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The future in action: neurophysiological and behavioral evidence of anticipatory motor simulation

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The purpose of brains is to produce future. Paul Valery

CHAPTER 1

General Introduction

Anticipation is a crucial phenomenon in nature allowing both animals and humans'survival. An effective individual's functioning in the physical and social environment needs to be guided by anticipatory mechanisms that are used both for the self and others' behavior. In a broad sense, anticipatory or also predictive, processing refers to any type of processing which not only provide information about the past or the present but also generates information about the future states of the body or the environment (Bubic et al., 2010). There are many benefits of being able to anticipate the immediate future of own and others' actions such us enabling the agent to control goal-directed behavior and intervening if necessary when unexpected events occur, to learn from the physical environment in which an action takes place, to infer the consequences and the intentions behind others'actions, in general to prepare appropriate motor responses when interacting with other conspecifics and the environment. There is compelling evidence that our own motor system is not only merely devoted to action planning and execution, but is also intrinsically involved in perceptual and cognitive functions concerning for example: i) specific sensorimotor transformations for action in the space (within and outside the peripersonal space, PPS), ii) action perception and understanding (Rizzolatti et al., 2002).

THE ROLE OF THE MOTOR SYSTEM IN ACTION PERCEPTION AND UNDERSTANDING

Prominent studies support the notion that the motor system of both monkeys and humans, tends to be activated when observing others'actions. This so called "motor resonance" or "motor simulation" prompted by action observation, essentially reflects the motor program that the observer would have to execute to perform the observed action. This suggests that each time one observes an action, the visual representation of that action is mapped onto the motor representation of the same action. It has been suggested that such coupling between action perception and execution leads to action understanding (Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). The clearest evidence of these phenomena comes from the discovery of a particular class of monkey's frontoparietal neurons, called "mirror neurons", that fire both when the animal executes a certain action but also when it perceives the same action performed by others (di Pellegrino et al., 1992; Gallese et al., 1996; Fogassi et al., 2005). Seminal single-cell recording studies in monkeys have defined a frontoparietal network of areas containing these mirror neurons. The monkey's Mirror neuron system (MNS) comprehends three areas (Keysers & Perrett, 2004; Rizzolatti & Craighero, 2004): area F5 in the premotor cortex (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al.,1996a; Umiltà, et al., 2001), area PF/PFG in the inferior parietal cortex (Gallese et al., 2002; Fogassi et al., 2005; Rozzi et al., 2008) and the superior temporal sulcus (STS) in the temporal cortex (Perrett et al., 1989,1990; Jellema & Perrett, 2006; see figure 1.1).

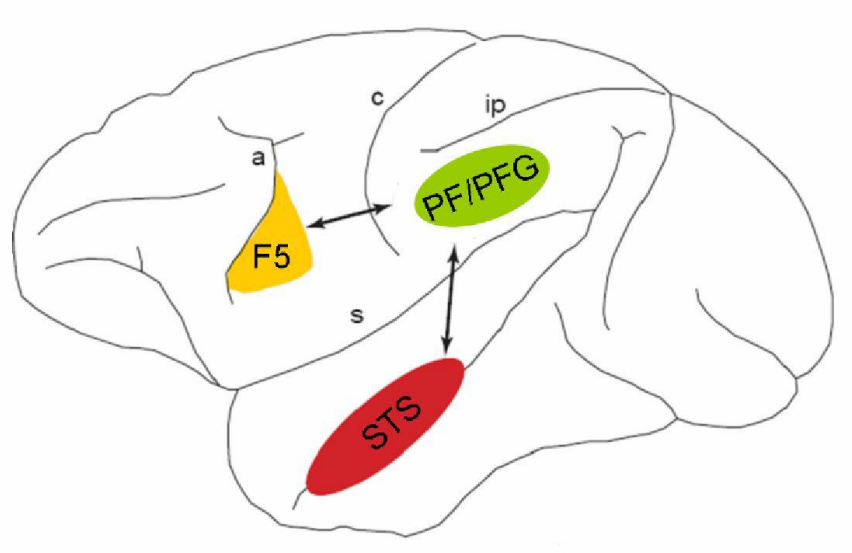

Figure 1.1. The figure illustrates the frontoparietal monkey's AON shown on a lateral view of the macaque brain. The area F5 in ventral premotor cortex, area PF/PFG of the inferior parietal lobule and the superior temporal sulcus (STS) and their anatomical connections (arrows). (a, arcuate sulcus; c, central sulcus; ip, intraparietal sulcus; s, sylvian sulcus). Adapted from Keysers & Perrett, 2004.

The areas reported in figure 1.1 have reciprocal connections. In the monkey brain, area F5 is reciprocally connected to area PF (Luppino et al., 1999) creating a premotor-parietal mirror neuron system (MNS) and STS is reciprocally connected to area PF (Harries & Perrett, 1991; Seltzer & Pandya, 1994) providing a sensory input to the MNS (Keysers & Perrett, 2004). Specifically, STS responds only to the sight of action and do not respond to action execution of any kind (Keysers & Perrett, 2004), whereas PF/PFG and F5 contain mirror neurons, visuomotor neurons which respond to both action execution and observation. It is worth noting that the "mirroring" it is not only limited to the above-cited circuit, but it is likely a more widespread property of the brain (Rizzolatti & Sinigaglia, 2010). Recently it was shown that other parietofrontal circuits in the monkey's brain possibly contain mirror neurons (Shepherd et al., 2009; Ishida et al., 2009). Interestingly, these studies highlight that the function of mirror neurons are closely related to the motor properties of the areas in which they are located. For example the ventral intraparietal area (VIP) contains neurons encoding tactile and visual stimuli delivered in the PPS of the monkey (Colby et al., 1993;

Duhamel et al., 1998), mirror neurons recently found in this area also respond to stimuli presented in the PPS of an individual located 1m from the monkey and facing it (Ishida et al., 2009).

There is now overwhelming evidence that a similar system of motor resonance may also exists in humans and that is activated by action observation and execution (Rizzolatti et al., 1996; Decety et al., 1997; Buccino et al., 2001; Gazzola & Keysers, 2009). Previous research identified a fronto-temporo-parietal action observation network (AON) encompassing the inferior frontal cortex (IFC, which includes the ventral premotor cortex and the posterior part of the inferior frontal gyrus, IFG), STS and the inferior parietal lobule (IPL) (Gazzola & Keysers, 2009; Grafton, 2009; Van Overwalle & Baetens, 2009; Caspers et al., 2010). These regions resemble the three 'core' areas of the human AON, supporting a possible homology between the system for the two species. The anatomical pattern of connectivity between these areas shows analogies to that found in monkeys (Figure 1.2).

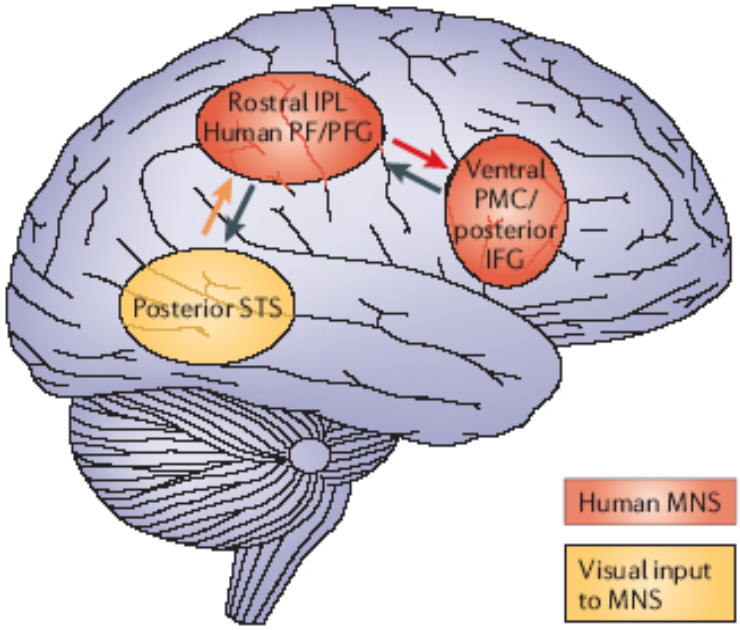

Figure 1.2. The figure illustrates a schematic representation of the frontoparietal human's AON with its main visual input, shown on a lateral view. IFG: inferior frontal gyrus; vPMc: ventral premotor cortex; IPL: inferior parietal lobule; STS: superior temporal sulcus. Adapted from Iacoboni & Dapretto, 2006.

It has been suggested that the AON and in particular its frontal node, the IFC, may support action perception and understanding (Rizzolatti & Sinigaglia, 2010; Aglioti & Pazzaglia, 2011; Avenanti & Urgesi, 2011). Direct evidence of the close link between the motor system and the mechanisms supporting the perception and understanding of actions, come from studies showing that lesions in the areas plausibly involved in this mechanism, brought about a reduction of subjects' performance in tasks requiring an interaction between perception and understanding of actions. In particular, recent studies have reported that a real or "virtual" lesion induced by TMS in the IFC worsens the performance in tasks requiring: a) the visual discrimination of two similar actions (Moro et al. 2008; Urgesi et al. 2007); b) to judge whether the actor is trying to deceive the observers concerning the real weight of a lifted box (Tidoni et al., 2012) or to explicitly make a weight estimation of objects during the observation of lifting actions (Pobric & Hamilton, 2006); c) to judge if a transitive or intransitive gesture was correctly performed (Pazzaglia et al., 2008a); d) to match an observed action with its typical sound (Pazzaglia et al., 2008b); e) to order, in a temporal sequence, pictures of different phases of human actions (Fazio et al., 2009). The link between these evidence from lesion studies and those describing motor resonance during action observation, was provided by the result that suppression of IFC, by means of rTMS, also disrupts the motor simulation of observed actions (mirror-like activity) in the motor system (Avenanti et al., 2007). Moreover, there is also direct evidence that the stimulation of IFC is able to influence action perception (Cattaneo et al., 2011; Avenanti & Urgesi, 2011). These findings thus suggest a pivotal role of IFC for action perception and the internal representation of others' actions.

The mirroring, can be considered one example of the sensorimotor transformations occurring within the motor system and particularly within the premotor cortex (as shown in the above-cited studies) since it transforms sensory representations of others' action into motor representations of the same action in the observer's brain (Rizzolatti & Sinigaglia, 2010). The discovery of mirror neurons in the premotor cortex has notably emphasized their involvement in action understanding, obviously the mirroring is not the only property of the motor system and the actions of others are not the only

sensory events processed in the motor system. Studies in monkeys and humans have outlined that the premotor cortex represents a multimodal station, since it receives afferents from parietal and somatosensory areas (Rizzolatti & Luppino, 2001), in which sensory information about the space and stimuli surrounding the body are analyzed and transformed into specific motor programs. Neurophysiological studies in monkey have shown that the premotor cortex is intrinsically involved in space perception and in sensorimotor transformations of visual and auditory stimuli in the PPS into specific body movements (within that space) (Rizzolatti et al., 2002). The finding that the human premotor cortex is active both during the execution of actions and also during i) presentation of tactile, auditory, and visual moving stimuli (Bremmer et al., 2001); ii) observation of tools (Grafton et al., 1997); and iii) observation of object-related actions performed by others (Buccino et al., 2001), has suggested that also in humans this region may be involved in sensorimotor transformations.

However direct information about how the motor system is modulated by the PPS representation in humans is poor. The chapter 2 of the thesis addressed this issue while the chapter 3 sheds light on the direct role of the premotor cortex on the effect of PPS representation on the motor (see below in the paragraph 'Overview of the thesis').

THE MOTOR SYSTEM ACTS AS AN "ANTICIPATORY DEVICE"

There is a consistent number of studies suggesting that the brain is a future-oriented system. Psychophysical studies suggest that human perceptual systems are projected into the immediate future, as in the phenomenon of the "representational momentum" (Freyd & Finke, 1984) which constitutes a systematic error in visual perception of moving objects. Instead of being recognized in their exact location, moving objects are perceived a bit further along their trajectory. This phenomenon has been extensively investigated and occurs when perceiving real, apparent or implied motion. Similarly, the "flash lag illusion" or "flash-lag effect" is a visual illusion in which a

flash adjacent to a continuously moving object is perceived to lag behind it (MacKay, 1958; Nijhawan, 1994). These findings therefore suggest that perception is a predictive activity and the perceptual system tends to extrapolate the future of seen events beyond what they are actually perceived (Wilson & Knolich, 2005).

These anticipatory perceptual phenomena occur in the early visual cortices, however anticipatory mechanisms are also part of the basic functioning of the motor system because of their important role in motor control. For example when pointing for a target with the arm, feed-back models propose that the pattern of muscle activation that is required to point to the target is not defined prior to the onset of movement, but rather during the course of arm displacement. Thus, the motor command is generated in real time through an error signal that continuously compares the relative locations of the hand and target. On the other hand, feed-forward models propose that a motor command is defined in advance of the onset of movement, through this mechanism the brain integrates information from all senses to detect imminent perturbations and adjust the movement online. However, in order to achieve the best online control of movement the motor system needs to integrate predictive central feed-forward and peripheral sensory feed-back signals.

Perceptual predictions are not only limited when perceiving simple stimuli or when executing a movement, they also occur for the perception of more complex movement patterns as the body's actions. Studies in humans and monkeys support the view that the motor system acts as an "anticipatory device" during action perception (Wolpert et al., 2003). In particular neurophysiological evidence have shown that activations of the motor system contingent upon observation of others' actions may: i) occur prior to the observation of a predictable motor act (Umiltà et al., 2001; Fogassi et al., 2005; Kilner et al., 2004; Aglioti et al., 2008; Avenanti et al., 2009); and ii) show an anticipatory bias in the simulation of the upcoming phases of observed actions (Gangitano et al., 2004; Borroni et al., 2005). This suggests that the mere knowledge of a forthcoming movement is sufficient to activate the motor system, thus allowing the individual to anticipate others' actions. More importantly it has been demonstrated that observing implied human

actions, namely static pictures of ongoing and incomplete actions, also engenders an activation of the motor system (Urgesi et al., 2006; Candidi et al., 2010). Crucially, the motor system is maximally activated when observing the initial and middle phases of the observed implied action rather than the final phases (Urgesi et al., 2006, 2010). Taken together these findings indicate that the motor system is preferentially activated by the anticipatory simulation of future phases of an action. However a direct evidence on the critical role of the IFC in this mechanism is still lacking, the experiments described in the chapter 4 of his thesis addressed this issue providing new insights on the differential role of frontal and temporal nodes of the AON, namely IFC and STS, in the anticipatory simulation of others' actions.

MODELS ON ACTION PERCEPTION AND PREDICTION

Hence, the above reviewed studies support the notion that perception of other people's actions influences the motor system by triggering an internal motor representation of how our body would perform those same actions (Rizzolatti & Craighero, 2004). Importantly, this internal simulation of others' actions is also anticipatory and would underlie our ability to "read" the goal of the observed actions and infer the intentions of the agent performing that action. These notions are supported by a series of theoretical models suggesting a predictive coding of the observed actions (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Schubotz, 2007). In particular the model proposed by Wilson & Knoblich (2005) starts from the assumption that the motor activation prompted by the observation of others' actions contributes to the perception of the behavior of conspecifics. It posits that the processes of motor resonance/simulation prompted by the perception of actions in turn influence the perceptual processing of the actions. The motor system, for its characteristics of "anticipatory device", would generate top-down expectations thus constraining predictions of the observed ongoing actions, that would in turn influence the perception. This way the mechanisms of motor simulation would help the perception by filling in the lacking or ambiguous aspects often

present in the visual scene. This model has two important implications: first, the motor system would generate representations of others' actions by projecting the course of ongoing actions into the future; and second, predictions about the future course of others' actions serve as feedback for the visual system thus exerting a top-down influence on action perception, this mechanism allows to complete missing information. Similarly, Schutz-Bosbach and Prinz (2007) also propose a model for anticipatory motor-based perception of actions. These authors assume that the representations of an event do not only contain information about its present state, but also about past and future states. In particular, their account is focused on the role of "perspective coding" of events, that is the predictive mechanisms of perception and the generation of an event. They suggest that the predictive power of the motor system could be exploited not only in the production, but also in the perception of sensory events. As said before, the prediction is much more advantageous than the simple reaction. A future-oriented perception would allows to select the most appropriate responses ahead of the realization of an event and would be essential to flexibly adapt to new situations, in order to optimally interact with the physical and social environment. Finally, the model of Schubotz (2007) proposes a new framework in which the motor system would not only be involved in the prediction of others' actions but also in the prediction of event dynamics in general. This model aims at generalizing the predictive account of the sensorimotor system from action to event perception by assuming that the sensorimotor system is used by default in the simulation of any kind of observable events. According to this view, prediction of events is achieved by the aid of sensorimotor-driven forward models.

The predictive coding accounts of action, therefore not only support an active role of the motor system in action perception, but also emphasize that this activity is predictive in nature. The prediction of others' actions is made possible by integrating the ongoing situation with prior knowledge, and using internal forward models, normally used to predict the consequences of our own actions. These accounts also presuppose that the motor knowledge is simultaneously used for simulating others' actions and planning own actions.

A further account is the predictive model proposed by Kilner and colleagues (2007a, 2007b) with the aim to answer the question: how the motor system, and the AON, enable the individual to infer the intention of an observed action from the movement kinematics? In the classical view of this mechanism, it has been suggested that the visual information was transformed as it was passed by forward connections from visual areas in the temporal lobe, via inferior parietal areas until the mirror neurons in the premotor area F5 were activated (Rizzolatti & Craighero, 2004; see figure 1.3 a). The model of Kilner, offers a view of how the visual information from an observed action maps onto the observers' own motor system and how the goal of that action is then inferred. An observed action can be understood at many levels, as proposed by Hamilton & Grafton (2007), there are at least four levels through which an action can be described: a) the intention level, b) the goal level c) the kinematic level and the muscle level. However every time one observes an action only the kinematic level is accessible from vision. The predictive coding model of the AON (Kilner et al., 2007a, 2007b, Kilner, 2011, Press et al., 2011) is based on the idea that information about errors and predictions are continuously exchanged between the various levels of cortical hierarchy. Each level of the hierarchy predicts representations in the level below by means of backward connections. These predictions (generative models) are then compared with the representation at the sub-ordinate level creating a prediction error. The prediction error would be in turn sent to a higher level, via forward connections, to update the representation. Minimizing the prediction error at all levels of the hierarchy, allows to recognize what actions others are performing and to infer the intentions behind these actions (figure 1.3 b). This model is in line with the previous approaches but adds the notion that the predictive activity of motor system not only helps perception (Wilson & Knoblich, 2005) but can also account for our ability to infer the intention behind an observed action. Based on this approach, observing a movement made by another and capturing the kinematic information about the movement, we are able to predict the goal of the observed action relying on the predictions generated in our motor system, such predictions will be updated and modified if they are incompatible with what we are seeing. The control of own motor system is based on a similar

organization, with the only difference that we already know the goal of our movements, thus the prediction will be directed on the consequences of movement we intend to perform. On the contrary, we need to predict the goal of action performed by others with a greater possibility of discrepancies between our predictions and the visual scene.

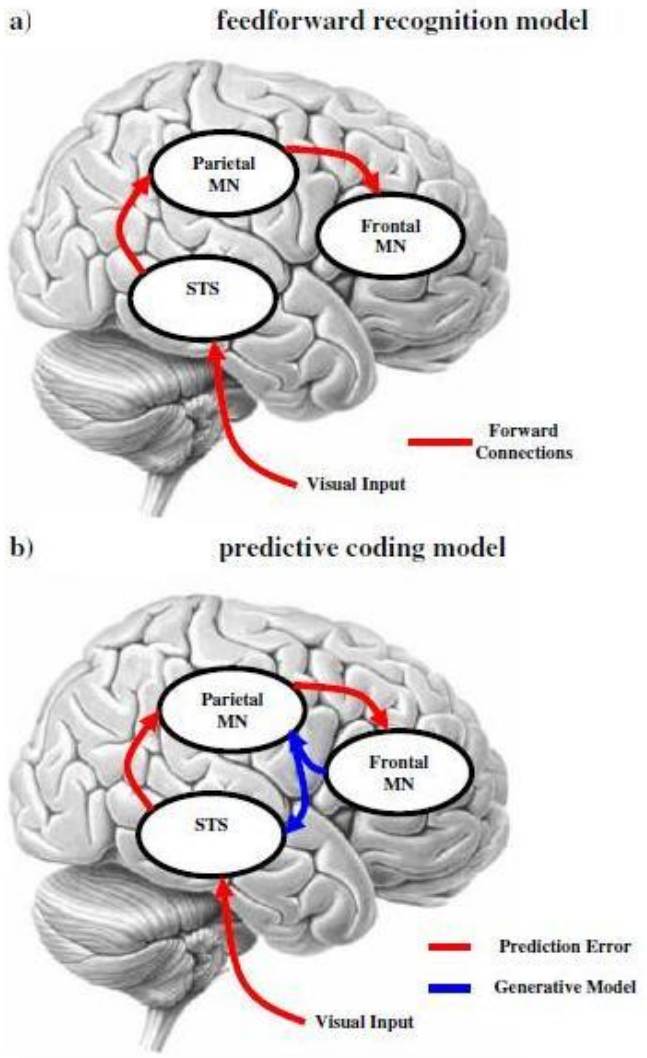

Figure 1.3. The figure illustrates a schematic view of the AON as a feedforward recognition model (a), and the alternative predictive coding model (b) In this model the lower level of cortical hierarchy is temporal node, followed by the parietal and frontal nodes. (Adapted from Kilner et al., 2007a).

These models of action perception may represent a link between the classical account of the mirror activity and the approaches of the so called "embodied cognition". The embodied cognition typically refers to those theories in cognitive science emphasizing the importance of action and the role played by bodily states for cognition (Borghi & Cimatti, 2010). These approaches consider

cognitive processes as deeply rooted in the body's interactions with the world (Wilson, 2002). The perceptual and motor systems would be part and parcel of the cognitive system and they would integrate an input - output system strictly linked to cognition. The subsequent approach of "grounded cognition" proposed by Barsalou (2008) goes forward adding that cognition is typically grounded in multiple ways, including simulation, situated action and on occasion, bodily states. According to this account the simulation is the reenactment of perceptual, motor, and internal states acquired during experience with the world, body and mind, thus our memory stores multimodal representations of each experience occurring across life. The perception of an event (objects, people "in action") would therefore call into play all the multimodal aspects associated with it, activating a very complex simulative system. Simulative processes would thus form the core of computations in the cognitive system.

An interesting issue deals with the origin and development of perceptual and predictive mechanisms of the motor system. A couple of theoretical accounts put forward by Heyes (2001) and Keysers and Perrett (2004) postulate that they are not completely hard wired but they are acquired and modeled through sensorimotor associations (see also Heyes et al., 2010 for a review). According to these views, the perception-action couplings emerge and develop in children from Hebbian plasticity of pathways connecting sensory and motor regions discharging simultaneously during imitation or self movement observation. Once the association is formed, perceiving the action would be sufficient to retrieve the sensorimotor network that became strengthened with experience. These accounts thus suggest that acquired sensorimotor representations of action vary as a function of experience, which in turn have the power to reconfigure the neural systems involved in their processing (Press et al., 2011). These theroretical models are supported by studies demonstrating that direct sensorimotor experience can strengthen action simulation mechanisms, for example within a given sport domain (Calvo-Merino et al., 2005; Fourkas et al., 2008) and this leads to more effective perceptual and predictive mechanisms (Aglioti et al., 2008; Urgesi et al., 2012). Importantly, it has been shown that the brain activity within the AON is crucially modulated by motor expertise in expert dancers and

athletes (Calvo Merino et al., 2006; Cross et al., 2006, 2008; Reithler et al., 2007; Abreu et al., 2012). Additionally, it has been demonstrated that the motor system can be reconfigured with specific visuo-motor trainings during action observation (Catmur et al., 2007, 2011) and these effects likely depend on plastic changes within the AON (Catmur et al., 2008).

SUMMARY

From the abovementioned brief review of the literature it essentially emerges that: the motor system is not only a system merely designed to action planning and execution, but it is inherently involved in the perception and understanding of others' actions and also devoted to sensorimotor transformations of sensory events occurring in the space around us. It has been suggested that the activity of the motor system is future-oriented, stressing the importance of a system which is able to anticipate the consequences of both own and others' actions, in order to smoothly interact with physical and social environment. It has been shown that these capacities of the motor system may be supported by a frontoparietal network of areas encompassing the IFC, the PPC and STS. Additionally a particularly relevant role in the motor simulation of others' action has been assigned to the IFC, considered the true "orchestra" of the network. However, it is still unclear whether this area exerts a pivotal role in the anticipatory motor simulation of others' actions and more in depth, whether this area is necessary in predicting the final end state of others' actions. It has been also suggested that this frontoparietal network is modified by subjective levels of sensorimotor experience acquired across the life-span. This experience seems to influence perceptual and predictive mechanisms of the motor system. An uninvestigated issue deals with how different levels of sensorimotor experience could affect action perception and prediction. The motor system is also importantly involved in sensorimotor transformations since the premotor cortex represents a multimodal station in which sensory events and space representations converge in order to be transformed in potential motor responses. However it is still unclear how the motor system could be

differentially modulated by sensory events occurring within and outside the PPS. This capacity to integrate external sensory events with appropriate motor reactions seems to depend from the same frontoparietal circuits involved in the motor representation of others' actions. However, it is not provided yet direct evidence of the crucial role of the two key node of these circuits, namely the premotor cortex (PMC) and the posterior parietal cortex (PPC) in the motor in the motor mapping of sensory events occurring within and outside the PPS.

All these unanswered issues constitute the focus of each study described in the present thesis, an overview of the whole work is presented in the following paragraph.

OVERVIEW OF THE THESIS

The aim of the present thesis is to explore some unanswered issues about the anticipatory mechanisms occurring in the motor system, in two main instances: i) when processing sensory events within the PPS, and ii) when perceiving and predicting others' actions. The experiments described in the Chapter 2 investigate for the first time, the reactivity of the motor system studied at rest while processing sensory events (auditory stimuli) presented within and outside the PPS. The following chapter 3 sheds light on the neural basis of the effects found in the previous experiments (Chapter 2) investigating the crucial role of the two key nodes of the PPS frontoparietal network, namely premotor cortex (PMc) and posterior parietal cortex (PPc) in the motor mapping of sensory events occurring within and outside the PPS.

The experiments of chapter 4 aim at investigating whether the anticipatory motor coding of others' actions critically relies on the activity of the frontoparietal AON. In particular, the chapter is focused on the critical role of two key nodes of the AON, the IFC and the STS in the anticipatory motor simulation of others' implied actions. By using a "perturb and measure" approach (see methods section of chapters 3, 4 and 5) we provide direct evidence that the IFC plays a critical role in the anticipatory motor simulation. In the following chapter 5, three experiments tackle a second

question of whether the IFC, crucially involved in perception, understanding and anticipatory motor simulation of others' action, is also necessary when predicting the future end state of others' actions compared to non-biological actions. Finally, the experiment presented in the chapter 6 has the purpose to examine how and to what extent the ability to predict others' actions could be influenced by the absence of a limb, to this aim both congenital and traumatic upper limb amputees were tested in two action prediction tasks. The results of each study will be discussed independently (see the Discussion section for each experimental chapter). Further, in a general discussion section (Chapter 7) the present findings will be considered comprehensively.

CHAPTER 2

Motor Properties of Peripersonal Space in Humans[1]

ABSTRACT

A stimulus approaching the body requires fast processing and appropriate motor reactions. In monkeys, fronto-parietal networks are involved both in integrating multisensory information within a limited space surrounding the body (i.e. peripersonal space, PPS) and in action planning and execution, suggesting an overlap between sensory representations of space and motor representations of action. In the present study we investigate whether these overlapping representations also exist in the human brain. We recorded from hand muscles motor-evoked potentials (MEPs) induced by single-pulse of transcranial magnetic stimulation (TMS) after presenting an auditory stimulus either near the hand or in far space. MEPs recorded 50 ms after the near-sound onset were enhanced compared to MEPs evoked after far sounds. This near-far modulation faded at longer inter-stimulus intervals, and reversed completely for MEPs recorded 300 ms after the sound onset. At that time point, higher motor excitability was associated with far sounds. Such auditory modulation of hand motor representation was specific to a hand-centred, and not a body-centred reference frame. This pattern of corticospinal modulation highlights the relation between space and time in the PPS representation: an early facilitation for near stimuli may reflect immediate motor preparation, whereas, at later time intervals, motor preparation relates to distant stimuli potentially approaching the body.

[1] *Published.* Serino, A., Annella, L., Avenanti, A., (2009). Motor properties of peripersonal space in humans. *PLoS ONE* 4:e6582.

INTRODUCTION

We can immediately and physically interact with stimuli in the external world when they occur within a limited space around us, reachable by our limbs and known as the Peripersonal Space (PPS). We might want to grab an interesting object placed in front of us or to retract a part of our body from an approaching, possibly dangerous, stimulus, such as a bee buzzing around. In order to realize these basic behaviours, our brain needs to integrate visual and auditory information about the external stimulus together with tactile and proprioceptive information about our body parts, and the result of this integration needs to be transformed into an appropriate motor plan.

In the monkey, multisensory neurons in fronto-parietal areas, integrate somatosensory information about the body with visual and acoustical information within the PPS. These neurons respond both to tactile stimuli on the monkey's arm, face or torso, and to visual and acoustic stimuli presented close, but not far (i.e. at more than 30 cm) from the corresponding body part (Rizzolatti et al., 1981; Graziano et al., 1994; Duhamel et al., 1998). Notably, neural responses of these multisensory cells decrease as a function of stimulus distance (Graziano et al., 1997). Somatosensory and visual receptive fields (RFs) are spatially in register: if the body part where the tactile RF is anchored moves, the visual RF shifts congruently. These neurons can therefore mediate a body-part centred multisensory representation of PPS. It has been shown that such a PPS representation has not only a sensory function, but also a motor function. Electrical microstimulation of multisensory neurons evokes a wide range of motor acts mimicking normal monkey behaviour in response to potential threats (Cooke et al., 2003). Thus, in the monkey, fronto-parietal areas representing PPS link together a multisensory representation of space with a motor representation of potential acts within that space.

In humans, neuropsychological (di Pellegrino et al., 1997; Làdavas & Serino 2008), behavioural (Spence et al., 2008), neuroimaging (Bremmer et al., 2001; Makin et al., 2007) and

electroencephalography (Sambo & Forster 2009) studies support the existence of neural systems representing the PPS. Although sensory components of human PPS representations have been extensively investigated, information about the possible motor features of human PPS representation is meagre. In the present study we explored hand-centred modulation of auditory space in the human motor cortex.

We recorded motor-evoked potentials (MEPs) induced by TMS to left motor cortex as a measure of the excitability of the corticospinal hand motor representation. MEPs were compared when identical sounds were presented either close to the subjects hand (at 5 cm; NEAR Sounds) or in distant space (at 100 cm; FAR Sounds). NEAR sounds, but not FAR sounds evoke a representation of the PPS around the hand (see Serino et al., 2007). Thus, a differential effect on MEPs associated with NEAR sounds compared to FAR sounds would reflect a modulation of corticospinal excitability of the hand motor representation due to the PPS representation.

Effective motor reactions to stimuli approaching the body need to be fast. In monkeys' multisensory areas, both neural responses elicited by sensory stimuli and body movements evoked by electrical stimulation show typically short latencies (up to 10–30 ms) (Graziano & Cooke 2006). In order to study the time-course of human corticospinal motor excitability due to PPS representation, we delivered TMS pulses at four time intervals following the auditory stimuli (50, 100, 200, and 300 msec). In a second experiment, we asked whether proprioceptive information coding hand position was critical for modulating the motor cortex during processing of NEAR and FAR auditory stimuli. Sounds were administered in the same positions as in the previous experiment, but subjects rotated their arm so that it was off to their side, pointing slightly backwards. This way, sound to head spatial distance was kept identical to Experiment 1, but both types of sound were in the far space with respect to subjects' hand. Thus if space dependent modulation of corticospinal excitability is coded in a hand-centre reference frame, in Experiment 2 MEPs associated with NEAR sounds should not be different to those associated with FAR sounds.

MATERIALS AND METHODS

Participants

A total of 24 healthy subjects, all students from University of Bologna, took part in the study. Twelve participants were assigned to Experiment 1 (8 females, mean age 25 y, range 22–28) and 12 to Experiment 2 (7 females, mean age 25 y, range 23–28). All subjects reported no abnormalities of touch or hearing and were right- handed. All subjects gave their written informed consent to participate in the study, which was performed with approval of the University of Bologna - Department of Psychology - ethics committee and in accordance with the Declaration of Helsinki (1964).

Transcranial Magnetic stimulation

MEPs induced by TMS were recorded from first right dorsal interosseus (FDI, in the region of the index finger) and abductor digiti minimi (ADM, in the region of the little finger) by means of a Biopac MP-150 (BIOPAC, U.S.A.) electromyograph. EMG signals were band-pass filtered (20 Hz–1.0 kHz, sampled at 5 kHz), digitized and stored on a computer for off-line analysis. Pairs of Ag-AgCl surface electrodes were placed in a belly-tendon montage on each muscle, with further ground electrodes on the wrist. A figure-of-8 coil connected to a Magstim Rapid2 stimulator (Magstim, Whitland, Dyfed, U.K.) was placed over the left motor cortex. The intersection of the coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45u angle away from the midline. In this way, the current induced in the underlying neural tissue was directed approximately perpendicular to the line of the central sulcus and was optimal for trans-synaptic activation of the corticospinal pathway (Brasil-Neto et al., 1992; Mills et al., 1992). Using a slightly suprathreshold stimulus intensity, the coil was moved over the left hemisphere to

determine the scalp position from which maximal amplitude MEPs were elicited from the FDI and the ADM muscles. The optimal position of the coil was then marked on the scalp with a pen to ensure correct coil placement throughout the experiment.

Different TMS intensities may disclose different neurophysiological modulations (Manganotti et al., 1997; Facchini et al., 2002), since they recruit different neural population within the motor cortex (Chen et al., 1997). We did not have any a-priori hypothesis about the critical TMS intensity necessary to study motor cortex modulation by PPS representation; therefore during the experiments, we used two different intensities of magnetic pulses eliciting MEPs, namely at 120% and at 140% of the resting motor threshold (rMT). The rMT was defined as the minimal intensity of the stimulator output that produced MEPs with amplitudes of at least 50 mV with 50% probability in the muscle with the higher threshold (Rossini et al., 1994), which in most cases corresponded to the ADM muscle. Mean values (S.D.) of rMT were 60.2 (8.3) in Experiment 1 and 59.4 (5.01) in Experiment 2. The two motor thresholds did not differ from one another ($p = 0.37$). The absence of voluntary contractions was continuously verified by visually monitoring of the EMG signal.

Procedure

Each subject was seated on a comfortable chair with the right arm placed on an arm rest. Two identical loudspeakers were placed in front of the subject and to the right, either in a NEAR position, at <60 cm from the subject head, or in a FAR position, 100 cm away from the near position, thus at <165 cm from the subject head (see Figure 2.1). In Experiment 1, the subjects right hand was placed close to the NEAR loudspeaker: therefore the distance between the hand and the sound sources was <5 cm for the NEAR loudspeaker and <100 cm for the FAR loudspeaker. In Experiment 2, the subject's right arm was rotated and pointed slightly backward, and therefore the

subject's right hand was placed at <80 cm from the NEAR loudspeaker and <180 cm from the FAR loudspeaker. In this way, both in Experiment 1 and in Experiment 2 the two types of auditory stimuli were close to or far from the subject's head, but only in Experiment 2 were both of them far from the hand. Participants were blindfolded during the whole duration of the experiment and oriented their heads towards the front. To maintain attention throughout the experimental session, subjects were requested to monitor the right hand for the infrequent occurrence of specific tactile stimuli (see below). On each trial an auditory stimulus (NEAR or FAR) was presented and TMS-induced MEPs were simultaneously recorded from the FDI and the ADM muscles. These two muscles were chosen to explore whether the possible modulation of corticospinal excitability due to PPS representations affected the motor representation of the whole hand (FDI and ADM) or was specific for the muscle that was contiguous to the source of auditory stimulation (ADM). Indeed, in the Experiment 1 set up, the NEAR sound was closer to the ADM muscle than to the FDI muscle. The inter-trial interval randomly varied between 10 and 12 sec. The choice of this long inter-trial interval was based on a study demonstrating that TMS pulses delivered for 1 h at 0.1 Hz frequency did not induce any change in motor excitability (Chen et al., 1997). Subjects were instructed to ignore any auditory stimulation and to focus only on the tactile stimulation administered to their right hand during the inter-trial intervals.

In order to study the time course of the motor changes evoked by auditory stimulation, TMS pulses were given at 4 different intervals: at 50, 100, 200 and 300 ms after the sound presentations.

Thus, the overall experimental design included a random combination of 2 sound locations (NEAR and FAR) and 4 TMS Delays (50, 100, 200, 300 ms), and a blocked combination of 2 TMS Intensities (120% and 140% of rMT). Each combination was randomly repeated 12 times, resulting in a total of 192 trials distributed across 6 experimental blocks, 3 with a TMS intensity at 120% rMT and 3 with a TMS intensity at 140% rMT. The order of the blocks was randomized. Two baseline blocks of 12 trials at 120% rMT and 140% rMT were recorded before (PRE) and after

(POST) the experimental session. During the baseline trials neither auditory nor tactile stimulation occurred.

Auditory stimulation

Inspection of phono-spectral waves, as recorded by a comput- erized software from the two loudspeakers, assured the sounds to be equal at their origin. Before each experimental block, the two loudspeakers were calibrated with a phonometer such that the intensity of sounds from both the NEAR and the FAR loudspeakers was identical at the subject's head (70 dB, 150 ms). We chose this relatively low intensity to avoid inducing any startle responses in the EMG signal (Lang et al., 1990). Indeed, loud auditory stimuli presented binaurally through headphones are known to suppress MEPs recorded after 30–60 ms from both distal and proximal muscles (Kuhn et al., 2004; Fisher et al., 2004), an effect likely due to cortico-reticular projections to the spinal cord. Auditory stimuli normally used to induce startle responses are quite louder (90–100 db) than those used in the present study (Lang et al., 1990). An equal proportion of NEAR and FAR sounds was administered unpredictably.

Tactile stimulation

Tactile stimuli were delivered via three miniaturized solenoids (M & E Solve, Rochester, UK; http://www.me-solve.co.uk), placed on the middle of the dorsal surface of the right hand at a distance of 5 mm one from each other. In different trials, either a single solenoid was briefly (5 ms) activated (weak stimulus) or all solenoids were activated together (strong stimulus): subjects had to respond, lifting the tip of their left foot, only to the strong stimulus. Tactile targets were rare, comprising 20% of total trials (equally frequently preceded by a NEAR or a FAR sound). An experimenter visually monitored subjects' responses. Tactile stimuli were administered in the inter-

trial interval at least 4–5 sec apart from TMS pulses to avoid MEP contamination due to tactile stimuli or motor responses (Terao et al., 1995; Classen et al., 2000). Error rates (false alarm, miss) were very low (2%) and were constant throughout the experiment.

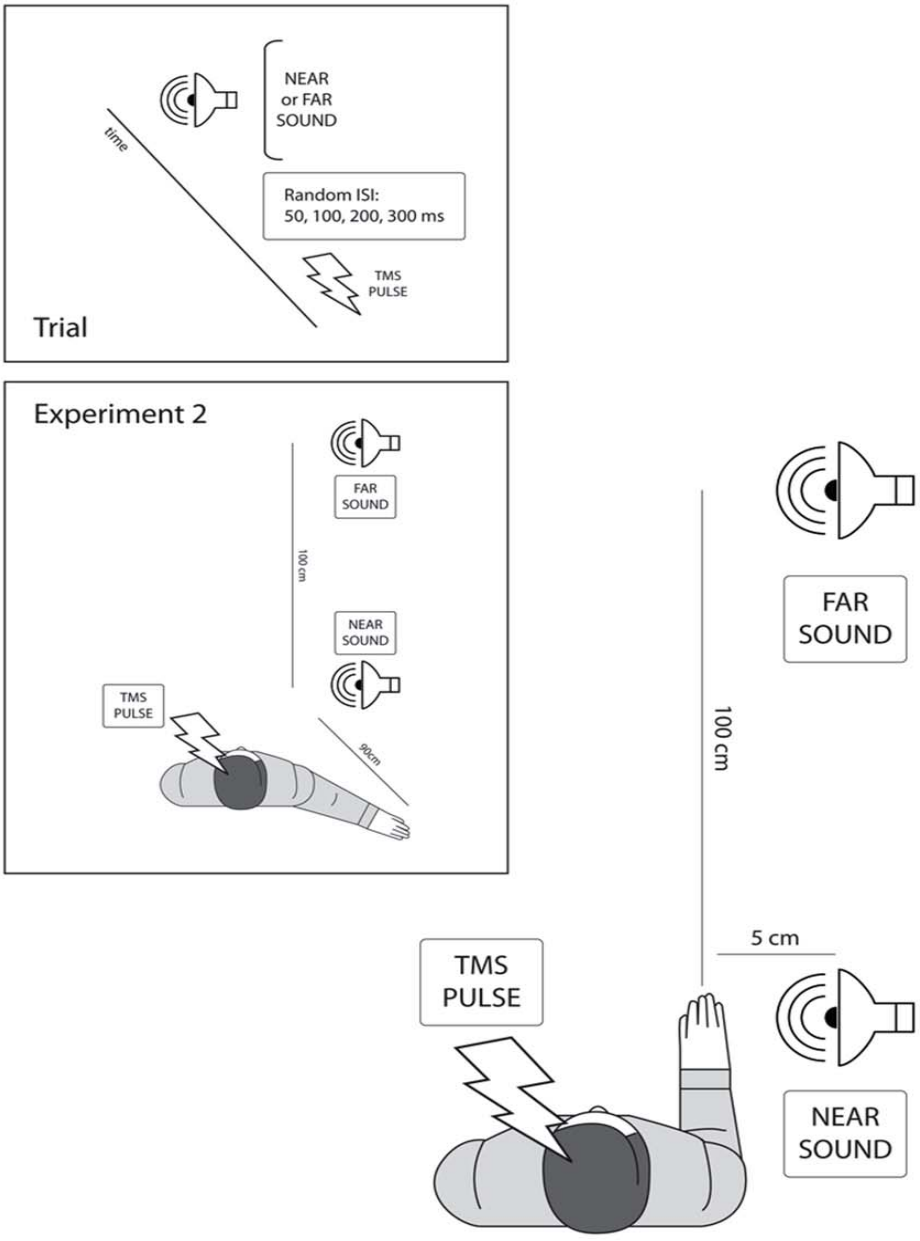

Figure 2.1 .Experimental set up. The main panel represents the experimental set up and a typical subject during Experiment 1. The small upper panel represents the sequence of events in each trial. The small lower panel represents a typical subject during Experiment 2, when participants placed their right arm to the side, with the hand pointing backwards (far from the source of near sounds). (Adapted from Serino et al., 2009).

Data analysis

Neurophysiological data were processed off-line. Trials with EMG activity prior to TMS were discarded from the analysis (less than 5% in each subject). Mean MEP amplitude values in each condition were measured peak-to-peak (in mV).

The amplitudes of raw MEPs recorded during baseline blocks were analyzed by means of a mixed-model ANOVA, with Muscle (FDI and ADM), TMS Intensity (120% and 140% of rMT) and Session (PRE and POST) as within-subjects factors, and with Experiment (arm forwards, EXP1, and arm backwards, EXP2) as a between-subjects factor.

The MEPs evoked during both PRE and POST baselines were averaged and used to compute an index of MEP modulation (MEPi), calculated as the ratio between the averaged MEPs recorded in each experimental condition and the averaged MEPs recorded in the baseline session, multiplied by 100. In this way, a MEPi = 100% indicates no modulation, MEPi.100% indicates an enhancement and a MEPi,100% indicates a reduction of corticospinal excitability with respect to the baseline.

MEPi data were entered in a mixed-model ANOVA with Muscle (FDI, ADM), TMS Intensity (120%, 140% of rMT), Delay (50, 100, 200, 300 ms) and Space (NEAR, FAR) as within-subjects factors and Experiment (EXP1, EXP2) as a between-subjects factor. When a significant quadruple or triple interaction was found, further analyses were performed by splitting the analysis into separate ANOVAs. Greenhouse-Geisser corrections were used to overcome possible violation of Sphericity assumption (Keselman et al., 2001).

RESULTS

The preliminary Muscle x TMS Intensity x Session x Experiment ANOVA on raw MEPs recorded during baseline blocks revealed a significant effect of TMS Intensity only ($F_{2,18} = 45.57$, $p < 0.00001$). As expected, amplitudes of MEPs induced by stronger TMS pulses (140% of rMT) were higher (mean ± s.e.m.: 1.42mV±.12) than those recorded with lower TMS pulses (120% of rMT; 0.88 mV±.11). This effect was equally present in the two experiments, for both the recorded muscles, and before and after each experiment, since no significant interaction between Intensity and the other factors was found ($p_s > .35$). Importantly, neither the main effect of Session ($p = .35$), nor any other interaction with Session were significant ($p_s > .38$), thus indicating that the overall excitability of the corticospinal system did not change over the course of the experiments. No other effects were significant ($p_s > .20$).

Baseline MEPs were averaged and used to compute an index of MEP modulation (MEPi) during the experimental session with auditory stimulation. The ANOVA on MEPis revealed a significant four-way interaction between Space, Intensity, Delay and Experiment ($F_{3,66} = 2.76$, $p < .05$). To further analyze this interaction, two separate Muscle x Space x Intensity x Delay ANOVAs were performed for each Experiment. The ANOVA run on Experiment 1 data revealed a triple Space x Intensity x Delay interaction ($F_{3,33} = 7.40$, $p < .0008$); thus we run two separate Muscle x Space x Delay ANOVAs for each Intensity. ANOVA on MEPi recorded with the lower TMS intensity (120% rMT) revealed a significant main effect of Space ($F_{1,11} = 5.81$, $p < .04$) and Time ($F_{3,33} = 5.05$, $p < .01$) and most importantly, a highly significant Space x Delay interaction ($F_{3,33} = 7.56$, $p < .003$; see Figure 2.2A). Post-hoc comparisons (Newman-Keuls Test) showed that MEPis recorded 50 ms after sounds occurrence were significantly enhanced when sounds were administered in the NEAR (mean MEPi ± s.e.m.: 113% ± 9) rather than in the FAR (97% ± 7; $p < .03$) space. This effect disappeared when TMS pulses were administered 100 and 200 ms after

sound presentations, and MEPis were not-significantly higher when FAR (122% ± 9 and 124% ± 10 for 100 ms and 200 ms of delay respectively) rather than NEAR (116% ± 11 and 113% ± 10) sounds were presented (p_s>.46). At a delay of 300 ms from sound presentation, the MEPi modulation found at 50 ms was completely reversed: at the long delay, the MEPis were significantly higher when FAR (117% ± 8) rather than NEAR (92% ± 9; p<.005) sounds were presented. Thus, MEPs were modulated by the presentation of NEAR and FAR sounds, and the direction of the effect depended on the time delay between MEP recording and sounds presentation. The interaction Muscle x Space x Delay was not significant ($F_{3,33}$ = 0.52, p = .64), indicating that the two muscles were similarly modulated as a function of space and time. Examples of raw MEPs recorded from the FDI and ADM muscle in these conditions (Experiment 1, 120% rMT) are shown in figure 2.3.

In Experiment 1, when TMS pulses were administered at 140% of rMT (figure 2.2 panel B), MEP amplitude values associated to NEAR auditory stimuli were numerically higher than those related to FAR stimuli (figure 2.2 panel B); however, no significant main effects, nor interactions, were found in the Muscle x Space x Delay ANOVA (p_s>.14).

The Muscle x Space x Intensity ANOVA performed on MEPis recorded in Experiment 2 did not show any significant main effect or interaction (p_s>.12). Therefore, as Figure 2.4 clearly shows, no relevant modulation of MEPs was recorded when participants rotated their arm backwards, thereby placing their hand quite distant from the previously NEAR loudspeaker.

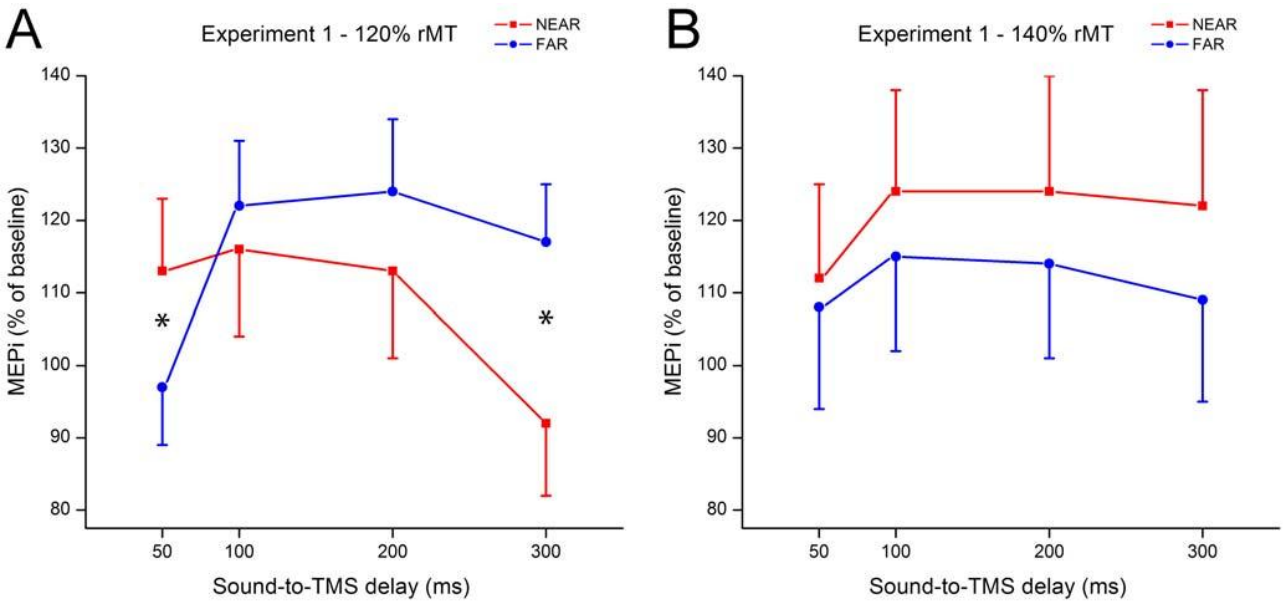

Figure 2.2. Mean MEP amplitude with respect to baseline (MEPi) recorded when sounds were presented NEAR (red lines) and FAR (blue lines) from the subjects' right hand (Experiment 1). (A) MEPi recorded with lower (120% rMT) TMS pulse intensity. (B) MEPi recorded with higher (140% rMT) TMS pulse intensity. Error bars denote s.e.m. Asterisks indicate a significant NEAR-FAR comparison ($p<.05$). (Adapted from Serino et al., 2009).

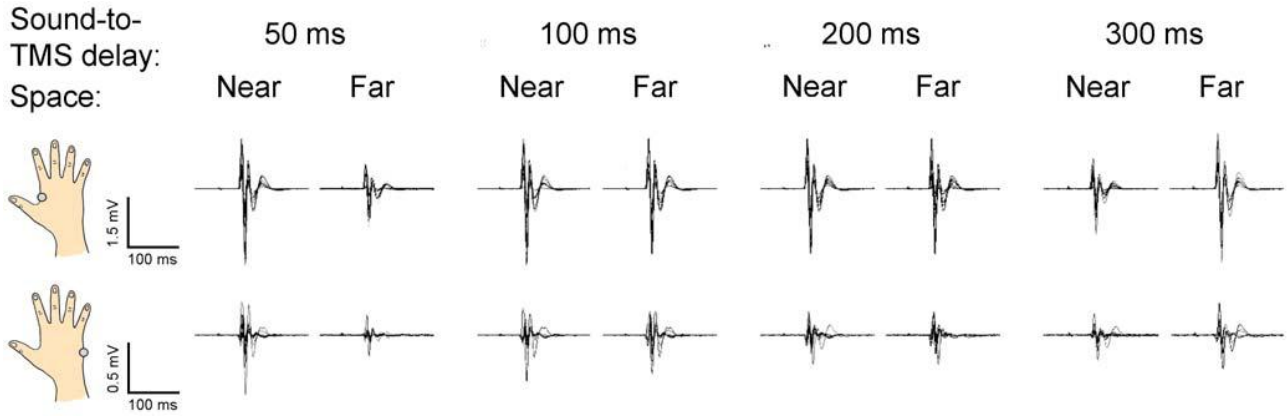

Figure 2.3. Raw MEPs amplitudes recorded from the FDI (top) and the ADM muscle (bottom) in one representative subject from Experiment 1 (only 120% rMT blocks are shown). (Adapted from Serino et al., 2009).

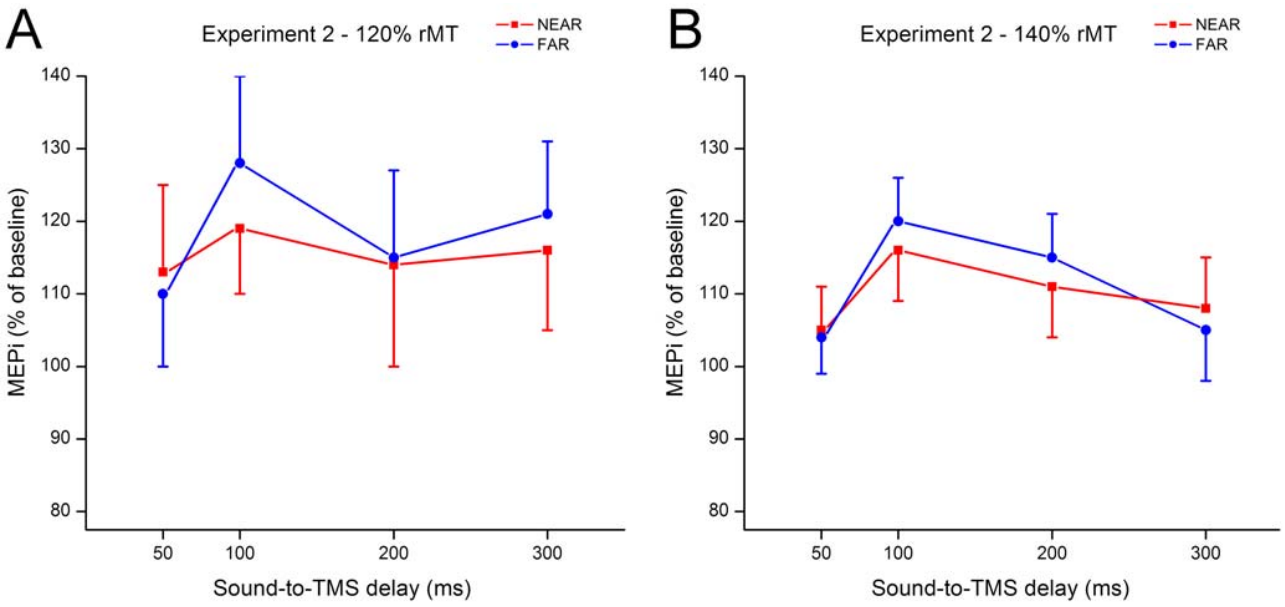

Figure 2.4. Mean MEP amplitude with respect to baseline (MEPi) recorded when sounds were presented NEAR (red lines) and FAR (blue lines) from the subjects' body (Experiment 2). (A) MEPi recorded with lower (120% rMT) TMS pulse intensity. (B) MEPi recorded with higher (140% rMT) TMS pulse intensity. Error bars denote s.e.m. (Adapted from Serino et al., 2009)

DISCUSSION

In the present study we show for the first time that the PPS representation in humans modulates neural activity within the motor system. We used MEPs evoked by single pulse TMS to assess the excitability of the hand representation in the motor cortex during the presentation of identical task-irrelevant auditory stimuli, administered either in near or far space. Stimulus distance was defined relative to a hand-centred reference frame.

In Experiment 1 we found that an auditory stimulus presented near the hand resulted in a specific modulation of the hand motor representation in comparison with an identical stimulus presented far from the hand. This effect was intensity dependent, since the near-far difference was present with TMS pulses delivered at 120% rMT and absent with higher (140%) intensities (see below).

Crucially, the different motor modulation for near and far stimuli detected at lower TMS intensities dynamically varied as a function of time. MEPs recorded 50 ms after presenting the sound close to the hand were enhanced in comparison to when the sound was administered far from the hand. This effect faded when MEPs were recorded 100 and 200 ms after sound presentation, and it was completely reversed for MEPs recorded at 300 ms: at that time delay, sounds administered far from the hand enhanced MEPs compared to sounds administered close to the hand.

Importantly, the different effects associated with near and far sounds were linked to hand-centred reference frames (Makin et al., 2007; Serino et al., 2007). When subjects placed their arm backwards, thus moving the hand away from the source of near sounds, while keeping constant the distance between the sounds and the rest of their body, MEPs associated to near and far sounds were comparable. This finding suggests that hand proximity, and not head or body proximity, was critical in modulating the excitability of the hand motor representation. This finding is also important in excluding the possibility that the changes in hand corticospinal excitability found in Experiment 1 were simply due to differential levels of arousal evoked by hearing a sound near or far from the body, and it further hints at the existence of a hand-centred representation of the auditory space (Serino et al., 2007; see also Makin et al., 2008 for a similar finding in the case of visual peri-hand space). Furthermore, the differential effects found in Experiment 1 and in Experiment 2 also suggest that the present results are not due to a startle response (Lang et al., 1990; Kuhn et al., 2004; Fisher et al., 2004), since this effect should have been quite similar in both experiments.

Thus, taken together these findings show first, that hand-centred PPS representation modulates the excitability of the hand corticospinal motor representation, and second, that such modulation acts with a definite time-course. An auditory stimulus presented within the peri-hand space enhances motor system excitability in a very short time window, whereas, in a later time window, a far sound has a greater facilitatory effect than a near sound. These findings are strongly related to each other

and can be interpreted in the light of the view that PPS ultimately has a motor function (Graziano & Cooke 2006; Rizzolatti et al., 1997).

In monkeys, bimodal neurons representing PPS were first described in the ventral premotor cortex, specifically in area F4. (Rizzolatti et al., 1981; Graziano et al., 1994; Fogassi et al., 1996; Graziano et al., 1999), which contains neurons representing specific body parts movements (Matelli & Luppino 2000; Rizzolatti et al., 2002; Graziano & Aflalo 2007). Electrical stimulation of such portions of the monkey VPM cortex results in complex motor acts, basically consisting of defensive behaviours (Cooke et al., 2003; Graziano & Cooke 2006; Graziano et al., 2002). Bimodal neurons are also present in area VIP (Duhamel et al., 1998; Bremmer et al., 2001; Colby et al., 1993; Bremmer et al., 2002), which is largely interconnected with VPM cortex (Matelli & Luppino 2001), and electrical stimulation of VIP also results in defensive motor behaviours. Thus, the very same areas integrating multisensory information in a limited space around given body parts also underlie the motor responses of those body parts, meaning that sensory representations of space and motor representations of action overlap in the monkey's bimodal regions. The findings of the present study, which demonstrate that an auditory representation of PPS around the hand results in an immediate modulation of the motor representation of the hand, suggest that a similar overlap between action and spatial processing exists in the human brain as well.

In humans, neural clusters in the ventral premotor cortex and in the inferior parietal sulcus (IPS) have been shown to be more strongly activated when visual or auditory stimuli approach the hand (Makin et al., 2007) or the face (Bremmer et al., 2001). These areas are likely to underlie PPS representation in humans and may functionally (Bremmer et al., 2001) and anatom- ically (Grefkes & Fink 2005) correspond to the VPM and VIP areas in the monkey (Rizzolatti et al., 2002). Moreover, human VPM and IPS are involved in sound localization (Maeder et al., 2001) and motor planning (Jeannerod 1997; Koch & Rothwell 2009). Importantly, TMS studies indicate that these areas exert action-related facilitatory influence on corticospinal excitability (Koch & Rothwell 2009; Avenanti et al., 2007; Davare et al., 2008; Koch et al., 2008).

We posit that the fronto-parietal network involved in multisensory integration may be the origin of the modulation of corticospinal excitability found in the present study. The pattern of connectivity of the monkey brain also supports this view. VPM and VIP cortices are strongly interconnected with each other (Luppino et al. 1999) and contain a high number of cells responding to auditory stimuli with early latency of response (10–40 ms) (Graziano et al., 1999; Schlack et al., 2005). VPM sends direct connections to the primary motor cortex (Cerri et al., 2003) and also direct connections to the spinal cord (Dum & Strick 2002). Electrical micro-stimulation of VPM and VIP neurons evokes motor responses with short latency (between 10 and 100 ms) (Cooke et al., 2003; Graziano & Cooke 2006; Dum & Strick 2002). Therefore, this pattern of fast connectivity would account for the increase of hand motor excitability found in our study 50 ms after the presentation of sounds near the hand. The early facilitation of motor cortex for near, but not far, auditory stimuli may have the function of preparing an immediate motor response for stimuli occurring within the PPS.

Fast sensory-motor transformations should apply to near stimuli potentially requiring an urgent motor reaction, whereas a far stimulus could in principle be processed at later stages and thus may later affect the motor system. We found that the specific MEP enhancement for near sounds disappeared 100 and 200 ms after sound onset, and that at 300 ms the effect fully reversed, so that far auditory stimuli were associated with motor facilitation. At that time delay, auditory stimuli near the hand are likely to be fully processed and evaluated as irrelevant to the body, at least when auditory stimuli carry no consequences, as in our experimental conditions. In contrast, a stimulus in far space is potentially relevant for the body at 300 ms, since external objects often move through space. As a consequence, 300 ms after onset, the far stimulus might potentially require a motor response and thus be associated with higher MEPs. The location of an external stimulus in space is not fixed, but varies in time as the subject and the external objects move relative to each other. The time-dependent modulation of corticospinal excitability due to near and far stimuli found in the present study captures this relationship between space and time in PPS representation.

We are aware that the effect reported in the present study has been obtained using static sounds, whereas, in everyday life, subjects face with moving stimuli, approaching or receding from the body. Future experiments are needed to explore the relationship between PPS representations and motor responses in more ecological conditions. It should be noted, however, that static stimuli allowed us to describe the time-course of the effect under more controlled experimental conditions. This information is critical to investigate the properties of moving sounds critical for activating PPS representations.

Two more issues need to be discussed before concluding. First, such space and time dependent MEP modulation was present when TMS pulses were delivered at 120% rMT but not at a higher intensity (140% rMT). These results are in keeping with previous findings showing that MEP modulation contingent upon the perception of tactile stimuli is stronger at low than at high TMS intensities (Manganotti et al., 1997). High intensity TMS pulses delivered to the motor cortex hand area are known to recruit less excitable corticospinal neurons within the motor hand area and/or neurons spatially further from the hand area (Facchini et al., 2002; Hallet et al., 1999) .Our data suggest that these neurons are less affected by the near-far modulation; it is possible that the excitation of such neural populations induced by 140% rMT pulses may have masked the activity of low-threshold motor neurons. Our findings confirm that lower TMS intensities are particularly adapt to disclose sensorimotor integrative effects in the human corticospinal system (Manganotti et al., 1997).

Finally, near and far auditory stimuli exerted comparable influence on MEPs recorded from the ADM and the FDI muscle, although in our experimental setup the former was closer to the near sound than the latter. The lack of a difference for the effects on these two muscles is not surprising considering that most of bimodal neurons in VPM normally have large RF covering the whole hand (Rizzolatti et al., 1981; Graziano et al., 1994). Furthermore, electrical stimulation of VPM bimodal neurons results in complex movements of the hand and the arm, and not in contraction of single muscles.

In conclusion, our findings suggest that in humans, as in monkeys, the representation of the PPS has an immediate effect on the motor system. Processing a stimulus close to the body can result directly in motor preparation. Stimulus distance is defined in a body part-centred reference frame. The effect of PPS representation on the motor system takes into account that spatial relationships between an external stimulus and the subject's body vary in time. These findings support the view that (multi)sensory and motor representations overlap in PPS and suggests that spatial representations are strongly bound up with temporal representations.

CHAPTER 3

Suppression of premotor cortex disrupts motor coding of peripersonal space[2]

ABSTRACT

Peripersonal space (PPS) representation depends on the activity of a fronto-parietal network including the Premotor cortex (PMc) and the Posterior Parietal cortex (PPc). PPS representation has a direct effect on the motor system: a stimulus activating the PPS around the hand modulates the excitability of hand representation in the primary motor cortex. However, to date, direct information about the involvement of the PMc-PPc network in the motor mapping of sensory events occurring within PPS is lacking. To address this issue, we used a "perturb-and-measure" paradigm based on the combination of transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) techniques. Cathodal tDCS was applied to transiently suppress neural activity in PMc, PPc and primary visual cortex (V1; serving as an active control site); single-pulse TMS was used to induce motor-evoked potentials (MEPs) from hand muscles and so to measure the excitability of the hand motor representation. MEPs were compared when a sound was presented either near the hand or at a distance. In experimental sessions performed after sham-tDCS and after tDCS over the control area V1, we found a spatially dependent modulation of the hand motor representation: sounds presented near the hand induced an inhibitory motor response as compared to sounds presented far apart. Critically, this effect was selectively abolished after tDCS

[2] *Published:* Avenanti, A., Annella, L., Serino, A. (2012c): Suppression of premotor cortex disrupts motor coding of peripersonal space. *Neuroimage* Oct 15; 63 (1): 281-288. Epub 2012 Jul 6.

suppression of neural activity in PMc, but not when perturbing the activity of PPc. These findings suggest that PMc, has a critical role in mapping sensory representations of space onto the motor system.

INTRODUCTION

When interacting with the external world, our brain integrates multisensory cues about environmental stimuli with information about the body in a coherent representation of Peri-Personal Space (PPS). In monkeys, a network of fronto-parietal regions, involving area F4 in the premotor cortex (PMc; Rizzolatti et al., 1981; Graziano et al., 1994; Fogassi et al., 1996; Graziano et al., 1997; 1999) and the ventral intraparietal area (VIP; Duhamel et al., 1997; Avillac et al., 2005; Schlack et al., 2005) in the posterior parietal cortex (PPC), support this function, since neurons in these regions integrate somatosensory stimuli from the body surface with visual and acoustic stimuli in the space immediately surrounding the body (Graziano & Cooke, 2006). Neuroimaging studies support the existence of a similar fronto-parietal network with homologous functions in the human brain. Portions of PMc and PPc respond to tactile stimuli on the face (Bremmer et al., 2001) and on the hand (Gentile et al., 2011) and to visual and auditory stimuli presented near the same body part (Makin et al., 2007). Moreover, suppression of PMc and PPc activity with Transcranial Magnetic Stimulation (TMS) impairs audio-tactile interaction within the PPS around the hand (Serino et al., 2011; see also Serino et al., 2007; Bassolino et al., 2010). Taken together, these findings suggest that in human and non-human primates a network of frontoparietal areas underlies a multi-sensory representation of PPS. PPS representation has not only a sensory but also a motor function. In monkeys, electrical stimulation of PPS neurons in F4 and VIP results in arm or head movements (Cooke et al., 2003; Graziano et al., 2002). In humans, auditory (Serino et al., 2009) or visual (Makin et al., 2009) stimuli presented near or far from the hand differentially modulate the excitability of the hand representation in the motor cortex (M1). More specifically, using single-

pulse TMS we showed that sounds presented within PPS transiently reduce M1 excitability as compared to sounds presented in extrapersonal space, within a specific temporal-frame (Serino et al., 2009). A nearby sound, by activating PPS mechanism, might cause a defensive-like freeze, resembling that found during the presentation of noxious stimuli (Farina et al., 2001; Urban et al., 2004) or potential threats (Cantello et al., 2000; Furubayashi et al., 2000), thereby reducing the excitability of the motor cortex. This effect suggests that sensory events occurring near the body primes motor reactions, and therefore that, in humans just as in monkeys, PPS representation is functionally linked to the motor system. However, to date it is not clear whether such spatially-dependent motor modulation relies on the activity of the same fronto-parietal areas involved in the sensory representation of PPS. To test this hypothesis, we designed a perturb-and-measure paradigm (Avenanti et al., 2007, 2012a) in which transcranial direct current stimulation (tDCS) was applied to transiently inhibit target PPS regions in PMc and PPc, whilst motor-evoked potentials (MEPs) to single-pulse TMS over M1 were recorded as a measure of corticospinal excitability during presentation of task-irrelevant sounds near and far from the hand. Based on the strong functional and anatomical link between PMc and M1 (Matelli & Luppino, 2001; Koch et al., 2006), we hypothesized that suppression of PMc would specifically affect the spatially-dependent modulation of M1 due to sound presentation. To test this hypothesis, in a first experiment, we compared MEPs from hand muscles after presentation of a near or a far sound following inhibitory tDCS over PMc or sham tDCS over the same area. In a second experiment, we tested whether not only PMc, but also PPc was involved in motor mapping of sensory events in PPS. To this aim, we compared MEPs associated to near and far sounds after inhibitory tDCS over PPc and over primary visual cortex, (V1), chosen as an active control site.

MATERIALS AND METHODS

Participants

Thirty neurologically healthy subjects were tested in the study. Sixteen volunteers (7 females, mean age 22.8 years, range 20-32) were assigned to Experiment 1 and fourteen to Experiment 2 (9 females, mean age 23.2 years, range 21-25). All subjects were right-handed, reported no abnormalities of touch or hearing and met the safety criteria for TMS and tDCS (Rossi et al., 2009; Poreisz et al., 2007). All the participants were naïve to the procedures and to the purpose of the experiments. A written informed consent, approved by the University of Bologna‟s Department of Psychology ethics committee, was obtained prior to participation. The study was conducted in accordance with the Declaration of Helsinki (1964).

Design

In two experiments, we used a "perturb-and-measure" paradigm (Avenanti et al., 2007; Avenanti et al., 2012a) in which neural activity is assessed with single-pulse TMS (measure) within or outside the inhibitory temporal window created by cathodal tDCS over target cortical sites (perturb). In both experiments, TMS was applied to left M1 to elicit MEPs from the first dorsal interosseus (FDI) muscle of the right hand; thus MEPs were taken as a measure of excitability of the hand representation in M1. TMS was delivered 50, 175 or 300 ms after a white-noise burst that was presented either at ~5 cm from the hand (Near sound) or at ~100 cm from the hand (Far sound). In Experiment 1, MEP recording was performed in two post-tDCS sessions that were carried out after 15 min of either Real- or Sham-tDCS over the left PMc. In Experiment 2, MEP recording was performed in two post-tDCS sessions that were carried out after 15 min of either Real-tDCS over

the left PPc (target site) or Real-tDCS over the visual cortex (V1, serving as an active control site, not involved in PPS representation). Experiments 1 and 2 were conducted on two different samples of subjects. In order to minimize carry-over effects, the two post-tDCS sessions of each experiment were performed on two different days, with an inter-session interval of at least 1 week. The order of the sessions was counterbalanced between subjects. Target sites and types of tDCS apart, procedure and stimuli were the same for the two experiments.

We predicted that different MEPs amplitude would be associated with near and far sounds after the two control conditions, Sham-tDCS (Experiment 1) and Real tDCS over V1 (Experiment 2). In contrast, if PMc and PPc are both necessary for a motor representation of PPS, little (or no) MEPs modulation due to sound position should be found after Real tDCS over these target areas. If PMc is necessary, and PPc is not, Real tDCS over the former, and not the latter, area should affect the spatial modulation of MEPs.

Procedure and stimuli

Each subject sat on a chair with their right arm placed on an arm rest. Two loudspeakers were placed to the right of the subject: one was positioned close to the subject, at ≈ 5 cm from the right hand (at ≈50 cm from the subject's torso and at ≈60 cm from the subject head); the other was positioned far from the subject, at 100 cm away from the near loudspeaker (at ≈150 cm from the subject's torso and ≈160 cm from the subject's head). Subjects were blindfolded, were asked to keep their eyes closed during the whole experiment and their head oriented towards their front. We recorded MEPs from the right FDI muscle induced by TMS just after presenting an auditory stimulus generated either from the near loudspeaker or from the far loudspeaker. TMS pulses were delivered at 120% of resting motor threshold (rMT; see below), at one of three possible time delays after the sound onset, i.e., at 50, 175, and 300 ms (see Figure 3.1). The inter-trial interval varied

between 10 and 12 seconds. To maintain attention throughout the experimental session, subjects were requested to monitor the right hand for the infrequent occurrence of specific tactile stimuli (see below). Subjects were explicitly instructed to not pay attention to any auditory stimulation during the experimental sessions. MEPs were recorded during two experimental blocks of 42 trials each; each trial resulted in a random combination of: a sound (near or far), a time delay between the sound and the TMS pulse (50, 175, 300 ms). The order of the blocks was randomized.

Auditory stimulation

Auditory stimuli consisted in 300 ms bursts of white noise, generated by two identical loudspeakers. The intensity of the near and far sounds was set to be equal (≈70 dB) as measured by a phonometer above the subject's head (over the vertex). Inspection of phono-spectral waves (recorded by a computer) from the two loudspeakers ensured that the sounds were equal at their origin for emitted frequencies.

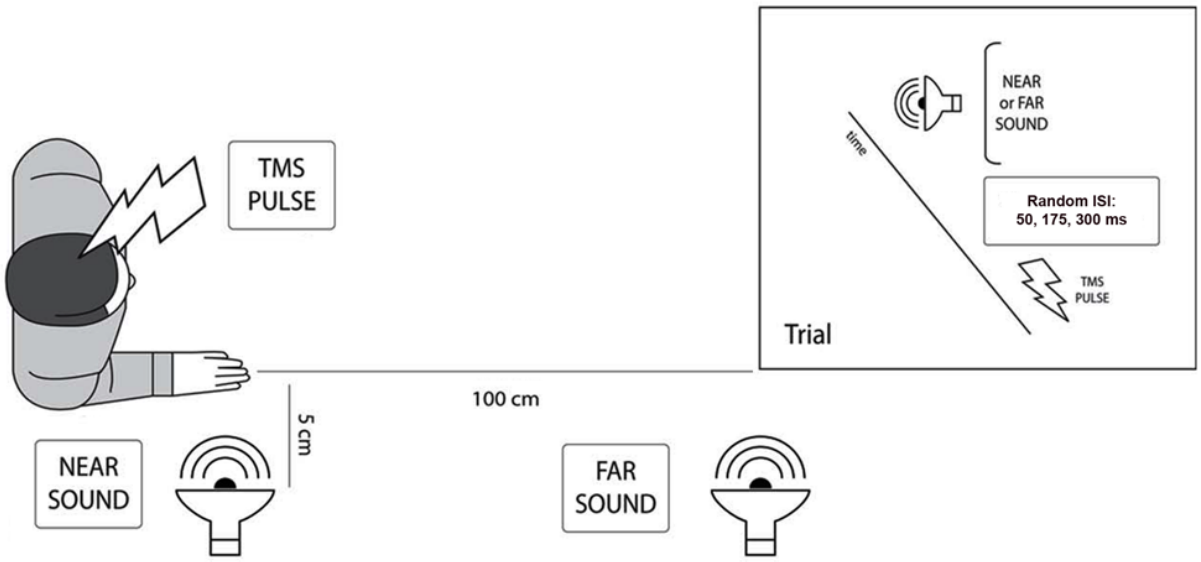

Figure 3.1. Schematic representation of the experimental set up and temporal sequence of events (right panel). (Adapted from Avenanti et al., 2012c).

We used white noise samples as auditory stimuli to activate PPS representation based on our previous studies on neural bases of PPS system in healthy humans (Serino et al., 2009; Serino et al., 2011; see also Serino et al., 2007; Bassolino et al., 2010) and on previous studies on auditory PPS in monkeys (Graziano et al., 1999) and in brain damaged patients (Farnè & Làdavas, 2002). Graziano et al., (1999) showed that white noise bursts administered close to the monkeys' body induced strong responses in F4 neurons, comparable to those elicited by more ecological sounds, such as jingling keys, claps, crinkling paper, whereas artificial sine waves of various frequencies were ineffective (see also Schlack et al., 2005). The same difference between white noise, eliciting a strong PPS response, and pure tones, not eliciting specific response, was reported by Farnè and Làdavas, (2002), in brain damaged patients suffering crossmodal extinction. Thus, although in principle more ecological sounds (see e.g. Tajadura et al., 2010) might induce even stronger effects, we were confident that white noise bursts were able to reliably activate the PPS system and therefore modulate the motor system.

Tactile stimulation

Tactile stimuli were delivered by means of three miniaturized solenoids (M&E Solve, Rochester, UK; http://www.me-solve.co.uk) placed under the palm of the right hand at a distance of 5mm from one another. During inter-trial intervals, either a single solenoid was briefly activated (weak stimulus) or all solenoids were activated simultaneously (strong stimulus). Subjects were asked to only respond to the strong stimulus, by lifting the front of their left foot. Strong stimuli were rare and comprised 20% of the total trials. Subjects' responses were visually monitored by an experimenter. Tactile stimuli were administered in the inter-trial interval at least 4-5 sec apart from TMS pulses to avoid MEP contamination due to tactile stimulation or motor responses (Terao et al., 1995; Classen et al., 2000).

Electromyography and Transcranial Magnetic Stimulation

MEPs were recorded from the first dorsal interosseus (FDI, in the region of the index finger) muscle of the right hand by means of a Biopac MP-150 (BIOPAC, U.S.A.) electromyograph. EMG signals were band-pass filtered (30-500 Hz and sampled at 5kHz), digitized and stored on a computer for off-line analysis. Electromyographic (EMG) recordings were performed through surface Ag/AgCl electrodes placed in a belly-tendon montage on the FDI muscle, with further ground electrodes on the wrist. TMS was performed by means of a figure-of-8 coil connected to a Magstim Rapid² stimulator (Magstim, Whitland, Dyfed, U.K.). The coil was placed over the left M1. The intersection of the two coil's wings was placed tangentially to the scalp with the handle pointing backward and laterally 45° away from the midline. In this way, the current induced in the underlying neural tissue was directed approximately perpendicular to the line of the central sulcus and was optimal for trans-synaptic activation of the corticospinal pathway (Brasil-Neto et al., 1992; Mills et al., 1992). During the recording sessions the coil was positioned in correspondence with the optimal scalp position (OSP), defined as the position from which MEPs with maximal amplitude were elicited from FDI muscle. The OSP was detected by moving the intersection of coil in 1cm steps around the hand motor area of the left M1 and by delivering TMS pulses with a slightly suprathreshold stimulus intensity. Participants wore a bathing cap on which the OSP of the coil was marked with a pen to ensure correct coil placement throughout the experiments. TMS intensity was calibrated at 120% of resting motor threshold (rMT) defined as the minimal intensity of the stimulator output that produces MEPs in the target muscle (the FDI) with amplitudes of at least 50 μV with 50% probability (Rossini et al., 1994). We selected this pulse intensity among the two levels of stimulation used in our previous study (i.e., 120% and 140% of rMT; see Serino et al., 2009), in order to reduce the experimental conditions and the total length of the experimental blocks. We focused on the lower level of stimulation (120% of rMT) because this intensity showed the greatest space-dependent modulatory effects in Serino et al. (2009) and was also closer to that

used by other studies investigating motor coding of PPS (e.g., Makin et al., 2009; Cardellicchio et al., 2011). It should be noted that in the present study we computed rMT by considering the target muscle FDI, while in our previous study, MEPs were collected also from the abductor digiti minimi (ADM) and rMT was computed on such muscle that showed higher threshold. Thus, in the present experiment systematically lower stimulation intensity was used to assess corticospinal excitability, although this was closer to that used in other studies investigating motor excitability changes during processing of potentially threatening visual (Cantello et al., 2000; Makin et al., 2009) or auditory stimuli (Furubayashi et al., 2000). Different TMS intensities may recruit neural populations with different activation thresholds (Chen et al., 2008). Based on previous results (Cantello et al., 2000; Furubayashi et al., 2000; Makin et al., 2009; Serino et al., 2009), low TMS intensity used in the present study is more likely to reveal inhibitory, rather than excitatory neural effects. Values of rMT were comparable in Experiment 1 (mean % of maximal stimulator output ± st.dev: 59% ± 11) and Experiment 2 (55% ± 7; $t_{28} = 1.09$, $p = 0.28$); thus any differential effects in the two experiments cannot be ascribed to differences in corticospinal excitability. The absence of voluntary contractions was continuously verified by visual monitoring of the EMG signal.

Transcranial direct current stimulation (tDCS) and Neuronavigation

A battery-driven, constant, direct current stimulator was used to apply tDCS (Eldith; www.eldith.de). A pair of surface conductive rubber electrodes (35 cm^2) were placed in two saline soaked sponges and positioned over the target areas. Rubber bandages were used to hold the electrodes in place during the stimulation. For active stimulation (Real-tDCS), cathodal tDCS was applied to PMc (Experiment 1) and to PPc and V1 (Experiment 2) with the cathode positioned above the target area and the anode over the contralateral orbit. The duration of each session of tDCS was 15 minutes and the intensity was set at 1mA (fade in/out duration: 20 sec). This type of

stimulation is known to induce a transient suppression of cortical excitability (mainly due to neural hyperpolarization and long-term depression-like mechanisms) which in turn may disrupt the function of the stimulated site (Nitsche et al., 2003a; Nitsche et al., 2003b). It has been demonstrated that the effects of tDCS on neuronal excitability last for up to 90 minutes after a stimulation of 13 minutes only (Nitsche et al., 2001). Thus we assumed that 15 minutes of tDCS ensured a large inhibitory window along which we run the MEP recording session. For the Sham-tDCS, the electrodes were placed on the same locations as for Real-tDCS and the current was turned off after 15s of stimulation (fade in/out: 20 sec). This stimulation is known to induce skin sensations indistinguishable from real tDCS. These parameters for sham stimulation were chosen based on previous reports that the perceived sensations on the skin, such as mild local tingling (associated with the onset of stimulation), usually fade out in the first few seconds of tDCS (Nitsche et al., 2003c; Paulus et al., 2003) The stimulation sites for correct positioning of the tDCS electrodes were identified on each participant's scalp by means of a SofTaxic Navigator system (Electro Medical Systems, Bologna, Italy) as in previous research (Avenanti et al., 2007; Bertini et al., 2010; Serino et al., 2011; Avenanti et al., 2012a). Skull landmarks (nasion, inion, and two preauricular points) and about 100 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitiZer (Northern Digital Inc, Ontario, Canada). Coordinates in Talairach space (Talairach & Tournoux, 1988) were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template. In Experiment 1, the PMc was targeted in the ventral aspect of the precentral gyrus (ventral premotor cortex) at the border with the posterior part of the inferior frontal gyrus (pars opercularis) (searched coordinates: x = -52, y = 8, z = 25, corresponding to Brodmann' s area 6/44 in the inferior frontal cortex). Individual‟s Talairach coordinates corresponding to the projection of the PMc target site on brain surface were automatically estimated through the neuronavigation system. Mean PMc ± SD brain surface coordinates (corresponding to the center of the cathodal tDCS electrode placed on the scalp) were: x = -55.7 ± 2.4; y = 7.6 ± 1.1; z = 23.8 ± 3.1 (Figure 3.2).

In Experiment 2, the PPc was targeted within the anterior part of the intraparietal sulcus (x = -39, y = -40, z = 43, corresponding to Brodman's area 40). These locations were chosen by averaging the coordinates of PMc and PPc sites found in previous neuroimaging studies on PPS in humans (Bremmer et al., 2001; Makin et al., 2007; see Figure 3.2); we have previously demonstrated that repetitive TMS over these sites disrupts multisensory audio-tactile representation of PPS (Serino et al., 2011). In Experiment 2, the active control site V1 was targeted on the scalp location that corresponded best to the visual cortex (x = 19, y = -98, z = 1, Brodmann's area 17, in the middle occipital gyrus). Talairach coordinates corresponding to the projection of PPc and V1 target sites on brain surface were x = -49.1 ± 1.4; y = -42.3 ± 1.1; z = 48.0 ± 1.8 and x = -18.7 ± 0.9; y = -98.2 ± 0.7; z = 0.2 ± 0.7, respectively (figure 3.2).

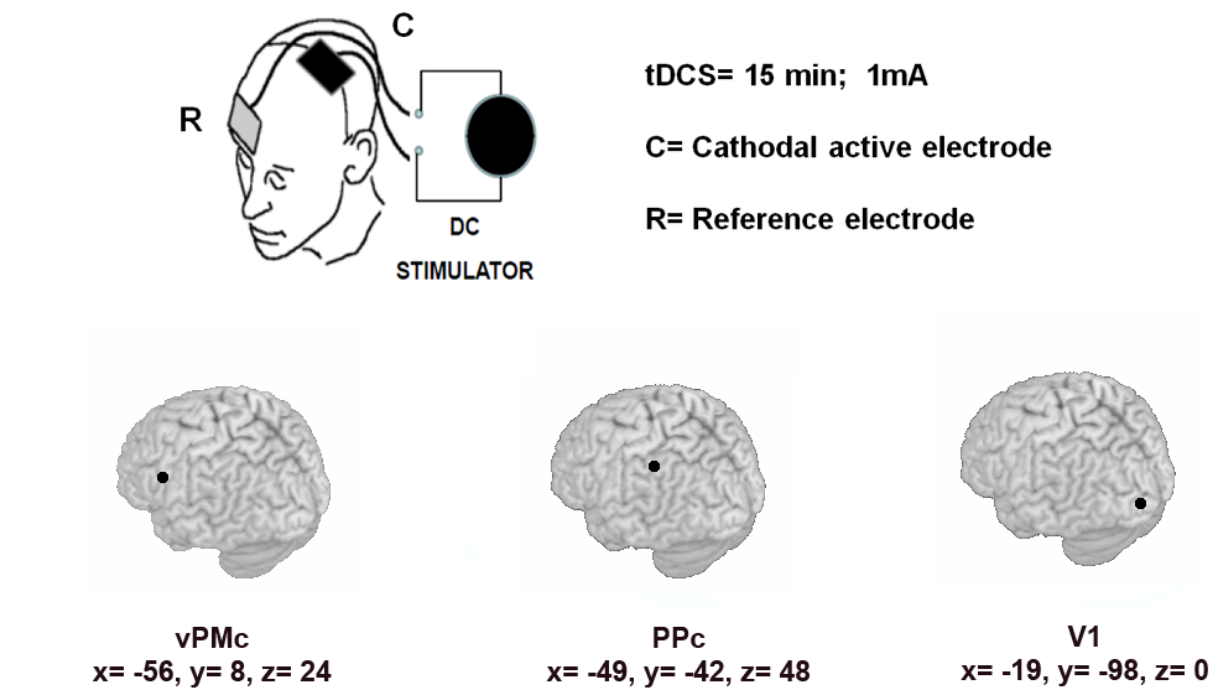

Figure 3.2. The upper part of figure shows transcranial direct current stimulation (tDCS) parameters and electrodes positioning. The lower part shows surface brain locations of tDCS.

Data analysis

MEPs were analyzed off-line with AcqKnowledge (v 4.10) software. The presence of background EMG activity prior to TMS was visually inspected. Trials with EMG activity preceding TMS were discarded from the analysis. Mean peak-to-peak MEP amplitudes (in mV) were computed for each experimental condition. In Experiment 1, we compared MEPs after Real-tDCS over PMc (test) or after Sham-tDCS over the same site (sham control), when near or far sounds were presented and were followed by a TMS pulse at 50, 175 or 300 ms. Mean raw MEP amplitudes were entered in a three-way repeated-measures ANOVA with Session (Real-tDCS PMc, Sham-tDCS PMc), Location of Sound (Near, Far), and TMS Delay (50, 175, 300 ms) as within-subjects factors. In Experiment 2, we compared the effect of Real-tDCS over PPc and V1. Mean raw MEP amplitudes were analyzed by means of a three-way repeated-measures ANOVA with Session (Real-tDCS PPc, Real tDCS V1), Location of sound (Near, Far), and TMS delay (50, 175, 300 ms). Post hoc comparisons were performed using the Duncan's test in order to correct for multiple comparisons. A further analysis was conducted on MEP differences (near-far) recorded after the critical conditions of Real-tDCS over PMc and Real-tDCS over PPc relative to the control conditions of Sham-tDCS over PMc and Real-tDCS over V1. In this way we directly compared motor reactivity to near/far sounds across the two experiments.

RESULTS

In Experiment 1, the Session x Location x TMS delay ANOVA revealed a main effect of Location ($F_{1,15}$= 5.33, $p < 0.05$), with lower amplitudes for MEPs recorded after near sounds (mean amplitude ± s.e.m.: 1.72 mV ± 0.03) relative to far sounds (1.84 mV ± 0.04), and a main effect of TMS delay ($F_{2,30}$= 5.49, $p < 0.01$), with greater amplitudes for MEPs recorded at 175 ms (1.89 mV ± 0.03) relative to MEPs recorded at 50 (1.75 mV ± 0.03) and 300 ms (1.71 mV± 0.05; all ps < 0.01). Importantly, the three-way interaction was significant ($F_{2,30}$= 4.03, $p < 0.05$), indicating that in the two tDCS sessions, MEPs were differently modulated as a function of the location of sounds and of the time of TMS administration. In order to identify the source of the three-way interaction, two separate Location x TMS delay ANOVAs were carried out, one for each tDCS session. In the Sham-tDCS Session, the ANOVA conducted on MEPs revealed a significant Location x TMS delay interaction ($F_{2,30}$ = 4.10, $p < 0.05$). Post-hoc comparisons showed that MEPs recorded 300 ms after a sound's occurrence were significantly lower when sounds were presented near the hand (1.66 mV± 0.26) than at a distance (2.00 mV ± 0.32; $p < 0.0001$), thus replicating the inhibitory modulation of corticospinal excitability due to near sounds, as shown in Serino et al. (2009) (Figure 3.3A, Table 3.1). No similar near-far difference in amplitude was found for MEPs recorded at 50 ($p = 0.07$) and 175 ms ($p = 0.41$). Critically, tDCS over PMc disrupted the space-dependent pattern of corticospinal modulation found after sham stimulation: in the Real-tDCS over PMc Session, the Location x TMS delay interaction was not significant ($p = 0.26$). Only the main effect of TMS delay was significant ($F_{2,30}$ = 5.55, $p < 0.01$), and post-hoc comparisons showed that MEPs recorded at 300 ms after sound presentation were lower (1.59 mV ± 0.01) as compared to those recorded at 175 ms (1.80 mV ± 0.01; $p < 0.01$), but not to those recorded at 50 ms (1.69 mV ± 0.03; $p = 0.12$), whereas MEPs recorded at 50 ms and 175ms were comparable ($p = 0.09$) (Figure 3.3B).

EXPERIMENT 1				
	SHAM tDCS (Left PMc)		*REAL tDCS (Left PMc)*	
DELAY (ms)	NEAR SOUNDS	FAR SOUNDS	NEAR SOUNDS	FAR SOUNDS
50	1.74 ± 0.28	1.87 ± 0.31	1.60 ± 0.24	1.78 ± 0.28
175	1.95 ± 0.31	2.02 ± 0.31	1.77 ± 0.27	1.84 ± 0.26
300	1.66 ± 0.26	2.00 ± 0.32	1.63 ± 0.27	1.55 ± 0.22
EXPERIMENT 2				
	Real tDCS (Left PPC)		*REAL tDCS (Left V1)*	
50	1.25 ± 0.12	1.33 ± 0.11	1.18 ± 0.15	1.11 ± 0.16
175	1.42 ± 0.14	1.52 ± 0.17	1.24 ± 0.17	1.28 ± 0.18
300	1.14 ± 0.14	1.35 ± 0.17	1.04 ± 0.15	1.19 ± 0.16

Table 3.1. MEP amplitudes (in mV) ± SEM recorded from FDI muscle in Experiment 1, after sessions of Real tDCS over PMc and Sham tDCS over PMc, and Experiment 2, after sessions of Real tDCS over PPC and the Real tDCS over V1.

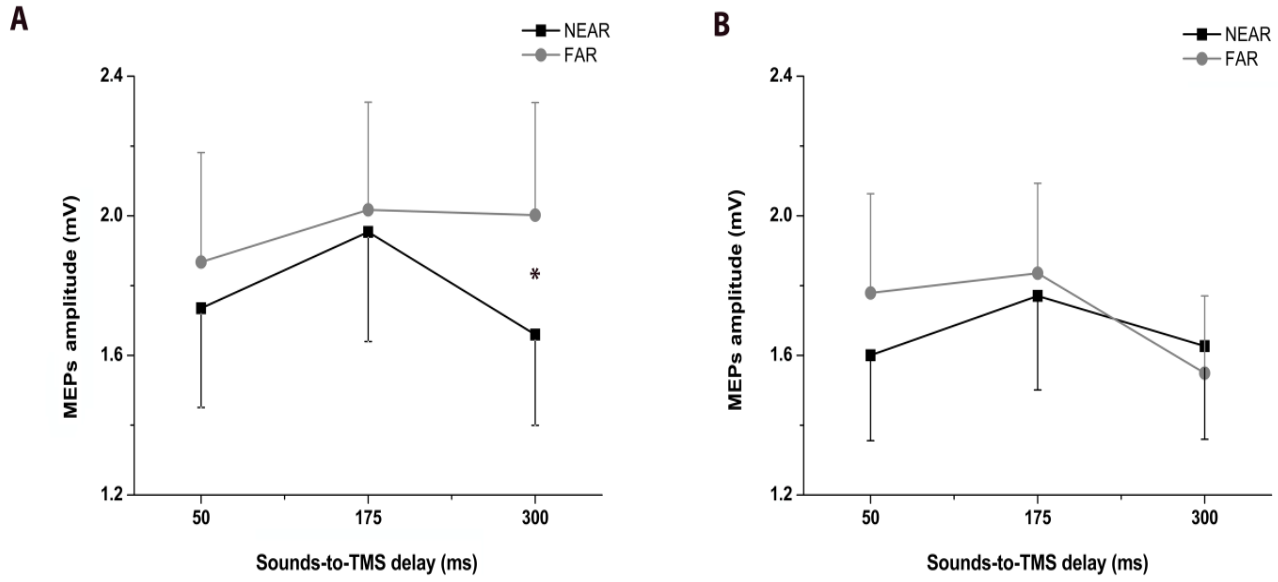

Figure 3.3. Raw mean MEPs amplitude recorded during Experiment 1, after the Sham tDCS session over the left PMc (A) and after the Real tDCS session over the left PMc (B), when sounds were administered NEAR (black lines) and FAR (grey lines) from the subject's right hand. Error bars denote SEM. Asterisks indicate significant comparisons. (Adapted from Avenanti et al., 2012c).

In contrast to Experiment 1, the Session x Location x TMS delay ANOVA conducted on MEPs recorded during Experiment 2 showed a significant two-way Location x TMS delay interaction ($F_{2,26} = 3.29$, $p < 0.05$), but not a three-way interaction ($p = 0.73$). These effects indicate that in both tDCS sessions, MEPs were similarly modulated as a function of the location of sound presentation and the time of TMS pulse administration. Post-hoc analysis of the two-way Location x TMS delay interaction showed that MEPs recorded at 300ms from sound onset were lower when a near sound was presented (1.09 mV ± 0.12), as compared to a far sound (1.27 mV ± 0.13; $p < 0.001$), similarly to what occurred after Sham-tDCS in Experiment 1 (Figure 3.4, Table 3.1). Moreover, no near-far difference in amplitude was found for MEPs recorded at 50 ($p = 0.93$) and 175 ms ($p = 0.16$). These results show that the spatially-dependent modulation of M1 excitability (due to sound presentation) was not disrupted by interfering with neural activity in either the control area, V1, or the target area, PPc.

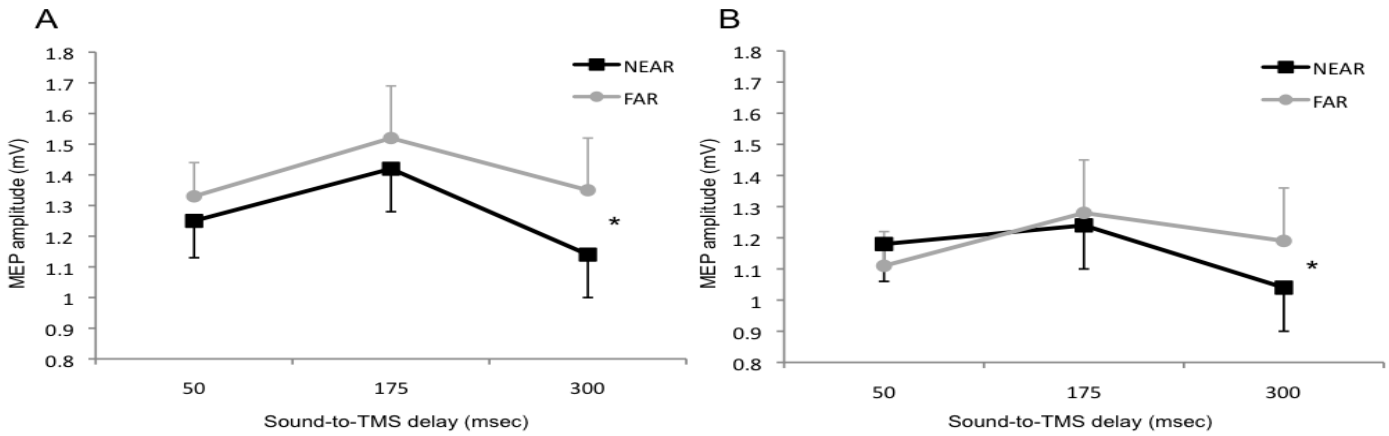

Figure 6.4. Raw mean MEPs amplitude recorded during Experiment 2, after the Real tDCS sessions over the left PPc (A) and over the left V1 (B), when sounds were administered NEAR (black lines) and FAR (grey lines) from the subject's right hand. Error bars denote SEM. Asterisks indicate significant comparisons. (Adapted from Avenanti et al., 2012c).

In sum, in the Sham-tDCS session of Experiment 1 and in both sessions of Experiment 2, MEPs recorded at 300 ms were lower when near sounds were presented as compared to when far sounds were presented. In contrast, such time-specific spatial modulation of MEPs was disrupted when Real-tDCS was applied to PMc (Experiment 1). In order to directly compare the effect of tDCS over the critical PPS areas PMc and PPc on motor reactivity to near/far sounds, we computed an index of spatial modulation of MEPs. For each tDCS session, we subtracted MEP values recorded 300 ms after administration of far sounds from those recorded 300 ms after administration of near sounds (Space-Index, SI). In this way, we could directly compare spatial effects on motor cortex excitability across the two experiments. We considered Real-tDCS sessions over PMc (Experiment 1) and over PPc (Experiment 2) as target conditions, and Sham-tDCS (Experiment 1) and RealtDCS over V1 (Experiment 2) as respective control conditions. We entered SI at 300 ms in a 2 x 2 mixed-model ANOVA with Condition (Target, Control) as the within-subjects factor and Experiment (Exp1, Exp2) as the between-subjects factor. The two-way interaction was significant ($F_{1,28} = 4.34$, $p < 0.05$). As Figure 3.5 shows, SI was negative, indicating a spatial modulation of MEPs, with lower MEPs following near sounds, for both control conditions (Sham tDCS = -0.34 mV ± 0.11; Real tDCS over V1 = -0.15 mV ± 0.12), as well as for the Real-tDCS over PPc condition (-0.21 mV ± 0.08). These values were not different from each other (all $p_s > .25$). On the contrary, no spatial modulation was evident after Real-tDCS over PMc (0.07 mV ± 0.08), and SI in this condition was significantly different from the two control conditions (all $p_s < .05$), and also, critically, from the other target condition of Real-tDCS over PPc ($p < .05$).

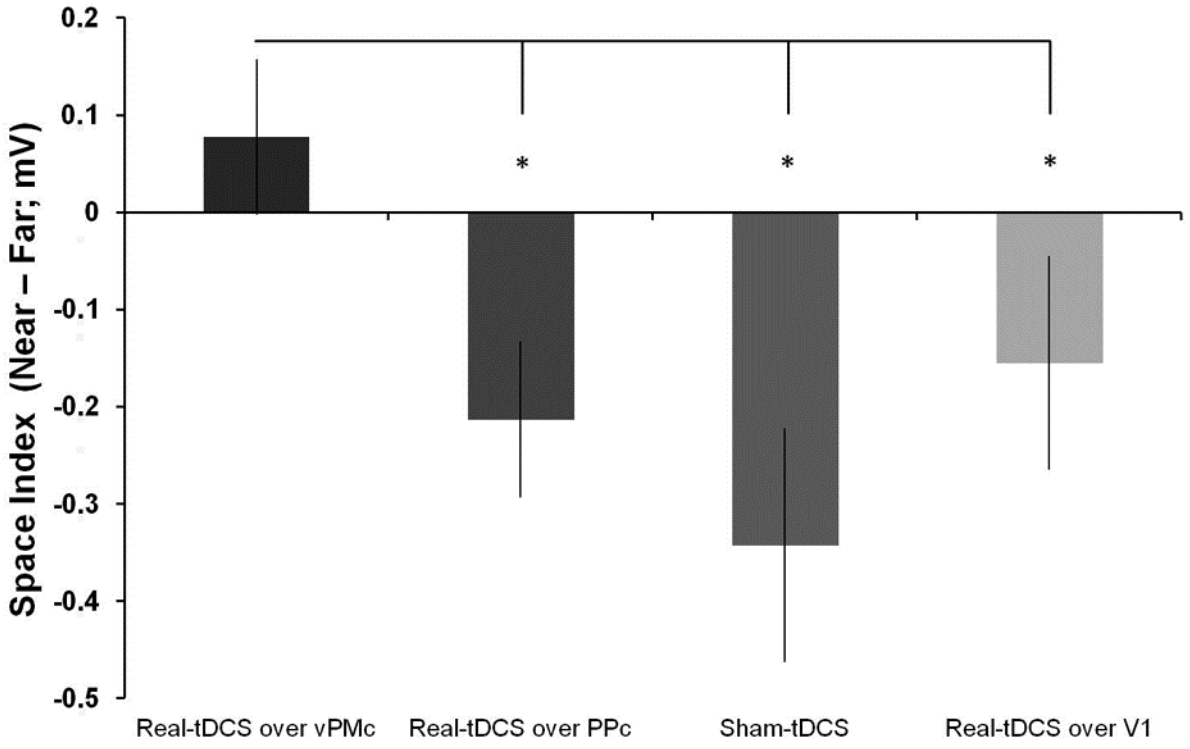

Figure 3.5. Indices of spatial modulation (SI) of MEPs (raw MEPs recorded 300 ms after near sounds– raw MEPs recorded 300 ms after far sounds) following the critical Real tDCS sessions over PMc and over PPc and the control sessions, Sham tDCS over PMc and Real tDCs over V1. Error bars denote SEM. Asterisks indicate significant comparisons. (Adapted from Avenanti et al., 2012c).

DISCUSSION

The brain has evolved an efficient sensorimotor mechanism, mapping sensory stimuli in the space immediately surrounding the body (i.e., in PPS) onto potential motor responses (Rizzolatti et al., 1997; Graziano & Cooke, 2006). In humans, the activation of PPS representation upon visual or auditory stimulation near the hand is associated with reduced corticospinal excitability than when stimuli are presented at a distance (Serino et al., 2009; Makin et al., 2009). This inhibitory, freezing-like, response resembles that found during the presentation of noxious stimuli (Farina et al., 2001; Urban et al., 2004) or unexpected events and potential threats, including loud acoustic stimuli (Furubayashi et al., 2000), unexpected visual flashes (Cantello et al., 2000) or motion (Schütz-Bosbach et al., 2009) or visual stimuli depicting pain in others (Avenanti et al., 2009; Minio-

Paluello et al., 2006), suggesting that motor mapping of sensory events occurring near the body primes defensive reactions (Graziano & Cooke, 2006). Using a perturb-and-measure approach (Avenanti et al., 2007, 2012a, 2012b), in the present study, we investigated the neural bases of this spatially-dependent modulation of motor excitability, by testing whether it relies on the fronto-parietal regions underlying multisensory representation of PPS, namely PMc (in particular its ventral sector) and PPc (Serino et al., 2011; Bremmer et al., 2001; Makin et al., 2007; Gentile et al., 2011; Brozzoli et al., 2011). We measured the excitability of the hand representation in M1 when a sound was presented either near or far from the hand, after inhibiting the target cortical sites of PMc and PPc, and V1 as a control site. In line with previous findings (Serino et al., 2009; see also Makin et al., 2009), when no neural perturbation was applied (Sham-tDCS), the hand representation in M1 was modulated as a function of sound location: MEPs recorded from the FDI muscle at 300 ms after the onset of a sound were lower if the sound was presented near the subjects' hand rather than at a distance. Analogous results were obtained when Real-tDCS was applied to the control site, V1. Importantly, the differential effect of near and far sounds on MEPs was abolished after inhibitory tDCS over PMc, showing that this area plays a critical role in the motor coding of sensory events occurring within PPS. In contrast, inhibitory tDCS over PPc did not disrupt the spatially-dependent modulation of motor excitability, since in this case, MEPs recorded at 300 ms were lower after a near than after a far sound, similarly to what occurred in the control sessions (Sham-tDCS; Real-tDCS over V1). These findings highlight the role of PPS network in modulating the human motor system when sensory stimuli are presented near or far from the body. A previous study targeting the very same brain areas showed that virtual lesions to PMc and PPc (not to V1) disrupt audio-tactile interactions within PPS (Serino et al., 2011), suggesting that in humans these two regions are similarly involved in a multisensory representation of PPS. The present data critically expand this notion by demonstrating that the two nodes of the fronto-parietal network representing PPS have partially dissociable functions, being PMc, rather than PPc, mainly involved in mapping sensory representations of space onto the motor system.

Our findings are consistent with the notion that premotor neurons are critically involved in sensory-to-motor transformations (Rizzolatti et al., 1997; Rizzolatti et al., 2002; Avenanti et al., 2007; Avenanti & Urgesi, 2011) supporting motor and cognitive functions. However, they may appear only partially in line with neurophysiological data in monkeys. In non-human primates, prolonged intra-cortical stimulation of both F4 (in the ventral sector of the PMc) and VIP (in PPc) areas results in overt motor behaviours, resembling defensive responses to threatening stimuli approaching the body in ecological conditions (Graziano et al., 2002; Cooke et al., 2003; Graziano & Cooke, 2006; Stepniewska & Kaas, 2005). This would suggest that monkey premotor and parietal areas are similarly involved in implementing defensive behaviour, whereas the results from the present study suggest that in humans, only PMc - and not PPc - is critically involved in processing motor reactions to sensory events occurring in the PPS.

It might be possible that the motor properties of the PPS network differ between the two species, despite the strong correspondence between the sensory properties of the posterior-parietal and premotor areas in the monkey and in the human brain (Bremmer et al., 2001). However, several pieces of evidence suggest that also in monkey, the posterior node of the fronto-parietal PPS network might be more involved in sensory processing, whereas the anterior node might be more involved in motor output (Graziano & Cooke, 2006; Fogassi & Luppino, 2005). Firstly, F4 sends direct projections to the spinal cord (He et al., 1993; 1995; Geyer et al., 2000; Rizzolatti & Luppino, 2001; Dum & Strick, 2002; 2005) as well as to M1, whereas VIP is strongly connected to PMc (Matelli & Luppino, 2001; Cavada & Goldman-Rakic, 1989), but the existence of direct connection from VIP to M1 is not well established (Petrides & Pandya, 1984; Luppino et al., 1999; Rozzi et al., 2006). Second, multimodal neurons in F4 are also active during movements of the body part where their sensory receptive fields are anchored (Rizzolatti et al., 1981), whereas evidence of motor activity associated with VIP neurons is limited to the intracortical microstimulation studies cited above (Cooke et al., 2003; Fogassi & Luppino, 2005). Third, even in the case of intracortical stimulation, evoking a motor response is much easier for F4 as compared to VIP areas: the current

threshold for evoking a response is lower in F4 than in VIP; moreover in F4, but not in VIP, a response can be evoked also in an anaesthetized animal; finally, responses are evoked on every trial after stimulation of F4, whereas the response generated by VIP stimulation quickly decays over repeated trials. Taken together these data suggest that in monkeys, just as in humans (Koch et al., 2010), PMc projections to the motor system are more robust and direct than PPc projections. These features fit with the results of the present study showing the necessity of PMc in mediating sensory to motor representations of PPS. It is possible that information about sounds in space is processed both in PMc and in PPc cortex, through direct connections from acoustic areas. In addition, acoustic input might also modulate PMc activity through an indirect projection from PPc neurons. However, only PMc can directly modulate the motor system, via the primary motor cortex (Matelli & Luppino, 2001; Rizzolatti & Luppino, 2001) and/or via direct projections to the spinal cord (He et al., 1993; 1995; Geyer et al., 2000; Rizzolatti & Luppino, 2001; Dum & Strick, 2002; 2005). Thus, when PMc cortex is inactivated, information related to the position of sounds in space cannot modulate the motor system, while when PPc is blocked, direct projections from the auditory cortex reach PMc, which in turn can affect the motor system.

An alternative hypothesis might be that stimulation of PPc through tDCS was less effective in abolishing the spatially-depended modulation of MEP, because task-relevant neurons lay in the depth of the intraparietal sulcus and tDCS was unable to target such neurons. While we cannot completely rule out this possibility, it should be noted that other brain stimulation studies using tDCS (Bolognini et al., 2010a; 2010b) or TMS (Serino et al., 2011) successfully modulated multisensory integrative processing in PPc, suggesting, on the one hand, that non-invasive stimulation techniques can affect intraparietal neurons and, on the other hand, supporting the view of a greater involvement of PPc in (multi)sensory, relative to motor, processes. Neural responses to near body stimuli in monkey area F4 and VIP are mainly excitatory (Colby & Duhamel, 1996; Rizzolatti et al., 2002; Graziano & Cooke, 2006), whereas, in the present study, inhibitory motor responses were detected. This is not surprisingly as activation of premotor or parietal regions may

result not only in increased, but also in reduced motor output (Tokuno & Nambu, 2000; Baldissera et al., 2001; Davare et al., 2009; Avenanti et al., 2009). The present data do not exclude that other facilitatory responses may occurs for stimuli near the body. It may be possible that other sectors of the motor system (e.g. controlling proximal muscles or the contralateral limb) may show increased excitability for stimuli near the hand and such facilitatory responses may occur simultaneously with the freezing-like response of hand muscles, similarly to what happens during processing of real or potential noxious stimuli (Urban et al., 2004; Avenanti et al., 2009). Future studies are needed to directly test these possibilities.

It is worth noting that in our previous TMS study (Serino et al., 2009), beside the inhibitory effect associated to near sounds at 300 ms, we had also found an earlier facilitatory response, detected at 50 ms after presenting near sounds (Serino et al., 2009). The failure to replicate that excitatory effect in the present study is likely to depend on the different TMS intensity used in the two studies (see Methods section). It is known that different TMS intensities may recruit neural populations with different activation thresholds (Chen et al., 2008). Therefore, it is possible that the relatively lower TMS intensity used in the present study could have disclosed the activity of inhibitory, more than excitatory neural units, which are both present in the motor cortex (Chen et al., 2008; Serino et al., 2009). While both these populations of neurons might be involved in the motor coding of sensory stimuli in the PPS, it is possible that early excitatory effects due to near stimuli could be detected only with higher TMS intensities (as in Serino et al., 2009), whereas inhibitory effects can be recorded also with intensities used in the present experiment or even lower (e.g. at 110% of rMT; see Cantello et al., 2000; Furubayashi et al., 2000; Makin et al., 2009).

There is an additional possible limitation in the present study that it is fair to highlight when commenting our conclusions. Although we centered our stimulation over the ventral premotor cortex and intraparietal sulcus, sites shown be active or critical for PPS representation by previous fMRI (Bremmer et al., 2001; Makin et al., 2007) and TMS studies (Serino et al., 2011), it is possible that additional sectors of PMc or PPc were influenced by tDCS due the relatively poor

spatial resolution of this technique (Nitsche et al., 2008; Datta et al., 2009; Priori et al., 2009). Brain stimulation techniques can also modulate activity in remote interconnected regions (Stagg et al., 2009; Keeser et al., 2011; Avenanti et al., 2012b). Thus, it is possible that regions interconnected to the premotor cortex were influenced by tDCS and may have contributed to the observed effects. At any rate, our study shows a clear dissociation between the anterior (PMc) and posterior (PPc) nodes of the PPS networks in mapping sensory representations of space onto the motor system.

In conclusion, the results from the present study confirm that, if the PPS network is intact, stimuli presented near the hand inhibits the motor representation of the hand in M1, as compared to stimuli presented at a distance. This spatially-dependent modulation of the motor system depends on the activity of the PMc; inducing a "virtual lesion" to this area abolished this inhibitory effect, thus highlighting the critical role of PMc in the motor coding of PPS. It is tempting to propose a model in which the PPc and the PMc constitute two critical nodes of a parieto-frontal network underlying a sensorimotor representation of space along a postero-anterior functional gradient: the parietal node might be more involved in multisensory processing of space, whereas the premotor node is necessary to trig or inhibit, potential, appropriate, motor responses to stimuli near the body, by projecting to the motor cortex and/or through direct connections to spinal cord motoneurons. The present study offers initial support to this model, as it provides evidence for a simple dissociation in the PMc-PPc network, with the PMc, but not the PPc, being critical for implementing freezing-like responses in the motor system. A stronger support for the model would come from concurrent evidence of the opposite dissociation, that is a manly sensory dysfunction following selective lesion to the PPc. Preliminary data from our laboratory show that structural lesions to PPc, and not to PMc, affect awareness of multisensory stimuli presented within PPS in right brain damaged patients suffering crossmodal extinction (Serino, Tomaiuolo, Quinquinio & Làdavas, Neural correlates of Peripersonal Space representation in humans: evidence from patients with crossmodal extinction, under revision). Providing strong evidence for such a double dissociation would definitely clarify

the relationship between sensory-motor functions of PPS and their neural correlates in PMc-PPc areas.

CHAPTER 4

Effects of induced short-term plasticity in the Action Observation Network: the role of the IFC and STS in anticipatory simulation of observed actions[3]

ABSTRACT

Observation of snapshots depicting ongoing motor acts increases corticospinal motor excitability. Such motor facilitation indexes the anticipatory simulation of observed (implied) actions and likely reflects computations occurring in the parietofrontal nodes of a cortical network subserving action perception (action observation network, AON). However, direct evidence for the active role of AON in simulating the future of seen actions is lacking. Using a perturb-and-measure transcranial magnetic stimulation (TMS) approach, we show that off-line TMS disruption of regions within (inferior frontal cortex, IFC) and upstream (superior temporal sulcus, STS) the parietofrontal AON transiently abolishes and enhances the motor facilitation to observed implied actions, respectively. Our findings highlight the critical role of IFC in anticipatory motor simulation. More importantly, they show that disruption of STS calls into play compensatory motor simulation activity, fundamental for counteracting the noisy visual processing induced by TMS. Thus, short-term plastic changes in the AON allow motor simulation to deal with any gap or ambiguity of ever-changing perceptual worlds. These findings support the active, compensatory, and predictive role of frontoparietal nodes of the AON in the perception and anticipatory simulation of implied actions.

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INTRODUCTION

Perceiving and understanding what other people do are crucial for effective social functioning. Mounting evidence suggests that this ability may be underpinned by frontal, parietal, and temporal areas that respond when seeing human actions (hereafter referred to as action observation network, AON) (Gazzola & Keysers 2009; Grafton 2009; Caspers et al. 2010; Van Overwalle & Baetens 2009). The inferior frontal (ventral premotor cortex and inferior frontal gyrus, hereafter referred to as ‘‘inferior frontal cortex,’’ IFC) and parietal cortices are important nodes of the AON (Chong et al. 2008; Etzel et al. 2008; Kilner et al. 2009; Oosterhof et al. 2010) coupling action perception and execution. Monkey studies indicate that a proportion of neurons in these frontoparietal regions increase their firing rate during both action perception and execution (so called ‘‘mirror neurons’’) (di Pellegrino et al. 1992; Gallese et al. 1996; Fogassi et al. 2005) and may implement a mechanism that matches perceived actions with one’s own motor representation of similar actions (Rizzolatti & Craighero 2004). Strong evidence for a motor simulation of seen actions in humans comes from single-pulse transcranial magnetic stimulation (spTMS) studies showing that seeing others’ actions increases the excitability of the corticospinal motor circuits involved in performing the same actions (Fadiga et al. 2005; Aglioti et al. 2008; Sartori et al. 2011). Relevant to the present study is that virtual lesions of IFC disrupt action observation-related motor facilitation (Avenanti et al. 2007) hinting at the crucial role of this structure in mediating action simulation in the motor cortex (M1). Theoretical models of action perception have emphasized the predictive nature of the frontoparietal AON activity (Wilson & Knoblich 2005; Kilner et al. 2007; Schütz-Bosbach & Prinz 2007; Gazzola & Keysers 2009; Friston et al. 2011; Press et al. 2011; Schippers & Keysers 2011) and have suggested that action perception relies on forward internal models that predict the future course of others’ motor acts. In keeping, neurophysiological studies have reported that M1 shows an anticipatory bias in the motor response to observed actions (Gangitano et al. 2004; Kilner et al. 2004; Borroni et al. 2005; Aglioti et al. 2008; Avenanti, Minio-Paluello, Sforza, et al. 2009). Using

motor-evoked potentials (MEPs) induced by spTMS, it has been demonstrated that M1 is activated during perception of static pictures of ongoing but incomplete human actions (implied actions, Urgesi et al. 2006; Candidi et al. 2010). Crucially, motor facilitation was greater for images depicting hand actions in their initial--middle phases than final phases (Urgesi et al. 2006, 2010). Thus, motor reactivity to implied actions likely reflects the anticipatory simulation of future phases of the observed implied action (Wilson & Knoblich 2005; Urgesi et al. 2010). While studies suggest that activation of M1 during action observation stems from activity within the frontoparietal AON (Avenanti et al. 2007; Koch et al. 2010; Catmur et al. 2011), direct evidence for the involvement of IFC in simulating the future of seen actions is lacking.

Moreover, no studies have addressed the issue of whether the anticipatory motor coding of the observed action 1) is linked to an active crucial role of frontoparietal AON (hypothesis A) (Wilson & Knoblich 2005; Kilner et al. 2007; Aglioti & Pazzaglia 2011; Friston et al. 2011) or 2) merely and passively reflects computations carried out in connected visual nodes of the AON (e.g., in the superior temporal sulcus, STS) as a consequence of learned Pavlovian-like visuomotor associations (Hickok 2009) (hypothesis B). During action observation visual information is thought to reach the frontoparietal AON via the STS (Rizzolatti &Luppino 2001; Nishitani & Hari 2002; Nishitani et al. 2004; Nelissen et al. 2011), a high-order visual area containing neurons that encode real or apparent biological motion stimuli (Keysers & Perrett 2004) and respond also to static images of body postures implying an action (Peigneux et al. 2000; Jellema &Perrett 2003). While neurons in STS may show anticipatory response to observed actions (Perrett et al. 2009) they do not respond to action execution and thus lack ‘‘classical’’ mirror properties. One way of directly addressing the issue of the functional relation between the frontoparietal and the visual nodes of the AON in mediating action prediction is to test the motor facilitation to implied action after perturbation of neural processing either within (IFC) or upstream (STS) the frontoparietal AON. While both hypothesis A and B may predict that anticipatory action simulation in M1 can be disrupted by perturbation to IFC, they make opposite predictions regarding the effect of perturbation to STS. If

the AON is organized as a ‘‘passive’’ feed-forward system, where the frontoparietal AON nodes passively reflect computations carried out in STS due to sensory--motor pairing (hypothesis B), then suppression of STS should reduce the flow of information reaching the frontoparietal AON and thus decrease simulation activity in the network (and consequently in M1). The alternative view (hypothesis A) predicts an ‘‘active’’ compensatory increase of action simulation after STS suppression. According to this hypothesis, the AON is organized as a dynamic control system where information initially flows from visual (STS) to visuomotor (frontoparietal) nodes and then back to visual regions (Schippers & Keysers 2011). In this vein, motor simulation activity occurring in frontoparietal regions is automatically called into play to solve fundamental computational challenges posed by action perception like completing missing information or making the best sense of ambiguous information (Wilson & Knoblich 2005; Schütz-Bosbach & Prinz 2007; Aglioti & Pazzaglia 2011; Avenanti & Urgesi 2011). An increment of noise in perceptual representation of actions would require the increase of filling-in function based on internal models of action (Kilner et al. 2007; Gazzola & Keysers 2009; D’Ausilio et al. 2011; Friston et al. 2011 Schippers & Keysers 2011). Thus, the disruption of visual processing in STS should trigger an increase of activity in the frontoparietal AON.

This effect would be reflected in an increased M1 facilitation. A direct test of these hypotheses would require to investigate how manipulation of neural activity in a given area (IFC or STS) influences responses in another (M1). Studies in the nonhuman primate have used such ‘‘perturb-and-measure’’ approach by showing that using a cooling procedure to inactivate temporarily a higher order visual area (middle temporal, MT) disrupted single-cell activity in the primary visual cortex (V1) and thus proved that the former area has a causal influence on the latter (Hupé et al. 1998). While the invasive nature of the direct interference approach limits its application to animal models, TMS allows to explore directly but noninvasively how transient inhibition of a target brain region (obtained by administration of repetitive TMS, rTMS) modifies neural responses in M1 (measured using spTMS) (Avenanti et al. 2007, 2012). Thus, thanks to this approach, it is possible

to test directly in humans the causative connectivity between different nodes of a given neural network (Paus 2005). Here, we used a perturb-and-measure TMS paradigm, which offers the unique possibility to 1) suppress neural activity in IFC or STS using low-frequency rTMS (to perturb and create ‘‘transient virtual lesions’’) and 2) assess the consequent functional modulation of corticospinal motor reactivity to observed actions via spTMS of M1 (Avenanti et al. 2007). Anticipatory action simulation processes in M1 were assessed by recording MEPs from the right hand during the observation of static pictures depicting a fine grasping performed with the index finger and the thumb (implied action stimuli). As a control, we presented images of a still hand and 2 nonbody static (icefall) and implied motion (waterfall) control visual stimuli. Based on electromyography (EMG) recording performed during action execution (Urgesi et al. 2010), we expected that in normal physiological conditions watching a fine grasping would increase the cortical excitability of the first dorsal inter- osseous (FDI, controlling index finger movements) but not of the abductor digiti minimi (ADM) muscle that is not involved in fine grasping. To test the role of IFC and STS in anticipatory action simulation, functional modulation of M1 contingent upon the perception of still and implied motion stimuli was assessed in 3 different sessions that were collected either within (In-win) or outside (Out-win, baseline) the transient inhibitory window created by low-frequency rTMS over the left IFC or left STS.

MATERIALS AND METHODS

Participants

Thirty-three participants took part to the study. Seventeen participants (8 females) aged between 22-29 years (mean: 25 st.dev 2.2) were tested in the TMS experiment. Sixteen participants were right handed and one participant was left handed according to a standard handedness inventory (Oldfield 1971). A group of additional sixteen right handed participants (8 females) aged between 20-33 years (mean: 24.8 st.dev 4.0) were tested in the psychophysics study. Participants received University course credit for their participation and gave their written informed consent. None of them had neurological, psychiatric, or other medical problems, or had any contraindication to TMS (Rossi et al. 2009). The protocol was approved by the local ethics committee at University of Bologna and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Visual stimuli

Stimuli were color pictures taken with a digital camera and modified by means of the Adobe Photoshop software (Adobe Systems, San Jose, CA). Images subtended a 18.53° x 12.19° region and showed: (i) a static hand laying on a table (still hand); (ii) a right hand in the middle of a fine grasping movement involving the index finger and the thumb (implied motion hand); (iii) a frozen waterfall (still object); (iv) a flowing waterfalls (implied motion object). To minimize habituation to the images and loss of attention, two different exemplars of body and non-body stimuli were presented for each condition. Body stimuli represented the right-hand of a male and a female actor during a pincer grip movement. To rule out that the mere observation of graspable objects would activate per se the motor system (Chao and Martin 2000; Nelissen et al. 2005), none of the action

snapshots contained any object. For each body or non-body category, corresponding still and motion stimuli were roughly matched for colour, luminance, and viewing perspective. Stimuli were adapted from a previous study (Urgesi et al. 2006, experiment 3; see figure 4.1).

Figure 4.1. Visual stimuli (On the top from left to right : Still hand 1, Still hand 2, Hand-implied motion 1, Hand-implied motion 2, Icefall 1, Icefall 2, Waterfall (implied motion) 1, Waterfall (implied motion) 2).

Study design

The experiment included three spTMS sessions in which MEPs were recorded during the observation of the different snapshots (Figure 4.2): 1) a baseline session outside the inhibitory influence of rTMS (Out-win); 2) a session immediately following inhibitory rTMS over the IFC ('In-win IFC'); and 3) a session immediately following inhibitory rTMS over the STS ('In-win STS'). The three sessions were separated by 90 minutes (to minimize carry-over effect of rTMS across sessions) and their order was counterbalanced across subjects. After the TMS sessions (at least 60 minutes from the last rTMS) participants provided subjective judgments about the stimuli.

Still hand and implied action stimuli depicted a right hand. Action simulation effects detected with TMS are largely contralateral with respect to the observed effectors (Aziz-Zadeh et al. 2002), thus we hypothesized that stimulation of left M1 (with spTMS) and left IFC (with rTMS, in the In-win IFC session) would have been optimal to explore motor reactivity to right hand actions.

Moreover, to avoid unwanted effects of hemispheric differences, in the In-win STS session, we stimulated the left STS. The choice of left STS was also based on a recent meta-analysis on 37 fMRI experiments that explored neural activity during observation of a right hand action (Caspers et al. 2010). It was shown that while seeing right hand actions activates a largely bilateral occipitotemporal network, the STS region was specifically active in the left not in the right hemisphere.

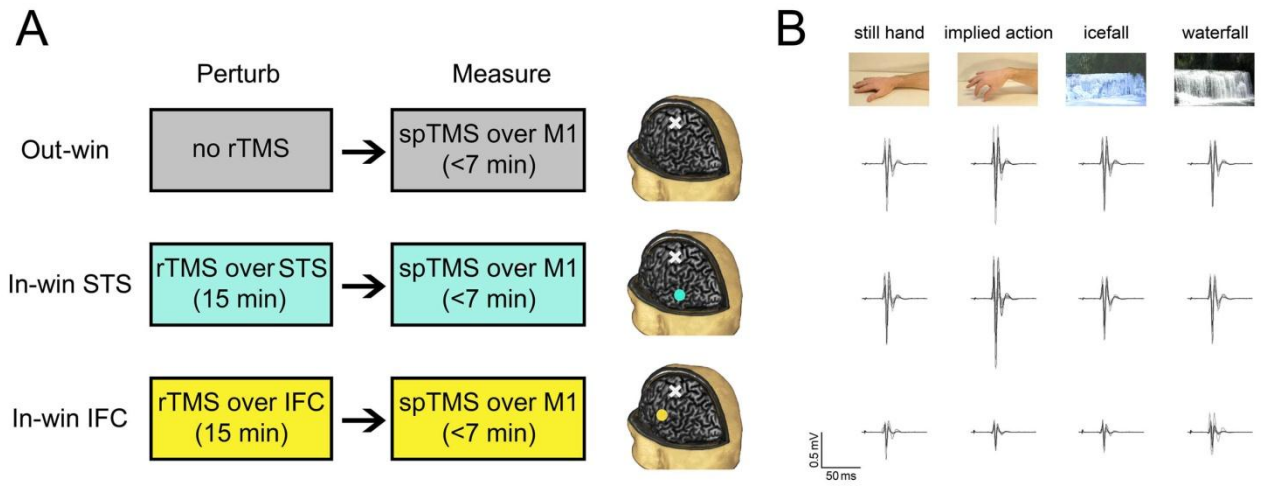

Figure 4.2. (A) Schematic representation of experimental design and TMS perturb-and-measure protocol. MEPs were recorded by means of spTMS during the observation of the visual stimuli. MEP recording was performed in 3 spTMS sessions, 1 outside (Out-win session, first row) and 2 within (In-win sessions, middle and lower rows) the influence of rTMS. In the In-win sessions, virtual lesions were applied using 1 Hz rTMS over the IFC or the STS. Talairach coordinates corresponding to the projection of the IFC or STS sites on brain surface were estimated through a neuronavigation system (IFC mean surface coordinates ± SEM: x = 58.6 ± 0.5, y = 9.4 ± 0.5, z = 23.6 ± 0.4; STS: x= 62.9 ± 0.5, y = 52.5 ± 0.1, z = 9.4 ± 0.6; white blobs in the head model). In all sessions, spTMS was performed by stimulating the hand representation in M1 (FDI OSP: x = 38.2 ± 2.9, y = 19.5 ± 1.8, z = 56.9 ± 2.0; white crosses in the head model). (B) MEPs recorded from the FDI muscle of a representative subject during the observation of the 4 categories of stimuli. Top, middle, and low rows represent Out-win, In-win STS, and In-win IFC sessions, respectively. (Adapted from Avenanti et al., 2012b).

Electromyography and spTMS recordings

During visual stimuli presentation, MEPs induced by spTMS were recorded simultaneouslyfrom the right FDI and ADM muscles by means of a Biopac MP-150 (Biopac Corp, Goletta, CA.)electromyograph. EMG signals were band-pass filtered (20 Hz-1.0 kHz, sampled at 5 kHz),digitized and stored on a computer for off-line analysis. Pairs of silver/silver chloride surface electrodes were placed in a belly/tendon montage. Two grounds electrodes were placed on the ventral surface of the right wrist. TMS was performed with a figure-of-8 coil connected to a Magstim Rapid2 stimulator (Magstim, Whitland, Dyfed, U.K.) placed over subjects' left M1. The coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45˚ angle away from the midline. In this way, the current induced in the underlying neural tissue was

directed approximately perpendicular to the line of the central sulcus and was optimal for trans-synaptic activation of the corticospinal pathways (Brasil-Neto et al. 1992). By using a slightly suprathreshold stimulus intensity, the coil was moved over the left hemisphere to determine the optimal scalp position (OSP) from which MEPs of maximal amplitude were recorded from FDI. The OSP was then marked on a bathing cap worn by subjects to ensure correct coil placement throughout the experiment. During the experimental spTMS sessions, the intensity of magnetic pulses was set at 120% of the individual resting motor threshold (rMT), defined as the minimal intensity of the stimulator output that produces MEPs with amplitudes of at least 50 μV with 50% probability in the muscle with the higher threshold (Rossini et al. 1994). This way a stable signal could be obtained in both muscles. Mean values (% of maximum stimulator output ± standard deviations) of rMT were 58.5 ± 9.2 %. The absence of muscle contractions was continuously verified on-line by visually monitoring the EMG signal. Each spTMS session (Out-win, In-win IFC, In-win STS) included 16 trials for each condition (64 trials in total per session) presented in a randomized order. In each session, a central cross (1,000 ms) indicated the beginning of a trial. On each trial, a magnetic pulse was randomly delivered between 800 and 100 ms before the end of the visual stimulus (lasting 1,500 ms) to avoid any priming effects that could affect MEP size. A blank screen was shown for 3,500 ms in the intertribal intervals. Each spTMS session lasted 6.4 min each. The two In-win spTMS sessions started 1 min after the cessation of the rTMS, and thus, in the In-win sessions, all MEPs were recorded within 7.4 min after the end of rTMS. The 1 min pause between rTMS and spTMS allowed changing the stimulating coil and setting the TMS pulse intensity. The experiment was programmed using a C++ software to control sequence and duration of images and to trigger TMS and EMG recording.

rTMS and Neuronavigation

The two In-win sessions were preceded by 15 min of 1Hz rTMS (900 stimuli in total) over the target area (either left IFC or left STS). This low-frequency rTMS protocol is known to reduce the excitability and disrupt the functions related to the target area for at least 50% of the time of stimulation (Walsh & Pascual-Leone 2003; O'Shea et al. 2007; Serino et al. 2011; Avenanti et al. 2012a). Since the entire In-win sessions were performed within 7.4 min after the end of rTMS, all MEPs in such sessions were recorded well within the temporal window of reduced excitability created by 1Hz rTMS. A subthreshold stimulation intensity was used (90% of rMT) and subjects were asked to keep their muscles as relaxed as possible during the rTMS as contraction may reduce the inhibitory effect of rTMS on motor excitability (Touge et al. 2001).

Coil position was identified on each participant's scalp with the SofTaxic Navigator system (EMS, Italy) as in our previous TMS research (Avenanti et al. 2007; Urgesi et al. 2007; Bertini et al. 2010; Serino et al. 2011). Skull landmarks (nasion, inion, and two preauricular points) and about 60 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra Optical Tracking System (NDI, Canada). Coordinates in Talairach space were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template. The IFC was targeted in the anterior-ventral aspect of the precentral gyrus (ventral premotor cortex) at the border with the pars opercularis of the inferior frontal gyrus (coordinates: x = -52, y = 10, z = 24), corresponding to Brodmann's area 6/44 (Avenanti et al. 2007; Gazzola et al. 2007; Mayka et al. 2006; Van Overwalle et al. 2009; Caspers et al. 2010; Urgesi et al. 2007). The STS was targeted in its posterior aspect (x = -52, y = -53, z = 9, corresponding to Brodmann's area 21; Van Overwalle et al. 2009; Caspers et al. 2010). Scalp positions were identified by means of the SofTaxic Navigator system and marked on the bathing cap with a pen. Moreover, the neuronavigation system was used to estimate the projections of the TMS sites (IFC, STS, M1) on the brain surface (Figure 4.2). No adverse effects during (subthreshold) 1Hz rTMS were reported or noticed in any subjects.

Psychophysical testing

At least 1 h after the last TMS session (thus outside the influence of rTMS), all the experimental stimuli were presented in a randomized order and participants were asked to rate the strength of the implied motion sensation induced by each image. The 1-h interval was adopted to be sure that rTMS effects had faded away and could not influence subjective ratings. Subjects rated the stimuli by marking a vertical, 10 cm visual analogue scale (VAS) with 0 cm indicating "no effect" and 10 cm "maximal effect imaginable". Stimuli were presented for 1.5 sec. each on the same monitor as in the TMS experiment. To further assess implied motion in the absence of any rTMS, an additional group of sixteen healthy subjects not participating to the TMS experiment was asked to rate along a VAS the strength of the implied motion sensation induced by the visual stimuli.

Data Analysis

Neurophysiological data were processed off-line. Trials with EMG activity exceeding 50 μV in a window of 100 ms prior to the TMS pulse were discarded from the analysis (< 4%). One subject was removed from the analysis due to a high number of pre-contractions artefacts (~40%); thus all the analyses were carried out on a sample of 16 subjects. The removal of the left handed subject from this sample did not change the pattern of results (not shown in the paper). Mean MEP amplitude values in each condition were measured peak-to-peak (in mV). Outliers (± 2.0 SD of individual mean) were identified for each muscle and in each condition and removed (< 2%). Raw MEPs values were analyzed by means of a four-way repeated measures ANOVA with Session (Out-win, In-win STS, In-win IFC), Muscle (FDI, ADM), Object (Hand, Fall) and Motion (Still, Implied-motion) as within-subjects factors. To quantify the amount of 'resonant' facilitation in the Out-win and In-win sessions, an action observation facilitation index was computed [(implied action – static hand)/(static hand)] for each session and muscle, separately. To assess how rTMS perturbation affected corticospinal responses to implied actions, a Session x Muscle ANOVA on the

action facilitation index was performed. VAS measures were submitted to Object x Motion ANOVAs. In all ANOVAs, post-hoc analysis was carried out using Duncan test correction for multiple comparisons. A correlational analysis was performed between action facilitation indices and VAS judgments (implied action – static hand) in the three different sessions using the Pearson's r coefficient.

RESULTS

Suppression of IFC, but not of STS activity, reduces corticospinal excitability

In three spTMS sessions (Out-win, In-win STS, In-win IFC), participants were asked to observe still hand, implied action (fine grasping), icefall and waterfall visual stimuli and MEPs were simultaneously recorded from the right FDI and the ADM muscle (see Figure 4.2 A). The Session x Muscle x Object x Motion ANOVA on MEP amplitudes revealed a main effect of Muscle ($F_{1,15}$ = 6.92, p = 0.02; higher amplitudes in the FDI than in the ADM, mean ± s.e.m.: 0.93 mV ± 0.16 vs. 0.60 mV ± 0.12). Importantly, a significant main effect of Session ($F_{2,30}$ = 5.84, p = 0.007) was also found. This effect was accounted for by the lower MEP amplitude recorded in the In-win IFC (0.59 mV ± 0.09) than in the Out-win (0.83 mV ± 0.15; p = 0.02) and the In-win STS sessions (0.89 mV ± 0.16; p = 0.008), which in turn did not differ from one another (p = 0.5; see Table 4.1). Thus, overall, rTMS over IFC induced a reduction of M1 excitability. This inhibitory effect was equally present in the FDI and the ADM since the interaction Session x Muscle was not significant (p = 0.9). These findings confirm that suppression of IFC reduces the excitability of hand representation in M1 (Avenanti et al. 2007) and suggest that at rest, the IFC may exert a facilitatory influence on M1 (Shimazu et al. 2004).

Table 4.1. Effect of rTMS on corticospinal excitability (across visual conditions)

	Out-win	In-win STS	In-win IFC
FDI	1.00 ± 0.20	1.07 ± 0.21	0.73 ± 0.10
ADM	0.65 ± 0.14	0.71 ± 0.16	0.44 ± 0.11

Note: MEP amplitude (in millivolts) ± SEM recorded from the 2 muscles in the 3 different sessions. In both muscles, MEPs recorded in the In-win IFC sessions were lower than MEPs recorded in the other 2 sessions indicating that suppression of IFC brought about a reduction of hand corticospinal excitability. (Adapted from Avenanti et al., 2012b).

Effect of rTMS on motor reactivity to visual input

The ANOVA also showed higher-order interactions, including the quadruple Session x Muscle x Object x Motion interaction ($F_{2,30} = 6.00$, $p = 0.006$). To further analyze this interaction two follow-up Session x Object x Motion ANOVAs were carried out separately for the two muscles. The ANOVA performed on MEPs recorded from the ADM muscle (control) revealed only a main effect of Session ($F_{2,30} = 3.42$, $p = 0.05$; Table 4.1) but no other main effects or interactions (all $p_s > 0.2$), indicating a lack of modulation due to the different observational conditions. In contrast, the ANOVA on MEPs recorded from the FDI muscle (target) showed the main effect of Session ($F_{2,30} = 3.39$, $p = 0.05$; Table 4.1) and Motion ($F_{1,15} = 8.47$, $p = 0.01$). Crucially, the triple interaction Session x Object x Motion was significant ($F_{2,30} = 9.04$, $p = 0.0008$; Figure 4.2 B). Post-hoc analysis showed that in the Out-win (Baseline) session (Figure 4.3 A) MEPs recorded from the FDI muscle were higher during observation of implied action than when watching static hand ($p = 0.02$), icefall ($p = 0.05$) and waterfall ($p = 0.02$) stimuli, which in turn did not differ from one another (all $p_s > 0.6$). Similar but stronger modulations were found in the In-win STS session (Figure 4.3 B): MEPs from the FDI were higher during observation of implied actions than during observation of static hand ($p < 0.0001$), icefall ($p = 0.0002$) and waterfall stimuli ($p = 0.0001$), which in turn did not differ from one another (all $p_s > 0.4$). Notably, pairwise comparisons between the Out-win and

the In-win STS sessions revealed that MEPs during implied actions were greater after suppression of STS than in the baseline session (all $p_s < 0.004$); MEPs in the two sessions were comparable for the other three control conditions (all $p_s > 0.3$). In the In-win IFC sessions (Figure 4.3 C) MEPs from the FDI were in general lower than in the other two sessions (for all pairwise comparisons, $p < 0.002$) and, importantly, they were not modulated by the different observational conditions (all $p_s > 0.2$). In sum, as expected, the observation of implied body actions in the absence of any rTMS interference with the activity of IFC or STS (Out-win baseline session), selectively facilitated the corticospinal representation of the muscle (FDI) that would be recruited during performance of the observed motor act, but not of a hand muscle (ADM) that was not involved in the observed motor act (Urgesi et al. 2010). Importantly, suppression of STS induced a motor facilitation greater than in the baseline session which strikingly contrasts with the lack of motor facilitation induced by suppression of IFC. No modulation was found during the observation of static or implied-motion non-body stimuli either in the Out-win or in the In-win sessions.

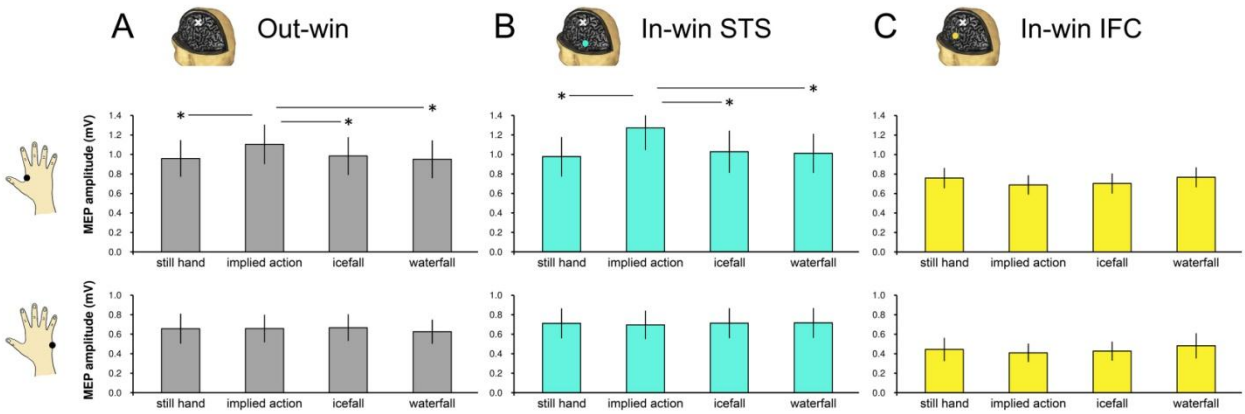

Figure 4.3. MEPs recorded from the FDI (top) and the ADM (bottom) muscle in the 3 different spTMS sessions. (A) Out-win, (B) In-win STS, and (C) In-win IFC. Asterisks indicate significant post hoc comparisons. Only within sessions, comparisons are represented, see main text for further pairwise comparisons between sessions. Error bars denote SEM. (Adapted from Avenanti et al., 2012b)

Effect of rTMS on anticipatory action simulation

The main analysis indicates that STS disruption increases the motor facilitation to implied actions. To quantify the amount of changes in motor facilitation due to IFC and STS perturbation, a further analysis was conducted on facilitation ratios [(implied action – still hand) / still hand] computed in the three sessions. Facilitation ratios were calculated for the FDI (target) and, to test muscle specificity, for the ADM muscle (control). These indices were entered into a repeated measure Muscle x Session ANOVA (Figure 4.4). The analysis showed a main effect of Session ($F_{2,30}$ = 10.43, p = 0.0004), a main effect of Muscle ($F_{1,15}$ = 9.09, p = 0.009) and, importantly, a significant Muscle x Session interaction ($F_{2,30}$ = 6.20, p = 0.006). The facilitation of the FDI muscle (Figure 2.4A) in the Out-win session (mean facilitation ratio ± s.e.m.: 17% ± 5) was greater than in the In-win IFC session (-8% ± 5; p = 0.02). Crucially, in the In-win STS session the facilitation (38% ± 6) was greater than in the Out-win (p = 0.02) and In-win IFC (p < 0.0001) sessions. Thus, disruption of IFC neural activity reduced motor facilitation more than 1 S.D. as compared its baseline level (large effect size, d = 1.27), while STS activity increased motor facilitation more than 1 S.D. than its baseline level (large effect size, d = 0.90). No modulation was found in the facilitation index computed on the ADM muscle (p > 0.3; Figure 4.4B).

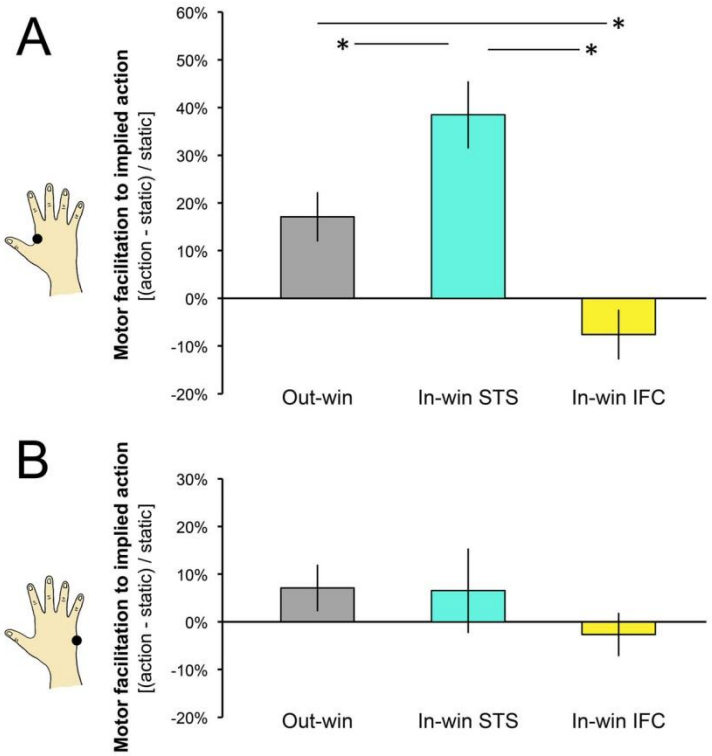

Figure 4.4. Motor facilitation to implied action stimuli recorded from the (A) FDI and (B) ADM muscle in the 3 different sessions. Asterisks indicate significant post hoc comparisons. Error bars denote SEM. (Adapted from Avenanti et al., 2012b).

Subjective data

At least 1 h after the last TMS session (thus outside the influence of rTMS), participants used VAS to rate the strength of the movement sensation induced by the visual stimuli. The Object x Motion ANOVA on VAS ratings of implied motion sensation showed a significant main effect of Motion ($F_{1,15}$ = 132.00, p < 0.0001) indicating that implied-motion stimuli (mean VAS rating ± s.e.m.: 6.93 cm ± 0.37) were rated as more 'dynamic' than still stimuli (1.47 cm ± 0.25); this effect was present for both the hand and fall stimuli as evinced by the non significant Object x Motion interaction (p = 0.9). The main effect of Object was not significant (p = 0.09; Table 4.2). These findings were replicated in a further psychophysical experiment conducted on an additional group of 16 subjects who did not participate in the TMS experiment (Main effect of Motion: $F_{1,15}$ = 263.59, p < 0.0001; no main effect or interaction with factor Object: p > 0.3; Table 4.2). Moreover, a further mixed-

model Group x Object x Motion ANOVA (including the group of subjects tested after TMS and the one tested only in the psychophysical experiment) revealed only a main effect of Motion ($F_{1,30}$ = 349.81, $p < 0.0001$) but no main effect or interaction with factor Group ($p > 0.3$). This rules out that subjective ratings in the TMS experiment were the results of the long exposure to the visual stimuli or of brain stimulation.

Table 4.2: Subjective report of implied motion

	Still hand (body static)	Implied action (body implied motion)	Icefalls (nonbody static)	Waterfalls (nonbody implied motion)
TMS experiment	0.94 ± 0.29	6.45 ± 0.47	2.00 ± 0.53	7.41 ± 0.60
Psychophysical experiment	1.44 ± 0.36	6.34 ± 0.52	1.55 ± 0.54	7.37 ± 0.45

Note: Mean VAS ratings (in centimeters) ± SEM. The top row reports data collected in TMS experiment (1h after the end of the last TMS session). The bottom row reports data collected in the psychophysical experiment. (Adapted from Avenanti et al., 2012b).

In the TMS experiment we also investigated the relation between motor response to observed pictures of implied actions and the strength of the movement sensation induced by such images. Correlations between action simulation indices (facilitation ratios computed separately for each session and muscle) and VAS ratings of implied motion were not significant ($-0.04 < r < 0.39$, $p > 0.1$). However after the removal of one outlier (with standard residuals > 2 sigma) we found a significant positive relation between action simulation index (FDI facilitation ratios) and subjective ratings. In the Out-win session, stronger FDI facilitation was found for those subjects who attributed more implied motion to hand stimuli ($r = 0.72$, $p = 0.003$; Figure 4.5A). A similar relation was found in the In-win STS session ($r = 0.56$, $p = 0.03$; Figure 4.5B) but not in the In-win IFC session ($r = 0.22$, $p = 0.4$; Figure 4.5C). No significant correlations were found between ADM modulations and subjective ratings of implied motion ($-0.11 < r < 0.28$, $p > 0.3$).

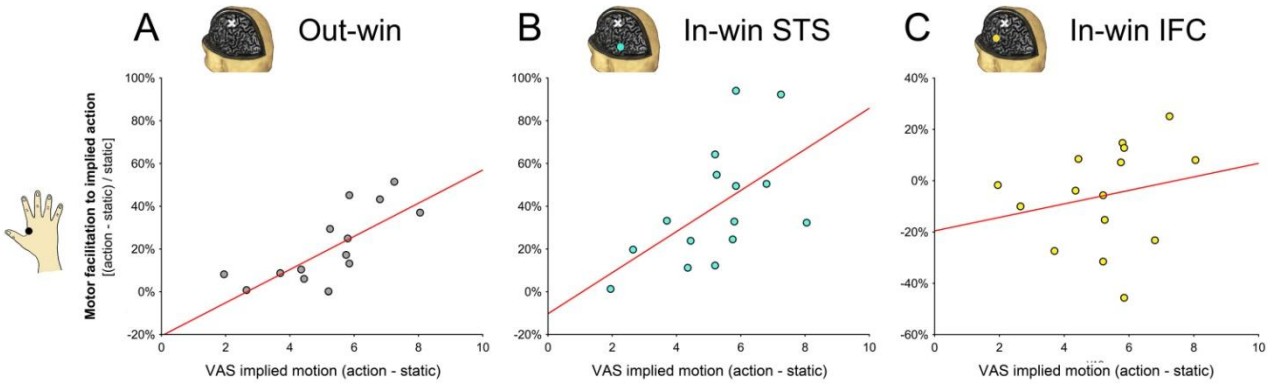

Figure 4.5. Relation between FDI motor facilitation to implied action and subjective perception of implied motion. Facilitation index computed in (A) Out-win, (B) In-win STS, and (C) In-win IFC sessions. (Adapted from Avenanti et al., 2012b).

DISCUSSION

Frontal and parietal cortices are activated during both action observation and execution. Unlike what happens during action execution, observing actions activates neurons in the temporal region, STS, thought to be crucial for biological motion perception and for providing the frontoparietal AON with high-order visual representations of the observed actions (Rizzolatti & Craighero 2004; Keysers & Perrett 2004; Nelissen et al. 2011). While previous 'virtual' or real lesion studies have shown that both IFC (Probic & Hamilton 2006; Urgesi et al. 2007; Avenanti et al. 2007; Pazzaglia et al. 2008; Moro et al. 2008; Tidoni et al. 2012) and STS (Grossman et al. 2005; Saygin 2007; Candidi et al., 2011) are essential in observed action representation, the specific role of the frontal and temporal areas in the process of implied action simulation remains unclear. We explored this issue by using a perturb-and-measure paradigm based on the combination of rTMS and spTMS. Low-frequency rTMS was applied to transiently suppress cortical activity either within (IFC) or upstream (STS) the fronto-parietal AON. SpTMS was used to assess the reactivity of the

corticospinal system during observation of implied action stimuli either within (In-win sessions) or outside (Out-win) the influence of the 'virtual lesions' induced by rTMS. We found that the motor facilitation contingent upon observation of implied stimuli was disrupted by the suppression of IFC, demonstrating that the anticipatory simulation in M1 is critically linked to the activity of the anterior node of the AON. Importantly, our paradigm allowed testing two alternative hypotheses about the functional architecture of the AON. In striking contrast to a 'passive' feedforward architecture model (hypothesis B in the introduction), we found that the disruption of STS region resulted in an enhanced motor simulation which clearly hints at an active role of the frontoparietal AON in action simulation (hypothesis A in the introduction). Thus, we provide direct causative evidence of a functional interplay between IFC/STS and M1 during extrapolation of dynamic action-related information from static images. These findings provide neurophysiological support to the predictive theories of action perception (Wilson & Knoblich 2005; Schubotz 2007; Schütz-Bosbach & Prinz 2007; Kilner et al. 2007; Gazzola & Keysers 2009; Press et al. 2011; Friston et al. 2011; Schippers & Keysers 2011) according to which the AON is organized as a dynamic control system where information can flow not only from visual (STS) to visuo-motor (fronto-parietal) nodes but also in the opposite direction, i.e. from IFC to STS. In this vein, watching an action activates stored motor representations (in fronto-parietal nodes) that provide an internal forward model of the ongoing action. These representations are likely used for predicting the future course of the observed action and for achieving a degree of perceptual stability sufficient to deal with any perceptual ambiguity derived from discontinuities in the sensory input. These theories predict that a gap of visual information would require increased activity in the motor system in order to guarantee stable action perception (Wilson & Knoblich 2005; Avenanti & Urgesi 2011; Aglioti & Pazzaglia 2011; Friston et al. 2011; Schippers & Keysers 2011).

Perception of implied actions triggers the simulation of their future

Influential theoretical models suggest that the human motor system is designed to work as an 'anticipation device' and that humans predict forthcoming actions by using their own motor system as an internal forward model (Wolpert 2003; Schütz-Bosbach & Prinz 2007; Gazzola & Keysers 2009). In keeping, human and monkey evidence suggests activations of the motor system contingent upon action observation may: i) occur prior to the observation of a predictable motor act (Umiltà et al. 2001; Fogassi et al. 2005; Kilner et al. 2004; Aglioti et al. 2008; Avenanti et al. 2009); and ii) show an anticipatory bias in the simulation of the upcoming phases of observed actions (Gangitano et al. 2004; Borroni et al. 2005). Anticipatory simulation is particularly evident during processing of implied actions where muscle-specific motor facilitation is maximal for static images depicting initial and middle phases of a given action (that correspond to the initial muscular involvement during the actual execution of the action) and reduced for its final posture (that corresponds to the maximal muscular involvement during execution) (Urgesi et al. 2006; Urgesi et al. 2010). These findings indicate that motor facilitation is maximal during extrapolation of dynamic information about the upcoming action phases and suggest that M1 is preferentially activated by the anticipatory simulation of future action phases. In keeping, the Out-win session of the present study (outside the inhibitory effect of rTMS), shows that watching static pictures of an ongoing fine grasping increased the amplitude of MEPs recorded from the FDI muscle which is recruited during execution of the very same action (Fadiga et al. 2005; Urgesi et al. 2010). Importantly, greater muscle-specific motor facilitation was found in participants who provided greater ratings of implied motion, suggesting a link between neurophysiological markers of action simulation and the subjective perception of implied motion. Tellingly, no motor modulation was found when observing static (icefall) or implied motion (waterfall) non-body stimuli, although a comparable modulation of implied motion ratings was found for non-body and hand stimuli. This suggests that the recruitment of the motor system during implied action perception does not reflect a

non-specific response to the presence of implied motion in the scene (i.e. in non-human entities) but the process of deriving dynamic information from static images that imply ongoing human body actions. Our perturb-and-measure paradigm highlights the IFC as a critical neural locus for this selective processing, as outlined in the next paragraph.

Suppression of IFC disrupts anticipatory action simulation

Monkeys' premotor cortices are known to modulate corticospinal activity through indirect cortico-cortical connections (Shimazu et al. 2004) as well as direct corticospinal connections (Dum and Strick 1991; Kraskov et al. 2009). In humans the functional contribution of the IFC on M1 activity is evident during action preparation and execution (Uozomi et al. 2004; Davare et al. 2009); moreover, studies suggest that during precision grasping the IFC sends muscle-specific signals to M1 in order to execute the grasp (Cattaneo et al. 2005; Davare et al. 2009). Similar cortico-cortical neural interactions are thought to be at play during covert motor simulation (Fadiga et al. 2005; Fourkas et al. 2008; Avenanti et al. 2009a; Catmur et al. 2010; Kock et al. 2010). It is also worth noting that action observation, execution, and imitation bring about a comparable, sequential activation of IFC and M1 (Nishitani and Hari 2002; Nishitani et al. 2004). Importantly, real (Saygin 2007; Pazzaglia et al. 2008; Moro et al. 2008; Fazio et al. 2009) or 'virtual' lesions (Pobric and Hamilton 2006; Urgesi et al. 2007; Tidoni et al. 2012) of the IFC have been shown to disrupt action recognition (Avenanti & Urgesi 2011) and imitation (Heiser et al. 2003), highlighting the critical role of the frontal node of the AON in the internal representation of observed actions. While providing evidence for a clear role of motor regions in visual action perception and imitation, the above studies do not clarify the specific functional influence of IFC on the motor mapping of implied actions.

Based on the notion that IFC and other motor regions are activated by implied action observation (Johnson-Frey et al. 2003; Nishitani & Hari 2002; Proverbio et al. 2009), in the present study we applied low-frequency rTMS to IFC and tested any modulation of corticospinal motor reactivity consequent to implied action stimuli. We found that motor facilitation occurring during observation of static images of hand conveying action information was abolished by rTMS over IFC. Moreover, after IFC-rTMS, motor response to implied actions was not correlated to the perceived sensation of motion implied in such stimuli. The lack of MEP modulation after suppression of IFC shows that the activity of the frontal node of the AON is crucial for encoding implied action stimuli in the observers' motor system. This result complements and extends previous studies showing that IFC is selectively involved in visual discrimination of biological dynamic (Pobric and Hamilton 2006; Saygin et al. 2007; Tidoni et al. 2012) and implied actions (Urgesi et al. 2007; Moro et al. 2008), and indicates that the anterior node of the AON plays a critical role in the basic visuo-motor encoding of action information extrapolated from static body postures. It is likely that other neural regions coupling action perception and execution (e.g. parietal regions) may participate to this predictive motor coding and further perturb-and-measure studies would directly test this hypothesis. It should be noted that suppression of IFC but not of STS also induced a general reduction of MEP amplitude from both the FDI and ADM muscles, in keeping with evidence that the former but not the latter region contains a hand motor representation functionally related to M1 (Rizzolatti & Luppino 2001; Uozumi et al. 2004; Davare et al. 2009). These findings support the notion that inhibiting hand representations in premotor regions reduces hand corticospinal excitability (Gerschlager et al. 2001; O'Shea et al. 2007) and further establish the facilitatory functional connectivity between IFC and M1 (Shimazu et al. 2004; Avenanti et al. 2007). The disruption of action simulation observed after IFC-rTMS, however, is unlikely to be due to the indirect inhibitory effect of IFC-rTMS on M1 activity. Indeed, we have previously shown that although both IFCrTMS and M1-rTMS induce a reduction of corticospinal excitability, suppression of IFC but not of M1 disrupts the action observation motor facilitation (Avenanti et al. 2007). Moreover, stimulation of

IFC, but not of M1, may influence action perception (Cattaneo et al. 2011; Avenanti and Urgesi 2011). Taken together these findings provide direct causative evidence for the notion that action simulation mechanisms in M1 passively reflect computations carried out in the AON, and in particular in its frontal node (Fadiga et al. 2005; Avenanti et al. 2007; Schütz-Bosbach et al. 2009).

Suppression of STS enhances anticipatory action simulation

A major point of novelty of the present study concerns the functional interplay between fronto-temporal brain regions involved in action perception and motor simulation in M1. Middle/superior temporal cortices are typically activated during the visual experience of real, illusory, or implied motion of animate as well as inanimate entities (Tootell et al. 1995; Senior et al. 2000; Kourtzi & Kanwisher 2000). In particular, the activity of STS has been selectively associated to the processing of biological motion (Keysers & Perrett 2004; Grossman et al. 2000; Peelen et al. 2006) and of implied body movements (Jellema & Perrett 2003, Peigneaux et al. 2000). Studies suggest that STS integrates body form and motion information from ventral and dorsal pathways (Giese & Poggio 2003; Vaina et al. 2001) to create a high-order visual representation of others' actions. This representation is visual in nature as neurons in STS do not respond to action execution (Rizzolatti & Craighero 2004; Keysers & Perrett 2004). Importantly neurons in STS seem to be able to compute action anticipation based on visual information alone (Perrett et al. 2009).

A plausible scenario is that during action observation, visually derived movement-related information is sent from STS to parietal and IFC regions where visuo-motor coupling takes place. The output of such computational process is then sent to M1 (Nishitani & Hari 2002; Nishitani et al. 2004) and can feed back in perceptual systems (Wilson & Knoblich 2005; Schippers & Keysers 2011). While it is held that the fronto-parietal AON receives action-related visual information processed in STS, no previous studies have directly explored action simulation in M1

(reflecting the anticipatory activity of fronto-parietal AON) after the inhibition of STS. Our findings speak against the hypothesis that the AON is organized as a pure feed-forward system where fronto-parietal regions passively reflect computations occurring in STS (hypothesis B; Hickok 2009) and rather support the notion that the AON is a dynamic control system (hypothesis A) where the fronto-parietal nodes actively compute anticipatory action simulations de novo. We found that disruption of STS leads to an increase of corticospinal reactivity to implied actions, in keeping with the notions that involvement of motor system is greater when perceptual information is noisy (d'Ausilio et al. 2011) and internal models of action may contribute to filling-in missing or ambiguous perceptual information (Kilner et al. 2007; Gazzola & Keysers, 2009; Friston et al. 2011; Schippers & Keysers, 2011). This result suggests that given the rTMS induces noise in STS, the frontal node of AON compensates for any gap of implied action-related visual information by enhancing its anticipatory simulative properties. Such an active, compensatory function indicates that visual perception of actions may be sustained by the simulative computations likely occurring in the frontal node of the AON (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Aglioti & Pazzaglia, 2011; Avenanti & Urgesi, 2011). In keeping, while neuromagnetic studies have reported that during action observation there is a sequential cortical activation from STS to parietal and frontal regions (Nishitani & Hari, 2002; Nishitani et al. 2004), a recent fMRI study suggests that information within the AON may also flow from IFC to parietal and STS regions (Schippers & Keysers, 2011). Such action-related information flow may be particularly relevant for compensating the noisy STS processing induced by rTMS and reflect the predictive information flow from premotor to STS regions hypothesized by forward models. Before accepting this interpretation, a critical methodological issue needs to be discussed. Suprathreshold TMS over STS can activate the temporal fascia muscle and may induce discomfort, at least in some subjects (Cattaneo et al. 2010). It may thus be that unspecific factor (e.g. increased vigilance due to STS stimulation) may explain the increase motor response to action stimuli in the In-win STS session. We find this alternative hypothesis unlikely. First, off-line rTMS is thought to minimize unspecific effects due to scalp

sensations (Walsh & Pascual-Leone, 2003) and in our study MEPs were collected after 1 min from the end of rTMS. Second, no discomfort or aversive effects of stimulation were reported or noticed in any subjects during rTMS, likely due to our subthreshold simulation intensity. Critically, also IFC stimulation may activate (facial) muscles and in principle result in increased vigilance. However, in the In-win IFC session, we found a disruption, not an enhancement, in the MEP facilitation to implied action. Moreover, in a previous perturb-and-measure TMS study we found that 1Hz rTMS over IFC (using even higher stimulation intensity) disrupted MEP facilitation to biomechanically possible actions (i.e. actions that could be performed by the observers, like those used in the present study) but did not affect the MEP facilitation to actions representing extreme stretching movements (biomechanically impossible actions) (Avenanti et al. 2007) whose facilitation relied on the somatosensory cortex. These findings speak against the possibility that potentially discomforting scalp sensations due to rTMS result in an increase in motor reactivity and suggest that the enhancement of action simulation observed in the present experiment was specifically due to disruption of neural processing in STS.

The future of seen action in the AON

While we focused on two key nodes of the AON, other regions of the network may contribute to anticipatory action simulation. Low-frequency rTMS can modulate activity in remote interconnected regions (Paus, 2005; Gerschlager et al. 2001; O'Shea et al. 2007; Avenanti et al., 2012). Thus, it is possible that rTMS over STS or IFC modulated activity in other visual (for example area MT) or visuo-motor (e.g. intraparietal) interconnected regions and that these regions contributed to the observed effects. At any rate, our data demonstrate a clear dissociation in action simulation when virtual lesions are applied to the STS or IFC sites that are typically active during action observation (as indicated by brain imaging meta-analyses, Van Overwalle, Baetens, 2009;

Caspers et al., 2010). Interestingly, a recent TMS study has suggested that also a more anterior sector of STS may be critically involved in action perception (Cattaneo et al., 2010). Future perturb-and-measure studies are needed to test whether disruption of other sectors of STS (or IFC) may induce changes in action simulation similar to those observed in the present experiment. Our study supports the notion that the functional role of motor activation during action perception is based on predictive coding. This process may allow to understand the goal of an action and ultimately to perform an anticipatory read-out of the intention behind the action (Rizzolatti & Craighero, 2004; Fogassi et al. 2005; Friston et al. 2011; Press et al. 2011) as well as to anticipate the future phases of upcoming actions of others (Wilson & Knoblich, 2005; Schütz- Bosbach & Prinz, 2007; Aglioti & Pazzaglia, 2011; Avenanti & Urgesi, 2011).

Predictive theories of action perception propose that the observer's motor system generates anticipatory representations of others' actions by projecting the course of ongoing movements into the future. These predictions are then fed back into perceptual systems (e.g. in STS) that create topdown expectations and constrain visual perception. According to this view, action simulation mechanisms are called into play to solve the computational challenges posed by action perception, that is, to fill-in missing or ambiguous visual information and to provide an anticipatory representation of ongoing actions ahead of their realization (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Aglioti & Pazzaglia, 2011; Avenanti & Urgesi, 2011; Friston et al. 2011; Schippers & Keysers, 2011). By showing enhanced action simulation after suppression of visual processing in STS our study provides neurophysiological evidence for a role of frontoparietal AON in implementing compensatory action simulation mechanisms that may be fundamental for perceiving and predicting others' actions. Our study shows that dynamic, action-related information is extracted from static images and mapped onto the motor system to provide forward anticipatory representations of ongoing actions. Moreover, the study highlights the active, compensatory and predictive nature of the simulation triggered by perception of implied actions.

CHAPTER 5

Investigating the role of the IFC in predicting others' actions: tDCS studies[4]

ABSTRACT

Influential theoretical models suggest that the human motor system is designed to act as an anticipation device and that humans predict others' forthcoming actions by using their own motor system as an internal forward model. However to date evidence for a causative role of the motor system in predicting the future of observed actions is lacking. Here we used transcranial direct current stimulation (tDCS) to test the role of inferior frontal cortex (IFC) in predicting the end-state of an observed action. In an Action-Prediction (AP) task, participants observed the initial phase of a right-hand reaching-grasping action. The final phase of the action was masked and subjects had to guess which of two objects were going to be grasped by the hand. In a difficulty-matched control task, the Non-biological Prediction (NP) task, subjects observed similarly interrupted movements of a geometrical form approaching one of two targets. Participants performed both tasks in two separate sessions that were carried out after 15 minutes of inhibitory (cathodal) active- or sham-tDCS over the left-IFC (experiment 1) or the right-IFC (experiment 2). To test stimulation specificity, also excitatory (anodal) active- or sham- tDCS was applied over the left-IFC (experiment 3). Relative to sham stimulation, suppression of left-IFC but not of right-IFC brought about a selective reduction of accuracy in the AP-task. Importantly, anodal stimulation of left-IFC did not affect the accuracy of subjects in the two tasks, compared to sham stimulation. These

[4] *In preparation*: Annella, L., Di Tante, F., Mancini D., Tidoni, E., Avenanti, A. Perturbing the activity of the left IFC impairs action prediction of others' right hand actions.

findings indicate that left-IFC is necessary for extracting the future end-state of human actions based on the observation of the initial phases of the movement and suggest a left frontal lateralization in the predictive coding of others' right-hand actions.

INTRODUCTION

To successfully interact with the environment we must be able to monitor our actions and their consequences but also to make the best sense of the actions of others and predict their future behavior. There is widespread evidence that we use the same neural systems for planning and executing our own actions, and for perceiving and understanding the actions of others. Previous research identified a fronto-temporo-parietal network, the so called "action observation network" (AON) which may support action perception and understanding (Gazzola & Keysers, 2009; Grafton, 2009; Van Overwalle & Baetens, 2009; Caspers et al., 2010). The inferior frontal cortex (IFC, including the ventral premotor cortex and the posterior part of the inferiror frontal gyrus) is a key node of the AON which is involved in coupling action perception with execution. In the monkey, a proportion of neurons in this regions is directly involved in such coupling (so called mirror neurons) and may be critical for making sense of the action of others (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti & Sinigaglia, 2010). It has been suggested that a key function of the AON is to predict others' actions (Prinz, 1997, 2006; Grush, 2004; Kilner et al., 2004; Wilson & Knoblich, 2005; Alaerts et al., 2011; Avenanti & Urgesi, 2011). According to this view, humans use their motor system as an internal forward model to internally simulate and predict the future of the actions of others. In support of this account, a consistent number of studies in human and non-human primates have shown that activations of the motor system contingent upon observation of others' actions may: i) occur prior to the observation of a predictable motor act (Umiltà et al., 2001; Fogassi et al., 2005; Kilner et al., 2004; Aglioti et al., 2008; Avenanti et al., 2009); and ii) show an anticipatory bias in the simulation of the upcoming phases of observed

actions (Gangitano et al., 2004; Borroni et al., 2005). Evidence also indicates that the AON, and in particular its frontal node (the IFC), may be critical for perceiving and understanding the action of others (Rizzolatti & Sinigaglia, 2010; Aglioti & Pazzaglia, 2011; Avenanti & Urgesi, 2011). For example, when seeing an actor lifting a box, online interference with IFC activity selectively worsens the ability: i) to judge whether the actor is trying to deceive the observers concerning the real weight of the lifted box (Tidoni et al., 2012); ii) to explicitly quantify the weight of the box (Pobric and Hamilton, 2006). Moreover, IFC interference impairs the visual discrimination of pictures depicting different actions (Urgesi et al., 2007). Notably, off-line suppression of IFC also disrupts the neurophysiological markers of the anticipatory simulation of future phases of seen actions (Avenanti et al., 2012b; chapter 4 of the thesis), suggesting a pivotal role of IFC in the predictive motor coding of others' motor acts. However, to date, direct causative evidence that stimulation of the IFC alters the ability to make predictions about the future course of seen actions is lacking. Moreover, one fundamental yet unsolved issue is whether the IFC may be involved in the prediction of event dynamics in general or its involvement is specific for the prediction of human body movements (Schubotz, 2007). Indeed, although studies have shown that the AON respond more to human actions than non-biological movements (Press, 2011), evidence indicates that the IFC is also recruited during prediction of events in general (Schubotz & Von Cramon, 2004; Schubotz, 2007; Schubotz et al., 2010). However, these studies provide only correlational evidence and cannot establish a direct causal link between brain and function (Avenanti and Urgesi, 2011). Thus, causative methods are needed to establish whether the IFC is critical for action prediction and for prediction of non-human movements. To address this issue, in the present research we used transcranial direct current stimulation (tDCS) to induce short-term plastic changes in the IFC during prediction of human actions and of non-biological movements. tDCS is an extraordinary method for non-invasive cortical stimulation that allows to induce polarity-specific excitability changes in the underlying stimulated area. Using cathodal or anodal currents, tDCS can induce cortical inhibition or excitation and alter neural functioning for several minutes after the end of the stimulation.

Relative to TMS, tDCS is much more endurable for the subjects because it does not cause any muscular contraction during the stimulation and has mild local effects on the skin underlying the electrodes. Moroever, tDCS is more reliable in keeping subjects unable to distinguish between sham and active stimulation (Nitsche et al., 2003; Paulus, 2003; Gandiga et al., 2006). In the current study we used tDCS to alter IFC neural functioning to test whether this region is specifically tuned to the anticipatory simulation of human actions or involved in event prediction in general. We designed two novel tasks requiring the prediction of biological (Action Prediction task) and non-biological movements (Non-biological movement Prediction task) and tested whether tDCS-induced modulation of IFC activity may influence behavioral performance in the two tasks. The AP task required to predict the end state of a right hand reaching-grasping action based on the observation of the initial phases of the movement (e.g. reaching trajectory, finger pre-shaping before grasping). A difficulty-matched control NP task required to predict the end state of an abstract geometrical form whose movements roughly mirrored those of the hand, in terms of trajectory and dynamic configuration changes. In three experiments, we applied active or sham tDCS over the IFC immediately before execution of the two prediction tasks. In Experiment 1 and 2 we used cathodal (inhibitory) tDCS. We predicted that if IFC is critical for action prediction only, then suppression of IFC excitability with cathodal tDCS should impair the accuracy in the AP, but not in the NP task. In Experiment 1 and 2 the left and right IFC were stimulated, respectively. Seeing others' actions typically recruits the AON bilaterally and previous TMS studies have suggested that stimulation of both left and right IFC may impair the visual discrimination of actions (Urgesi et al., 2007; Candidi et al., 2008).However, imaging studies suggest that action simulation activity is greater in the hemisphere contralateral relative to the observed effector (Aziz-Zadeh et al., 2002; Shmuelof and Zohary, 2005; Gazzola and Keysers, 2009; Cabinio et al., 2010; Caspers et al., 2010). Hence, to test whether prediction of right hand actions critically relies on the activity of the hemisphere contralateral with respect to the observed effector, in Experiment 1 cathodal tDCS was applied over the left IFC. As a control, in Experiment 2 cathodal tDCS was applied over the

right IFC (ipsilateral to the observed effector). Notably, tDCS allows also to enhance cortical excitability and previous studies have shown that anodal tDCS over unimodal or multisensory regions can improve the underlying perceptual or motor functions (Bolognini et al., 2009, 2010). While increases in excitability may boost simple unimodal or multisensory mechanisms, this type of stimulation may be less effective in modulating more complex cognitive functions or may even been detrimental (as it may alter an optimal level of excitability). Interestingly, models of IFC functioning have emphasized either simple visuo-motor integrative mechanisms (e.g. Rizzolatti & Craighero, 2004; Hickock, 2009) or more complex dynamic control processing (Wilson & Knoblich, 2005; Schippers & Keysers, 2011; Avenanti et al., 2012b). Hence, tDCS-enhancement of IFC excitability may offer some insights into the type of neural processing implemented this area and how this relates to the ability to predict others' actions. Thus, in Experiment 3 we tested whether increases in left IFC excitability due to anodal tDCS may be associated to any change in accuracy in the AP or NP tasks.

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MATERIALS AND METHODS

Subjects

A total of 127 healthy right-handed volunteers took part to the study. Thirty-seven participants were tested in one of three tDCS experiments and ninety participants were tested in one of three pilot studies. Subjects gave their written informed consent. The protocol was approved by the local ethics committee at University of Bologna and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. Subjects in the tDCS experiments received University course credit for their participation. 13 subjects were assigned to Experiment 1 (6 females, mean age 23.4 ± 3.8 years, range 19-32), 12 to Experiment 2 (6 females, mean age 24.2 ± 2.5 years, range 21-29) and 12 to Experiment 3 (6 females, mean age 23.6 ± 3.6 years, range 20-30). All participants were

right handed and had normal or corrected-to-normal vision. None had a history of neurological, psychiatric illness, or any contraindication to tDCS or was on medication at the time of the experiments. All subjects were naïve to the purposes of the study. Information about the experimental hypothesis was provided only after the experimental tests were completed. No discomfort or adverse effects during tDCS were reported or noticed.

Design

In three tDCS experiments we tested the role of left and right IFC in predicting the end-state of observed movements. Using cathodal tDCS, we transiently suppressed neural activity in the left IFC (Experiment 1) or right IFC (Experiment 2) to test their crucial role in two tasks involving prediction of human biological (AP task) or non-biological movements (NP task). To test stimulation specificity, in Experiment 3 we used anodal tDCS over left IFC. In each experiment, subjects were tested in two sessions that were carried out immediately after 15 min of active (cathodal or anodal) or sham tDCS over the target region. The order of the sessions was counterbalanced across subjects and the two sessions were separated by at least 4 days.

Tasks and stimuli

In the Action Prediction (AP) task, participants observed 120 video-clips (640 x 480 pixels, 30 fps) depicting the initial phase of a reaching-grasping action. All stimuli subtended a 22.3° x 33.4° visual angle from the participant's viewing position.Videos started showing a still right-hand (on the right side of the screen) with two objects placed in front of it on the left side of the screen. After a variable delay (1000-2200 ms) the hand started to reach and grasp one of the two objects. The final phase of the action was masked and subjects had to guess which object was going to be grasped by the hand. A random-dot mask (150 ms duration, obtained by scrambling the final frame of the movie with a custom-made image segmentation software) interrupted the video and was

followed by a response screen showing the two objects and lasting until response. Participants provided their answer using two computer keys.

Video-clips in the AP task included 8 different actors (3 females; mean age ± S.D.; 23.6 ± 1.06) reaching and grasping 8 different couples of objects (Figure 5.1). The two objects in each couple were located in two closed positions in space and presented different affordances, thus implying slightly different hand trajectories and grips (e.g. power vs precision grips). In different trials, only 30-80% of the entire movement was shown and in none of the videos the hand-object interaction was visible. Indeed, prediction in the AP task involved the processing of hand trajectory and finger pre-shaping during the reaching phase.

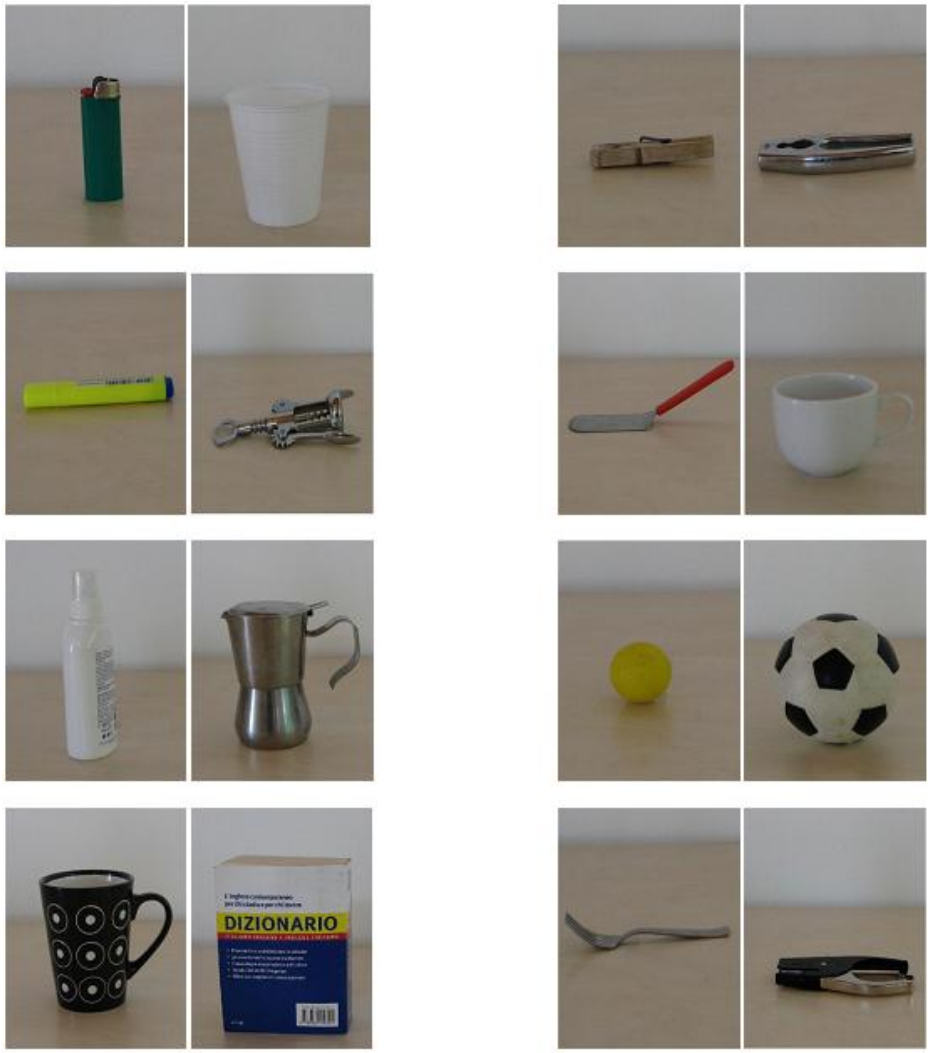

Figure 5.1. The eight couples of objects used for the video-clips of the AP task.

In a difficulty-matched Non-biological Prediction (NP) control task, subjects observed 120 similarly interrupted video-clips showing a non-biological geometrical forms approaching one of two targets and subjects had to guess which target was going to be hit by the stimulus. The NP videos (640 X 480 pixel, 30 fps) were animations created with Adobe Flash Professional software to match temporal and spatial features of AP stimuli. They showed incomplete movement (30-80% of the actual duration) of a geometrical form which moved from the right side of the screen in order to reach and fit with one among two different geometrical targets placed on the opposite side. The trajectory of the moving forms was roughly matched to that of the hands in the AP task. Moreover, the two targets presented different geometrical properties and, in analogy with the pre-shaping of the fingers (AP task), during the reaching phase the moving form changed configuration over time in order to fit to one of the two targets. Also for the NP video clips eight different couples of geometrical targets and eight objects (Figure 5.2) were used and random-dot image were used as masking.

Pilot studies

The final sets of 120 AP and 120 NP videos used in the two tasks was selected from an initial sample of ~1400 AP and ~1200 NP videos using a two steps procedure. Initially, we selected 180 stimuli for each task based on the performance of two groups of subjects. We presented the initial sample of AP stimuli to 30 subjects (15 female, mean age: 24.5 y ± 2.4) and the sample of NP stimuli to 30 other subjects (15 female, mean age: 24.2 y ± 2.6) and selected stimuli that were recognized with accuracy ~75% (range: 65-85%). This resulted in >300 stimuli per task and thus, a further selection was applied to reach 180 stimuli per task (90 and 90 stimuli for the upper and the lower object/target, respectively) in which the different actors/forms were similarly represented. To assure that the two tasks were matched for difficulty, 30 additional subjects (15 female, mean age: 23.9 y ± 2.9) were presented with 180 AP and 180 NP stimuli selected in the first step. Each video

was presented twice (720 trials in total). The final set of stimuli included 120 AP and 120 NP stimuli with accuracy ~75% (range: 65-85%). In both tasks, the hand/form reached both objects/targets with 50% probability. The percentage of the hand/form movement shown in the two tasks was matched (mean 45%, range 30-80%, p ~ 1.00). With this procedure we created two difficulty-matched tasks that were doable but not trivial.

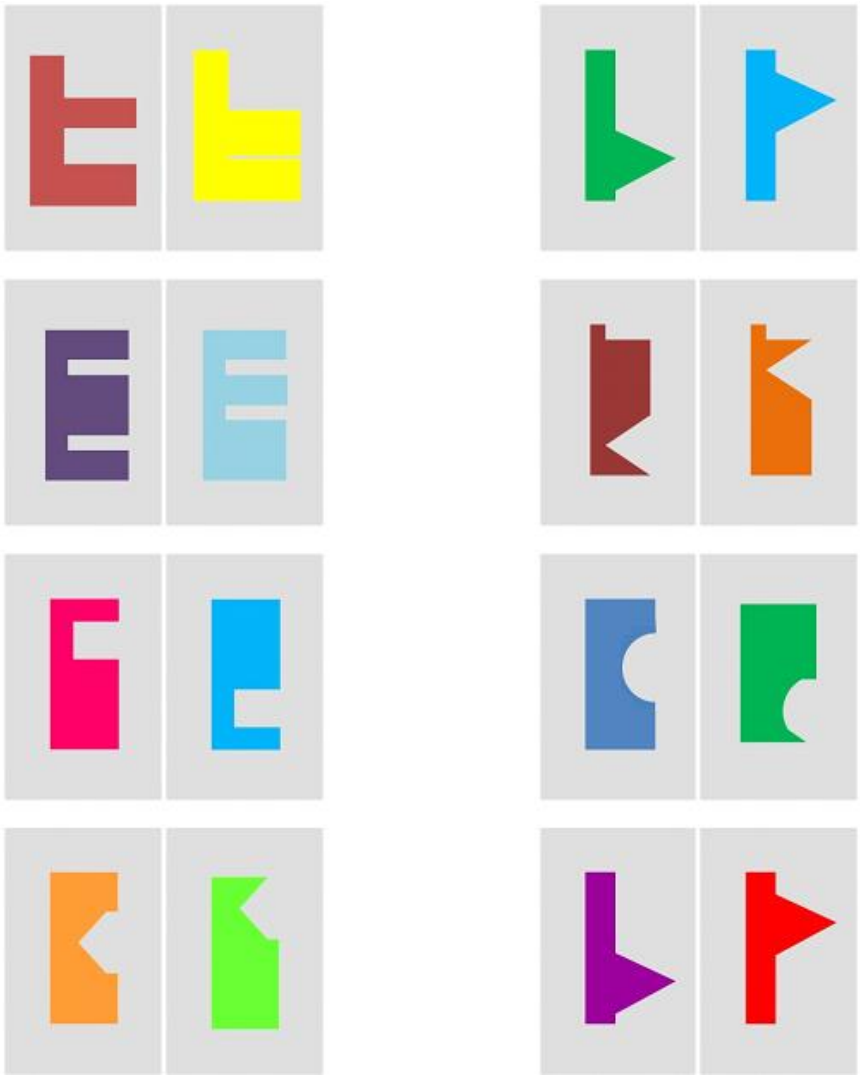

Figure 5.2. The eight couples of non-biological objects used for the video-clips of the NP task.

Transcranial direct current stimulation (tDCS) and Neuronavigation

tDCS was delivered using a battery-driven constant direct current stimulator (Eldith, Germany). A pair of surface sponge electrodes (35 cm^2) were soaked with a standard saline solution (NaCl 0.9%) and maintained in place by elastic rubber bands. In Experiment 1 the cathode electrode was applied over the Left IFC, in Experiment 2 the cathode electrode was applied over the Right IFC and in the Experiment 3 the anode electrode was applied over the Left IFC. In all experiments the reference electrode was placed over the contralateral deltoid muscle (Priori et al., 2008; Bolognini et al., 2010). Extra cephalic electrodes montages allow more focal stimulation and avoid the confounding effect from the reference electrode (Cogiamanian et al., 2007; see also Brunoni et al., 2011 for a review). Active tDCS was delivered with 2 mA intensity for 15 min. This protocol is known to affect cortical excitability for several minutes after the end of stimulation (Nitsche & Paulus, 2001). The current was constantly delivered for 15 min at 2 mA but it slowly increased to 2 mA from the onset of stimulation in a ramp-up like fashion over the first 40 sec, and then ramped down over the last 40 sec. For the sham stimulation the electrodes were placed on the same locations and the current was turned on for only 30 seconds at the beginning of the sham session and then was turned off in a ramp-shaped fashion (fade in/out: 20 sec), so that subjects experienced the sensations initially associated with the onset of stimulation (mild local tingling) without inducing any real effects. This procedure has been demonstrated to prevent subjects differentiating between real and sham stimulation (Nitsche et al., 2003; Paulus, 2003; Gandiga et al., 2006).

Electrodes position was identified on each participant's scalp with the SoftTaxic Navigator system (Electro Medical Systems, Bologna, Italy) as in previous research (Avenanti et al., 2007; Bertini et al., 2010; Serino et al., 2011; Avenanti et al., 2012a; Avenanti et al., 2012c). Skull landmarks (nasion, inion and two preauricular points) and ~100 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northern Digital Inc, Ontario,

Canada). Talairach coordinates were automatically estimated by the SofTaxic Navigator from an MRI-constructed stereotaxic template. For both hemispheres IFC was targeted in the anterior-ventral aspect of the precentral gyrus (ventral premotor cortex) at the border with the pars opercularis of the inferior frontal gyrus (coordinates: x = ± 54, y = + 10, z = + 24, corresponding to Brodmann's area 6/44). Individual's Talairach coordinates corresponding to the projection of IFC target site on the brain surface were automatically estimated through the neuronavigation system. Mean IFC ± SD surface coordinates were: x = -53.6 ± 1.47; y = 10 ± 0.59; z = 24 ± 0.45 in Experiment 1, x = 55.3 ± 1.68; y = 10 ± 0.58; z = 24.5 ± 0.78 in Experiment 2 and x = -54 ± 1.48; y = 10.1 ± 0.76; z = 24.2 ± 0.41 in Experiment 3.

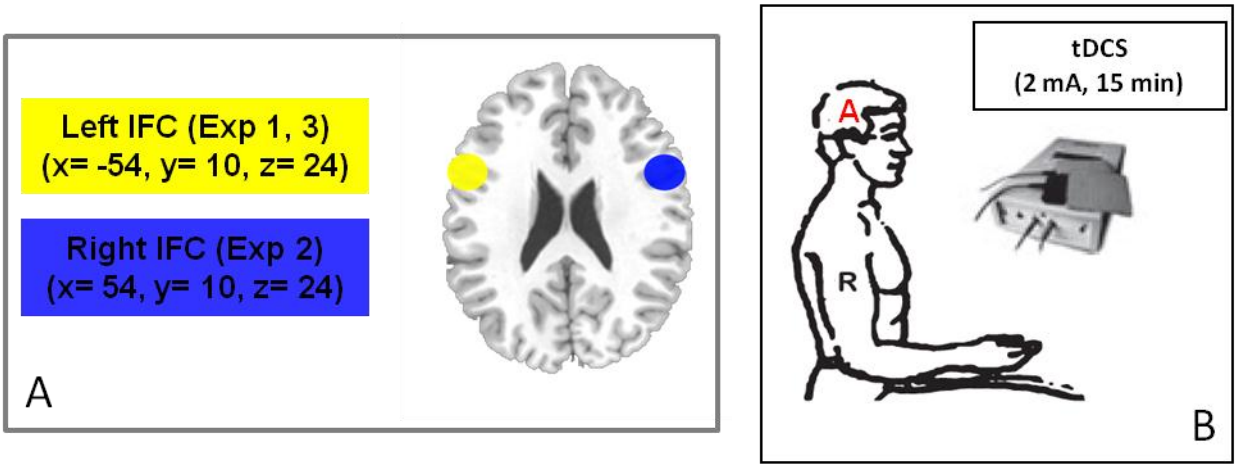

Figure 5. 3. The panel A illustrates the Talairach coordinates of IFC in both hemispheres. The panel B illustrates parameters of tDCS and a schematic representation of monopolar montage with the active electrode (A) positioned over the left or right IFC and the extracephalic reference electrode (R) over the contralateral deltoid muscle.

Procedure

Participants sat in front of a laptop (equipped with a 15.4-inches screen) located ~50 cm from their head in a dimly illuminated room. After neuronavigation and tDCS electrodes montage, participants received instruction and performed two training blocks (1 for each task, 30 trials each) in order to familiarize with the tasks. They were asked to respond as fast and accurately as possible by button press with the hand ipsilateral to the tDCS scalp site (left hand in Experiment 1 and 3, right hand in

Experiment 2). Trials used in the training were not included in the experimental blocks but had similar difficulty (~75% accuracy). If subject's accuracy was < 60% in one of the tasks, the corresponding instructions and training block were repeated. After the training participants received 15 min of active or sham-tDCS over the target sites (left or right IFC) and then performed four randomized blocks of 60 trials (2 blocks for each task). The order of the four blocks was randomized. One minute break was allowed between different blocks. Subjects completed the four blocks within 30 minutes after tDCS, thus well within the temporal window of cortical modulation induced by active tDCS (Nitsche et al., 2001). The sequences of video-clips were run by means of a software written in MATLAB 7 with a custom-made interface, which also allowed to record the accuracy trial by trial. To test whether sham or active tDCS induced different scalp sensations, after each session we asked participants to evaluate the discomfort caused by the stimulation using a 5-points Likert scale with 1 indicating "not unpleasant at all" and 5 "extremely unpleasant".

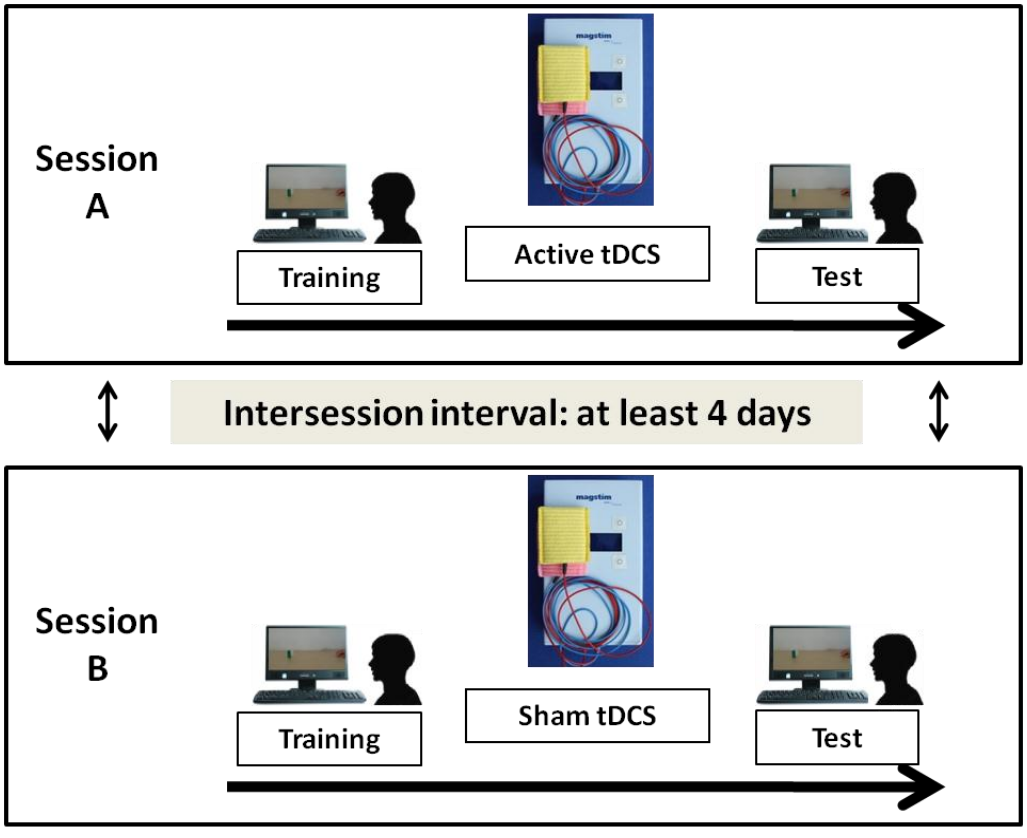

Figure 5.4. Schematic representation of the experimental procedure followed in the two separate tDCS sessions.

Data Analysis

For each participant, the accuracy rate in each task and session was calculated as the mean percentage of correct responses across the different blocks.. Mean accuracy were analyzed by means of a three-way mixed-model Analysis of Variance (ANOVA) with Task (two levels: NP, AP) and Stimulation (two levels: sham tDCS, active tDCS) as within-subjects factors and Experiment (three levels: Exp 1, Exp 2, Exp 3) as the between-subjects factor. To assess the amount of disruption of action prediction due to tDCS, for each experiment we computed the differences in AP task accuracy in sham-tDCS relative to active-tDCS session. A one-way ANOVA with the between-subjects factor Experiment was carried out on such tDCS-disruption index to directly compare the effect of cathodal tDCS over the left IFC on action prediction (Experiment 1) with the effect of cathodal tDCS over right IFC (experiment 2) and anodal tDCS over left IFC (Experiment 3). Subjective evaluation of discomfort caused by tDCS was analyzed with a two-way mixed-model ANOVA with Stimulation as within-subjects factor and Experiment as between-subjects factor. In all the ANOVAs, post hoc comparisons were performed using Newman-Keuls tests. Statistical analyses were carried out using STATISTICA 8.0 software (StatSoft, Inc.).

RESULTS

The Experiment x Task x Stimulation ANOVA conducted on accuracy rates revealed a significant three-way interaction ($F_{2,34}$ = 3.32, p= .04) indicating that the performance of subjects was differentially modulated by tDCS across the three experiments. To identify the source of the triple interaction, three separated Task x Stimulation ANOVAs were performed for each experiment. In Experiment 1, the ANOVA revealed a marginally significant main effect of Stimulation ($F_{1,12}$ = 4.33, p = 0.06) but no main effect of Task ($F_{1,12}$ = 0.41, p = 0.54). Importantly, the ANOVA also

showed a significant Task x Stimulation interaction ($F_{1,12} = 8.15$, $p = 0.01$) which was accounted for by the lower accuracy in the AP task after active tDCS (mean accuracy ± S.D.: 73% ± 7) relative to accuracy found in the other three conditions, namely AP task after sham tDCS (78.7% ± 4; $p = 0.039$), NP task after active tDCS (78.4% ± 8; $p = 0.029$) NP task after sham tDCS (76.7% ± 8; marginally significant difference, $p = 0.063$), which in turn did not differ from one another ($p > 0.38$). This indicates that suppression of activity in the left IFC selectively worsen participants' ability to predict human actions but not to predict non-biological movements (figure 5.5).

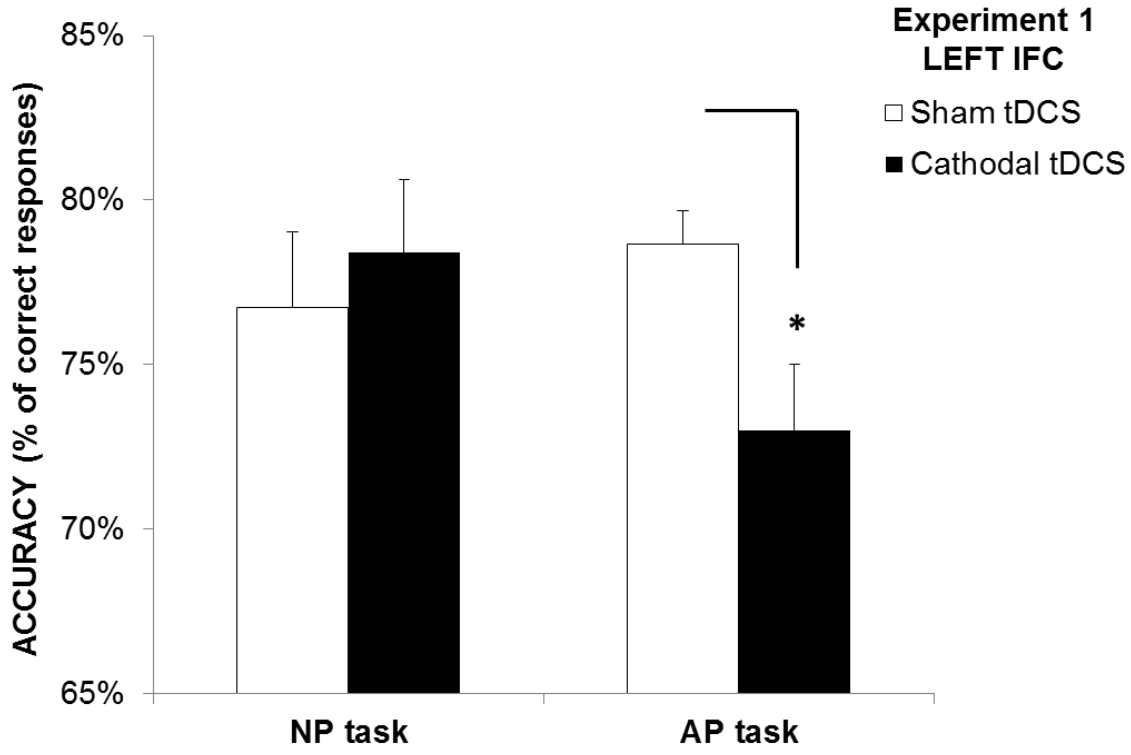

Figure 5.5. Accuracy of subjects in the NP task and AP task recorded after sham and active cathodal tDCS over the left IFC. Asterisk indicate significant post hoc comparisons. Error bars denote S.E.M.

In contrast to Experiment 1, the Task x Stimulation ANOVA conducted on accuracy data from Experiment 2 ($p > 0.40$) and Experiment 3 ($p > 0.70$) failed to reveal any significant main effect or interaction. Thus, the detrimental effect of active cathodal (inhibitory) tDCS over the left IFC on AP was absent after cathodal tDCS over the right IFC (figure 5.6) or anodal (excitatory) tDCS over the left IFC (figure 5.7).

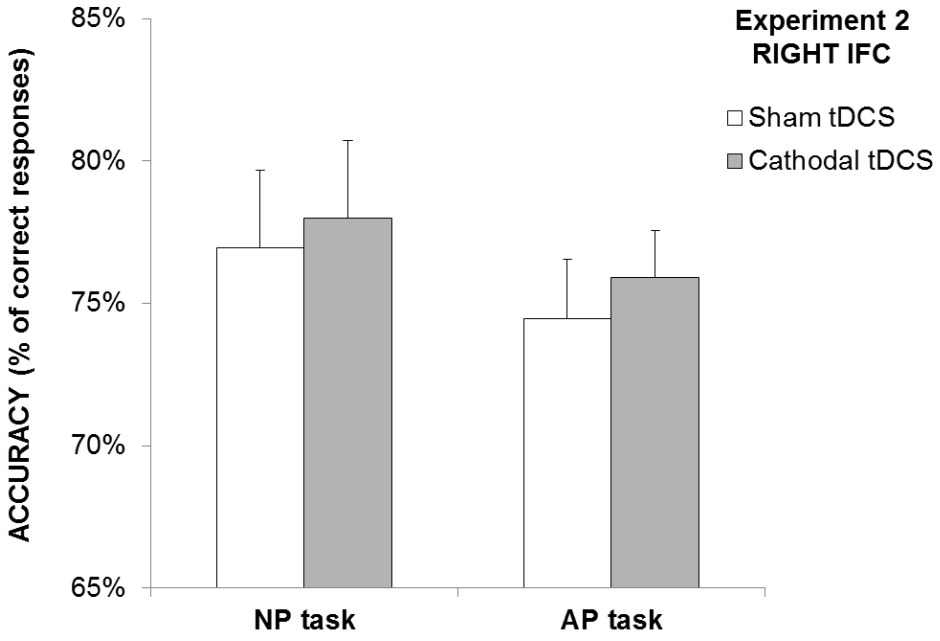

Figure 5.6. Accuracy of subjects in the NP task and AP task recorded aftersham and active cathodal tDCS over the right IFC. Error bars denote S.E.M.

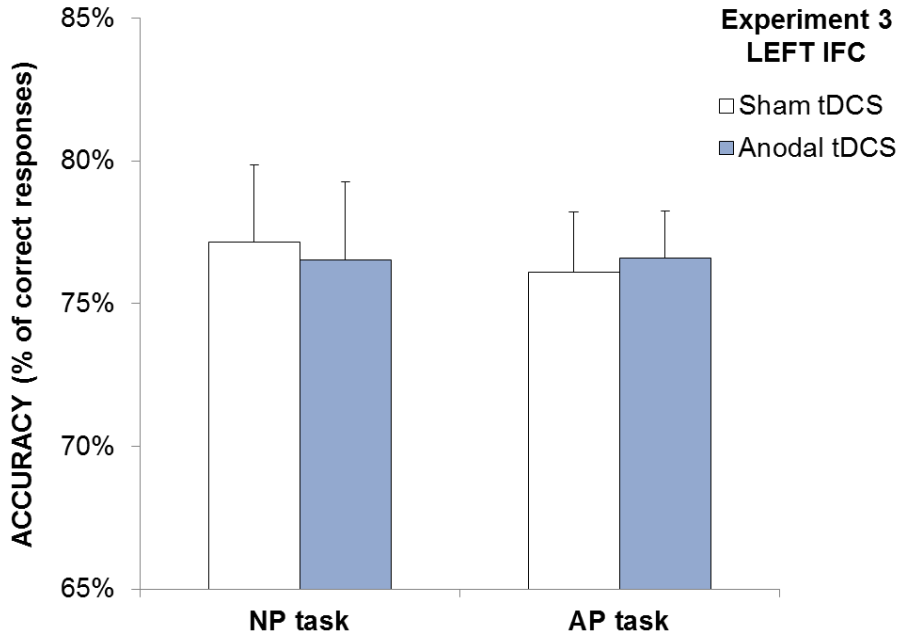

Figure 5.7. Accuracy of subjects in the NP task and AP task recorded after sham and active anodal tDCS over the left IFC. Error bars denote S.E.M.

To directly compare the amount of AP disruption after active tDCS in the three experiments we conducted a further analysis. For each experiment, we computed an index obtained by subtracting AP accuracy after active tDCS from that recorded after sham tDCS and entered such tDCS disruption index in a one-way ANOVA with the between-subjects factor Experiment. The ANOVA was significant ($F_{2,34}$ = 4.02, p = 0.03). As shown in figure 5.8 , tDCS-disruption values were negative only in Experiment 1 (-5.6% ± 6) and they were significantly lower than in Experiment 2 (1.5% ± 7; p = 0.04) and Experiment 3 (0.5% ± 7; p = 0.03); values were comparable in Experiment 2 and 3 (p = 0.73).

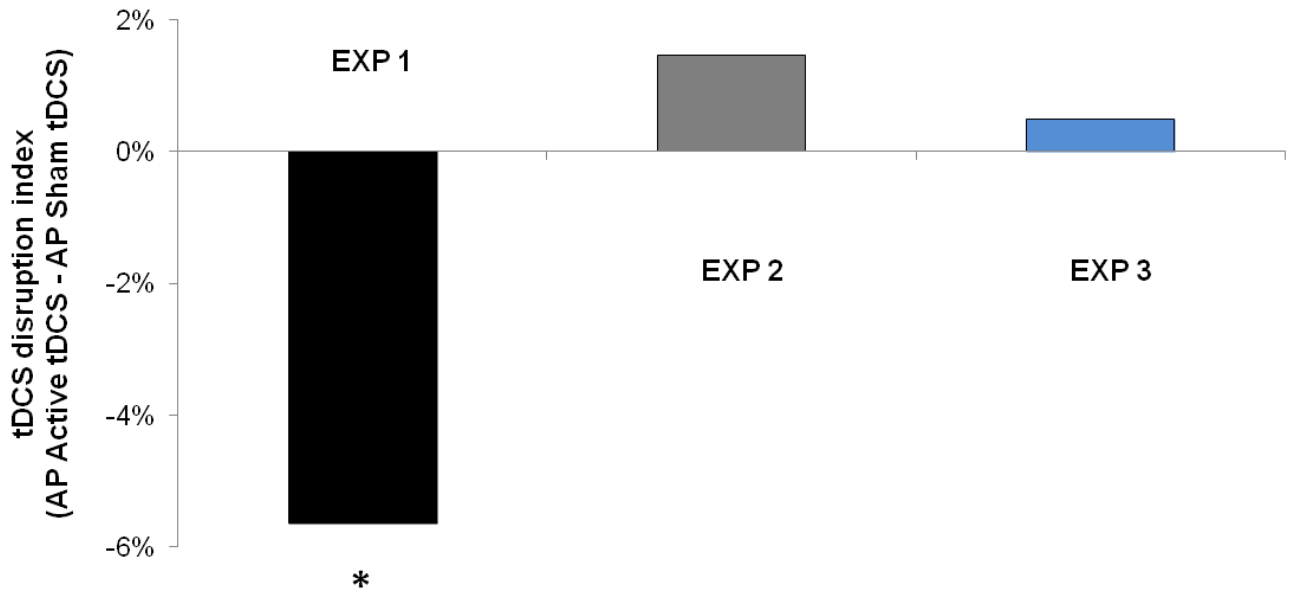

Figure 5.8. AP tDCS disruption index in the three experiments. Asterisk indicate significant post-hoc comparisons.

Importantly, discomfort during active or sham tDCS was very low and comparable across sessions and experiments as suggested by the lack of main effect or interaction in the Experiment x Stimulation ANOVA on subjective evaluation of scalp discomfort (all $p > 0.54$; Table 5.1).

Table 5.1 : Subjective evaluation of scalp discomfort

	Sham tDCS	Active tDCS
Experiment 1	1.62 ± 0.65	1.69 ± 0.63
Experiment 2	1.46 ± 0.66	1.46 ± 0.52
Experiment 3	1.62 ± 0.51	1.69 ± 0.48

Note: Mean values of the reported scalp discomfort after sham and active tDCS (expressed on a 5-point Likert scale with 1 indicating "not unpleasant at all" and 5 "extremely unpleasant") ± S.D. in the three experiments.

DISCUSSION

In the present study we tested whether non-invasive manipulations of activity in the frontal nodes of the AON alter the ability to predict human actions. Participants were presented with two tasks involving prediction of human actions or of non-biological movements, immediately after sham or active tDCS. In the AP task, subjects watched clips showing the initial phase reaching-grasping movement of a right hand toward one of two objects and had to predict the outcome of the action based on the initial kinematic cues of the hand (i.e. trajectory and finger pre-shaping). A difficulty-matched NP task was designed as a control task to assess prediction of non-biological movements. In Experiment 1, we found that cathodal tDCS over the left IFC impaired accuracy in the AP relative to sham tDCS. These effects were specific for the prediction of biological movements as cathodal tDCS did not alter accuracy in the NP task. Moreover, no changes in performance were found in Experiment 2 and 3 were cathodal tDCS over right IFC and anodal tDCS over left IFC

were applied, respectively. These findings demonstrate that stimulation of the left IFC, but not of the right IFC, impairs prediction of right-hand actions but not of non-biological movements. Moreover, they indicate that worsening of action prediction is specific when inhibitory (cathodal) not excitatory (anodal) tDCS is applied to the left IFC, which may suggest that in the intact brain non-invasive induction of plasticity can disrupt but not potentiate action prediction ability. Our data support the view that the IFC is a core region in the AON involved not only in planning and executing motor acts, but also in the perception and prediction of others' actions (Avenanti and Urgesi, 2011; Avenanti et al., 2012b) and suggest a left frontal lateralization in the predictive coding of others' right-hand actions.

Action perception and execution share a common neural network, which include frontal motor regions (i.e. the IFC) of the so called AON (Gazzola & Keysers, 2009; Grafton, 2009; Van Overwalle & Baetens, 2009; Caspers et al., 2010). These motor regions are thought to subserves perceptual and predictive purposes by relying on internal model of the action (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Kilner et al., 2007a; Kilner et al., 2007b; Avenanti and Urgesi, 2011). In this vein, seeing a motor act activates stored motor representations in the visuo-motor nodes of the AON. These motor representations provide an internal forward model of the ongoing seen action that is used for predicting its future course. Such anticipatory representation may be used to fill-in missing or ambiguous perceptual information derived from the discontinuity in the sensory input and thus may guarantee stable perception.

This hypothesis has been supported by studies in monkey showing the anticipatory firing of premotor mirror neurons before the observation of the relevant phase of the action (e.g. the hand-object interaction or the final outcome) or during its visual occlusion (Umiltà et al., 2001; Fogassi et al., 2005). In humans, activation of the motor system has been found before the observation of upcoming or expected hand actions (Kilner et al., 2004; Avenanti et al., 2009), or during the transient occlusion of an ongoing full-body actions (Stadler et al., 2011). Single-pulse TMS studies have shown an anticipatory bias in the simulation of the future phases of observed actions, both

when observing dynamic (Gangitano et al., 2004; Borroni et al., 2005; Aglioti et al., 2008) or static implied action stimuli (Avenanti et al., 2009; Urgesi et al., 2006, 2010; Borgomaneri et al., 2012). Importantly, TMS inhibition of left IFC disrupted this anticipatory action simulation activity (Avenanti et al., 2012b) indicating a critical role of IFC in the predictive coding of seen actions. In contrast, an increase of anticipatory action simulation activity was detected when TMS disrupted neural processing in an early visual node of the AON, the STS. These findings highlighted the active role and the compensatory functions of IFC in building anticipatory action simulation *de novo* when perceptual information are degraded.

All these studies support the predictive theories of action perception by showing that when seeing others' actions, the motor system, and IFC in particular, is actively involved in predicting the future of seen actions (Blakemore & Decety, 2001; Grush, 2004; Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Avenanti & Urgesi, 2011). However, none of the above mentioned studies have directly tested the critical prediction of such theories, namely that interference with IFC activity may impair the ability to predict the future of seen actions.

Our study provides direct causative evidence that left IFC plays a crucial role in predicting the end-states of human actions. Our results add to previous TMS on action perception. These studies suggested a role of IFC in processing temporal aspects of seen dynamic actions (Pobric & Hamilton, 2006; Tidoni et al., 2012) or configurational features of static body postures (Urgesi et al., 2007). In such studies, online TMS interference with IFC, but not with control regions, reduced performance in tasks requiring to discriminate between truthful or deceptive movements (Tidoni et al., 2012), to judge the weight of a box when seeing it being lifted by a human agent (Pobric & Hamilton, 2006); or to discriminate between pictures displaying different body postures (Urgesi et al., 2007). Our study is also in line with the recent study by Stadler and colleagues (2012). In that study, participants observed complex everyday whole-body actions that were transiently occluded. After each occlusion, participants indicated whether the time course of the action was coherent or had been manipulated (accelerated, decelerated) during the occlusion phase. It was found that

online TMS interference with the left dorsal premotor cortex immediately after the occlusion phase tended to reduce task accuracy.

Our experiments significantly expand such prior studies on several fronts. First, it demonstrates that IFC is not only required for processing temporal aspects of seen actions (Pobric & Hamilton, 2006; Stadler et al., 2012; Tidoni et al., 2012) or configurational features of static body postures (Urgesi et al., 2007) but it also necessary for correct estimation of the future phases of observed dynamic actions. Indeed, our data suggest that IFC is actively involved in the extrapolation of the action end-state based on the visual processing of the initial phases of the movement, which may include dynamic spatial (arm direction, trajectory) and configurational (finger pre-shaping) kinematic cues. Second, while the four above mentioned studies used frontal TMS during action observation, in the present study tDCS was applied before task execution (off-line stimulation). It should be noted that online frontal TMS, but not tDCS, may cause facial muscle contractions or unpleasant scalp sensation. Hence, our data cannot be accounted for by the potentially distracting effects of online frontal TMS during task execution. Moreover, subjective data show that in Experiments 1-3 participants felt very similar scalp sensations during active and sham tDCS, in keeping with the notion that tDCS provides a reliable sham stimulation condition (Jacobson et al., 2012). Third, by using a well-matched control task, our study clearly demonstrates that IFC is necessary for prediction of human actions, not of non-biological movements. Fourth, our paradigms indicate that only the inhibition of left IFC and not its excitation or the inhibition of the contralateral IFC is capable of worsening the ability to predict the future of hand actions.

The findings that suppression of IFC impairs prediction of human but not of non-human movements is in line with the notion that AON is biologically tuned, such that it responds more to the observation of human, than non-human, movement (Press, 2011). This tuning refers both to form and kinematic profile. For example, human bodies moving with a non-human kinematic activate the

AON less (Stevens et al.,, 2000; Dayan et al., 2007; Casile et al., 2010) and interference with IFC impairs perception (Candidi et al., 2008) and motor mapping of (Avenanti et al., 2007) human possible but not of biomechanically impossible body movements. Relevant to the present study, human body movements activate the anterior parts of the AON more than non-human movements, including geometrical stimuli (Kessler et al., 2006; Engel et al., 2008), inanimate objects (Costantini et al., 2005; Oberman et al., 2005), humanoid robots (Tai et al., 2004; Miura et al., 2010; Chaminade et al., 2010; Shimada, 2010) or virtual hands (Perani et al., 2001), even when all movements are matched for kinematic profile (but see Gazzola et al., 2007). While these studies suggest greater sensitivity of IFC for human actions, they cannot tell whether the IFC activity is necessary only for predicting human but not non-human movements. It has been found that the same sector of the IFC involved in action perception is also recruited during prediction of sequence of abstract events (Schubotz & Von Cramon, 2004; Schubotz et al., 2010). These findings have suggested that functions of the anterior node of the AON are not limited to the prediction of others' actions and may extend to event prediction more generally (Schubotz, 2007).

Our study sheds light on this issue by showing that suppression of IFC impairs prediction of human actions but not of non-biological movements. Importantly, similarly to the AP task, the NP required to process and predict the complex movement of a complex object (a geometrical form) that: i) followed a trajectory similar to the kinematic profile to the hand in the AP; ii) changed in its general configuration during the approaching phase in order to fit to one of the two target objects, in analogy to the pre-shaping of the fingers in the AP clips. Moreover, the two tasks were matched for difficulty based on a series of pilot studies on a large sample of participants. Thus, the absence of modulation of NP accuracy cannot be due to ceiling or floor effects (see Hamilton & Pobric, 2006). In sum, our data provide causative evidence that the AON is biologically tuned and may suggest that motor activations during non-biological event prediction may reflect task-irrelevant outflow into the motor system. Another relevant issue we addressed in our study, deals with the differential role of left IFC and right IFC in action prediction. Our data may suggest a lateralization in the

predictive coding of seen actions in the left hemisphere. On the other hand it should be noted that right hand actions were used in the AP task. Although the activity of the AON is bilaterally distributed (Van Overwalle & Baetens, 2009; Grosbras et al., 2012), studies using a variety of methods, including TMS, EEG MEG and fMRI, have shown gradient of lateralization in the AON which is dependent on the laterality of the observed body part movements (Aziz-Zadeh et al., 2002, van Schie et al., 2004, 2008; Shmuelof and Zohary, 2005; Gazzola and Keysers, 2009; Cabinio et al., 2010; Caspers et al., 2010). In particular during observation of right hand actions, AON activation tends to be stronger and can be detected earlier (Ortigue et al., 2010) in the left relative to the right hemisphere. Such (partial) lateralization may account for by the tDCS disruption found in Experiment 1 (left IFC) but not Experiment 2 (right IFC) for AP task. Further studies will test whether suppression of activity in the two IFC alter the ability to predict left hand actions.

Studies suggest that the polarizing effects of tDCS are generally restricted to the area under the electrodes (Nitsche, et al., 2003; 2004). Stimulation of motor, somatosensory, visual or prefrontal cortices all have been shown to deliver site-specific and differential effects on a range of behavioral and electrophysiological tests (Zaghi et al., 2010). Additionally, tDCS over the motor regions induces highly focal effects (Uy & Ridding, 2003), especially when a monopolar montage with extracephalic reference electrode is adopted, as in the present case (Brunoni et al., 2011). Although the effects of tDCS can be considered site-specific, they are not site-limited (Zaghi et al., 2010). Studies show that tDCS can modulate the excitability of distant interconnected regions (Boros, et al., 2008; Vines et al., 2008). While our study indicates that only left IFC and not right IFC is critical for accurate performance in the AP task, it likely that other interconnected regions within the AON may significantly contribute to action prediction. Thus, it is possible that cathodal tDCS over left IFC modulated activity in other visuomotor (e.g. intraparietal) or visual (e.g. STS) interconnected regions and that these regions contributed to the observed effects. Further studies will directly test such possibility. A final issue that needs to be discussed is related to the polarity dependent effects of tDCS. Since Experiment 1 and 2 clearly showed that AP performance relied on

the left IFC, in Experiment 3 we tested the possibility that enhancement of left IFC could lead to changes in the ability to predict others' actions. We reasoned that if the basic functioning of the IFC is to implement simple (visuo-motor) integrative mechanisms (e.g. Rizzolatti & Craighero, 2004) and action prediction relies on such processing, then increasing IFC excitability may lead to enhanced output and improved behavioral performance (note task accuracy was intentionally set at ~75%), similarly to what has been demonstrated for basic visual and motor functions during stimulation of unimodal or multisensory areas (Jacobson et al., 2012; Bolognini et al, 2010a , 2010b). On the other hand, it is possible that more complex functions are implemented in the IFC. If this is the case, as suggested by predictive theories of action perception, then IFC excitation would not necessarily result in improved performance in the AP task as the anodal/cathodal dichotomy does not apply for complex cognitive processing (Jacobson et al., 2012). Results from Experiment 3 offer some insights into the neural processing implemented in the left IFC. These findings may suggest that during action prediction, the functions of IFC are not limited to a coupling neural mechanism – a proposal that is in line with the predictive theories of action perception. These theories have suggested that the AON works a dynamic control system, where information initially flows from the visual (e.g. STS) to the visual-motor nodes of the AON and then flows back in visual regions (Wilson & Knoblich, 2005; Schippers & Keysers, 2011; Avenanti, Urgesi, 2011; Avenanti et al., 2012b). In this vein, the IFC would be actively involved in generating an anticipatory representation of seen actions by projecting the course of ongoing movements into the future. These predictions are then fed back into perceptual systems (e.g. in STS) to create top-down expectations and constrain visual perception in a top-down manner.

In summary, the present study allows to draw three main conclusions: i) the IFC is a crucial node of the AON involved in predicting the future phases of observed hand actions; ii) the involvement of IFC is specific for human actions and does not extend to prediction of non-biological movements; and iii) prediction of right hand actions relies on the left, not on the right IFC. Additionally, the result that left IFC excitation does not enhance AP performance may suggest that during action

prediction, the type of neural processing in IFC is more complex than a simple coupling mechanism. Taken together, these findings support theories of action perception that have emphasized the active role of the motor system in the predictive coding of others' actions.

CHAPTER 6

The role of sensorimotor experience on action prediction: the case of traumatic and congenital amputees[5]

ABSTRACT

According to predictive theories of action simulation, humans use their own motor system as an internal forward model to predict the future of others' actions. A direct sensorimotor experience of an observed action could make perceptual and predictive mechanisms more effective. How and to what extent, the absence of a limb and the contingent lack of sensorimotor experience, could affect perceptual and predictive mechanisms? In the present study we asked whether congenital and traumatic upper limb amputation might hamper the predictive coding of others' actions. To this aim, we examined the performance of both congenital and traumatic upper limb amputees (and a group of age-matched normally limbed controls) in two prediction tasks, in which video clips of both biological (Action Prediction, AP task) and non biological movements (Non-biological movement Prediction, NP task) were displayed. Participants were required to predict the end state of a right or left hand reaching-grasping action (AP task), while in a difficulty-matched control task (NP task) they were required to predict the end state of a left or right geometrical form whose movement roughly mirrored the trajectory of the hand in the AP clips. The results show that congenital but not traumatic amputees, were selectively impaired while predicting the final end state of hands corresponding to their affected side relative to hands corresponding to the intact side. These findings suggest that a successful prediction of others' actions is not prevented by the current

[5] *In preparation*

absence of a limb per se, rather by the lack of sensorimotor experience associated to that limb from the birth.

INTRODUCTION

It has been suggested that perceiving and understanding others' people actions is made possible through the same sensorimotor processes involved in action execution (Rizzolatti & Craighero 2004). Recent studies have identified an action observation network (AON) which includes frontal, temporal and parietal areas, and that represents the neural substrate of the coupling between action perception and execution (Gazzola & Keysers, 2009; Grafton, 2009; Van Overwalle & Baetens, 2009; Caspers et al., 2010; Rizzolatti & Sinigaglia, 2010). The recruitment of the AON during action observation, has been suggested to serve predictive purpose in support of action understanding (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Kilner et al. 2007a; Kilner et al. 2007b; Avenanti and Urgesi, 2011). Moreover, the anterior node of the AON, the inferior frontal cortex (IFC), is thought to mediate predictive mechanisms within this network (Annella et al., 2012 in preparation, chapter 5 of the thesis). It has been proposed that perceptual and predictive properties of the motor system are not entirely genetically prewired. Rather, they are largely acquired and modeled through sensorimotor associative learning (Brass & Heyes 2005; Heyes 2001, 2010; Keysers & Perrett 2004). These accounts support the notion that during the life-span, the experience related to the observation and execution of an action establishes links between sensory and motor representations of the same action, so that every time that action is observed, the corresponding motor representation is activated (Press, 2011).

In this framework, acquired sensorimotor representations vary as a function of experience, which in turn have the power to reconfigure the neural systems involved in their processing. Studies in professional athletes have extensively investigated the role of motor expertise in modeling action perception and prediction. It has been shown that the intensive and direct sensorimotor experience within a given sport domain can strengthen action simulation mechanisms (Calvo-Merino et al.,

2005; Fourkas et al., 2008) and improve the ability to recognize (Jackson et al., 2006; Sebanz & Shiffrar, 2009) and to make predictions (Aglioti et al., 2008; Urgesi et al., 2011) about specific actions within one's own domain of expertise.

Notably, evidence suggests that visual perception of others' actions is influenced by one's own motor experience even when no visual feedback is provided during action execution. Elegant psychophysics studies have shown that repetitive execution of a particular action may bias the way another person's action is perceived (Glenberg et al., 2010; Cattaneo et al., 2011), and learning new motor acts improves the ability to recognize those acts in point-light displays (Casile & Giese 2006). Moreover, imaging studies have suggested that motor more than visual expertise is crucial in modulating AON activity in expert dancers and athletes (Calvo Merino et al., 2006; Cross et al., 2006, 2009a; Reithler et al., 2007; Abreu et al., 2012).

On the other hand, also pure visual experience can induce plastic changes in the motor system. For example, it has been experimentally demonstrated that visual exposure to an observed action can promote movement-specific memory formation in the motor cortex (Stefan et al., 2005) and modulate practice-induced memory formation (Stefan et al., 2008). The specific coupling between action execution and observation seems particularly important for shaping brain activity within the AON. For example, , specific visuo-motor trainings can reconfigure motor system reactivity during action observation (Catmur et al., 2007, 2011), at least to a certain degree (Barchiesi and Cattaneo, 2012), and these effects likely depend on plastic changes within the AON (Catmur et al., 2008). Taken together, these studies suggest that the individual's sensorimotor experience and the acquired skills shape the AON and influence the ability to perceive and predict others' actions (Gallese et al., 2009). While all the above mentioned studies have suggested that new visual and motor experience may improve action perception, evidence that reduced sensorimotor experience reduces the ability to perceive and predict the action of others' is very scanty. Some studies have shown that hemiplegic relative to non-hemiplegic patients are specifically impaired in recognizing point-light displays of hand gestures when the display corresponds to their affected limb (Serino et al., 2010).

This evidence may suggest that loss or reduction of limb use due to hemiplegia may impair action perception in a body part specific fashion. However it is also very likely that perceptual impairment in hemiplegics may be driven by the specific brain lesions occurring in the motor system, more than by the consequent reduction in limb use, as brain damage patients with no hemiplegia but lesions in motor areas may show action perceptual impairments(see Pazzaglia et al., 2008; Moro et al., 2008).

Congenital amputees represent an extraordinary model to test how the absence of a limb from birth may affect perception. Previous single-cases imaging studies on three congenital amputees have documented a relatively spared activation of the AON (Gazzola et al. 2007, Aziz-Zadeh et al., 2012), but only when amputees were presented with actions they could perform with other body parts. No activation of the AON was found when amputees observed action they could not perform. However, it is unclear whether the ability to perceive the actions of others was affected.

It is believed that the majority of congenital patients do not develop a motor representation of the missing limb due to the total lack of sensorimotor experience with that body part (Melzack, 1997). This would suggest that such individuals may show impairments in action perception due to the lack of body part specific motor representations. The presence of (phantom) postural or movement sensations of the missing limb has suggested that, in some cases, an innate "body schema" may represent the missing limb even in the absence of experience (Brugger et al., 2000). Interestingly, in a previous study, two individuals born with not hands were tested during an action perception task in which they had to discriminate possible vs impossible human movements (Funk et al., 2005). A first amputee, who had experienced vivid phantom sensations from early youth, showed a performance similar to that of control subjects, while a second amputee, who had no experience of phantom sensation, showed reduced task performance. Although it is difficult to drawn strong conclusions from such single-cases studies, they suggest that altered or non-well developed sensorimotor representations in congential amputees may be associated to impairments in action perception. Moreover, these studies suggest that it may be important to assess the presence of phantom sensations as index of possible preserved sensorimotor representation of the missing limb.

Importantly, adult traumatic amputees represent an important control for congenital amputees because their loss of limb does not persist from the birth but has traumatically occurred in adulthood after normal development. Traumatic amputees thus allow to test whether the loss of sensorimotor experience after a normal development is sufficient to impair action perception.

While previous single-case studies have suggested that bilateral congential amputees may show reduced performance when dealing with tasks requiring to assess the speed of an observed action (Funk et al., 2005), no systematic studies have investigated action perception in unilateral congenital or traumatic amputees. In the present research we investigate the ability to perceive and predict others' actions of unilateral traumatic and congenital upper limb amputees. Amputees were tested in two prediction tasks involving both human (Action Prediction, AP task) and non-human movements (Non-biological movement Prediction, NP task). Participants had to predict the end state of a right or left hand reaching-grasping action (AP task), while in a difficulty-matched control task (NP task) they had to predict the end state of a left or right geometrical form whose movement roughly mirrored the trajectory of the hand in the AP clips. We have previously shown that, in normally developed healthy subjects, tDCS suppression of left but not right IFC impairs the ability to make predictions about right hand movements in the AP task, but does not alter performance in the NP task (Annella et al., in prep; chapter 5). These findings have suggested that transcranial disruption of normally developed cortical motor representations of a limb impairs the ability to visually process and predict observed actions performed with the same limb. Thus, since the AP, but not the NP task should rely on stored body part specific motor representations, we predict that amputees without a normal development of such representations – that is, congenital amputees – should show reduced performance in the AP task relative to the NP task and the reduced performance should be specific for the affected limb. Moreover, the investigation of a group of traumatic amputees allowed to test whether the current absence of a limb *per se* (and the consequent motor system reconfiguration and reduction in sensorimotor experience) in an otherwise normally developed motor system is sufficient to hamper action perception and prediction.

MATERIALS AND METHODS

Subjects

A total of 38 subjects participated in the study. The experimental sample consisted in three groups of subjects: the Congenital amputees group (CAG), the Traumatic Amputees group (TAG) and Controls group (CG). The CAG included six subjects (four women, mean age 34.3 ± 8.7 years, range 23-44 years). Three subjects had agenesis of the left limb whereas three of the right limb. The TAG included seven subjects (one woman, mean age 42.9 ± 15.7 years, range 23-62 years) who suffered from traumatic amputation of the left (N=4) or the right (N=3) upper limb. All had normal or corrected to normal vision. None of the subjects of the CAG had never experienced phantom sensations, while none of the TAG reported phantom limb pain at the moment of tests. The CAG and TAG's main demographic and clinical data are reported in tables 6.1 and 6.2. A group of 25 age-matched healthy subjects formed the CG. They were recruited among relatives and through posted advertisements (thirteen women, mean age 34 ± 16.8 years, range 22-63 years). All were right-handed and had normal or corrected to normal vision. Participants had no previous history of neurological or psychiatric disorders. The experimental protocol was explained to all subjects who gave their written informed consent prior to participating in the study which was approved by the Local Ethics Committee of Lyon Sud-Est IV, and conformed to the ethical aspects of the Declaration of Helsinki (1964). All subjects were paid 50 euros for their participation in the study.

	Demographic Data			Amputation			Prosthesis	
Subject	**Sex**	**Age (years)**	**Education (years)**	**Amputated side/ Dominant side**	**Level**	**Cause**	**Prosthesis (use and type)**	**Time (years)**
CA1	M	23	17	L/L	arm	agenesis	Aesthetic	5 (childhood)
CA2	F	39	17	L/R	forearm	in utero amputation	Aesthetic	35
CA3	M	38	14	L/R	forearm	in utero amputation	Aesthetic	34
CA4	F	44	12	R/R	forearm	agenesis	Aesthetic	2 (childhood)
CA5	F	38	17	R/R	forearm	in utero amputation	Aesthetic	35
CA6	F	24	17	R/R	forearm	agenesis	Aesthetic	24

Table 6.1. Main demographic and clinical features of the Congenital Amputees Group (CAG).

	Demographic Data			Amputation				Prosthesis	
Subject	**Sex**	**Age (years)**	**Education (years)**	**Amputated side/ Dominant side**	**Level**	**Time since amputation (months)**	**Cause**	**Prosthesis (use and type)**	**Time (years, months)**
TA1	M	61	12	L/R	arm	46	Bike Accident	No	X
TA2	M	29	8	L/R	forearm	113	Job Accident	Myoelectric	6
TA3	M	52	13	L/R	arm	108	Job Accident	Myoelectric	10
TA4	F	23	8	L/L	hand	131	Job Accident	Myoelectric	1
TA5	M	41	11	R/R	forearm	75	Job Accident	Aesthetic	2
TA6	M	62	11	R/R	shoulder	168	Aggression	No	X
TA7	M	32	8	R/R	hand	24	Job Accident	Myoelectric	8

Table 6.2. Main demographic and clinical features of the Traumatic Amputees Group (TAG).

Design

Participants performed two tasks involving prediction of human biological (Action Prediction task, AP) or non-biological movements (Non-biological Prediction, NP task). The AP task involved videos showing a hand reaching and grasping one of two objects. The final phase of the action was masked and subjects had to guess which object was going to be grasped by the hand. Similarly, in the NP task, subjects saw videos showing a non-biological geometrical form approaching one of two targets and subjects had to guess which target was going to be hit by the form.

To investigate the effect of laterality of upper limb amputation on the ability to predict others' actions, participants watched both left and right hand motor acts in the AP task. This way, we tested how amputees predict others' motor acts performed with the missing or the intact limb. Left- and right-side videos were tested in two separate sessions whose order was counterbalanced across subjects (see figure 6.1).

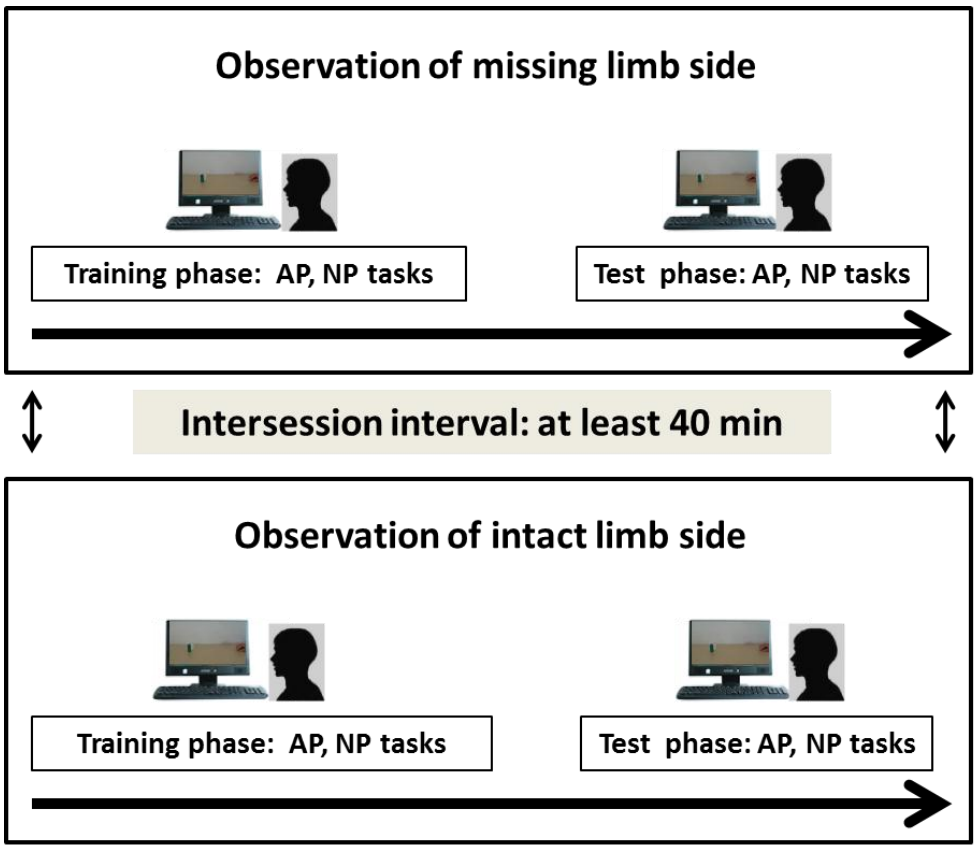

Figure 6.1. Schematic representation of the experimental design.

Tasks and stimuli

The stimuli set was adapted from a previous study (Annella et al., in preparation; chapter 5). Stimuli in the original set showed right hands and geometrical forms making right-to-left movements. To obtain a comparable set of left hand movies (and corresponding geometrical form control videos), all the original clips were edited and flipped horizontally using the VirtualDub software. In the Action Prediction (AP) task, participants observed 240 video-clips (120 and 120 videos for the right-side and left-side conditions, respectively 640 x 480 pixels, 25 fps) depicting the initial phase of a reaching-grasping action. Right-side AP videos started showing a still right-hand (on the right side of the screen) with two objects placed in front of it on the left side of the screen. After a variable delay (1000-2200 ms) the hand started to reach and grasp one of the two objects. The final phase of the action was masked and subjects had to guess which object was going to be grasped by the hand. A random-dot mask (150 ms duration, obtained by scrambling the final frame of the movie with a custom-made image segmentation software) interrupted the video and was followed by a response screen showing the two objects and lasting until response. Participants provided their answer using two computer keys. Left-side AP clips showed left hands making left-to-right reaching-grasping movements and were identical mirror-reversed copies of the right-side clips.

Video-clips in the AP task included 8 different actors (3 females; mean age ± S.D.; 23.6 ± 1.06) reaching and grasping 8 different couple of objects (see figure 5.1 of chapter 5). The two objects in each couple were located in two closed positions in space and presented different affordances, thus implying slightly different hand trajectories and grips (e.g. power vs precision grips). In different trials, only 30-80% of the entire movement was shown and in none of the videos the hand-object interaction was visible. Indeed, prediction in the AP task involved the processing of hand trajectory and finger pre-shaping during the reaching phase.

In the Non-biological Prediction (NP) control task, subjects observed 120 right-side and 120 left-side similarly interrupted video-clips showing a non-biological geometrical forms approaching one

of two targets and subjects had to guess which target was going to be hit by the stimulus. The NP videos (640 x 480 pixel, 30 fps) were animations created with Adobe Flash Professional software to match temporal and spatial features of AP stimuli. They showed incomplete movement (30-80% of the actual duration) of a geometrical form which moved from the right side of the screen (right-side movies) or from the left side of the screen (left-side movies) in order to reach and fit with one among two different geometrical targets placed on the opposite side. The trajectory of the moving forms was roughly matched to that of the hands in the AP task. Moreover, the two targets presented different geometrical properties and, in analogy with the pre-shaping of the fingers (AP task), during the reaching phase the moving form changed configuration over time in order to fit to one of the two targets. Also for the NP video clips eight different couples of geometrical targets and eight objects were used and random-dot image were used as masking (see figure 5.2 of chapter 5).

Procedure

All subjects completed a 2-h testing. Right-side and left-side clips were shown in two separate sessions whose order was counter balanced across subjects. In each session, AP and NP tasks were presented in 4 separate blocks of 60 trials each. The order of the blocks was randomized. In all participants, a total of 120 responses were collected for each task and side (480 trials in total). For CAG and TAG the experimental sessions was preceded by the collection of general clinical data and a semi-structured interview investigating clinical features about the amputation, nonpainful and painful phantom limb sensations, stump pain, as well as the treatment received for pain (Kooijman et al. 2000).

During the experimental sessions, participants faced a screen (15.4-inches) of a laptop located ~50 cm from their head in a dimly illuminated room. They first received experimental instructions and then performed a two blocks training session (1 for each task, 30 trials each). They were required to

look carefully to the video-clips and to respond as fast and accurately as possible by button press. If subject's accuracy was < 60% in one of the tasks, the corresponding instructions and training block were repeated. CAG and TAG were required to respond with their intact hand. For each amputee, 2 control participants were tested. To check for any possible laterality effects, each control provided their responses with the same hand used by the corresponding amputee. A preliminary analysis showed no difference between control subjects responding with the left or the right hands and thus data were collapsed.

After the training, participants performed two AP and two NP blocks.. The order of the four blocks was randomized. The sequences of video-clips were run by means of a software written in MATLAB 7 with a custom-made interface, which also allowed to record accuracy trial by trial.

Data Analysis

Data were processed offline. A preliminary analysis of variance (ANOVA) showed no significant differences between CG and the CAG and TAG with the respect to age (all $p_s > .16$). Accuracy was calculated as the proportion of correct responses and was analyzed using parametric tests, since data in the three groups were normally distributed (Shapiro Wilx tests:, all $p_s > .22$). In the CG (N= 25), thirteen subjects performed the tasks using their right hand while the other twelve their left hand. To control for the possible effects due to the hand preference, a preliminary three-way ANOVA with Observed side (two levels: left, right) and Task (two levels: NP, AP) as within subjects factors and Hand used (two levels: right, left) as between subjects factor, was conducted. The ANOVA revealed no significant effects (all ps >.48, see figure 6.2), thus data in the two subgroups using the left or the right hand were collapsed and their accuracy mean and standard deviation were used to convert CAG and TAG's performance into z-scores. None of the subjects showed accuracy below 2 SD relative to the control groups. The z-scores were analyzed by means of a four-way ANOVA with Group (two levels: congenital, traumatic amputees) and Side of amputation (two levels: right

and left) as between factors and Observed side (two levels: affected, intact) and task (two levels: NP, AP) as within subjects factors. Post hoc analysis of significant interactions were performed using Newman-Keuls Test. Moreover, a series of Analysis of Covariance (ANCOVA) were further conducted in order to control for the potential confounding effects of age and level of education between the CAG and TAG.

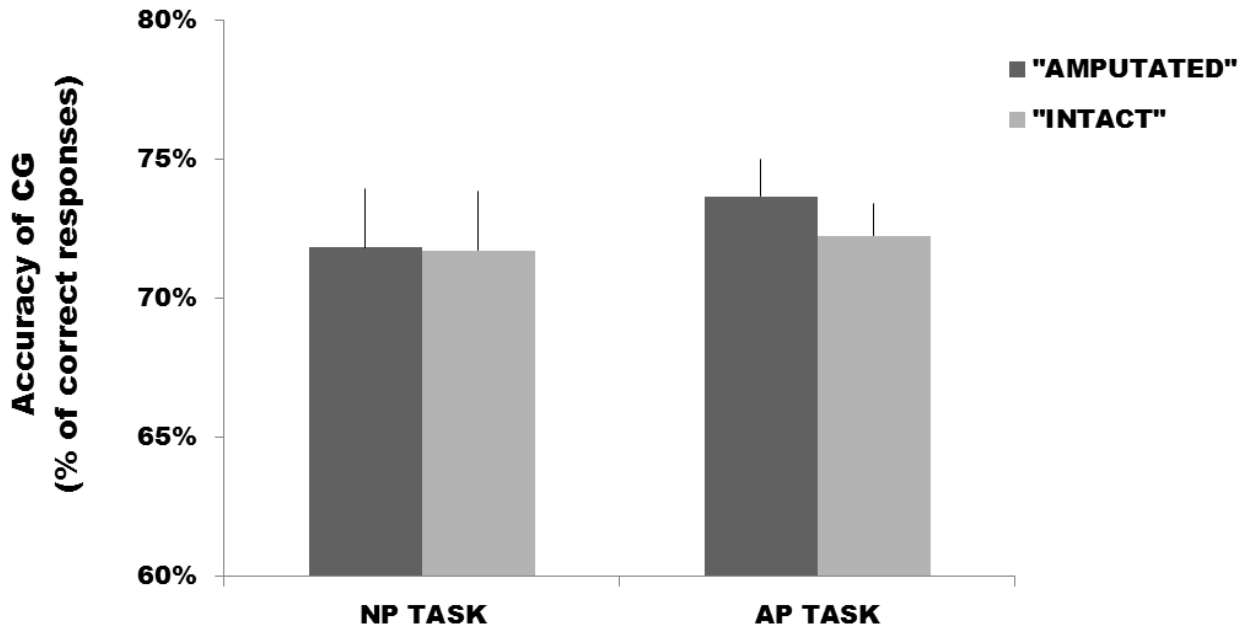

Figure 6.2. Accuracy of the Control Group (CG) in the NP and AP tasks. Error bars denote S.E.M.

RESULTS

Tables 6.1 and 6.2 illustrate demographic characteristics of the amputees. There were no difference between TAG and CAG for age, sex, laterality of the affected side (all $p_s > .06$). However, the two groups differed with respect to education with CAG showing more years of education relative to the TAG.

The four-way ANOVA (Group x Side of amputation x Observed side x Task) revealed a marginally significant main effect of Group ($F_{1,9}= 4.52$, $p= .06$) with CAG showing a trend toward greater

accuracy relative to TAG and a Observed side x Task interaction ($F_{1,9}$= 7.74, p= .02). Most importantly a three-way interaction Observed side x Task x Group ($F_{1,9}$ = 12.17, p= .007) was found. No other main effects or interactions were significant in the ANOVA (all p > 0.25).

To analyze the triple interaction, two separate Side of amputation x Observed side x Task ANOVAs were conducted, one for each group. The ANOVA conducted in the CAG revealed a significant two-way Observed side x Task interaction ($F_{1,4}$ = 21.74, p= .009) but no other effects (all p > 0.27). Post-hoc comparisons showed that CAG participants showed reduced performance in the AP task when predicting the final end-state of observed hands corresponding to their affected side (z = -0.17) relative to hands corresponding to the intact side (z = 0.77; p= .016). Moreover, AP accuracy in the affected side condition was lower than accuracy in the NP affected (z = 0.85, p = .019) and NP intact conditions (z = 0.55, p = 0.019). Accuracy in the AP intact side, NP affected and NP intact side conditions was comparable in the CAG (all p_s > 0.31; figure 6.3).

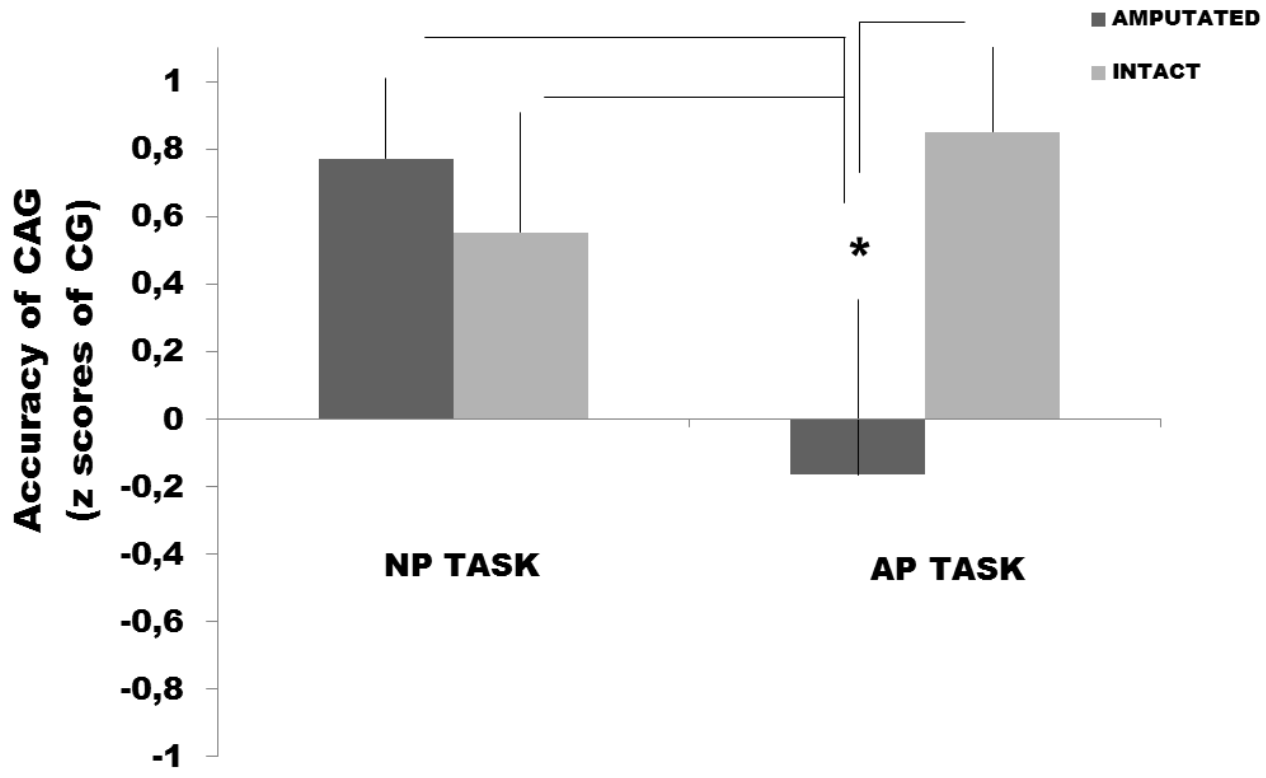

Figure 6.3. Accuracy of the Congenital Amputees Group (CAG) in the NP and AP tasks. Error bars denote S.E.M.

In striking contrast, the ANOVA performed on the TAG showed no significant interaction or main effect (all $p_s > 0.34$), suggesting that performance of TAG was comparable in both tasks and observed side conditions (all $p_s > 0.33$, see figure 6.4).

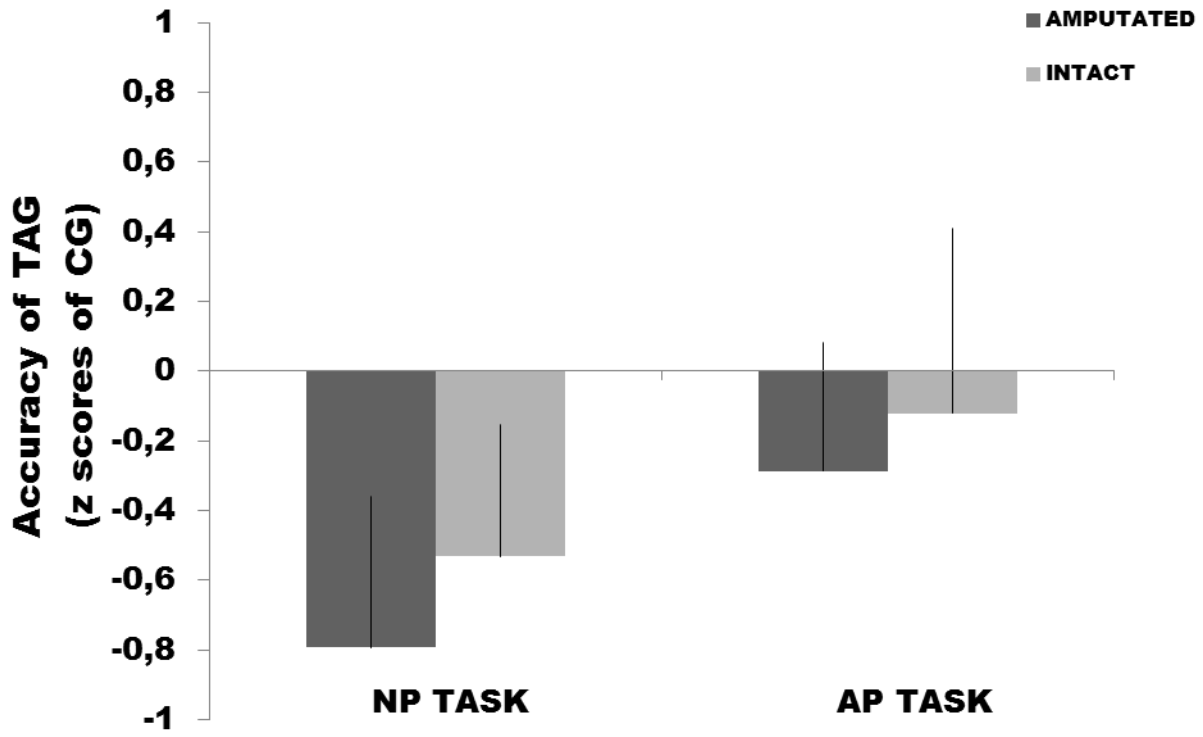

Figure 6.4. Accuracy of the Traumatic Amputees Group (TAG) in the NP and AP tasks. Error bars denote S.E.M.

To check whether age or years of education may have influenced the results in the main analysis, three further analyses were performed. Since TAG and CAG differed in terms of education, in a first analysis the variable education was entered as a covariate into a Group x Side of amputation x Observed side x Task ANCOVA. The ANCOVA confirmed the significance of the Group x Observed side x Task interaction ($F_{s1,8} = 10.61$, $p = 0.012$) found in the main analysis and showed no main effect of Group ($p = 0.32$). No other main effects or interactions were significant (all $p_s > 0.11$). The triple interaction was confirmed also in two further ANCOVAs in which the variable age or age and education were entered as covariates (all $p_s < 0.022$). No other significant effects were found in these analyses (all $p_s > 0.12$).

DISCUSSION

The present study was aimed at investigating whether the absence of a limb might affect action prediction in unilateral traumatic and congenital upper-limb amputees. Subjects were tested in two action prediction tasks in which human and non-human interrupted movements were displayed. In order to control for the limb loss side, subjects observed video clips displaying motor acts performed with the missing or intact limb (and corresponding left and right moving forms). Despite an overall "non-pathological" performance of both groups (none of the subjects showed accuracy below 2 SD relative to the control groups), we found that congenital but not traumatic amputees showed lower accuracy in predicting the future end-state of observed hands actions when these were performed with their missing hand compared to the intact one. Strikingly, their performance was also lower if compared to that obtained when predicting non-biological movements corresponding to both missing and intact limb side, thus indicating a body part specific impairment in the predictive coding of observed actions. These results clearly demonstrate that an optimal perception and prediction of the final end-state of an observed action is not hampered by the current absence of a limb per se, rather by its absence from birth and the contingent lack of sensorimotor experience with the limb performing that action. Outstanding evidence in the literature, points out that the motor system is crucially involved in action perception and is used as an internal forward model to predict others' actions, this ability critically relies on the activity of the fronto-parietal AON (Wilson & Knoblich, 2005; Schütz-Bosbach & Prinz, 2007; Kilner et al. 2007a; Kilner et al. 2007b; Avenanti and Urgesi, 2011; see also chapters 4 and 5 of the thesis). Two prominent models have been proposed for accounting the emergence of this system, subserving perception-action couplings, in typically developed subjects. The associative learning model suggests that this system is forged through sensorimotor learning acquired by observing and executing the same action, such repetitive experience endows the system with his matching properties (Heyes, 2001, 2010; Casile et

al., 2011). The second model posits that the matching between observing and executing the same action during the individual's development is realized following Hebbian learning rules according to which "neurons that fire together wire together" (Keysers & Perrett, 2004; Del Giudice et al., 2009). Both proposals therefore, strongly regard sensorimotor experience as crucial to the development of "mirroring" in humans. Our results allow to better understand that the mirroring of others' actions partly derives from the direct sensorimotor experience of self-produced movements. As a matter of fact, all along their development, congenital amputees can only benefit from a visual experience of the missing limb coming from the observation of others' movements and our findings entail that this is not sufficient to develop optimal predictive abilities while observing others' actions. In keeping , human infants tend to watch their own hands in motion, this visual bias has been suggested to promote the development of mirror neurons through sensorimotor learning (Del Giudice et al., 2009). Moreover, it has been shown that the observation of own movements is critical to the development of perceptual abilities especially for hand movements requiring long periods of maturation for achieving optimal motor skills (Casile et al., 2011). These possibilities clearly lack in congenitally but not traumatic amputees who until amputation followed a normal development of motor abilities, and may support the different results found in the current study.

Previous research on tipically developed subjects, pointed out that new visual and motor experience may improve action perception (Stefan et al., 2005, 2008; Casile & Giese, 2006; Catmur et al., 2007; 2011) Such data are also corroborated by neuroimaging studies showing that experience-dependent modulations occur at the neural level within the AON (Calvo Merino et al., 2006; Cross et al., 2006, 2009a; Reithler et al., 2007). Taken together these results suggest that the observer's own motor repertoire, with his personal sensorimotor experience, influences the way in which observed actions are encoded. Such claim is crucially evident in the case of elite athlete. Recent studies have shown that a high motor expertise leads to better performance in anticipating others' actions (Aglioti et al., 2008; Urgesi et al., 2011). The authors found that elite athletes were more

accurate than expert watchers and novices in predicting the future of displayed actions which belonged to their domain of expertise. These results allow to reconsider our data, and to rethink the CAG as "pure watchers" of their absent limb, since theirs visual experience only relies on the observation of other people using that limb. Such indirect experience of the limb they have never physically experienced could not allow to optimally develop perceptuo-motor representations that are used to predict others' actions.

In keeping, neurophysiological data from congenitally blind and deaf subjects show an impaired and atypical motor resonance in response to observed or heard others' actions, it has been suggested that not the lack of sight and audition per se, but rather their lifelong experience shaped by the lack of multimodal sensory abilities may account for the altered motor resonant responses (Alaerts et al., 2011).

Action perception in unilateral congenital and traumatic amputees has not been systematically investigated. Previous studies focused for example on action perception which implicitly requires motor imagery (Nico et al., 2004). The authors found that the absence of a limb per se did not prevent motor mental simulation but it makes the performance more difficult for the subjects, especially if they have lost their dominant limb and if wearing an aesthetic prosthesis. The congenital amputees showed no impairment in mental imagery when observing unnatural postures of the absent limb but did show this tendency for the present hand. This difference with our results, might strengthens the idea that the relative impairment of the CAG when predicting others' actions is specific for the anticipatory representations of action.

However, the differences between congenital and traumatic amputees in the motor cortex representation of the upper limb, have been extensively studied . After traumatic amputation of a limb, the modifications occurring in the sensorimotor system are frequently associated to experiences of vivid phantom limb sensations (Flor et al., 2006; Reilly & Sirigu, 2008). Transcranial magnetic stimulation (TMS) studies have shown that the stimulation of the motor cortex of traumatic amputees evokes phantom limb movements, indicating that the motor cortex

still contains a representation of the absent limb (Hess et al., 1986; Cohen et al., 1991; Pascual-Leone et al., 1996; Mercier et al., 2006). Such representations are still present when amputees voluntarily move their phantom limb and are differentially activated according to the movement that the subjects plan to perform, this would represent the proof that hand motor commands are preserved after amputation (Reilly et al., 2006). In striking contrast, TMS was showed to be ineffective in triggering phantom limb sensations in congenital amputees who do not report the presence of a phantom limb, suggesting that the representations of limb's movement needs the experience of movement to be expressed within the primary motor cortex (Reilly & Sirigu 2011).

This is in line with previous results showing that the absence of phantom limb sensations in congenitally amputees could lead to an impaired action perception performance (Funk et al., 2005). In keeping, the CAG tested in our study did not report phantom limb sensations.

Another relevant aspect of our results is the fact that the impairment of predictive abilities in congenital amputees was specific for the observed human action (AP task) and the observed absent effector. The motor system shows a distinct sensitivity to the observation of human than non-human movements both at form and kinematic level. Previous behavioral studies support this notion by showing that the observation of real human actions and not robotic actions, selectively interferes with the execution (Kilner et al., 2003; Gowen et al., 2008) and imitation (Brass et al., 2001; Press et al., 2005) of hand movements. Similar results were also obtained during the observation of movements with human rather than non-biological kinematics (Chaminade et al., 2005; Kilner et al., 2007a). Such a biological tuning has been confirmed also for the AON since it shows greater activation for human than non-biological movements (see Press, 2011 for a review). Furthermore, the effector-specific effect found in the current study is in line with the evidence that within the fronto-parietal AON there are spatially segregated representations of different effectors (Buccino et al. 2001). Moreover, action observation has been shown to increase primary motor cortex excitability in an effector-specific manner (Fadiga et al., 1995, 2005; Aziz-Adeh et al., 2002) and

fMRI studies also have shown effector-specific increases of activation within motor cortex (see Aziz-Adeh & Ivry, 2009 for a review).

As said in the introduction of this chapter, recent studies have shown that the integrity of the motor system seems to be crucial for achieving an optimal action perception and prediction. Studies on hemiplegic patients show that their performance in an action recognition task, was selectively impaired when observing actions performed with their affected limb or the corresponding limb of another person. Our results allow to strengthen the idea that action perception was not affected by the non-use of hemiplegic limb, but likely by their lesions to the motor system. Previous studies in patients showing similar impairments in action perception, confirm the critical involvement of the motor system in this processing (Pazzaglia et al., 2008; Moro et al., 2008; Eskenazi et al., 2009; see also Kalénine et al. 2010). In addition, recent evidence from our group (Annella et al. 2012 in prep, chapter 5 of the thesis) shows that the ability to predict others'actions critically relies on the integrity of the IFC.

Future perspectives

A point we did not tackle in the present study is the relationship between the time since traumatic amputation and the variations across time of perceptual and predictive abilities of the subjects. Indeed, the TAG was composed by subjects with a relatively recent amputation ($\leq$ 10 years from the test) and no individual differences emerged according to this variable. Interestingly, Diersch and colleagues (2011) have demonstrated that both age and extensive sensorimotor experience in specific domains lead to an expertise-related benefit in action prediction performance. The authors suggest that representations of actions naturally decline in the aging mind, but the role of expertise is crucial to enable experts to represent actions from their domain of expertise more precisely even

in older age. In keeping with this results, future research could investigate in traumatic amputees how the degree of sensorimotor experience with their limb before the amputation, could differentially affect perceptual and predictive abilities. One might hypothesize a temporal gradient of perceptual effectiveness depending on the time of amputation. According to this hypothesis: more time has elapsed from the amputation and more faded will be the sensorimotor experience related to the missing limb. This trend might lead to less accurate performance in tasks involving action perception and prediction. Put in simpler words: the more you use it the less you lose it.

CHAPTER 7

General Discussion

Seminal studies in monkey's brain have extensively investigated and described the organization principles and the properties of the motor system. It has been shown that : i) the motor cortex is constituted by a series of anatomically and functionally distinct areas; ii) like the motor cortex, the posterior parietal lobe is constituted by a multiplicity of areas with distinct anatomical and functional properties. Each parietal area is involved in the analysis of particular aspects of sensory information ; iii) Motor and parietal areas are reciprocally connected and form a series of specialized circuits working in parallel. These frontoparietal circuits are involved in specific sensorimotor transformations for action and represent the functional units of the motor system (Rizzolatti et al., 1998). In the architecture of the motor system, the ventral premotor cortex (vPMc) exerts a pivotal role in the sensorimotor transformations occurring within this system. This sector of the motor system is crucially involved in action planning and execution. vPMc exerts its role in action by sending fibers to the primary motor cortex (M1) but also with direct connections to the spinal cord. Importantly, the vPMc receives afferents from parietal and somatosensory areas (Rizzolatti & Luppino, 2001) and neurophysiological studies in monkey have well highlighted the sensory functions of vPMc. Indeed this region represents a multimodal station in which sensory information about the stimuli surrounding the body are analyzed and transformed into specific motor programs. Different populations of neurons have been identified in vPMc by means of single cell recordings.

a) In the caudal part of vPMc (monkey area F4), there are multimodal neurons involved in integrating somatosensory information about the body with visual and auditory events occurring within the peripersonal space (Rizzolatti et al., 2000). These cells have also motor properties and are recruited during specific body part (arm, neck, face or mouth) movements (Rizzolatti et al., 1981; Gentilucci et al., 1988). Therefore, these neurons are thought to be involved in transforming sensory information within peripersonal space into appropriate motor plan.

b) In the rostral part of vPMc (monkey area F5) visuomotor neurons which discharge during the execution of specific goal directed actions like grasping, holding, manipulating specific objects, have been found (Rizzolatti et al., 1988). A class of F5 neurons selectively respond to the observation of objects with pragmatic features coherent with the action motorically coded by the neuron, they were called "canonical neurons" (Rizzolatti & Luppino, 2001). Therefore vPMc may play a crucial role in transforming the visual properties of three-dimensional objects into hand shapes appropriate to interact with them.

c) Another class of neurons in F5 fire both when the monkeys perform a specific action and it observes another agent performing a similar action. These neurons were named "mirror neurons" and it has been suggested that they might be involved in the understanding of actions made by others (Gallese et al., 1996; Rizzolatti et al., 1996).

Taken together these findings allow to claim that the motor system can no longer be considered as a mere passive executive system of motor commands generated elsewhere in the brain. On the contrary it is deeply involved in perceptual and cognitive functions concerning for example: processing of spatial information and specific sensorimotor transformations for action in the space (within and outside the PPS), and action perception and understanding. Furthermore, the sensorimotor transformation of sensory information into potential motor acts needs to be anticipatory in order to trigger appropriate responses directed to the environment. In fact, it has been suggested that, as well as the perceptual systems, also the motor system is designed to act in a

predictive manner (see also General introduction section, chapter 1). The benefits of anticipation in perceptual and motor domain are straightforward, since it allows a smooth and effective interaction with the environment. This is true for both animals and humans.

Many studies have attempted to find homologies of the motor system organization and functioning between the two species. Findings reported in monkeys' studies are highly reliable since they come from single-neuron activity recordings. However the invasive nature of this methodology limits its application to animal models. A lot of techniques used in the study of human motor system mainly provide indirect evidence of the investigated phenomena, often leading to controversial and uncertain results which are difficult to compare with monkey findings. The TMS can be considered a non-invasive revolutionary technique in the study of motor system, it allows to study cortical functions by means of magnetic fields applied on the scalp. When applied to M1, TMS allows to probe the excitability of specific cortico-spinal motor representations of the body, with a striking muscle-specificity and temporal resolution; moreover, TMS allows to distinguish between inhibitory and excitatory mechanisms in the studied cortex. Therefore TMS is ideal to non-invasively probe the activity within the motor system. Furthermore, TMS is capable to induce transient "virtual lesions" of discrete brain regions in healthy subjects, providing direct insight into the causal role of a given area in human behavior. This technique has been chosen in 3 studies of this thesis (chapters 2, 3, and 4) with the aim to give more direct evidence of different mechanisms characterizing sensorimotor transformations occurring within the human motor system. Furthermore, also tDCS was used for the studies in chapter 3 and 5. Relative to TMS, tDCS is a less invasive technique with the interesting property to induce polarity-specific excitability changes in the human brain, and has been shown to be effective in altering physiological, perceptual and higher-order cognitive processes (Brunoni et al, 2011). Importantly, the non-invasive nature of this technique mostly relies in the optimal control between sham and real stimulation thanks to the unnoticeable difference of the local skin sensations.

Basing on the insights deriving from monkey studies and combining the methodological approaches described above, the present thesis aims to provide direct evidence on three main issues concerning the human motor system: 1) the anticipatory reactivity of the motor system studied at rest while processing sensory events occurring within the PPS (chapter 2) and the same anticipatory motor mechanisms when perceiving others' implied actions (chapter 4); 2) the functional connectivity and plasticity of premotor-motor circuits both during the motor mapping of sensory events occurring within the PPS (chapter 3) and when perceiving others' implied actions (chapter 4); and 3) the anticipatory mechanisms related to others' actions prediction (chapter 5 and 6). A critical and comprehensive discussion of the main results found in the present project, could be done in the light of these three above mentioned aspects.

The study described in the chapter 2 provides new insights on the differential corticospinal modulation occurring when presenting auditory stimuli within and outside the peri-hand space. By presenting sounds near and far from the body at different time intervals (50, 100, 200, 300 ms) this study explored the spatial and temporal dynamics of the corticospinal responses to auditory stimuli within the PPS.

The results show an enhancement of the motor reactivity (higher MEPs) 50 ms after presenting the sound within the peri-hand space in comparison to when the sound was administered far from the hand. This effect faded when probing motor reactivity 100 and 200 ms after sound presentation and it was completely reversed at 300 ms when the motor reactivity was enhanced by the presentation of far sounds relative to those presented near the hand. Importantly we also provide evidence suggesting that these effects associated with near and far sounds are linked to hand-centred reference frames, since only the hand proximity, and not the head or body proximity to the sound's source, was critical in modulating the excitability of the hand motor representation (see also Makin et al., 2007; Serino et al., 2007). These results, thus highlight the relation between space and time in the PPS representation: an early facilitation for near stimuli reflects immediate motor preparation,

whereas, at later time intervals, motor preparation relates to distant stimuli potentially approaching the body. Overall these modulations may both reflect anticipatory mechanisms in the basic reactivity of the motor system, because even at a resting state, the motor system is prompt to quickly generate potential motor responses to stimuli approaching the body. Hence, these results are in line with the view that the motor system need to be projected into the future in order to trigger effective motor reactions necessary for survival (see evidence reported in the introduction section, chapter 1).

Anticipatory motor responses were also explored when perceiving others' implied actions (Urgesi et al., 2006; 2010). The term implied motion refers to dynamic information extrapolated from static images. Psychophysical studies suggest that static images implying motion, are stored in memory as if the depicted object or living being were indeed moving. Specifically, instead of being recognized in their exact location, objects with implied motion are perceived a bit further along their trajectory. This phenomenon is called "representational momentum" (Freyd & Finke, 1984) and has been demonstrated with a large variety of stimuli (see also General introduction, chapter 1). In keeping with the literature, the study described in the chapter 4 replicates the evidence that in physiological conditions (Out-win session, see experimental design in chapter 4), the motor system shows an increased corticospinal excitability when observing static pictures of an ongoing action compared to a static hand. Importantly, this enhancement of motor reactivity is specific for the muscle recruited in the very same actions (Fadiga et al., 2005; Urgesi et al., 2010). No similar modulations were reported for the observation of static pictures depicting non-biological stimuli (icefall and waterfall). Such results suggest and confirm that the recruitment of the motor system during implied motion perception is specific for human actions and not merely deriving from the presence of implied motion in the visual scene.

Another main issue addressed in this thesis is the exploration of functional connectivity of premotor-motor circuits and their plasticity. Important insights come from experiments reported in

chapters 3 and 4 in which "perturb and measure" paradigms were used. This novel methodological approach derives from studies in nonhuman primates showing that using a cooling procedure to inactivate temporarily an area, disrupted single-cell activity in another area and thus proved that the former area has a causal influence on the latter (see Hupé et al. 1998 for an example). However the invasive nature of the direct interference approach limits its use to animal studies. TMS and tDCS both allow to non-invasively investigate how manipulation of the neural activity in a given area influences responses in another. More importantly the "perturb and measure" approach allows to directly test the causative connectivity between different nodes of a given neural network (Paus, 2005). In the studies of chapters 3 and 4, we induced plastic changes in the ventral premotor area by means of TMS (chapter 3) and tDCS (chapter 4) in order to observe remote effects in the responses of motor system (MEPs recorded by means of spTMS) (Avenanti et al., 2007; Avenanti et al., 2012).

Experiments of chapter 3 aimed at investigating whether the anticipatory reactivity of the motor system to stimuli occurring within the PPS (chapter 2) critically relies on the activity of PPS network. These studies thus shed light on the causative connectivity within the nodes of this network. The PPS network namely tDCS was applied to transiently inhibit the activity of the core regions of the PPS network , namely PMc and PPc (and a control area, V1), whilst motor-evoked potentials (MEPs) to single-pulse TMS over M1 were recorded as a measure of corticospinal excitability during presentation of task-irrelevant sounds near and far from the hand (we used the same experimental setting of experiments described in chapter 2). As shown in chapter 2, the auditory activation of PPS leads to specific modulations of the corticospinal motor system. The results found that the differential effect of near and far sounds on MEPs was selectively abolished after cathodal (inhibitory) tDCS over PMc (and not PPc and V1), showing that this area plays a critical role in the motor coding of sensory events occurring within PPS. These findings shed light on the causative connectivity between the PMc and M1, supported by the strong functional and anatomical link between the two areas (Matelli & Luppino, 2001; Koch et al., 2006). Moreover

these data are consistent with the notion that premotor neurons are critically involved in sensorimotor transformations (Rizzolatti et al., 1997; Rizzolatti et al., 2002; Avenanti et al., 2007; Avenanti & Urgesi, 2011) supporting motor and cognitive functions.

Experiments of chapter 4, give an important contribution to the study of causative connectivity between the nodes of the AON. Low-frequency rTMS was applied to transiently suppress the activity either within (IFC) or upstream (STS) the frontoparietal AON. SpTMS was used to assess the reactivity of M1 during observation of implied action stimuli either within (In-win sessions) or outside (Out-win sessions) the influence of the 'virtual lesions' induced by rTMS. We found that the motor facilitation contingent upon observation of implied action stimuli was selectively disrupted by the suppression of IFC, demonstrating that the anticipatory simulation in M1 is critically linked to the activity of the anterior node of the AON. More importantly, the suppression of STS region resulted in an enhanced reactivity of the motor system to implied action stimuli which clearly hints at an active role of the frontoparietal AON in action simulation. It should be noted that suppression of IFC but not of STS also induced a general reduction of MEP amplitude from both the FDI and ADM muscles, in keeping with evidence that the former but not the latter region contains a hand motor representation functionally related to M1 (Rizzolatti & Luppino 2001; Uozumi et al. 2004; Davare et al. 2009). These findings support the notion that inhibiting hand representations in premotor regions reduces hand corticospinal excitability (Gerschlager et al. 2001; O'Shea et al. 2007) and further establish the facilitatory functional connectivity between IFC and M1 (Shimazu et al. 2004; Avenanti et al. 2007). The disruption of action simulation observed after IFC-rTMS, however, is unlikely to be due to the indirect inhibitory effect of IFC-rTMS on M1 activity. Indeed, it has previously shown that although both IFC-rTMS and M1-rTMS induce a reduction of corticospinal excitability, suppression of IFC but not of M1 disrupts the action observation motor facilitation (Avenanti et al. 2007).

It should be noted that in the experiments of chapter 3, the suppression of activity in PMc did not induce a general reduction of MEP amplitude. This lack of modulation of excitability may be due to

a number of methodological factors. First, in the study of chapter 3, tDCS and not TMS was used to induce transient "virtual lesions" in the PMc. It could be that TMS is in general more adept to induce changes in the corticospinal excitability relative to tDCS. However, the absence of corticospinal modulation may be also related to the relatively low current intensity used in the study (i.e. 1 mA); this intensity may be sufficient to disrupt activity within the target cortical area but not enough powerful to influence the excitability of remote neural regions (e.g. M1). Moreover, the post-tDCS sessions were performed in two different days, with an inter-session interval of at least 1 week, while the three sessions of TMS experiments (Out-win and In-win sessions) were separated by 90 minutes.

Finally, this thesis adds notions to the topic of action perception and prediction. In particular, experiments in chapter 5 assessed whether the abilities to perceive and predict others' actions could be affected by induced plastic changes in the IFC. Notably, it has been shown that off-line suppression of IFC the anticipatory simulation of future phases of seen actions (Avenanti et al., 2012b; chapter 4 of the thesis), suggesting a pivotal role of IFC in the predictive motor coding of others' motor acts.

The activity of both left and right IFC was suppressed by means of cathodal tDCS in two experiments, while in a third experiment the activity of the left IFC was also enhanced by means of anodal tDCS. The results show that the inhibition of the left IFC, but not of the right IFC, impairs prediction of right-hand actions but not of non-biological movements. Moreover, they indicate that worsening of action prediction is specific when inhibitory (cathodal) not excitatory (anodal) tDCS is applied to the left IFC, which may suggest that in the intact brain non-invasive induction of plasticity can disrupt but not potentiate action prediction ability. These data support the view that the IFC is a core region in the AON involved not only in planning and executing motor acts, but also in the perception and prediction of others' actions (Avenanti and Urgesi, 2011; Avenanti et al., 2012) and suggest a left frontal lateralization in the predictive coding of others' right-hand actions.

Furthermore, the study described in the chapter 6 provides evidence of how a reduction of sensorimotor experience with a limb could affect the abilities to perceive and predict others' actions. To this aim, two emblematic models were tested: the congenital and the traumatic upper-limb amputees. The results show that, despite an overall "non-pathological" performance of both groups (compared to normally limbed subjects), only congenital amputees were impaired in predicting the future end-state of observed hands actions when these were performed with their missing hand compared to the intact one. Strikingly, this performance was also lower if compared to that obtained when predicting non-biological movements corresponding to both missing and intact limb side, thus indicating a specific impairment in the predictive coding of observed biological actions. These results clearly demonstrate that an optimal perception and prediction of others' actions is not hampered by the current absence of a limb per se, rather by its absence from birth and the contingent lack of sensorimotor experience with the limb performing that action.

Despite a long history of studies in the field of the "amputee brain", to our knowledge, these data represent a first attempt to investigate the predictive abilities in subjects suffering from congenital and traumatic upper-limb amputation.

REFERENCES

Abreu, A. M., Macaluso, E., Azevedo, R. T., Cesari, P., Urgesi, C., Aglioti, S. M. (2012). Action anticipation beyond the action observation network: a functional magnetic resonance imaging study in expert basketball players. *Eur J Neurosci*, 35, 1646-1654.

Aglioti, S.M., Cesari, P., Romani, M., Urgesi, C. (2008). Action anticipation and motor resonance in elite basketball players. *Nat Neurosci*, 11: 1109-1116.

Aglioti, S.M., Pazzaglia, M. (2011). Sounds and scents in social action. *Trends Cogn Sci*. 15:47-55.

Alaerts, K., de Beukelaar, T.T., Swinnen, S.P., Wenderoth, N. (2011). Observing how others lift light or heavy objects: Time dependent encoding of grip force in the primary motor cortex. *Psychol Res*. Sep 20. [Epub ahead of print].

Alaerts, K., Swinnen, S. P., Wenderoth, N. (2011). Action perception in individuals with congenital blindness or deafness: how does the loss of a sensory modality from birth affect perception-induced motor facilitation? *J cogn neurosci*, 23 (5): 1080-1087.

Avenanti, A., Annella, L., Candidi, M., Urgesi, C., Aglioti, S.M. (2012 b). Compensatory plasticity in the action observation network: virtual lesions of STS enhance anticipatory simulation of seen actions. *Cerebral Cortex*, Mar 16. [Epub ahead of print].

Avenanti, A., Annella, L., Serino, A. (2012c): Suppression of premotor cortex disrupts motor coding of peripersonal space. *Neuroimage*, 63(1): 281-288. Epub 2012 Jul 6.

Avenanti, A., Bolognini, N., Maravita, A., Aglioti, S.M. (2007). Somatic and motor components of action simulation. *Curr Biol*. 17: 2129-2135.

Avenanti, A., Coccia, M., Làdavas, E., Provinciali, L., Ceravolo, M.G. (2012a). Lowfrequence rTMS promotes use-dependent motor plasticity in chronic stroke: a randomized trial. *Neurology*, 78: 256-264.

Avenanti, A., Minio-Paluello, I., Bufalari, I., Aglioti, S.M. (2009). The pain of a model in the personality of an onlooker: influence of state-reactivity and personality traits on embodied empathy for pain. *Neuroimage*, 44: 275-283.

Avenanti, A., Minio-Paluello, I., Sforza, A., Aglioti, S.M. (2009b). Freezing or escaping? Opposite modulations of empathic reactivity to the pain of others. *Cortex*, 45: 1072-1077.

Avenanti, A., Urgesi, C. (2011). Understanding 'what' others do: mirror mechanisms play a crucial role in action perception. *Soc Cogn Affect Neurosci*, 6: 257-259.

Avillac, M., Deneve, S., Olivier, E., Pouget, A., Duhamel, J.R. (2005). Reference frames for representing visual and tactile locations in parietal cortex. *Nat Neurosci,* 8: 941-949.

Aziz-zadeh, L., Ivry, R.B. (2009). The human mirror neuron system and embodied representations. (D. Sternad, Ed.) *Adv Exp Med Biol*, 629: 355-376.

Aziz-Zadeh, L., Maeda, F., Zaidel, E., Mazziotta, J., Iacoboni, M. (2002). Lateralization in motor facilitation during action observation: a TMS study. *Exp Brain Res*, 144: 127-131.

Aziz-Zadeh, L., Maeda, F., Zaidel, E., Mazziotta, J., Iacoboni, M. (2002). Lateralization in motor facilitation during action observation: a TMS study. *Exp. Brain Res*, 144: 127–131.

Baldissera, F., Cavallari, P., Craighero, L., Fadiga, L. (2001). Modulation of spinal excitability during observation of hand actions in humans. *Eur J Neurosci, 13*, 190-194.

Barchiesi, G., Cattaneo, L. (2012). Early and late motor responses to action observation. *Soc Cogn Affect Neurosci*, May 27. [Epub ahead of print]

Barsalou, L. W. (2008). Grounded Cognition. *Annual Review of Psychology*, *59*, 617-645.

Bassolino, M., Serino, A., Ubaldi, S., Làdavas, E. (2010). Everyday use of the computer mouse extends peripersonal space representation. *Neuropsychologia*, 48: 803—811.

Bertini, C., Leo, F., Avenanti, A., Làdavas, E. (2010). Independent mechanisms for ventriloquism and multisensory integration as revealed by theta-burst stimulation. *Eur. J. Neurosci*, 31: 1791-1799.

Blakemore, S.J., Decety, J. (2001). From the perception of action to the understanding of intention. *Nat Rev Neurosci,* 2(8): 561-567.

Bolognini, N., Fregni, F., Casati, C., Olgiati, E., Vallar, G. (2010b). Brain polarization of parietal cortex augments training-induced improvement of visual exploratory and attentional skills. *Brain research*, 1349: 76-89.

Bolognini, N., Olgiati, E., Rossetti, A., Maravita, A. (2010a) Enhancing multisensory spatial orienting by brain polarization of the parietal cortex. *Eur J Neurosci*, 31(10): 1800-1806.

Borghi, A. M., Cimatti, F. (2010). Embodied cognition and beyond: acting and sensing the body. *Neuropsychologia*, *48*(3), 763-73.

Borgomaneri, S., Gazzola, V., Avenanti, A. (2012). Motor mapping of implied actions during perception of emotional body language. *Brain Stimul*, 5(2): 70-76. [Epub 2012 Mar 31].

Borroni, P., Montagna, M., Cerri, G., Baldissera, F. (2005). Cyclic time course of motor excitability modulation during the observation of a cyclic hand movement. *Brain Res*, 1065: 115-124.

Brasil-Neto, J.P., Cohen, L.G., Panizza, M., Nilsson, J., Roth, B.J., Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil

orientation, shape of the induced current pulse, and stimulus intensity. *J Clin Neurophysiol*, 9: 132-136.

Brass, M., Bekkering, G., Prinz, W. (2001). Movement observation affects movement execution in a simple response task. *Acta Psychol*, (Amst.) 106: 3-22.

Bremmer, F., Duhamel, J.R., Ben Hamed, S., Graf, W. (2002). Heading encoding in the macaque Ventral Intraparietal Area (VIP). *Eur J Neurosci*, 16: 1554-1568.

Bremmer, F., Schlack, A., Shah, N.J., Zafiris, O., Kubischik, M., Hoffmann, K., et al. (2001). Polymodal Motion Processing in Posterior Parietal and Premotor Cortex: A Human fMRI Study Strongly Implies Equivalencies between Humans and Monkeys. *Neuron*, 29: 287-296.

Brozzoli, C., Gentile, G., Petkova, V.I., Ehrsson, H.H. (2011). FMRI adaptation reveals a cortical mechanism for the coding of space near the hand. *J Neurosci*, 31: 9023-9031.

Brunoni, A.R., Nitsche, M.A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., Edwards, D.J., Valero-Cabre, A., Rotenberg, A., Pascual-Leone, A., Ferrucci, R., Priori, A., Boggio, P.S., Fregni, F. (2011). Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions. *Brain Stimul*, Apr 1. [Epub ahead of print].

Brugger, P., Kollias, S.S., Müri, R.M., Crelier, G., Hepp-Reymond, M.C., Regard, M. (2000). Beyond re-membering: phantom sensations of congenitally absent limbs. *Proc Natl Acad Sci U S A*, 97(11):6167-72.

Bubic, A., von Cramon, D. Y., Schubotz, R. I. (2010). Prediction, cognition and the brain. *Frontiers in human neuroscience*, 4 (March), 25.

Buccino, G., Binkofski, F., Fink, G.R., Fadiga, L., Fogassi, L., Gallese, V., et al. (2001). Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. *Eur J Neurosci*, 13: 400–404.

Cabinio, M., Blasi, V., Borroni, P., Montagna, M., Iadanza, A., Falini, A., Cerri, G. (2010). The shape of motor resonance: right- or left-handed? *Neuroimage*, May 15; 51(1): 313-23.

Candidi, M., Stienen, B.M., Aglioti, S.M., de Gelder, B. (2011). Event-related repetitive transcranial magnetic stimulation of posterior superior temporal sulcus improves the detection of threatening postural changes in human bodies. *J Neurosci*, 31: 17547-17554.

Candidi, M., Urgesi, C., Ionta, S., Aglioti, S.M. (2008). Virtual lesion of ventral premotor cortex impairs visual perception of biomechanically possible but not impossible actions. *Soc neurosci*, 3 (3-4): 388-400.

Candidi, M., Vicario, C.M., Abreu, A.M., Aglioti, S.M. (2010). Competing mechanisms for mapping action-related categorical knowledge and observed actions. *Cereb Cortex*, 20: 1018-1022.

Cantello, R., Civardi, C., Cavalli, A., Varrasi, C., Vicentini, R. (2000). Effects of a photic input on the human cortico-motoneuron connection. *Clin Neurophysiol*, 111: 1981-1989.

Cardellicchio, P., Sinigaglia, C., Costantini, M. (2011). The space of affordances: a TMS study. *Neuropsychologia*, 49:1369-1372.

Casile, A., Caggiano, V., & Ferrari, P. F. (2011). The mirror neuron system: a fresh view. *The Neuroscientist*, 17(5), 524-38.

Casile, A., Dayan, E., Caggiano, V., Hendler, T., Flash, T., Giese, M.A., (2010). Neuronal encoding of human kinematic invariants during action observation. *Cereb cortex*, 20 (7): 1647-1655.

Casile, A., Giese, M.A. (2006). Nonvisual motor training influences biological motion perception. *Curr biol*, 16 (1): 69-74.

Caspers, S., Zilles, K., Laird, A.R., Eickhoff, S.B. (2010). ALE meta-analysis of action observation and imitation in the human brain. *Neuroimage*, 50: 1148-1167.

Catmur, C., Gillmeister, H., Bird, G., Liepelt, R., Brass, M., Heyes, C. (2008). Through the looking glass: counter-mirror activation following incompatible sensorimotor learning. *Eur J neurosci*, 28 (6): 1208-15.

Catmur, C., Mars, R.B., Rushworth, M.F., Heyes, C. (2011). Making mirrors: premotor cortex stimulation enhances mirror and counter-mirror motor facilitation. *J Cogn Neurosci*, 23: 2352-2362.

Catmur, C., Walsh, V. (2007). Report Sensorimotor Learning Configures the Human Mirror System. *Curr Biol*, 1527-1531.

Cattaneo, L., Barchiesi, G., Tabarelli, D., Arfeller, C., Sato, M., Glenberg, A.M. (2011). One's motor performance predictably modulates the understanding of others' actions through adaptation of premotor visuo-motor neurons. *Soc Cogn Affect Neurosci*, 6: 301-310.

Cattaneo, L., Voss, M., Brochier, T., Prabhu, G., Wolpert, D.M., Lemon, R.N. (2005). A cortico-cortical mechanism mediating object-driven grasp in humans. *Proc Natl Acad Sci USA*. 102: 898-903.

Cavada, C., Goldman-Rakic, P.S. (1989). Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and limbic areas with the frontal lobe. *J Comp Neurol*, 287: 422-445.

Cerri, G., Shimazu, H., Maier, M.A., Lemon, R.N. (2003). Facilitation from ventral premotor cortex of primary motor cortex outputs to macaque hand muscles. *J Neurophysiol*, 90: 832-842.

Chaminade, T., Franklin, D.W., Oztop, E., Cheng, G. (2005). Motor interference between humans and humanoid robots: effect of biological and artificial motion. In: Proceedings of 2005 4th IEEE International Conference on Development and Learning , pp. 96–101.

Chaminade, T., Kawato, M. (2010). Mutual benefits of using humanoid robots in social neuroscience. In: Caccioppo, J., Decety, J. (Eds.), *Handbook of Social Neuroscience*, Oxford University Press, Oxford, pp. 974–991.

Chao, L.L., Martin, A. (2000). Representation of manipulable man-made objects in the dorsal stream. *Neuroimage,* 12: 478-484.

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E.M., Hallett, M., Cohen, L.G. (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, 48: 1398-1403.

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaicher, J.P., Magistris, M.R., Mills, K., Rösler, K.M., Triggs, W.J., Ugawa, Y., Ziemann, U. (2008). The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*, 119: 504-532.

Chong, T.T., Cunnington, R., Williams, M.A., Kanwisher, N., Mattingley, J.B. (2008). fMRI adaptation reveals mirror neurons in human inferior parietal cortex. *Curr Biol*, 18: 1576-1580.

Classen, J., Steinfelder, B., Liepert, J., Stefan, K., Celnik, P., Cohen, L.G., et al. (2000). Cutaneomotor integration in humans is somatotopically organized at various levels of the nervous system and is task dependent. *Exp Brain Res*, 130: 48-59.

Cogiamanian, F., Marceglia, S., Ardolino, G., Barbieri, S., Priori, A. (2007). Improved Isometric Force Endurance after Transcranial Direct Current Stimulation Over the Human Motor Cortical Areas. *Eur J Neurosci*, 26(1): 242-249.

Cohen, L.G., Bandinelli, S., Findley, T.W., Hallett, M. (1991). Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. *Brain*, 114 (Pt 1B): 615-627.

Colby, C.L., Duhamel, J.R.(1996). Spatial representations for action in parietal cortex. *Brain Res Cogn Brain Res*, 5(1-2):105-115.

Colby, C.L., Duhamel, J.R., Goldberg, M.E. (1993). Ventral intraparietal area of the macaque: anatomic location and visual response properties. *J Neurophysiol*, 69: 902-914.

Cooke D.F., Graziano, M.S. (2004). Sensorimotor integration in the precentral gyrus: polysensory neurons and defensive movements. *J Neurophysiol*, 91: 1648-1660.

Cooke, D.F., Taylor, C.S., Moore, T., Graziano, M.S. (2003). Complex movements evoked by microstimulation of the ventral intraparietal area. *Proc. Natl. Acad. Sci. USA*, 100: 6163-6168.

Cross, E. S., Hamilton, A. F. D. C., Grafton, S. T. (2006). Building a motor simulation de novo: observation of dance by dancers. *NeuroImage,* 31 (3): 1257–1267.

Cross, E. S., Hamilton, A. F. D. C., Kraemer, D. J. M., Kelley, W. M., Grafton, S. T. (2009a). Dissociable substrates for body motion and physical experience in the human action observation network. *Eur J Neurosci*, 30 (7), 1383–1392.

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., Bikson, M. (2009). Gyri -precise head model of transcranial DC stimulation: Improved spatial focality using a ring electrode versus conventional rectangular pad. *Brain Stimul*, 2: 201-207.

D'Ausilio, A., Bufalari, I., Salmas, P., Fadiga, L. (2011). The role of the motor system in discriminating normal and degraded speech sounds. *Cortex*, In press [Epub May 27 2011].

Davare, M., Lemon, R., Olivier, E. (2008). Selective modulation of interactions between ventral premotor cortex and primary motor cortex during precision grasping in humans. *J Physiol*, 586 (Pt 11): 2735-2742.

Davare, M., Montague, K., Olivier, E., Rothwell, J.C., Lemon, R.N. (2009). Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. *Cortex*, 45: 1050-1057.

Dayan, E., Casile, A., Levit-binnun, N., Giese, M. A., Hendler, T., Flash, T. (2007). Neural representations of kinematic laws of motion: Evidence for action-perception coupling. *PNAS*, *104* (51): 20582-20587.

Decety, J., Grèzes, J., Costes, N., Perani, D., Jeannerod, M., Procyk, E., Grassi, F., Fazio, F. (1997). Brain activity during observation of actions. Influence of action content and subject's strategy. *Brain*, 120 (Pt 10): 1763-77.

Del Giudice, M., Manera, V., Keysers, C. (2009). Programmed to learn? The ontogeny of mirror neurons. *Developmental science*, 12(2): 350-63.

Di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., Rizzolatti, G. (1992). Understanding motor events: a neurophysiological study. *Exp Brain Res*, 91: 176-180.

Di Pellegrino, G., Làdavas, E., Farné, A. (1997). Seeing where your hands are. *Nature*, 388: 730.

Diersch, N., Cross, E. S., Stadler, W., Schütz-Bosbach, S., Rieger, M. (2011). Representing others' actions: the role of expertise in the aging mind. *Psychol res*, Dec 24. [Epub ahead of print]

Duhamel, J.R., Colby, C.L., Goldberg, M.E. (1998). Ventral intraparietal area of the macaque: congruent visual and somatic response properties. *J Neurophysiol*, 79: 126-136.

Dum, R.P., Strick, P.L. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *J Neurosci*, 11: 667-689.

Dum, R.P., Strick, P.L. (2002). Motor areas in the frontal lobe of the primate. *Physiol Behav*, 77: 677-682.

Dum, R.P., and Strick, P.L. (2005). Frontal lobe inputs to the digit representations of the

motor areas on the lateral surface of the hemisphere. *J Neurosci*, 25: 1375-1386.

Engel, A., Burke, M., Fiehler, K., Bien, S., Rösier, F. (2008). Howmoving objects become animated: The human mirror neuron system assimilates non-biological move- ment patterns. *Soc Neurosc*, 3: 368–387.

Eskenazi, T., Grosjean, M., Humphreys, G. W., Knoblich, G. (2009). The role of motor simulation in action perception: a neuropsychological case study. *Psychol res*, 73 (4): 477-85.

Etzel, J.A., Gazzola, V., Keysers, C. (2008). Testing simulation theory with cross-modal multivariate classification of fMRI data. *PLoS One*, 311: e3690.

Facchini S., Romani, M., Tinazzi, M., Aglioti, S.M., (2002). Time-related changes of excitability of the human motor system contingent upon immobilisation of the ring and little fingers. *Clin Neurophysiol*, 113: 367-375.

Fadiga, L., Craighero, L., Olivier, E. (2005). Human motor cortex excitability during the perception of others' action. *Curr Opin Neurobiol*, 15: 213-218.

Farina, S., Valeriani, M., Rosso, T., Aglioti, S.M., Tamburin, S., Fiaschi, A., Tinazzi, M. (2001). Transient inhibition of the human motor cortex by capsaicin-induced pain. A study with transcranial magnetic stimulation. *Neurosci Lett*, 314: 97-101.

Farné, A., Làdavas, E. (2002). Auditory peripersonal space in humans. *J Cogn Neurosci*, 14: 1030-1043.

Fazio, P., Cantagallo, A., Craighero, L., D'Ausilio, A., Roy, A.C., Pozzo, T., Calzolari, F., Granieri, E., Fadiga, L. (2009). Encoding of human action in Broca's area. *Brain*, 132: 1980-1988.

Fisher, R.J., Sharott, A., Kuhn, A.A., Brown, P. (2004). Effects of combined cortical and acoustic stimuli on muscle activity. *Exp Brain Res*, 157: 1-9.

Flor, H., Nikolajsen, L., Staehelin Jensen, T. (2006). Phantom limb pain: A case of maladaptive CNS plasticity? *Nat Rev Neurosci*, 7: 873–881.

Fogassi, L., Ferrari, P.F., Gesierich, B., Rozzi, S., Chersi, F., Rizzolatti, G. (2005). Parietal lobe: from action organization to intention understanding. *Science*, 308: 662-667.

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Matelli, M., Rizzolatti, G. (1996). Coding of peripersonal space in inferior premotor cortex (area F4). *J Neurophysiol*, 76: 141-157.

Fogassi, L., Luppino, G. (2005). Motor functions of the parietal lobe. *Curr Opin Neurobiol*, 15: 626-631.

Fourkas, A.D., Bonavolontà, V., Avenanti, A., Aglioti, S.M. (2008). Kinesthetic imagery and tool-specific modulation of corticospinal representations in expert tennis players. *Cereb Cortex*, 18: 2382-2390.

Freyd, J. J., Finke, R. A. (1984). Representational momentum. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, 10: 126–132.

Friston, K., Mattout, J., Kilner, J. (2011). Action understanding and active inference. *Biol Cybern*, 104: 137-160.

Funk, M., Shiffrar, M., Brugger, P. (2005). Hand movement observation by individuals born without hands: phantom limb experience constrains visual limb perception. *Exp Brain Res*, 164(3): 341-6.

Furubayashi, T., Hanajima, R., Sakai, K., Machii, K., Mochizuki, H., Shiio, Y., Uesugi, H., Enomoto, H., Kanazawa, I. (2000). The human hand motor area is transiently suppressed by an unexpected auditory stimulus. *Clin. Neurophysiol*, 111: 178-183.

Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain*, 119: 593-609.

Gallese, V., Fogassi, L., Fadiga, L. & Rizzolatti, G. (2002). Action representation and the inferior parietal lobule. In W. Prinz and B. Hommel. (Eds.), *Common Mechanisms in Perception and Action: Attention and Performance* (Vol. XIX, pp. 247-266). New York, Oxford University Press.

Gallese, V., Rochat, M., Cossu, G., Sinigaglia, C. (2009). Motor cognition and its role in the phylogeny and ontogeny of action understanding. *Dev Psychol*, 45 (1): 103-13.

Gandiga, P.C., Hummel, F.C., Cohen, L.G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin Neurophysiol*, 117: 845-850. [Epub 2006 Jan 19].

Gangitano, M., Mottaghy, F.M., Pascual-Leone, A. (2004). Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements. *Eur J Neurosci*, 20: 2193–2202.

Gazzola, V., Keysers, C. (2009). The observation and execution of actions share motor and somatosensory voxels in all tested subjects: single-subject analyses of unsmoothed fMRI data. *Cereb Cortex*, 19: 1239-1255.

Gazzola, V., van der Worp, H., Mulder, T., Wicker, B., Rizzolatti, G., Keysers, C. (2007). Aplasics born without hands mirror the goal of hand actions with their feet. *Curr Biol*, 17: 1235-1240.

Gentile, G., Petkova, V.I., Ehrsson, H.H. (2011). Integration of visual and tactile signals from the hand in the human brain: an FMRI study. *J. Neurophysiol*, 105: 910-922. [Epub 2010 Dec8].

Gerschlager, W., Siebner, H.R., Rothwell, J.C. (2001). Decreased corticospinal excitability after subthreshold 1 Hz rTMS over lateral premotor cortex. *Neurology*, 57: 449-455.

Geyer, S., Matelli, M., Luppino, G. and Zilles, K. (2000) Functional neuroanatomy of the primate isocortical motor system. *Anat Embryol*, 202, 443–474.

Giese, M.A., Poggio, T. (2003). Neural mechanisms for the recognition of biological movements. *Nat Rev Neurosci*, 4: 179-192.

Glenberg, A.M., Lopez-Mobilia, G., McBeath, M., Toma, M., Sato, M., Cattaneo, L. (2010). Knowing beans: human mirror mechanisms revealed through motor adaptation. *Front Hum Neurosci*, 4; 4:206.

Gowen, E., Stanley, J., Miall, R.C. (2008). Movement interference in autism-spectrum disorder. *Neuropsychologia*, 7, 46 (4): 1060-1068.

Grafton, S.T. (2009). Embodied cognition and the simulation of action to understand others. *Ann N Y Acad Sci*, 1156: 97-117.

Graziano, M.S., Aflalo, T.N. (2007). Mapping behavioral repertoire onto the cortex. Neuron 56: 239-251.

Graziano, M.S., Cooke, D.F. (2006). Parieto-frontal interactions, personal space, and defensive behavior. *Neuropsychologia*, 44: 845-859.

Graziano, M.S., Hu, X.T., Gross, C.G. (1997). Visuospatial properties of ventral premotor cortex. *J Neurophysiol*, 77: 2268-2292.

Graziano, M.S., Reiss, L.A., Gross, C.G. (1999). A neuronal representation of the location of nearby sounds. *Nature*, 397: 428-30.

Graziano, M.S., Taylor, C.S., Moore, T. (2002). Complex movements evoked by microstimulation of precentral cortex. *Neuron*, 34: 841-51.

Graziano, M.S., Yap, G.S., Gross, C.G. (1994). Coding of visual space by premotor neurons. *Science*, 266: 1054-1057.

Grefkes C, Fink, G.R. (2005). The functional organization of the intraparietal sulcus in humans and monkeys. *J Anat*, 207: 3-17.

Grosbras, M.H., Beaton, S., Eickhoff, S.B. (2012). Brain regions involved in human movement perception: a quantitative voxel-based meta-analysis. *Hum Brain Mapp*, 33 (2): 431-54.

Grossman, E., Donnelly, M., Price, R., Pickens, D., Morgan, V., Neighbor, G., Blake, R. (2000). Brain areas involved in perception of biological motion. *J Cogn Neurosci*, 12: 711-720.

Grossman, E.D., Battelli, L., Pascual-Leone, A. (2005). Repetitive TMS over posterior STS disrupts perception of biological motion. *Vision Res*, 45: 2847-2853.

Grush, R. (2004). The emulation theory of representation: motor control, imagery, and perception. *The Behavioral and brain sciences*, 27 (3): 377-96; discussion 396-442.

Hallett, M., Chen, R., Ziemann, U., Cohen, L.G. (1999). Reorganization in motor cortex in amputees and in normal volunteers after ischemic limb deafferentation. *Electroencephalogr Clin Neurophysiol Suppl,* 51: 183-187.

Harries, M. H., Perrett, D. I. (1991). Visual processing of faces in the temporal cortex: Physiological evidence for a modular organization and possible anatomical correlates. *J Cogn Neurosci,* 3: 9–24.

He, S.Q., Dum, R.P., Strick, P.L. (1993). Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. *J Neurosci,* 13: 952-980.

He, S.Q., Dum, R.P., Strick, P.L. (1995). Topographic organization of corticospinal projections from the frontal lobe: motor areas on the medial surface of the hemisphere. *J. Neurosci,* 15: 3284-3306.

Heiser, M., Iacoboni, M., Maeda, F., Marcus, J., Mazziotta, J.C. (2003). The essential role of Broca's area in imitation. *Eur J Neurosci,* 17: 1123-1128.

Hess, C.W., Mills, K.R., Murray, N.M. (1986). Magnetic stimulation of the human brain: facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee. *Neurosci Lett,* 71: 235-240.

Heyes C. (2001). Causes and consequences of imitation. *Trends Cogn Sci,* 5: 253-261.

Heyes, C. (2010). Where do mirror neurons come from? *Neuroscience and biobehavioral reviews,* 34 (4), 575-583.

Hickok, G. (2009). Eight problems for the mirror neuron theory of action understanding in monkeys and humans. *J Cogn Neurosci*, 21: 1229-1243.

Hupé, J.M., James, A.C., Payne, B.R., Lomber, S.G., Girard, P., Bullier, J. (1998). Cortical feedback improves discrimination between figure and background by V1, V2 and V3 neurons. *Nature,* 394: 784-787.

Ishida, H., Nakajima, K., Inase, M., Murata, A. (2009). Shared mapping of own and others' bodies in visuotactile bimodal area of monkey parietal cortex. *J Cogn Neurosci*, 22: 83–96.

Jackson, R. C., Warren, S., Abernethy, B. (2006). Anticipation skill and susceptibility to deceptive movement. *Acta psychologica*, 123 (3): 355-71.

Jacobson, L., Koslowsky, M., Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Experimental brain research*, 216 (1): 1-10.

Jeannerod, M. (1997). *The Cognitive Neuroscience of Action*. Oxford, Blackwell. 256 p.

Jellema, T., Perrett, D.I. (2006). Neural representations of perceived bodily actions using a categorical frame of reference. *Neuropsychologia*, 44: 1535-46.

Jellema, T., Perrett, D.I. (2003). Cells in monkey STS responsive to articulated body motions and consequent static posture: a case of implied motion? *Neuropsychologia*, 41: 1728-1737.

Johnson-Frey, S.H., Maloof, F.R., Newman-Norlund, R., Farrer, C., Inati, S., Grafton, S.T. (2003). Actions or hand-object interactions? Human inferior frontal cortex and action observation. *Neuron*, 39: 1053-1058.

Kalénine, S., Buxbaum, L.J., Coslett, H.B. (2010). Critical brain regions for action recognition: lesion symptom mapping in left hemisphere stroke. *Brain*, 133 (11): 3269-80.

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., Brunelin, J., Möller, H.J., Reiser, M., Padberg, F. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J Neurosci*, 31 (43): 15284-93.

Keselman, H.J., Algina, J., Kowalchuk, R.K. (2001). The analysis of repeated measures designs: a review. *Br J Math Stat Psychol*, 54: 1-20.

Kessler, K., Biermann-Ruben, K., Jonas, M., Siebner, H.R., Baumer, T., Munchau, A., Schnitzler, A. (2006). Investigating the human mirror neuron system by means of cortical synchronization during the imitation of biological movements. *Neuroimage*, 33: 227–238.

Keysers, C., Perrett, D.I. (2004). Demystifying social cognition: a Hebbian perspective. *Trends Cogn Sci*, 8: 501-507.

Kilner, J.M., Friston, K.J., Frith, C.D. (2007b). Predictive coding: an account of the mirror neuron system. *Cogn Process*, 8: 159-166.

Kilner, J.M., Friston, K.J., Frith, C.D. (2007b). The mirror-neuron system: a Bayesian perspective. *Neuroreport*, 18 (6): 619-23.

Kilner, J.M., Neal, A., Weiskopf, N., Friston, K.J., Frith, C.D. (2009). Evidence of mirror neurons in human inferior frontal gyrus. *J Neurosci*, 29: 10153-10159.

Kilner, J.M., Paulignan, Y., Blakemore, S.J. (2003). An interference effect of observed biological movement on action. *Curr Biol*, 18; 13 (6): 522-525.

Kilner, J.M., Vargas, C., Duval, S., Blakemore, S.J. Sirigu, A., (2004). Motor activation prior to observation of a predicted movement. *Nat Neurosci*, 7: 1299-1301.

Koch, G., Cercignani, M., Pecchioli, C., Versace, V., Oliveri, M., Caltagirone, C., Rothwell, J., Bozzali, M. (2010). In vivo definition of parieto-motor connections involved in planning of grasping movements. *Neuroimage*, 51: 300-312.

Koch, G., Fernandez Del Olmo, M., Cheeran, B., Schippling, S., Caltagirone, C., et al. (2008). Functional interplay between posterior parietal and ipsilateral motor cortex revealed by twin-coil transcranial magnetic stimulation during reach planning toward contralateral space. *J Neurosci*, 28 (23): 5944-5953.

Koch, G., Franca, M., Del Olmo, M.F., Cheeran, B., Milton, R., Alvarez Sauco, M., Rothwell, J.C. (2006). Time course of functional connectivity between dorsal premotor and contralateral motor cortex during movement selection, *J. Neurosci*, 26: 7452-7459.

Koch, G., Rothwell, J.C. (2009). TMS investigations into the task-dependent functional interplay between human posterior parietal and motor cortex. *Behav Brain Res*, 202: 147-152.

Koch, G., Versace, V., Bonnì, S., Lupo, F., Lo Gerfo, E., Oliveri, M., Caltagirone C. (2010). Resonance of cortico-cortical connections of the motor system with the observation of goal directed grasping movements. *Neuropsychologia*, 48: 3513-3520.

Kooijman, C.M., Dijkstra, P.U., Geertzen, J.H., Elzinga, A., van der Schans, C.P. (2000). Phantom pain and phantom sensations in upper limb amputees: an epidemiological study. *Pain*, 87 (1): 33-41.

Kourtzi, Z., Kanwisher, N. (2000). Activation in human MT/MST by static images with implied motion. *J Cogn Neurosci*, 12: 48-55.

Kraskov, A., Dancause, N., Quallo, M.M., Shepherd, S., Lemon, R.N. (2009). Corticospinal neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? *Neuron*, 64: 922-930.

Kuhn, A.A., Brandt, S.A., Kupsch, A., Trottenberg, T., Brocke, J., Irlbacher, K., et al. (2004). Comparison of motor effects following subcortical electrical stimulation through electrodes in the globus pallidus internus and cortical transcranial magnetic stimulation. *Exp Brain Res*, 155: 48-55.

Làdavas, E., Serino, A. (2008). Action-dependent plasticity in peripersonal space representations. *Cogn Neuropsychol*, 25: 1099-1113.

Lang, P.J., Bradley, M.M., Cuthbert, B.N. (1990). Emotion, attention, and the startle reflex. *Psychol Rev*, 97: 377-395.

Luppino, G., Murata, A., Govoni, P., Matelli, M. (1999). Largely segregated parietofrontal connections linking rostral intraparietal cortex (areas AIP and VIP) and the ventral premotor cortex (areas F5 and F4). *Exp Brain Res*, 128:181-187.

MacKay, D. (1958). Perceptual stability of a stroboscopically lit visual field containing self luminous objects. *Nature*, 181: 507-508.

Maeder, P.P., Meuli, R.A., Adriani, M., Bellmann, A., Fornari, E., Thiran, J.P., Pittet, A., Clarke, S. (2001). Distinct pathways involved in sound recognition and localization: a human fMRI study. *Neuroimage*, 14: 802-816.

Makin, T.R., Holmes, N.P., Brozzoli, C., Rossetti, Y., Farnè, A. (2009). Coding of visual space during motor preparation: Approaching objects rapidly modulate corticospinal excitability in hand-centered coordinates. *J Neurosci*, 29: 11841-11851.

Makin, T.R., Holmes, N.P., Zohary, E. (2007). Is that near my hand? Multisensory representation of peripersonal space in human intraparietal sulcus. *J Neurosci*, 27: 731–740.

Manganotti, P., Zanette, G., Bonato, C., Tinazzi, M., Polo, A., Fiaschi, A. (1997). Crossed and direct effects of digital nerves stimulation on motor evoked potential: a study with magnetic brain stimulation. *Electroencephal Clin Neurophysiol*, 105: 280-289.

Matelli, M., Luppino, G. (2000). Parietofrontal circuits: parallel channels for sensory-motor integrations. *Adv Neurol*, 84: 51-61.

Matelli, M., Luppino, G. (2001). Parietofrontal circuits for action and space perception in the macaque monkey. *NeuroImage*, 14: S27-32.

Mayka, M.A., Corcos, D.M., Leurgans, S.E., Vaillancourt, D.E. (2006). Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage*, 31: 1453-1474.

Melzack, R., Israel, R., Lacroix, R., Schultz, G. (1997). Phantom limbs in people with congenital limb deficiency or amputation in early childhood. *Brain*, 120 (Pt 9): 1603-1620.

Mercier, C., Reilly, K.T., Vargas, C.D., Aballea, A., Sirigu, A. (2006). Mapping phantom movement representations in the motor cortex of amputees. *Brain*, 129 (Pt 8): 2202-2210.

Mills, K.R., Boniface, S.J., Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalogr Clin Neurophysiol*, 85: 17–21.

Minio-Paluello, I., Avenanti, A., Aglioti, S.M. (2006). Left hemisphere dominance in reading the sensory qualities of others' pain? *Soc Neurosci*, 1: 320-33.

Miura, N., Sugiura, M., Takahashi, M., Sassa, Y., Miyamoto, A., Sato, S., Horie, K., Nakamura, K., Kawashima, R. (2010). Effect of motion smoothness on brain activity while observing a dance: an fMRI study using a humanoid robot. *Soc Neurosci*, 5: 40–58.

Molnar-Szakacs, I., Iacoboni, M., Koski, L., Mazziotta, J. C. (2005). Functional segregation within pars opercularis of the inferior frontal gyrus: evidence from fMRI studies of imitation and action observation. *Cereb. Cortex*, 15, 986–994.

Moro, V., Urgesi, C., Pernigo, S., Lanteri, P., Pazzaglia, M., Aglioti, S.M. (2008). The neural basis of body form and body action agnosia. *Neuron*, 60: 235-246.

Nelissen, K., Borra, E., Gerbella, M., Rozzi, S., Luppino, G., Vanduffel, W., Rizzolatti, G., Orban, G.A. (2011). Action observation circuits in the macaque monkey cortex. *J Neurosci*, 31: 3743-3756.

Nelissen, K., Luppino, G., Vanduffel, W., Rizzolatti, G., Orban, G.A. (2005). Observing others: multiple action representation in the frontal lobe. *Science*, 310: 332-336.

Nico, D., Daprati, E., Rigal, Ë., Parsons, L., Sirigu, A., Lucia, F. S., De, I., et al. (2004). Left and right hand recognition in upper limb amputees. *Brain*, 127 (1): 120-132.

Nijhawan, R. (1994). Motion extrapolation in catching. *Nature*, 370: 256-7.

Nishitani, N., Avikainen, S., Hari, R. (2004). Abnormal imitation-related cortical activation sequences in Asperger's syndrome. *Ann Neurol*, 55: 558-562.

Nishitani, N., Hari, R. (2002). Viewing lip forms: cortical dynamics. *Neuron*, 36: 1211-1220.

Nitsche, M.A., Cohen, L.G., Wassermann, E.M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P.S., Fregni, F., Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art. *Brain Stimul*, 1: 206-223.

Nitsche, M.A., Liebetanz, D., Antal, A., Lang, N., Tergau, F., Paulus, W. (2003a). Modulation of cortical excitability by weak direct current stimulation-technical, safety and functional aspects. *Suppl Clin Neurophysiol*, 56: 255–276.

Nitsche, M.A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., Paulus, W. (2003c). Safety criteria transcranial direct current stimulation (tDCS) in humans. *Clin Neurophysiol*, 114: 2220–2222 [author reply 2222–2223].

Nitsche, M.A., Niehaus, L., Hoffmann, K.T., Hengst, S., Liebetanz, D., Paulus, W., Meyer, B.U. (2004). MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clin Neurophysiol*, 115 (10): 2419-23.

Nitsche, M.A., Nitsche, M.S., Klein, C.C., Tergau, F., Rothwell, J.C., Paulus, W. (2003b). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clin Neurophysiol*, 114: 600-604.

Nitsche, M.A., Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, 57: 1899–1901.

O'Shea, J., Johansen-Berg, H., Trief, D., Gobel, S., Rushworth, M.F.S. (2007). Functionally specific reorganization in human premotor cortex. *Neuron*, 54: 479-490.

Oberman, L.M., Hubbard, E.M., McCleery, J.P., Altschuler, E.L., Ramachandran, V.S., Pineda, J.A. (2005). EEG evidence for mirror neuron dysfunction in autism spec- trum disorders. *Cogn Brain Res*, 24: 190–198.

Oldfield, R.C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, 9: 97-113.

Oosterhof, N.N., Wiggett, A.J., Diedrichsen, J., Tipper, S.P., Downing, P.E. (2010). Surface-based information mapping reveals crossmodal vision-action representations in human parietal and occipitotemporal cortex. *J Neurophysiol*, 104: 1077-108.

Ortigue, S., Sinigaglia, C., Rizzolatti, G., Grafton, S. T. (2010). Understanding actions of others: the electrodynamics of the left and right hemispheres. A high-density EEG neuroimaging study. *PloS one*, *5*(8), e12160.

Pascual-Leone, A., Peris, M., Tormos, J.M., Pascual, A.P., Catalá, M.D. (1996). Reorganization of human cortical motor output maps following traumatic forearm amputation. *Neuroreport*, 7(13):2068-2070.

Paulus, W. (2003). Transcranial direct current stimulation (tDCS). *Suppl Clin Neurophysiol*, 56: 249-254.

Paus, T. (2005). Inferring causality in brain images: a perturbation approach. *Philos Trans R Soc Lond B Biol Sci*, 360: 1109-1114.

Pazzaglia, M., Smania, N., Corato, E., Aglioti, S.M. (2008). Neural underpinnings of gesture discrimination in patients with limb apraxia. *J Neurosci*, 28: 3030-3041.

Peelen, M.V., Wiggett, A.J., Downing, P.E. (2006). Patterns of fMRI activity dissociate. Overlapping functional brain areas that respond to biological motion. *Neuron*, 49: 815-822.

Peigneux, P., Salmon, E., van der Linden, M., Garraux, G., Aerts, J., Delfiore, G., Degueldre, C., Luxen, A., Orban, G., Franck, G. (2000). The role of lateral occipitotemporal junction and area MT/V5 in the visual analysis of upper-limb postures. *Neuroimage*, 11: 644-655.

Perani, D., Fazio, F., Borghese, N. a, Tettamanti, M., Ferrari, S., Decety, J., Gilardi, M. C. (2001). Different brain correlates for watching real and virtual hand actions. *NeuroImage*, *14*(3), 749-758.

Perrett, D.I., Harries, M.H., Bevan, R., Thomas, S., Benson, P.J., Mistlin, A.J., et al. (1989). Frameworks of analysis for the neural representation of animate objects and actions. *J Exp Biol*, 146: 87–113.

Perrett, D.I., Mistlin, A.J., Harries, M.H., & Chitty, A.J. (1990). Understanding the visual appearance and consequence of hand actions. In M.A. Goodale (ed.), *Vision and action: the control of grasping* (pp. 163–180). Norwood, New Jersey: Ablex Publishing.

Perrett, D.I., Xiao, D., Barraclough, N.E., Keysers, C., Oram, M.W. (2009). Seeing the future: Natural image sequences produce "anticipatory" neuronal activity and bias perceptual report. *Q J Exp Psychol*, (Colchester), 62: 2081-2104.

Petrides, M., Pandya, D.N. (1984). Projections to the frontal cortex from the posterior parietal region in the rhesus monkey. *J Comp Neurol*, 228: 105-116.

Pobric, G., Hamilton, A.F. (2006). Action understanding requires the left inferior frontal cortex. *Curr Biol*, 16: 524-529.

Poreisz, C., Boros, K., Antal, A., Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res Bull*, 72: 208-214.

Press, C., Bird, G., Flach, R., Heyes, C. (2005). Robotic movement elicits automatic imitation. *Brain Res Cogn Brain Res*, 25 (3): 632-40.

Press, C., Heyes, C., Kilner, J.M. (2011). Learning to understand others' actions. *Biol Lett*, 7: 457-460.

Prinz, W. (1997). Perception and action planning. *Eur J Cogn Psychol*, 9(2), 129-154.

Prinz, W. (2006). What re-enactment earns us. *Cortex*, 42(4), 515–517.

Priori, A., Hallett, M., Rothwell, J.C. (2009). Repetitive transcranial magnetic stimulation or transcranial direct current stimulation? *Brain Stimul*, 2: 241-245.

Priori, A., Mameli, F., Cogiamanian, F., et al. (2008). Lie-specific involvement of dorsolateral prefrontal cortex in deception. *Cereb Cortex*, 18: 451-455.

Proverbio, A.M., Riva, F., Zani, A. (2009). Observation of static pictures of dynamic actions enhances the activity of movement-related brain areas. *PLoS One*, 4:e5389.

Reilly, K. T., Mercier, C., Schieber, M. H., Sirigu, A. (2006). Persistent hand motor commands in the amputees' brain. *Brain*, 129 (Pt 8): 2211-2223.

Reilly, K.T., Sirigu, A. (2008). The motor cortex and its role in phantom limb phenomena. *Neuroscientist*, 14:195–202.

Reilly, K.T., Sirigu, A. (2011). Motor cortex representation of the upper-limb in individuals born without a hand. *PLoS One*, 6 (4): e18100.

Reithler, J., van Mier, H. I., Peters, J. C., Goebel, R. (2007). Nonvisual motor learning influences abstract action observation. *Curr Biol*, 17 (14): 1201–1207.

Rizzolatti, G., Camarda, R., Fogassi, L., Gentilucci, M., Luppino, G., Matelli, M. (1988). Functional organization of inferior area 6 in the macaque monkey. II. Area F5 and the control of distal movements. *Exp Brain Res*, 71(3): 491-507.

Rizzolatti, G., Craighero, L. (2004). The mirror-neuron system. *Annu Rev Neurosci*, 27: 169-192.

Rizzolatti, G., Fadiga, L., Fogassi, L., & Gallese, V. (1996a). Premotor cortex and the recognition of motor actions. *Brain res Cogn Brain res*, 3: 131–141.

Rizzolatti, G., Fadiga, L., Fogassi, L., Gallese, V. (1997). The space around us. *Science*, 277: 190-191.

Rizzolatti, G., Fogassi, L., & Gallese, V. (2002). Motor and cognitive functions of the ventral premotor cortex. *Curr Opin Neurobiol*, (12): 149-154.

Rizzolatti, G., Fogassi, L., Gallese, V. (2002). Motor and cognitive functions of the ventral premotor cortex. *Curr Op Neurobiol*, 12: 149-154.

Rizzolatti, G., Luppino, G. (2001). The cortical motor system. *Neuron*, 31: 889-890.

Rizzolatti, G., Scandolara, C., Matelli, M., Gentilucci, M. (1981). Afferent properties of periarcuate neurons in macaque monkeys. II. Visual responses. *Behav Brain Res*, 2: 147-163.

Rizzolatti, G., Sinigaglia, C. (2010). The functional role of the parieto-frontal mirror circuit: interpretations and misinterpretations. *Nat Rev Neurosci*, 11(4): 264-74.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A. (2009). Safety of TMS Consensus Group,. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*, 120: 2008-2039.

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., Maertens de Noordhouti, A.L., Marsden, C.D., Murray, N.M.F., Rothwell, J.C., Swash, M., Tomberg, C. (1994). Noninvasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol*, 91: 79-92.

Rozzi, S., Calzavara, R., Belmalih, A., Borra, E., Gregoriou, G.G., Matelli, M., Luppino, G. (2006). Cortical connections of the inferior parietal cortical convexity of the macaque monkey. *Cereb Cortex*, 16(10): 1389-417.

Rozzi, S., Ferrari, P.F., Bovini, L., Rizzolatti, G., Fogassi, L. (2008). Functional organization of inferior parietal lobule convexity in the macaque monkey: electrophysiological characterization of motor, sensory and mirror responses and their correlation with cytoarchitectonic areas. *Eur J Neurosci*, 28: 1569-1588.

Sambo, C.F., Forster, B. (2009). An ERP Investigation on Visuotactile Interactions in Peripersonal and Extrapersonal Space: Evidence for the Spatial Rule. *J Cogn Neurosci*, 21: 1550-1559.

Sartori, L., Cavallo, A., Bucchioni, G., Castiello, U. (2011). Corticospinal excitability is specifically modulated by the social dimension of observed actions. *Exp Brain Res*, 211: 557-568.

Saygin, A.P. (2007). Superior temporal and premotor brain areas necessary for biological motion perception. *Brain*, 130: 2452-2461.

Schippers, M.B., Keysers, C. (2011). Mapping the flow of information within the putative mirror neuron system during gesture observation. *Neuroimage*, 57: 37-44.

Schlack, A., Sterbing-D'Angelo, S.J., Hartung, K., Hoffmann, K.P., Bremmer, F. (2005) Multisensory space representations in the macaque ventral intraparietal area. *J Neurosci*, 25: 4616-4625.

Schubotz, R. I., Anwander, A., Knösche, T. R., von Cramon, D. Y., & Tittgemeyer, M. (2010). Anatomical and functional parcellation of the human lateral premotor cortex. *NeuroImage*, *50*(2), 396-408.

Schubotz, R.I. (2007). Prediction of external events with our motor system: towards a new framework. *Trends Cogn Sci*, 11(5): 211-218.

Schubotz, R.I., Von Cramon, D.Y. (2004). Sequences of abstract nonbiological stimuli share ventral premotor cortex with action observation and imagery. *J Neurosci*, 16; 24(24): 5467-5474.

Schütz-Bosbach, S., Avenanti, A., Aglioti, S.M., Haggard, P. (2009). Don't do it! Cortical inhibition and self-attribution during action observation. *J Cogn Neurosci*, 21: 1215-1227.

Schütz-Bosbach, S., Prinz, W. (2007). Prospective coding in event representation. *Cogn Proc*, 8:93-102.

Sebanz, N., Shiffrar, M. (2009). Detecting deception in a bluffing body: the role of expertise. *Psychon bull rev,* 16 (1): 170-5.

Seltzer, B., Pandya, D.N. (1994). Parietal, temporal, and occipital projections to cortex of the superior temporal sulcus in the rhesus monkey: a retrograde tracer study. *J Comp Neurol*, 343 (3): 445-63.

Senior, C., Barnes, J., Giampietro, V., Simmons, A., Bullmore, E.T., Brammer, M., David, A.S. (2000). The functional neuroanatomy of implicit-motion perception or representational momentum. *Curr Biol*, 10: 16-22.

Serino, A., Annella, L., Avenanti, A. (2009). Motor properties of peripersonal space in humans. *PLoS ONE*, 4:e6582.

Serino, A., Bassolino, M., Farnè, A., Làdavas, E. (2007). Extended multisensory space in blind cane users. *Psychological Science*, 18, 642-648.

Serino, A., Canzoneri, E., Avenanti, A. 2011. Fronto-parietal Areas Necessary for a Multisensory Representation of Peripersonal Space in Humans: An rTMS Study. *J Cogn Neurosci*, 23(10):2956-67.

Serino, A., De Filippo, L., Casavecchia, C., Coccia, M., Shiffrar, M., Làdavas, E. (2010). Lesions to the motor system affect action perception. *J Cogn Neurosci*, 22 (3): 413-26.

Shepherd, S. V., Klein, J. T., Deaner, R. O., Platt, M. L. (2009). Mirroring of attention by neurons in macaque parietal cortex. *Proc Natl Acad Sci USA*, 106: 9489–9494.

Shimada, S. (2010). Deactivation in the sensorimotor area during observation of a human agent performing robotic actions. *Brain Cogn*, 72, 394–399.

Shimazu, H., Maier, M.A., Cerri, G., Kirkwood, P.A., Lemon, R.N. (2004). Macaque ventral premotor cortex exerts powerful facilitation of motor cortex outputs to upper limb motoneurons. *J Neurosci*, 24: 1200-1211.

Shmuelof, L., Zohary, E. (2005). Dissociation between ventral and dorsal fMRI activation during object and action recognition. *Neuron*, Aug 4;47(3):457-70.

Spence, C., Pavani, F., Maravita, A., Holmes, N.P. (2008). Multisensory interactions. In: Lin MC, Otaduy MA, editors. Haptic rendering: Foundations, algorithms, and applications. Wellesley: AK Peters LTD. pp. 21-52.

Stadler, W., Ott, D. V. M., Springer, A., Schubotz, R. I., Schütz-Bosbach, S., Prinz, W. (2012). Repetitive TMS suggests a role of the human dorsal premotor cortex in action prediction. *Frontiers in human neuroscience*, *6*(February), 20.

Stadler, W., Schubotz, R. I., von Cramon, D. Y., Springer, A., Graf, M., Prinz, W. (2011). Predicting and memorizing observed action: differential premotor cortex involvement. *Human brain mapping*, *32*(5), 677-87.

Stagg, C.J., O'Shea, J., Kincses, Z.T., Woolrich, M., Matthews, P.M., Johansen-Berg, H. (2009). Modulation of movement-associated cortical activation by transcranial direct current stimulation. *Eur J Neurosci*, 30: 1412-1423.

Stefan, K., Cohen, L. G., Duque, J., Mazzocchio, R., Celnik, P., Sawaki, L., Ungerleider, L., et al. (2005). Formation of a motor memory by action observation. *J neurosci*, 25 (41): 9339-46.

Stefan, K., Gentner, R., Zeller, D., Dang, S., Classen, J. (2008). Theta-burst stimulation : Remote physiological and local behavioral after-effects. *NeuroImage*, 40: 265 - 274.

Stepniewska, I., Fang, P.C., Kaas, J.H. (2005). Microstimulation reveals specialized subregions for different complex movements in posterior parietal cortex of prosimian galagos. *Proc Nat Acad Sci USA*, 29: 4878-4883.

Stevens, J. a, Fonlupt, P., Shiffrar, M., Decety, J. (2000). New aspects of motion perception: selective neural encoding of apparent human movements. *Neuroreport*, 11 (1): 109-15.

Tai, Y. F., Scherfler, C., Brooks, D. J., Sawamoto, N., Castiello, U. (2004). The Human Premotor Cortex Is "Mirror" Only for Biological Actions. *Curr Biol*, 14 (2): 117-120.

Tajadura-Jiménez, A., Väljamäe., A, Asutay, E., Västfjäll D. (2010). Embodied auditory perception: the emotional impact of approaching and receding sound sources. *Emotion*, 10(2):216-29.

Talairach, J., Tournoux, P. (1988). *Co-planar stereotaxic atlas of the human brain*. New York, Thieme Medical.

Terao, Y., Ugawa, Y., Uesaka, Y., Hanajima, R., Gemba-Shimizu, K., Ohki, Y., Kanazawa, I. (1995). Input-output organization in the hand area of the human motor cortex. Electroencephalogr. *Clin Neurophysiol*, 97: 375–381.

Tidoni, E., Borgomaneri, S., di Pellegrino, G., Avenanti, A. (2012). Action simulation plays a critical role in deceptive action recognition. In revision.

Tokuno, H., Nambu, A. (2000). Organization of nonprimary motor cortical inputs on pyramidal and nonpyramidal tract neurons of primary motor cortex: An electrophysiological study in the macaque monkey. *Cereb Cortex*, 10(1): 58-68.

Tootell, R.B., Reppas, J.B., Kwong, K.K., Malach, R., Born, R.T., Brady, T.J., Rosen, B.R., Belliveau, J.W. (1995). Functional analysis of human MT and related visual cortical areas using magnetic resonance imaging. *J Neurosci*, 15: 3215-3230.

Touge, T., Gerschlager, W., Brown, P., Rothwell, J.C. (2001). Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? *Clin Neurophysiol*, 112: 2138-2145.

Umiltà, M.A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C., Rizzolatti, G. (2001). I know what you are doing. a neurophysiological study. *Neuron*, 31:155-165.

Uozumi, T., Tamagawa, A., Hashimoto, T., Tsuji, S. (2004). Motor hand representation in cortical area 44. *Neurology*, 62: 757-761.

Urban, P.P., Solinski, M., Best, C., Rolke, R., Hopf, H.C., and Dieterich, M. (2004). Different short-term modulation of cortical motor output to distal and proximal upper-limb muscles during painful sensory nerve stimulation. *Muscle nerve*, 29: 663-669.

Urgesi, C., Candidi, M., Ionta, S., Aglioti, S.M. (2007). Representation of body identity and body actions in extrastriate body area and ventral premotor cortex. *Nat Neurosci*, 10: 30-31.

Urgesi, C., Maieron, M., Avenanti, A., Tidoni, E., Fabbro, F., Aglioti, S.M. (2010). Simulating the future of actions in the human corticospinal system. *Cereb Cortex*, 20: 2511-2521.

Urgesi, C., Moro, V., Candidi, M., Aglioti, S.M. (2006). Mapping implied body actions in the human motor system. *J Neurosci*, 26: 7942-7949.

Urgesi, C., Savonitto, M. M., Fabbro, F., Aglioti, S. M. (2011). Long- and short-term plastic modeling of action prediction abilities in volleyball. *Psychol res*, Nov 2. [Epub ahead of print].

Uy, J., Ridding, M.C. (2003). Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation. *J Neurosci Methods*, Aug 15; 127(2):193-7.

Vaina, L.M., Solomon, J., Chowdhury, S., Sinha, P., Belliveau, J.W. (2001). Functional neuroanatomy of biological motion perception in humans. *Proc Natl Acad Sci USA*, 98: 11656-11661.

Van Overwalle, F., Baetens, K. (2009). Understanding others' actions and goals by mirror and mentalizing systems: a meta-analysis. *Neuroimage*, 48:564-584.

van Schie, H.T., Koelewijn, T., Jensen, O., Oostenveld, R., Maris, E., Bekkering, H. (2008). Evidence for fast, low-level motor resonance to action observation: an MEG study. *Soc Neurosci*, 3(3-4): 213-28.

van Schie, H.T., Mars, R.B., Coles, M.G.H., Bekkering, H. (2004). Modulation of activity in medial frontal and motor cortices during error observation. *Nat Neurosci*, 7(5): 549-554.

Vines, B.W., Cerruti, C., Schlaug, G. (2008). Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. *BMC Neurosci*, 9: 103.

Walsh, V., Pascual-Leone, A. (2003). *Transcranial magnetic stimulation. A neurochronometrics of mind*. MIT Press.

Wilson, M., (2002). Six views of embodied cognition. *Psychon Bull Rev*, 9 (4): 625-36.

Wilson, M., Knoblich, G. (2005). The case of motor involvement in perceiving conspecifics. *Psychol Bull*, 131: 460-473.

Wolpert, D.M., Doya, K., Kawato, M. (2003). A unifying computational framework for motor control and social interaction. *Philos Trans R Soc Lond B Biol Sci*, 2358:593-602.

Zaghi, S., Acar, M., Hultgren, B., Boggio, P.S., Fregni, F. (2010). Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. *Neuroscientis*,(3): 85-30.

Glossary

ADM: Abductor Digiti Minimi

AON: Action Observation Network

AP: Action Prediction

CAG: Congenital Amputees Group

CG: Control Group

EEG: Electroencephalography

EMG: Electromyography

FDI: First Dorsal Interosseus

fMRI: functional Magnetic Resonance Imaging

IFC: Inferior Frontal Cortex

M1: Primary motor area

MEP: Motor Evoked Potential

MNS: Mirror Neuron System

NP: Non-biological Prediction

OSP: Optimal Scalp Position

PMC: PreMotor Cortex

PPC: Posterior Parietal Cortex

PPS: Peripersonal Space

RF: receptive field

rMT: resting Motor Threshold

rTMS: repetitive Transcranial Magnetic Stimulation

spTMS: single-pulse Transcranial Magnetic Stimulation

STS: Superior Temporal Sulcus

TAG: Traumatic Amputees Group

tDCS: transcranial Direct Current Stimulation

TMS: Transcranial Magnetic Stimulation

V1: Primary visual cortex

VIP: Ventral Intraparietal

VPM: Ventral Premotor

vPMc: ventral PreMotor cortex