2012). However, when presented with static body pictures depicting a typical body or a body with scrambles gross anatomy, such as arms replaced with legs, infants show a visual preference for scrambled body shapes, appearing able to discriminate between the two stimuli, only from the age of 18 months (Slaughter et al., 2002). If presented with the same scrambled bodies animated to move in a biologically possible way, even 9-and 12-month-old infants are able to discriminate between the two stimuli, showing that the presence of biological motion contributes to infants' recognition of the human form (Christie and Slaughter, 2010).

Different findings were obtained by using faces. Infants as young as few days or few months are able to discriminate between typical and scrambled static configuration of a human face; therefore, a preference for typical configurations of a human face is shown even in absence of motion (Goren et al., 1975; Johnson et al., 1991; Slaughter et al., 2002).

Recent studies with 2-day-old newborns indicate that newborns are able to discriminate between point-light displays depicting a non human biological agent (e.g. a hen) moving either in a biological or non biological manner (i.e. inverted displays or random patterns) (Simion et al., 2008; Bardi et al., 2010).

A further study from Craighero and colleagues (2011) in newborns suggests that sensorimotor associations are already present in the first days of life. During their intrauterine life, foetuses develop motor skills and seem to have experience of goal-directed actions. In particular, from the 14th week of gestation, about the two third of the foetal hand movements are directed to targets, such as body parts or wall of the uterus or umbilical cord, that are explored and manipulated (Sparling et al., 1999). Then, starting from about 22 weeks of gestation, the kinematics patterns appear to be affected by the action goal, with different peak velocity when the action is directed to the eyes or the mouth (Zoia et al., 2007) or, in the case of twin foetuses, toward the own body or the one of the sibling (Castiello et al., 2010). Two days after birth, newborns are able to visually discriminate between goal-directed and not-goal-directed actions (Craighero et al., 2011). Given the motor experience that foetuses have in their intrauterine life with goal-directed actions, it has been suggested that primitive sensory-motor associations might be already present at birth (Craighero et al., 2011).

This ability to match motor and sensory representations might be facilitated by the fact that infants in the first months of life are also capable of intermodal perception, transferring information acquired in a specific sensory modality to another one, for instance recognizing visually objects previously explored manually (Streri and Spelke, 1988) or orally (Melzoff and Borton, 1979).

So far, it is still unknown whether at birth newborns are also able to discriminate between possible and impossible simple, not-goal-directed, hand movements. To address this issue, the present study investigated whether 2-day-old newborns are able to discriminate between a simple hand movement (i.e. non-directed action) and another one that is not anatomically plausible. The newborns' ability to discriminate between possible and impossible stimuli was assessed by means of an infant-controlled visual preference paradigm, which consists in presenting two stimuli at the same time and recording the length of time the infant looks at each (Fantz, 1958). If infants are able to discriminate between them, the total looking time spent to look at each of them will be different. The stimulus that infants watch for the longest amount of time can be inferred to be the one infants find to be the most interesting, for instance because novel as compared to a familiar one.

In four different experiments we explored: 1) the role of sensorimotor experience in discriminating between possible and impossible movements (experiments 1 and 3), and 2) whether this discrimination occurs even in the absence of motion information, extending to the observation of static gestures (experiments 2 and 4).

5.2 Experiment 1

Experiment 1 explored whether 2-day-old newborns were able to discriminate between an observed plausible hand movement that they are already able to perform, and a similar movement which violates the constraints of human anatomy. Newborns were presented with either a biomechanical possible or impossible movement of the hand closing in a full hand grip. Given that newborns are already able to perform such a movement, we hypothesized that newborns, if able to discriminate between the two stimuli, would have looked longer at the impossible stimulus, because they perceived it as novel.

Methods

Participants. Fourteen, full-term, healthy 2-day-old newborns (8 females, mean age=45 hours, range 20h-96h, mean weight at birth=3160gr, range=2530-3720gr) were enrolled in the study. Only infants with an Agpar score (Apgar, 1953) of at least 8 at 5 minutes were recruited. Twelve additional infants were tested, but discarded from the final sample because they changed their state during the testing session (N=10), watched only one trial out of two (N=1), or manifested a position bias, looking towards one direction for over 85% of their looking time (N=1). The protocol was carried out in accordance with the ethical standards of the Declaration of Helsinki (BMJ 1991; 302: 1194), and was approved by the ethical committees of the San Gerardo Hospital (Monza), and the University Milan-Bicocca. Parents gave their written informed consent. Infants were tested when they were in an alert and attentive state.

Stimuli, apparatus and procedure. Newborns were presented with two dynamic stimuli showing a hand moving on a black background. One stimulus showed a hand closing in a possible manner, as in a whole-hand grip, with the fingers moving towards the palm. The other stimulus depicted a hand moving in an impossible manner, with the fingers moving backward toward the back of the hand, violating the biomechanical properties of the phalangeal joints. Each stimulus consisted of 7 frames and lasted 4 seconds. Luminance, contrast, hue, and saturation were kept constant between frames in each video-clip, and between different video-clips. In both stimuli, the first frame depicted a hand with the fingers straight up, and the palm facing the viewer. In the second frame, the hand made a 90° rotation around the vertical axis, presenting the hand on its side with the thumb in front and the other fingers oriented upward. In the following 5 frames, the fingers would either close gradually towards the palm (possible movement) or move unnaturally towards the back of the hand (impossible movement) (Figure 1). The dimension of the hands, at a distance of 30 cm, were at a visual angle of 13°-16° height and 6°-9° width. The distance of each stimulus from the centre was 8 cm.

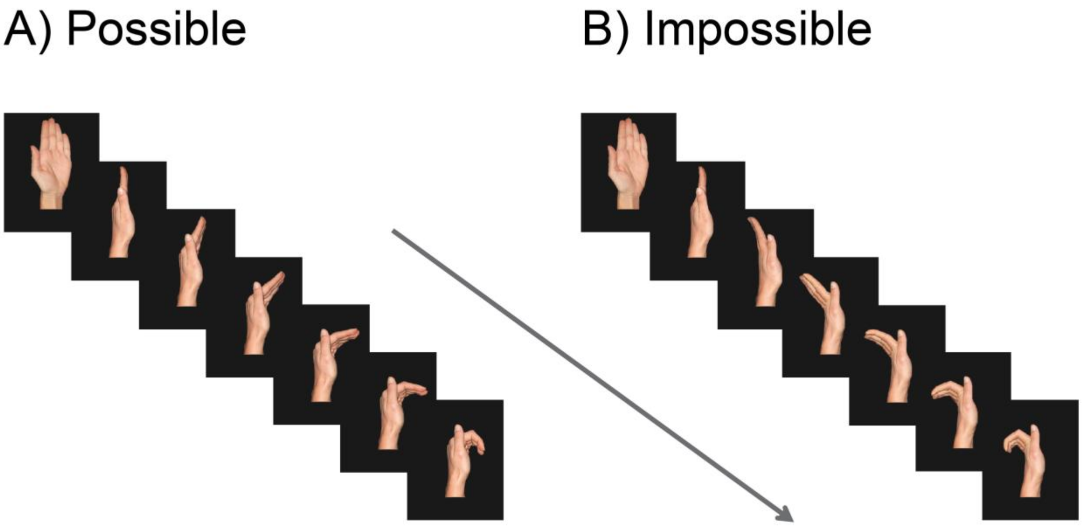

Figure 1. Experimental stimuli presented in Experiment 1. Video-clip frames depict the two dynamic stimuli presented: a) Possible movement; b) Impossible movement.

Newborns sat on an experimenter’s lap at a distance of about 30 cm from a 27” monitor (1920 x 1080 pixel resolution, refresh rate of 60 Hz). To catch the attention of the newborn, at the beginning of the experimental session, we presented a red circle (2 cm diameter), at the centre of the monitor against a black background and flickering at a frequency of 300 ms (on and off). Stimuli presentation was controlled by a second experimenter through E-prime 2 (Psychology Software Tools). The experimenter monitored newborn’s gaze through a video-camera placed above the monitor on which stimuli were presented. Newborn’s gaze was recorded and stored for off-line coding. The experiment took place in a dimly lit room. Dark curtains and black panels around the monitor and the video-camera were use to minimize distractions during the testing session.

An infant-controlled visual preference paradigm was used (Fantz, 1958). When the newborn was seated on the experimenter's lap facing the monitor, the other experimenter started the experimental session by sending the red flashing circle. As soon as the newborn looked at it, the first trial started. Each participant was presented with two trials. In each trial the two stimuli (possible, impossible gestures) were shown at the same time, one on the left and the other one on the right of the screen, and then their position on the screen was switched in the following trial. The order of presentation was counterbalanced across participants. Stimuli were shown continuously, in a loop, and each trial ended when each stimulus was watched at least once for 5 s minimum and when the newborn looked away for more than 10s. At the end of the first trial, the fixation point was presented again to attract newborns' attention. The experimental session was considered valid only if the newborn watched both trials.

The experimenter coded the duration of each newborns' fixation, by pressing either the left or right button of the mouse accordingly to which side of the screen newborns were looking at. The experimenter could only see the movement of infants' eyes but did not know the position of the stimuli. Videos of the newborns' gaze direction were also coded offline by another experimenter. Agreement between the two observers was very high, as measured on a sample of seven participant with Pearson correlation on newborns' cumulative looking time ($r = 0.97$, $p < 0.001$).

Results

Newborns’ total looking time was analysed by using a repeated-measures analysis of variance (ANOVA) with Type of stimulus (possible, impossible) and Order of presentation (first, second) as within-subjects factors. A significant main effect of Type of stimulus ($F_{1,13}$=5.7; p=0.03) showed that newborns looked longer at the impossible hand movement (Mean=108 s, Standard Deviation ± 44.66) as compared to the possible one (81.25 s, ± 56) (Figure 2). Neither the main effect Order of presentation, nor their interaction were significant (all ps>0.4). Newborns’ preference for the impossible stimulus (57.9%) was significantly different from chance (one-sample t-test, t_{16}=2.4, p<0.03). Moreover, the preference for the impossible stimulus was present in 11 out of 14 neonates (binomial test, p=0.02).

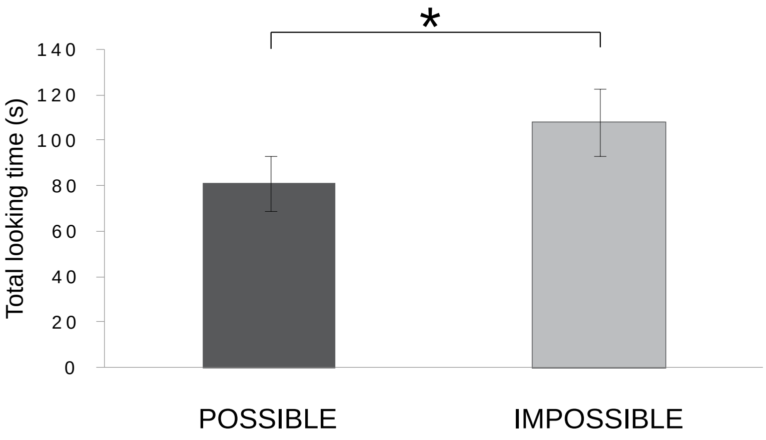

Figure 2. Total looking time for the possible and impossible dynamic stimuli in Experiment 1. Error bars indicate SEM.

These results indicate that newborns discriminated between the possible and impossible movements: since they looked longer at the impossible movement, as compared to the possible, it is plausible to hypothesize that they recognize the first as familiar, and perceived the latter as novel and unexpected.

5.3 Experiment 2

In order to assess the role of motion on newborns' ability to discriminate possible and impossible hand movement, we ran a second experiment in which newborns were presented with static instead of dynamic stimuli. Newborns and infants are able to discriminate between a typical and a scrambled static human face, but seem to be unable to discriminate between static possible and scrambled body shapes (e.g. Slaughter et al., 2002). It is possible that face may be a particularly salient stimulus, and that it is only later in development that infants become sensitive to violations of the body schema. Nevertheless, newborns seem to be attracted by their own hands, and they tend to explore them during their awake time (White et al., 1964; von Hofsten, 2004), even executing movements in order to keep them within the light beam where they can see them (Van der Meer, 1997), and therefore the hand might represent a particularly salient stimulus for newborns. However, if in the first experiment newborns were able to discriminate between the two stimuli due to a link between the motor representation of a movement, and its visual consequence, such a preference would occur only in the presence of dynamic stimuli.

Methods

Participants. Fourteen, full term, healthy 2-day old newborns (9 females, mean age=49h, range=22h-97h; mean weight=3320gr, range=2600gr-4050gr, Apgar score≥8 after 5 min), were recruited at the Neonatal Ward of San Gerardo Hospital.

Other 15 neonates were tested, but were eventually excluded from the final sample because they changed their state during the testing session (N=7), watched only one trial (N=5), and showed a position bias by looking to one side of the screen for over 85% of their total looking time (N=3).

Stimuli, apparatus and procedure. Newborns were presented with two static stimuli corresponding to the penultimate image (i.e. frame number 6) of the dynamic stimuli used in Experiment 1. Thus, one stimulus depicted a hand in a possible static gesture, and the other one an impossible static posture. The stimuli depicting the two static gestures were presented in videos of 4 s continuously, in a loop.

The procedure was the same as for Experiment 1. Inter-rater agreement, calculated with Pearson coefficient on a sample of seven participants on their cumulative looking times, revealed a high inter-observer agreement (r=0.91, p<0.001).

Results

A repeated-measures ANOVA was conducted on the newborns' total looking time, with Type of stimulus (possible, impossible) and Order of presentation (first, second trial) as within-subjects factors. Neither significant main effects nor interaction were found (all ps>0.1). Therefore, at variance with Experiment 1, newborns did not show any visual preference for the possible (85.82 s, SD ± 29.45) or the impossible (84.77 s, SD ± 39.04) static postures (Figure 3). One sample t-test of the preference for the impossible stimulus (54.9%) against chance level at 50% was not significant (t=-1.77, p=0.1). Similarly, a binomial test also did not yield any significance, with 9 out of 14 participants who preferred the possible stimulus (p>0.1).

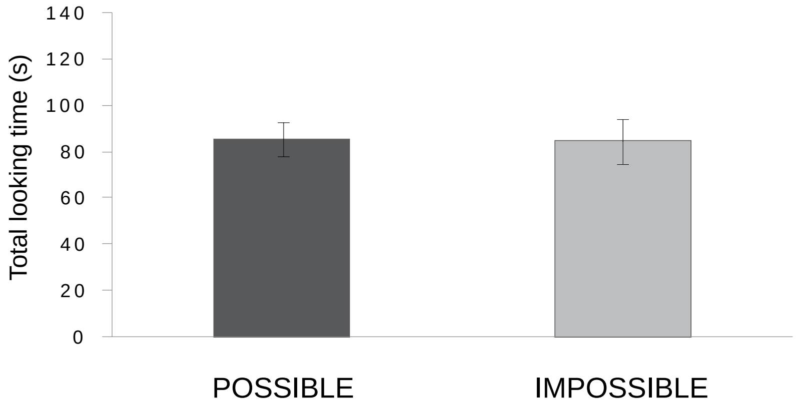

Figure 3. Total looking time for the possible and impossible static stimuli in Experiment 2. Error bars indicate SEM.

These results show that newborns were not able to discriminate simple possible and impossible hand gestures when they were presented as static images. Together with the results from Experiment 1, motion information appears to play a crucial role in newborns' ability to distinguish between possible and impossible hand gestures.

5.4 Experiment 3

In a third experiment, we investigated whether newborns' ability to discriminate between possible and impossible movements could also be modulated by their own experience to perform a movement (see Buccino et al., 2004; Fack-Ytter et al., 2006). In particular, we explored whether newborns were able to discriminate between a possible movement which they are not able to perform yet (i.e. a pincer grip) and a similar one, which is anatomically impossible to execute. Hence newborns were presented with two stimuli, either possible or impossible, which are both novel to them, since newborns are not able to perform neither of the two. As a consequence, newborns should not show a preference for the impossible gesture, given that the stimuli are both unfamiliar to them. On the other hand, the presence of motion might still help them to discriminate the plausibility of the biomechanics of the human body (Slaughter et al., 2002; Christie and Slaughter, 2010).

Methods

Participants. Fourteen, full term, healthy 2-day-old newborns (9 females, mean age=43h, range=22h-75h, mean weight=3249gr, range=2470-3690gr, Apgar score after 5 min≥8), took part in the study. Another 16 newborns were tested, but excluded from the final sample because they changed their state (N=12), watched only one trial (N=3), manifested a bias toward a spatial position, looking to the left or to the right for over 85% of their total looking time (N=1).

Stimuli, apparatus and procedure. Newborns were presented with two dynamic stimuli of moving hands on a black background. One video showed an index finger and a thumb closing in a precision grip. In the other video, the index and the thumb were closing unnaturally backwards, against the biomechanics of the phalanges. Each stimulus consisted of seven frames, and paired stimuli were played in a 4 s video, continuously, in a loop. As in Experiment 1, the first two frames were the same for the two stimuli, with the first frame showing a hand in a vertical position facing the experimenter and the second with the hand rotated by 90° on the vertical axis, with the thumb in front and the other fingers aligned vertically. In the remaining five frames, the thumb and the index finger moved gradually towards each other in a precision grip in a either a possible or impossible (i.e. with the fingers moving backwards) manner (Figure 4).

Apparatus and procedure were the same used in Experiment 1 and 2. Pearson correlation revealed a high inter-observer agreement on infants' cumulative looking time (r=0.98, p=0.000).

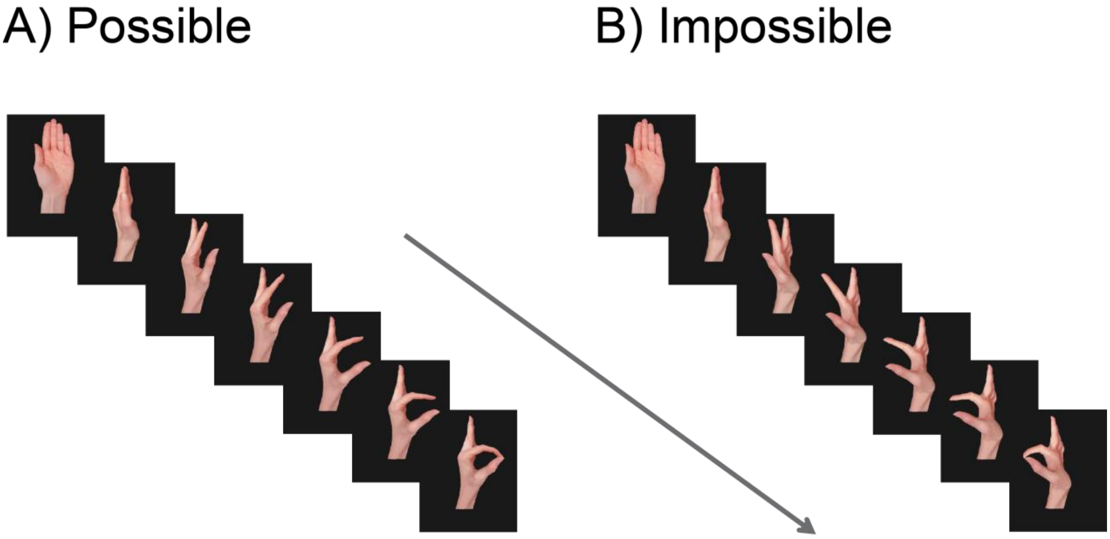

Figure 4. Experimental stimuli presented in Experiment 3. Video-clip frames depict the two dynamic stimuli presented: a) Possible movement; b) Impossible movement.

Results

The total looking time was analysed via a repeated-measures ANOVA with Type of stimulus (possible, impossible) and Order of presentation (first, second) as within-subjects factors. A significant main effect of Type of stimulus ($F_{1,13}=10.95$; $p<0.01$) showed that newborns looked longer at the possible stimulus (99.73s ± 39.5), as compared to the impossible one (71.90 s ± 23.14) (Figure 5). No main effect of the factor Order of presentation nor the interaction were significant (all ps>0.7).

A one-sample t-test showed that newborns' preference for the possible movement (57.3%) was significantly different to the chance level of 50% (t=2.84, p≤0.01). Moreover, 13 out of 14 participants looked longer at the plausible movement (binomial test, p<0.001).

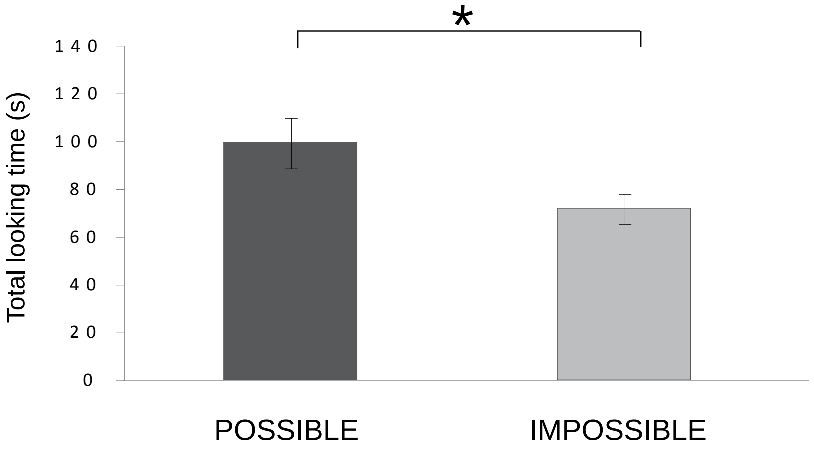

Figure 5. Total looking time for the possible and impossible dynamic stimuli in Experiment 3. Error bars indicate SEM.

These results indicate that when newborns view a possible movement never experienced before, and an anatomically implausible version of the same movement, they show a visual preference for the possible one. A pincer grip movement indeed involves fingers moving individually, which is a motor skill established only later in development (e.g., Berthier and Keen, 2006). However, newborns might show a preference for the possible movement only because it is triggered by a natural inclination towards biological motion (see for instance Simion et al., 2008); this account is addressed in the last experiment.

5.5 Experiment 4

In order to investigate whether motion information helps newborns in the discrimination between possible and impossible pincer grips, static pictures of possible and impossible precision grip gesture were now used.

Methods

Participants. Fourteen, full term, healthy 2-day-old newborns (7 females, mean age=37h, range 19h–80h, mean weight=3570gr, range 2890-4100gr) took part in the study. Twelve additional participants were tested but not included in the final sample because they changed their state during the testing session (N=7), watched only one trial out of two (N=3), showed a position bias towards one side of the monitor for over 85% of their looking time (N=2).

Stimuli, apparatus and procedure. Newborns were presented with two static stimuli corresponding to the penultimate image (i.e. frame number 6) of the dynamic stimuli used in Experiment 3.

Apparatus and procedure were the same as those used in Experiment 1. Inter-rater reliability was measured on infants' cumulative looking times, and applied on a sample of seven newborns. Pearson correlation on total looking times revealed a high inter-rater reliability (r=0.980, p<0.001).

Results

A repeated measures ANOVA was conducted on newborns' cumulative looking time, with Type of stimulus (possible, impossible) and Order of presentation (first, second trial) as within-subjects factors. No significant main effect or interaction were found (all ps>0.5), hence indicating that newborns spent a similar amount of time in looking at the static possible (82.12 s ± 36.08) and at the impossible pincer grip postures (87.75 s ± 15.66) (Figure 6). Newborns' preference for the possible image (50.7 %) did not differ from the chance level of 50% (One-sample t-test, t=0.11, p>0.9). Moreover, a binomial test on the number of newborns preferring the plausible pincer grip image (N=6), as compared to the ones who preferred the impossible one (N=8), also did not reveal any significance (p= 0.18).

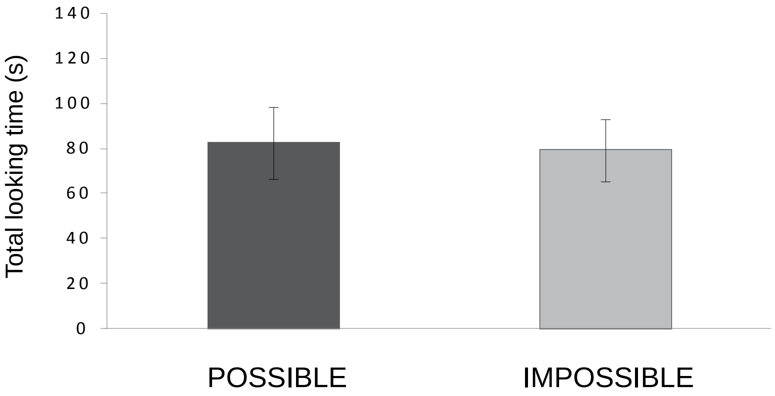

Figure 6. Total looking time for the possible and impossible static stimuli in Experiment 4. Error bars indicate SEM.

5.6 Discussion

Using a preferential looking paradigm, we explored whether 2-day-old newborns were able to visually discriminate between an observed plausible hand movement and a similar one that violates the constraints of human anatomy.

In Experiment 1, newborns were presented with two videos of a hand closing in a possible (i.e. the fingers moving towards the palm) or impossible manner (i.e. the fingers moving backward toward the back of the hand, violating the biomechanical properties of the phalangeal joints). The possible movement is already experienced by newborns in their intrauterine life. Newborns showed a visual preference for the impossible movement, suggesting that they discriminated between the possible and impossible movements, recognizing the first as familiar, while perceiving the latter as novel and unfamiliar. Previous studies have shown that infants, when presented with a movement they are able to perform and a similar one that violates body's biomechanical constraints, tend to look more at the impossible, novel one and this ability correlates with their motor skills (Reid et al., 2005; Morita et al., 2012). These results support the notion that the infant's own motor skills may relate to their understanding of the human body (Reid et al., 2005).

Conversely, in the case of a hand movement not experienced before (i.e., pincer grip, Experiment 3), newborns preferred the possible movement. In this case, given that newborns have no experience with neither the possible movement or the impossible one, the two movements are both novel to them. The mirror neuron system is supposed to involve a direct matching between the observed action and the internal motor representation of that action, showing a stronger activation for actions that belong to the observer's motor repertoire (e.g. Buccino et al., 2004; Calvo-Merino et al., 2005). Since newborns have not yet experienced the pincer-grip movement, they may have been attracted by fingers' movement that matches the biomechanical properties of biological possible movement, hence showing a preference that merely reflects a preference for a biological possible movement, regardless to its connection to the newborns previous sensorimotor experience (e.g. Simion et al, 2008; Bardi et al., 2010). Experiment 2 and 4 show that newborns did not manifest any visual preference for static possible and impossible movements, suggesting that the view of a body posture, in the absence of motion, is not a sufficient cue to differentiate between the two stimuli. Similar results were observed by Bertenthal and colleagues (1984) in 3- and 5-month-old infants presented with point light displays of a walking person presented upright or upside-down. Infants were able to discriminate the two displays when they were moving, but not when they were presented as static displays, indicating that the presence of biological motion was a necessary cue for discriminating the two displays. Newborns are able to distinguish between static typical and scrambled facial stimuli (e.g. Goren et al., 1975), but they are not able to discriminate between typical and scrambled bodies until their second year of life (Slaugher et al., 2002). Our results further show that newborns are not able to discriminate between possible and impossible hand gestures without the help of motion.

Overall, the present experiments suggest that mechanisms linking motor and visual representations of movements are already present a few hours after birth. This visuo-motor link is active also for simple movements, which differ only for their anatomical plausibility.

6. General Discussion

Overall, the present research shows that mechanisms linking motor and visual representations of actions are present from birth, and that the observer's sensorimotor experience plays a crucial role in action perception. The first study (Chapter 2) demonstrates that in infants the motor system is recruited during action observation, and it is selectively modulated by the observed action goal. In 6- and 9-month-old infants, but not in 3-month-olds, electromyographic (EMG) activity, recorded from muscles involved in mouth opening, increases during the observation of an agent grasping an object and bringing it to the mouth, as compared to when the object is brought onto the head. Hence, from 6 months of age, the observed action can be internally simulated, activating the corresponding motor program up to the peripheral muscles that would be involved in the execution of the observed action. Motor simulation is present in 6- and 9-month-old infants, who are already able to execute the observed action, while it is absent in 3-month-old infants, who have no or little experience with the observed action. These findings support previous evidence that only actions belonging to the observer's motor repertoire are mapped onto the observer's motor system (Buccino et al., 2004). Moreover, while 6-month-old infants simulate on-line the time course of the action, in 9-month-olds the muscles responsible for the action final goal increase their activity as soon as the action starts, hence indicating that in older infants motor simulation may allow them to anticipate the goal of the observed action, by recruiting the corresponding motor program of the whole observed action before it is concluded. Given that such an ability is present only in older infants, it is plausible to hypothesize that it is influenced by sensorimotor experience: the greater is the experience with the observed action, the better is the ability to anticipate its goal.

The second study (Chapter 3) shows that the observation of a familiar action performed by an unusual effector elicits a mirror motor facilitation, as measured by motor evoked potentials (MEPs) induced by Transcranial Magnetic Stimulation (TMS), not only in the same muscles that would be involved in the observed action, but also in the muscles normally used to achieve the same goal. This evidence indicates that during action observation there is both a mapping of the observed movements onto the observer's motor repertoire, and the recruitment of the typical motor program involved to reach the goal of the observed action, in accordance with the observer's motor expertise.

The third study (Chapter 4) demonstrates, by means of eye tracking technique, that 6-month-old infants are able to discriminate between biomechanically possible and impossible grasping actions, and that they interpret both actions as goal directed, as measured by predictive gazes towards the to-be-grasped object. However, when the possible action is presented as first, infants make more predictive gazes in the possible than in the impossible condition, indicating that infants' experience with the observed possible action may play a role in the discrimination of the two actions.

Finally, in Chapter 5, it is demonstrated that 2-day-old newborns are able to discriminate between biomechanically possible and impossible hand movements. In particular, using a preferential looking paradigm, newborns show a novelty effect for an impossible hand movement, as compared to a possible movement, which is experienced during the intrauterine life; this result further supports the key role of sensorimotor experience in shaping mechanisms linking action execution and perception. However, when presented with a possible hand movement never experienced before and an impossible one, newborns show a visual preference for the possible hand movement. Given that in this case newborns cannot make an association between their own motor ability to perform a movement and its visual counterpart, their preference for the possible movement might be driven by newborns'preference for a biological motion (e.g. Simion et al., 2008).

According to the direct matching hypothesis (e.g. Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004), the process of action understanding derives from mapping the observed actions onto the observer's motor repertoire: by internally simulating the observed action, the observer can identify the goal that generated the action. Critically, motor simulation requires that the observed action is already available in the observer's motor repertoire, which implies that the interpretation of an action will strongly be influenced by the development of the observer's ability to perform it (e.g. Buccino et al., 2004; Falck-Ytter et al., 2006).

Previous evidence in infants supports the hypothesis that infant's motor skills determine the way in which observed actions are interpreted (e.g. Sommerville et al., 2005; Reid et al., 2005; Falck-Ytter et al., 2006; Bertenthalet al., 2006; Hauf et al., 2007; van Elk et al., 2008; Libertus and Needham, 2010; Kanakogi and Itakura, 2011). For instance, the ability to perform sophisticated goal-directed reaching actions emerges at about 6 months of age (e.g. Rochat, 1989), and it is at this same age that infants start to perceive reaching and grasping actions performed by others as being goal-directed (e.g. Woodward et al., 1998). Moreover, infants who have better tuned motor skills present a stronger *mu rhythm* desynchronization during the observation of actions they are skilled at (van Elk et al., 2008), and, in turn, they are better at discriminating between biomechanically possible and impossible actions (Reid et al., 2005). Therefore, infants' agentive experience may facilitate the learning of the biomechanical constraints of the human anatomy. Similarly, in our research, 6-month-old infants, which are able to perform reach-to-grasp actions, are able to make predictive gazes towards the goal of the action, and to discriminate between biomechanically possible and impossible actions (Chapter 4). During the observation of more complex actions, such as motor chains involving reach-to-grasp-to-bring, only older infants (i.e. 9-month-olds), that are more familiar with those actions, showed an anticipatory modulation of their own motor activity (Chapter 2). It might be argued that infants' interpretation of actions is not modulated by sensorimotor experience, but rather both the emergence of new motor skills and changes in action interpretation are influenced by some unspecific maturational processes. However, previous evidence that motor trainings in infants influence the interpretation of actions (e.g. Sommerville et al., 2005; Libertus and Needam, 2010) indicates that the opportunity to experience new actions affects action perception, and thus that sensorimotor experience exerts a strong influence in the interpretation of actions over and above unspecific maturational processes.

It has been hypothesized that the mirror neuron system develops gradually through the repeated, contiguous and contingent experience of observing and executing the same actions (e.g. Heyes et al., 2001; 2010a; Del Giudice et al., 2009). According to this hypothesis, mechanisms linking action production and perception would emerge and be refined throughout the entire life. Even in adults these mechanisms are modulated by individual expertise and motor trainings (e.g. Calvo-Merino et al., 2005; Haslinger et al., 2005; D'Ausilio et al., 2006; Cross et al., 2006; Press et al., 2007; Catmur et al., 2007; 2008; Aglioti et al., 2008). The observation of an action which is familiar to the observer would activate the corresponding motor program in the observer, and the more time an outcome has been associated with a certain action, the stronger will be the association between a motor code and its effect (Hommel et al., 2001, Paulus et al., 2011). The study described in Chapter 3 shows that the observation of a familiar action performed by an atypical effector induces not only a motor facilitation of the muscles involved in the observed action, but also the recruitment of the motor program of the effector typically used to reach the observed goal, and thus that is associated with the achievement of the observed goal.

Typically, in our daily lives we observe actions performed by agents which have effectors and biological constraints similar to ours, and as a consequence we observe actions in which the goal is achieved by the same means we habitually use. The observation of human actions indeed induces in the observer stronger resonance responses, elicits more automatic imitation, and a better prediction of the outcome of the observed action, as compared to actions performed by non-human agents (e.g. Stevens et al, 2000; Kilner et al, 2003; Tai et al., 2004; Press et al., 2005; 2007). Studies in infants from about 6 months of age show that infants tend to represent familiar actions as goal-directed when performed by humans, but not when performed by mechanical devices (e.g. Meltzoff, 1995; Woodward et al., 1998; Kanakogi and Itakura, 2011; Daum and Gredebäck, 2011) or when the actions are unfamiliar (Woodward et al., 1999). The familiarity with the observed action seems to play a role in its interpretation: a familiar action performed by human agents can be internally simulated by the observer through mirror mechanisms (e.g. Buccino et al., 2004). However, it has been hypothesized that the attribution of goal-directedness to human and non human actions may be driven not by mechanisms of motor simulation, but by a system which detects efficient actions (e.g. Csibra, 2007;

Gergely and Csibra, 2007). Infants are constantly exposed, in daily life, to human actions followed by a salient effect, and this can help them in interpreting those actions as goal-directed. Even if unusual, infants perceive as goal directed also biomechanically impossible actions, as long as they are physically efficient in reaching a familiar goal (Souhtgate et al., 2008). This result has been interpreted in favor of the hypothesis that the action goal is not derived from infants' own sensorimotor experience through a direct matching between an observed action and the observer's own motor system (Rizzolatti et al., 2001), but rather it is inferred from the recognition of a specific event structure. In this view, an action is interpreted as goal-directed when it is judged to be an efficient means towards its end state within the situational constraints (Souhtgate et al., 2008; see also Csibra, 2007; Gergely and Csibra, 2007).

In the study illustrated in Chapter 4, 6-month-old infants were presented with biomechanically possible and impossible grasping actions, which were equally efficient in reaching the goal of the action, and thus, for the *teleological stance* (Csibra et al., 1999; 2003; 2007; Csibra and Gergely, 2007), they should be both interpreted as goal-directed. According to this hypothesis, our results demonstrated that infants were able to anticipate the goal of both actions. However, an analysis on the looking time to the hand showed that infants were able to discriminate between the two actions, and gaze behavior patterns showed that when the possible action was presented as first, it was interpreted as goal-directed more than the impossible one. These results suggest that motor and visual experience with the possible, but not with the impossible, actions may play a role in discriminating between the two types of actions, thus helping infants to understand which movements fingers can or cannot perform. The internal action simulation (Rizzolatti et al., 2001), along with the experience with both self-produced and observed actions (Woodward, 1998), may facilitate infants in interpreting the observed actions, hence allowing them to notice the violation of the biomechanical constraints.

Unlike 6-month-old infants, adults make goal predictions even about actions performed by inanimate agents, and they show a similar pattern of gaze behavior during the observation of grasping actions performed by both human and non-human agents such as mechanical claws (Kanakogi and Itakura, 2011). Even in adults the mirror system is modulated by sensorimotor experience and can be activated, after training, even by the observation of actions performed by inanimate agents (e.g. Press et al., 2007). It is possible that the ability to predict the goal of actions performed by human agents may be generalized even to tools such as mechanical claws, due to experience with tools acquired during life (Kanakogi and Itakura, 2011). Similarly, in monkeys, mirror neurons, which normally do not fire for the observation of action performed by tools, can become responsive to tool-use actions after trainings (Ferrari et al., 2005; Umiltà et al., 2008).

Sensorimotor experience seems to play a role in influencing action perception very early in life, and even before birth. A study by Craighero and colleagues (2011) has shown that 2-day-old newborns are able to discriminate between goal-directed and non-goal-directed actions, indicating that a link between action execution and perception may be present from birth. Based on ultrasound imaging studies, it seems that, from about 14-22 weeks of gestation, fetuses have experience of goal-directed actions such as tactile exploration of the uterine wall and of their own body, and manipulation of the umbilical cord (e.g. Sparling et al., 1999; Zoia et al., 2007; Castiello et al., 2010). Fetuses' motor activity may allow them to explore properties and constraints of the body, and the association between movements and their sensory counterpart (e.g. Zoia et al., 2007). For instance, grasping and manipulating the umbilical cord can induce heart rate deceleration (Petrikovsky et al., 1993), and thus fetuses can immediately experience proprioceptive consequences of their actions.

Even if fetuses does not have experience of the visual consequences of movements they perform, in newborns the ability to transfer information acquired through the sensory-motor experience into its visual representation is present from birth, as shown by the study described in Chapter 5, where 2-day-old newborns are shown to be able to discriminate between a biomechanically possible hand movement they have experienced during their fetal life and an impossible hand movement. It is plausible that fetuses' sensorimotor experience during their intrauterine life might help them in recognizing violations of the biomechanical constraints of the phalangeal joints.

It has been suggested that the main role of the mechanisms linking action execution and perception is allowing the observer to understand the goal of the observed action (e.g. Rizzolatti et al., 2001), which means figuring out the intentions of the agent who is performing the action. In social situations it is crucial to be able to predict what the others are doing before the actions are concluded, in order to have the opportunity to intervene in time if necessary. Our studies show that infants who have experience with the observed action tend to predict its goal before the action is concluded, as measured by means of EMG recordings (Chapter 3) and gaze behaviour (Chapter 4). Previous studies in both children and adults have shown that, during action observation, the motor program of the observed action is recruited as a whole at the beginning of the action (Gangitano et al., 2004; Cattaneo et al., 2007), suggesting that mechanisms of motor simulation might have a predictive nature (Kilner et al., 2007a; 2007b; Gazzola and Keysers 2009; Press et al., 2011; Avenanti et al., 2012). For instance, EEG studies have shown that when adults or infants as young as 9 months observe predictable movements, motor activations begin before the observation of the action (Kilner et al., 2004; Southgate et al., 2009). Similarly, when observing a cyclic repetition of the same movement, the excitability of the muscle involved in the observed movement is modulated with the same rate at which the movement is executed, but this modulation precedes the cycle of the observed movement (Borroni et al., 2005). Moreover, in adults the motor system is activated even by the observation of static snapshots depicting ongoing actions, but not completed actions, possibly because the motor system is involved in simulating the future phases of the implied actions (Urgesi et al., 2006; Candidi et al., 2010).

It has been proposed that the motor system is used to generate predictions about the observed actions according to a Bayesian statistical approach (Kilner et al., 2007a; 2007b). According to this hypothesis, when we execute an action, we predict its kinematics and its sensory consequence from its goals on the basis of generative or internal forward models (Wolpert et al., 1995; 2003; Wolpert and Miall, 1996). Kilner and collegues (2007a; 2007b) propose that the same models used for action execution and control can be inverted to generate predictions about the causes of the observed actions, given visual inputs. An action can be described at the level of the intention that drives the action, the goal (i.e. short-term goals required to reach the long-term intention), the kinematics, and the muscles involved in executing the action (Hamilton and Grafton, 2007). A sensory input is not associated uniquely to only one cause (i.e. the same movements can be caused by different goals, and the same goal can be associated with different kinematics patterns). Thus, understanding the intention or the goal of the observed action requires inferring the goal or intention levels from a visual description of the kinematics of the action. The predictive model proposed by Kilner and colleagues (2007a; 2007b) has a hierarchical structure, and it is thought to minimize prediction error at all levels (i.e. intentions, goals, kinematics), between the observed action and the predicted action. During action observation, we infer the goal of the action and, given this prior expectation about the goal, our motor system predicts the motor commands and the kinematics of the agent of the observed action. A prediction error is generated by the comparison between the predicted and the observed kinematics, and it is used to update or correct the predictions about the goal of the action. Each level of the hierarchy utilizes a generative model to predict the representations in the level below. During action observation, the most likely cause of the observed action is inferred by continuously minimizing the prediction error at all levels of the motor hierarchy (intention, goal, kinematics; Kilner et al., 2007a; 2007b).

The range of possible intentions and goals to ascribe to the observed action may derive from, and be constrained by previous sensorimotor experience of the observer, such as specific expertise, and such information may act as Bayesian priors in the estimation of the most likely cause of the observed action. Thus, making inferences about the observed actions is based on an interaction between the sensory information available by movement kinematics, and the observer’s prior expectations acquired from past experience (i.e., the prior; Chambon et al., 2011; see also Avenanti et al., 2012). When it is not possible to unequivocally infer the cause of the observed movements, as for instance in novel or unfamiliar situation, action understanding might be achieved also with the help of sensory information related to the context in which the movement takes place. In such cases, sensory information about the context may operate as clues for inferring the intention of the agent of the action (e.g. Brass et al., 2007; Kilner et al., 2008); in this cases, action understanding may be primarily mediated by an inferential interpretative system, such as teleological reasoning, rather than by motor simulation (see also Csibra et al., 2007; Csibra and Gergely, 2007). In the other cases, observing an action is supposed to activate stored sensorimotor representations of that action (e.g. Elsner, 2007) that can be used to predict the future phases of the ongoing action. In particular, more precise predictions can be made during the observation of actions that are well practiced and learned though sensorimotor experience, which in turn presumably strengthens the associations between sensory inputs and their potentials causes (e.g. Bertenthal and Longo, 2008).

Our results are compatible with this proposal, given that only 9-month-old infants, which have more experience with the observed and predictable action, showed a modulation of their EMG activity during action observation at the onset of the action, anticipating its end state rather than simulating it on-line, as found in 6-month-olds (Chapter 2). Moreover, 6-month-old infants were able to predict the goal of both a biomechanically possible and impossible observed grasping action, as proved by the presence of predictive gazes toward the goal of the action (see Flanagan and Johansson, 2003; Falck-Ytter et al., 2006), and this ability seems to be modulated by the experience with the observed action. In a further study, the ability to predict the goal of biomechanically possible and impossible observed actions may be investigated in younger infants, for instance 4-month-olds. Four-month-old infants are starting at about this age to perform reach-to-grasp actions, and thus do not typically perform the efficient goal-directed reaches of older infants (e.g. von Hofsten, 1984), and, coherently, do not typically perform predictive gazes toward the to-be-grasped object of an observed action (e.g. Woodward, 1998; Kanakogi and Itakura, 2011). Independent measures of reaching and grasping ability and gaze behavior may be calculated and correlated for each infant. A study in this group of age, in which reaching and grasping abilities are very variable from one infant to another, may allow to explore whether individual motor ability influences the ability to predict the outcome of the observed action and to discriminate between actions on the basis of their anatomical plausibility.

In conclusion, the results of the present research indicate that mechanisms linking motor and visual representations of movements are present from birth, and develop during life, likely through sensorimotor experience. The growing experience with the observed actions allows the observer to anticipate the goal, and likely to understand the intentions that drive such actions.

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