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CICLO XXIX

COMPLEMENTARY ACTIONS:

EXPLORING THE FLEXIBILITY OF THE ACTION OBSERVATION SYSTEM

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«The observation of actions performed by other agents generates in the brain of the observer representations similar to those of the agents. This circular process, from the self to action and from action to other selves, has as a consequence that action representations can be shared by two or more people. These new findings have radically changed the traditional view of the motor system as an executive system that merely follows instructions elaborated somewhere else. Instead, the motor system now stands as a probe that explores the external world, for interacting with other people and gathering new knowledge.»

Marc Jeannerod

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADM: Abductor Digiti Minimi muscle
aIPS: anterior Intraparietal Sulcus
AOI: Area of Interest
AOS: Action Observation System
APS: Action Planning System
BA: Brodmann Area
BOLD: blood-oxigen-level-dependent
CE: Corticospinal excitability
CS: Corticospinal
cTBS: continuous Theta Burst Stimulation
EEG: Electroencephalography
EMG: Electromyography
FCU: Flexor Carpi Ulnaris muscle
FDI: First Dorsal Interosseous muscle
fMRI: Functional Magnetic Stimulation
fNIRS: Functional Near-Infrared Spectroscopy
IFG: Inferior Frontal Gyrus
IOR: Inhibition of Return
IPL: Inferior Parietal Lobule
M1: Primary Motor Cortex
MEG: Magnetoencephalography
MEP: Motor Evoked Potential
MNI: Montreal Neurological Institute
MNS: Mirror Neurons System
OP: Opponens Pollicis muscle
OSP: Optimal Scalp Location
PET: Positron Emission Tomography
PG: Precision Grip

PMv: Ventral Premotor cortex

QF: Quadriceps Femoris muscle

rMT: Resting Motor Threshold

SMA: Supplementary Motor Area

SPL: Superior Parietal Lobule

spTMS: single pulse TMS

S-R: Stimulus-Response

STS: Superior Temporal Sulcus

TMS: Transcranial Magnetic Stimulation

WHG: Whole-hand Grasp

SYNOPSIS

Social interaction is an essential part of the human experience and actions are the primary means by which humans interact with the surrounding world. The ability to recognize and understand other people's actions is necessary for an efficient interaction with other agents. Our motor system can promptly and accurately coordinate these forms of interactions in our daily activity. However, how the brain is able to produce such appropriate output has yet to be fully understood. The present work aims at unveiling the processes taking place in the motor system while observing actions of interactive agents and action calling for an (interactive) involvement of the observer. The core argument is to explore the flexibility of the motor system when preparing identical and non-identical responses in complex realistic situations, and to test the automaticity of these processes by directly investigating the role played by visuospatial attention during action observation.

The introductory section of this thesis will first provide an overview of the state of the art regarding the mechanism that could be at the basis of the comprehension of other's actions, that is the 'mirror mechanism' (Chapter 1). *Mirror neurons* are neural cells which activate both during the execution of an action and during the observation of the same action performed by another individual. From their first discovery in the premotor cortex of the macaque brain in the early nineties (Di Pellegrino, Fadiga, Fogassi, Gallese and Rizzolatti, 1992), these visuomotor neurons have been extensively studied both in primates and in humans. Chapter 1 will review evidence on the existence of such mirror mechanism and on its basic properties and anatomy, with a particular focus on the human literature concerned with the Action Observation System (AOS). Convergent evidence suggests that actions are coded in the observer's brain in such a way that resembles the actual execution of the action. As a result, an embodied simulation (i.e., from the inside) would allow to understand the observed action through the onlooker's own motor experience. However, to simulate the actions of other people is not always the best strategy to interact with them. Indeed, we are often required to perform actions which differ from those observed. Chapter 2 will summarize recent neurophysiological findings suggesting that the human brain is able to overcome the imitative bias in favor of non-identical responses which are appropriate to the context requirements. A particular focus will be given to the literature on *complementary actions*, namely a type of social interactions in which the involved agents have to perform incongruent responses to reach a common goal. Put simply, it seems that while the human motor system is prone to simulate other's action, this imitative tendency can be modulated according to the context. However, whether top-down factors might play a role in determining the embodied simulation is still controversial. In Chapter 3 a review of the literature in which the automaticity of the visuo-motor transformation has been questioned will be presented. Overall, it emerges that top-down factors, such as visuospatial attention, may influence the motor simulation of observed actions.

The second part of the present thesis concerns the experimental work I undertook. Chapter 4 provides a description of the general methodology common to the experimental studies conducted with transcranial magnetic stimulation (TMS) coupled with electromyographic (EMG) registration to measure corticospinal excitability modulations during action observation. In the first experiment (Chapter 5) a novel paradigm to study complementary actions at the level of multiple effectors will be described. Participants were presented with a soccer player kicking a ball toward them, thus implicitly requiring their response as to parry the approaching ball. Control conditions showing lateral kicks, mimicked kicks, and the ball still in penalty area were also included. This paradigm was adopted to investigate the time-course of imitative and complementary responses in lower and upper limbs. In a subsequent experiment presented in Chapter 6, a similar paradigm was adopted, but crucially it included a condition in which the imitative and the complementary responses were simultaneously elicited in the observers' motor system. This allowed to disentangle the contribution of different levels of motor coding – namely, kinematic, predictive and response coding – during action observation. Chapters 7 and 8 will report on two experiments aimed at clarifying the influence of spatial attention allocation during observation of actions eliciting (or not) a complementary response. In particular, in Chapter 7 participants were presented with action sequences evoking a complementary gesture, such as picking up a mug placed in the video foreground, when an individual was inviting them to do so. Notably, the observed and required actions were mismatched in order to investigate the observers' spontaneous unfolding of different motor activations. Crucially, spatial attention allocation toward specific parts of the visual scene was manipulated by means of the sudden appearance of a red dot, and responses were recorded both at a behavioral (eye-tracking) and at a neurophysiological level. In a further experiment (Chapter 8), the allocation of attention toward parts of the visual scene was modulated by means of the actor's gaze direction, which represents a more ecologically-valid manipulation. This experimental work shows the role of spatial attention in simulation and reciprocity, thus promoting a more complete and integrated understanding on the role of top-down factors in action observation.

A general discussion (Chapter 9), contextualizing the results obtained by the studies presented in the present thesis will follow. Taken together, these studies will help to better define how the motor system flexibly and dynamically modulates its activity during the course of action observation. Moreover, the present work could broadening our view on action observation processes in social contexts, towards the definition of a more complete account.

SINOSSI

Le interazioni sociali rappresentano un aspetto essenziale dell'esperienza umana, e le azioni rappresentano il mezzo principale attraverso cui gli esseri umani interagiscono con il mondo circostante. L'abilità di riconoscere e comprendere le azioni altrui è necessaria per garantire un'efficace interazione con altri individui. Nella vita quotidiana il sistema motorio è in grado di coordinare queste forme di interazione sociale con prontezza e accuratezza. Tuttavia, come il nostro cervello sia capace di produrre risposte così appropriate deve essere ancora pienamente compreso. L'obiettivo della presente tesi consiste nell'indagare i processi che hanno luogo nel sistema motorio durante l'osservazione di azioni, e in particolare nel caso di azioni che richiedono un coinvolgimento (interattivo) dell'osservatore. L'argomento principale che verrà trattato riguarda proprio la flessibilità del sistema motorio nel preparare azioni simili o dissimili rispetto a quanto osservato in situazioni realistiche. Inoltre, il presente lavoro ha lo scopo di verificare l'automaticità di questi processi, esaminando direttamente il ruolo giocato dall'attenzione visuospaziale durante l'osservazione di azioni.

La parte introduttiva della presente tesi fornirà una panoramica sullo stato dell'arte riguardo il meccanismo che potrebbe essere alla base della comprensione delle azioni altrui, ossia il 'meccanismo specchio' (Capitolo 1). I *neuroni specchio* sono cellule neurali che si attivano sia durante l'esecuzione, che durante l'osservazione di una stessa azione compiuta da un altro individuo. Dalla loro prima scoperta nella corteccia premotoria della scimmia (*Macaca nemestrina*) all'inizio degli anni Novanta (Di Pellegrino, Fadiga, Fogassi, Gallese and Rizzolatti, 1992), questi neuroni visuo-motori sono stati estensivamente studiati sia nei primati che nell'uomo. Nel Capitolo 1 verrà quindi presentata una rassegna delle prove a favore dell'esistenza di tale meccanismo specchio, nonché sulle sue proprietà e basi anatomiche. Una particolare attenzione verrà data alla letteratura relativa agli studi condotti sull'uomo, approfondendo le evidenze riguardanti il Sistema di Osservazione dell'Azione (AOS) acquisite tramite l'uso di diverse metodologie. Risultati convergenti suggeriscono che le azioni vengano codificate nel cervello dell'osservatore in modo tale da replicare l'effettiva esecuzione dell'azione. Pertanto, una simulazione incarnata (dall'interno) permetterebbe di comprendere le azioni osservate tramite l'esperienza motoria propria dell'osservatore. Tuttavia, simulare le azioni altrui non è sempre la migliore strategia per interagire con essi. Spesso, invece, è necessario mettere in atto azioni che differiscono da quelle osservate. Nel Capitolo 2 saranno riassunte evidenze in ambito neurofisiologico che suggeriscono come il cervello umano sia in grado di superare il *bias* imitativo in favore di risposte dissimili, che sono tuttavia appropriate alle esigenze dettate dal contesto. Un'attenzione particolare verrà data alla letteratura riguardante le *azioni complementari*, un tipo di interazioni sociali nelle quali gli individui coinvolti devono eseguire azioni dissimili o opposte a quelle osservate, al fine di perseguire uno scopo comune. Riassumendo, il sistema motorio umano risulta essere incline a simulare le azioni altrui, ciò nonostante questa tendenza imitativa pare possa essere modulata in funzione del contesto in cui l'azione avviene. Tuttavia, qualora fattori *top-down* abbiano un ruolo nel determinare la simulazione incarnata è ancora un argomento dibattuto. Nel Capitolo 3 verrà presentata una rassegna della letteratura in cui l'automaticità della trasformazione visuo-motoria è stata messa in discussione. Complessivamente, emerge come fattori *top-down*, quali l'attenzione visuospaziale, possano influenzare il processo di simulazione motoria delle azioni osservate.

Nella seconda parte della tesi verrà descritto il lavoro sperimentale da me svolto. Il Capitolo 4 fornirà una generale descrizione della metodologia adottata e comune a tutti gli studi in cui è stata utilizzata la stimolazione magnetica transcranica (TMS) accoppiata con la registrazione elettromiografica (EMG) per misurare modulazioni dell'eccitabilità corticospinale durante l'osservazione di azioni. Nel primo esperimento (Capitolo 5) è stato utilizzato un nuovo paradigma per lo studio delle azioni complementari che coinvolge effettori multipli. Ai partecipanti è stata presentata un'azione ritraente un calciatore lanciare una palla verso di loro, richiedendo implicitamente una loro risposta al fine di parare la palla in avvicinamento. Ulteriori condizioni di controllo in cui calci laterali, calci mimati, o in cui la palla veniva presentata ferma in area di rigore sono state utilizzate. Questo paradigma è stato adottato per studiare l'andamento temporale dell'insorgere delle risposte imitative e complementari in effettori inferiori e superiori. In un successivo esperimento presentato nel Capitolo 6 è stato adottato un paradigma simile, tuttavia esso includeva una condizione nella quale le risposte imitative e complementari venivano simultaneamente elicitate nel sistema motorio dell'osservatore. Ciò ha permesso di disambiguare il contributo di differenti livelli di codifica motoria – nello specifico, cinematico, predittivo e di codifica della risposta – in funzione durante l'osservazione di azioni. I Capitoli 7 e 8 riguarderanno invece due esperimenti aventi lo scopo di chiarificare l'influenza dell'attenzione spaziale durante l'osservazione di azioni capaci di elicitare o meno una risposta complementare. In particolare, nel Capitolo 7, ai partecipanti venivano mostrate sequenze di azioni che potevano evocare una risposta complementare, quale afferrare una tazza posizionata in primo piano, quando una persona li invitava a farlo. Va notato che il movimento osservato presentava caratteristiche muscolari diverse da quello richiesto, al fine di studiare lo spontaneo manifestarsi di attivazioni motorie differenti negli osservatori. Un aspetto cruciale è che l'allocazione dell'attenzione spaziale verso specifiche parti della scena visiva veniva manipolata tramite la rapida presentazione di un pallino rosso, e le risposte sono state registrate sia a livello comportamentale (eye-tracking) che neurofisiologico. In un successivo esperimento (Capitolo 8), l'allocazione di risorse attentive verso parti della scena visiva è stata modulata tramite la direzione dello sguardo dell'attore, utilizzando quindi una manipolazione che presenta una migliore validità ecologica. Questo lavoro sperimentale ha indagato il ruolo dell'attenzione nei processi di simulazione e reciprocità, promuovendo così la definizione di una più completa e integrata comprensione del ruolo di fattori *top-down* nell'osservazione di azioni.

Seguirà una discussione generale (Capitolo 9) volta a contestualizzare i risultati ottenuti dagli studi presentati in questa tesi. Nel complesso, questi studi aiuteranno a definire meglio come il sistema motorio sia in grado di modulare la sua attività in maniera flessibile e dinamica durante l'osservazione di azioni. Inoltre, il presente lavoro di ricerca può contribuire ad ampliare la nostra conoscenza dei processi in atto durante l'osservazione di azioni in contesti sociali, in direzione di una più completa definizione del fenomeno.

PART 1

THEORETHICAL BACKGROUND

CHAPTER 1

THE NEURAL UNDERPINNINGS OF ACTION OBSERVATION

Understanding others' actions is a fundamental ability for survival in a social context. A peculiar class of neurons discovered in monkeys from a Parma's team of neuroscientists in early nineties could be at the basis of this ability. In 1992 Di Pellegrino, Fadiga, Fogassi, Gallese and Rizzolatti published a seminal report on the discovery of a new class of neurons with visuomotor properties in area F5 of the monkey's premotor cortex. These neurons activate both during the execution of an action and during the observation of another individual (human or conspecific) performing the same action. They have been named 'mirror neurons' (Rizzolatti, Fadiga, Gallese and Fogassi, 1996) for their capacity to reflect the observed actions within the observer's motor system. Since then, many studies contributed to the definition of the characteristics and the neural bases of the mirror mechanism in monkeys and humans, paving the way to speculations on its possible functions.

The present chapter will provide an overview on the state of the art regarding the Mirror Neuron System (MNS) or, by using a more general term, the Action Observation System in both human and non-humans primates. Evidence on the basic characteristics of the mirror mechanism and on its neural basis will be provided, with a major focus on the literature adopting the Transcranial Magnetic Stimulation (TMS) as a tool for the study of MNS activity in humans.

1.1 THE MIRROR NEURONS SYSTEM IN NON-HUMAN PRIMATES

Neural cells that activates both during action execution and during observation of a similar action performed by another individual have been discovered in area F5 of the monkey's (*Macaca nemestrina*) premotor cortex (Di Pellegrino et al., 1992). Neurons with similar properties to those of F5 (i.e., mirror neurons) have been subsequently identified also in the inferior parietal cortex (PF; e.g., Gallese, Fogassi, Fadiga and Rizzolatti, 2002). It has been hypothesized that areas F5 and PF, together with the inputs provided by other brain areas such as the superior temporal sulcus (STS) cortex, form the MNS in primates

(Fig. 1.2; Keysers and Perrett, 2004). The following paragraph will focus on the experimental evidence showing the basic 'mirror' properties of F5 and PF.

1.1.1 Basic properties of Mirror Neurons

The electrical activity of single neurons ('units') in monkeys is studied by means of a scientific technique called 'single unit recording'. This technique requires microelectrode probes to be inserted in the brain while the animal is performing a task. The recorded signal allows to measure action potentials, also referred as 'spikes', that indicate the discharging rate or activation of specific neurons during the task. By adopting this procedure, Rizzolatti and colleagues extensively studied the motor properties of monkey F5 neurons. Neural cells in area F5 respond during execution of a motor act (i.e., movements with a specific goal, like grasping a piece of food), independently from the used effector (e.g., the hand or the mouth), and its laterality (e.g., the right or the left hand; Rizzolatti and Gentilucci, 1988). Neurons of F5 can be classified in terms of what motor act they encode: "Grasping-with-the-hand-and-the-mouth", "Grasping-with-the-hand", "Holding", "Reaching", "Precision grip" or "Whole hand prehension" (Rizzolatti, Camarda, Fogassi, Gentilucci, Luppino and Matelli, 1988). Interestingly, F5 neurons' activity is correlated with specific goal-related motor acts rather than with single movements performed by the animal, so that the same type of movement (e.g., index flexion) activates a specific neuron during a motor act (e.g., grasping), but not during another motor act (e.g., scratching).

Crucially, two classes of neurons, which present also visual properties have been discovered in area F5. These neurons present peculiar visual properties that allow classifying them in *canonical* and *mirror* neurons. The 'canonical neurons' in monkey F5 area respond during passive observation of objects of particular shape and dimension, and they typically show congruence between the type of act they encode (e.g., precision grip; PG) and the object's physical properties (e.g., small object; Rizzolatti et al., 1988; Murata, Fadiga, Fogassi, Gallese, Raos and Rizzolatti, 1997). The 'mirror neurons', instead, discharge both during execution of a given motor act and during the observation of a motor act with a similar goal performed by another individual (see Fig. 1.1). Mirror neurons, unlike canonical neurons, do not activate during the mere visual presentation of an object nor when the action is executed in absence of it (i.e., intransitive or mimicked action; Rizzolatti and Craighero, 2004). Therefore, only object-related actions (i.e., transitive actions) trigger a discharge within this class of neurons. Mirror neurons present a large degree of generalization by responding to different visual stimuli as long as they represent the same action. They indeed discharge both when the action is executed by a conspecific and an experimenter, irrespective of whether it is executed near or far from the animal (Rizzolatti and Craighiero, 2004).

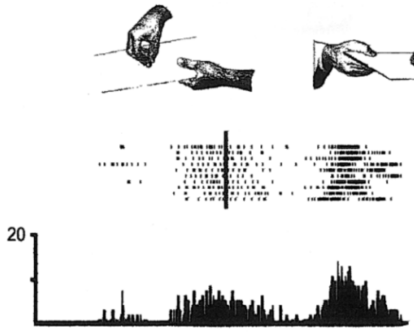

Fig. 1.1: Example of visual and motor responses of a monkey's mirror neuron (unit) during observation of the experimenter grasping a piece of food with his/her hand (upper left part) and during execution of the same movement by the monkey itself (upper right part). The neuron discharged during grasping observation (left part of the histogram, the vertical bar represent the moment in which the experimenter grasped the food), was silent when the food was moved (middle part of the histogram) and discharged again when the monkey grasped the food by itself (right part of the histogram). (Modified from Gallese and Goldman, 1998)

Mirror neurons can also be classified according to the motor act they visually encode: most of F5 mirror neurons code for just a type of motor act (e.g., grasping, holding, manipulating), but others seem less selective and discharge for two, or rarely three, motor acts (Rizzolatti and Sinigaglia, 2006).

Another classification is based on the congruence between the motor responses mirror neurons code for and the visual actions they respond to. In this respect they can be subdivided into 'strictly congruent' and 'broadly congruent' mirror neurons (Gallese, Fadiga, Fogassi and Rizzolatti, 1996). The former (about one third of F5 mirror neurons) show an exact correspondence between observed and executed action both in terms of goals (e.g., grasping) and means to reach the goal (e.g., PG); the latter (about two third of F5 mirror neurons), instead, discharge when the motor acts they code are connected but not identical to the observed ones. In particular, broadly congruent mirror neurons respond to the execution of a specific motor act (e.g., grasping with a PG), but to the observation of different forms of the same act (e.g., grasping with a whole-hand prehension, WHG), or the observation of different actions performed to obtain a logically-related goal (e.g., grasping with the hand or mouth), or the observation of different hand actions (e.g., object manipulation; Gallese et al., 1996). Broadly congruent neurons thus respond during observation and execution of actions that are not exactly identical.

From the evidence provided it appears that mirror neurons activity depends on general characteristics of the action, like the action goal. As suggested by Rizzolatti, Fogassi and Gallese (2001) the function of mirror neurons is to transform visual information into knowledge. It has indeed been proposed that the functional role of mirror neurons could be action understanding (Rizzolatti et al., 2001; but see Hickok, 2014 for a critical view). Two studies seem to corroborate this hypothesis by proving that the monkey could understand the meaning of the observed action even in absence of the visual features that typically activate mirror neurons (Kohler, Keysers, Umiltà, Fogassi, Gallese and Rizzolatti, 2002; Umiltà, Kohler, Gallese, Fogassi and Fadiga, 2001). The study from Kohler and colleagues (2002) demonstrated that 15% of mirror neurons in F5 that responded to a noisy observed action (e.g., ripping a piece of paper) discharged also when presented to the associated sound alone. This population of neurons, defined 'audio-visual mirror neurons', represents an evidence that the activity of mirror neurons depends on abstract features of the motor act. Along these lines, Umiltà et al. (2001) demonstrated the existence of mirror neurons in area F5 which were activated by action observation even when the critical part of the observed motor act (i.e., grasping and holding) was hidden from view. This activation did not significantly differ from the full-vision condition and it emerged only with a transitive action. To summarize, it appears that mirror neurons activity can code the observed action even in the absence of direct visual stimulation.

Along with F5, other two areas are considered as part of the MNS: PF and the superior temporal sulcus (STS). Neurons in PF region within the inferior parietal lobe are functionally heterogeneous: 90% of them respond to visual stimuli, whereas just half of them have motor properties discharging during execution of specific movements or actions (Fogassi, Gallese, Fadiga and Rizzolatti, 1998). Among the PF neurons that respond to visual stimuli, about 40% of them are specifically activated during observation of actions like hand-object interactions, and about two thirds of them have mirror properties (Gallese et al., 2002; Rizzolatti and Craighero, 2004).

Neurons within the STS region, instead, differ from those of PF and F5 for not being activated by action execution. However, they present important visual properties that contribute to the functioning of the MNS by means of the connection with PF and indirectly with F5. STS neurons are indeed activated by a large variety of biological movements, including walking, head, limbs and torso movements, and goal-directed interactions with objects (Keysers and Perrett, 2004; Perrett et al., 1990).

As pointed out by Keysers and Perrett (2004), all the three areas (STS, F5 and PF) allow for an accurate discrimination of the observed actions and they present a relative invariance to change in illumination, color, distance and identity of the agent. However, PF and STS, unlike F5, show a response preference for the performing hand. Moreover, PF and F5 cells show a specialization to hand and mouth actions directed to object, instead STS appear to code for a wide range of movements. As for F5 neural cells, also STS contains neurons that discharge to action sound as well as to stimuli that are not completely visible (e.g., a man behind a screen; Baker, Keysers, Jellema, Wicker and Perrett, 2001; Umiltà et al., 2001). Therefore, both F5 and STS appear to integrate the visual input by using non-visible cues inferred from the context. In general, all these three areas seem to respond when presented to the vision or to the sound of an action, whilst only PF and F5 are activated during action execution and thus present mirror properties.

1.1.2 The Mirror Neurons anatomical circuit

Anatomically, F5, PF and STS areas form a network of interconnected areas (see Fig. 1.2) where STS is connected with PF, which in turn shows strong connections with F5 (Keysers and Perrett, 2004; Seltzer and Pandya, 1994). All these three areas form a functional circuit in which observed biological actions are described in visual terms and matched to the corresponding actions by our own motor neurons. According to Keysers and Perrett (2004), a simple physiological learning rule – namely the Hebbian learning (i.e., "neurons that fire together wire together") – is at the basis of the monkey's ability to understand others' actions. Notably, the mirror activity from F5 and PF is concomitant to the inactivity of STS neurons, and this in turn may allow to distinguish oneself's from others' actions. It is the synergic contribution of each area that allows an efficient functioning of the MNS.

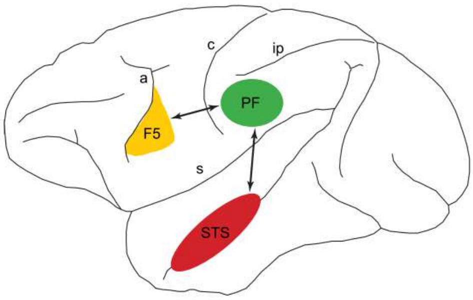

Fig. 1.2: Schematic representation of the MN circuit in the macaque brain from a left hemisphere view. Localization of area F5 in ventral premotor cortex, area PF of the inferior parietal lobule and the superior temporal sulcus (STS) are reported in the image together with their anatomical connections (arrows). Abbreviations: a, arcuate sulcus; c, central sulcus; ip, intraparietal sulcus and s, sylvian sulcus. (Modified from Keysers and Perrett, 2004)

1.2 THE MIRROR NEURONS SYSTEM IN HUMANS

A circuit resembling the one identified in monkeys' areas F5-PF-STS is thought to exist also in humans (Fig. 1.3). A large body of data provides evidence, mainly indirectly, that homologous areas in the human brain are activated during the observation of others actions. Cytoarchitectonic studies identify the equivalent of monkey F5 in the pars opercularis of the inferior frontal gyrus (IFG) or Brodmann area 44 (BA 44), located anteriorly of the premotor area (BA 6; Petrides and Pandya, 1997; Rizzolatti and Arbib, 1998). The homologous region of PF monkey area is instead identified in the inferior parietal lobule (IPL) and in particular in area BA 40 (Rizzolatti and Sinigaglia, 2006). As regards the STS area being part of the MNS, in humans it corresponds to the medial and caudal part of STS (Keysers and Perrett, 2004). The putative human MNS appear to be more extended than the monkey's one. However, methodological differences could explain these findings given that the human MNS is mainly studied indirectly by means of neuroimaging and neurophysiological techniques. The following paragraphs will introduce researches adopting various methodologies to investigate whether the existence of a system similar to the one studied in monkeys – able to match observed with executed actions – exists also in humans.

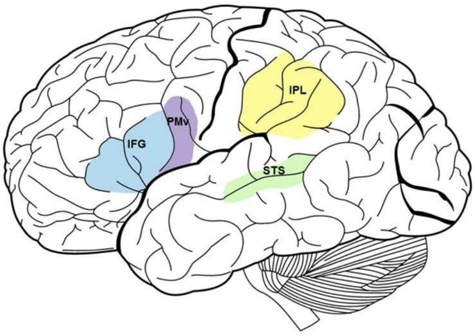

Fig. 1.3: Schematic representation of the putative mirror neuron circuit in the human brain from a left hemisphere view. Localization of the inferior frontal gyrus (IFG), the ventral premotor cortex (PMv), the inferior parietal lobule (IPL) and the superior temporal sulcus (STS) are highlighted in the image. (From Werner, Cermak and Aziz-Zadeh, 2012)

1.2.1 Electrophysiological studies

The first indirect evidence for the existence of a putative MNS in the human brain date back to the half of the twentieth century. Gastault and Bert (1954) adopted electroencephalography (EEG), a technique that allows to register the brain electric activity through electrodes from the scalp, to perform a pioneering experiment. They reported a rolandic mu-rhythm desynchronization in the electric activity of individuals observing actions performed by others. Notably, the mu rhythm is an index of motor relaxation and it is desynchronized during action execution. The finding that this cerebral rhythm desynchronizes during action observation, when subjects are completely relaxed, suggests the existence of a mirror mechanism similar to that studied in monkeys. Subsequent researches confirmed these findings showing desynchronization while both observing and executing movements (Cochin, Barthelemy, Lejeune, Roux and Martineau, 1998; Cochin, Barthelemy, Roux and Martineau, 1999). Further evidence came from studies investigating the post-stimulus rebound, namely an activity with a frequency of about 20 Hz in the precentral cortex induced by median nerve stimulation (Salmelin and Hari, 1994). This activity is abolished both during object manipulation and action observation (Schnirzler, Salenius, Salmelin, Jousmaki and Hari, 1997). More direct confirmations came from the adoption of magnetoencephalography (MEG), a technique that measures the magnetic fields associated with electrical brain activity. Nishitani and Hari (2000) showed an initial activation of BA 44 followed by the primary motor cortex during action execution; this sequence was similar also in observation and imitation contexts. Overall, these findings provide further evidence for a mechanism that respond similarly to action execution and action observation.

1.2.3 Neuroimaging studies

Neuroimaging studies, unlike the neurophysiological ones, are characterized by a better spatial resolution which allows to make inferences on anatomical localization of the Action Observation System in humans (i.e., overlapping activations of the neural network while both observing others' actions and executing them; Avenanti, Candidi and Urgesi, 2013). Techniques like Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) that measures changes in metabolic brain activity (e.g., changes in blood flow, increase of oxygen and glucose consumption) have been adopted to identify brain areas active during both action observation and execution.

Initial PET studies in humans showed that hand-grasping actions produced activations in regions which have analogous distribution and properties as those described in monkey MNS. These regions comprised IPL, IFG and STS (Rizzolatti et al., 1996; Grafton, Arbib, Fadiga and Rizzolatti, 1996). Following fMRI studies confirmed these findings (Caspers, Zilles, Laird and Eickhoff, 2010). However, other evidence suggests the involvement of other areas during action observation beyond the core regions of the MNS (i.e., activations were also found in dorsal premotor, supplementary motor, somatosensory cortices, dorsal posterior parietal cortex and cerebellum; Keysers, Thioux and Gazzola, 2013). Interestingly, Buccino and colleagues (2001) performed an fMRI study to localize activations loci when the observed action was performed with different effectors. Participants were asked to observe transitive or intransitive actions performed with the mouth (i.e., biting an apple or chewing), with the hand (i.e., reaching and grasping a mug or a ball, or their mimicked performance), or with the foot (i.e., kicking a ball or pushing a brake, or their mimicked performance). As controls, static images of a face, a hand and a foot were presented. Results showed that the premotor cortex of observers was activated in a somatotopic fashion, where the different areas coding for actions executed by different effectors followed the classical motor organization of Penfield's Homunculus (Penfield and Rasmussen, 1952; Fig. 1.4). The dorsal part thus represented the foot actions, whereas more ventrally were represented the hand and below the mouth actions. Furthermore, from the results it emerged that for the transitive action condition there was an additional and still somatotopically organized activation of the posterior parietal lobe, suggesting that a further analysis process took place when object-related interactions were observed. More recent findings by Pelphrey, Morris, Michelich, Allison and McCarthy (2005) revealed that also different sectors of the STS region codes for biological motion of eye, mouth and hand in a somatotopic fashion.

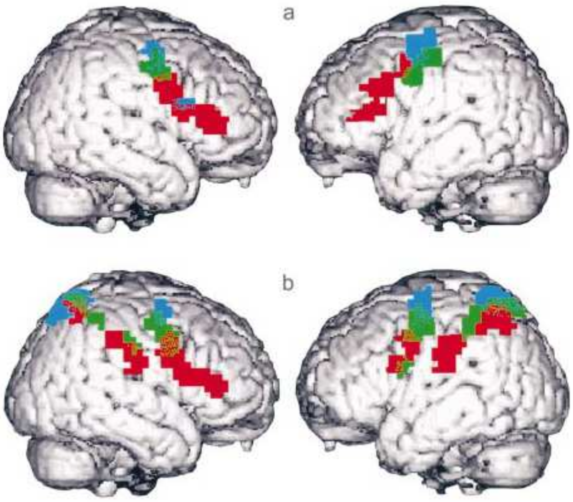

Fig. 1.4: Representation of somatotopically organized activation foci of premotor and parietal cortices referring to observation of intransitive actions (a) or object-related transitive actions (b), performed with the foot (blue), the hand (green) and the mouth (red); overlap of colors indicates activation foci present during observation of actions made by different effectors. The activation foci are projected on the lateral surface of a standard brain (Montreal Neurological Institute, MNI). (Modified from Buccino et al., 2001)

Overall, the results obtained by Buccino et al. (2001) represent a further confirmation that observing others' actions activates in the observer's brain the same motor representations involved in the actual execution of the action. A key evidence on the existence of 'mirror regions' in the human brain came from an fMRI study by Turella, Erb, Grodd and Castiello (2009) which directly tested overlapping activity for both observation and execution of actions. Participants underwent both observation and execution conditions where they had to observe and to perform grasping actions toward a spherical object, respectively. Results showed that a network of areas including premotor, parietal and temporal cortices was active in the two conditions, together with a number of areas which exceeded the classical MNS. Likewise, Gazzola et al. (2007) found similar activations for executed and observed actions, even if they were performed by a human or a robotic agent (Gazzola, Rizzolatti, Wicker and Keysers, 2007; but see Tai, Scherfler, Brooks, Sawamoto and Castiello, 2004 for a different view). These results also support the primary role of the action goal, irrespective of differences in observed kinematic, for the mirror activity to emerge.

To summarize, converging data suggest that humans can recognize a large variety of actions performed by others by matching the observed actions onto the observer's motor system (i.e., 'direct matching'; Buccino, Binkofski and Riggio, 2004), a visuo-motor matching mediated by the MNS.

However, a crucial difference between the classical neurophysiological studies in monkeys and the techniques adopted in humans is the lack of spatial resolution. Neuroimaging studies, indeed, do not allow to determine the behavior of single neurons. Instead, with neuroimaging and neurophysiological techniques it is possible to measure activity of populations of neurons. These methods can test mirror-like properties within a neural system, but only intra-cortical recording would provide a straightforward demonstration of the existence of neurons having mirror properties in humans. The findings obtained from the only study that explored the existence of a MNS in humans with single cell recording are reported in the next paragraph.

1.2.4 A direct recording study

Mukamel and colleagues in 2010 published the first paper in which mirror neuron activity in humans was recorded through single-cell recordings (Mukamel, Ekstrom, Kaplan, Iacoboni and Fried, 2010). Twenty-one patients undergoing surgical treatment for pharmacologically intractable epilepsy were studied. As part of the clinical procedure, electrodes were implanted in the patients' brains to localize the focus that triggered the epilepsy for surgical removal, providing the opportunity to record activity from single neurons. In this study the activity of 1177 neurons were recorded. Patients were asked to observe video clips representing grasping actions (PG and WHG) and to perform the same actions. Crucially, Mukamel et al. (2010) identified some neural cells that responded during both execution and observation of the same action (*action observation/execution matching neurons*) thus providing the first proof of the existence of mirror neurons in the human brain. This discovery resembles monkey data, however the regions in which this visuo-motor matching cells were recorded in humans are different from the studied areas in monkeys. In fact, the ventral premotor cortex (PMv) and inferior parietal lobule (IPL), where mirror neurons have been recorded in monkeys, are rarely focus of epileptic seizures and Mukamel and colleagues (2010) positioned the electrodes based purely on clinical considerations. For this reason the neural activity from PMv and IPL has not been recorded, whereas neurons having mirror properties have been identified in supplementary motor area (SMA) and in hyppocampus, parahippocampal gyrus and entorhinal cortex of the medial temporal lobe. To my knowledge, experiments in which the homologues of these areas have been targeted in monkeys have not yet been performed, so the presence of mirror neurons in similar locations in monkeys cannot be excluded. These results suggest that considering few areas as 'mirror neuron regions' can be incorrect. According to Keysers and Gazzola (2010), mirror neurons are a minority of neurons but spread in many brain regions. Consistently, as suggested by the Hebbian learning account for mirror neurons origins (Keysers and Perrett, 2004; Keysers and Gazzola, 2010), the motor neurons involved in action execution and those involved in seeing, hearing and feeling during action performance could be wired together through the repeated temporal co-occurrence of these events. In this way the Hebbian learning could promote the development of many mirror neurons, not only in PMv and IPL but also in areas such the medial frontal and medial temporal lobes, to be excitable both while performing an action and while seeing or hearing someone else performing the same action.

The study of Mukamel et al. (2010) provided also another very interesting finding: they found 'anti-mirror' cells, namely neurons that increased their firing rate during action execution, but decreased their firing rate during the observation of the same action. This type of cells could possibly mediate information to the primary motor cortex (responsible for the execution of voluntary movements), sending excitatory inputs during action execution and inhibitory inputs during action observation. This in turn could help disambiguating our own actions from those of others and limiting automatic imitation during action observation.

1.2.5 Transcranial Magnetic Stimulation studies

A technique that has been extensively adopted to directly study the putative MNS in humans is Transcranial Magnetic Stimulation (TMS). TMS is a non-invasive technique for electromagnetic stimulation of the nervous system. When applied over the primary motor cortex (M1), TMS generates action potentials along efferent nerves producing motor-evoked potentials (MEPs) that can be recorded with electromyography (EMG) from peripheral contralateral muscles. The amplitude of the MEPs is considered as indicative of the level of excitability of the corticospinal (CS) system and it is modulated by the behavioral context. MEPs are typically recorded from participants during action observation, and their amplitude is compared with control (baseline) conditions. The MEP increase during action observation is commonly interpreted as evidence of a mirror response under the rationale that observing an action triggers mirror neurons, which in turn lead to an increase in M1 excitability and thus to an increased muscular response (see Naish, Houston-Price, Bremner and Holmes, 2014, for a recent review).

Fadiga, Fogassi, Pavesi and Rizzolatti (1995) were the first to adopt TMS to study the human MNS. During the experiment, single-pulse TMS (spTMS) was delivered over the left M1 and MEPs were recorded from the right hand and arm muscles of participants that were required to observe an experimenter grasping an object. As control conditions, participants were required to observe meaningless arm gestures, 3-D objects and to detect a dimming light. The results showed that the observation of both transitive (object-directed) and intransitive (without object) actions determined an increase of MEPs amplitude with respect to the other conditions (Fig. 1.5a,b). Crucially, this increase concerned specifically those muscles that participants would have adopted to actually perform the action (Fig. 1.5c). Further studies have subsequently confirmed this muscle-specific effect, showing, for example, facilitation in the first dorsal interosseous (FDI; involved in index finger abduction) muscle and not in the abductor digiti minimi (ADM; involved in little finger abduction) muscle during index finger movements observation, and the opposite for little finger movements observation (e.g., Romani, Cesari, Urgesi, Facchini and Aglioti, 2005; Urgesi, Candidi, Fabbro, Romani and Aglioti, 2006; Catmur, Mars, Rushworth and Heyes, 2007). The mirror response in humans, unlike in monkeys, is sensible also to the observation of intransitive actions like meaningless arm movements, finger abduction-adduction, movements of the wrist, and complex movement stimuli like pinching (e.g., Loporto, McAllister, Edwards, Wright and Holmes, 2012), squeezing (e.g., Lepage, Saint-Amour and Théoret, 2008), lifting objects (e.g., Alaerts, Senot, Swinnen, Craighero, Wenderoth and Fadiga, 2010), tools actions (e.g., Cattaneo, Maule, Barchiesi and Rizzolatti, 2013) and also sporting actions (e.g., Aglioti, Cesari, Romani and Urgesi, 2008). Overall, these studies confirm that action observation causes an increase in MEPs amplitude in the observer similar to that recorded during action execution.

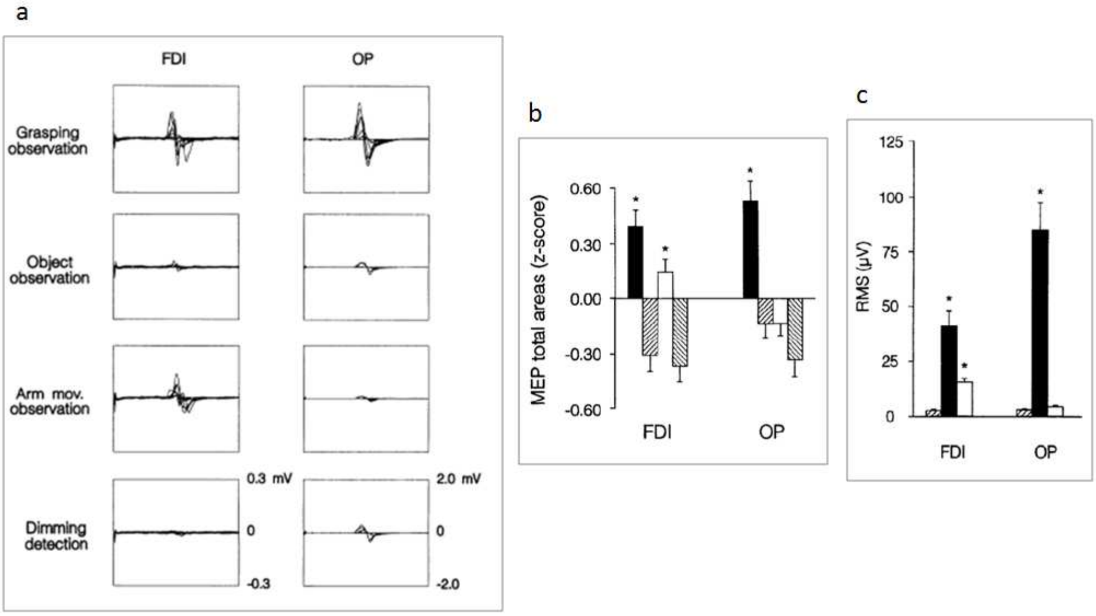

Fig. 1.5: Results obtained in the Fadiga et al. (1995) study for the first dorsal interosseus (FDI) and opponens pollicis (OP) muscles of the right hand. a) MEPs acquired from the eight stimuli presentation for each muscle and experimental condition from a representative participant. b) Mean normalized MEPs values in the four experimental conditions, where grasping and arm movements observation are represented in black and white, respectively. c) Mean values of EMG activity while participants performed grasping (black), arm movements (white) and were resting (grey). (Modified from Fadiga et al., 1995)

Crucially, another property of the putative human MNS has been demonstrated by means of the TMS: MEPs are modulated in a fashion strictly resembling the time-course of the observed actions. Gangitano, Mottaghy and Pascual-Leone (2001) recorded MEPs from hand muscles at different time intervals while participants observed grasping movements. From the results it emerged that MEPs amplitude followed the temporal phases of the observed action, increasing during the finger aperture and becoming smaller during the hand closure phase. The adoption of several stimulation time points is an important tool to investigate the time course of cortical facilitation during action observation. However, every experimental stimulus (e.g., a grasping action) is usually repeated several times, and participants are quickly able to predict the time course of the action. This prediction effect clearly emerged in a study from Urgesi and colleagues (2010) who found increased MEPs in the muscle involved in the observed action while observing hand postures in the starting and middle action phase compared to the final phase (Urgesi, Maieron, Avenanti, Tidoni, Fabbro and Aglioti, 2010). This suggests that the human MNS may play an important role in predicting and anticipating the action of others individuals, which represents a key ability to efficaciously interact with them.

The temporal matching of corticospinal excitability (CE) modulation during observation and execution of actions has been confirmed also by Borroni, Montagna, Cerri and Baldissera (2005). They adopted, in conjunction with spTMS, a peripheral nerve stimulation technique to stimulate the medial nerve and evoke a Hoffman (H-) reflex in the muscles. By recording both MEP and H-reflex it is possible to measure the excitability modulation following action observation either in the observer's cortical and spinal motor pathways, respectively. Indeed, the H-reflex gives an indication of the excitability level in the pathway between the spinal cord and the muscle. This study showed that watching a sinusoidal flexion-extension of the wrist elicited a sinusoidal excitability modulation in the motor pathways (measured as changes in amplitude of either the H-reflex or MEP) of the observer's wrist muscles. The results thus suggested that the time course of the observed movement was tightly correlated with the subliminal modulation of the motor pathways, and slightly predictive, with muscle activation advanced with respect to the movement (Borroni et al., 2005). By adopting the same methodology, Baldissera et al. (2001) further investigated the spinal cord excitability contribution during action observation. What they found is that the spinal cord excitability, tested by eliciting the H-reflex in a finger muscle, varied in accordance with the observed movements but in the opposite direction to that occurring when executing them (Baldissera, Cavallari, Craighero and Fadiga, 2001). They suggest that the emerged inhibitory pattern in the spinal cord could allow the motor system to replicate the observed action internally while preventing its overt replica. Consistent with the discovery of 'anti-mirror' neurons in the human brain (Mukamel et al., 2010), it is possible that our action observation system contains mechanisms that, while allowing to resonate with others actions, still ensure to not imitate them when it is not required. However, other inhibitory mechanisms to prevent this kind of involuntary behaviors could be present also in other part of the brain, like the frontal cortex. The effect of the impairment of these inhibitory gates emerge dramatically in neuropsychological pathologies, such as the utilization behavior, in which patients with lesions associated to the frontal lobes (Lhermitte, 1983) are unable to inhibit stereotyped actions elicited by the sight of an object, even if the action is inappropriate to the context. The fact that usually we do not overtly and compulsively imitate others actions indeed indicates the existence of a supervisor system that ensures that only appropriate behaviors in a given situation are executed, while the others should be inhibited (Shallice, Burgess, Schon and Baxter, 1989).

As concerns the neural basis of the subliminal resonant mechanism, it appears to be mediated by the activity of a key site of the MNS, namely the premotor cortex, through cortico-cortical connections to the primary motor cortex (Strafella and Paus, 2000). Repetitive TMS (which interferes with the functioning of a restricted brain area) over the premotor cortex, but not over M1, reduced muscle-specific facilitation following action observation (Avenanti, Bolognini, Maravita and Aglioti, 2007), thus suggesting a functional contribution of the premotor cortex in the action-related corticospinal mapping. Indeed, given that corticospinal projections from premotor cortex are few in numbers, it is likely that the motor resonant mechanism could be mediated by the abundant connections between the premotor and the primary motor cortex (Cerri, Shimazu, Maier and Lemon, 2003; Shimazu, Maier, Cerri, Kirkwood and Lemon, 2004).

To summarize, TMS allows to study the mirror mechanism in humans, showing that the motor cortex dynamically replicates the observed actions as if they were executed by the observer. Furthermore, TMS permits to measure corticospinal modulations induced by action observation with a very high temporal resolution. In addition, in virtue of the possibility to evoke MEPs in specific muscles, TMS allows to determine fine-grained correspondences with the muscular pattern presented in the observed action. However, it should be noted that the MEPs amplitude reflect the final output of the motor pathways, thus it indicates the resulting excitatory and inhibitory effect exerted by the action observation system during action perception, not its origins. Overall, considering the increasing body of literature deriving from the application of TMS in action observation contexts, TMS represents a valuable tool to study the motor resonant response in humans.

CHAPTER 2

FROM MOTOR RESONANCE TO COMPLEMENTARY ACTIONS

As introduced in Chapter 1, humans appear remarkably efficient in resonating with others actions. Indeed, a multitude of studies has shown that a ‘motor resonant’ mechanism allows us to match observed actions with corresponding motor representations. The neural substrate of this ability is identifiable in the activity of the MNS that appears to be shared with our monkey ancestors. However, not only phylogenetically, but also ontogenetically we are prone to process others actions. Newborn babies are able to imitate facial and manual gestures, showing the ability to equate their own behavior with the observed one (Meltzoff and Moore, 1977, 1983). Strikingly, even twin fetuses by the 14th week of gestation display interactive movements specifically directed to the co-twin (Castiello et al., 2010). It thus appears that human beings are hard-wired to socially interact.

The ability to interact with other people is indeed critical for our success in a social environment, since working in concert with others in order to achieve common goals is an everyday need. From carrying objects or cooking together to activities like sports, dance and music, we constantly need to coordinate our actions with those of others. Nonetheless, the basic mechanisms underlying perception and action in social contexts are still poorly understood. The traditional approach of studying cognitive processes in individuals – and their minds and brains – in isolation has only recently been challenged (Sebanz, Bekkering and Knoblich, 2006; Richardson et al., 2012). Indeed, it has been demonstrated that the presence of another individual during a task may significantly change our performance (e.g., Sebanz, Knoblich and Prinz, 2003; Atmaca, Sebanz, Prinz and Knoblich, 2008; Sartori, Becchio, Bulgheroni and Castiello, 2009). Interacting with someone else, in fact, requires the ability to adapt our behavior (i.e., action plans, movements, intentions) and adjust it to be able to coordinate our actions with the other person’s ones. Therefore, a new branch of research is now specifically focusing on *joint actions*, that is all those interactions in which two (or more) people coordinate their actions in space and time to produce a joint outcome (Sebanz et al., 2006; Knoblich, Butterfill and Sebanz, 2001).

Understanding the goal of an observed action is an essential step to successfully interact with it. It has been demonstrated that humans, when required to imitate an observed action, tend to spontaneously reproduce the goal rather than the means (the scope of an action, rather than the specific movement; e.g., Wohlshlager, Gattis and Bekkering, 2003; Gergely, Bekkering and Király, 2002). Then, the very same action executed with different aims could manifest peculiar differences that could be decoded from the observer's action observation system. The ability to decipher these differences is surely crucial to understand others behavior. Evidence that the MNS can distinguish between different intentions underlying a specific behavior according to the context in which the action is embedded has been found both in monkeys (Fogassi, Ferrari, Gesierich, Rozzi, Chersi and Rizzolatti, 2005) and humans (Iacoboni, Molnar-Szakacs, Gallese, Buccino, Mazziotta, and Rizzolatti, 2005). Interestingly, kinematic studies (in which action execution is quantified in terms of position and displacement of body segments and their velocities and acceleration patterns) demonstrated that the intentions guiding the action (e.g., grasping to cooperate or to compete with another person) translate into specific motor outputs that are detected by the observer and can influence her action (Becchio, Sartori, Bulgheroni and Castiello, 2008a, 2008b; Sartori, Becchio and Castiello, 2011a). Furthermore, Sartori and colleagues (2009) have demonstrated that, while participants were performing a simple reach-to-grasp action, the unexpected presentation of an empty hand asking for the object was able to cause an early deviation of the participants' hand trajectory (i.e., after 165 ms from the hand presentation), even though they were asked to ignore the social request. Even more interestingly, in some trials (~20%) the social request was able to override the agent's initial motor plan and induce a complementary response (i.e., handing the object to the person asking for it), regardless of the instructions (Sartori et al., 2009). Indeed, it is interesting to note that in social situations to imitate what other people do is not always the best strategy to successfully interact with them. Rather, we are often required to perform dissimilar actions to the ones observed. For example, if someone hands us a mug by its handle we will automatically grab the mug with a power grip (the most appropriate grasp in this particular situation), the two gestures thus differ and are incongruent, but they are nevertheless appropriate and complementary. The present chapter will focus on this specific type of joint actions, namely *complementary actions*, in which, the action to perform differs from the observed one (Sebanz et al., 2006; Sartori, Betti and Castiello, 2013a; Sartori and Betti, 2015). As a working definition, complementary actions refer to "any form of social interaction wherein two (or more) individuals coordinate and mutually complete their incongruent actions, rather than performing imitative behaviors" (Sartori and Betti, 2015). In these situations, the tendency to imitate the observed action has to leave room to the inclination to carry out a non-identical response that is more appropriate to the context. It thus emerges that the putative human MNS may play a role in this process given its function in action observation and execution. However, acting in a complementary fashion might require the recruitment of neural systems beyond the MNS.

The present chapter will provide an overview on findings that bring new evidence – by means of behavioral, neuroimaging and neurophysiological paradigms – on the processes and neural bases underlying complementary actions.

2.1 Behavioral studies

A variety of studies have explored the flexibility of the action observation system and its inclination to perform a non-matching behavior following action observation. In many of them participants were explicitly instructed to imitate or to complement a virtual co-actor's grasp toward a manipulandum which could be grasped in two ways, namely using PG to grasp the top-part and a WHG to grab the bottom-part (van Schie, van Waterschoot and Bekkering, 2008; Newman-Norlund, van Schie, van Zuijlen and Bekkering, 2007; Poljac, van Schie and Bekkering, 2009). As expected, when asked to imitate, participants were faster in initiating a congruent action to the observed one, whereas they were relatively delayed when a dissimilar action was observed. However, this pattern was completely reversed in the complementary action task in which, instead, they were faster in responding to dissimilar rather than similar observed actions. These results suggest that, according to the task, participants can overcome imitative tendencies and develop appropriate stimulus-response (S-R) associations in a flexible manner. By adopting a 3-D motion-capture system, Ocampo and Kritikos (2010) studied spatial and temporal movement parameters in a similar imitative vs. complementary situation. They demonstrated that identical responses were improved in the imitative context, while the opposite was true for the complementary context. They demonstrated that action observation may alter the kinematic profiles of executed manual responses depending on contextual factors. Therefore it appears that the cognitive system allows to flexibly set appropriate S-R associations according to the task and to the goal of the action in order to optimize the performance. Similarly, Heyes and colleagues (Heyes, Bird, Johnson and Haggard, 2005) demonstrated that automatic effects of imitation can be temporarily abolished after incompatible training. According to the *associative sequence learning* (ASL) theory (Heyes, 2001), perception and execution of actions are closely linked, with responses given to a certain stimulus depending on the associations that the stimulus has developed through experience. Then, the correlated experience of observing and executing a particular action reinforce the S-R association and this matching can explain facilitation/interference effects when performing identical or non-identical responses, respectively. In this regard, Faber, van Elk and Jonas (2016) have recently shown that hand gestures such an hand-shaking can prime observers to respond in a complementary fashion, with the incongruent rather than the specular effector (see also Liepelt, Prinz and Brass, 2010). A lifetime experience of hand shaking can indeed drive our spontaneous responses and reverse classical facilitation effects. Taken together, these data challenge the idea that action observation automatically brings to imitation in the observer (i.e., direct matching hypothesis). Rather, they suggest that observed actions can prime non-identical responses depending on the context.

2.2 Neuroimaging studies

Very few studies have examined the neural underpinnings behind complementary actions. A complete picture of the involvement of the MNS circuitry together with the contribution of other brain areas is still lacking.

Newman-Norlund and colleagues (2007), in a pioneering fMRI experiment, have specifically investigated the role of the MNS while participants prepared to execute imitative or complementary actions. Brain activity was recorded during action preparation toward a manipulandum graspable in two ways – either with a PG or a WHG – after watching a virtual actor gripping it. Increased blood-oxygen-level-dependent (BOLD) signal was found in two core components of the MNS, namely the right IFG and bilateral IPL, when preparing complementary actions compared to imitative ones. The authors suggested that this finding might reflect the relative contribution of strictly and broadly congruent mirror neurons (see Chapter 1, par. 1.1.1 for a description). The former respond to identical observed and executed actions, whereas the latter respond to non-identical observed and executed actions, allowing to associate the observed action with a different, but related, motor response. However, it is also possible that canonical neurons, which respond both during action execution and during observation of objects linked to the performed behavior (Murata et al., 1997; see also Chapter 1. par. 1.1.1), might play a role in this situation. The need to grasp, in the non-identical condition, an object part eliciting a different grasp might determine an interplay between mirror and canonical neurons (Sartori, 2016). Social interactions may then imply the involvement of different sets of mirror neurons to integrate observed and executed action to achieve a joint goal in a complementary fashion.

The idea that the putative MNS would link perceived actions with appropriate motor plans was confirmed in another fMRI study (Ocampo, Kritikos and Cunnington, 2011). The authors investigated if performing actions that are dissimilar to those observed is mediated by core regions of the human MNS (namely, IFG and IPL) or by general cognitive control mechanisms responsible for selecting and preparing conflicting responses. They adopted a naturalistic situation in which participants observed videos depicting an actor reaching and grasping a wineglass using either a PG (at the stem) or a WHG (at the rim), or a condition in which upwards or downwards-pointing arrows were instead presented. As expected, activity within the right IFG and the right IPL was greatest in the imitative context when participants performed similar responses to the observed hand action. Interestingly, activity within those regions also increased when dissimilar responses were cued by the directional arrows, reflecting a non-specific increase while dealing with a response conflict (i.e., a remapping of the S-R association), possibly involving other control mechanisms. Shibata et al. (Shibata, Inui and Ogawa, 2011) also found greater activity in the right IFG (together with the right cerebellum) during observation of incongruent rather than congruent actions, thus suggesting their role in understanding complex social situations. Taken together, these findings seem to suggest that two processes might be involved in mediating the connection between perception and action when non-identical actions have to be performed, both supported by fronto-parietal brain regions (Ocampo et al., 2011). The first process seems to match perceived actions onto motor representations in contexts that require similar responses. Whereas the second allows to inhibit the response that it is compatible with the stimulus in favor of the response that is compatible with the task demands.

A more integrated description of the neural circuitry underlying complementary actions have been outlined by Kokal and colleagues (Kokal, Gazzola and Keysers, 2009; Kokal and Keysers, 2010), who asked their participants to get involved in a real-time cooperation task with an experimenter while being scanned in fMRI. They mapped the brain areas of the putative MNS by looking for areas active during both action observation and execution. Crucially, they also mapped the brain areas specifically involved in joint actions, namely voxels exceeding the sum of execution and observation. The results showed additional activations adjacent to the putative MNS in the prefrontal, posterior parietal and temporal lobe. The authors identified two anatomical networks at the basis of joint actions: one set of areas, including the putative MNS, which seems to transform observed and executed action into a common code; and another set of areas that utilizes this common code to flexibly integrate our own action with those of others. Strikingly, even if the putative MNS regions were activated during joint actions, the flexible remapping required to perform joint actions seems to be performed outside the putative MNS.

More recently, Sacheli and colleagues (Sacheli, Candidi, Era and Aglioti, 2015) further explored the neural basis of complementary actions by using repetitive continuous inhibitory Theta Burst Stimulation (cTBS), a technique that, when coupled with neuronavigation, allows to study the causal role of a target area of the cerebral cortex by temporarily deactivate it (i.e., 'virtual lesion' approach). They inhibited two key nodes of the human fronto-parietal network, namely the left anterior intraparietal sulcus (aIPS) and the left ventral premotor area (PMv), to study their causal role in the execution of imitative and complementary actions while participants were interacting with a human-avatar. They demonstrated that the inhibition of the left aIPS selectively impairs the performance during complementary interactions. Sacheli et al. (2015) then provided evidence on the neural underpinnings underlying complementary action by adopting a dynamic joint action task.

Results from the outlined neuroimaging studies on complementary actions are graphically summarized in Figure 2.1.

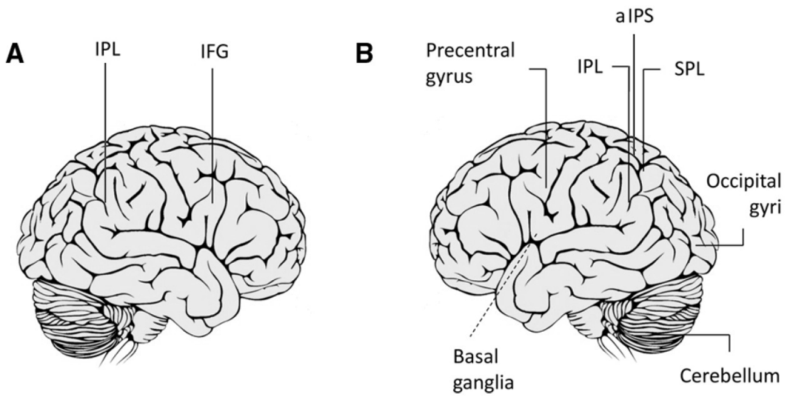

Fig. 2.1: Graphic representation of the areas resulted involved in complementary actions in several neuroimaging studies from a right (A) and left (B) hemisphere view. A) Right inferior frontal gyrus (IFG) and inferior parietal lobule (IPL) are not only involved in imitation, they activate also when responding in a complementary fashion (Newman-Norlund et al., 2007, 2008; Ocampo et al., 2011; Shibata et al., 2011). B) Other studies support the involvement of a larger network including IFG, IPL, superior parietal lobule (SPL), anterior intraparietal sulcus (aIPS), precentral gyrus, basal ganglia, middle and temporal occipital gyri and cerebellum to flexibly integrate one's own actions to those of others (Kokal et al., 2009; Kokal and Keysers, 2010; Sacheli et al., 2015). (Modified from Sartori and Betti, 2015)

2.3 Neurophysiological studies

A serious limitation of the previously described experimental paradigms is that participants were explicitly asked to act in an imitative or in a complementary fashion and employed tasks that are not – or not completely – associated with any meaningful behavior in real life, or that are likely to uncover dispositions formed throughout the experimental task itself (e.g., in imitation vs. complementary blocks) rather than spontaneous tendencies. Sartori and colleagues, in a series of neurophysiological studies, overcome those limits by showing to participants actions embedded in a naturalistic setting able to elicit (or not) a complementary response in the observers (Sartori, Bucchioni and Castiello, 2012a, 2013b; Sartori, Cavallo, Bucchioni and Castiello, 2011b, 2012b; Sartori, Begliomini, Panozzo, Garolla and Castiello, 2014). By using spTMS (see also Chapter 1, par. 1.2.4) they were able to investigate under-threshold corticospinal (CS) excitability while participants were observing actions, without requiring any explicit response. An increase in MEPs induced by TMS in specific muscles indicates that observers are specifically attuned to the observed action. In this manner it is possible to uncover spontaneous tendencies to react to the observed actions in social contexts.

In order to investigate whether the CS facilitation is modulated in function of the observed action but is also influenced by contextual factors, Sartori et al. (2011b) asked participants to watch video sequences in which an actress grasped a fruit (e.g., an almond) to place it within a tray, and eventually unfolded her hand in a request gesture as to ask for a further piece of fruit located out of her reach, but placed near the observer. In control conditions the same action sequences were adopted, but the nearby object was not presented. The results showed that only when the request gesture was shown together with the object, the MEPs amplitude reflected the intention to accept the request (i.e., grasping the object) rather than the tendency to resonate with the observed action. A mirror response (i.e., the activation of the muscles specifically involved in the observed action) was shown in the initial part of the action (i.e., when the model grasped for the object), whereas MEPs recorded at the time the actress unfolded the hand in the request gesture reflected a complementary motor preparation. To ascertain if the effect was intrinsically social or whether it could also be elicited by non-social cues like an arrow pointing toward the object, another experiment was performed. The results showed that the arrow determined a MEP activation, but not as strong as that recorded in presence of the biological request gesture (Sartori et al., 2011b). This study provided evidence that, at a neurophysiological level, the mechanisms underlying action observation are flexible and they can be spontaneously modulated by contextual factors above and beyond imitation.

To further deepen this finding, Sartori et al. (2012b) devised another study to explore the shift from the congruent to the incongruent activation in participants' CS excitability, what they called *functional switch*. In this case, participants were presented with a double-step action in which an actress: 1) grasped an object (e.g., a thermos), performed an action with it (i.e., she poured coffee in coffee cups) and then 2) she stretched out her arm toward an object out of her reach (e.g., a cup located on the right corner of the screen). Crucially, the type of grasp observed (i.e., a WHG on the thermos) was specifically mismatched with the type of grasp required to act on the nearby object in order to interact with the actor (i.e., a PG on the coffee cup). Results showed that imitative activations were evident at the beginning of the action sequence, and crucially they turned into complementary activations when the request for a reciprocal action was evident at the end of the action sequence (*functional shift*). This complementary preparation was evident only when the out-of-reach object on the right corner of the screen was shown on the visual scene.

A subsequent study was then performed to explicitly investigate the time-course of this functional switch, allowing the preparation of a complementary response. When does the functional switch occur exactly? To answer this question Sartori and colleagues (2013b) adopted five TMS stimulation time-points that were specifically identified according to kinematic landmarks characterizing the observed action. TMS was then delivered: when the actress's hand made contact with the object (T_1), when she finished pouring (T_2), when she raised her hand from the cup/mug after it (T_3), at the onset of the complementary request gesture (T_4) and, lastly, at the end of it (T_5; see Fig. 2.2.). It emerged that a variation in MEP amplitude in the direction of a complementary activation emerged as soon as the actress' hand trajectory began to significantly move toward the out-of-reach object (T_4, Fig. 2.2). Participants were then able to detect subtle kinematic cues that allowed them to successfully discriminate between an action driven by a social goal from one that was not, and to anticipate the future course of an action. In the control condition, the actor's hand was instead shown moving back to its initial position at the end of the sequence, without expressing any social request. The presence of the out-of-reach object still visible in the visual scene in both conditions allowed to control for motor affordances elicited by the mere presence of the object (Sartori et al., 2013a, 2013b). In this respect, whereas *affordances* can be defined as action possibilities, guided by associations between environmental properties and abilities (Gibson, 1979), with the term *complementary affordances* are defined all those possibilities for interaction provided by others aimed to bring a joint goal to completion (Sartori and Betti, 2015). In this way, complementary requests can be processed in terms of their interactive potential, even in situations in which the involvement does not occur.

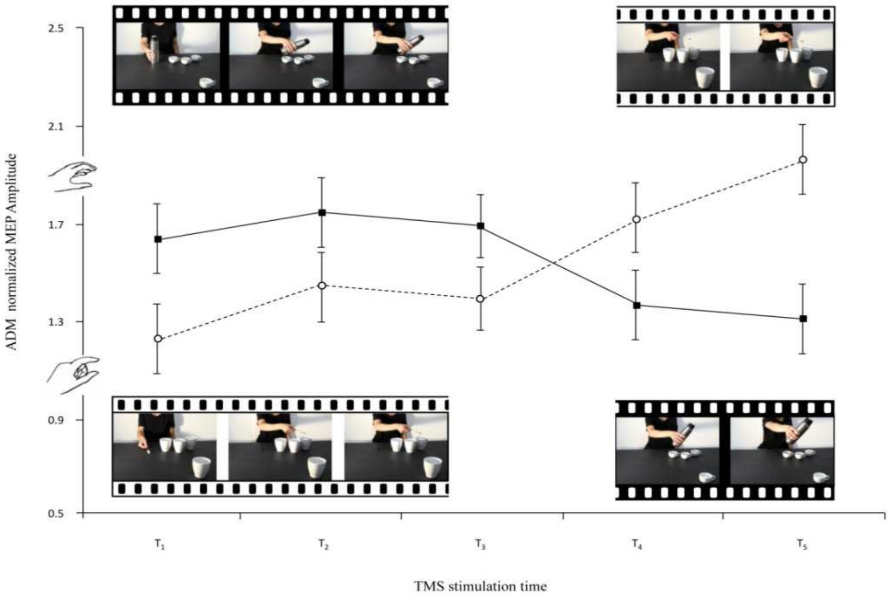

Fig. 2.2: Experimental stimuli and corresponding results from the Sartori et al. (2013b) experiment exploring the time-course of the functional shift from simulation to preparation of a complementary response. spTMS was delivered at five time points (T_1, T_2, T_3, T_4, T_5) corresponding to kinematic landmarks characterizing the action. Normalized mean MEP amplitude for the ADM muscle are represented, social PG movements requiring a WHG are represented in white, whereas in black are social WHG movements requiring a PG. Bars represent the standard error of means.

The same experimental paradigm was then adopted to test whether the observer's handedness may influence the complementary activation. Sartori et al. (2014) reported that in social contexts action preparation is remapped according to the dominant hand, so that a complementary activation emerges in the effector most functional for performing the requested gesture (i.e., the left hand for left-handers and the right hand for right-handers). In terms of action execution, a kinematic study has recently measured the effect of performing a grasping action while observing another action eliciting a different, complementary gesture (temporal overlap). Results showed that the social request interfered with the motor output, causing a general delay in the grasping and reaching components and an increased trajectory variance (Chinellato, Castiello and Sartori, 2015). Overall, these results highlight that the adoption of these stimuli, beyond their ecological validity, may shed light on the processes that our motor system implements to interact with others in a flexible manner.

The adoption of TMS has also demonstrated that the tendency to imitate can be reversed when training participants to perform a different action in relation to the one observed (Catmur, Walsh and Heyes, 2007). This reversal of the congruency effect, however, may not reflect spontaneous tendencies.

The use of more naturalistic experimental stimuli is surely needed to investigate the cognitive mechanisms – and the neural basis – of interactive performance to ensure a good ecological validity. Social behavior, indeed, requires the ability to quickly and constantly modify our behavior as to adapt it to a constantly changing environment (i.e., 'behavioral flexibility'; Easton, 2005).

To summarize, when interacting with another person, successful complementary actions are founded on the abilities: i) to simulate his/her movements, ii) to predict his/her future action(s), iii) to produce an appropriate incongruent response which differ, while interacting, with observed ones, iv) to complete the social interaction by integrating the predicted effects of one's own action with his/her one.

The study of the interactive behavior and of the mechanisms underlying the capacity to adapt our actions to the others to pursue a common goal is fascinating. And the attempt to shed light on the motor system involvement in this processes is indeed the aim of the research work illustrated in the present thesis.

CHAPTER 3

THE ROLE OF ATTENTION DURING ACTION OBSERVATION

A large body of evidence has shown that the observation of actions performed by others evokes a subliminal activation in the motor pathways of observers (i.e., 'motor resonance'), a mechanism that is thought to be mediated by the mirror neuron system activity (see Chapter 1 for a review). According to the 'direct matching hypothesis' (Iacoboni et al., 1999), the tendency to execute the observed action is triggered automatically and inevitably. The automaticity of this process is stated by Rizzolatti and Craighero (2004): "Each time an individual sees an action done by another individual, neurons that represent that action are activated in the motor cortex. This automatically induced motor representation of the observed action corresponds to what is spontaneously generated during active action and whose outcome is known to the acting individual" (p. 172).

Thus it might well be that humans have the tendency to unconsciously imitate the behavior of other people (e.g., Chartrand and Bargh, 1999). This disposition emerges from studies demonstrating that covert motor simulation follows action observation, thus providing support to the automaticity of the visuo-motor matching (e.g., evidence from TMS and EEG techniques; Fadiga et al., 1995; Cochin et al., 1998). Moreover, behavioral paradigms reveal the emergence of involuntary response priming during action observation (e.g., Brass, Bekkering and Prinz, 2001; Sturmer, Aschersleben and Prinz, 2000). Specifically, 'automatic imitation' tasks show an instruction-independent tendency to perform movements that are topographically similar to those observed (Heyes, Bird, Johnson and Haggard, 2005; Heyes, 2011). In this kind of tasks participants responses are faster and more accurate while observing congruent, rather than incongruent movements (e.g., responding with an open hand cued by an open, rather than a close, hand stimulus). Crucially, these effects emerge when participants are not explicitly required to attend or to respond to the observed actions.

Overall, a large body of literature seems to suggest that the activation of motor representations in the observer motor system is direct and automatic (e.g., Gallese et al., 1996; Rizzolatti and Craighero, 2004). However, the role of top-down factors in the visuo-motor transformation process has been largely ignored.

Given that information is selected on the basis of the actions to perform, selective processes are needed to behave in a coherent, but still flexible manner (Allport, 1989). Furthermore, they should deal with the capacity limits of the human cognitive system and prevent from information overload (Broadbent, 1958). From this perspective, a noteworthy question is whether attentional processes are needed for motor resonance to occur.

In the next section, following a paragraph covering some basic concepts related to visuospatial attention, studies in which the automaticity of the visuo-motor transformation process has been challenged will be reported.

3.1 Visuospatial attention: basic mechanisms

In broad terms, attention is defined as the mechanism through which certain information is selected for further processing while other information is discarded (e.g., Desimone and Duncan, 1995). The attended information is then processed more efficiently compared to the non-attended one. The world in which we live is indeed a complex environment, characterized by the presence of many individuals and objects. Nonetheless, we have only a limited amount of cognitive resources. A selective mechanism designed to enhance processing of relevant information and to limit distracting information is therefore evolved.

Selective attention allows to selectively concentrating on goal-relevant information and to discard what can interfere with our actions or intentions. In this line, visuospatial attention is the ability to focus on specific stimuli in a visual environment. Attention can be shifted toward specific parts of the visual scene either voluntarily, also referred as to endogenous attention, or automatically, which is referred as to exogenous (or stimulus-driven) attention (James, 1890). Exogenous visual attention is captured by salient physical properties of the visual stimuli like bright colors. Conversely, endogenous visual attention is voluntarily directed toward the stimulus, thus depending on the current task and on the individual's goal (for instance, attention can be focused on the color of an object to discriminate it among an array of visual stimuli). Moreover, attention orienting can be overtly or covertly allocated toward a stimulus (Posner and Petersen, 1990). In the first case eye-head-body movements are directed toward it (to bring the stimulus at the fovea, where visual acuity reaches its peak), whereas cover orienting occurs independently from eye movements. Therefore, in everyday life, the processing of objects and locations in space is influenced by the dynamic interaction between top-down factors, such as knowledge, expectation and current goals, and bottom-up influences from the environment based on sensory stimulation (Corbetta and Shulman, 2002).

Traditional views of human cognition have distinguished automatic from controlled processes. Whereas controlled processes are voluntary, relatively slow and require attention (Cohen, Dunbar and McClelland, 1990), automatic processes typically occur without awareness, are triggered involuntarily, are relatively fast and do not need attention for their execution (Bargh, 1992). Whether action observation is an automatic process and whether it is then resistant to top-down mechanisms such as attention is the topic covered in the following paragraphs.

3.2 Behavioral studies

A large amount of literature on the action observation system suggests that vision is automatically converted into motor representations. However, the inevitability of this process has been poorly investigated. Many studies were indeed conducted in absence of a secondary task, asking participants to observe the action and thus directing their attentional focus right on it. To better clarify the role of attention in the process that convert vision into action, Tipper, Paul and Hayes (2006) asked participants to respond with the left or right hand to the presentation of door handles oriented leftward or rightward. Crucially, they asked them either to attend the handle shape or its color. Because the shape plays a role in determining how the object should be grasped, it has a direct relationship with the action. The results showed that only when participants attended the action-relevant features a compatibility effect emerged (i.e., faster reaction time when the handle direction was compatible with the response hand: for example, when a handle pointing to the right was presented and a right hand response was required). In a subsequent experiment, Bach, Peatfield and Tipper (2007) demonstrated similar effects but only when participants focused their attention to a compatible effector. In a first experiment participants observed pictures of two actions, one involving a right foot (i.e., kicking a ball; Fig. 3.1, top panel) and the other involving a right hand (i.e., typing on a keyboard). Colored target dots were superimposed to the photographs and could appear either on the site relevant for action (the hand or the foot) or on a neutral site (the head). Participants were instructed to discriminate between the two colors (red or blue) by pressing a foot key or a finger key. Results demonstrated that the allocation of attention on the action-relevant stimulus feature is necessary for compatibility effect to emerge (i.e., quicker response times to the kicking action while responding with the foot compared to the hand, and the opposite for the observed hand action). In a second experiment the compatibility effect was still confirmed when all cues for the action and the objects were removed from the stimuli, thereby a person was presented either standing on a field or sitting next to a table without performing any kicking or typing action, respectively (Fig. 3.1, bottom panel). Bach et al. (2007) then demonstrated the crucial role played by attention allocation on motor facilitation. They also verified that not mere action perception, but rather attention to a body site seems to be the critical boundary for imitative tendencies to emerge. The results from Bach et al. (2007) suggest that only those body parts that are selectively attended, and therefore included within the spotlight of attention, are processed.

Fig. 3.1: Examples of the stimuli used in the experiment from Bach et al. (2007). The upper row refers to Experiment 1 and depicts kicking (left column) and typing (right column) actions. The bottom row refers to Experiment 2, showing the same person and situation, but objects and cues to actions were removed. Colored targets (red or blue) were superimposed over the foot, the hand or the head as a neutral position. (Modified from Bach et al., 2007)

Chong and colleagues (2009) adopted a Go/NoGo paradigm in which participants had to grasp an object with either a precision grip (PG) or a whole-hand grasp (WHG) while movement parameters were measured (Chong, Cunnington, Williams and Mattingley, 2009). By presenting a hand in the posture of a PG or a WHG as a 'Go' signal, they replicated the response compatibility effect (i.e., shorter response time for compatible rather than incompatible stimuli). Then, by superimposing a colored (red or blue) shape on the hand serving as a 'Go' signal, they demonstrated that when attention is directed away from the hand stimulus, the compatibility effect disappears. Automatic imitation no longer occurs when participants do not attend the critical feature of the observed hand. Overall, these findings suggest that selective attention plays a critical role for the visuo-motor matching process to take place, thus arguing against its automaticity. These results, together with evidence showing that also experience and prior exposure may influence the visuo-motor matching (e.g., Heyes et al., 2005, see also Chapter 2), suggest that this process is not as automatic as previously thought.

3.3 Neuroimaging studies

Chong and colleagues (2008) also performed an experiment aimed at exploring whether the strategic allocation of selective attention was capable of modulating the brain activity correlated with action observation (Chong, Williams, Cunnington and Mattingley, 2008). They ran an fMRI study in which participants were shown a set of stimuli which could either involve a natural reach-to-grasp action toward an object (PG or WHG) or a non-biological movement. A diamond silhouette partially interrupted by gaps was superimposed on each class of stimuli. The attentional load was manipulated by varying the relative spatial sizes of the gaps, which it could be large and thus easier to discriminate (Low Attentional Load) or small, thus more difficult to distinguish (High Attentional Load). A non-specific fronto-parietal activation was shown throughout the experiment. However, activity in the left pars triangularis (part of the IFG) was consistently suppressed under condition of High Attentional Load. The authors suggested that the left IFG – susceptible to attentional manipulation – may be the locus in which task-irrelevant actions are filtered during ongoing behavior to prevent unnecessary processing. These findings provide further evidence against the automaticity of the visuo-motor transformation process.

3.4 Electrophysiological and Neurophysiological studies

The studies illustrated so far required overt responses by the participants, thus potentially biasing action tendencies evoked by the experimental manipulation. Schuch, Bayliss, Klein and Tipper (2010) overcame these limits by using EEG to measure motor system activation during action observation. They registered the mu rhythm (i.e., an oscillatory activity in the alpha frequency range, 8-15 Hz), which has been associated with the MNS activity since it changes both during action execution and observation (see Chapter 2, par. 1.2.1). Specifically, mu rhythm over sensorimotor cortex is enhanced at rest and suppressed during movement production and observation. Schuch et al. (2010) asked participants to observe videoclips depicting a hand grasping a mug (either with a PG or a WHG) while simultaneously a grey 'X' superimposed on it changed its color into either blue or green. The participants' task was to make a subsequent judgement about the grasp or the color in separate blocks. Therefore, the observed videos were the same and the conditions differed only with respect to the task participants had to perform, namely to attend or not movement-relevant features of the stimulus. From the results it emerged that the suppression of the mu rhythm was observed not only when participants were attending to the action aspects of the stimuli, but also when they were detecting the color change. However, when directly comparing the two, greater suppression was found when the grasp was relevant for the task and therefore attention was focused on the action. Furthermore, more inhibitory rebound (i.e., increased synchronization in the alpha band) emerged after attending the action rather than the color, suggesting that greater levels of activation are followed by greater level of inhibitory feedback to prevent overt behavior. To summarize, Schuch et al. (2010) demonstrated that, though the recruitment of the motor system during action observation can be automatic, the extent of its activation is modulated by the task. Besides mu rhythm activity, also cortical oscillations in the beta (15-35 Hz) frequency band are attenuated by both the execution and observation of actions (e.g., Babiloni et al., 2002), and thus considered as a marker of MNS activity in human. Muthukumaraswamy and Singh (2008) used MEG recordings to measure brain oscillations while participants were passively observing hand movements or when they were actively watching the same movements to imitate them afterwards. They found that beta band sensorimotor desynchronization was enhanced when the task required active attention compared to passive viewing. They then provided additional support to the fact that brain activity during movement observation can be modulated by attention.

Saucedo Marquez, Ceux and Wenderoth (2011) adopted a dual-task approach to test the attentional costs related to action observation. They hypothesized that, in parallel with action observation, a second demanding task would create interference due to capacity limitations of the attentional system. By means of TMS, they detected changes in primary motor cortex excitability while participants observed simple motor actions either alone or in parallel with a secondary task. In the dual task condition they required participants to perform either an auditory or a visual discrimination task. They found that M1 excitability was perturbed (i.e., no action-specific CE modulations emerged) in case of the visual, but not the auditory, discrimination task – that is, when the second task was in the same visual sensory modality. These results suggest that the interference effects are not cross-modal and this can be explained by the presence of separate attentional resources for each sensory modality (Alais, Morrone and Burr, 2006). To summarize, visual distractors appear to interfere with M1 excitability. However, when the secondary task was in a different modality, the observer motor system responded in a near-automatic manner.

A further TMS study by Leonetti and colleagues (2015) investigated whether presenting an action in peripheral vision compared to central vision could modulate motor excitability. They then dissociated attentional and visual focuses in observers asking them to observe action sequences while fixating away from it. They hypothesized a decreasing precision of motor resonance from central to peripheral vision, given that the accuracy of visual information in the latter is much lower than in central observation. The results indeed showed that, even if the action viewed in peripheral vision was effective in modulating the excitability of motor pathways, the accuracy of the motor response was low and rough. Then, although covert attention was possibly allocated toward the action, the authors suggested that the poor visual information provided by peripheral vision were not enough to evoke a distinct motor resonant response in the observer.

So far, the activation of the motor system following action observation has been considered as an essentially automatic process. Whereas, the influence of top-down factors in modulating the processing of observed action has been largely neglected. However, a recent series of studies has changed this view, suggesting that efficient mechanisms should deal with capacity limitations of the cognitive system and that top-down factors such as attention might play a role in determining the visuo-motor matching.

PART 2

THE EXPERIMENTS

CHAPTER 4

GENERAL METHODS

In this chapter the methods and the procedures which are common to all the TMS experiments included in the present thesis will be described.

4.1 Participants information

The participants recruited for all the presented studies were right-handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971) and had normal or corrected-to-normal vision. Participants were naïve to the purpose of the experiments. Prior to the experiment participants were screened to rule out any history of neurological, psychiatric and medical illness, or any contraindication to TMS (Rossi, Hallett, Rossini and Pascual-Leone, 2009; Wassermann, 1998). None of the participants reported experiencing discomfort or adverse effects during the experiments. All participants gave informed written consent in accordance with the ethics approval by the Institutional Review Board at the University of Padua, in accordance with the Declaration of Helsinki (Sixth revision, 2008).

4.2 TMS Procedure

The basic principle of TMS functioning is that a changing primary electric current in a stimulation coil produces a changing magnetic field, which in turn induces a secondary flow of electric current in nearby conductors (e.g., the cortical tissue) as prescribed by Faraday's law (Barker, Jalinous and Freeston, 1985). Therefore, through electromagnetic induction, TMS can cause a transient and non-invasive depolarization of neurons, with the electric current passing across the scalp and skull without physical contact (see Fig. 4.1A-B). Single-pulse TMS (pulse characteristics: 100 μs rise time, 1 ms duration) was delivered over the participant's head using a 70 mm figure-of-eight coil (Magstim polyurethane-coated coil) connected to a Magstim Bistim^2 stimulator (Fig. 4.2B; Magstim Co., Whitland, Dyfed, UK). Pulses were delivered to the left M1 and the coil was oriented with the handle pointing laterally and caudally, so that the flow of induced electrical current in the brain travelled in a posterior-anterior direction (see Fig. 4.1A; Mills, Boniface and Schubert, 1992; Brasil-Neto et al., 1992). The optimal scalp position (OSP) – the position at which a maximal MEP amplitude was produced in the target muscle with a minimal stimulation intensity – for TMS stimulation was determined by moving the intersection of the coil in approximately 0.5 cm steps around the target area until the position was reached. This position was marked on a tight-fitting cap that each participant was asked to wear. During the experimental sessions the coil was held over the OSP by a mechanical arm (see Fig. 4.2C; Manfrotto, Italy) and continuously checked by the experimenters to maintain constant positioning. Moreover, the position and orientation of the coil over the OSP was recorded and loaded into a neuronavigation system (Brainsight, Rogue Research, Montreal, QC) to maintain accurate placement of the coil throughout the experiment. For each participant, the resting motor threshold (rMT) – i.e., the minimum stimulation intensity at which TMS generate MEPs of at least 50 μV peak-to-peak amplitude in a relaxed muscle in 5 out of 10 consecutive trials – was determined (Rossini et al., 1994). The stimulation intensity was set at 120% of the rMT to record a clear and stable electromyographic (EMG) signal throughout the experiments.

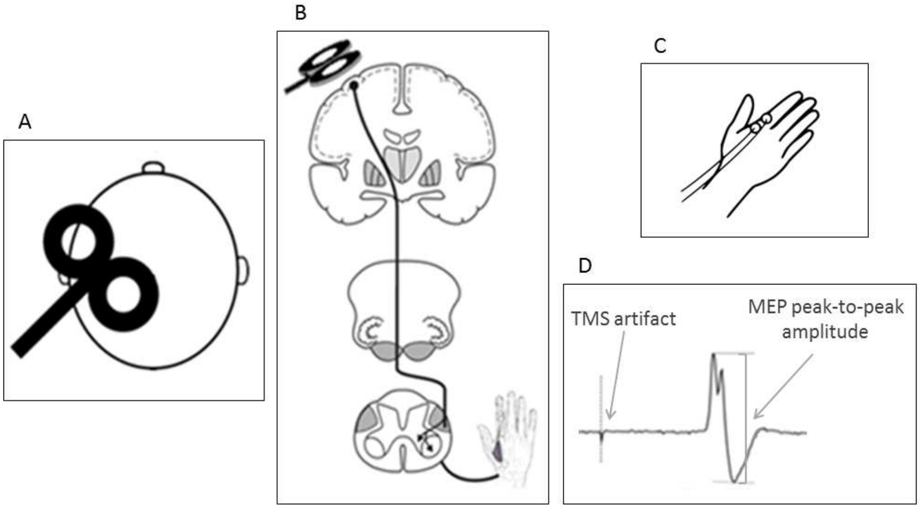

Figure 4.1: A schematic illustration of: A) posterior-anterior orientation using a figure-of-eight stimulating coil over left M1; B) TMS-induced corticospinal activation registered through C) pairs of electrodes in contralateral FDI muscle; D) EMG signal showing a TMS-induced motor-evoked potential (MEP). MEP peak-to-peak amplitude has been adopted as dependent variable in all TMS experiments.

4.3 EMG Recording

EMG activity was recorded through pairs of surface Ag-AgCl surface electrodes (9 mm diameter) placed in a belly-tendon montage, with the active electrodes over the muscle bellies and the reference electrodes over the ipsilateral metacarpophalangeal joint (Fig. 4.1C). The ground electrode was placed over an electrically neutral tissue (e.g., the dorsal part of the wrist for hand muscle recording). Prior to electrode application, the site was cleaned by means of an abrasive skin prepping gel and the electrodes were covered with a small amount of water soluble EEG conductive past. The skin impedance condition, evaluated at rest prior to beginning the experimental session, was considered of good quality when below the threshold level (5 Ohm). Electrodes were connected to an isolable portable ExG input box linked to the main EMG amplifier for signal transmission via a twin fiber optic cable (Fig. 4.2E; Professional BrainAmp ExG MR, Munich, Germany). The raw myographic signals were band-pass filtered (20 Hz - 1 kHz), amplified prior to being digitalized (5 KHz sampling rate), and stored on a computer for off-line analysis. EMG data were recorded for a 300 ms interval. The interval was time locked to the delivery of the TMS pulse and began 100 ms before the onset of stimulation and ended 200 ms after. Trials in which any EMG activity was present in the time window preceding the magnetic pulse were discarded to prevent contamination of MEP measurements by background EMG activity.

4.4 Experimental Procedure

Participants were tested individually in a sound-attenuated Faraday room. Each participant sat in a slightly raised armchair with both the hands and legs comfortably stretched and the head positioned on a fixed head rest (see Fig. 4.2). Participants were requested to remain still and relaxed and to watch the video clips presented on a monitor set at an eye level (eye-screen distance: 80 cm) placed in front of them (Fig. 4.2A).

The experimental protocol for each study will be discussed in the respective chapter.

Figure 4.2: The laboratory set-up for TMS experiments, showing: A) the screen from which the experimental videos were presented; B) the TMS figure-of-eight coil; C) the TMS coil holder tripod; D) the EMG electrodes; E) the EMG amplifier.

4.5 MEP Data Analysis

The corticospinal (CS) excitability of the target muscles was quantified at each stimulation point during each experimental condition by the MEP peak-to-peak amplitude (mV; see Fig. 4.1D). Individual peak-to-peak MEP amplitude was calculated offline and averaged for each participant and experimental condition using Brain Vision Analyzer software (Brain Products GmbH, Munich, Germany). To control for inter-individual variability in the absolute MEP values, MEPs amplitude values recorded during the experimental conditions were divided by MEPs amplitude values recorded during the baseline blocks (MEP ratio = MEP obtained / MEP baseline). MEP ratios were then analyzed.

CHAPTER 5

SIMULATION AND RECIPROCITY AT THE LEVEL OF MULTIPLE EFFECTORS[1]

5.1 Introduction

According to the MNS account, the comprehension of others' actions is mediated by the simulation of the perceived action in the observer's brain. Therefore, this 'motor resonance' (Rizzolatti and Craighero, 2004) or 'direct matching' (Iacoboni et al., 1999) mechanism is the subliminal activation of the same motor representations during both action observation and execution. It has been demonstrated that this visuo-motor matching mechanism requires that observed actions are already part of the observer's motor repertoire (Buccino et al., 2004, but see also Gazzola et al., 2007). Moreover, the observers' motor expertise plays a crucial role (e.g., Haslinger et al., 2005). For example, motor resonant activations in expert athletes compared to novices provide evidence on the superior abilities of athletes in perceiving and predicting motor actions (Aglioti et al., 2008; Abreu et al., 2012; Calvo-Merino, Glaser, Grézes, Passingham and Haggard, 2005, 2006; Fourkas, Bonavolontà, Avenanti and Aglioti, 2008). In neurophysiological terms, other studies have demonstrated that action observation selectively activates the muscles involved in the action being observed, retracing its temporal pattern (Fadiga et al., 1995; Urgesi, Candidi, Fabbro, Romani and Aglioti, 2006; Alaerts, Swinnen and Wenderoth, 2009; Borroni et al., 2005; Gangitano et al., 2001). However, the tendency to imitate observed actions is not always appropriate, as it happens in contexts requiring to perform different actions with respect to the observed ones (Hamilton, 2013). For example, in a sport setting it would be difficult to play if observing an action (e.g., throwing a ball) would lead to perform the same imitative behavior instead of different actions (e.g., catching the ball). This kind of situations is common during human social interactions, in which we are often required to perform dissimilar responses (i.e., *complementary actions*) to appropriately react to what is observed. As reviewed in the opening section of this thesis, a series of recent psychophysiological studies have specifically assessed CE modulations

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[1] *Published*: Sartori, L., Betti, S., Perrone, C. and Castiello, U. (2015). Congruent and Incongruent Corticospinal Activation at the Level of Multiple Effectors. *Journal of Cognitive Neuroscience*, *27*(10), 2063-2070.

occurring when the tendency to perform imitative symmetrical simulation gives way to reciprocity, according to the context (for a review see Sartori et al., 2013b; Sartori and Betti, 2015). However, in these studies both the imitative and the complementary actions tendencies were elicited in the same effector (i.e., the hand) therefore partially overlapping their temporal unfolding. Capitalizing on this literature, here I adopted an action sequence able to uncover spontaneous congruent and incongruent CE modulation in different limb muscles. In particular, I presented videos of penalty kicks in frontal view given the well-established association between observing someone kicking a ball straight in our direction far above ground, and the spontaneous reaction to lift the upper limbs as to parry the ball. The effectiveness of this type of stimuli in modulating motor responses in the observers has been recently confirmed in a fake detection task by Tomeo, Cesari, Aglioti and Urgesi (2013). In the present experiment I filmed penalty kicks performed by a soccer player who ran up to a ball and then kicked it straight in the direction of the camera. Given that the action was filmed from the goal, it then represents the perspective of a goalkeeper. Additional control conditions were also included, consisting in penalty kicks not directed toward the observers (i.e., lateral kicks) or that were only mimicked (i.e., kicked without the ball). A third control condition with the image of a static ball in the penalty area was also added to control for the motor coding of object affordances (Gibson, 1979). Indeed, it has been demonstrated that visual information linked to objects are transformed into appropriate motor commands even in absence of an explicit intention to act (Jeannerod, 1994; Tucker and Ellis, 1998). In this case, the soccer ball represented a unique control condition for our purposes since it can be used both with legs (i.e., for kicking) and hands (i.e., for throwing), unlike in other sports such as basketball or baseball, where the ball is only associated with hand movements.

Single-pulse TMS was used to assess CS excitability of participants' hand and leg muscles as they watched the videos. Depending on the action progression, it was then possible to disentangle the time-course of imitative and complementary responses at the level of multiple effectors. In particular, I hypothesized that if observing a soccer player running and kicking the ball elicits a motor resonant response in the observers, then an activation of their lower limb muscle was expected in the first part of the video. In addition, if facing a central penalty kick primes in the observer a complementary motor response, then an activation of participants' upper limb was expected when the ball was approaching them in the second part of the video. Along these lines, it could be hypothesized that the presentation of a lateral/mimicked kick would produce a simple motor resonant response in lower limbs, whereas observing a ball alone in the penalty area would elicit a stable activation of both limbs due to the motor coding of object affordance. To test these predictions, an extensor leg muscle and an abductor hand muscle have been specifically selected because they are typically recruited during kicking and parrying actions, respectively.

5.2 Methods

The methods employed in this study followed the procedure outlined in Chapter 4, pp. 55-58.

5.2.1 Participants

Thirty individuals (11 males and 19 females, mean age 23 years) took part in the experiment. All the participants were right-handed and reported right-foot dominance. As their greater action simulation abilities could have biased the results, individuals with any specific motor expertise in playing soccer were excluded from the experiment by means of a screening procedure. In fact, it has been demonstrated that athletes present superior abilities in predicting and anticipating other player's actions (Abernethy, Zawi and Jackson, 2008; Aglioti et al., 2008; Calvo-Merino et al., 2005; Makris and Urgesi, 2014; Tomeo et al., 2013; Urgesi, Savonitto, Fabbro and Aglioti, 2012). Furthermore, to be included in the study each participant was prescreened to check whether a reliable motor evoked potential could be elicited from the leg muscle in five out of ten consecutive trials. Participants were financially compensated for their time.

5.2.2 Experimental Stimuli

The stimuli were four digitally video clips recorded in an ecological setting showing penalty kicks performed by a professional soccer player (Fig. 5.1): a) central kick: the player kicked the ball into the goal straight toward the camera; b) lateral kick: the player kicked the ball clearly to the left of the camera; c) mimicked kick: the player mimicked a kick without the ball; d) ball: a static soccer ball was shown. All the videos were taken frontally, from the goal point of view. A 1800 ms sequence was extracted from each of the four videos, which included the player's initial run and the ball's trajectory until it disappeared. The player's foot made contact with the ball approximately 1350 ms after the video began, and the ball trajectory reached its highest peak approximately 400 ms later (1750 ms after the video onset). The ball was travelling at a velocity of approximately 10 m/s during its trajectory. An animation effect was obtained by presenting a series of single frames each lasting 25 ms (resolution = 720 x 576 pixels, color depth = 24 bits) after the first frame which lasted 500 ms.

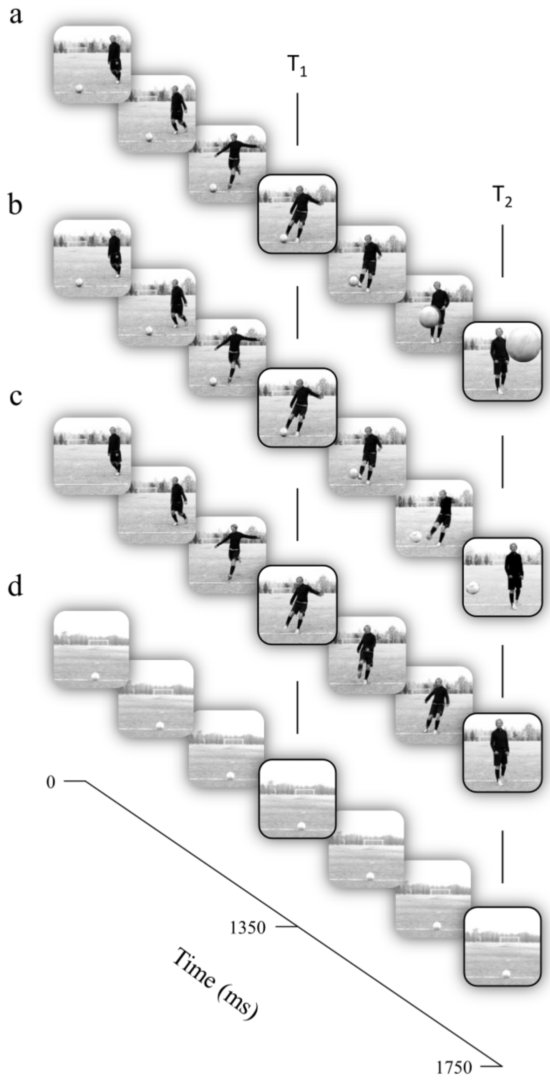

Fig. 5.1: A schematic representation of the sequence of events for each condition: a) Central kick; b) Lateral kick; c) Mimicked kick; d) Ball. The vertical lines denote the time points when single TMS pulses were delivered: at T_1, when the player's foot made contact with the ball, and at T_2, at the end of the video clip.

5.2.3 Procedure

The participants were tested individually in a sound-attenuated Faraday room during a single experimental session lasting approximately one hour and consisting of two blocks (upper limb, lower limb). Participants were instructed to watch the video clips that were presented on a 19” monitor (resolution = 1280 x 1024 pixels, refresh frequency = 75 Hz, background luminance of 0.5 cd/m^2) set at an eye level while remaining still and relaxed. In order to ensure attention to the video clips, they were told they would be questioned at the end of the experimental session about the stimuli presented. TMS-induced MEPs were acquired from the right opponens pollicis (OP) and quadriceps femori (QF) muscles. Upper and lower limb activity was recorded in separate blocks to precisely identify the OSP for stimulating each muscle and avoid loss of modulation involving the less stimulated muscle. The order in which the two blocks were presented was counterbalanced across the participants. A single TMS pulse was released during each video presentation at one of two specific time points: i) during the frame showing the player’s foot making contact with the ball (T_1, 1350 ms) and ii) during the frame showing the highest peak of the ball’s trajectory (T_2, 1750 ms; Fig. 5.1). The first time point (T_1) was chosen to evaluate the motor resonant response, whereas the second time point (T_2) was set at the highest peak of the ball’s trajectory before it disappeared in order to maximize the complementary reaction to the stimulus. The order of the videos and of the two different TMS stimulation times was randomized within each of the two blocks. A total of 160 MEPs (2 muscles x 4 conditions x 2 time points x 10 repetitions) were recorded for each participant. Prior and after the experimental block, each participant’s baseline CE was assessed by acquiring 10 MEPs while they passively watched a white-colored fixation cross on a black background presented on the computer screen. By comparing the MEP amplitudes recorded during the two baseline periods (20 MEPs in total) it was possible to check for any CE changes related to TMS per se. The average amplitude of the two series was then utilized to set each participant’s individual baseline for data normalization procedure. An interpulse interval lasting 10 s was presented between trials to avoid any short-term conditioning effect (Classen et al., 2000). During the resting period, a message reminding the participants to remain fully relaxed appeared on the screen for the first 5 seconds, followed by a fixation cross that was presented for the remaining 5 seconds. Stimuli presentation, timing of TMS stimulation and EMG recordings were managed by E-Prime V2.0 software (Psychology Software Tools, Pittsburgh, PA) running on a PC.

5.2.4 Data Recordings

The TMS procedure was identical to that reported in Chapter 4 in paragraph 4.3 and EMG recordings were collected as outlined in paragraph 4.2. MEPs were recorded from the OP muscle of the right hand and from the QF muscle of the right leg. EMG activity was recorded through pairs of surface Ag/AgCl electrodes placed in a belly-tendon montage. The ground electrode was placed over the dorsal part of the wrist during the 'upper limb' block and over the patella of the leg during the 'lower limb' block. Single-TMS pulses were delivered to the left M1 corresponding to the hand and leg regions during the upper limb and lower limb blocks, respectively. rMT ranged from 30% to 62% (mean = 49.2%, *SD* = 6) of the maximum stimulator output in the upper limb block and from 54% to 78% (mean = 64.7%, *SD* = 7.2) in the lower limb one.

5.2.5 Data Analysis

The data analysis followed the procedure outlined in Chapter 4, paragraph 4.5. The CE of OP and QF muscles were quantified at each stimulation point during each experimental condition by the MEP peak-to-peak amplitude (mV). Those amplitudes deviating more than 3 SDs from the mean and trials contaminated by muscular pre-activation were excluded as outliers (< 5%). A paired sample *t*-test (two-tailed) was used to compare the amplitude of MEPs recorded during the two baseline periods carried out at the beginning and at the end of each block. The normalized MEP amplitudes (MEP ratio) were entered in a repeated-measures ANOVA with muscle (OP, QF), type of action (central kick, lateral kick, mimicked kick, ball) and stimulation time (T_1, T_2) as within-subjects factors. The sphericity of the data was verified before performing statistical analysis (Mauchly's test, $p > 0.05$). Post-hoc pairwise comparisons were carried out using *t*-tests and Bonferroni correction was applied to control *p*-values for multiple comparisons. A significance threshold of $p < 0.05$ was set for all statistical analyses run with SPSS software package (SPSS Inc., Chicago, IL, USA).

5.3 Results

The mean raw MEP amplitude recorded for each participant at the beginning and at the end of the experimental blocks were not significantly different for either the OP (t_{29} = 1.42, $p = 0.17$) and QF (t_{29} = 0.04, $p = 0.97$) muscles. Therefore, it could be excluded that TMS per se induced changes in CE that could have biased the results. The ANOVA on normalized MEP amplitudes showed a statistically significant main effect of time ($F_{1,29}$ = 7.21, $p < 0.01$, η^2_p = .20), a two-way interaction of muscle x time ($F_{1,29}$ = 4.26, $p < 0.05$, η^2_p = 0.13) and a three-way interaction of muscle x type of action x time ($F_{3,87}$ = 7.27, $p <$ 0.01, η^2_p = 0.20). The results obtained from the post-hoc contrasts exploring the source of the significant three-way interaction are outlined as follows.

Direct matching in lower limb

In terms of direct matching, observing a soccer player kicking a ball elicited an higher activation of the lower limbs, but just when the foot made contact with the ball. Specifically, MEP amplitude in QF muscle of the lower limb was higher at the beginning of the video (T_1) with respect to the end (T_2) in the 'central', 'lateral' and 'mimicked' kick conditions ($p_s < 0.05$). However, this decreased activation did not appear in the 'ball' condition in which no modulation emerged across stimulation time points ($p > 0.05$; Fig. 5.2). Furthermore, the statistically significant decrease in the three kicking condition (central, lateral and mimicked) at T_2 was confirmed when comparing each of them with the ball condition ($p_s < 0.05$). Overall, this indicates that observing kicking actions leads to a replicative activation in the observers' lower limbs, which is followed by a reduction of the CS excitability when the player is shown still after kicking (T_2). On the other hand, the motor coding of object affordance keeps unchanged across time points.

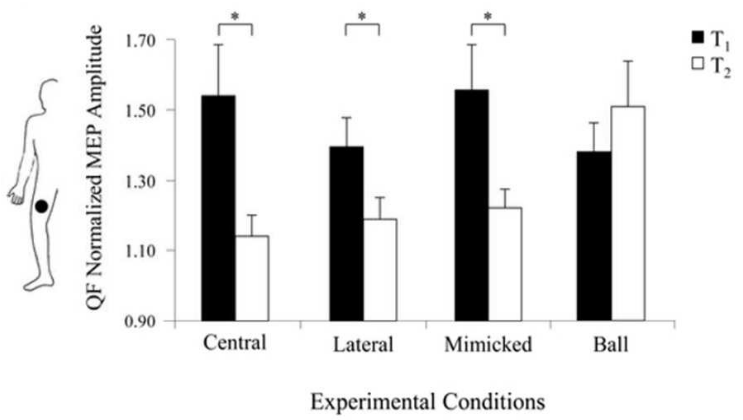

Figure 5.2: Results showing normalized mean MEP amplitude for the quadriceps femori (QF) muscle of the leg in the 'central', 'lateral', 'mimicked' kick and 'ball' conditions. The graph represents MEP acquired at T_1 and T_2 in black and white, respectively. Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons ($p < 0.05$).

Complementary motor activations in upper limb

As concern upper limb activations, the mean MEP amplitude of the OP muscle was higher at T_2 than T_1 only for the central kick condition ($p < 0.05$; Fig. 5.3), when the ball was approching the observer. Post-hoc comparisons at T_2 confirmed this increased activation with respect to the lateral and mimicked conditions ($p_s < 0.05$). Conversely, there was no difference between T_1 and T_2 for the control conditions ('lateral', 'mimicked' kick, 'ball'; $p_s > 0.05$). Furthermore, when the soccer ball was presented alone in the penalty area (ball condition), a significant corticospinal activation was found at T_2 with respect to the lateral and mimicked conditions ($p_s < 0.05$). The ball presentation indeed activated object-related affordances in the observers' hand muscle which, as also previously seen for the leg muscle, were kept unchanged across time points. To summarize, it seems that a complementary motor preparation emerged when the observed action required an appropriate reaction (i.e., parry the approaching ball). Moreover, a motor coding of object affordance appeared when observers were presented with the soccer ball alone in the penalty area.

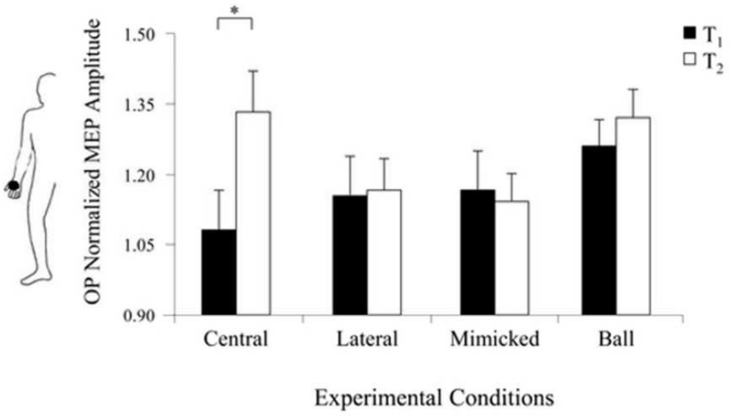

Figure 5.3: Results showing normalized mean MEP amplitude for the opponens pollici (OP) muscle of the hand in the 'central', 'lateral', 'mimicked' kick and 'ball' conditions. The graph represents comparisons between MEP acquired at T_1 and T_2 in black and white, respectively. Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons ($p < 0.05$).

Interaction between direct matching and complementary action preparation

Interestingly, a dissociation emerged for the central kick condition, when comparing the leg and hand CE activations. Specifically, post-hoc analysis showed a statistically significant increase in the QF muscle at T_1 (i.e., while seeing the soccer player kicking the ball) compared to the OP muscle ($p < 0.05$). Conversely, at T_2 (i.e., while seeing the ball approaching the observer), post-hoc analysis showed an increase in the OP muscle activation compared to the leg muscle ($p < 0.05$). The interplay between the two muscular activations is showed in Fig. 5.3.

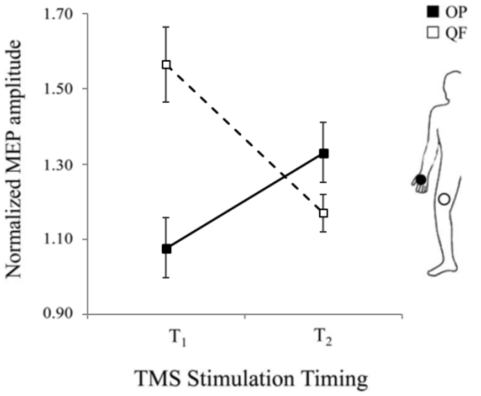

Figure 5.3: Interplay between normalized MEP amplitudes recorded from the OP (black) and QF (white) muscles at T_1 and T_2 for the 'central kick' condition. Bars indicate standard error of the means.

5.4 Discussion

This study aimed at examining the modulation of corticospinal excitability contingent on observation of actions priming identical and non-identical responses on multiple effectors. The results showed that observing a kicking action increased the leg muscle activation, and observing the approaching ball increased the hand muscle activation. It thus emerged an interesting dissociation between upper and lower limbs depending on the context.

Simulating the observed action (i.e., producing an internal replica) is useful during the first part of the action sequence (T_1) to understand its meaning and to predict its consequences (Kilner, Friston and Frith, 2007). Conversely, when the observer feels engaged in a potential interaction with the player who kicked the ball in his/her direction (T_2), the preparation of an appropriate response is primed. According to Ocampo et al. (2011), a two-step process is at the basis of this ability: i) the observed action is mapped onto the observer's motor system, and ii) this activation is inhibited to allow for the preparation of an incongruent response. The inhibition of an imitative response in favor of a complementary reaction can be useful in particular when the observed effector and the effector required to interact are exactly the same. If this is the case, in fact, interference effects generated from the observed action would occur (e.g., Chinellato et al., 2015). In the present experiment, I disentangled this two-step process by investigating congruent and incongruent activations at the level of different effectors. In line with the two-step hypothesis, the absence of leg activation at the end of the central kick could be explained as the byproduct of inhibitory mechanisms implemented to facilitate the complementary response in the upper limbs. Alternatively, this decrease could be related to the fact that the player was shown still after kicking the ball. In fact, a decrease in the leg muscle was registered in all the kicking conditions, not only when a complementary action was explicitly called for. These results suggest that a motor resonant activation, reproducing the muscle involved in the observed action, occurred. The present data also provide evidence that in humans, differently from monkeys (Rizzolatti and Craighero, 2004), object-directed (i.e., transitive) and mimicked (i.e., intransitive) actions elicit comparable mirror-like activations. As regards the complementary response, the upper limb muscle was specifically activated when the ball was kicked towards the observer, thus requiring his/her reaction as to parry the ball. No activation of upper limb was instead found when the ball trajectory was clearly lateral - and thus not suitable for any actions toward it - or when the action was mimicked - then excluding any possible effects of motor imagery. As concerns the ball condition, a muscle-unspecific activity in both upper and lower limb effectors was found, reflecting the activation of object-related affordances about the possible actions that could be performed with it (e.g., kicking or parrying). The general activation of both effectors is consistent with the findings from Lago and Fernandez-del-Olmo (2011), which described a general enhancement of participants' CE in muscles that would be required to interact with an object when presented with a static image of an effector in front of it. They further reported that only when the effector-object interaction took place, a muscle-specific activation emerged (Lago and Fernandez-del-Olmo, 2011). In the present situation, however, the object was presented alone. In neural terms, the activation of canonical neurons - which respond during the execution of a specific type of action but also during the perception of objects related to this particular action (Rizzolatti et al., 1988; Rizzolatti and Craighero, 2004) - might underlie this pattern of result.

Taken as a whole, this study's findings suggest that the action observation system is able to adapt to context demands in a flexible manner. Adopting spTMS, allowed to track these adaptive changes with a high temporal resolution. Moreover, the adoption of an ecologic setting as well as the exploration of spontaneous reactions in the observers (i.e., no task was given to participants), make this paradigm a useful tool to study the involvement of the motor system in everyday-life interactive situations. Crucially, the results presented here take research on the action observation system a step further by showing that an interplay between congruent and incongruent motor activations at the level of multiple effectors might occur, depending on the context.

The open question is whether these two types of divergent activations are mutually exclusive or they may functionally coexist, and this will be addressed in the following chapter.

CHAPTER 6

THE MULTIFORM MOTOR CORTICAL OUTPUT: KINEMATIC, PREDICTIVE AND RESPONSE CODING[1]

6.1 Introduction

Corticospinal excitability facilitation induced by action observation has been proven to be an effective method to study the mapping of the observed actions in the onlooker's motor cortex (e.g., Fadiga et al., 1995). Nevertheless, a still debated issue in this research area regards what is actually reflected by the motor system's output during action observation. In particular, a univocal view on the level of motor coding carried out during action observation is still unspecified. For example, many studies report that observed actions are reflected in the motor system in a muscle-specific fashion strictly related to the action kinematic pattern (e.g., Fadiga et al., 1995; Romani et al., 2005; Borroni et al., 2005; Cavallo, Becchio, Sartori, Bucchioni and Castiello, 2012). However, also more general aspects of the action like its final goal have been shown to be effective in modulating this matching response (e.g., Cattaneo, Maule, Barchiesi and Rizzolatti, 2013; Cattaneo, Caruana, Jezzini and Rizzolatti, 2009). A more integrated view considering the reciprocal contribution of these coding levels suggests that factors like the information provided to the observer about the action's goal (Mc Cabe, Villalta, Saunier, Grafton and Della-Maggiore, 2015), the different processing stage of the action (Cavallo, Bucchioni, Castiello and Becchio, 2013), or even the symbolic value of the action (Betti, Castiello and Sartori, 2015) may influence the motor outcome. Moreover, it has been demonstrated that the action observation system activity reflects the tendency to anticipate the future course of an action, showing higher responses for ongoing and still incomplete actions (Urgesi et al., 2010). Many studies also demonstrated that an observer can anticipate the outcome of an action, for example by correctly identifying the fate of a basketball shot (Aglioti et al., 2008) or the direction of a penalty kick (Tomeo et al., 2013) as well as whether an action heralds a competitive or a cooperative interaction (Sartori et al., 2011a). This predictive attitude is surely essential while dealing with a constantly changing environment in which we have to adapt our behavior according to the many requests coming from other individuals. This scenario is common for instance in sports, in which athletes have to

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[1] *Published*: Sartori, L., Betti, S., Chinellato, E. and Castiello, U. (2015). The multiform motor cortical output: Kinematic, predictive and response coding. *Cortex*, *70*, 169-178.

control their own actions (for example, in basketball trying to throw the ball in the basket) while monitoring actions of others and predicting their future outcomes (e.g., avoiding that an opponent would catch the ball), and this in turn would allow the player to adapt his behavior accordingly (e.g., to complete the shot successfully avoiding the opponent's attack).

The focus on the preparation of an action in response to an interactive agent is provided by a recent line of research centered on complementary actions (for a recent review see Sartori and Betti, 2015; see also Chapter 2). In particular, it has been demonstrated that observing a social request is able to generate divergent forms of motor activations (i.e., activation of muscles not primarily involved in the observed action) in the onlooker's motor system (e.g., Sartori et al., 2013a). Therefore, the matching activation following action observation appears to be overridden by the preparation of a social response when needed (e.g., Sartori et al., 2012; 2013a; 2013b; see also Chapter 5).

These findings suggest that different coding levels develop during action observation. A kinematic coding level, which operates by replicating the observed action; a predictive coding level, which anticipates the upcoming actions; and a response coding level, which allow the observer to prepare a response that is compatible with the task demands. To date, an integrated view of these three levels has yet to be proposed, since a single effector could only be activated in one or the other modality in a given moment. The present experiment was specifically designed to disentangle the relative contribution of these coding levels during action observation by measuring CE in multiple effectors at different timings. Single-pulse TMS was adopted to assess CE of participant's arm and leg muscles as they watched videoclips depicting penalty kicks performed by a soccer player. Participants then observed a soccer player performing: i) a penalty kick straight to the observer and then coming to a full stop ('Still'; Fig. 6.2a); ii) a penalty kick straight to the observer and the continuing to run ('Run'; Fig. 6.2b); iii) a penalty kick to the side and then continuing to run ('Side', Fig. 6.2c).

If motor coding purely reflects what is observed, then a motor resonant activation in the onlookers' leg muscle should be found in all conditions (Fig. 6.1a). Conversely, if a predictive coding is guiding action observation, then leg activation should not be found when the soccer player is going to stop (Fig. 6.1b). Moreover, if motor coding reflects the preparation of a divergent response in the upper limb, then an activation in the arm muscle should be found at the end of the action sequence when the ball is seen approaching the observer (Fig. 6.1c). The adoption of the present paradigm would allow to test these hypothesis by dissociating the different coding levels simultaneously in different effectors.

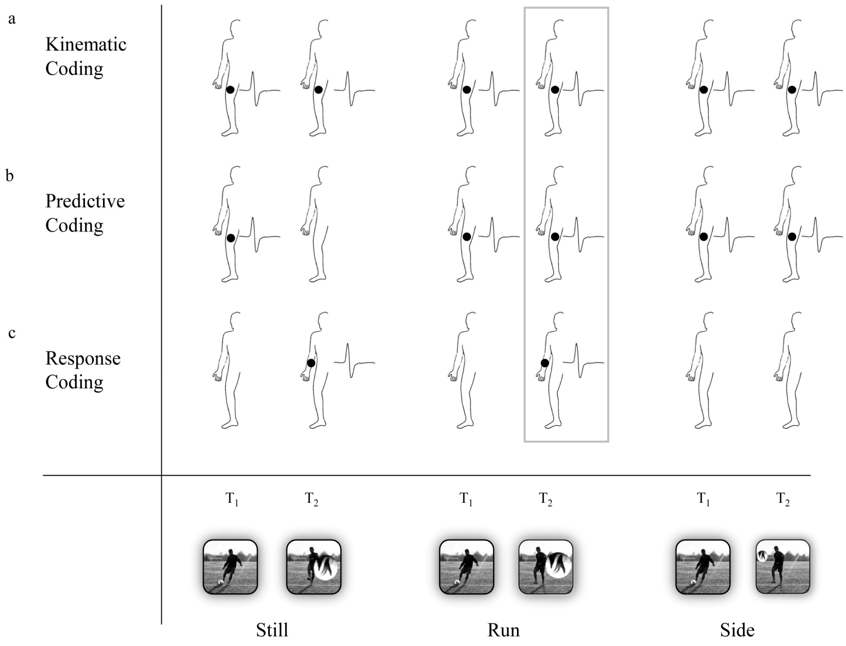


Figure 6.1: Schematic representation of the expected muscular dissociations for the three levels of motor coding: a) Kinematic, b) Predictive and c) Response coding for the three experimental conditions (Still, Run and Side; respectively for each column) and the two TMS stimulation times (T_1, T_2; on the left and on the right of each column, respectively). For example, it is hypothesized that while at T_1 the CE should be equal in all conditions showing an activation of the leg muscle while observing a player kicking a ball, in the Run condition (see the gray rectangular area) at T_2 it is expected a leg activation according to the observed action (Kinematic coding) or the subsequent one (i.e., continuing to run; Predictive coding), together with the activation of the upper limb muscle as to parry the approaching ball (Response coding).

6.2 Methods

The methods employed in this study followed the procedures outlined in Chapter 4, pp. 55-58.

6.2.1 Participants

Thirty individuals were recruited and took part in the experiment, however the data from one participant could not be used in the analysis due to technical problems. Twenty-nine participants (8 males and 21 females, mean age 23 years) were then included in the final analysis. All the participants were right-handed and reported right-foot dominance. As reported in Chapter 5, individuals with any motor expertise in playing soccer were excluded from the experiment by means of a prescreening procedure to avoid a bias in predicting and anticipating other player's actions (Abernethy et al., 2008; Aglioti et al., 2008; Calvo-Merino et al., 2005; Makris and Urgesi, 2014; Tomeo et al., 2013; Urgesi et al., 2012). Furthermore, in order to be included in the study each participant was prescreened to check whether a reliable motor evoked potential could be elicited from the leg muscle in five out of ten consecutive trials. Participants were financially compensated for their time.

6.2.2 Experimental Stimuli

The stimuli were three video clips recorded in an ecological setting showing a professional soccer player: a) kicking a ball into the goal straight toward the observer and then coming to a full stop (*still condition*, Fig. 6.2a); b) kicking a ball into the goal straight toward the observer and then continuing to run (*run condition*, Fig. 6.2b); c) kicking a ball clearly to the left side of the camera and then continuing to run (*side condition*, Fig. 6.2c). All of the videos were taken from a frontal view. A 1800 ms sequence was extracted from each of the videos which included the player's initial run and the ball's trajectory until it disappeared. The player's foot made contact with the ball approximately 1350 ms after the video began, and the ball trajectory reached its highest peak approximately 400 ms later (1750 ms after onset of the video). The ball was travelling at a velocity of approximately 10 m/s during its trajectory. An animation effect was obtained by presenting a series of single frames each lasting 25 ms (resolution = 720 x 576 pixels, color depth = 24 bits) after the first frame which lasted 500 ms.

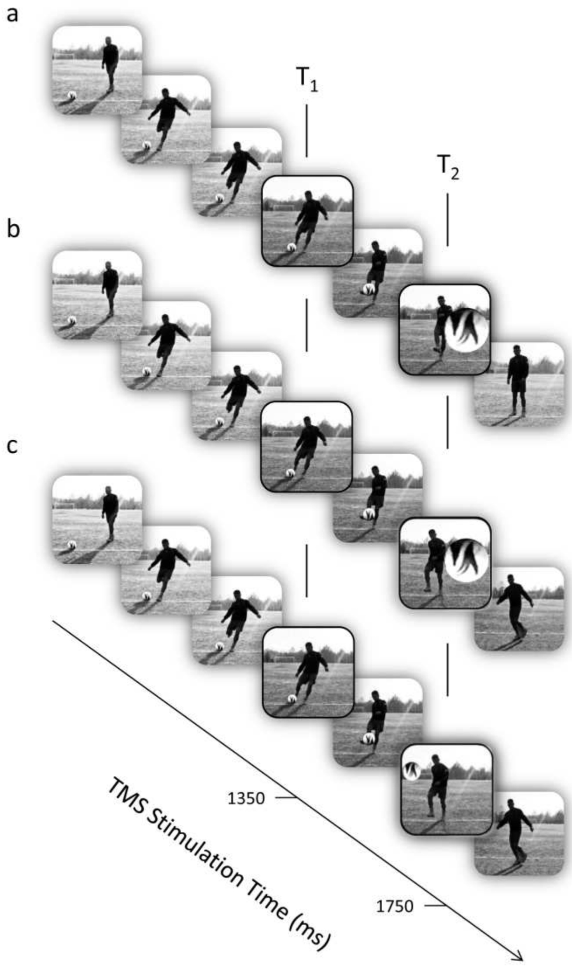


Figure 6.2: Schematic representation of the sequence of events taking place for each condition: a) Still, b) Run, c) Side. The vertical lines denote the time points when the single TMS pulse were delivered, namely at T_1 (i.e., when the player's foot made contact with the ball) and at T_2 (i.e., when the ball trajectory reached its highest peak).

6.2.3 Procedure

The participants were tested individually in a sound-attenuated Faraday room during a single experimental session lasting approximately one hour and consisting of two blocks (upper limb, lower limb). Participants were instructed to remain still and relaxed and to watch the video clips that were presented on a 19" monitor (resolution = 1280 x 1024 pixels, refresh frequency = 75 Hz, background luminance of 0.5 cd/m^2) set at an eye level. In order to ensure attention to the video clips, they were told they would be questioned at the end of the experimental session about the stimuli presented. TMS-induced MEPs were acquired from the right flexor carpi ulnaris (FCU) and right quadriceps femoris (QF) muscles. Upper and lower limb activity was recorded in separate blocks to precisely identify the OSP for stimulating each muscle and avoid loss of modulation involving the less stimulated muscle. The order in which the two blocks were presented was counterbalanced across the participants. A single TMS pulse was released during each video presentation at one of two specific time points: i) during the frame showing the player's foot making contact with the ball (T_1, 1350 ms) and ii) during the frame showing the highest peak of the ball's trajectory (T_2, 1750 ms). The first time point (T_1) was chosen to evaluate the motor resonant response, whereas the second time point (T_2) was set at the highest peak of the ball's trajectory before it disappeared in order to maximize the complementary reaction to the stimulus. The observer could not know before the player kicked the ball whether the he would continue to run or would stop, neither whether he would kick straight or to the side. All the videos implied the same amount of perceived movement, and the leg at T_2 was equally lifted (i.e., approximately 20 cm from the ground) in all conditions (see Fig. 6.2). Therefore, no difference was expected in the leg muscle activity across conditions. The order of the three videos and the two different TMS stimulation times were randomized within each of the two blocks. A total of 120 MEPs (2 muscles x 3 conditions x 2 time points x 10 repetitions) were recorded for each participant. Prior and after the experimental block, each participant's baseline CE was assessed by acquiring 10 MEPs while they passively watched a white-colored fixation cross on a black background presented on the computer screen. By comparing the MEP amplitudes recorded during the two baseline periods (20 MEPs in total) it was possible to check for any CE changes related to TMS per se. The average amplitude of the two series was then utilized to set each participant's individual baseline for data normalization procedure. An interpulse interval lasting 10 s was presented between trials to avoid any short-term conditioning effect (Classen et al., 2000). During the resting period, a message reminding the participants to remain fully relaxed appeared on the screen for the first 5 seconds, followed by a fixation cross that was presented for the remaining 5 seconds. Stimuli presentation, timing of TMS stimulation and EMG recordings were managed by E-Prime V2.0 software (Psychology Software Tools, Pittsburgh, PA) running on a PC.

6.2.4 Data Recording

The TMS procedure was identical to that reported in Chapter 4 in paragraph 4.3 and EMG recordings were collected as outlined in paragraph 4.2. MEPs were recorded from the FCU muscle of the right hand and from the QF muscle of the right leg. EMG activity was recorded through pairs of surface Ag/AgCl electrodes placed in a belly-tendon montage. The ground electrode was placed over the dorsal part of the wrist during the 'upper limb' block and over the patella of the leg during the 'lower limb' block. Single-TMS pulses were delivered to the left M1 corresponding to the forearm and leg regions during the upper limb and lower limb blocks, respectively. rMT ranged from 34% to 59% (mean = 46%, *SD* = 6.19) of the maximum stimulator output in the upper limb block and from 50% to 65% (mean = 57%, *SD* = 4.45) in the lower limb one.

6.2.5 Data Analysis

The data analysis followed the procedure outlined in Chapter 4 in paragraph 4.5. The CE of FCU and QF muscles were quantified at each stimulation point during each experimental condition by the MEP peak-to-peak amplitude (mV). Those amplitudes deviating more than 3 *SD* from the mean and trials contaminated by muscular preactivation were excluded as outliers (< 6%). A paired sample *t*-test (two-tailed) was used to compare the amplitude of MEPs recorded during the two baseline periods carried out at the beginning and at the end of each block. The normalized MEP amplitudes (MEP ratio) were entered into a repeated-measures ANOVA with muscle (FCU, QF), condition (still, run, side) and stimulation time (T_1, T_2) as within-subjects factors. The sphericity of the data was verified before performing statistical analysis (Mauchly's test, $p > 0.05$). Post hoc pairwise comparisons were carried out using *t*-tests and Bonferroni correction was applied to control *p*-values for multiple comparisons. A significance threshold of $p < 0.05$ was set for all statistical analyses run with SPSS software package (SPSS Inc., Chicago, IL, USA).

6.3 Results

The mean raw MEP amplitude recorded at the beginning and at the end of each experimental block were not significantly different for the FCU muscles ($t_{28} = 1.42$, $p >$

0.05) or the QF (t_{28} = -0.04, $p > 0.05$). Therefore, it can be concluded that TMS per se induced no unspecific changes in CE during the experiment that could have influenced the results. The ANOVA on normalized MEP amplitudes showed a statistically significant two-way interaction of muscle x condition ($F_{2,56} = 6.42$, $p < 0.05$, $\eta^2_p = 0.19$), a significant two-way interaction of muscle x time ($F_{1,28} = 9.67$, $p < 0.05$, $\eta^2_p = 0.26$) and a significant three-way interaction of muscle x time x condition ($F_{1,28} = 5.19$, $p < 0.05$, $\eta^2_p =$ 0.16). The results obtained from the post-hoc contrasts exploring the source of the significant three-way interaction are outlined as follows.

Kinematic coding

The mean MEP amplitude of the QF muscle was higher at T_1 than at T_2, but only in the still condition, namely when the player was stopping after kicking the ball ($p = 0.043$; Fig. 6.3a). When the player was kicking the ball (T_1), the same activation for the leg muscle was found when comparing the 'still' condition with the 'run' ($p = 0.941$) and the 'side' conditions ($p = 0.771$; Fig. 6.3). This result seems to reflect a motor resonant activation which reflects the observed movements in a muscle-specific fashion.

Predictive coding

Post-hoc comparisons for the QF muscle at T_2, during the final part of the action sequence, showed a higher activation both in the 'run' ($p = 0.001$) and the 'side' conditions ($p = 0.036$) compared to the 'still' condition (Fig. 6.3). No differences were instead found when comparing QF activity between T_1 and T_2 for either the 'run' ($p =$ 0.604; Fig. 6.3b) and the 'side' conditions ($p = 0.381$; Fig. 6.3c), then suggesting that the leg muscle was equally activated across time points. Therefore, given that the leg was moving and lifted from the ground in all conditions (still, run, side) when TMS was delivered at T_2, this pattern of activation seems to suggest that the prediction of the upcoming action (i.e., coming to a full stop) resulted in a decrease of leg corticospinal excitability. Conversely, when the player was seen continuing to run after the kicking action, the CS activation was maintained also in the second phase of the action sequence.

Response coding

As concerns the pattern of motor activation observed for the upper limb, the mean MEP amplitude of the FCU muscle was higher at T_2, when the ball was approaching the observer, compared to initial phase of the action at T_1 both for the 'still' ($p = 0.040$; Fig. 6.3a) and the 'run' ($p = 0.045$; Fig. 6.3b) conditions, but not for the 'side' condition ($p = 0.561$; Fig. 6.3c). Indeed, while in both the 'still' and the 'run' conditions the ball was kicked toward the observer, in the 'side' condition it was kicked to the left. Therefore, the results suggest that the corticospinal activation in the upper limb emerged only when the perceived action directly involved the observer. Post-hoc comparisons at T_2 confirmed this effect showing an increased activity in the FCU muscle in the 'still' ($p = 0.029$) and 'run' conditions ($p = 0.021$) compared to the 'side' one, whereas at T_1 no significant differences emerged across conditions ($p_s > 0.05$; Fig. 6.3).

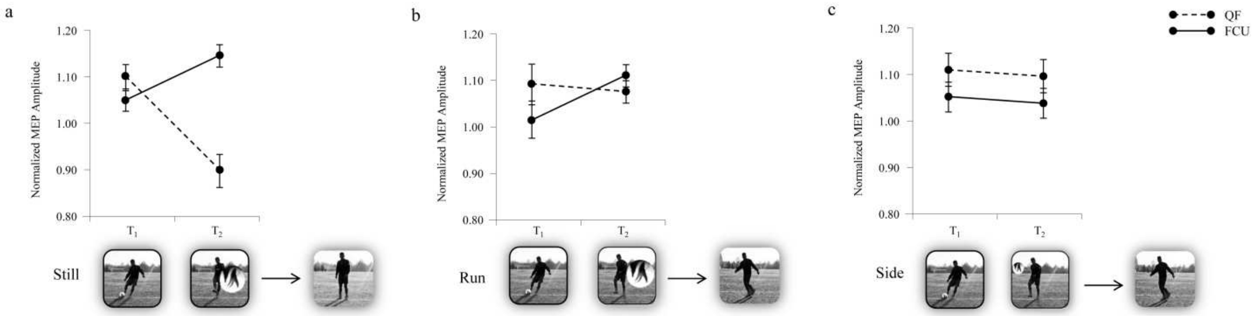


Figure 6.3: Results showing normalized mean MEP amplitudes for the lower limb QF muscle (dotted line) and the upper limb FCU muscle (black line) in the a) 'Still', b) 'Run' and c) 'Side' conditions. Bars indicate standard error of the means. The following post-hoc contrasts were significantly different: QF MEP amplitudes were higher at T_1 compared to T_2 in the 'Still' condition ($p < 0.05$), whereas QF MEPs were higher at T_2 both in the 'Run' and 'Side' conditions compared to the 'Still' condition ($p_s < 0.05$). FCU MEP amplitudes were higher at T_2 compared to T_1 both in the 'Still' ($p < 0.05$) and the 'Run' ($p < 0.05$) conditions, but not for the 'Side' condition.

6.4 Discussion

The present experiment aimed at disentangling the relative contribution and combination of different levels of motor coding during action observation. The adopted experimental design allowed to determine whether the modulation of the corticospinal response – according to the phase of the observed action – either reflected the action that was seen, was modulated by what was expected, or showed a non-matching activation required to appropriately react in response to the context demands. The results showed a modulation of the quadriceps femoris muscle consistent with the observed kicking action during the first part of the action sequence. This leg activation emerged in all three conditions ('still', 'run', 'side') thus supporting the kinematic coding of the observed actions. The motor resonant activation of the lower limb protracted in the second part of the action only when the soccer player continued to run after having kicked the ball. This modulatory pattern, receptive to the following phase of the action, seems to suggest the influence of a predicting coding in modulating the CS output. Crucially, during the final phase of the motor sequence, and only when the ball was seen approaching the observer, a motor activation of the flexor carpi ulnaris emerged. The complementary motor activation in the upper limb muscle suggests the influence of a response coding level, allowing to prepare the onlooker to respond to the observed action (i.e., parrying the ball). The study of the activation pattern of an extensor leg muscle and an abductor of the wrist muscle – that are typically recruited during kicking and parring actions, respectively – allowed to provide evidence of the existence of parallel levels of motor coding taking place while observing others' actions.

Different aspects of behavior are represented hierarchically in the motor system (Grafton and Hamilton, 2007). Therefore, by moving from a basic kinematic level up to a goal and an intentional level, aspects like how an action is performed, the aim of the motor act and the overall reason for executing it are defined in the motor system (Kilner, 2011). As pointed out by Grafton and Hamilton (2007), using action observation may represent a useful tool for determining the organization of action representations in humans. In particular, the emergence of multiple levels of representation is expected during action observation likewise action execution. This in turn would reflect corresponding functional gradations in the action observation system activations according to which level of the action is observed (Grafton and Hamilton, 2007). Although the study presented in this chapter does not allow to shed light on the neural basis generating the differentiated responses recorded in the observer's CS output (i.e., the relative contribution of different areas of the MNS, or beyond the MNS, which generates it), it provides novel insights on the presence of different levels of motor coding taking place, in a non-mutually exclusive way, during action observation.

The results of the present study confirm previous literature by showing corresponding motor activations in the observer's motor system during action observation (e.g., Fadiga et al., 1995; Gangitano et al, 2001; Borroni et al., 2005; Romani et al., 2005; Urgesi et al., 2006; Montagna et al., 2005) and the presence of an anticipatory modulation according to the final end of the perceived movement (Urgesi et al., 2010; Betti et al., 2015). Moreover, the present results further extend the literature on complementary actions (e.g., Newman-Norlund et al., 2007; van Schie et al., 2008; Ocampo and Kritikos, 2010; Sartori et al., 2011b, 2012a, 2012b, 2013b) and confirm the findings reported in Chapter 5 proving that when the observation of an action requires an appropriate response with a different effector to the one observed, an incongruent motor preparation indeed emerged (i.e., an activation of the upper limbs in response to an action performed with the lower limbs). Strikingly, the present experiment also allows to extend previous literature by demonstrating that the preparation of the complementary response in the upper limbs can co-exist with a motor resonant activation in the lower limb. Indeed, it appears that the preparation of a non-matching response does not necessarily cause an inhibition of the observed action internal replica (Ocampo et al., 2011; Sartori et al., 2013b), rather, the inhibitory activity is more probably occurring when both activations cannot coexist, like in the case in which they are both related to the same effector. So, when the congruent and incongruent motor responses involve different effectors, their parallel activation could actually occur.

According to Chinellato, Ognibene, Sartori and Demiris (2013), congruent and incongruent motor activations would emerge following a two-level competition between the Action Observation System (AOS) and the Action Planning System (APS). Whereas the AOS is in charge of monitoring the actions of others, mainly by matching them to the onlooker's own motor repertoire (low-level coding), the APS accounts for the planning and monitoring the executions of actions (high-level coding). Therefore, the dynamic interplay between these two systems may support the results reported in this chapter. Indeed, automatic imitation (Heyes, 2011) and mirroring effects (e.g., Fadiga, Craighero and Oliver, 2005), as well as the increased CE for congruent motor responses emerged from this study, indicate that the AOS, by producing a matching response to the observed behaviors, seems to control by default the motor system. However, when a complementary action is expected or required, the APS takes control over the AOS. Nonetheless, when this functional switch takes place, the monitoring of the other person's action is still performed by the AOS and could directly affect on-line execution. This is particularly relevant when considering complex social interactions, in which an individual needs to keep both simulative and complementary tendencies simultaneously active to efficiently process both observed actions and their relative responses. Previous tasks used to investigate these phenomena were not able to unveil such parallel processing.

The present results show that observers were able to contemporary resonate with the observed leg action while preparing to respond to it with the arm muscle as to parry the ball. However it is worth noting that since onlookers cannot foveate both the player's leg and the approaching ball, attentional processes should be considered while interpreting the outlined results. Indeed, the contemporary processing of the action and the object to which interact was possibly undertaken by splitting attention to the two locations of interest (e.g., Castiello and Umiltà, 1992).

In conclusion, the present study allowed to stratify the motor representation following action observation into distinct levels of motor coding. The results delineate a sophisticated mechanism able to dynamically modulate the observer's motor reactions at the level of different limbs in a functional manner.

CHAPTER 7

DIFFERENTIAL ROLE OF SPATIAL ATTENTION DURING ACTION OBSERVATION: THE CASE OF COMPLEMENTARY ACTIONS

7.1 Introduction

A large body of literature suggests that the mapping of the observed actions onto the observer's motor system is direct and automatic, however most studies have not explicitly challenged the automaticity of the visuomotor transformation process. To date, whether or not this visuomotor transformation process is automatic is currently under debate (Heyes, 2001; Liepelt, von Cramon and Brass, 2008). Automatic processes, as opposed to controlled ones, are judged against the fact that are immune to interference, may lack in awareness and occur without intentionality (Neumann, 1984). They are thus triggered involuntarily and do not require attention for their execution (e.g., Posner, 1978; Bargh, 1992). In this perspective, if direct matching is automatic, then a perceived action should be processed in any case, even in the absence of attentional resources (Catmur, 2016). If attention is, instead, critical for direct matching to occur, motor activation following action observation should diminish whenever a person's attention is diverted from an observed movement. Support to the latter comes from behavioral (Bach et al., 2007; Chong et al., 2009), neuroimaging (Chong et al., 2008) and neurophysiological (Schuch et al., 2010) studies (see Chapter 3). These findings suggest that motor system activation during action observation seems not purely automatic, but could be influenced by top-down factors, such as attention. Crucially, a possible role played by attentional filters could be the limiting of unnecessary processing and mimicry of observed actions (e.g., Chong et al., 2008; but see Catmur, 2016 for an alternative hypothesis).

The question of whether attention is required for direct matching to occur is relevant also when considering special classes of stimuli with particular biological and social valence. Social stimuli such as familiar faces, for instance, requires less attention to be processed than other type of stimuli (Lavie, Ro and Russell, 2003), eliciting neural activity despite observers' attention is directed toward distractors (Vuilleumier, Armony, Driver and Dolan, 2001; Williams, McGlone, Abbott and Mattingley, 2005). To date, it still remains unclear whether social stimuli, such as interactive body movements, can be processed without attention. In this respect, the choice of an experimental paradigm that involve *complementary actions* (i.e., any form of social interaction wherein individuals complete each other's action according to a common aim; see Chapter 2) provides an ideal opportunity to investigate the role of spatial attention during social interactions. Therefore, an established paradigm for inducing complementary activations in the observers' muscle (Sartori et al., 2011b, 2012b, 2013b) has been adopted in the experiments outlined in the present chapter. In the first experiment, eye-tracking procedures were adopted to measure the spontaneous allocation of overt spatial attention during passive observation of video clips showing the interactive and non-interactive action sequences. In a second eye-tracking experiment, attentional-capturing dots were superimposed within the scenes to manipulate the allocation of overt spatial attention. Then, in the third experiment spTMS was delivered on M1 to test whether diverting attention from the salient parts of a scene was able to affect motor preparation. Taken together, these studies may provide some hints on the role played by a top-down factor, such as spatial attention, in influencing covert motor activations during the observation of social and non-social interactions.

7.2 Eye-tracking Experiments

7.2.1 Experiment 1: The spontaneous allocation of overt spatial attention during action observation

7.2.1.1 Methods

7.2.1.1.1 Participants

Nineteen right-handed individuals (8 males and 11 females, mean age 24.8 years) took part in the experiment. All participants had normal or corrected-to-normal vision and they were naïve as to the purpose of the experiment. All participants gave written informed consent in accordance with the ethics approval by the Institutional Board at the University of Padova, in accordance with the Declaration of Helsinky (Sixth revision, 2008).

7.2.1.1.2 Experimental Stimuli

Two videoclips (7.2 s duration each) were used as experimental stimuli for the 'Request' and 'No-Request' conditions, respectively.

a) *Request condition*: In this condition an actress is seen grasping a sugar spoon placed on a small starting box, then pouring sugar into a mug placed next to her on a table and eventually stretching out her arm towards a mug out of her reach, but strategically placed near the observer. Thus, in a real life situation, requiring the observer intervention to lift the mug and complete the complementary action (Fig. 7.1).

b) *No-Request condition*: In this condition the actress is seen grasping the sugar spoon placed on a small starting box, pouring sugar into a mug placed next to her on the table and eventually moving back the spoon to its initial position on the starting box (Fig. 7.1).

The videoclips were edited in order to hide the actress' head, since seeing a face looking at an object causes a rapid and spontaneous shift of spatial attention towards the same target (Driver, Davis, Ricciardelli, Kidd, Maxwell and Baron-Cohen, 1999; Langton, O'donnell, Riby and Ballantyne, 2006). Gaze can act indeed as a confounding factor in biasing the participants' attentional orientation (see Frischen, Bayliss, and Tipper, 2007, for a review).

Figure 7.1: Each row represents the sequence of events for each experimental condition: 'Request' on the top and 'No-Request' on the bottom. The overlaid colored rectangular areas represent the AOIs adopted in Experiment 1: the dynamic Hand AOI for the Request (orange) and Non-Request (green) conditions, and the static Mug AOI for the Request (violet) and Non-Request (red) conditions. Both AOIs had the same dimensions and lasted for the entire duration of the video stimuli.

7.2.1.1.3 Procedure

Participants were seated at a distance of 65 cm from the eye-tracker monitor (1280 x 1024 pixels) and they were asked to carefully observe the experimental stimuli (AVI format videos, 25 frames per second) presented on the screen. Each trial started with a 3 seconds presentation (inter-trial interval) of a white fixation cross at the center of the black screen to ensure that all participants would start observing the video stimuli from the same origin point. Each video was presented once to each participant in a randomized order. The experimental session lasted approximately ten minutes.

7.2.1.1.4 Data Recording

Eye movements were recorded by means of an infrared T120 Eye Tracker (Tobii Technology, Danderyd, Sweden). Eye position was sampled at 120 Hz with a spatial accuracy of 0.5 degrees of visual angle. A calibration procedure using a standard five-points grid was performed at the beginning of the experiment and repeated when necessary. The eye-tracker was set to head-free mode to allow for head movements and correct for them while remaining very accurate in measuring eye movements.

7.2.1.1.5 Data Analysis

The eye-tracking data were processed by means of the software Tobii Studio 3.2. Areas of Interest (AOIs) were created to investigate fixations targeted to specific regions. A fixation event was considered when gaze remained within 0.5 degree of visual angle for at least 100 ms. For each video two AOIs of the same dimension (217 x 327 pixels) were identified: a) Hand AOI: a dynamic area which included the model's hand while manipulating the sugar spoon (Fig. 7.1); and b) Mug AOI: a static area which included the mug placed next to the observer in the right corner of the screen (Fig. 7.1). Both AOIs were presented for the entire duration of the video stimuli. The total Fixation Duration, namely the average duration in seconds for all fixations within the AOI, was considered for gaze data analysis. A repeated-measure analysis of variance (ANOVA) was conducted on Fixation Duration with condition (Request, No-Request) and AOI (Hand, Mug) as within-subjects factors. A subsequent analysis has been performed in order to investigate the temporal distribution of fixations towards the Mug AOI over time. This experimental design allowed to test whether the adopted social manipulation (i.e., the implicit request to the observer to potentially interact with the mug to fulfil the action) was able to influence gaze deployment and attention allocation over the salient object. Crucially, temporal information on attentional resources deployment toward the mug was also measured. Based on the fact that the two action sequences were identical for the first part, a difference in gaze parameters was expected just in the last part of the action, namely when the nearby mug acquired a social valence (Request condition) or not (No-Request condition). To do so, the analysis of the Fixation Duration has been divided into three periods (see Fig. 7.2): i) *T1*, a period that comprised the time elapsing between the start of the action (i.e., hand laying still on the table) and the PG on the sugar spoon; ii) *T2*, the time between the PG on the spoon and the end of the act of pouring sugar into the first mug; iii) *T3*, the time between the end of pouring and the end of the action, namely the maximum extension of the model's arm toward the observer as to pour sugar into the out-of-reach mug (Request condition) or the hand lying again on the table in the initial position after moving back the spoon to the small starting box (No-Request condition). A repeated-measure ANOVA was then conducted for the Mug AOI on Fixation Duration with condition (Request, No-Request) and time (T1, T2, T3) as within-subjects factors. The Partial Eta Square (η^2_p) value was calculated as an estimate of effect size. In presence of significant interactions, post-hoc comparisons were performed using the Bonferroni correction. The significance level was fixed at $p < 0.05$ for all statistical analysis carried out with SPSS software package (SPSS Inc., Chicago, IL, USA).

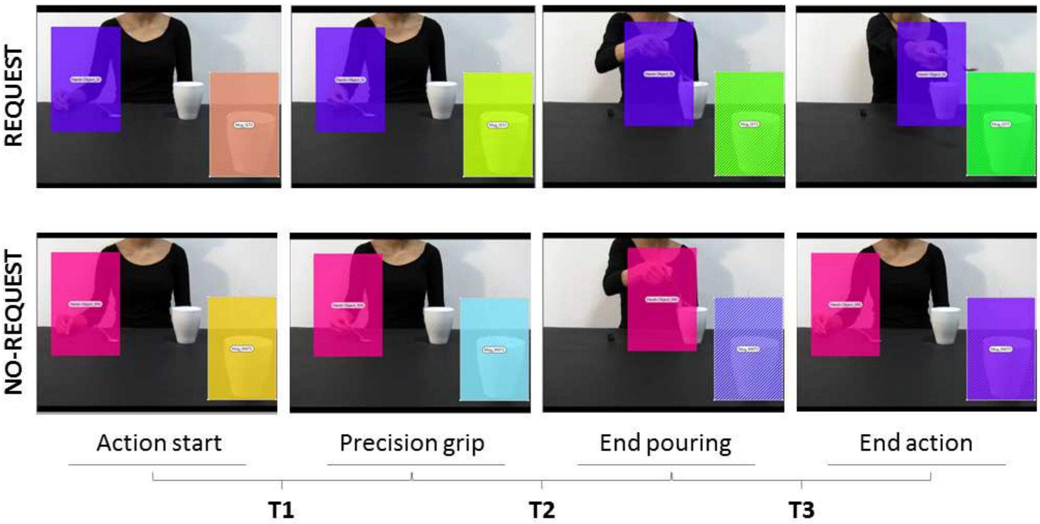


Figure 7.2: Representation of the three time periods adopted to analyze temporal information on Fixation Duration for the Mug AOI. Each row represents an experimental condition: 'Request' on the top and 'No-Request' on the bottom. Each column represents a key phase of the action, from the left: the start of the action with the model's hand placed still on the table (first column); the PG on the sugar spoon (second column); the end of pouring sugar into the first mug (third column); the end of the action, that is the model's hand stretched out toward the nearby mug in the Request condition, or the hand moved back in the initial position in the No-Request condition (fourth column). Temporal information in eye gaze data for the Mug AOI have been analyzed by using the three time periods that elapsed between these events (T1, T2, T3, see bottom of the figure).

7.2.1.2 Results

Spatial allocation of overt attention toward the observed action and the salient object

The ANOVA on the mean Fixation duration (i.e., the time spent fixating within the AOI for each videoclip) showed significant main effects for both condition ($F_{1,18} = 7.29$, $p = 0.015$, $\eta^2_p = 0.29$) and AOI ($F_{1,18} = 550.75$, $p < 0.001$, $\eta^2_p = 0.97$), and a significant interaction of condition by AOI ($F_{1,18} = 33.11$, $p < 0.001$, $\eta^2_p = 0.65$). The results obtained from the post-hoc contrasts exploring the interaction showed significantly longer fixation times for the Hand AOI compared to the Mug AOI for both the Request and the No-Request conditions ($p_s < 0.001$). This result is in accordance with the salience of the observed moving hand in attracting overt attention during the observation of the two types of actions. However, statistically significant longer fixation duration for the

Mug AOI for the Request condition with respect of the No-Request condition (*p* < 0.001) was found. Participants looked longer the mug placed next to them when a social request was embedded in the action. Accordingly, in the Request condition participants fixate shorter the Hand AOI compared to the No-Request condition (*p* = 0.019). Results are graphically summarized in Fig. 7.3.

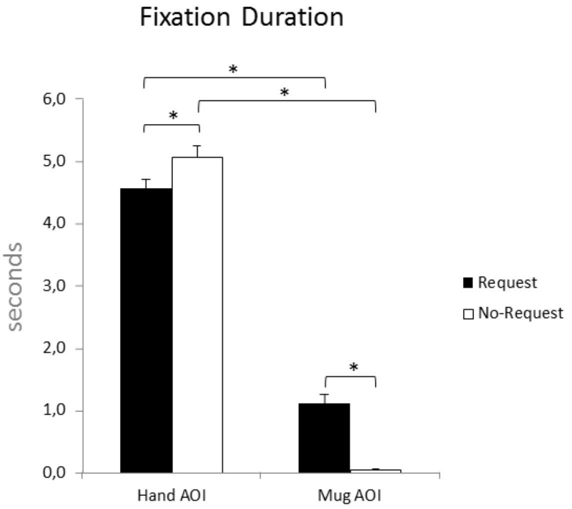


Figure 7.3: Results for total Fixation Duration on the Hand and Mug AOIs for the Request (black) and No-Request (white) conditions. Bars indicate the standard error of the means. Asterisks indicate statistically significant comparisons (*p* < 0.05).

Temporal pattern of spatial attention allocation toward the salient object

The present analysis aimed at measuring the temporal aspects of gaze deployment when directed towards the nearby object (i.e., the mug on the right corner of the screen) during the observation of interactive and non-interactive action sequences. The ANOVA on the mean Fixation duration showed a significant main effect of both condition ($F_{1,18}$ = 62.73, $p < 0.001$, $\eta^2_p = 0.78$) and time ($F_{2,36} = 67.47$, $p < 0.001$, $\eta^2_p = 0.79$), and a significant interaction of condition by time ($F_{2,36} = 60.66$, $p < 0.001$, $\eta^2_p = 0.77$). The results obtained from the post-hoc contrasts exploring the interaction showed significantly longer fixation time for the Request condition at the T3 time period compared to either the T1 and the T2 ($p_s < 0.001$). Furthermore, results show a significant longer fixation time at T3 for the Request compared to the No-Request condition (*p* < 0.001). Overall, participants fixate longer the Mug AOI when the implicit request to interact is unfolded (i.e., T3, when the hand is stretched toward the out-of-reach mug) compared to the earlier phases of the action. Crucially, in this final part of the action it is only in the interactive context that attention is spontaneously shifted toward the mug, that is the object to which interact to complete the complementary request. Results for Fixation Duration on Mug AOI in T1, T2 and T3 are graphically summarized in Fig. 7.4. In addition, Fig. 7.5 shows the spatial and temporal distribution of fixations over time (gaze plot) for both the Request and No-Request conditions from a representative participant.

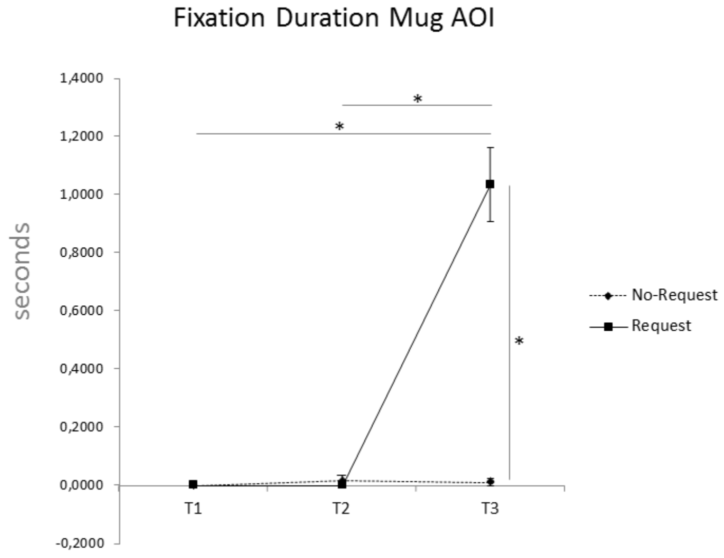
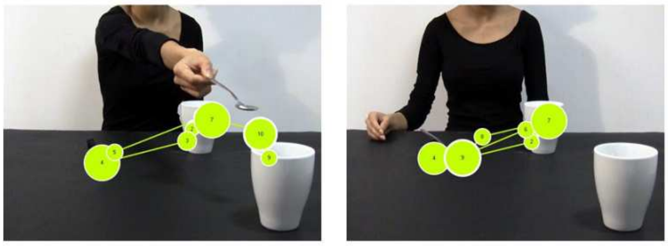


Figure 7.4: Results for total Fixation Duration on the Mug AOI for the Request (black line) and the No-Request (dotted line) conditions in the three key period considered for the analysis of temporal information: T1, T2 and T3. Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons (*p* < 0.001).



REQUEST NO-REQUEST

Figure 7.5: Example of gaze plots (i.e., the representation of the sequence of eye movements, displaying order and duration of fixations) for the Request (left column) and the No-Request (right column) conditions from a representative participant. The lines represent saccades, while the circles represent gaze fixations and circle areas are proportional to fixation lengths.

7.2.2 Experiment 2: Spatial attention allocation in interactive and non-interactive contexts during exogenous attentional manipulation

7.2.2.1 Methods

The method in Experiment 2 was identical to Experiment 1 with the following exceptions regarding the experimental sample, the type of stimuli and the data analysis process.

7.2.2.1.1 Participants

Twenty-one different right-handed participants (9 male and 12 female, mean age 25.05 years, *SD* = 6.35) with normal or corrected-to-normal vision took part in the experiment.

7.2.2.1.2 Experimental Stimuli

The same videoclips as for the Experiment 1 were adopted. Crucially, a red dot (note that from now on it would be regarded as 'dot') was superimposed on the videos and briefly presented in order to elicit the shift of the observer's exogenous attention to different locations in the visual scene. The dot (40 x 40 pixels, 120 ms duration) was presented at the end of both action sequences (4460 ms from video onset) in either one of two specific locations: i) *'left side'* (Fig. 7.6, left column), over the actress's hand moving back to the initial position on the left side of the screen (No-Request condition) or on the same spatial location over the small starting box (Request condition); and ii) *'right side'* (Fig.

7.6, right column), over the out-of-reach mug located on the right side of the screen (for both the Request and the No-Request conditions). In particular, the location of the 'right side' dot have been selected on the basis of the findings obtained from Experiment 1, showing that for the Request video the Mug AOI was significantly more attended by the observers, and this happened exactly in the last part of the action sequence. Each video lasted 5540 ms and was preceded by the presentation of a white fixation cross on a black background for 3000 ms to ensure participants would start the observation from a neutral and fixed position. Each video has been presented five times in a random order. The experimental session lasted approximately fifteen minutes. A pilot study (N = 31, with the same characteristics of the participants of Experiment 2) was performed to verify whether the dots were detected. Participants were asked to report at the end of each video presentation whether they saw the dots or not. The accuracy of the responses (99% of correct detections) allowed to conclude that, even if presented briefly, the 'dot' manipulation was effective in attracting attention to specific sides of the visual scenes. However, the aim of Experiment 2 was to test whether the 'dot' manipulation were able to attract *overt* visuospatial attention (i.e., directing the eye gaze toward the stimulus) toward the target area where the dots were presented.

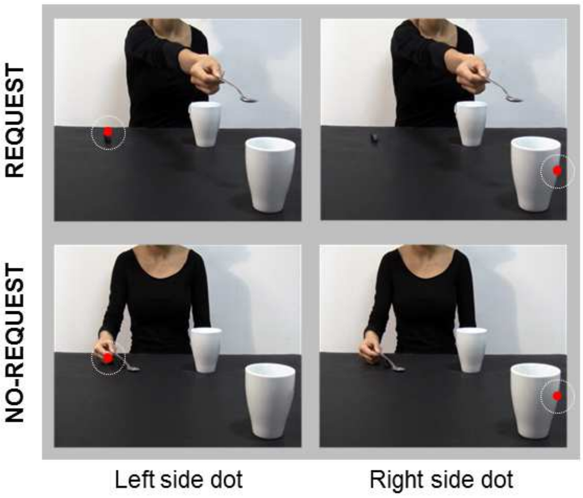


Figure 7.6: Experimental stimuli adopted in eye-tracking Experiment 2 and TMS Experiment 3. An attentional-capturing red dot was briefly presented in either one of two specific positions: 'left side' (see left column), over the starting block for the Request condition and over the actress's hand moving back to the initial position for the No-Request condition; and 'right side' (see right column), over the out-of-reach mug located on the right side of the screen (Request and No-Request conditions). White dotted circles indicate the red dot position.

7.2.2.1.3 Data Analysis

As for Experiment 1, eye gaze data have been analyzed by means of the software Tobii Studio 3.2. Areas of Interest have been adopted to investigate fixations targeted to the areas of the visual scenes in which the dot was presented. Specifically, for each video two static AOIs of the same dimension (188 x 237 pixels) were created: a) 'Left dot space', including the area in which the left dot was presented, that is the hand grasping the sugar spoon in the No-Request condition or the same spatial location over the small starting box in the Request condition (see Fig. 7.7, left column); b) 'Right dot space', including the area in which the right dot was showed, namely the mug placed near to the observer in the right corner of the screen (see Fig. 7.7, right column). The analysis of eye gaze for each video presentation were carried out in a time window that started with the dot's appearance (dot presentation duration: 120 ms) and ended after 320 ms. This temporal window has been adopted to prevent the Inhibition of Return (IOR) phenomenon (i.e., the inhibition to reorient attention to a previously explored location; Posner and Cohen, 1984; Klein, 2000). In order to detect the orienting of attention to a specific location induced by the brief presentation of an exogenous attention-capturing dot, Fixation Count (the number of fixations within the AOI) has been considered. In fact, for the aim of the present experiment and given the short time window adopted, the total Fixation Duration (the average duration for all fixations within the AOI) would not represent the most sensible parameter to use. A repeated-measure ANOVA was conducted on Fixation Count with condition (Request, No-Request), dot location (left side, right side) and AOI (Left dot space, Right dot space) as within-subjects factors. The Partial Eta Square (η^2_p) value was calculated as an estimate of effect size. In presence of significant interactions, post-hoc comparisons were performed using the Bonferroni correction. Significance threshold was set at $p < 0.05$ for all statistical analysis carried out with SPSS software package (SPSS Inc., Chicago, IL, USA).

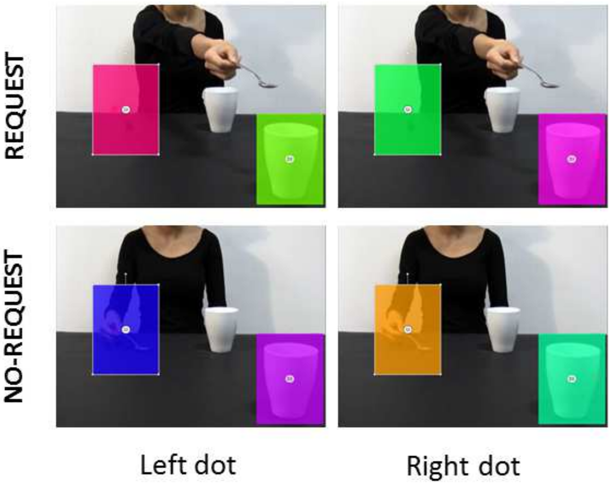


Figure 7.7: Each row represents an experimental condition: 'Request' on the top and 'No-Request' on the bottom. The overlaid colored rectangular areas represent the AOIs adopted in Experiment 2: 'Left dot space' AOI, including the area in which the left dot appeared, when present (see the rectangle on the left on each figure); 'Right dot space' AOI, including the area in which the right dot appeared, when present (see right rectangle on each figure). Eye gaze data were acquired for 320 ms starting from red-dot presentation.

7.2.2.2 Results

The ANOVA on the Fixation Count showed a significant main effect of condition ($F_{1,20} = 73.62$, $p < 0.001$, $\eta^2_p = 0.79$), AOI ($F_{1,20} = 132.65$, $p < 0.001$, $\eta^2_p = 0.87$), a significant two-way interaction of condition by AOI ($F_{1,20} = 79.31$, $p < 0.001$, $\eta^2_p = 0.80$) and of dot location by AOI ($F_{1,20} = 22.88$, $p = 0.008$, $\eta^2_p = 0.30$) and a three-way interaction of condition by dot location by AOI ($F_{1,20} = 5.71$, $p = 0.027$, $\eta^2_p = 0.22$). The results obtained from the post-hoc contrasts exploring the source of the significant three-way interaction are outlined as follows.

The attention-capturing dot attracts overt attention in the interactive context

For the Request condition, when the dot was presented on the left side (i.e., over the small starting box) participants eye gaze fixated more frequently the Left dot space AOI compared to the Right dot space AOI ($p = 0.033$) and compared to the same Left AOI when the dot appeared on the right side over the out-of-reach mug ($p = 0.005$; Fig. 7.8a). Similarly, Fixation Count in the Right dot space AOI was higher when the dot appeared on the right side ($p = 0.005$) and compared to the same Right AOI when the dot was instead presented on the left ($p = 0.005$; Fig. 7.8a). To summarize, in the interactive context the dot manipulation was able to attract overt attention toward the area of dot presentation.

Overt attention is anchored on biological movement in the non-interactive context

Results for the No-Request condition showed that Fixation count was higher in the Left AOI compared to the Right AOI regardless of dot location ($p_s < 0.001$; Fig. 7.8b). So, in the non-interactive context participants tended to remain anchored to the action without overtly attending the dot when presented on the contralateral side. In fact, even if an increasing tendency to look to the Right AOI when the dot appeared on the right side is present (0.1 vs. 0.6), it did not reach significance ($p = 0.11$; Fig. 7.8b). These results are consistent with the fact that motion is a powerful exogenous cue, able to attract attention to the moving part of the stimulus (e.g., Posner and Cohen, 1984). Moreover, due to this anchoring effect, when comparing the Non-Request and the Request conditions it emerges that, irrespective of dot position, Fixation Count is higher in the Left AOI for the former condition ($p_s < 0.001$) and in the Right AOI for the latter ($p_s < 0.001$).

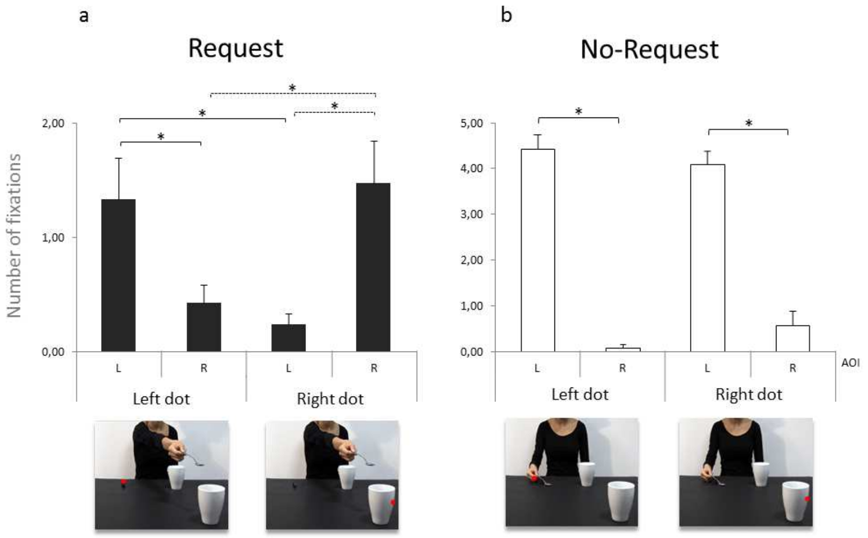


Figure 7.8: Results for Fixation Count for the Request (left panel) and No-Request (right panel) conditions for the left dot space AOI and the Right dot space AOI ('L' and 'R', respectively; y-axis) and dot location ('left dot' and 'right dot'; y-axis). Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons ($p < 0.05$).

7.3 Experiment 3: TMS

7.3.1 Methods

The methods employed in this study followed the procedure outlined in Chapter 4, pp. 55-58.

7.3.1.1 Participants

Thirty right-handed individuals (8 males and 22 females, mean age 23 years) participated in the experiment and they were financially compensated for their time.

7.3.1.2 Stimuli

The same stimuli as for Experiment 2 were adopted (see Fig. 7.6), namely the Request and the No-Request action sequences in which attention-capturing dots were superimposed on the actions in either one of two specific locations (i.e., 'left side', 'right side'). These experimental stimuli were designed to study the role of spatial attention during action observation in social and non-social contexts and to allow investigating the influence of spatial attention allocation on the observer's CE. Crucially, in both videoclips the model grasps the sugar spoon with her right hand using a PG, whilst the mug requires the use of a WHG in order to be handled. Therefore, the type of grasp observed (i.e., a PG) and the one that was required to interact in the Request context (i.e., a WHG) were reciprocally mismatched. It follows that the 'left side' dots were always associated with precision grips (i.e., the observed PG on the spoon in the No-Request condition and the small starting box eliciting a PG in the Request condition; Fig. 7.6, left column), whereas 'right side' dots positioned on the nearby mug were associated with a whole-hand grasp (Fig. 7.6, right column). This experimental designed allows to disentangle different types of motor preparations and to control for object-related motor affordances (Gibson, 1979). Each video lasted 5540 ms, and the animation effect was obtained by presenting a series of single frames each lasting 30 ms (resolution 1920 x 1080 pixels, color depth 32 bits), following the first frame lasting 800 ms.

7.3.1.3 Procedure

Participants were tested individually in a sound-attenuated Faraday room during a single experimental session lasting approximately one hour. Participants were asked to remain as still and relaxed as possible and to watch the video clips that were presented on a 24” monitor (resolution = 1920 x 1080 pixels, refresh frequency = 120 Hz) set at an eye level. In order to ensure attention to the video clips, participants were told they would be questioned at the end of the experimental session about the stimuli presented. TMS-induced MEPs were acquired from the participants’ FDI and ADM muscles of the right hand. These muscles were chosen because of their differential activation during the execution of PG versus WHG, namely they are characterized by a higher activation of FDI for PG and of ADM for WHG (see Davare, Rothwell and Lemon, 2010). A single TMS pulse was released during each video presentation at 4610 ms, after 150 ms from the dot’s presentation on the left or the right side of the visual scene. This timing was chosen based on previous literature showing a fast visuomotor mapping corresponding to the observed motor act at around 150 ms (Ubaldi, Barchiesi and Cattaneo, 2015). Each video was preceded by the presentation of a fixation cross to ensure participants would start observing the experimental stimuli from a neutral gaze position in each trial. The order of presentation of the videoclips was randomized across participants. A total of 80 MEPs (2 muscles x 2 conditions x 2 dot positions x 10 repetitions) was recorded for each participant. Before and after the experimental session, each participant’s baseline CE was assessed by acquiring 10 MEPs during passive observation of a white fixation cross on a black screen. The average amplitude of the MEP amplitudes recorded during the two baseline periods (20 MEPs in total) was utilized for data normalization procedures (MEP ratio; see Chapter 4, paragraph 5). Furthermore, by comparing the MEP amplitudes in the two baseline blocks it is possible to control for variation in CE related to TMS per se. An inter-pulse interval of 10 s (i.e., 5 seconds in which on the screen appeared a message reminding participants to remain relaxed followed by other 5 seconds of a fixation cross presentation) was adopted between trials to avoid any short-term conditioning effect (Classen et al., 2000).

Stimuli presentation, timing of TMS stimulation and EMG recordings were managed by E-Prime V2.0 software (Psychology Software Tools, Pittsburgh, PA) running on a PC.

7.3.1.4 Data Recording and Data Analysis

The TMS procedure was identical to that reported in Chapter 4 in paragraph 4.3 and EMG recordings were collected as outlined in paragraph 4.2. MEPs were recorded from the FDI and ADM muscles of the right hand. Single-TMS pulses were delivered over the optimal scalp location to stimulate the hand region on the left M1. rMT ranged from 34% to 61% (mean = 47%, *SD* = 7) of the maximum stimulator output. Individual peak-to-peak MEP amplitude (mV) was averaged for each participant and experimental condition for data analysis. MEP amplitudes deviating more than 3 *SD* from the mean for each subject and trials contaminated by muscular pre-activation were excluded as outliers (< 5%). A paired sample *t*-test (two-tailed) was used to compare the amplitude of MEPs acquired during the two baseline periods. The normalized MEP amplitudes (MEP ratio) were entered into a repeated-measures ANOVA with condition (Request, No-Request), dot position (left side, right side) and muscle (FDI, ADM) as within-subjects factors. The Partial Eta Square (η^2_p) value was calculated as an estimate of effect size. In presence of significant interactions, post-hoc comparisons were performed using the Bonferroni correction. Significance threshold was set at $p < 0.05$. Statistical analyses were performed with SPSS software package (SPSS Inc., Chicago, IL, USA).

7.3.2 Results

The mean raw MEP amplitudes recorded at the beginning and at the end of the experimental session were not significantly different for either the FDI ($t_{29} = -0.287$, $p = 0.783$) or the ADM ($t_{29} = 0.638$, $p = 0.529$) muscles. Therefore, TMS per se did not induce any nonspecific change in CE that could have biased the results. The ANOVA on normalized MEP amplitudes showed a significant muscle by condition interaction ($F_{1,29} = 7.350$, $p = 0.011$, $\eta^2_p = 0.202$) and a 3-way interaction of muscle by condition by dot position ($F_{1,29} = 7.436$, $p = 0.011$, $\eta^2_p = 0.204$). The results obtained from the post-hoc contrasts exploring the significant interactions are outlined as follows.

Attention allocation modulates direct matching

In the No-Request condition, when the dot was presented on the model's hand (left side), the MEP amplitude of the observers' FDI muscle (i.e., the muscle involved in PG) was significantly greater than ADM (i.e., the muscle involved in WHG; $p = .035$) and greater than in the Request condition, though the dot was located on the same left spatial location ($p = 0.030$; Fig. 7.8a). This effect of direct matching was instead reduced when the dot was located on the out-of-reach mug (right side) in which the FDI muscle activity was significantly decreased with respect to when the dot was presented on the model's hand ($p = 0.010$; Fig. 7.8a) and was not statistically different from ADM activity ($p = 0.849$). As suggested by previous literature, direct matching seems to depend on attentional allocation directed to body parts (Bach et al., 2007; Chong et al., 2009).

Attention allocation do NOT affect social motor preparation

As concerns the ADM muscle activity, results show that ADM MEP amplitude is higher in the Request compared to the No-Request condition ($t_{59} = 2.15$, $p = 0.035$; Fig. 7.8b). Moreover, in the Request condition ADM activity was not statistically different when the dot was positioned on the out-of-reach mug and when it was positioned on the left side of the screen (1.194 vs. 1.185 mV, respectively; $p = 0.856$). Therefore, diverting attention did not influence the preparation of a complementary response.

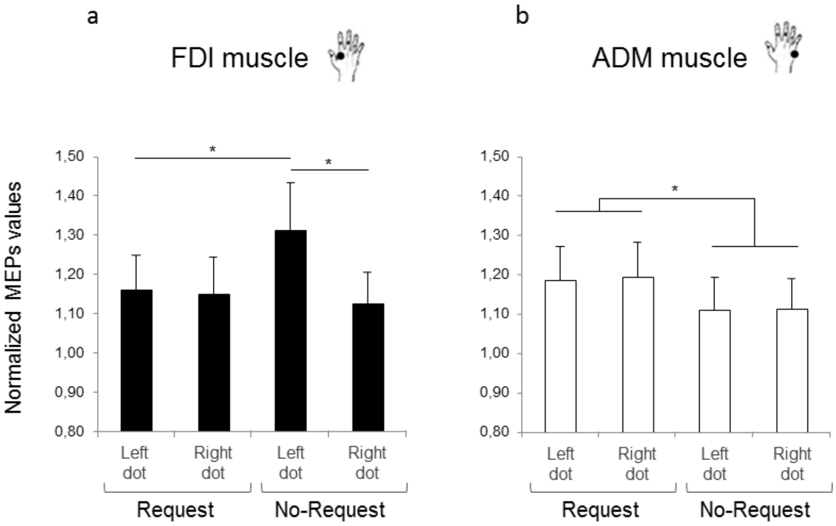


Figure 7.8: Results showing corticospinal activation for the 'Request' and 'No-Request' conditions. The graph represents the normalized mean MEP amplitudes recorded from a) FDI and b) ADM muscles, respectively. Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons ($p < 0.05$).

7.4 Discussion

The current study aimed at clarifying the influence of spatial attention on direct matching and complementary action preparation during action observation. The analysis of eye-tracking data during the observation of the adopted experimental stimuli (Experiment 1) allowed to understand how attention was spontaneously displaced while observing interactive and non-interactive action sequences. This put the basis for manipulating attention toward specific parts of the visual scene (Experiment 2) in order to test whether exogenous attentional manipulation was able to modulate corticospinal excitability following action observation (Experiment 3).

Participants observed two action sequences depicting a model grasping a tablespoon, pouring some sugar into her mug and then: i) stretching toward an out-of-reach mug which was placed close to the participant (Request condition), ii) moving back to the starting point (No-Request condition). Eye-tracking data showed that observers' attention was spontaneously captured by the mug placed next to them only when the observed action implied a social request. Moreover, this attentional shift toward the salient object happened just in the final phase of the action, namely when the model, while grasping a spoon filled with sugar, stretched her arm toward the observer. In a real-life situation, the socially appropriate response in this context would be picking up the mug and offer it to the model in order to allow her to pour the sugar in it. In a previous study, Sartori and colleagues (2013b) adopted a similar paradigm to study motor activations following action observation. Crucially, they used several time points based on kinematic landmarks characterizing the observed action. What they found was that a complementary motor preparation emerged when the model's hand trajectory began to significantly move toward the out-of-reach mug. The eye-tracking results obtained from Experiment 1 demonstrated that not only kinematic cues provided by the model's action, but also the object to which the action was directed has to be attended for the complementary effect to emerge. This in turn allows for *complementary affordances* (i.e., all the action possibilities in which suitable motor programs aiming to bring a joint goal to completion are activated, Sartori and Betti, 2015; see also Chapter 2, p. 41) to be triggered, enabling complementary requests to be understood in terms of the potential for the interactive involvement they elicit, which can be express by an action toward an object, and even when the interaction does not really take place.

Attentional deployment during exogenous attentional manipulation

The analysis of spontaneous allocation of spatial attention in the Request and No-Request contexts (Experiment 1) provided the basis to achieve the main aim of the present research: to investigate the role of spatial attention in the observation of action stimuli evoking imitative and complementary gestures. This was pursued by adopting an exogenous attentional manipulation by presenting attention-capturing dots in key parts of the visual scene at the end of the action sequences. Previous studies (e.g., Bach et al., 2007; Wiggett and Tipper, 2015) already used colored-dots to attract attention toward body parts to study body compatibility effects during actions and body postures observation. The results from Experiment 2 showed that in the No-Request condition participants remained anchored to the observed action (i.e., the model grasping the sugar spoon) without directly looking at the mug when exogenous attention was manipulated in order to attract attention toward it. Conversely, in the Request condition participants were more receptive to the presentation of the dot in either sides of the visual scene by looking toward it when presented. The reason for this difference could be found in the fact that, in the No-Request condition, the nearby mug was not relevant for the observer and no events happened in that area of the visual scene. In the Request condition, instead, both areas of interest covered potentially relevant information: namely, the model's peripersonal space, in which she could potentially act (left dot space AOI), and the observer's peripersonal space, where the mug to which potentially interact was located (right dot space AOI). It is possible that when the observers were kept involved in the action through the model's social request (i.e., to pick up the mug and offer it to her) they became more sensitive in detecting possible informative cues. In fact, by avoiding missing potential informative details, observers would be facilitated in predicting action consequences and to interact accordingly. Whereas, in a non-interactive context, the only relevant source of information was represented by the acting hand, since no implicit interaction was elicited. Interactive behaviors indeed require specific perceptual, cognitive and motor abilities to allow individuals to coordinate their actions and predict their joint outcome (Knoblich et al., 2011). For example, the sensitivity to detect subtle changes in kinematic patterns and to direct one's attention to where an interaction partner is attending, provide a mechanism for understanding shared representations of objects and events (Sartori et al., 2013b; Sartori et al., 2009; Frischen and Tipper, 2004). However, the tendency to spend more time on the acting hand compared to the nearby mug appeared clearly also from Experiment 1 data. Body movements are indeed powerful exogenous cues, which could attract attention toward them (Posner and Cohen, 1984).

To summarize, based on the context engendered by the action, the same exogenous attentional manipulation superimposed on the visual scene seems to be differentially effective in attracting overt attention. Nonetheless, when just asked to report whether a dot was presented in the visual scene (pilot study), participants were accurate in reporting it (99% accuracy). Then, it could be argued that overt (i.e., with eye movement) and covert (i.e., without eye movements) mechanisms of attention are possibly differently involved in these kinds of situations.

Attention modulates direct matching

Previous evidence suggests that direct matching depends on attentional allocation directed to body parts (Bach et al., 2007; Chong et al., 2009). Attending to goal-directed actions primes movements involving the corresponding body part (Bach et al., 2007) and facilitate performance of the same action (Chong et al., 2009). In addition, stronger activations of the motor system were reported when an observed grasping action was selectively attended since relevant to the observer's task (Schuch et al., 2010). In line with this literature, the results obtained in Experiment 3 confirm and extend previous findings to the neurophysiological domain. Specifically, for the No-Request condition a CE increase compatible with the observed movement (i.e., a precision grip) was registered in the observers' muscles, but only when the dot was presented on the hand. Instead, when diverting attention to the contralateral space with respect to the moving hand by means of a dot, the matching activations were significantly diminished.

Diverting attention does not affect a complementary response

Strikingly, a CE modulation compatible with a complementary response (i.e., a whole-hand grasp) was shown in the observers' muscles when the observed action was calling for a joint intervention (i.e., Request condition). More importantly, this activation was not affected by diverting attention toward the contralateral side of the scene by means of the dot presentation. These findings therefore suggest that social motor preparation is resistant to modulation by top-down mechanisms such as spatial attention. This would be analogous to the processing of biologically relevant stimuli (e.g., faces) which are reported to be processed despite participants' attention is directed toward distractor stimuli (Vuilleumier et al., 2001; Williams et al., 2005). Present data suggest that the automatic decoding of others' actions influences our behavior beyond attentional involvement, maximizing the efficiency of our socially appropriate responses (Hamilton, 2013; Hogeveen and Obhi, 2012). Moreover, the present study support and extend previous data showing that when gaze cues – which represent the primary source of information to predict others' action goals – are not available, participants orient their attention to the others' actions as a secondary source of information (Letesson, Grade and Edwards, 2015; Sartori et al., 2011a). Notably, people shift their attention towards what they expect other people will look at (Joyce, Schenke, Bayliss and Bach, 2015) and these prediction biases can lead to similar attentional shifts as directly perceived gaze (Teufel, Alexis, Clayton and Davis, 2010). According to the predicting coding of social perception (Kilner, Friston and Frith, 2007), here biological movement and prediction of others' gaze behavior seem to drive the observer's attention regardless cue manipulation. It could be argued that this effect is due to the presence of a biological movement close to the object, without the effect being intrinsically social. If this were the case, then a simple arrow cue pointing towards the object would produce similar findings. However, results from previous studies in which the social request was substituted by an arrow did not provided support for this view (Sartori, Cavallo, Bucchioni and Castiello, 2011; Flach, Press, Badets and Heyes, 2010). Rather, we suggest that the motor system is preferentially tuned to meaningful actions of interactive partners.

Dissociation between eye gaze pattern and motor activation

An interesting finding that emerged in this study is the dissociation between eye gaze behavior and CE activations during observation of the action sequences adopted for the Request and the No-Request conditions. In fact, CE in the No-Request context was selectively modulated in function of the attentional manipulation: direct matching occurred only when the dot was over the action, whilst decreased when the dot was presented contralaterally. Interestingly, however, eye-tracking data showed that overt attention was not modulated by the dot presentation, with the participants keep looking at the action without moving the eyes toward the mug when the dot appeared over it. Then it seems that even if overt attention was displaced over the action, the dot presented on the mug placed next to the observer modulated attentional displacement (i.e., covertly) causing motor facilitation to decrease. Conversely, in the Request condition eye tracking data showed that the dot was always overtly attended by participants, but the preparation of the complementary response was *not* affected by the attentional manipulation. Indeed, the increased CE for the little-finger muscle (i.e., the muscle required to perform whole-hand grasps) reflected the preparation of a complementary response (i.e., WHG) irrespective of dot presentation, either appearing over the mug or on the small starting box on the other side of the visual scene.

There are two main limitations of this study that should be considered when interpreting this dissociative data. The first is that it was not possible to record eye gaze measures together with the registration of CS excitability. This would have allowed a better picture of the reciprocal role of these parameters in the same sample, thus adopting a within-subjects design. Although the two samples recruited in Experiment 2 and 3 are representative of the population, it cannot be excluded that the outlined dissociation between gaze and motor data could be due to differences in the samples. The second limitation is that the dot presentation appeared in specific parts of the visual scene (i.e., left, right) that were carefully selected based on the muscular activation they were able to elicit, namely a PG for the left dot and a WHG for the right one. However, in the Request condition the dot located on the left, over the small starting box eliciting a PG, could have been less effective in interfering with the complementary preparation activated by the request action, compared to the No-Request condition in which the distracting dot appeared on the nearby mug. The starting box, in fact, is a small object that poorly contrast with the surrounding colors, whereas the mug is a big white object on a black table. Having said that, the results from the Experiment 2 together with the results coming from the pilot experiment suggest that the adopted attentional manipulation was able to attract attention, either overtly or covertly. The muscular pattern of activations that emerged from the TMS study seems to be coherently interpreted considering the influence exerted by spatial attention allocation on CE modulations.

Few studies have investigated the role played by attentional manipulation over the action observation system (e.g., Leonetti et al., 2015; Chong et al., 2008; Donaldson, Gurvich, Fielding and Enticott, 2015). A study from Leonetti et al. (2015) specifically tested whether action stimuli presented in peripheral vision – then covertly attended – would have been able to elicit direct matching in the observer's motor system. In action observation experiments, in fact, stimuli are usually presented in central vision, where visual acuity reaches its peak. Leonetti et al. (2015) then hypothesized that a decreasing precision pattern of direct matching from central to peripheral vision would have emerged. They indeed found a reduced kinematic specificity reflected by the motor resonant response. It seem plausible, in fact, that when an action appears in peripheral vision the natural response might be to either shift one's gaze so as to observe the action in central vision (Wilson and Knoblich, 2005), or to keep it in periphery if a different action is already engaging the action observation network. However, when participants have to adopt a strategic allocation of attention in central vision, while concentrating on a presented action or on a superimposed object to perform a discrimination task, selective attention has been demonstrated to modulate motor responses, both at a behavioral and a neural level (Chong et al., 2008, 2009). In particular, the decreased activity in the action observation system while a secondary task is imposed during action observation (see also Saucedo Marquez et al., 2011), suggests that selective filters are at work to limit processing of task-irrelevant stimuli. This filtering activity might play a role in inhibiting indiscriminate imitation of the many gesture we perceive in daily life. Furthermore, Donaldson and colleagues (2015) recently demonstrated a positive relationship between motor cortical facilitation and gaze pattern (i.e., fixation counts in areas of biological motion) during the observation of grasping actions. Here, the seemingly incongruence between gaze pattern and motor facilitation could be due to the fact that in the No-Request condition, even if participants did not explicitly explored the area in which the dot appeared, the attentional manipulation was still effective in covertly distracting attention when the dot was incongruent with the observed action. Indeed, it has been proved that irrespective of gaze direction, the visuomotor transformation process is influenced by the strategic allocation of attention (Leonetti et al., 2015; Chong et al., 2008, 2009). In the Request condition, instead, a complementary motor preparation emerged whether or not the mug was explicitly attended.

Several studies have suggested that attention can be distributed and observers can select sensory information independently from separate locations (e.g., Castiello and Umiltà, 1990, 1992; Cavanagh and Alvarez, 2005; Frey, Schmid, Murphy, Molholm, Lalor and Foxe, 2014). A recent study from Fagioli and Macaluso (2016) stressed on the adoption of more naturalistic scenes to study attentional deployment with a better ecological validity. Notably, visual search in complex natural situations can be remarkably fast and efficient despite the overwhelming amount of information (Peelen and Kastner, 2011; Wolfe, Alvarez, Rosenholtz, Kuzmova, and Sherman, 2011). Fagioli and Macaluso (2016) then suggest that two factors may influence attentional control in these contexts: i) real-world objects are recognized more quickly when they are familiar object ('ultra-rapid categorization'; Thorpe, Fize and Marlot, 1996), being categorized in a pre-attentive manner with little requirements of top-down control (Li, VanRullen, Koch, and Perona, 2002); ii) prior knowledge about the spatial arrangement of objects within natural scenes may influence visual search and recognition ('contextual cueing effect'; Chun, 2000). In the present experiment, the contribution of these factors may have led to an easier and more efficient processing of the salient object by the observer, particularly when the action was calling for a social interaction.

To summarize, the present research suggests a causal role of attention allocation in the process of topographically mapping the observed action (direct matching). However, attention manipulation was ineffective in modulating the process of preparing an appropriate response to an observed action. In this respect, this work provides the first evidence regarding the role of attention in social motor preparation.

CHAPTER 8

THE ROLE OF GAZE DIRECTION DURING ACTION OBSERVATION

8.1 Introduction

While interacting with another agent, eye gaze and body movements represent relevant sources of information for social communication (Emery, 2000; Porciello et al., 2014). Accordingly, people have the tendency to attend to an interacting agent's gaze (e.g., Conty, Tijus, Hugueville, Coelho and George, 2006; Langton, Watt and Bruce, 2000), from infancy and early childhood (Maurer and Salapatek, 1976; Farroni, Csibra, Simion and Johnson, 2002). Kobayashi and Koshima (2001) demonstrated that the humans' eyes, compared to the primates' ones, present a unique morphology (i.e., the widely exposed white sclera surrounding the darker colored iris) which allows to easily discern gaze direction when looking to someone's face. Gaze direction, indeed, provides information regarding where and what one is paying attention to (Bukowski, Hietanen and Samson, 2015), it may activate 'joint attention' between two agents (Butterworth, 1991) and it also induces reflexive shifts of attention in the onlooker (Driver et al., 1999; Friesen and Kingstone, 1998, Lagton and Bruce, 1999). Indeed, in gaze cuing paradigms, participants are typically faster at detecting or identifying an object when it appears in direction of an observed gaze, compared to when it is presented in the opposite side (for a review see Frischen et al., 2007). Moreover, the gaze of another person may give access to his/her intentions, like the willingness to act upon objects (e.g., Pierno et al., 2006; Castiello, 2003). Therefore, eye movements may provide information related to interests and desires of other people and hence to their future behaviors (Baron-Cohen et al., 1995; Sartori et al., 2011a; Becchio, Bertone and Castiello, 2008c).

In terms of action execution, proactive eye movements directed to the target object are required both for planning and controlling the performance (e.g., Johansson, Westling, Bäckström and Flanagan, 2001; Land, Mennie and Rusted, 1999). Interestingly, similar predictive gaze profiles are found both when participants perform an object-directed action and when they merely observe it (Flanagan and Johansson, 2003). These findings are consistent with the direct matching hypothesis, for which observed and executed actions are matched in the observer's motor system (Gallese et al., 1996; see also Chapter 1 for a review). They also further extend knowledge on this mechanism suggesting that information regarding eye movements is crucially tied to it. Along this line, a recent study from Coudé et al. (2016) showed the existence of mirror neurons in monkeys' ventral premotor cortex which specifically modulate their activity according to the gaze direction of an observed agent during a grasping action. In particular, these gaze-modulated neurons showed preference for a situation in which the gaze direction was congruent with the action direction, but also when the gaze was incongruent with the actor's movements. These very interesting results demonstrate that an integrated representation of other's actions, together with gaze direction, is coded at a single neuron level in the premotor cortex (Coudé et al., 2016). Jellema and colleagues (2000) also reported the existence of a population of cells within the superior temporal sulcus (STS; an area that provides visual input to the MNS) preferentially activated by the congruence between the direction of a reaching action and the direction of both the gaze and head of the actor (Jellema, Baker, Wicker and Perrett, 2000). Overall, these results suggest that action and gaze are tightly linked, even at a neural level. Indeed, the coupling between action and gaze direction is crucial for our everyday interactions, since it helps us to understand and predict the behavior of other people. Innocenti and colleagues (2012) tested the role of gaze direction and request gesture in affecting the kinematics of an onlooker's object-directed action in a social context (Innocenti, De Stefani, Bernardi, Campione and Gentilucci, 2012). Participants were requested to reach, grasp and lift a bottle of juice in the presence of an empty glass. Crucially, when another agent, sat on the opposite side of the table, held the empty glass while directing gaze to the participants (i.e., a request gesture), the kinematic pattern of their performed action was affected. Therefore, observing a request gesture while performing an action activated a social motor program (i.e., pouring), which interfered with the action to perform (i.e., lifting), but only when it occurred simultaneously with a direct gaze indicating an interactive attitude. The modulatory effect of gaze is indeed crucial in social meaningful situations (Hamilton, 2015).

A point worth noting is that the ability of gaze direction to influence the motor preparation for a social action has never been tested at the level of corticospinal excitability. In the present study I then adopted the same experimental paradigm presented in Chapter 7, depicting a situation in which an agent expresses (or not) a social request toward the observer, but rather than manipulating visuospatial attention by mean of a cue I used an actor's gaze direction. Gaze cuing of attention triggers fast and automatic shifting of attention toward the gazed location, thus resembling the effect of sudden onset cues (Tipper, 2010). As discussed above, gaze also presents an intrinsic social valence. Generally speaking, the present experiment aimed at further deepening the findings presented in Chapter 7 by exploring the role of attentional allocation during motor resonance and reciprocity by adopting a more ecologically-valid manipulation. The results presented in Chapter 7 showed that direct matching is dependent on attentional allocation toward the actor's hand while preparing the same action, in line with previous literature (Bach et al., 2007; Chong et al., 2008, 2009; Schuch et al., 2010; see also Chapter 3). Conversely, the preparation of a complementary action in response to the observed request resulted unaffected by attentional manipulation. Here I adopted TMS over M1 combined with EMG recordings to test whether, compared to the dot presentation utilized in the experiment reported in Chapter 7, gaze direction would be more effective in modulating motor preparation depending on the nature of the observed action.

8.2 Methods

The methods employed in this study followed the procedure outlined in Chapter 4, pp. 55-58.

8.2.1 Participants

Thirty right-handed individuals (13 males and 17 females, mean age 21.8 years) took part in the experiment.

8.2.2 Experimental Stimuli

Two videoclips were used as experimental stimuli for the 'Request' and the 'No-Request' conditions, respectively:

a) *Request condition*: the actor grasps a sugar spoon placed on a small starting block, he pours sugar into a mug placed next to him on a table and then he stretches out his arm towards a mug out of his reach, but strategically placed near the observer, thus requiring his/her intervention to lift the mug and complete the complementary action (Fig. 8.1, top row).

b) *No-Request condition*: the actor grasps the same sugar spoon placed on a small starting block, pours sugar into the mug placed next to him on a table and then he moves back the spoon to its initial position (Fig. 8.1, bottom row).

Note that in both video clips the actor grasped the sugar spoon with his right hand using a PG (i.e., the opposition of the thumb to the index finger). Whereas the other object presented in the scene, namely the mug, requires the use of a WHG (i.e., the opposition of the fingers to the palm) in order to be handled. This kind of action sequences has been chosen based on several previous studies on complementary actions (e.g., Sartori et al., 2012a, 2012b, 2013b; see also Chapter 2, paragraph 2.3).

Attentional allocation to specific parts of the scene was manipulated by means of the actor's gaze direction. The actor was instructed to follow his hand movements in a natural way with the gaze and the head, then, after pouring the sugar into the first mug, he was required to gaze at one of two possible locations: i) on the right side of the screen (*right gaze*), toward the out-of-reach mug located on the corner of the screen and placed next to the observer (Non-Request and Request conditions, Fig. 8.1, right column); or ii) on the left side of the screen (*left gaze*), toward the small starting box (Request and No-Request conditions, Fig. 8.1, left column). Notably, the left gaze was always associated with a PG (i.e., the grip required to grasp small objects like the starting box) and the right side gaze with a WHG (i.e., a power grip required to grasp big objects like the mug). It is worth noting that the conditions in which gaze and action were compatible (i.e., request on the right/right gaze; moving back to the left/left gaze) are similar to what we usually experience in daily life, whereas the incongruence between these two indexes (request on the right/left gaze; moving back to the left/right gaze) is less common. Therefore, whereas in the former situations gaze would keep attention to the action sequence, the latter would divert attention away from the action in favor of an object placed on the other side of the scene. Notably, the two salient objects in the scene (i.e., small starting box and big mug) were carefully selected to allow a mismatch in terms of affordances (i.e., PG for small objects and WHG for big ones).

Each video lasted 5690 ms and the animation effect was obtained by presenting a series of single frames each lasting 30 ms (resolution 1920 x 1080 pixels, color depth 32 bits) following the first frame lasting 500 ms.

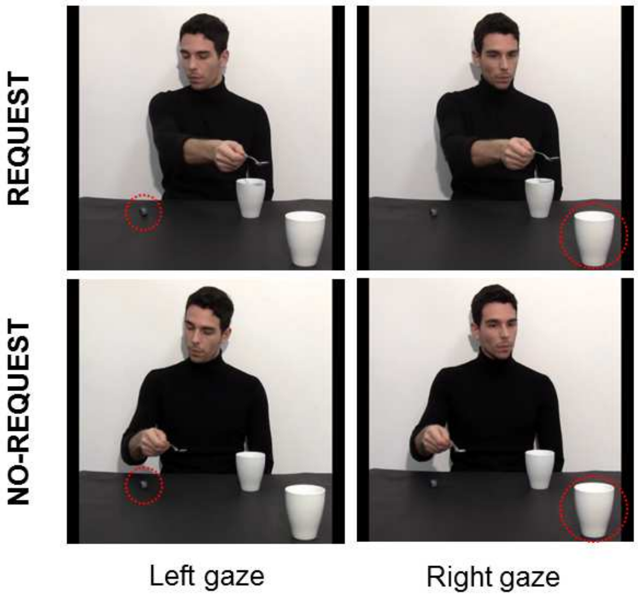


Figure 8.1: Each row represents an experimental condition: 'Request' on the top and 'No-Request' on the bottom. Attentional allocation to either one of two specific positions in the visual scene was manipulated by means of the actor's gaze direction: 'left gaze' (see left column) when the actor looked at the starting block; 'right gaze' (see right column) when the actor looked at the out-of-reach mug located on the right side of the screen. Red dotted circles indicate the object to which gaze was directed.

8.2.3 Procedure

Participants were tested individually in a single experimental session lasting approximately one hour. They were instructed to watch the video clips that were presented on a 24" monitor (resolution 1920 x 1080 pixels, refresh frequency 120 Hz) set at eye level while remaining as still and relaxed as possible. No specific task was given to participants; however, in order to ensure attention to the video clips, they were told that at the end of the experiment they would be questioned about the stimuli presented (post-experiment questionnaire). TMS-induced MEPs were acquired from the participants' right first dorsal interosseous (FDI) and adductor digiti minimi (ADM) muscles of the right hand. A single TMS pulse was released during each video presentation at 4700 ms, namely when the actor arm was stretching out toward the out-of-reach mug (Request conditions; Fig. 8.1, top row) or when he was moving back the sugar spoon toward the initial position on the small starting box (Non Request conditions; Fig. 8.1, bottom row). Notably, the actor's hand movements were identical for each condition, only the actor's gaze direction differed (see Figure 8.1). The order of the video clips was randomized across participants.

A total of 120 MEPs (2 muscles x 2 conditions x 2 gaze directions x 15 repetitions) was recorded for each participant. Prior and after the experimental block, each participant's baseline corticospinal excitability was assessed by acquiring 15 MEPs while they passively watched on the computer screen a white-colored fixation cross on a black background. Possible variations in corticospinal excitability related to TMS per se were assessed by comparing the MEP amplitudes recorded during the two baseline periods (30 MEPs in total). Their average amplitude was then utilized to set each participant's individual baseline for data normalization procedures. An inter-pulse interval lasting 10 s (i.e., 5 s during which participants were reminded to remain fully relaxed, followed by other 5 s of fixation cross presentation) was adopted between trials in order to minimize the potential risk of carryover effect of a TMS pulse on the subsequent one. The presentation of a fixation cross before each trial ensured that participants always started the trial by observing the videos from a neutral gaze position. Stimuli presentation, timing of TMS stimulation and EMG recordings were managed by E-Prime V2.0 software (Psychology Software Tools Inc., Pittsburgh, PA, USA) running on a PC.

Post-experiment questionnaire

At the end of the experimental session participants were asked to rate four sentences regarding each experimental condition on a five-point Likert scale (ranging from 'I totally disagree' to 'I totally agree'). The order of the conditions to which the sentences referred was counterbalanced between participants. Hereafter the four sentences adopted: 1) "I felt involved in the action"; 2) "At the end of the video I looked at where the actor was gazing at"; 3) "At the end of the video the actor's gaze distracted me from the action"; 4) "At the end of the video I would have grabbed the nearest mug". These sentences were adopted to quantify the subjective experience of involvement experienced by each participant after the experiment. The responses to the questionnaire were correlated with the mean normalized MEP values to explore whether the participants' perceived experience corresponded to this motor excitability index.

8.2.4 Data Recordings

The TMS procedure was identical to that reported in Chapter 4 in paragraph 4.3 and EMG recordings were collected as outlined in paragraph 4.2. Single-TMS pulses were delivered in order to stimulate the hand region on the left M1. MEPs were recorded simultaneously from the FDI and ADM muscles of the participants' right hand. These muscles were chosen because of their differential activation during the execution of PG versus WHG (i.e., a higher activation of FDI for PG and of ADM for WHG; see Davare, Montague, Olivier, Rothwell and Lemon, 2009). The experimental stimuli were in fact created in order to have a mismatch between the observed type of grasp and the one that would be required to carry out the social request (i.e., a PG performed by the model on the spoon and a WHG to act on the mug). The EMG signal was recorded by means of electrodes placed in a belly-tendon montage, with the ground electrode positioned over the participant's left wrist. rMT ranged from 30 to 56% (mean = 41%, *SD* = 6.2) of the maximum stimulator output.

8.2.5 Data Analysis

The data analysis followed the procedure outlined in Chapter 4, paragraph 4.5. Data were analyzed offline using Brain Vision Analyzer software (Brain Products GmbH, Munich, Germany) and SPSS 22 (SPSS Inc., Chicago, IL, USA). The MEP peak-to-peak amplitude (mV) for the FDI and the ADM muscles was determined as a measure of participants' corticospinal excitability. Trials contaminated by muscular pre-activation were excluded as outliers (< 1 %). A paired sample *t*-test (two-tailed) was used to compare the amplitude of MEP recorded during the two baseline trials carried out at the beginning and at the end of each block. The normalized MEP amplitudes (MEP ratio) were entered into a repeated-measures ANOVA conducted on the MEP ratios with condition (Request, No-Request), gaze direction (left, right) and muscle (FDI, ADM) as within-subjects factors. One sample *t*-tests to compare normalized MEP amplitude and baseline were conducted. Furthermore, for each experimental condition, Pearson's correlations were performed between the mean MEP values for each muscle and the results of the post-experiment questionnaire (*p*-values were Bonferroni corrected for multiple comparisons). A significance threshold of $p < 0.05$ was set for all statistical analysis.

8.3 Results

The mean raw MEP amplitudes recorded at the beginning and at the end of the experimental session were not significantly different for either the FDI ($t_{29} = 0.946$, $p = 0.352$) or the ADM ($t_{29} = 0.086$, $p = 0.932$) muscles. Therefore, TMS *per se* did not induce any nonspecific change in corticospinal excitability that could have biased the results.

The ANOVA on normalized MEP amplitudes showed a significant main effect of gaze ($F_{1,29} = 7.271$, $p = 0.012$, $\eta^2_p = 0.20$) and muscle ($F_{1,29} = 16.329$, $p < 0.001$, $\eta^2_p = 0.36$). The ADM MEP amplitude (1.05 mV) was significantly lower than the FDI MEP amplitude (1.26 mV). As a consequence, the MEP amplitude for the Right gaze condition (1.11 mV), when the gaze pointed to the salient mug, was significantly lower than for the Left gaze condition (1.19 mV), when the gaze was directed to the small starting box. This suggests a generalized inhibition of the little finger abductor (i.e., the muscle involved in the social response of grasping the mug). MEP amplitudes for the ADM muscle showed indeed a general inhibitory profile, with no activations above baseline throughout all conditions ($p_s > 0.05$). According to my hypothesis, I predicted a differential role of gaze direction in social and non-social contexts. Consequently, a significant 3-way interaction Condition x Gaze x Muscle was expected. However, such interaction was not significant ($F = 0.11$). Given the specific predictions on differences between MEP amplitudes across conditions and given that an examination of the interaction alone would not provide the most sensitive test of these predictions[1], I chose to perform planned *t*-test comparisons (see Villiger, Chandrasekharan and Welsh, 2011 for a similar approach). Post-hoc contrasts were then conducted separately for each muscle. This choice has been made taking into consideration both the significant muscle main effect and the fact that looking into the two muscles separately may allow to minimize the potential influence of the adoption of a unique hotspot for both the FDI and the ADM stimulation, which could have advantaged the FDI motor representation at the expense of the ADM (Cavallo, Sartori and Castiello, 2011; Hétu, Taschereau-Dumouchel, Meziane, Jackson and Mercier, 2016). The results from the planned *t*-test comparisons conducted separately for each muscle (FDI, ADM) are reported as follows. The normalized MEP amplitude for the FDI

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[1] The significant interaction would reveal whether there are significant differences between the differences of the experimental conditions, but not necessarily whether there are (or there are not) significant differences between individual conditions (Villiger et al., 2011).

muscle was significantly lower for the Request condition when the actor was looking at the out-of-reach mug compared to when he was looking at the small starting box (t_{29} = 2.76; p = 0.010) and compared to the No-Request condition, when the actor was looking to the box while bringing the spoon back (t_{29} = 2.05; p = 0.049; Figure 8.2 left graph). These data suggest that the combination of social request and convergent gaze was effective in priming a complementary response instead of a motor facilitation in the corresponding muscle.

In the Request condition, the ADM muscle activity was lower when the actor looked at the out-of-reach mug than when his gaze was directed on the small starting box (t_{29} = 2.22; p = 0.035; Fig. 8.2 right graph). Since no activations above baseline were shown for this muscle throughout all conditions (p_s > 0.05), this result is likely due to an inhibition (for the right gaze condition) rather than to an enhancement effect (for the left gaze condition).

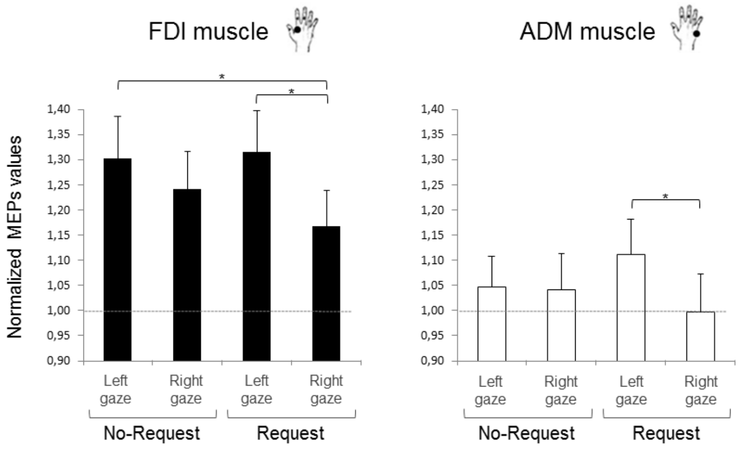

Figure 8.2: Results showing corticospinal activation for the No-Request and the Request conditions when the actor was looking leftward (Left gaze) and rightward (Right gaze). The plot represents the normalized mean MEP amplitudes recorded from the FDI (left graph) and the ADM (right graph) muscles. The horizontal dotted lines indicate MEP baseline. Bars indicate standard error of the means. Asterisks indicate statistically significant comparisons (p < 0.05).

Overall, the present results seem to suggest that the muscle involved in preparing the complementary response (i.e., ADM for a WHG on the mug) showed a general inhibitory activity. In particular, this inhibition emerged when the actor's gaze was coupled with the request gesture and was generalized to both muscles of the hand (ADM and FDI). It is tempting to suggest that this inhibitory activity might reflect the refrain to produce an overt response given that participants were instructed to remain still and relaxed.

Correlations between the MEP amplitude and the responses to the post-experiment questionnaire

A strong support to the inhibitory hypothesis comes from the correlations between the CE values and the responses to the post-experiment questionnaire (see Fig. 8.3 for an overview of the mean results to the statements of the questionnaire). Indeed, the ADM MEP amplitude for the Request condition when the gaze was rightward was negatively correlated with the responses to the 4th statement of the questionnaire, asking for the tendency to grasp the mug at the end of the action sequence ($r_{28} = -0.47$; $p = 0.034$; Fig. 8.4a). The more the participants were willing to respond, the lower were the MEP amplitudes. Then, the subjective evaluation regarding the willingness to act toward the mug was actually correlated with an inhibition of the motor response in the observers' muscle.

As concern the No-Request condition, a positive correlation between the responses to the 2nd statement of the questionnaire (asking for the tendency to look at the actor's gazed location) for both the FDI and the ADM muscles was shown ($r_{28} = 0.47$; $p = 0.04$ and $r_{28} = 0.52$; $p = 0.01$, respectively; Fig. 8.4b, c). So, when gaze direction was compatible with the observed action (i.e., the actor gazed at the small box while moving back the sugar spoon), an increase in the MEP amplitudes for both the hand muscles was correlated with the perception of being captured by the actor's gaze. Notably, this is a generalized effect of attention cueing and since the No-Request condition was not involving any response by the observer, MEP inhibition was not necessary.

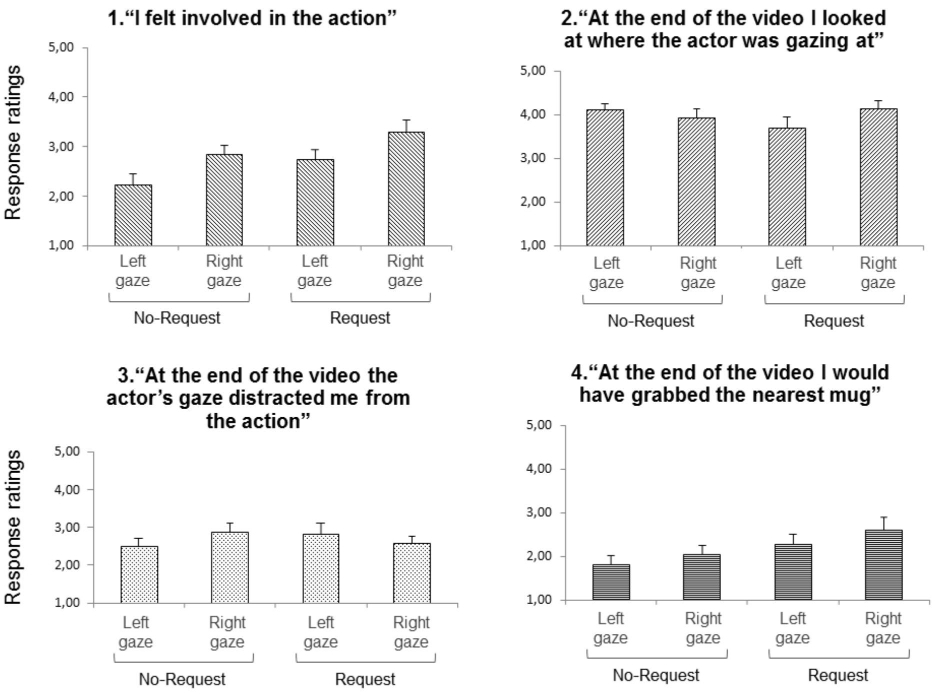

Figure 8.3: Average responses to the post-experimental questionnaire. Each graph represents the mean response rating of all participants to each statement in a five-point rating scale, from 1 (‘not at all’) to 5 (‘much’), and referring to each experimental condition (No-Request, Request) and gaze direction (left, right).

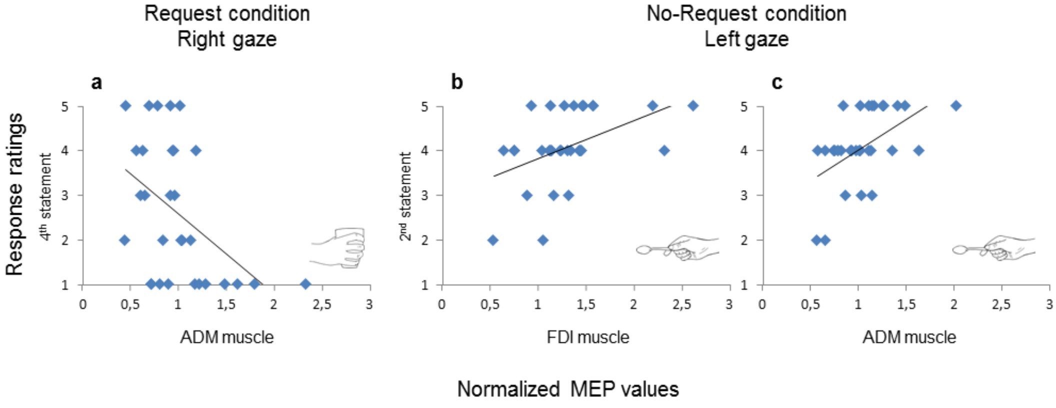

Figure 8.4: Results showing the statistically significant correlations between the response rating to the questionnaire statements (y-axes) and the normalized MEP values (x-axes). The graphs, from left to right, refer to: a) the ADM muscle activity in the Request condition with the actor gazing to the mug (right gaze), which was negatively correlated with the questionnaire responses for the 4th sentence (“At the end of the video I would have grabbed the nearest mug”); b) the FDI and c) the ADM muscles activity in the No-Request condition with the actor gazing at the starting box (left gaze), which were positively correlated with the questionnaire results for the 2nd sentence (“At the end of the video I looked at where the actor was gazing at”).

To summarize, these data seem to suggest that gaze direction plays a role in modulating the onlookers' CE. In particular, when the actor's gaze pointed to a mug placed next to the observer while unfolding a request gesture toward it, a decrease in the MEP amplitude was measured. This inhibition has been recorded both for the ADM and the FDI muscle, then resulting related to the effector, and not being muscle-specific. However, a general inhibitory activity emerged in particular for the ADM compared to the FDI muscle, thus involving the muscle required to interact with the nearby mug (i.e., the ADM with a WHG).

8.4 Discussion

The present research aimed at studying the role of the agent's gaze direction on the motor response elicited by action observation with particular reference to a social context evoking complementary actions. The current experiment capitalized on the study presented in Chapter 7 in which, during action observation of motor sequences eliciting (or not) a complementary response in the onlooker, attention to specific parts of the visual scene was manipulated by means of the appearance of attention-capturing dots. In the current study, visuospatial attention was directed toward the same parts of the scene, but through the actor's gaze direction. Indeed, it has been extensively demonstrated that perceived gaze shifts could influence attention orienting (see Frischen et al., 2007 for a review). The results from the present study show that gaze manipulation was able to influence the observers' corticospinal excitability, but the direction of this influence was unexpected. Indeed, a general inhibition was found in the muscle involved in responding to the social request (i.e., ADM). This is a novel result in the field of neurophysiological studies on complementary actions, given that an increased pattern of activation is usually found when observing an actor's arm unfolding a social request toward the observer (e.g., Sartori et al., 2011b, 2012b, 2013a, 2013b). Notably, however, in the previous studies gaze cues were not provided to the participants since the video clips did not show the actor's face.

In terms of motor resonance, I showed a facilitatory profile on the observer's muscle corresponding to the action observed (i.e., FDI), consistent with the replication of the observed action (i.e., a PG on the sugar spoon) in the No-Request condition. This facilitation was higher when the actor's gaze was pointing to the left – in line with the movement direction – compared to when it was pointing to the mug placed next to the observer. Therefore, the CE of the observers' index finger muscle reflected an activation compatible with the actor's focus of attention. Whereas, when a social request was presented and the gaze was pointing to the object with which the observer was required to interact (i.e., the nearby mug), a decrease in the MEP amplitude occurred, signaling the suspension of direct matching (in favor of a complementary motor preparation). Similarly, when the social request and the gaze direction were congruent, a decreased activity in the ADM muscle was measured. This inhibitory pattern of activity can be interpreted as a mechanism preventing the tendency to react to the salient action observed. A kinematic study from Sartori and colleagues (2009) demonstrated that a sudden request (i.e., a give-it-to-me hand gesture) was able to override a previously planned action, regardless of the given instructions. In the present experiment, even if participants were required to remain still and relaxed while watching the actions, it is possible that a similar reactive response preparation took place in the motor system, therefore requiring inhibition in order to prevent any overt behavior. In fact, the inhibition could also reflect a rebound effect (Jurkiewicz, Gaetz, Bostan and Cheyne, 2006; Salmelin, Hámáaláinen, Kajola and Hari, 1995). A 'post-stimulus rebound effect' has been also registered in a EEG study from Schuch et al. (2010). The authors found that the more the mirror system was activated (i.e., greater desynchronization of the mu rhythm over sensorimotor cortex during action observation), the more the motor system was subsequently inhibited (i.e., greater synchronization of the mu rhythm). Crucially, a connection between mu power and MEPs have been provided by some studies which demonstrated that increased mu oscillations are associated with a reduction of MEP amplitude (Hummel, Andres, Altenmüller, Dichgans and Gerloff, 2002; Sauseng, Klimesch, Gerloff and Hummel, 2009; Zarkowski, Shin, Dang, Russo and Avery, 2006). Overall, these evidences suggest that the inhibitory activity registered at the level of the ADM muscle could have been the byproduct of an increased activation. However, given that I adopted one stimulation timing for MEP registration, this procedure could not have been sufficiently sensitive to unveil a possible preceding activation. Notably, this inhibition was confined to the little finger muscle (not the index finger muscle) thus pointing to a specific effect of social response preparation. A study from Pfurtscheller, Neuper, Andrew and Edlinger (1997) reported that when participants were required to perform a foot action, the mu power in the hand motor area increased, thus reflecting suppression of processing in the non-responding hand area while having to move the foot. It may be possible that here a similar muscle-selective inhibition took place, but the fact that it was present in both the Request and the No-Request conditions irrespective of gaze direction (in all conditions the MEP amplitude did not differ with the baseline value) make it hard to interpret this result.

An interesting finding of the present experiment is the correlation between the perceived impulse to grasp the mug (as reported in the post-experiment questionnaire) and the motor excitability measurements. Indeed, whereas in the Request condition, when the gaze was pointing to the mug, the participants referred the willingness to grab the mug at the end of the action sequence, the MEP amplitude reflected a significant inhibition. This may provide further support to the hypothesis discussed above regarding the possible inhibition of the ADM muscle to prevent any overt response elicited by the salient request. On the other hand, the fact that the CE was not consistent with the participants' subjective report is in line with a recent study showing that the observers' own intentions and motivations during the experiment are not able to influence motor excitability during action observation (Naish and Obhi, 2015).

In conclusion, the present results extend what emerged in the study presented in Chapter 7. It is important to note that in previous neurophysiological studies on complementary actions (see Chapter 2, paragraph 2.3) the head of the actor was never included in the scene. Having added head and gaze of the actor in the stimuli of the present experiment have led to different effects compared to the appearance of a flashing dot. This could be due to the fact that the observed gaze may have added 'social noise' to the visual scene. In fact, Bunday et al. (2016) have recently demonstrated that the presentation of a whole person performing an action – compared to the presentation of the only hand – abolished grasp-specifics effects in motor resonance, without the results being due the relative size of the observed hand (Bunday, Lemon, Kilner, Davare and Orban, 2016). So far, the available literature on the role of attention on action observation was confined to limited paradigm adopting simplified stimuli (e.g., a big hand over a neutral background) that, although boosting subtle responses in the neural activity (e.g., Chong et al., 2008), may lack in their ecological validity. Here I tried to find a compromise between the use of a realistic situation and the adoption of a controlled experimental design. The use of gaze direction to manipulate attention allocation goes in this direction. Indeed, in our everyday life we usually observe action embedded in moving bodies where information deriving from gaze direction is provided and it is crucial for action understanding (Becchio et al., 2008c).

The present study may represents a starting point for future research that will clarify the origins and will further explore the temporal aspects of the inhibitory activation emerged in this set of data. The effort to design experiments that may allow to unveil the motor system functioning in realistic situations should be a challenge for future studies in this field.

CHAPTER 9

GENERAL DISCUSSION

Even if taking part in a joint action – like grabbing an object when someone is handing it to us – appears to be a trivial and ordinary task, it requires the implementation of several abilities. Indeed, while observing the action of an interactive agent, we are required to recognize the underlying goal and intention, to make predictions about the other's person behavior and to properly coordinate our actions accordingly in order to produce an appropriate response. Therefore it is important to understand the cognitive mechanisms that allows us to make sense of observed actions and to produce socially-adequate responses.

The experimental work included in the present thesis aimed at investigating this issue within the framework provided by complementary actions, namely those form of social interactions wherein the interacting individuals complete reciprocal actions, rather than performing imitative behaviors, to bring a joint goal to completion (see Chapter 2; for review, Sartori and Betti, 2015). In particular, the flexibility of the motor system in preparing non-identical actions in response to an interactive agent's behavior, together with the involvement of spatial attention in modulating those motor responses, has been investigated.

An overview of the obtained results and their implications for our understanding of the role of the motor system during action observation is outlined below.

The flexibility of the Action Observation System

Recent advances in human motor cognition have been obtained from the study of mirror neurons, first discovered in nonhuman primates, by which observed actions are matched onto the observer's motor system without being executed (Rizzolatti and Craighero, 2004). The existence of an AOS in humans, mediated by the mirror mechanism activity and able to simulate observed gestures, has been considered at the basis of our ability to understand others' actions (Rizzolatti et al., 2001; Gallese, Keysers and Rizzolatti, 2004). Wilson and Knoblich (2005) defined the activation of the motor system when movements are only observed (i.e., motor resonance) as a "neurological extravagance", but they also recognize it as an "elegant solution to a perceptual problem". Indeed, they identified in the imitative motor activations following action observation the mechanism by which we simulate the perceptual incoming signals in a predictive fashion. Therefore, covert imitation may serve to track another person's action in real time, allowing the onlooker to interpret the behavior of the interactive agent and to quickly react. In the framework provided by Wilson and Knoblich (2005), the motor involvement in perceiving others' actions acts as an *emulator*, internally simulating the ongoing perceived behavior. The activity of the emulator would allow other's actions and our own actions to be rapidly integrated in real time, therefore allowing individuals to engage in joint actions. As noted by Sebanz and colleagues (2006), although social interactions sometimes require imitative kinds of movement, in other situations imitating the others' actions could be counterproductive. Instead, complementary movements should be selected to successfully accomplish a joint goal. From this, many studies have attempted to explore the flexibility of the AOS and its role in guiding the implementation of non-matching behavior following action observation (see Chapter 2 for a review). In particular, Sartori and colleagues performed a series of neurophysiological studies in which the excitability of the motor system was assessed during observation of actions evoking imitative and complementary gestures (e.g., Sartori et al., 2012b, 2013a). It emerged that, according to the context and to the action's phase, the corticospinal excitability reflected the tendency to prepare imitative simulation of the observed action, which however could leave room for the preparation of a dissimilar response when needed (*functional shift*). The experimental studies presented in Chapter 5 and 6 of this thesis allow to bring research on complementary actions a step further by demonstrating for the first time that imitative and complementary activations could be elicited at the level of different effectors. Indeed, by adopting an experimental paradigm in which the observation of a soccer player kicking a ball could trigger (or not) a complementary response in the upper limbs as to parry the approaching ball, it was possible to register identical and non-identical elicited motor responses not only in different muscles, but also in different effectors. The results from the study presented in Chapter 5 provided evidence that the functional switch from the corticospinal facilitation reflecting an imitative response in the leg muscle to a complementary activation in the upper limb took place only when the ball was seen as approaching the onlooker. Indeed, when the ball followed a lateral trajectory away from the observer, or when the ball was not present and the action was only mimicked, just an emulative activation in the lower limb emerged. Strikingly, the adoption of this paradigm in a following experiment (Chapter 6) allowed to further extend these findings by testing whether simulation and reciprocity could co-exist in the motor system. Therefore, participants were presented with a soccer player performing a penalty kick toward the observer, but the player continued to run after the kick. This allowed to test whether the tracking of the observed action at the level of the leg muscles was kept activated in the motor system together with the preparation of an incongruent reaction elicited at the level of the arm muscles. Control conditions in which the player stopped after kicking or in which he send the ball laterally were also included. Crucially, this experiment allowed to demonstrate that the observation of another person's movements can prompt three different levels of motor coding in a not mutually exclusive way. In particular, the internal replica of the observed action (kinematic coding), the predictive activation (predicting coding) and the preparation of a non-identical reaction (response coding) emerged to be processed in parallel in different effectors. The fact that actions can be coded at many different levels had already been suggested (Grafton and Hamilton, 2007; Kilner et al., 2007). For example, Kilner and colleagues (2007) identified four levels through which actions could be described, namely an intentional level (which defines the long-term goal of an action), a goal level (which defines the short-term goals necessary to achieve the long-term intention), a kinematic level (which describes the movements in space and time) and a muscle level (which describes the pattern of muscle activity required to execute the action). The authors believe that the function of the MNS should be considered within a predictive coding framework, through which the cause of an observed action can be estimated from the visual representation of the observed movements. Even though the paradigm adopted in Chapter 6 was not specifically designed to explore the higher-levels of the action hierarchy (i.e., the intentional and goal levels underlying the observed action), it allowed to introduce a further coding level (i.e., the response level) related to the preparation of a response to the observed action (for a review see Hamilton, 2013). This is particularly relevant when considering observed actions within a social framework, in which actions of interactive agents reciprocally influence each other since they may require a reaction in response. Overall, it seems that running different coding levels while processing an observed action would be helpful to prepare a correct reaction. As hypothesized by Sartori and colleagues (2013a), the mirror system function could be similar to that of a working memory, but specifically adapted for actions. Therefore, as in the case of working memory in which distinct elements are kept on-line while a larger structure is being processed (Baddeley, 1992), simulative and complementary tendencies could be kept simultaneously active in order to process both the observed action and its relative response. This would represent a crucial ability in case of involvement in complex social interactions, allowing to flexibly adapt our behavior according to the context requests.

The automaticity of the Action Observation System

A growing body of literature suggests that the matching between observed and executed actions is not necessarily straightforward, and that action observation does not inevitably lead to a matching motor facilitation. The motor system is indeed capable to overcome the imitative tendencies (direct matching) in favor of non-identical response preparation depending on the context requirements (e.g., Heyes et al., 2005; Newman-Norlund et al., 2007; see Chapter 2 for a review). Furthermore, an increasing number of evidence suggests that the motor system activation following action observation appears to be dependent on the allocation of attentional resources (e.g., Bach et al., 2007; Chong et al., 2008, 2009; see Chapter 3 for a review). Attention to the action seems indeed to be necessary for the visuo-motor matching to occur. Taken together, these data challenge the idea that action observation automatically leads to imitation in the observer. To date, the role of attention during observation of actions eliciting a complementary response has never been tested. The experiments presented in Chapters 7 and 8 have indeed been designed to study the influence of spatial attention on direct matching and complementary action preparation. To do so, an established paradigm for inducing (or not) complementary activations in the onlookers' muscle (e.g., Sartori et al., 2011b, 2012b, 2013b) was adopted. Participants were then presented with action sequences in which an actor was signaling a request referenced to a salient object out of his reach, but strategically placed near the observer (i.e., pouring some sugar in an out-of-reach mug). In a real life situation this would require the observer's intervention to grasp the nearby object (i.e., the mug) and lift it to fulfill the request. In another condition a similar sequence of movements was adopted, but no social request was presented. Noticeably, the observed movement (i.e., a PG on the sugar spoon) was specifically mismatched with the one required to interact in a complementary fashion (i.e., a WHG on the mug). Doing so, the imitative and the complementary action preparation could be elicited and measured in different hand muscles (i.e., the index finger muscle should reflect the observed PG and the little finger muscle should reflect the preparation for the WHG). In Chapter 7 attention toward crucial parts of the scene was manipulated by means of the brief appearance of an attention-capturing dot. The results showed that the simulative muscular activation was modulated by attentional allocation. Specifically, corticospinal excitability significantly decreased when attention was distracted from the actor's movements. This result confirmed previous findings on the role of attention on direct matching (e.g., Bach et al., 2007; Chong et al., 2008, 2009; Schuch et al., 2010) and extended their validity also to the neurophysiological domain. More interestingly, the complementary action activation was not influenced by the attentional manipulation. Corticospinal excitability significantly increased irrespective of whether attention was attracted toward the salient object or away from it. These results are among the first to provide evidence that social motor preparation is impervious to attentional interference. Moreover, the adoption of eye-tracking procedures – allowing to assess gaze direction and to measure fixations duration – shed light on the possible relationship between corticospinal excitability and overt attention allocation. Even if further studies should better clarify the reciprocal influence of these indexes by simultaneously measuring eye gaze behavior and corticospinal excitability (in the present study, on the basis of the available facilities, it was not possible to do so; but see Donaldson et al., 2015 for a paradigm in this direction), this study provides some preliminary evidence of the relationship between them. In particular, it emerged that overt orienting of spatial attention and corticospinal excitability during action observation appeared to be unrelated. In fact, when the attentional manipulation was able to distract eye gaze from the salient object, the preparation for a complementary response was not affected; on the other hand, even if the dot presentation was not able to distract attention from the actor's movements, direct matching was compromised. Therefore, it is likely that the interplay between overt and covert attention (i.e., mediated by or without eye movements, respectively) might have determined this pattern of results. To summarize, these findings regarding the dissociation between attentional and motor excitability patterns during observation of actions evoking (or not) an interaction might contribute to shed light on the peculiar characteristics of the processing concerned with social stimuli. Indeed, it has been reported that top-down factors such as attention appear to differently influence the processing of stimuli characterized by an intrinsic social valence with respect to other stimuli (Vuilleumier et al., 2001; Williams et al., 2005). It is worth noting that a certain level of selection of the incoming information is important to allow for an efficient behavior and to prevent an information overload due to capacity limitation of our cognitive system. However, some automaticity in the decoding of others' social actions could be crucial for maximizing the efficiency of our appropriate responses (Hamilton, 2013): a key ability in a social environment.

In the last experiment presented in Chapter 8, attention to parts of the visual scene was manipulated by means of the actor's gaze direction, which is a more ecologically-valid index of attentional allocation in real-life interactions. Eye, head and hand movements are indeed of mutual importance in everyday tasks, and gaze usually serves as a precursor of subsequent manual actions (e.g., Hayhoe and Ballard, 2005) and is used to signal communicative intents (Emery, 2000). Crucially, the observation of someone's gaze direction can trigger in the onlooker a shift of attention toward the gazed location (e.g., Friesen and Kingstone, 1998). From the results of the experiment (Chapter 8) it emerged an unexpected inhibitory modulation on the muscle that would be involved in the preparation of the complementary response (i.e., the little finger muscle involved in preparing a WHG on the mug). This inhibitory effect was higher when both gaze direction and the request gesture were directed to the object to which interact, namely the mug located near the observer. This result has been interpreted as an inhibition of motor activity, reflected in the decreased corticospinal excitability, as to prevent an overt behavior in the observer. This interpretation was supported by an inverse correlation between the reported willingness to act toward the mug (on the basis of the responses to a questionnaire) and the measured corticospinal excitability in the observer's muscle. Therefore, the more the participants were willing to act on the object, the more inhibited was the activity in the muscle required to interact with it. The fact that this kind of inhibitory pattern of activity is not always measured in action observation studies could be dependent on the type of stimuli adopted. Indeed, as suggested by Naish et al. (2014), it is possible that some types of actions or observation environments are more likely to elicit action imitation compared to others, and that these conditions consequently necessitate of a suppression mechanism. Naish and colleagues (2014) also developed a model in which a description of how corticospinal excitability is modulated during action observation is proposed and that can account for the results obtained in the present study. According to their model, inhibitory processes would follow an early increase of corticospinal excitability due to action observation, in order to prevent the production of overt imitation of the observed movements. The authors propose that the inhibitory mechanism might occur either (or both) in parallel with excitatory processes, or be triggered when the level of excitation reaches a certain threshold (Naish et al., 2014). The results of my study seemingly reflect what the model from Naish et al. (2014) predicted. This inhibitory pattern of activity represents a novel result in the field of neurophysiological studies on complementary actions, in which motor facilitation was usually recorded when observing an individual expressing a social request toward the observer (see Sartori and Betti, 2015 and Sartori et al., 2013a, for reviews). Nonetheless, in those studies the actor's face was never included in the visual scene, therefore gaze direction is likely to be the source of the inhibitory modulation emerged from the study presented in Chapter 8. Even if the present results cannot provide a definitive answer on the role played by gaze direction in action observation, they suggest that the adoption of more realistic experimental paradigms is needed to get closer to the understanding of how the human brain works in real-life situations.

Overall, this experimental work provides some evidence on the role of attention in social motor preparation.

Considerations on the neural mechanisms underlying complementary actions

In terms of neural substrates supporting the ability to code for imitative behaviors and the selection of appropriate incongruent actions, Sebanz et al. (2006) suggested they could be similar neurocognitive mechanisms. Results of subsequent researches seem to suggest that the MNS could play a crucial role underlying the ability to code similar and dissimilar responses in the motor system. In particular, the interplay between strictly congruent and broadly congruent mirror neurons (see Chapter 1, par. 1.1.1 for a description) may allow to resonate with the observed action and to activate a different, but related, motor response. Different sets of mirror neurons may then be involved in the integration between observed action and the preparation of a dissimilar response to achieve a joint goal in a complementary fashion.

The involvement of the MNS in supporting social behavior has been suggested by Rizzolatti and Craighero (2004). Heyes and colleagues (e.g., Catmur et al., 2007; Heyes, 2010), instead, consider the MNS as “a product, as well as a process, of social interaction” (Heyes, 2010). Indeed, Heyes and colleagues proposed that mirror neurons may be a byproduct of associative learning, in which the repeated co-occurrence of action execution with the observation of one’s own or other’s actions bring the motor neurons to develop the visuo-motor association characteristic of mirror neurons. Accordingly, in case an observed action is constantly paired with the execution of a different action, the mirror neuron response could reflect a complementary action. This, according to the mirror neurons classification provided by Gallese and colleagues (1996), would likely reflect the properties of the above mentioned broadly-congruent mirror neurons. Ocampo and colleagues (2011) have suggested that fronto-parietal brain regions lies behind the processes of both matching perceived action into motor representation and preparing a different response on the basis of the task requirements (see also Sacheli et al., 2015). However, Kokal and colleagues (2009, 2010) proposed that the engagement of areas beyond the MNS might permit the flexible integration of our own actions to those of others, which, in turn, would allow joint interactions to take place smoothly. Going back to the working memory proposal from Sartori et al. (2013a), it is possible that while the mirror system is involved in keeping action-related information on hold, other brain areas may be engaged to extract the meaning of the observed action so as to achieve the joint goal.

At present, a complete picture of the neural underpinnings governing the preparation of complementary actions is still lacking and future research is needed to contribute to its definition. Nonetheless, the available research seems to suggest that the MNS, together with the possible involvement of areas beyond it, appear to underlie the abilities to decode actions of others and to respond to them accordingly.

9.1 Conclusive remarks

Observed actions are consistently, flexibly and dynamically simulated by the human motor system. In everyday life, the integration of different sources of information for the understanding of others' behavior occurs effortlessly. In this respect, the central advance of the present research work is manifold. I attempted to challenge the flexibility of the action observation system by probing the spontaneous tendencies to respond to observed actions calling for a social interaction. I demonstrated that the interplay between motor resonance and the preparation of non-identical appropriate responses occurs at the level of multiple effectors simultaneously. I investigated the role of spatial attention in the preparation of complementary responses revealing how in some circumstance the motor system can be impermeable to top-down factors.

Taken together, the present work could contribute to one of the goal of social neuroscience, that is to understand how the brain is able to orchestrate the kind of social interactions which constantly take place in every individual's life.

9.2 Future directions

Although the properties of the spTMS technique used here make it a very useful tool for the study of action observation in interactive contexts, future studies should aim at identifying the mechanisms underlying the processing of complementary actions and to further explore their neural basis by adopting combined methodologies. Tracking the motor excitability pattern in conjunction with the measurement of the interactive behavior, as provided by kinematics recordings, for instance, would help to better investigate *real* social interactions. Moreover, the adoption of neuroimaging techniques able to provide ecologically-valid testing environment (e.g., MEG or functional near-infrared spectroscopy - fNIRS, which allow the subject to be surrounded by other persons), should be adopted to investigate the neural underpinning of interactive behavior.

In this connection, to obtain a far more detailed (and generalizable) picture of the considered phenomenon, more ecologically valid paradigms should be adopted to determine how the motor system cope with complex forms of dynamic interactions. In particular, the adoption of *real* social interactions should be the reference standard for future studies in social neuroscience (Schilbach et al., 2013; Reader and Holmes, 2016). Innovative approaches should be developed to probe social cognition and to provide a better understanding of social interaction as it occurs in naturalistic contexts.

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PUBLICATIONS AND PRESENTATIONS ASSOCIATED WITH THE THESIS

Full peer review journal articles

Sartori, L., **Betti, S.**, Perrone, C., & Castiello, U. (2015). Congruent and incongruent corticospinal activations at the level of multiple effectors. *Journal of Cognitive Neuroscience*, *27*(10), 2063-2070.

Sartori, L., **Betti, S.**, Chinellato, E., & Castiello, U. (2015). The multiform motor cortical output: kinematic, goal and response coding. *Cortex*, *70*, 169-178.

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Submitted

Betti, S., Castiello, U., Guerra, S. & Sartori, L. Overt orienting of spatial attention and corticospinal excitability during action observation are unrelated. *PLoS one.*

Proceedings

Sartori, L., **Betti, S.**, & Castiello, U. (2015). Complementary Actions: Novel perspectives. *Cognitive Processing*, 16, S26. doi: 10.1007/s10339-015-0732-7

International academic conference presentations

Betti, S., Castiello, U. & Sartori, L. Differential role of spatial attention on motor resonance and complementary actions. European Society for Cognitive and Affective Neuroscience (ESCAN), Porto, June 23rd-26th, 2016. Invited oral presentation (Symposium).

Betti, S., Castiello, U. & Sartori, L. Attention in (Joint) Action. 2016 Meeting of the Social and Affective Neuroscience Society, New York, April 28th-30th, 2016. Poster.

Betti, S., Castiello, U. & Sartori, L. The role of spatial attention during action observation: the case of complementary actions. Cognitive Science Arena, Brixen, February 19th-20th, 2016. Oral presentation.

Sartori, L., **Betti, S.**, & Castiello, U. Complementary Actions: Novel perspectives. VI International Conference on Spatial Cognition, Rome, September 7th-11th 2015. Oral presentation.

Betti, S., Castiello, U. & Sartori, L. The role of spatial attention on motor resonance: the case of complementary actions. 6th Joint Action Meeting, Budapest, July 1st-4th, 2015. Poster.

Sartori, L., **Betti, S.** & Castiello, U. Symbolic actions modulates motor cortex excitability. 4th Nijmegen Gesture Centre Workshop, Nijmegen, June 4th-5th, 2015. Invited oral presentation.