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The neural correlates of grasping actions: observation and execution

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Table of contents

TITLE PAGE	1
TABLE OF CONTENTS	3
 CHAPTER 1	
GENERAL INTRODUCTION	
1.1 The neural underpinnings of action observation	9
1.2 Monkey AOS	10
1.2.1 Ventral premotor cortex: region F5 and mirror neurons	11
1.2.2 Inferior parietal lobule: region PF/PFG and mirror neurons	16
1.2.3 STS and biological motion responding neurons	19
1.2.4 Extending the boundary of the AOS and the ‘mirror’ system: monkey studies	21
1.3 The Human Action Observation System	25
1.3.1 Human ‘extended’ AOS	28
1.3.2 Human ‘mirror’ system	32
1.4 Summary	34
1.5 The present research	35

CHAPTER 2

GENERAL METHODS

2.1 Participants' characteristics	37
2.2 Stimuli	37
2.3 Procedures and task	38
2.4 Data acquisition	38
2.5 Pre-processing of fMRI data	38
2.6 Measures of interest	39
2.7 fMRI data analysis	40
2.8 Localization of activated brain regions	40

CHAPTER 3

INVESTIGATING THE NEURAL CIRCUIT UNDERLYING THE OBSERVATION OF GRASPING MOVEMENTS: A STUDY ON MULTIPLE SCLEROSIS PATIENTS

Abstract	43
3.1 Introduction	41
3.2 Materials and Methods	42
3.2.1 Participants	43
3.2.2 Stimuli	44
3.2.3 Procedures	45
3.2.4 Data analysis	45
3.3 Results	46
3.4 Discussion	48

CHAPTER 4

EXPLORING THE NEURAL CIRCUIT UNDERLYING THE OBSERVATION OF GRASPING MOVEMENTS: THE EFFECT OF ACTION TRANSITIVITY

Abstract	53
4.1 Introduction	54
4.2 Methods	55
4.2.1 Participants	55
4.2.2 Stimuli	55
4.2.3 Procedures and task	57
4.2.3.1 AOS localiser	57
4.2.3.2 Main experiment	58
4.2.4 Data analysis	61
4.2.4.1 AOS localiser	61
4.2.4.2 Main experiment	61
4.3 Results	61
4.3.1 AOS localiser	61
4.3.2 Main experiment	62
4.4 Discussion	63
4.4.1 Right Posterior Temporal Sulcus	63
4.4.2 Anterior Intraparietal Sulcus	65

CHAPTER 5

THE ‘MIRROR’ SYSTEM IN HUMANS

Abstract	67
5.1 Introduction	68
5.2 Materials and Methods	68
5.2.1 Participants	68
5.2.2 Apparatus	68
5.2.3 Stimuli	69
5.2.3.1 Action Observation	69
5.2.3.2 Action Execution	70
5.2.4 Procedures and task	70
5.2.4.1 Action Observation	70
5.2.4.2 Action Execution	71
5.2.5 Data analysis	71
5.2.5.1 Action Observation	72
5.2.5.2 Action Execution	72
5.2.5.3 Conjunction analysis	72
5.3 Results	73
5.3.1 Action Observation	73
5.3.2 Action Execution	74
5.3.3 Conjunction analysis	75
5.4 Discussion	76

CHAPTER 6

THE RESPONSE OF THE HUMAN VENTRAL PREMOTOR 'MIRROR' REGION TO THE TYPE OF OBSERVED AGENT

Abstract	77
6.1 Introduction	78
6.2 Materials and Methods	79
6.2.1 Participants	79
6.2.2 Stimuli	79
6.2.3 Procedures and task	80
6.2.4 Data analysis	80
6.3 Results	81
6.4 Discussion	82

CHAPTER 7

GENERAL CONCLUSIONS

7.1 Conclusive remarks	85
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ACKNOWLEDGEMENTS	89
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REFERENCES	91
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Appendix A	105
Appendix B	107
Appendix C	110
Appendix D	112

1. General introduction

1.1 The neural underpinnings of action observation

The discovery of ‘mirror’ neurons (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992), visuomotor neurons responding both to the observation and the execution of grasping action, had a great impact on the study of the motor system and on neuroscience in general. The possibility that the motor system is not merely involved in motor planning and execution, but it might play an important role in cognitive processing, led to a complete rethinking of its role within the brain.

Since the discovery of mirror neurons there has been a great deal of speculation regarding the existence of a similar system in humans and the functions it may serve. Reasonable proposals have suggested that they mediate action observation and understanding (Rizzolatti, Fogassi, & Gallese, 2001). Whereas, intriguing, but more speculative proposals suggest that they mediate imitation (Iacoboni, 2005), understanding of intention (Iacoboni et al., 2005), speech processing (Rizzolatti & Craighero, 2004), music processing and misperception of emotion in music (Gridley & Hoff, 2006, Molnar-Szakacs & Overy, 2006), empathy (Leslie, Johnson-Frey, & Grafton, 2004), cigarette addiction (Pineda & Oberman, 2006), and sexual preference (Ponseti et al., 2006). However, at present, compelling experimental evidence for the possible involvement of the ‘mirror’ system in any one of these functions is relatively weak.

The present thesis aims at investigating the functional role and the peculiar properties of the cerebral areas involved in observing actions. In the following sections,

I shall provide an overview of the neural substrates underlying action observation termed as Action Observation System (AOS) in both non-human primates and humans. Then I shall report on a series of human neuroimaging studies which have been designed as to better characterize the human AOS.

1.2 Monkey AOS

Single-cell recording studies have classically defined the AOS as comprehending three areas (Keysers & Perrett, 2004; Rizzolatti & Craighero, 2004): the superior temporal sulcus (STS) in the temporal cortex (Perrett et al., 1989; Perrett, Mistlin, Harries, & Chitty, 1990; Jellema & Perrett, 2006), area F5 in the premotor cortex (di Pellegrino et al., 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti, Fadiga, Fogassi, & Gallese, 1996a; Umiltà, et al., 2001) and area PF/PFG in the inferior parietal cortex (Gallese, Fadiga, Fogassi, & Rizzolatti, 2002; Fogassi et al., 2005; Rozzi, Ferrari, Bonini, Rizzolatti, & Fogassi, 2008; see Figure 1.1 for an anatomical localization within macaque monkey brain).

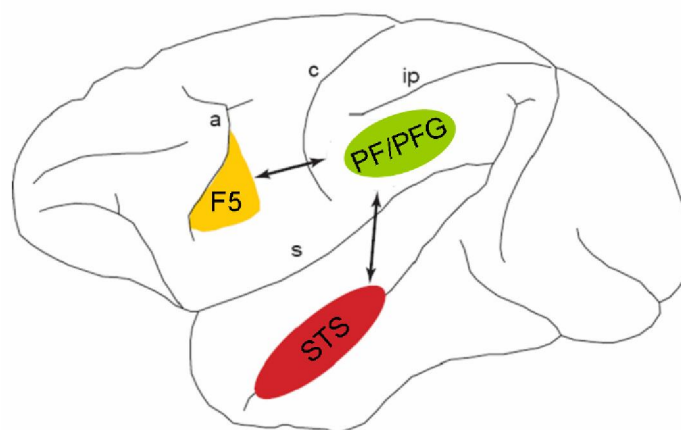


Figure 1.1. Monkeys AOS. Illustration of the location of area F5 in ventral premotor cortex, area PF/PFG of the inferior parietal lobule and the superior temporal sulcus (STS) together with their anatomical connections (arrows) shown on a lateral view of the macaque brain. Abbreviations: a, arcuate sulcus; c, central sulcus; ip, intraparietal sulcus; s, sylvian sulcus. (Modified from Keysers & Perrett, 2004).

In the next sections, I shall describe the particular features of the neurons studied within each of these areas. Then I shall introduce the latest findings demonstrating the involvement of additional areas in action observation which fall outside the classically defined AOS.

1.2.1 Ventral premotor cortex: region F5 and mirror neurons

Region F5 is located within the ventral premotor cortex (see Figures 1.1 and 1.2 for localization) and it is involved in the control of hand and/or mouth movements (Rizzolatti et al., 1988).

Firing of neurons within F5 is correlated with specific goal-related motor acts performed using either hands or the mouth, such as grasping, tearing, holding, manipulating. The execution of a simple movement involving exactly the same muscles, but for a different aim, fails to activate these neurons.

As the present thesis is related to grasping actions, I shall further describe only those neurons responding to actions performed using the hand. Within this category of motor acts, the most represented category is grasping and most neurons are selective for one type of grip: e.g., precision grip, finger prehension or whole hand prehension.

A recent study (Belmalih et al., 2009) demonstrated that region F5 comprehends three architectonically distinct subregions, probably corresponding to functionally different areas (see Figure 1.2). Two of these sub-regions are located within the inferior postarcuate bank, one more posteriorly (F5p) and the other more anteriorly (F5a). The third sub-region occupies most of the postarcuate convexity (F5c).

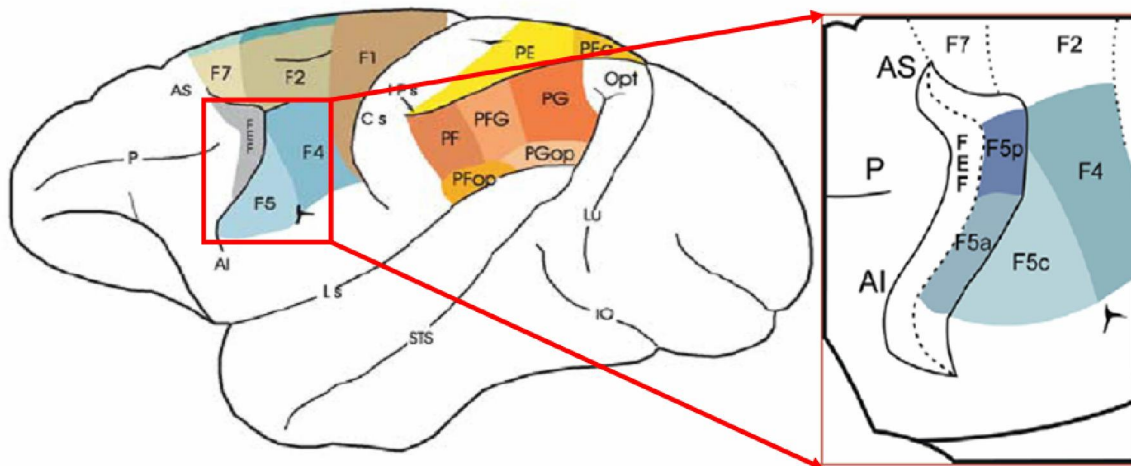


Figure 1.2. Parcellation of region F5. Cytoarchitectonic parcellation of the agranular frontal cortex (areas indicated with F and arabic numbers) and of the parietal lobe (areas indicated with P and progressive letters). The enlargement of the frontal region (rectangle on the right) shows the parcellation of area F5 buried within the arcuate sulcus. Abbreviations: intraparietal sulcus. AIP, anterior intraparietal area; IP, intraparietal sulcus; LIP, lateral intraparietal area; MIP, medial intraparietal area; POs, parieto-occipital sulcus; As, superior arcuate sulcus; Ai inferior arcuate sulcus; C, central sulcus; Ca, calcarine fissure; CG, cingulate cortex; FEF, frontal eye field; L, lateral sulcus; Lu, lunate sulcus; P, principal sulcus; STS, superior temporal sulcus. (Modified from Rizzolatti & Fabbri-Destro, 2008).

Neurons within all the sub-regions of F5 share the same motor properties, but some of them also show visual properties. Up to now, two categories of visuo-motor neurons have been described in F5:

- (i) ‘canonical’ neurons, responding to the sight of graspable objects, mainly present in area F5p (see Figure 1.2; Murata et al., 1997; Raos, Umiltá, Murata, Fogassi & Gallese, 2006). Please note that given the focus of the present thesis on action observation ‘canonical’ neurons will not be discussed at great length;
- (ii) ‘mirror’ neurons, which are mainly reported in area F5c (see Figure 1.2, di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a; Umiltà et al., 2001). The peculiarity of mirror neurons is that they discharge both when the monkey performs a specific goal-directed hand action and when it observes another monkey (or the experimenter) performing the same or a very similar action (see Figure 1.3). It has been proposed that these neurons may match the properties of the observed action with the representation of the motor acts within the observer’s

premotor cortex. For this reason, they were considered as the first evidence of a system subtending an action observation/execution matching system (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a).

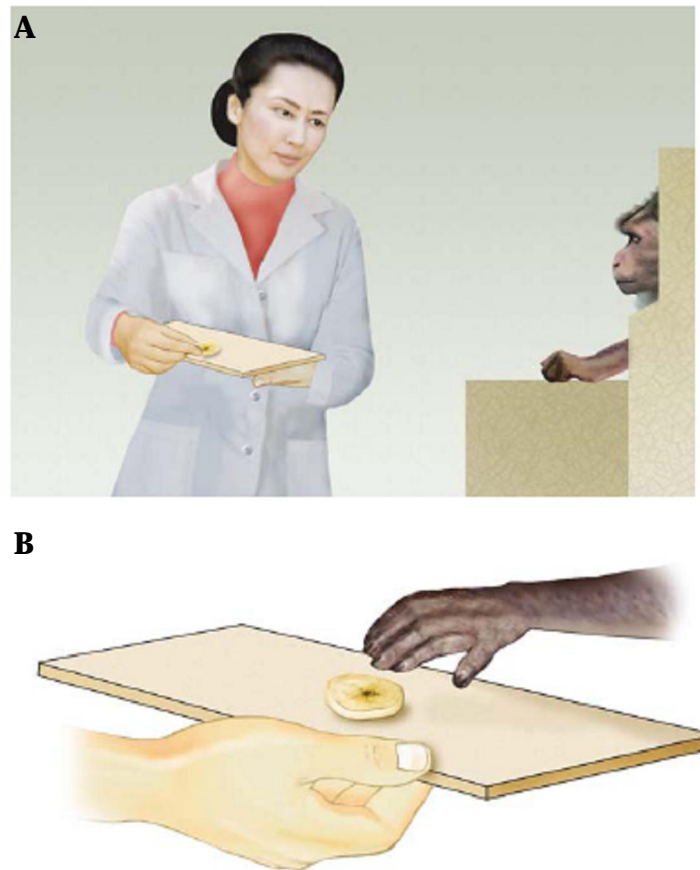


Figure 1.3. Visual and motor responses of a mirror neuron in area F5. The panels represent the experimental conditions triggering activation in mirror neurons. A) The neuron discharges during observation of a grasping movement done by the experimenter and B) during monkey grasping movements. (Modified from Fabbri-Destro & Rizzolatti, 2008).

Mirror neurons show different levels of congruence between the properties of the representation of the executed and the observed actions. In some cases (i.e. ‘strictly congruent’ neurons), a response is elicited while executing a specific motor act and while observing only the same action both in terms of goal (e.g. grasping) and means (e.g. precision grip). In other cases (i.e. ‘broadly congruent’ neurons), cells are active when the observed action possesses the same goal (e.g. grasping) achieved either using the same or a different mean (e.g. both precision grip and whole hand prehension). This

seems to suggest that there are two levels of possible matching between the observed and the executed action: one linked to both the goal and the means of the observed action; the other, more abstract, based only on the goal.

In order to have a better comprehension of the role mirror neurons might play some of their features have to be described. These cells are not active when the action is executed in absence of an object (i.e. intransitive actions) or during the mere visual presentation of an object (as ‘canonical neurons’). This means that only object-related actions (i.e. transitive actions) trigger a discharge within these neurons.

Strong evidence in favour of the claim that mirror neurons are involved in action understanding came from a study by Umiltà et al. (2001). These authors adopted an elegant experimental design in order to investigate the properties of the representation of the observed actions. The procedure took advantage of the fact that mirror neurons respond only during the observation of transitive actions, but not during the observation of intransitive ones.

The neurons were tested in two basic conditions (see Figure 1.4). In the first condition, the monkey was shown a fully visible object-related action (see Figure 1.4A, ‘full vision grasping’ condition). In the second condition, the monkey saw the same action but with its final part hidden (see Figure 1.4C, ‘hidden grasping’ condition). In these ‘hidden’ trials, the experimenter performed a reach-to-grasp movement towards an object placed behind a screen. At the beginning of the trial the object was fully visible, then was covered by a screen and subsequently grasped by the experimenter.

All mirror neurons responded to the observation of a visible grasping action (‘full vision grasping’ see Figure 1.4A) and some of them also responded when the final part of the action was hidden, but the monkey knew that an object was present behind the partition (‘hidden grasping’ see Figure 1.4C). As already reported, the observation of a

fully visible intransitive action ('full vision pantomime' see Figure 1.4B) did not activate the neuron. Finally, the same neuron did not discharge when observing a mimed action even when the last part of the action was hidden ('hidden pantomime' see Figure 1.4D). This last control condition strongly supports the claim that mirror neurons might be responsible for understanding the meaning of an action, allowing the discrimination between transitive and intransitive actions simply relying on previous information provided by the environment.

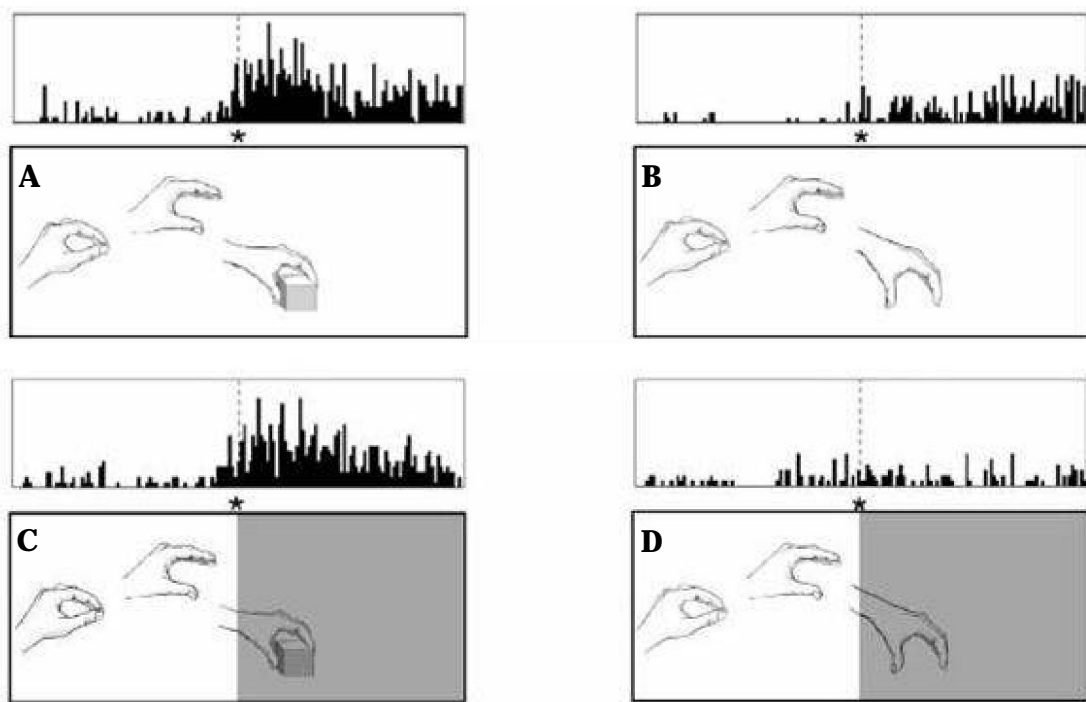


Figure 1.4. Response of a mirror neuron in the four conditions of the study by Umiltà & colleagues (2001). Mirror neuron responses to action observation in full vision (A and B) and in hidden condition (C and D). The upper panel shows the discharge of the neuron. The lower part of each panel illustrates schematically the experimenter's action as observed from the monkey's vantage point. The asterisk indicates the location of a stationary marker attached to the frame. For the hidden conditions the experimenter's hand started to disappear from the monkey's vision when crossing this marker. Histograms are aligned with the moment at which the experimenter's hand was closest to the marker. Histograms bin width = 20 ms. The ordinate is in spike/s. (Modified from Umiltà et al. 2001).

This classical description of mirror neurons should end reporting that they discharge only when a biological effector (e.g. the hand) interacts with an object; if the actor uses a tool (e.g. a plier) the neuron does not discharge. But, a recent study by Ferrari, Rozzi & Fogassi (2005) demonstrated that some mirror neurons respond during the

observation of actions performed using tools (e.g. using a plier to take an object). This was evident only after a long exposure to the observation of the experimenter using a tool. The motor response of such cells was related to the execution of grasping movements, because the monkey can not utilize the tool. These ‘tool-responding’ mirror neurons seem to show that the representation of observed actions might be modified according to the experience of the monkeys and may incorporate actions which lie outside the motor repertoire of the monkey. In this case, the proposed matching mechanism seems to work only in relation to the goal of the action, because the means by which this goal is achieved are completely different.

1.2.2 Inferior parietal lobule: region PF/PFG and mirror neurons

Mirror neurons were later discovered in a region of the inferior parietal lobule, area PF/PFG (see Figure 1.1 for localization). A recent study (Rozzi et al., 2008) investigated in details the distribution and properties of the neurons within the subdivisions of the inferior parietal lobule in macaque (PF, PFG, PG). Each subdivision is characterized by the presence of neurons with specific motor and sensory properties.

For what concerns mirror neurons, they have been found mainly in area PF/PFG and their general features appear to be very similar to those found within the ventral premotor cortex. In particular, the majority of these cells present the same pattern of response as the neurons in F5c. They discharge both for action execution and action observation. As in F5c there are PF/PFG mirror neurons for hand and mouth action and there are strictly and broadly congruent neurons.

Fogassi et al. (2005) investigated neurons with motor properties and mirror neurons of area PF/PFG. The neurons recorded from these areas were tested in two main conditions (see Figure 1.5). In the first condition, the animal performed a reach-to-grasp

movement towards an object located in front of it (see Figure 1.5, condition 1) and brought it to its mouth (see Figure 1.5, condition 2A). In the second condition, the monkey reached-to-grasp an object (see Figure 1.5, condition 1) and subsequently placed it into a container (see Figure 1.5, condition 2B).

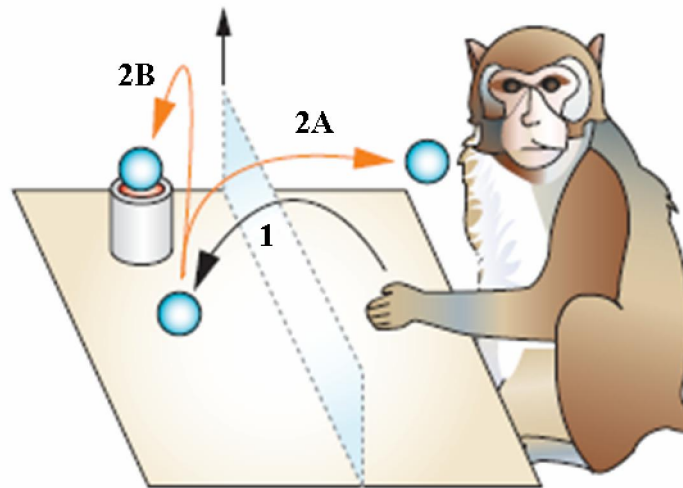


Figure 1.5. Action-constrained neurons in the monkey IPL. Apparatus and paradigm used for a task designed to demonstrate action-constrained neurons. The monkey starts from the same position in all trials, reaches for an object (1) and brings it to the mouth (2A) or places it into a container (2B). (Modified from Rizzolatti, Fabbri-Destro & Cattaneo, 2009).

The results showed that the majority of the recorded neurons discharged during the initial grasping action with a different intensity according to the final goal of the action (e.g. eating or placing, see Figure 1.6). These cells were defined ‘action-constrained’ neurons, because the same action (i.e. grasping) did elicit or not a response depending on the motor act (e.g. eating or placing) in which it was embedded.

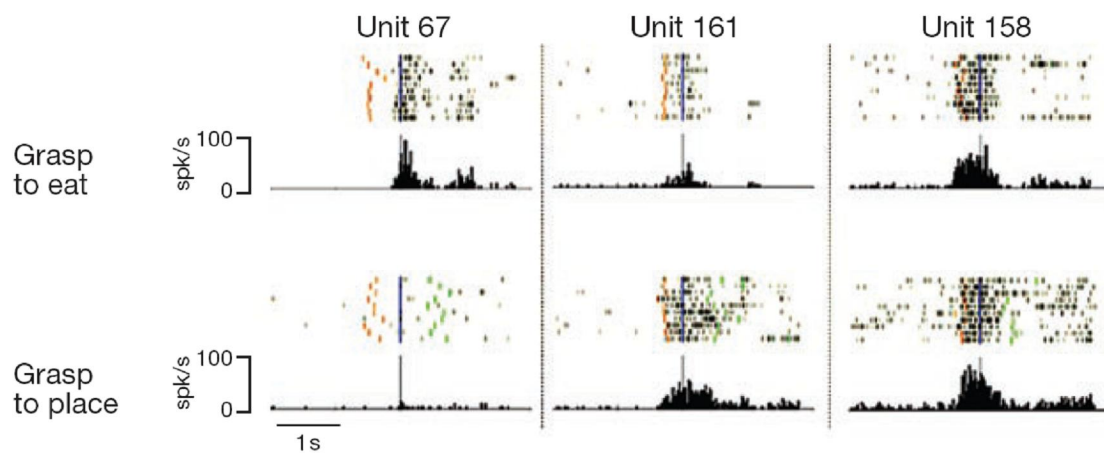


Figure 1.6. Example of motor properties of PF/PFG neurons. Activity of three PFG neurons during the motor task in conditions 2a (grasp to place) and 2b (grasp to eat). Raster histograms are synchronized with the moment at which the monkey touched the object to be grasped. Unit 67 fires during grasping to eat and not during grasping to place. Unit 161 is selective for grasping to place. Unit 158 does not show any task preference. (Modified from Rizzolatti, Fabbri-Destro & Cattaneo, 2009).

Most of these ‘action-constrained’ neurons have mirror properties. Almost all of them selectively discharge when the monkey observes motor acts embedded in a specific action (e.g. grasp-to-eat but not grasp-to-place, see Figure 1.7).

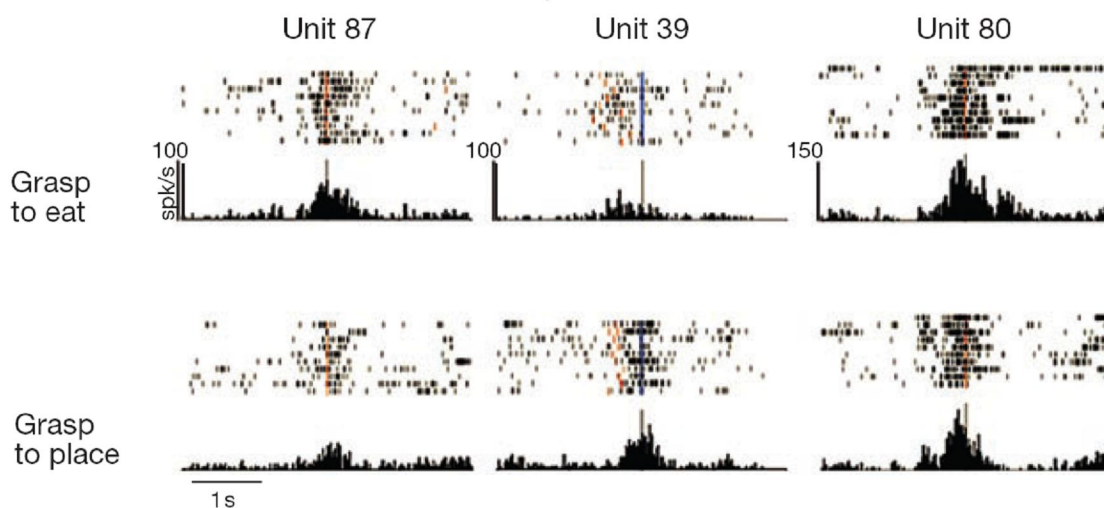


Figure 1.7. Example of visual properties of PF/PFG mirror neurons. Visual responses of PFG mirror neurons during the observation of grasping to eat and grasping to place performed by an experimenter. Unit 87 is selective for grasping to eat, unit 39 is selective for grasping to place and unit 80 does not display any task preference. (Modified from Rizzolatti, Fabbri-Destro & Cattaneo, 2009).

The activation of action-constrained mirror neurons seems to code not only a generic grasp movement, but specific grasps such as ‘grasping for eating’ or ‘grasping

for placing' (see Figure 1.17). This coding implies that when the monkey observes a grasping action done by another it is able to understand, and maybe predict, what the individual's next motor act will be on the basis of the information provided by the environment. To conclude, the animal is able to comprehend the 'motor' intentions behind the observed motor act based on environmental cues (e.g. presence of the container in the grasping-to-place condition).

1.2.3 STS and biological motion responding neurons

Single cell recording studies within STS reported on neural populations having complex response properties (see Figure 1.1 for localization). These experiments revealed response to visual information related to the shape and posture of the fingers, hands, arms, legs and torso. In addition face-selective-neurons responding to facial details such as the shape of the mouth and the direction of gaze were also found (Desimone, Albright, Gross, & Bruce, 1984; Wachsmuth, Oram, & Perrett, 1994; Perrett et al., 1989, 1990; Jellema, Backer, Wicker, & Perrett, 2000; Jellema & Perrett, 2006).

Of particular interest for the purpose of this thesis are the STS neurons responding to different type of goal-directed hand action, such as reaching for grasping, tearing and manipulating objects (Perrett et al. 1989, 1990; Jellema et al., 2000; Jellema & Perrett, 2006). For instance, some cells discharge to the sight of object-related grasping action, but not to a pantomime of the same action, or if there is no interaction between the hand and the object. In this case, the neurons do not respond even when the pantomime is observed nearby the object. These neurons are also sensitive to the form of the effector performing the grasping action, not responding to the sight of a tool used to execute a similar action (Perrett et al. 1989, 1990; Jellema et al., 2000; Jellema & Perrett, 2006).

This sensitivity of STS cells to the relation between the biological effector and the object allow these cells to detect causal relations (Perrett et al., 1989).

Recently, Barraclough, Keith, Xiao, Oram, & Perrett (2009) reported an interesting property of some cells responding to the observation of grasping and placing actions. The response of these neurons was modulated by transitivity, i.e. the presence of an object as the target of the action. These neurons discharge during transitive actions, but they also respond, but to a lesser extent, during the observation of the pantomime for the same action (intransitive) (see Figure 1.8).

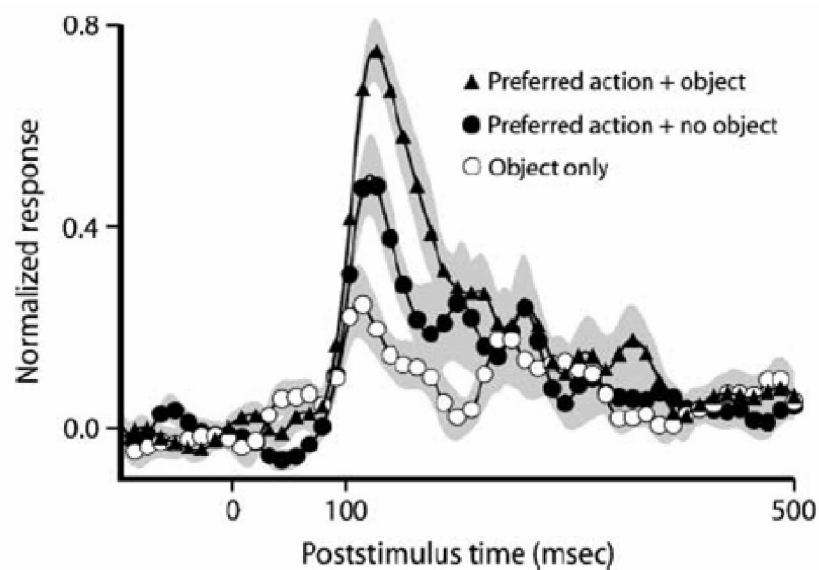


Figure 1.8. Response of action-selective STS neurons. Responses of the average cell to the preferred action with and without the presence of the object, and when the object is presented alone. SDFs (grey = SEM) is presented alone averaged across cells. SDFs (grey = SEM) from responses of 15 cells, of which 4 preferred grasping actions and 11 preferred placing actions. (Modified from Barraclough et al., 2009)

A final consideration on STS cells is that, although extensively tested, they do not discharge during action execution of any kind (Keysers & Perrett, 2004).

1.2.4 Extending the boundary of the AOS and the ‘mirror’ system: monkey studies

Recent neuroimaging evidence has indicated that other areas, outside the three AOS core regions (F5c, PF/PFG, STS), may be part of an ‘extended’ AOS comprehending prefrontal, premotor, motor, somatosensory and posterior parietal cortices (Raos, Evangeliou, & Savaki, 2004, 2007; Nelissen, Luppino, Vanduffel, Rizzolatti, & Orban, 2005; Evangeliou, Raos, Galletti, & Savaki, 2009).

Nelissen et al. (2005) used fMRI to map the activation of the anterior part of the frontal lobe during action observation, and in particular of the regions near the arcuate sulcus. In addition to F5, other regions involved in action observation were revealed (areas 45 and 46).

Furthermore these authors applied a region of interest (ROI) analysis to four regions which were found significantly activated at the level of whole brain analysis. The four regions were part of area 45, namely area 45B, and the three subregions of F5: F5c, F5p and F5a (see Figure 1.9A for activation and 1.9B for localization).

Interestingly area F5c, in which mirror neurons are usually found, responded only when an individual grasped an object, whereas merely seeing a hand alone (detached from a body) grasping an object did not elicit a response.

The authors suggested that the view of the agent performing the action is necessary to trigger a response in this area. However, the other three areas 45B, F5p and F5a, responded both to the observation of a hand alone or of an entire person grasping an object.

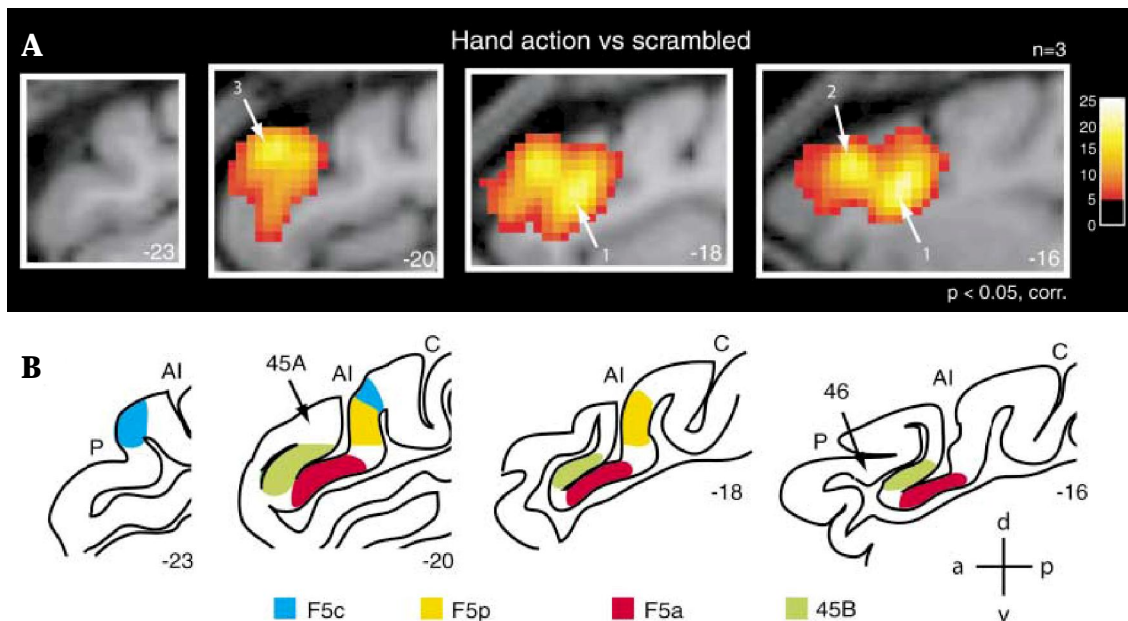


Figure 1.9. Activation of the areas near the arcuate sulcus and regions of interest. A) SPMs plotting voxels (coloured red to yellow), which were significantly ($P < 0.05$, corrected for multiple comparisons) more active when the monkeys viewed actions of the isolated hand compared with viewing their scrambled counterpart (in the group of three monkeys (M1, M3, M5) superimposed on sagittal sections through the left hemisphere of M3, at levels ranging from -23 to -16). B) Schematic representation of the four ROIs on four sagittal sections through M3's brain at same levels as in A) and B). P, principal sulcus; AI, arcuate sulcus inferior ramus; C, central sulcus; d, dorsal; v, ventral; a, anterior; and p, posterior. In (A), arrows point to local maxima in the arcuate sulcus (arrow 1), area 46 (arrow 2); and 45A (arrow 3). (Modified from Nelissen et al., 2005).

Area F5 seemed to host two different representations of hand action: the first was more related to the agent acting, requiring visual or context-dependent information; the second was more abstract and required less visual detailed or context-dependent properties. This brought to the conclusion that different representations of action, with different functions, were stored within frontal areas.

Recent studies (Raos et al., 2004, 2007; Evangelidou et al., 2009), using the deoxyglucose quantitative autoradiographic method, investigated the role of motor and parietal cortices in hand grasping execution and observation. The main results of these studies (see Figures 1.10 and 1.11) were that for action observation (in green), and not only for action execution (in red, see Figures 1.10 and 1.11), widespread activation was present within motor and parietal regions. Furthermore, a close inspection to the results concerned with the frontal cortex (see Figure 1.10), permits to observe a widespread

recruitment of regions within premotor and motor cortex for action observation. A finding which supports and extends the finding by Nelissen et al. (2005). In addition, the approach adopted in these studies allowed the authors (Raos et al., 2007) to define ‘mirror’ areas, as regions recruited both for observation and execution of action (in yellow, see Figures 1.10 and 1.11). An interesting result is that overlapping activity for action observation and execution (in yellow in Figure 1.10) was not limited to ‘mirror’ area F5c, but comprehended the entire F5, and other premotor areas such as dorsal premotor cortex (F2) and primary motor cortex (F1).

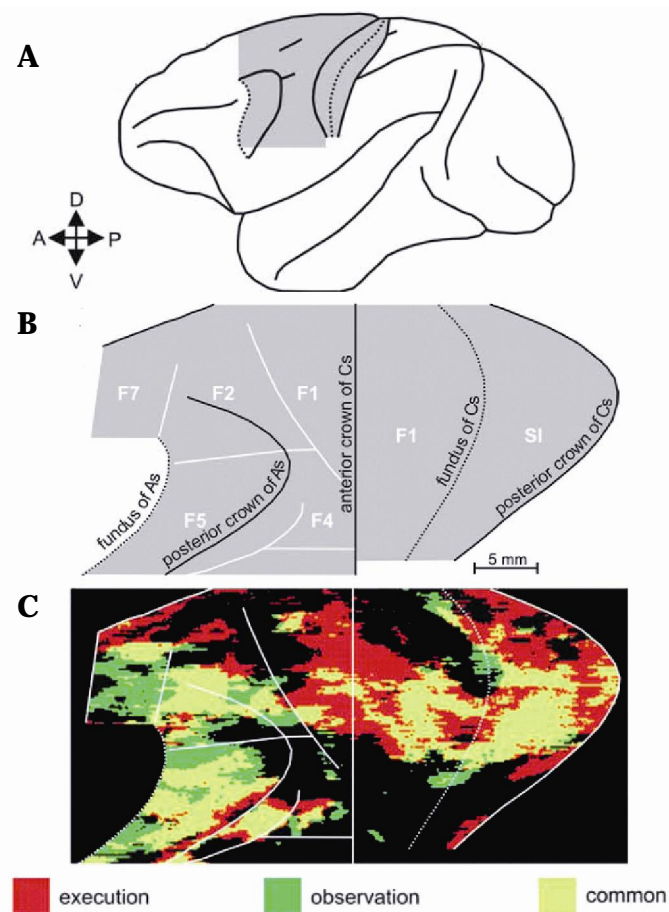


Figure 1.10. Quantitative 2D maps of metabolic activity in the lateral-frontal cortex. A) Lateral view of a monkey brain with the central sulcus (Cs) and the posterior bank of the arcuate sulcus (As) unfolded. Dotted lines depict the fundus of the As and that of the Cs. Shaded area indicates the reconstructed cortex. Abbreviations: A, Anterior; D, dorsal; P, posterior; V, ventral. B) Schematic illustration of the geometrically normalized reconstructed cortical field. Black lines correspond to surface landmarks and white lines to cytoarchitectonically identified borders of the labeled cortical areas. C) Red and green represent activations induced by grasping execution and grasping observation, respectively. Yellow stands for activations induced by both execution and observation of the same action. White lines correspond to the surface landmarks and the cytoarchitectonic borders illustrated in B). (Modified from Raos et al., 2007).

With respect to the parietal cortex, Evangeliou et al. (2009) demonstrated a widespread involvement of the superior and the inferior parietal lobuli and intraparietal cortex during action observation (see Figure 1.11). Results regarding ‘mirror’ activity seem to point to the direction of a network of parietal regions recruited for both action perception and action execution, including the ‘mirror’ region PF/PFG discovered using single cell recording. As evident in Figure 1.11, there is a widespread ‘mirror’ activity within the intraparietal cortex, but also in the superior and inferior parietal lobuli.

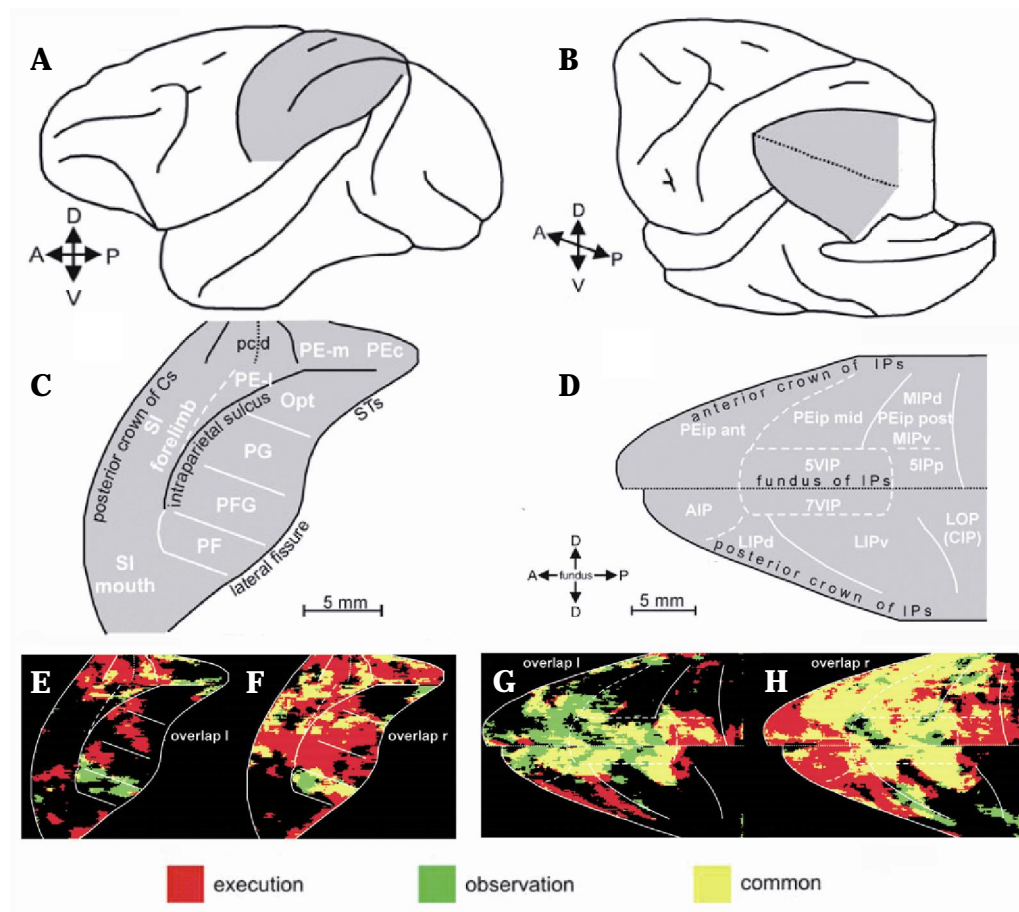


Figure 1.11. Quantitative 2D-maps of metabolic activity in the parietal cortex. A) Lateral view of the of a monkey brain. Shaded area indicates the reconstructed cortex around the intraparietal sulcus (IPs), surrounded by the central (Cs) and superior temporal sulci (STs) and the lateral fissure. B) Postero-lateral view of the partially dissected hemisphere of a monkey brain. Shaded area represents the reconstructed medial and lateral banks of the intraparietal cortex. Abbreviations: A, anterior; D, dorsal; P, posterior; V, ventral. C-D) Schematic representation of the geometrically normalized reconstructed cortical field. Black lines correspond to surface landmarks, solid white lines to cytoarchitectonically identified borders of the labeled cortical areas. E-H) Red and green represent activations induced by action execution and action observation, respectively. Yellow stands for activations induced by both execution and observation of the same action. (Modified from Evangeliou et al., 2009).

In conclusion, a widespread network of parietal and frontal regions, that we can term ‘extended’ AOS, is recruited during action observation. A subset of these areas seems to be recruited for both action observation and execution. These ‘mirror’ regions are conceptually different from mirror neurons, because overlapping activity found in neuroimaging studies does not imply necessarily the presence of neurons with visuomotor properties within a region. It might well be that two separate populations of neurons, one visual and one motor are intermingled within such area. Another possibility is that a neural population, or more than one, might be responsible for coding a common process related to both behaviours.

1.3 The Human Action Observation System

The use of neuroimaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) has provided the possibility to map the cortical areas involved in action observation in humans. The first two neuroimaging studies which investigated the areas involved in the observation of hand grasping, were conducted by Rizzolatti’s and Grafton’s groups (Rizzolatti et al., 1996b; Grafton, Arbib, Fadiga, & Rizzolatti, 1996).

In the first study, Rizzolatti et al. (1996b) used PET to localize brain regions that are active during the observation and execution of grasping movements. Subjects were tested under three conditions: in the first condition they observed grasping movements of common objects performed by the experimenter; in the second condition, they reached and grasped the same objects. These two conditions were compared with a third condition consisting in object observation. The contrast between action observation and object observation revealed that grasp observation activated the left superior temporal sulcus and the left inferior frontal gyrus.

In a second PET study, Grafton et al. (1996) investigated the areas involved in observation and imagination of grasping movements. In a condition, subjects saw precision grasping of common objects done by the experimenter. Whereas in a second condition, subjects imagined themselves grasping the objects without performing any movement. A third condition consisted in object observation. The contrast between grasping observation and object observation confirmed the results by Rizzolatti et al. (1996b). The authors found activation within the left inferior frontal gyrus and the superior temporal sulcus, but also within the left and right posterior inferior parietal areas and the right premotor cortex. To sum up, these two studies revealed regions in humans which have an analogous distribution and similar properties as those described in monkeys.

The role of each area involved in action observation has been subsequently investigated in a series of fMRI studies looking at differential loci of activation depending by the type of observed moving effector (e.g., Buccino et al., 2001; Pelphrey, Morris, Michelich, Allison, & McCarthy, 2005).

The observation of body part movements (transitive and intransitive actions) activated the premotor cortex in a somatotopically fashion (Buccino et al., 2001). That is, different areas coded actions executed by different effectors (i.e. hand, mouth, leg), following the classical motor organization of Penfield's Homunculus (see Figure 1.12A, B, C, D).

Furthermore, seeing an object-related action activates also the posterior parietal cortex following a somatotopic organization (see Figure 1.12Ab, Bb, Cb, Db). In general it was proposed that the mirror system in humans is not restricted to mouth (A) and hand (B) actions, but includes also foot (C). Interestingly, the present results seem

to demonstrate a peculiar property of the human AOS, or at least part of it: the response to intransitive actions (see Figure 1.12Aa, 1.12Ba, 1.12Ca, 1.12Da).

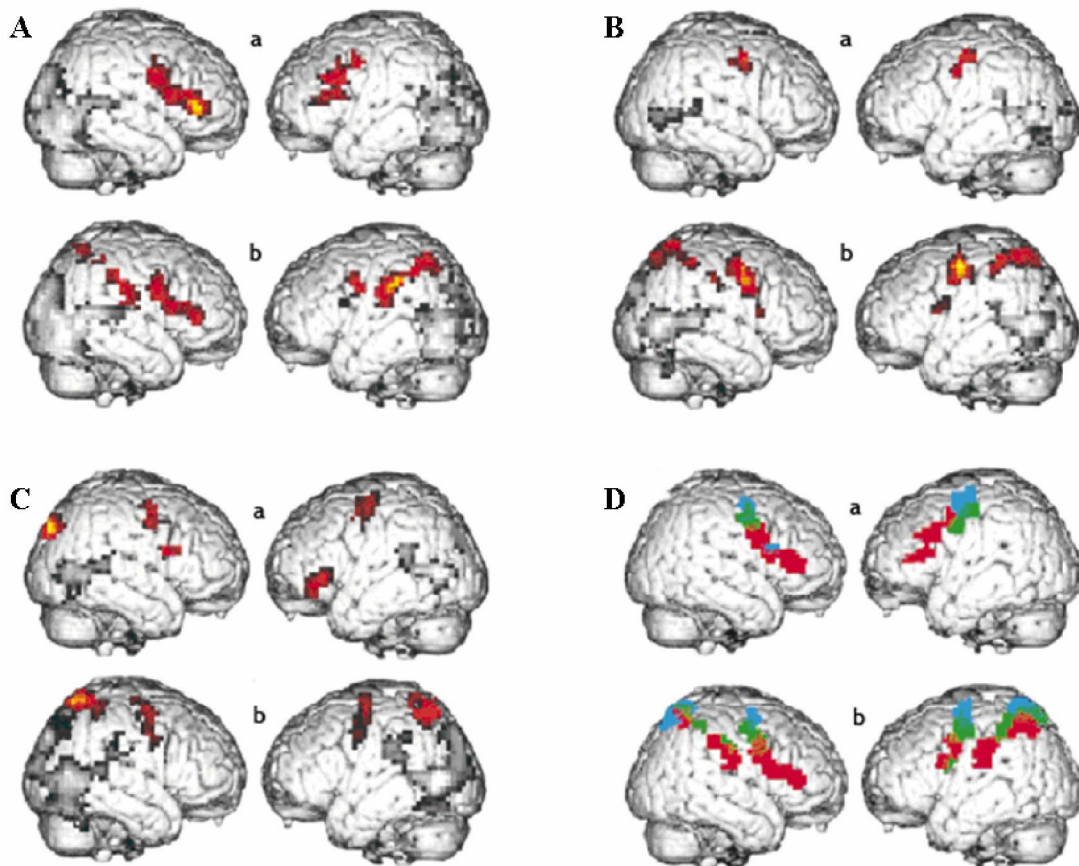


Figure 1.12. Somatotopic representation of observed actions. A) Observation of mouth actions. Projections of the activation foci on the lateral surface of a standard brain [Montreal Neurological Institute (MNI)] during the observation of non-object-related (chewing: a) and object-related (biting an apple: b) mouth actions. B) Observation of hand actions. Projections of the activation foci on the lateral surface of a standard brain (MNI) during the observation of non-object related (mimicking grasping of a cup or a ball, without object: a) and object-related (grasping a cup or a ball: b) hand actions. C) Observation of foot actions. Projections of the activation foci on the lateral surface of a standard brain (MNI) during the observation of non-object related (mimicking kicking a ball or pushing a brake, without the object: a) and during the observation of object-related foot actions (kicking a ball or pushing a brake: b) foot actions. D) Somatotopy of premotor and parietal cortices as revealed by action observation. a) Observation of non-object-related actions. b) Observation of object-related actions. Activation foci, shown in detail in the first three part of the Figure (1.12A,1.12B,1.12C) are projected on the lateral surface of a standard brain (Figure 1.12D). Red activation corresponds to the observation of mouth movements; green activation corresponds to the observation of hand movements; blue, activation corresponds to the observation of foot movements. Overlap of colours indicates activation foci present during observation of actions made by different effectors. (Modified from Buccino et al., 2001).

More recently Pelphrey et al. (2005), tested the hypothesis that also in the STS region there was a somatotopically representations of observed movements performed with different body parts: eyes (red in Figure 1.13), mouth (blue in Figure 1.13) and hand (green in Figure 1.13). As found for the brain areas targeted by Buccino et al.

(2001), it was revealed that different sectors of the STS region codes biological motion in an effector dependent manner (see Figure 1.13).

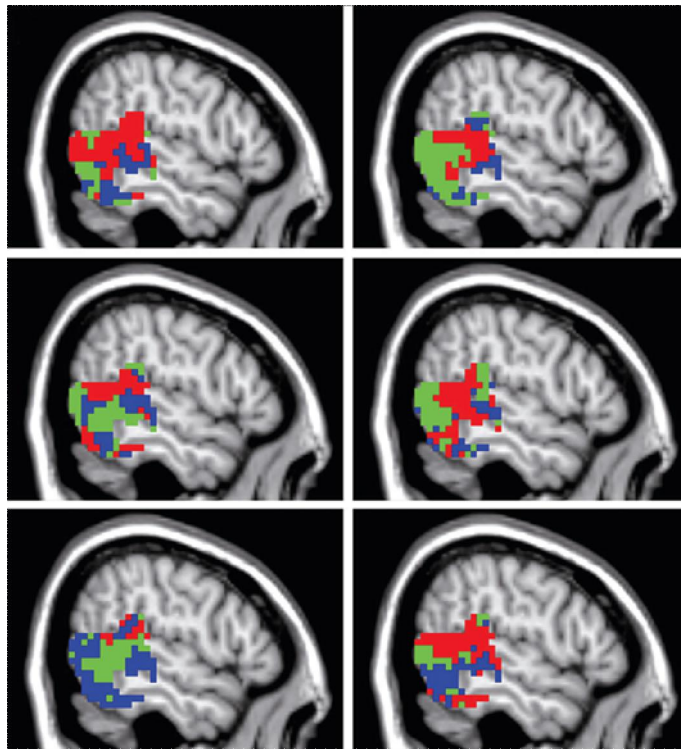


FIGURE 1.13. Greatest response topography maps in posterior superior temporal sulcus for six single subjects. Each panel represent a single subject. Red voxels exhibited the greatest response to Eyes, blue voxels to Mouth, and green voxels to Hand . (Modified from Pelphrey et al., 2005).

To summarize, an action observation system seems to be present also in humans and it is composed by at least three groups of areas: a) the superior temporal sulcus, b) the ventral precentral cortex and the inferior frontal gyrus, c) the posterior parietal cortex. These regions resemble the three ‘core’ components of the monkey AOS, supporting a possible homology between the system for the two species.

1.3.1 Human ‘extended’ AOS

A common result stemming from recent fMRI neuroimaging studies (Brass & Heyes, 2005) is the involvement of areas during action observation which exceeds the above mentioned ‘core’ regions. These data point to the direction of a human ‘extended’

AOS, similar to that uncovered in monkeys, comprehending frontal and parietal regions. This ‘extended’ AOS includes the following regions: inferior frontal gyrus, ventral and dorsal premotor cortex, posterior parietal cortex (superior, inferior and intraparietal cortices) and the superior temporal sulcus (see Figure 1.14).

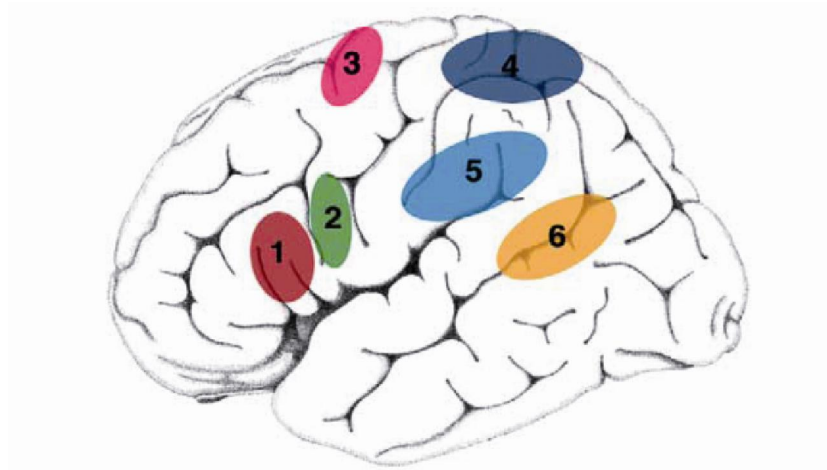


Figure 1.14. Human ‘extended’ AOS. Areas of activation during movement observation. A schematic, lateral view of the human cortex showing areas that have consistently been found to be active during passive observation of biological motion. (1) the pars triangularis and the pars opercularis of the inferior frontal gyrus, (2) the ventral premotor cortex, (3) the dorsal premotor cortex, (4) the superior parietal lobule and the intraparietal cortex (5) the inferior parietal cortex, and (6) the posterior superior temporal sulcus. (Modified from Brass & Heyes 2005).

A recent series of fMRI studies (Hamilton & Grafton, 2006, 2007, 2008) adopting ‘repetition suppression’ technique has begun to widen our knowledge regarding the precise role of the different areas characterizing the AOS.

Repetition suppression (RS), also known as ‘fMRI-adaptation’, is based on a trial by trial reduction of a physiologic response to repeated stimuli. Repetition of a stimulus often results in a reduction of signal within the brain areas that encode that stimulus. Suppression occurs when two successive stimuli are represented within the same neural population, and release from suppression occurs when two successive stimuli are represented in different populations. Thus, the method assumes the existence of population coding within brain regions, for which there is extensive evidence in many parts of the cortex. In summary, RS effects allow to demonstrate regions selective for

certain properties of the stimuli or to identify changes within a class of stimuli rather than between classes of stimuli.

Hamilton & Grafton (2006, 2007, 2008) used this method to identify possible topologies in the AOS corresponding to different features of a simple hand-object grasping actions. In Figure 1.15, there is a schematic representation of video-clips used to test the properties of the AOS in the three studies (see Figure 1.15B, 1.15C, 1.15D) and of the predicted response and RS effects (see Figure 1.15A, 1.15E).

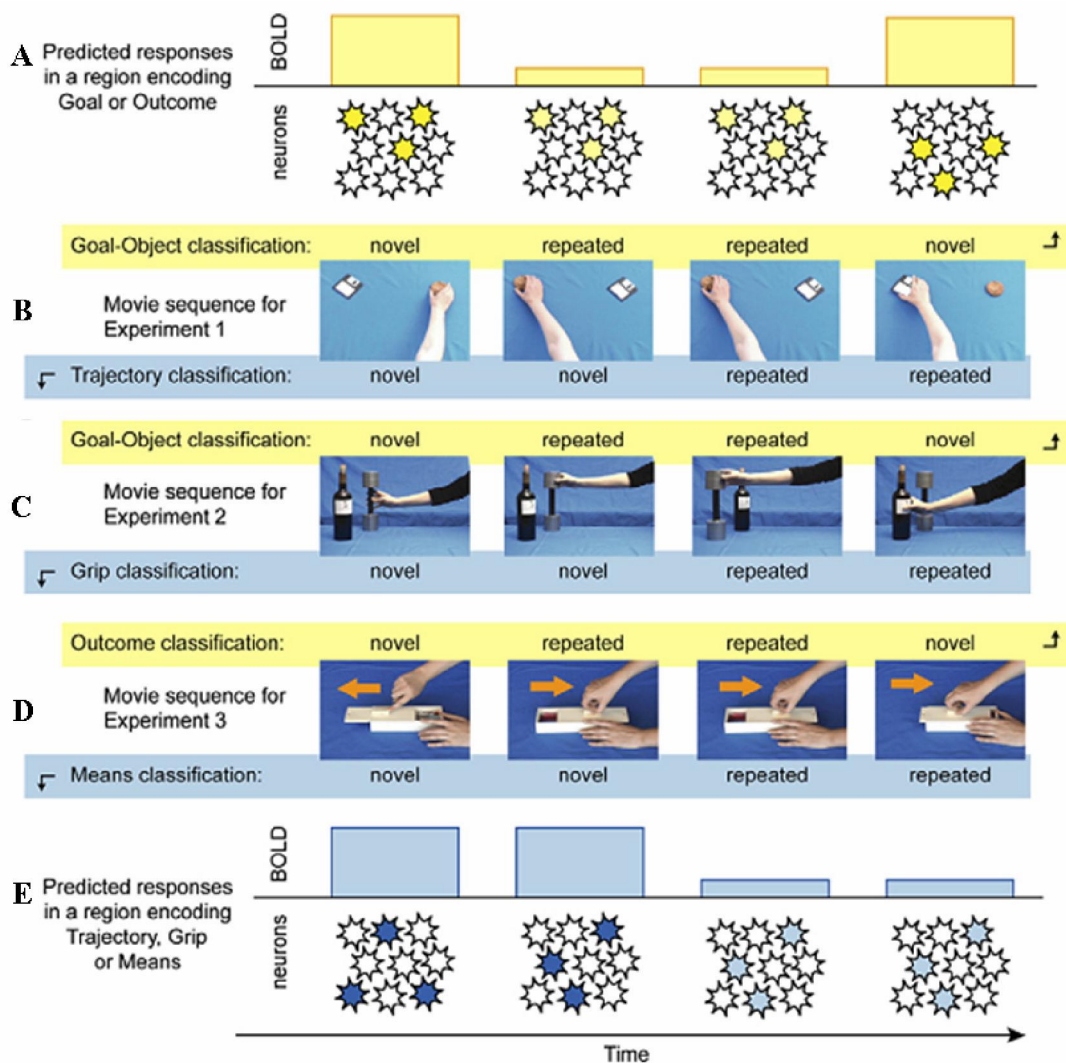


Figure 1.15. Stimuli and expected RS effects in the three experiments by Hamilton & Grafton (2006, 2007, 2008). In a given sequence, when a stimulus of a specific class, such as an object, is repeated (Row B), there is reduction of fMRI related responses (Row A). Similar effects apply for different types of objects (Row C). The prediction is that different areas will show RS effects for outcomes of actions (Row D) and these can be separated from RS effect (Row E) of lower level features such as trajectory, grip, and means (respectively Rows B, C, D). (Modified from Grafton & Hamilton, 2007).

The results of these three studies (Hamilton & Grafton, 2006, 2007, 2008) point to the direction of a distributed network of areas within the AOS devoted to processing different features of the observed actions. Specifically, selective processing within the AOS has been identified for basic perceptual features indicating ‘how’ an observed action has been performed (e.g., kinematics, type of grip, trajectory) and for its goal (i.e., ‘what’ has been observed).

In details, it has been demonstrated that in terms of how an observed grasping action is performed, the coding of its basic kinematic features (e.g., movement trajectories) might occur within the lateral occipital and the superior precentral sulci (see Figure 1.16, experiment 1). Whereas, the trajectory assumed by the observed hand during reaching appears to be coded within the inferior and the middle occipital cortices, together with the middle intraparietal cortex, the supplementary motor area, the middle frontal gyrus and the inferior frontal gyrus (see Figure 1.16, experiment 2). In terms of the goal subtending the observed action the suggestion is that the locus of such processing varies depending on the level of goal complexity. Sensitivity to the goal of a ‘simple’ action (e.g., grasping a cookie) has been identified mainly within the left intraparietal cortex, but also to a lesser extent within the right intraparietal cortex and left inferior frontal gyrus (Hamilton & Grafton, 2006, 2007 see Figure 1.16, Experiments 1 and 2). Sensitivity to the goal of more complex actions, which implies the modification of the state of an object in order to obtain a change in the environment (i.e., an outcome), has been associated with activity within the bilateral intraparietal cortex and the inferior frontal gyrus, particularly within the right hemisphere (Hamilton & Grafton, 2008, see Figure 1.16, Experiment 3).

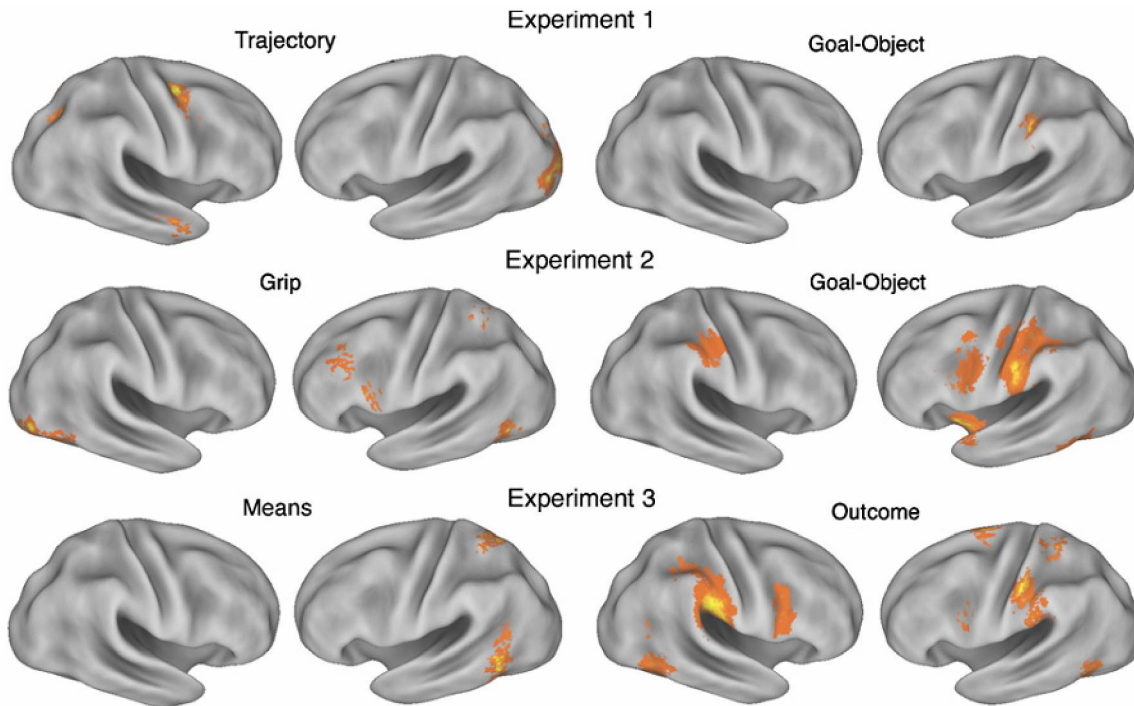


Figure 1.16. AOS regions showing sensitivity for different properties of the observed actions. Data are adapted from three separate RS experiments (Hamilton & Grafton, 2006, 2007, 2008). Repetition suppression (RS) effects for low-level kinematics, such as hand trajectory, grip size and object movement are localized within the visual association cortex including the inferior occipital cortex and the posterior superior parietal cortex (parietal reach region). RS effects for object-centred goals strongly modulate the anterior intraparietal sulcus and the left ventral premotor cortex. Sensitivity to the outcome of an action overlaps to a certain degree with goal-object areas, but also strongly modulates activity within the bilateral inferior parietal lobule and the right ventral premotor cortex. (Modified from Grafton & Hamilton, 2007).

Altogether these findings resemble those by Raos and Evangeliou (Raos et al., 2004, 2007; Evangeliou et al., 2009), showing a widespread network of frontal and parietal regions recruited for action observation. In addition, these studies demonstrate a distributed processing of the basic motor features and of the goal of an observed action within different regions of the AOS, supporting a different role for different parts of the system.

1.3.2 Human ‘mirror’ system

A recent fMRI study investigated the possibility of defining ‘mirror’ regions in humans using a paradigm similar to the paradigm adopted in monkeys single cell recordings experiments (Gazzola, Rizzolatti, Wicker, & Keysers, 2007; see also

Gazzola & Keysers, 2009). Specifically, the authors tested the same subjects in two tasks: one involving action execution and the other involving observation of the same action.

Comparing the activation for the two tasks, the results showed overlap within an extensive network of regions comprehending the dorsal and ventral premotor cortex, the inferior frontal gyrus, the posterior parietal cortex and the posterior temporal cortex. These findings were consistent even when applying a single-subject analysis approach (using both smoothed and unsmoothed data) in addition to a customary random effects fMRI analysis (see Figure 1.17).

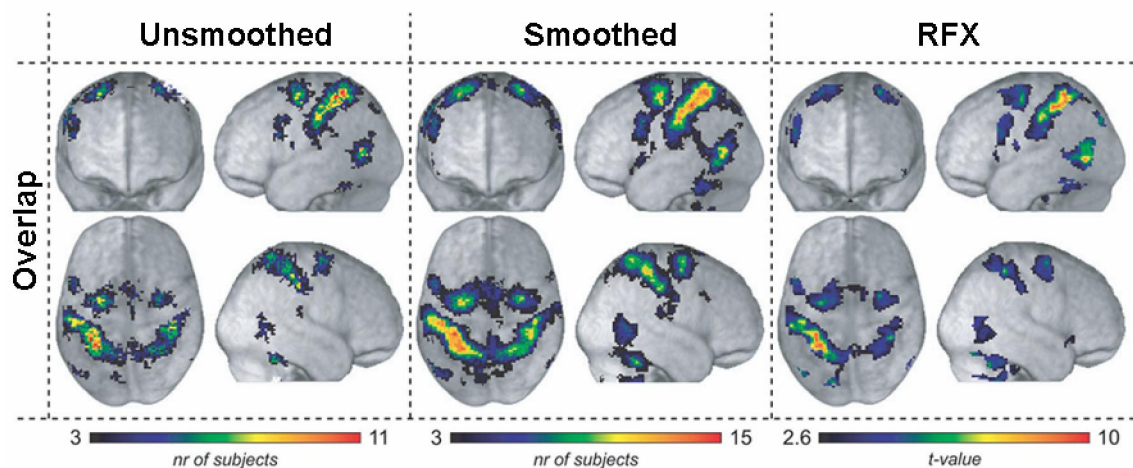


Figure 1.17. Human ‘mirror’ regions. Images within the left and the middle columns show the number of subjects showing ‘mirror’ regions using unsmoothed and smoothed data respectively. Images within the right column show the t-values of a traditional random effect analysis (RFX) using smoothed data. (Modified from Gazzola & Keysers, 2009).

Having identified ‘mirror’ regions in humans, Gazzola et al. (2007) then investigated the properties of these areas during action observation. The main result was that even observing a robotic hand grasping an object activated the human ‘mirror’ system. This occurred despite the kinematics of the robotic movement was completely unnatural with respect to the kinematics normally exhibited by a human hand. This was taken as the evidence that the system might allow to understand a wide range of actions,

including those performed by non-biological agents. And that at least some regions within this network may be more sensitive to the goal of an action rather than the means by which it is achieved. Altogether these findings provide further support the idea that subsets of the regions characterizing the AOS are recruited for both action execution and observation (Raos et al., 2004, 2007; Evangeliou et al., 2009).

1.4 Summary

From the above brief literature review it emerges that three cortical regions, STS, PF/PFG and F5 (Keysers & Perrett, 2004) might be chiefly involved in the processing of action observation in monkeys, forming the so-called Action Observation System (AOS). Specifically, STS responds only to the sight of action, whereas PF/PFG and F5 contain mirror neurons, visuomotor neurons which respond to both action execution and observation.

Recent neuroimaging investigations (Raos et al., 2004, 2007; Nelissen et al., 2005; Evangeliou et al., 2009) support the idea of an involvement in action observation of other areas within the parietal (Evangeliou et al., 2009) and the frontal (Raos et al., 2004, 2007; Nelissen et al., 2005) cortices. These results point to the existence of an ‘extended’ AOS, exceeding the three ‘core’ regions defined using single cell recordings. In addition, these studies show that ‘mirror’ kind of activity exceeds the regions in which mirror neurons were discovered using single-cell recordings.

Neuroimaging evidence has shown that an extended AOS, similar to that found in monkeys, is also present in humans. In details, this ‘extended’ human AOS comprehends various regions, i.e., the ventral and the dorsal premotor cortices, the inferior frontal gyrus, the superior temporal sulcus and the posterior parietal cortex.

The present research is nested within a novel approach to the investigation of the AOS in humans which aims to unveil the specific properties of each region within the system (e.g., Gazzola et al., 2007; Hamilton & Grafton, 2006). In particular, this line of research tries to assign to different regions specific roles such as the coding of basic kinematics ('how' an action is performed) and action goals ('what' has been performed).

1.5 The present research

The present thesis aims at investigating several unexplored issues regarding the human AOS with respect to the specific role played by the regions belonging to such system. The first experimental part (Chapters 3) describes a research on multiple sclerosis (MS) patients which may provide a suitable experimental model for testing some properties of the AOS. The second experimental part (Chapter 4) is concerned with the effect of transitivity, i.e. the presence of an object as target of an action. In contrast to monkeys, the human AOS responds to both intransitive and transitive actions. In particular, this study was designed as to understand how object-related and not-object-related actions are represented and coded within the human AOS. The third experimental part (Chapter 5) will describe a research testing the possibility of defining human 'mirror' regions. Finally, in the last experimental part (Chapter 6) I shall explore the properties of the human homologue region of mirror area F5c as to test whether its activity is modulated by the type of agent performing the action. The results for each study will be discussed independently (see the 'Discussion' section for each experimental chapter). Further, in a general discussion section the present findings will be considered globally in light of the most recent theoretical tenets on action observation (Chapter 7).

2. General methods

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

2.1 Participants' characteristics

The participants recruited for all the presented studies were right-handed. They all had normal or corrected-to-normal vision and had no history of neurological problems.

A further screening was adopted in order to ensure that the participants could enter a magnetic resonance (MR) scanner. Finally, informed consent was obtained from all subjects before the testing session in accordance with the declaration of Helsinki.

2.2 Stimuli

Either static pictures or video-clips were adopted as stimuli in the studies reported in the present thesis (a detailed description will be provided within each experimental chapter). Video clips were edited using Adobe Premiere (Adobe Systems, San Jose, CA, www.adobe.com), whereas pictures were edited with Adobe Photoshop (Adobe Systems, San Jose, CA, www.adobe.com). All stimuli were presented by means of a laptop PC that ensured synchronization with the MR scanner using either E-prime (Psychology Software Tools Inc, Pittsburgh USA, www.pstnet.com) or Presentation (Neurobehavioral Systems, Albany, CA, www.neuro-bs.com). An LCD computer-controlled projector was employed to present the stimuli on a screen positioned outside the bore of the magnet of the MR scanner.

2.3 Procedures and task

For all experiments, participants lay inside an MR scanner. Data regarding their brain activity (functional data) were acquired while they performed specific tasks. As a general instruction, participants were requested to keep their head still during scanning and to pay attention to the presented stimuli. To minimize head motion, cushions and pads specifically designed to restrain head translations and rotations within the head coil were utilized.

2.4 Data acquisition

Functional data were acquired for each subject together with high quality T1-weighted image (structural data) of the brain. For a detailed description of the MR sequences adopted in the studies please refer to Appendix A.

2.5 Pre-processing of fMRI data

Here I shall provide a general overview of pre-processing and analysis of functional data. Functional data were analysed using Statistical Parametric Mapping software (SPM5, www.fil.ion.ucl.ac.uk/spm/, see Friston et al., 1995), implemented in MatLab (version 7.3, www.mathworks.com). Pre-processing of functional data started with the realignment of the collected functional images using a two-step procedure. The images were registered to the first functional volume of each series and then to the mean image of the entire sequence in order to correct for any head movement occurring during the entire investigation. For each subject, a high quality structural image was co-registered to the mean EPI image and segmented. The co-registered grey matter segment was normalized onto the grey matter template (provided within SPM) and the resulting normalization parameters applied to all functional images (resampling the voxels at

2x2x2 mm). Finally, data were spatially smoothed using 8 mm FWHM (Full Width at Half Maximum) Gaussian kernel.

2.6 Measures of interest

Functional magnetic resonance imaging (fMRI) measures the functional activity of the brain. The most commonly used contrast technique in fMRI is the so-called BOLD (blood oxygenation level-dependent contrast) technique. In details, BOLD fMRI technique basically measures changes in the inhomogeneity of the magnetic field, which are the result of changes occurring in the level of oxygen present in the blood (blood oxygenation) (Ogawa, Lee, Kay, & Tank, 1990; Ogawa et al., 1992).

The function of the BOLD fMRI signal against time in response to a temporary increase in neuronal activity is known as the haemodynamic response function (HRF) (see Figure 2.1). Following this initial decrease (initial dip), there is a large increase in the BOLD fMRI signal which reaches its maximum after approximately 6 seconds. Finally, the BOLD signal returns to normal and until it has reached its original baseline level after an initial undershoot after approximately 24 seconds. This function of the BOLD fMRI signal against time is also known as the BOLD response and it is the response that is estimated in the analysis of fMRI data.

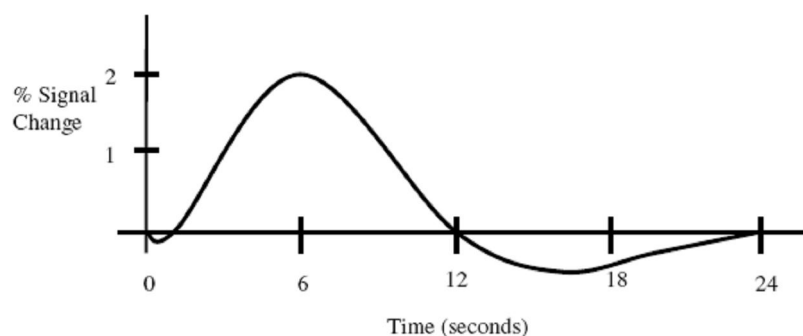


Figure 2.1. Time course of the HRF.

2.7 fMRI data analysis

Following pre-processing, functional data were subsequently analyzed by applying a General Linear Model (GLM) separately for each individual (first level analysis). In order to produce the model, regressors were defined based on the timing of presentation for each of the experimental conditions and were modelled using a delta function (event-related studies) or a box car function (block design) convolved with the HRF. Additional regressors of no interest were modelled to account for translation and rotation along the three possible dimensions as measured during the realignment stage of the pre-processing. Parameters of the model are estimated for each subject. Then in order to draw inferences on the population, a second level analysis must be adopted. The implementation in SPM of the second level analysis implies taking the parameters estimated from the first-level (fixed-effect) analysis and entering them into a second-level (random-effect) analysis. In the second-level analysis, contrasts between the different conditions of interest are performed.

2.8 Localization of activated brain regions

Different and complementary methods were used in order to have a clear localization of the activated brain regions. As a general neuroanatomical reference I adopted two neuroanatomical atlases (Duvernoi & Bourgouin, 1999; Mai, Assheuer, & Paxinos, 2004). Further, the SPM Anatomy Toolbox (version 1.6; Eickhoff et al., 2005), based on three-dimensional probabilistic cytoarchitectonic maps, was used to determine the probability of the peak activity voxels and of the clusters. For premotor, motor and somatosensory cortices the position of the cluster and the peak was ascertained with the data of the meta-analysis by Mayka, Corcos, Leurgans, & Vaillancourt (2006).

3. Investigating the neural circuit underlying the observation of grasping movements: a study on multiple sclerosis patients¹

Abstract

Recent fMRI evidence indicated that both the execution and the observation of hand actions determine, in multiple sclerosis (MS) patients, an increased recruitment of a portion of the human ‘mirror’ system. However, it remains unclear whether this is the expression of a compensatory mechanism for the coding of observed action or whether such a mechanism represents a rather unspecific functional adaptation process. Here I used fMRI on early relapsing remitting MS (RRMS) patients to clarify this issue. Functional images of 15 early RRMS patients and of 15 sex- and age-matched healthy controls were acquired using a 1.5T scanner. During scanning, participants simply observed images depicting a human hand either grasping an object or resting alongside an object. When compared to controls, RRMS patients revealed a robust increase of activation in an extensive network of brain regions including frontal, parietal, temporal and visual areas usually activated during action observation. However, this pattern of hemodynamic activity was completely independent of the type of observed hand-object interaction. These findings are in line with previous fMRI evidence demonstrating cortical reorganization in MS patients during action observation. However, they add to this literature suggesting that such functional cortical changes may be the expression of a generalized and unspecific compensatory mechanism which not necessarily is involved in action understanding.

¹ *Published.* Pierno, A.C., Turella, L., Grossi, P., Tubaldi, F., Calabrese, M., Perini, P., Barachino, L., Morra, A., Gallo, P., & Castiello, U. (2009). Investigation of the neural correlates underlying action observation in multiple sclerosis patients. *Experimental Neurology* 217, 252-7.

3.1 Introduction

Research showing that grey matter demyelination starts early and can be extensive in MS (Geurts & Barkhof, 2008; Pirko, Lucchinetti, Sriram, & Bakshi, 2007), stimulated the use of fMRI to investigate this disease. For instance, a wealth of studies conducted on MS patients indicated that, compared to healthy controls, the execution of motor tasks causes a markedly increased recruitment not only of motor and sensory regions, but also of some frontal and parietal areas usually associated with grasping and manipulating objects (Lee et al., 2000; Filippi et al., 2002; Filippi, Rocca, & Mezzapesa, 2004; Reddy et al., 2000; Rocca, Falini, Colombo, Scotti, & Filippi, 2002; Rocca et al., 2005; Pantano et al., 2005). This altered pattern of activation has been postulated to be an expression of a functional cortical reorganization put in place to compensate for the impact determined by MS-related injuries and to maintain an apparently normal level of functioning.

A recent study tested the intriguing hypothesis that the over-activation mechanism reported in MS patients during action execution tasks may extend to action observation and action understanding situations (Rocca, Tortorella, & Ceccarelli, 2008). Results from this study indicated that, compared to healthy controls, the observation of a video clip representing flexion-extension movements of the last four fingers of a human hand elicited in MS patients an increased activation of a number of areas known to be involved during hand action observation (Buccino et al., 2001; Gazzola et al., 2007). Although the findings from this study are of great interest for the understanding of the cortical plasticity mechanism operating in MS, an important issue remains unsolved. Specifically, it is still unclear whether the reported increased cortical activation in action observation-related brain areas is the result of a specific deficit in understanding the

observed actions or it reflects a rather unspecific and generalized response of the human brain to structural damage of the central nervous system.

The aim of the present study was to address this issue by comparing patterns of hemodynamic activity measured in early RRMS patients and in normal controls during the observation of a human hand either interacting with an object or simply resting close to an object. To do so, I conducted a whole-brain fMRI experiment in which subjects were scanned while they observed two types of display depicting a human hand either grasping an object (hand grasping condition), or resting alongside an object (hand resting condition).

3.2 Materials and Methods

3.2.1 Participants

Fifteen early relapsing remitting MS (RRMS) patients (7 males and 8 females, mean age = 30.6 years, range = 19-44; see Table B1 in Appendix B) have been recruited for the present study. Their average disease duration was 16.2 months (range = 3-34 months) and the mean Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) score was 1.5 (range = 1-3). All patients were relapse-free for at least two months and steroid-free for at least one month, when the anatomical and functional MRI scans were performed. The main inclusion criteria were maximum disease duration of 36 months and the absence of clinical impairment in the visual system and the upper limbs, which could affect the performance of the requested task. Three patients presented a single episode of optic neuritis with complete recovery at the onset of disease. At the time of image acquisition, ten patients were under treatment with immunomodulatory drugs (8 interferon beta, 2 glatiramer acetate) and five patients were therapy free. Fifteen

volunteers were recruited as controls (8 males and 7 females, mean age = 34 years, range = 24-54; see Table B1 in Appendix B).

3.2.2 Stimuli

Different types of black and white digital photographs (bitmap format, resolution 1024x768 pixels), which proved to be effective in eliciting activation within areas of the AOS (Johnson-Frey et al., 2003), were utilized as stimuli. During acquisition of functional volumes participants were presented with images depicting: i) a human right hand grasping an object positioned on a dark surface ('hand grasping condition', see Figure 3.1A) or ii) a human right hand resting alongside an object with the palm adjacent to the dark surface ('hand resting condition', see Figure 3.1B). Note that for the control condition any sort of hand-object interaction was avoided. For all conditions the same set comprising eleven objects were utilized (e.g., a glass, a tin box, a candle, a can, a jar, a tennis ball, etc., see Figure 3.1).

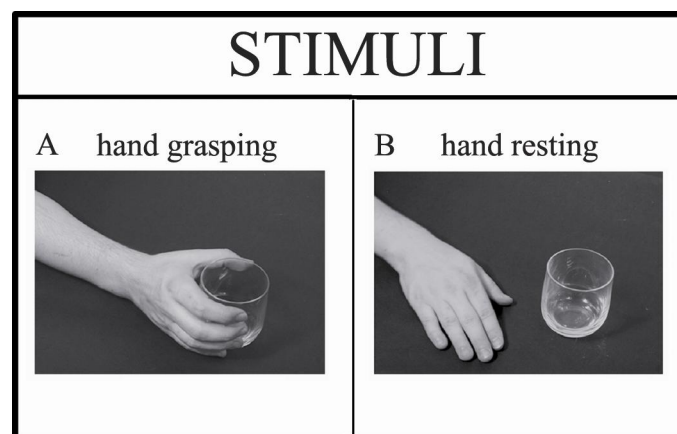


Figure 3.1. Example of the stimuli used in the present study.

3.2.3 Procedures

The experimental conditions were presented in a block design in which two different types of block (corresponding to the experimental conditions) were implemented. Within each block eleven static images were displayed on the screen for 1100 ms and were separated by 290 ms intervals of blank screen yielding a block duration of 15 s. Consecutive blocks were separated by a 15 s rest period consisting of a blank screen with a white fixation cross. The experiment was split into four functional runs. Within each run eight periods of activation were alternated with nine periods of rest. The two experimental conditions were presented four times per run resulting in a total of sixteen repetitions throughout the entire experiment.

3.2.4 Data analysis

Data were analyzed by applying a General Linear Model (GLM) separately for each individual. All conditions were modelled using a box-car function convolved with the HRF and contrasts were defined in order to pick out the main effects of each experimental condition. These contrasts were subsequently entered into a second level random effects analysis (2x2 ANOVA) in which 'condition' (grasping or control) was manipulated as within-subjects factor, and group (healthy controls and RRMS patients) served as a between-subjects factor. The main effects and the interactions were then tested by specifying appropriately weighted linear contrasts. Unless specified, the voxel-level threshold for these second-level contrasts was set at $p < 0.01$ (False Discovery Rate, FDR, corrected for multiple comparisons; see Genovese, Lazar, & Nichols, 2002) and the extent threshold was of at least 15 contiguous voxels.

3.3 Results

First I tested for possible differences between RRMS patients and controls independently from the type of observed stimuli by exploring the main effect of the factor 'group'. Results from this contrast [(RRMS patients/hand grasping + RRMS patients/hand resting) – (Controls/hand grasping + Controls/hand resting)] indicated robust differential activation in a widespread network of areas including occipital, parietal, temporal and frontal regions (see Figure 3.2 and table B1 in Appendix B). In the occipital lobe, RRMS patients were more activated than controls in two extensive clusters which also extended to the parietal cortex. Specifically, RRMS patients showed increased activations in a number of areas including the fusiform gyrus, the inferior, the middle and the superior occipital gyri, and the calcarine gyrus. All these activations were bilateral. In the parietal lobe RRMS patients were more activated than controls in both the inferior and the superior parietal lobule bilaterally including the angular and the supramarginal gyri. In the temporal lobe RRMS patients showed increased activation in the inferior and the middle temporal gyri bilaterally and in the left superior temporal gyrus. Increased activation for RRMS patients was also reported in the insular cortex and in the hippocampus bilaterally. In the frontal and prefrontal cortex differential activations were found in the precentral gyrus (premotor cortex), in the paracentral lobule, and in both the inferior and the superior frontal gyri. Finally, RRMS patients also showed increased activation in the left amygdala and in the cerebellum. The reverse contrast [(Controls/hand grasping + Controls/hand resting) - (RRMS patients/hand grasping + RRMS patients/hand resting)] did not bring to any significant activations. This signifies that for neurologically healthy controls no areas were more activated than RRMS patients.

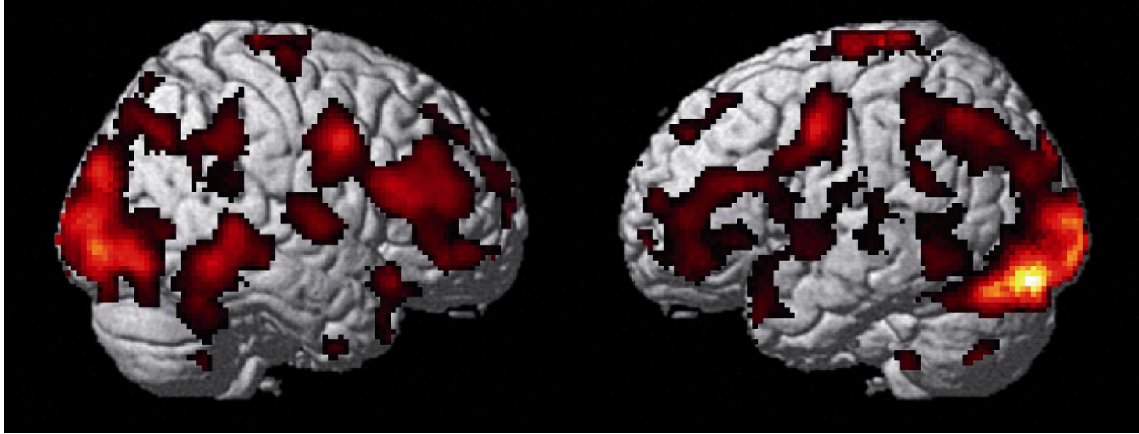


Figure 3.2. Regions of increased activation for the main effect of 'group'.

I next investigated the effects of viewing pictures depicting transitive grasping actions by exploring the main effect of the factor 'condition'. Specifically, for both groups I compared activation elicited by the 'hand grasping' condition with that elicited by the 'hand resting' condition $[(\text{Controls/hand grasping} - \text{Controls/hand resting}) + (\text{RRMS patients/hand grasping} - \text{RRMS patients/hand resting})]$. As shown Figure 3.3 (see Table B2 in Appendix B), this contrast revealed significant differential activation in a number of occipital, parietal and frontal areas classically known to be activated during action observation (AOS; Buccino et al., 2001; Gazzola et al., 2007). With respect to the 'hand resting' condition the sight of a human hand grasping an object determined increased activation within the inferior and the middle occipital gyri, the superior and the inferior parietal lobule including the intraparietal sulcus and the postcentral gyrus, and finally within ventral premotor cortex, the precentral and the inferior frontal gyri. All these activations were bilateral except for the precentral gyrus which was confined to the left hemisphere. The reverse contrast $[(\text{Controls/hand resting} - \text{Controls/hand grasping}) + (\text{RRMS patients/hand resting} - \text{RRMS patients/hand grasping})]$ revealed significant differential activation only in the left calcarine gyrus (primary visual cortex, see Table B3 in Appendix B). Finally the interaction aimed at localizing the modulating

effect of the factor 'group' on the factor 'condition' was not significant even when explored at a more liberal threshold (i.e., $p < .001$ uncorrected).

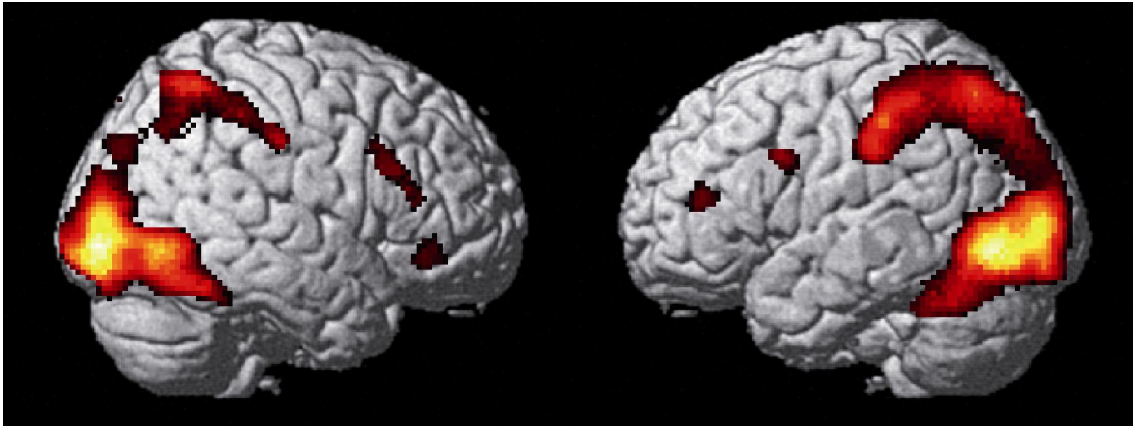


Figure 3.3. Regions of increased activation for the main effect of 'condition'.

3.4 Discussion

The aim of the present work was to ascertain whether possible differences in cortical activation between MS patients and controls during the viewing of other people actions. The main question was whether such differences could be genuinely interpreted as the expression of a specific deficit in the ability to understand observed actions or represent a rather unspecific and generalized adaptive response of the human brain to compensate for structural damage.

A previous action observation study conducted on MS patients (Rocca et al., 2008) revealed that observing hand actions elicited in MS patients significantly higher activation than controls within the inferior frontal gyrus and the superior temporal sulcus, two neural markers of action observation. These results are important because they extend to action observation situations the notion that during the execution of actions MS patients tend to show an increased recruitment of neural resources in terms of both the level of activity and the number of involved areas (Filippi et al., 2004;

Reddy et al., 2000; Rocca et al., 2002; Pantano et al., 2005). If the above mentioned activation pattern in action observation areas is the expression of a difficulty in performing such function, this should be carefully considered at both clinical and rehabilitative levels.

However, before these findings can be fully accepted, there is a fundamental issue which needs to be clarified. In particular, it is unclear whether the reported effects are due to a compensatory mechanism specific for the understanding of observed actions or whether such mechanism represents a less specific functional adaptation process. The study by Rocca et al. (2008) does not provide a definite answer to this question because a control condition aside from simple rest was missing in their experimental design. Indeed, simply comparing neural activation of MS patients following the observation of an action with the neural activation found in healthy controls performing the same task (Rocca et al., 2008) does not allow to fully ascertain whether the reported differences can be ascribed to functional changes which reflect action understanding mechanisms. In order to draw such conclusion what is needed is a control situation in which patients observe the hand and an object, but presented in a non interactive fashion. In such circumstances no differences amongst groups (MS patients and controls) should emerge.

The results of the present study have the potential to provide some answers to this question. If, as it is tempting to assume, we are in the presence of a compensatory mechanism specifically tailored as to contribute to the maintenance of a suitable level of action understanding, then such over-activation mechanism should have been found only for my 'hand grasping' but not for my 'hand resting' condition. This is because the adopted 'hand resting' condition does not entail any hand-object interaction. The present results clearly indicate that this is not the case. Although the experimental

design was able to reveal robust main effects of both the factors ‘group’ and ‘condition’, any significant ‘group’ by ‘condition’ interaction was found. Specifically, results from the main effect of the factor ‘group’ (RRMS patients vs controls) revealed a widespread network of areas in which activation was indeed greater for early RRMS patients than for healthy controls. Furthermore, results from the main effect of the factor ‘condition’ (grasping hand > resting hand) indicated that in early RRMS patients, as well as in healthy controls, observing a human hand grasping an object as compared to the same hand resting nearby the object triggered differential significant activation in areas known to play a pivotal role in action understanding such as the inferior frontal gyrus, the precentral gyrus, and both the inferior and the superior sectors of the parietal cortex including the intraparietal sulcus. The most important result, however, is the lack of any interaction between the two manipulated factors (‘group’ and ‘condition’). This indicates that the difference in terms of hemodynamic activation between observing a hand grasping an object and a hand resting in proximity of an object is similar for early RRMS patients and healthy controls. In other words, action understanding mechanisms seem to operate in a comparable fashion across the two tested groups. This is suggestive that the robust increased activation showed by early RRMS patients regardless of the type of the observed hand-object interaction does not represent a compensatory mechanism put in place as to overcome a specific deficit in action understanding. Conversely, it is more likely to be the expression of rather unspecific adaptive functional cortical changes which may contribute to maintain a normal level of function despite the presence of brain tissue damages.

The possible neurophysiological causes for such over-activation might be mainly related to two interrelated factors. The first factor relies on the evidence that MS causes demyelination of axons and which consequently determines a deficit in neuronal

transmission (e.g., Franklin, & ffrench-Constant, 2008). The second factor is concerned with the continuous repairing mechanism process triggered by the pathology (e.g., Franklin, & ffrench-Constant, 2008). Both a deficit in neural transmission and repairing require higher metabolism with respect to a normal brain and therefore may account for the abnormal level of functioning reported for these patients.

Altogether these findings demonstrate the presence in RRMS patients of a robust increased recruitment of neural resources within an extensive and widespread network of brain regions including visual, parietal, temporal and frontal regions. Although some of the over-activated areas do play a role during the observation of other people actions, the present results clearly indicate that such increase in neural activity is not specifically related to action observation.

4. Exploring the neural circuit underlying the observation of grasping movements: the effect of action transitivity²

Abstract

In the present study I investigated the influence that transitivity - i.e. the presence of an object as the target for an action - might have on the AOS. Participants were requested to observe grasping actions ending behind a partition knowing in advance whether a target-object would be present or absent. Importantly, independently from the presence of the target-object, the observed actions were exactly the same in terms of movement kinematics and contextual information. The ‘action’ conditions were compared with ‘control’ conditions in which a stationary hand was presented either alone or together with the target-object hidden behind the partition. I found that the presence/absence of the target-object modulated activity within the right posterior superior temporal sulcus and the anterior intraparietal sulcus bilaterally for the ‘action’ but not for the ‘control’ conditions. These findings provide strong evidence that information regarding the transitivity of an action is sufficient to influence the level of activity within the neural system subtending action observation. Further, they add to the notion that in humans this system can rely solely on previous knowledge regarding the action goal (i.e., presence or absence of a target-object) as to discriminate between transitive and intransitive actions.

² *Submitted.* Turella, L., Tubaldi, F., Erb, M., Grodd, W. & Castiello, U. Transitivity modulates activity within the action observation system.

4.1 Introduction

Here, I exploit the sensitivity of the human AOS by testing for the first time whether this system is modulated by action transitivity. In other words, I am testing whether action goals implying a physical interaction between an effector and an object can be distinguished from goals which do not imply such interaction. Some evidence that this might be the case comes from a study on monkeys suggesting that the AOS could be profoundly influenced by the transitivity of an observed action (Umiltà et al., 2001). Specifically, this study revealed that some F5c neurons responded even when the final part of an observed grasping action was hidden behind a partition. The prerequisite for this to happen was that the monkey knew about the presence of an object behind the partition (Umiltà et al., 2001). The same neurons did not discharge when the monkey observed a pantomime of the same action. That is, when the animal knew that there was no object behind the partition. These findings, therefore, seem to suggest that previous knowledge regarding the presence of a target-object might suffice as to distinguish between transitive and intransitive actions.

I capitalize on the paradigm used by Umiltà et al. (2001) to ascertain a modulation within the AOS depending on the transitivity of the observed action. Participants were requested to observe a model performing a grasping action towards an object which was hidden behind a partition, or the very same action in the very same contextual conditions, but in the absence of the target-object (i.e., pantomimed grasp). Information about the presence or absence of the target-object was conveyed by a prime presented before the stimulus representing either a real or a pantomimed grasp. This procedure might permit a specific investigation of whether the presence/absence of a target-object, intended as the goal of the action, modulates activity within the AOS during the observation of partially hidden actions. In turn, this will allow inferences to be drawn on

how the abstract representation of a goal is coded within the AOS by eliminating the subtle confounds related to ‘how’ an action is performed. If the same visual stimulus has the ability to modulate activity within the AOS on the basis of previous knowledge regarding the presence of an unseen target-object (i.e. transitivity), then it could be argued that the system is able to discriminate between transitive and intransitive actions.

4.2 Methods

4.2.1 Participants

Nineteen right-handed paid volunteers (9 male, mean age 27.2 years, range 20-37) were recruited for the present study.

4.2.2 Stimuli

Video-clips (AVI format, Xvid codec compression, resolution 320x240, 25 frames per second) were used as stimuli for the present study, namely the ‘AOS localizer’ and the ‘main experiment’. The stimuli used for the ‘AOS localizer’ represented: i) the hand of a model grasping an object resting on a table (see Figure 4.1A); and ii) the hand of a model resting nearby an object (see Figure 4.1B). For the implementation of these video-clips two different individuals served as models and four objects were used as target stimuli for the models’ action (i.e., a sphere of 3.5 cm diameter; a sphere of 9 cm diameter; two parallelepipeds measuring 9x6x6 and 6x2.5x2.5 cm, respectively). Furthermore, object position was counterbalanced with respect to the participants’ point of view. For half of the video-clips the object was located to the left, whereas for the other half the object was located to the right side of the screen upon which the stimuli were presented. The combination of the considered factors (2 models x 4 objects x 2 positions) resulted in a total of sixteen videos.

The ‘main experiment’ considered three specific events: i) prime (duration: 1 second); ii) experimental condition (duration: 3 seconds); and iii) question (duration: 2 seconds). For the ‘prime’ event, the stimuli represented an object resting on a table, which was fully visible at the beginning of the video and then gradually covered by means of a grey partition (see Figure 4.1C); and the same visual scene in the absence of the object (see Figure 4.1D). For this event the stimuli were constructed starting from static frames depicting an object resting on a table or the same scene without the object. The moving grey partition was added using a video-editing technique. This partition entered the scene at the beginning of the video-clip and its motion ended when the object was fully hidden. When the object was absent the partition ended at the same position as when the object was present. A total of ten videos were adopted (eight video-clips with the object and two video-clips without the object). For the ‘experimental condition’ event the stimuli represented the hand of a model grasping an object hidden behind the partition (see Figure 4.1E); and the hand of a model maintained static nearby the partition (see Figure 4.1F). For this event the stimuli were at all cases edited versions of the video-clips used for the ‘AOS localizer’. Here a static grey partition was present for the entire duration of the video-clip. The stimulus for the ‘question’ event corresponded to a string of text appearing on the screen.


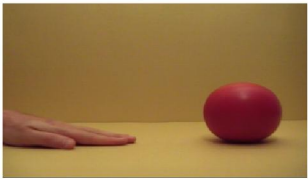
Localiser		Main Experiment	
Conditions		Primes	Experimental Conditions
A grasping		C object	E action
B control		D no object	F static

Figure 4.1. Types of stimuli. For the AOS localiser, two types of video-clips were adopted ('grasping' and 'control'). The represented frames (Figure 4.1A, B) were extracted from the central part of the video-clips. For the main experiment, four types of videos were adopted: two for the prime event ('object' and 'no object') and two for the experimental condition event ('action' and 'static'). The represented frames for both prime conditions (Figure 4.1C, D) and for the experimental conditions (Figure 4.1E, F) are extracted from the central part of the video-clips.

4.2.3 Procedures and task

4.2.3.1 AOS localiser

The 'AOS localizer' was performed as to identify a 'pure' action observation pattern of activity (for a similar procedure, see Buccino et al., 2001; Gazzola et al., 2007; Gazzola & Keysers, 2009). It consisted of a single run during which participants were requested to carefully observe the stimuli described above. This part of the experiment was always administered after the 'main experiment'. There were two experimental conditions: i) an 'object-related grasping' condition in which participants observed a model performing a grasping action and ii) a 'static' control condition, in which participants observed the hand of a model resting on a table nearby an object. The two experimental conditions were presented in an alternated block design. Within each block four video-clips were displayed for 3 s and were separated by 0.5 s intervals of blank screen (total block duration: 13.5 s). Consecutive blocks were separated by a 12.5

s rest period during which a grey screen with a black fixation cross was presented. Each condition was repeated sixteen times.

4.2.3.2 Main experiment

The procedures for the ‘main experiment’ largely relied on those previously used in a neurophysiological study by Umiltà and colleagues (2001). Naturally they were modified in order to fit the needs of an fMRI study. The sequence of events was as follows (for the specific trial timeline please refer to Figure 4.2). The first event was a ‘prime’. There were two types of prime: an ‘object’ prime, in which the stimulus represented an object resting on a table (Figure 4.2; red labelling) and a ‘no object’ prime, in which the object was absent (Figure 4.2; blue labelling). Following this, a grey partition entered the scene and covered either the part of the scene in which the object was present (‘object’ prime in Figure 4.2) or the corresponding empty area (‘no object’ prime; Figure 4.2). The second event ‘experimental condition’ varied depending on the type of prime. When the prime signalled the presence of an object located behind the partition (Figure 4.2; left upper panel) the stimuli for this event could be either (i) the hand of a model grasping the object (‘grasping’ option within the left bottom panel in Figure 4.2) or (ii) the model’s hand maintained static (‘object’ option within the right bottom panel in Figure 4.2). When the prime signalled the absence of an object behind the partition (Figure 4.2; right upper panel) the stimuli for the ‘experimental condition’ event were either (i) the hand of a model performing a pantomimed grasp (‘pantomime’ option within the left bottom panel in Figure 4.2) or (ii) the model’s hand maintained static (‘no object’ option within the right bottom panel in Figure 4.2). For the third event termed ‘question’ (see Figure 4.2) a text line appeared on the screen for two seconds and participants were requested to indicate whether during the two previous events the

object was absent or present. They pressed the left key of an MRI-compatible response box with the index finger of the right hand if the object was present or the right key with the middle finger if the object was absent. Trials were considered as successful only when the response was congruent with the prime. When the response was incorrect the trial was considered as an error and subsequently discarded. The mean percentage of errors across participants was about 3.3% (6.3 trials over a total of 192). In order to ensure that participants fully understood the task they attended a practice session outside the scanner.

The considered experimental conditions were embedded in a 2x2 factorial design, with two within-subjects factors: ‘observed task’ (action vs control) and ‘object’ (presence vs absence). The combination of the two factors brought to four conditions of interest:

i) ‘grasping’ condition, in which participants observed the model grasping an object hidden behind the partition. Participants knew from the type of prime that an object was present.

ii) ‘pantomime’ condition, in which participants observed the model performing a grasping action knowing from the type of prime that there was no object behind the partition.

iii) ‘object’ condition, in which participants observed a static hand and the partition knowing from the type of prime that behind the partition there was an object.

iv) ‘no object’ condition, in which participants observed a static hand and a partition knowing from the type of prime that there was no object behind the partition.

As reported above within the ‘stimuli’ section, only two sets of videos were used as stimuli for the experimental conditions: one set was used for the ‘action’ conditions (i.e., grasping and pantomime) and one set for the ‘control’ conditions (i.e., ‘object’ and

‘no object’). Within each of the three functional runs 64 video-clips were presented (16 for each experimental condition). The total duration for each trial was 14 seconds (seven TR). The time line for each trial was the following: the ‘prime’ event was presented only within the first two TRs, the ‘experimental condition’ event was presented only from the third to the fifth TRs and the ‘question’ event within the last two TRs (i.e., sixth and seventh). All the stimuli started on a variable schedule, determining a jittered inter-stimulus interval (ISI) between the three events of each trial (prime, experimental condition, question).

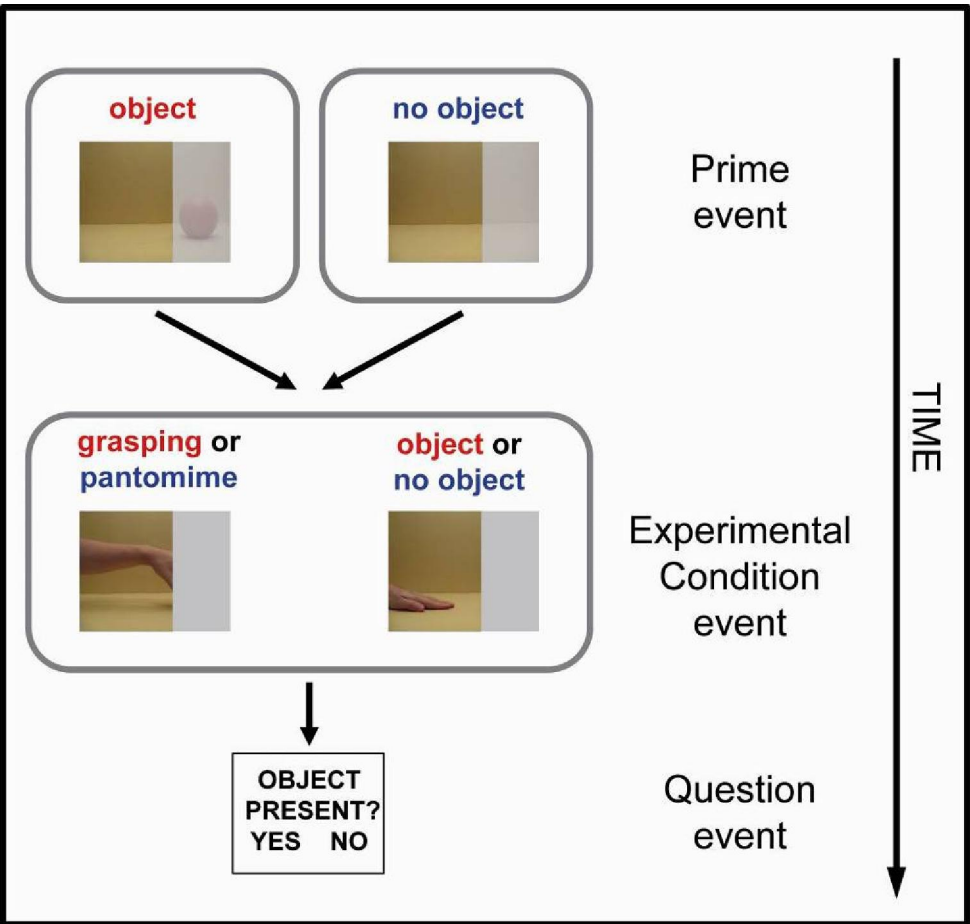


Figure 4.2. Timeline of the study. Graphic representation for the sequence of events. The represented frames for the prime conditions were extracted at the end of the video-clips. For the sake of clarity in order to show the difference between the two types of prime we rendered the partition transparent. For the experimental conditions, the pictures represent frames extracted from the central part of the video-clips.

4.2.4 Data Analysis

4.2.4.1 AOS Localiser

The analysis of the localiser data allows the definition of regions within the AOS. The images referring to the contrast between ‘action observation’ and ‘static control’ were extracted for each subject at first level and then tested in a second level random effect analysis using a one-sample t-test. The adopted statistical threshold was set at $p < 0.05$ FDR corrected (Genovese et al., 2002) and 25 contiguous voxels.

4.2.4.2 Main experiment

As my specific interest was to test the modulation within the AOS, a Region of Interest (ROI) analysis was performed only on the regions found activated within the localiser. This ROI analysis was performed on the mean percent signal change extracted from these regions using Marsbars SPM Toolbox (Brett, Anton, Valabregue, & Poline, 2002). I tested the main effects and interaction of the 2x2 factorial design (two within-subjects factors: ‘observed task’ and ‘object’) for each region. Within the regions, where the interaction was significant, paired t-tests were performed to test the following contrasts: ‘grasping’ vs ‘pantomime’ conditions and ‘object’ vs ‘no object’ conditions.

4.3 Results

4.3.1 AOS localiser

When contrasting the two conditions characterizing the AOS localiser (‘object-related grasping action’ > ‘static control’, see Figure 4.3) activation was found within the classically defined areas of the AOS. In details, activation was evident bilaterally within the premotor, parietal, temporal cortices and the occipital cortices (see Figure 4.3

and Table C1 in Appendix C). This is in line with previous literature demonstrating an automatic involvement of these areas during a ‘pure’ action observation task (Buccino et al., 2001; Gazzola et al., 2007; Gazzola & Keysers, 2009).

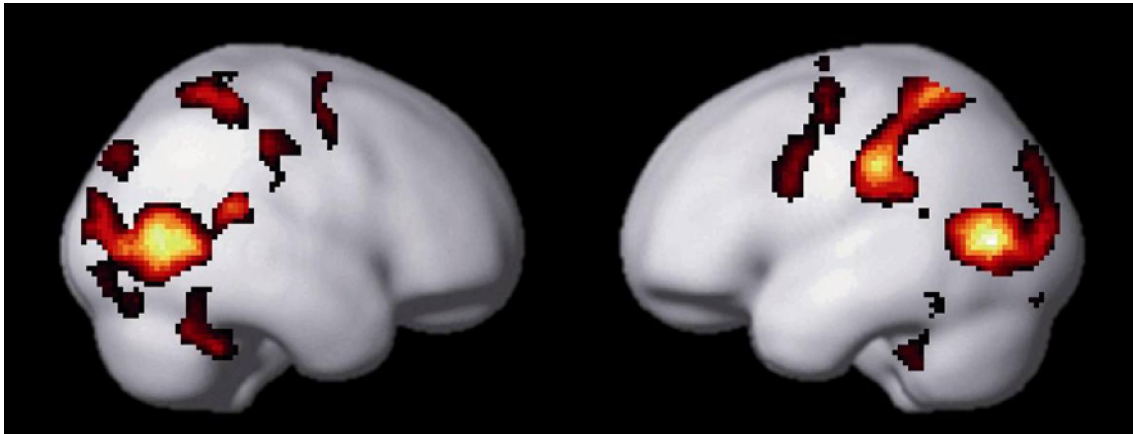


Figure 4.3. Activation maps for the AOS localizer.

4.3.2 Main experiment

ROI analysis was performed on those regions which were activated at the level of the AOS localiser. In all the tested regions the main effect of action observation was significant. I shall discuss only those regions in which the interaction was significant, i.e. the intraparietal sulcus (aIPS) bilaterally and the right posterior STS.

In order to understand the selective modulation within these areas due to the presence/absence of an object I performed paired t-tests contrasting the ‘grasping’ vs the ‘pantomime’ conditions and the ‘object’ vs the ‘no object’ conditions. The results indicated that activity within the bilateral aIPS and the right pSTS was higher when participants were exposed to the ‘grasping’ rather than to the ‘pantomime’ condition (see Figure 4.4 and Table C2 in Appendix C).

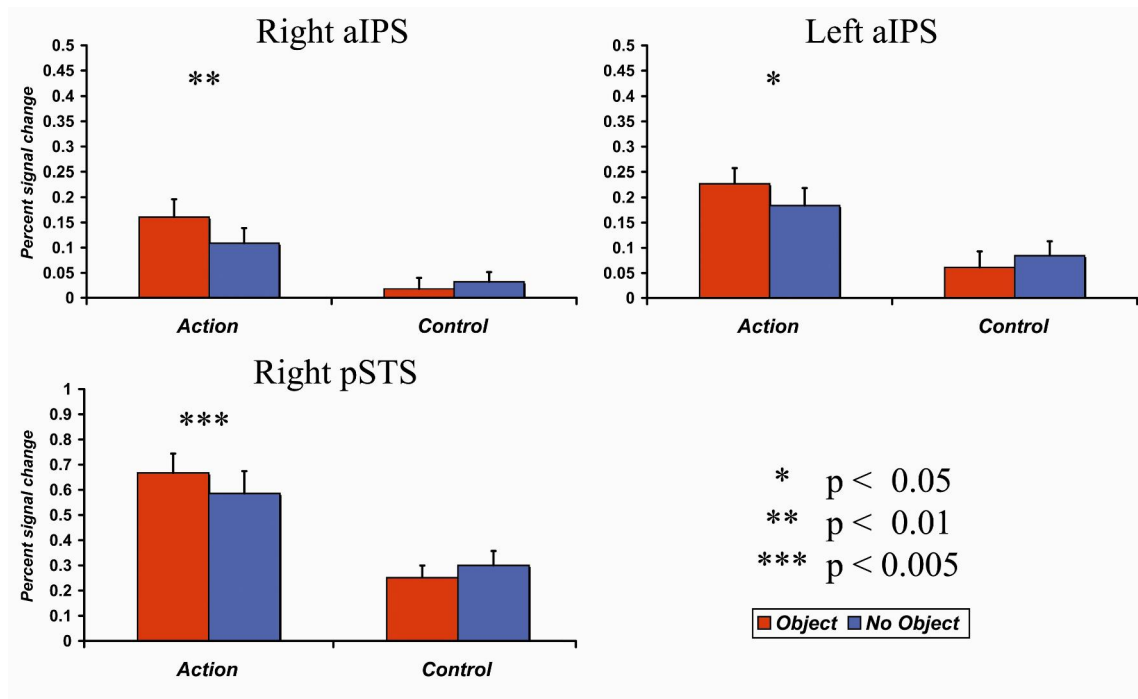


Figure 4.4. ROI results. Plots of the percent signal change extracted from the regions of interest. Paired t-test (two-sample) were performed on the conditions. I reported different p values using the following symbols: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. The exact t and p value of the contrasts are reported in Table C2 in Appendix C.

4.4 Discussion

The aim of the present study was to investigate whether activity within the AOS is modulated by the transitivity of an observed action, i.e. the presence/absence of a target-object. In general, my results suggest that the AOS is modulated by the nature of the observed action (transitive versus intransitive) and it has the ability to discriminate across different actions; even when their goal was not visually available to the observer. Below I report on the more specific aspects concerned with these findings by discussing the pattern of activity for each of the areas which were sensitive to the experimental manipulation.

4.4.1 Right Posterior Superior Temporal Sulcus

In a series of single cell studies, Perrett and colleagues (1989, 1990; Jellema et al., 2000; Jellema & Perrett, 2006) have demonstrated that some cells within the STS

respond to the sight of goal-related actions, such as a hand manipulating an object. The response was not present (or extremely reduced) during the observation of a pantomime or of a pantomimed grasp occurring in proximity of the object (Perrett et al., 1989, 1990). A recent study further investigated such modulation revealing that object-related actions elicited the highest response, whereas pantomimes triggered a much weaker response (Barraclough et al., 2009). However for both action conditions the level of activity differed from a baseline condition in which the object was presented alone. The present findings show a similar modulation within the pSTS indicating the highest response for transitive actions, a reduced response for intransitive actions and an even lower response for the control conditions.

Another aspect of the present results concerns the influence that knowledge regarding the presence/absence of a previously seen object (i.e. the prime) has on pSTS activity during action observation. Recent evidence in humans indicated that some form of knowledge such as ‘perceptual history’ (Jellema & Perrett, 2003) - events that have been recently witnessed - might modulate activity within the pSTS (Wyk, Hudac, Carter, Sobel, & Pelphrey, 2009). Here participants observed a model expressing a positive or a negative emotion towards one out of two objects, following which the model would then grasp one of the two objects. The relationship between the grasped object and the emotion expressed towards it, modulated activity within the pSTS. The present study extends this literature showing that, in humans, past contextual information regarding the transitivity of an action (presence or absence of a target-object) might modulate activity within the pSTS.

Altogether the present findings provide some evidence of a possible link between activity related to ‘perceptual history’ (Jellema & Perrett, 2003) and activity for goal-related movements (Perrett et al., 1989, 1990; Barraclough et al., 2009). In this view the

pSTS might act as a central node for the processing of goal-related actions by integrating the representation of a partially visible movement with the information coming from previously experienced events.

4.4.2 Anterior Intraparietal Sulcus

Recent evidence indicates that the aIPS is sensitive to the presence of an object which is the target of an action ('goal-object') (Hamilton & Grafton, 2006; Shmuelof & Zohary, 2005, 2006). Along these lines here it is shown that the most effective response in aIPS was evident when the model performed a real grasp, even when the object was not visible. Further, the present results go a step forward demonstrating that it is the knowledge regarding the transitivity of a partially hidden action which modulates activity within this area.

Another aspect of the aIPS results is that the pattern of activation suggests a differential processing for transitive and intransitive actions. Although the observation of transitive actions determined a level of activity which was higher than that observed for intransitive actions, the observation of intransitive actions still activated the aIPS. This result is in line with recent findings suggesting that in humans the aIPS might be activated by the view of intransitive movements (Wheaton, Thompson, Syngienotis, Abbott, & Puce, 2004; Dinstein, Hasson, Rubin, & Heeger, 2007; Dinstein, Gardner, Jazayeri, & Heeger, 2008; Lui, et al., 2008). Therefore it appears that in humans the aIPS is sensitive, though to a lesser extent, to actions in which no physical goal (i.e., the object) is present. Support to this contention comes from a recent study by Lui et al. (2008), showing that observation of mimed actions (i.e., pantomime of object-related actions) elicits an activation similar to the pattern of activation found in previous studies considering object-related actions (i.e. Buccino et al., 2001). However, these authors did

not consider a condition involving the observation of object-related actions, and therefore the ‘transitivity’ issue remained unsolved. The present study, in which transitive and intransitive actions are directly tested, provides compelling evidence that within the aIPS both types of actions are coded. In addition, it demonstrates that the aIPS might discriminate between different types of action on the basis of previous knowledge regarding the presence of a target-object.

5. The ‘mirror’ system in humans³

Abstract

In this study, I tested the possibility that regions of the human brain show overlapping activity for both observation and execution of action (‘mirror’ activation), adopting a highly conservative procedure and a paradigm which was similar to the paradigm used in monkey single cell recording studies. The same participants were requested to observe a grasping action (observation condition) and to perform a grasping action (execution condition). Results from whole-brain analyses indicate that overlapping activity for action observation and execution was evident in a broad network of areas including parietal, premotor and temporal cortices. A finding which is in line with the most recent proposals on the composition of the ‘mirror’ system.

³*Published.* Turella, L., Erb, M., Grodd, W. & Castiello, U. (2009). Visual features of an observed agent do not modulate human brain activity during action observation. *NeuroImage*, *46*, 844-53.

5.1 Introduction

Following the discovery of mirror neurons, many fMRI studies have been performed in order to uncover a similar system in humans. Amongst these studies the most convincing evidence of a ‘mirror-like’ system in humans comes from a study by Gazzola et al. (2007; Gazzola & Keysers, 2009) who tested both action execution and observation within the same individuals. In one day they asked participants to observe either a human model or an industrial robot performing a variety of actions and in a separate day to perform the actions. They found regions of overlap for action observation and execution in classic ‘mirror’ areas together with many areas which were not previously considered as mirror (Gazzola et al., 2007, Gazzola & Keysers, 2009). In the present study, I wanted to identify the mirror system in humans without the confound of the robotic hand which might have introduced ‘noise’ in the results. Therefore I used a strict experimental paradigm, as similar as possible to that originally adopted in neurophysiological studies.

5.2 Materials and Methods

5.2.1 Participants

Seventeen paid participants (10 female, mean age 27.8 years, range 21-39) were recruited for the present study. One subject was discarded due to head motion exceeding 3 mm (voxel size).

5.2.2 Apparatus

In order to investigate both execution and observation within the MR scanner I adopted a custom-built MRI-compatible apparatus consisting of two main parts (Figure 5.1). A lower part embedding a screen which served to present the video clips and an

upper part consisting of a pneumatic piston containing a stick with a spherical stimulus attached to it. The stimulus could be lowered down within the scanner at a reachable distance using compressed air. The use of a pneumatic/mechanical apparatus allowed for a precise control of the timing of the sequence of events and to avoid the interaction of the subject with the experimenter. The apparatus was positioned over the subject's legs and the subject's head was tilted in the coil (30 degrees) allowing both the screen and the stimulus (when lowered down) to be visually available by the subjects.

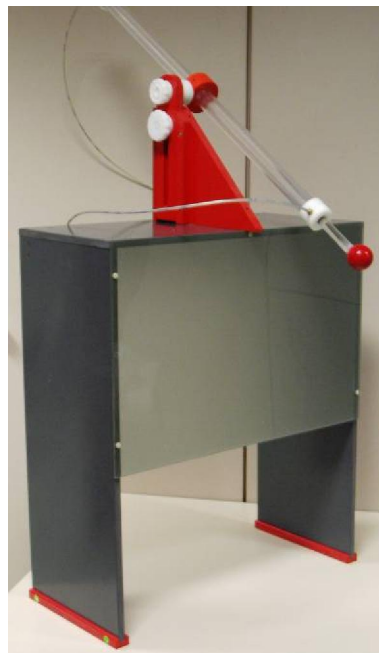


Figure 5.1. Apparatus.

5.2.3 Stimuli

5.2.3.1 Action observation

Two different types of video clips served as stimuli (4 s duration, AVI format, Xvid codec compression, resolution 360x240, 25 frames per second). As shown in Figure 5.2 these video clips could represent: i) a human model grasping an object ('model grasping', Figure 5.1A); ii) a static human model with the hand resting on the table in

the proximity of the object ('model control', Figure 5.1B). Two different models (a female and a male) performed the action for a total of 30 videos for each condition.

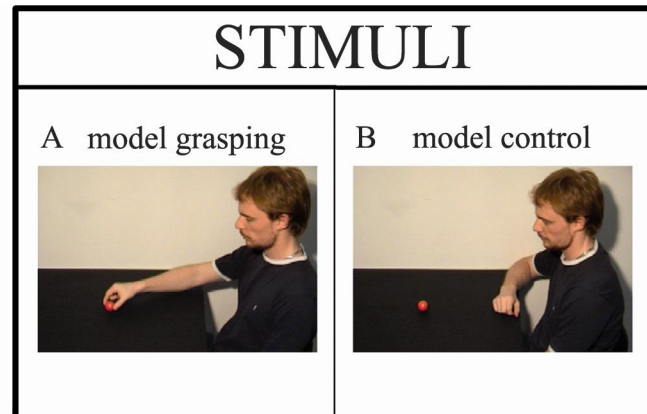


Figure 5.2. Stimuli. Frames extracted from the videos for each experimental conditions characterizing the 'observation' component of the experiment. From top left to bottom right the following conditions are represented: A) 'model grasping', B) 'model control'.

5.2.3.2 Action execution

The stimulus was a firm red sphere (diameter: 40 mm) attached to the stick inside the piston of the pneumatic apparatus (see Figure 5.1).

5.2.4 Procedures and task

5.2.4.1 Action observation

Subjects were asked to watch the videos carefully. Subjects were not asked to perform behavioural tasks within the scanner for the following reasons. First, to eliminate any possible confound due to the possible involvement of sharing/divided attentional processes (Chong, Williams, Cunnington, & Mattingley, 2008). Second, because it has been recently suggested that the 'action observation' system is mainly activated when no active inferential process is involved (Brass, Schmitt, Spengler, & Gergely, 2007). Third, because I wanted to test a 'pure' observation condition as in

monkeys' studies (Rizzolatti et al., 2001). In between stimuli presentation subjects were requested to fixate a cross presented in the middle of the screen.

5.2.4.2 Action execution

Subjects were requested to fixate a black cross presented on the grey background of the screen embedded within the apparatus. Then they were requested to reach towards and grasp the stimulus with a precision grip (see Culham et al., 2003, for a similar procedure) or to fixate the stimulus. Specifically the sequence of events was the following: i) subjects were requested to fixate the central cross (as for the observation part); ii) the central cross disappeared; iii) the stimulus was lowered down in the scanner and subjects were requested to fixate it. The time taken by the stimulus as to reach the pre-determined location was 1.5 s; iv) subjects were requested to fixate the stimulus for 1.5 s until the beginning of the experimental conditions. The latter 3 seconds of the event sequence were defined as 'preparation time'. Following 'preparation time', the subjects had 3 seconds either to perform the reach-to-grasp movement (if they hear a sound) or just to continue to fixate the ball (in case no sound was presented), afterwards the ball was removed from the subject's view. The task was performed by all subjects with the dominant (right) hand. Half subjects performed the execution task before the observation task, the other half performed the observation task before the execution task.

5.2.5 Data Analysis

Two whole-brain analyses, one for the observation and one for the execution part of the experiment were carried out by applying the General Linear Model (GLM). The

alpha level for these second-level analyses was set at $p < 0.0001$ uncorrected at voxel-level and at $p < 0.05$ corrected at cluster level.

5.2.5.1 Action observation

The contrast of the two conditions ('model grasping' > 'model control') was extracted for each subject at first level and then entered in a second level analysis.

5.2.5.2 Action execution

The contrast of interest, reach-to-grasp against object fixation, was extracted for each subject at first level and then tested in a second level analysis. In order to distinguish effects only related to the different tasks, 'preparation time' was modelled as a regressor of no interest. Specifically it was defined as the time from the moment the stimulus started to be lowered down up to the time the different tasks began. The tasks, 'reach-to-grasp' and 'object fixation', were modelled as regressors starting from the end of the preparation time regressor up to the time the stimulus started to re-enter within the piston. Both the task and the preparation time duration was 3 seconds. Errors were separately modelled as an 'error' regressor of no interest within the first level design matrix.

5.2.5.3 Conjunction analysis

To define regions in which activation was elicited by both grasping observation and execution a conjunction analysis between regions activated during action observation and execution was performed. In order to find common voxels we created a binary image overlapping the SPM t maps for the two contrasts of interest reported above. The statistical threshold of the two maps was the same reported for the single contrasts,

i.e. $p < 0.0001$ uncorrected at voxel-level and at $p < 0.05$ corrected at cluster level. Thus, we logically inferred that both action observation and execution would trigger activity within cerebral areas in which both contrasts of interest reached statistical significance (Nichols, Brett, Andersson, Wager, & Poline, 2005; Friston, Penny, & Glaser, 2005).

5.3 Results

5.3.1 Action observation

The contrast testing ‘model grasping’ vs ‘model control’ showed differential activation within AOS comprehending premotor, temporal and parietal cortices (Buccino et al., 2001; Gazzola et al., 2007; Gazzola and Keysers, 2009, see table D1 in Appendix D). Activation map related to action observation is overlaid on the mean image of the group (see Figure 5.3). Consistent with previous findings, significant differential activation was evident bilaterally within both the dorsal and the ventral sectors of the premotor cortex. Further, two bilateral clusters were activated within the caudal cingulate motor area. For the parietal cortex bilateral activation was evident within the superior and the inferior parietal lobules and the intraparietal sulcus. Parietal activity within the left hemisphere also spread within the postcentral gyrus. Bilateral activation was also evident in the superior, the middle and the inferior temporal gyri and in two clusters within the superior occipital cortex. Activity was also evident in the thalamus. Finally, activation within the cerebellum was evident at the level of the VI, VII and VIII lobules. The reverse t-contrast considering ‘static control’ versus ‘action observation’, did not show any significant activation.

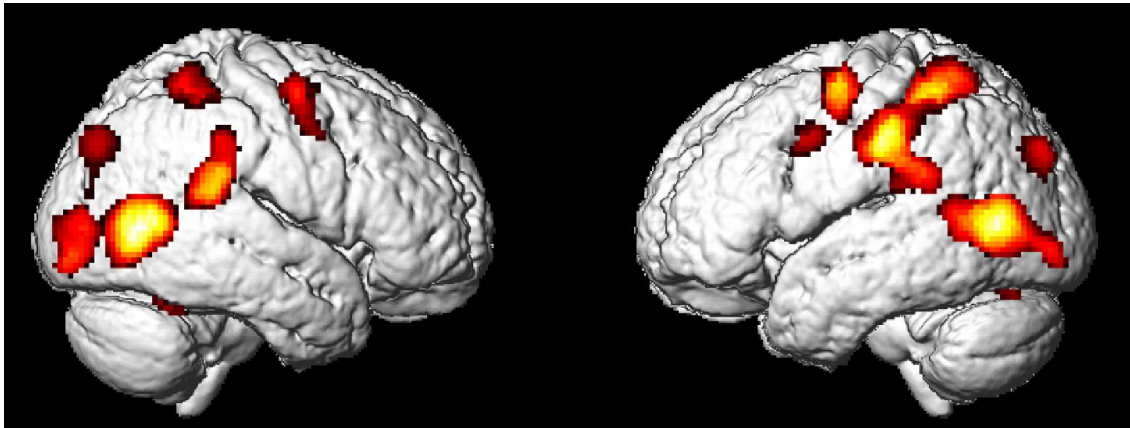


Figure 5.3. Regions of increased activation for the main effect of 'action observation'.

5.3.2 Action execution

Activations related to 'grasping execution' versus 'object fixation' are shown Figure 5.4 (see also Table D2 in Appendix D). In general, the revealed pattern of activation was similar to that reported in previous grasping studies (for review see Castiello, 2005; Castiello & Begliomini, 2008). Specifically, activation was significant within the dorsal and the ventral sectors of the premotor cortex, in the inferior frontal gyrus and in the insula. For the parietal cortex significant differential activation comprehended the postcentral gyrus, the superior and the inferior parietal lobule together with the intraparietal sulcus. Other activations closely associated with actual execution included SMA and caudal cingulate motor area together with the inferior and the middle temporal and the superior occipital cortices. An involvement of subcortical structures was also detected at the level of the basal ganglia and the thalamus. Whereas all the above mentioned brain loci were characterized by a bilateral pattern of activity there was an evident left lateralization of activity within the postcentral gyrus and precentral sulcus resembling somatomotor activation within regions related to the movement of the thumb, the index finger, the arm and the elbow (Lotze et al., 2000).

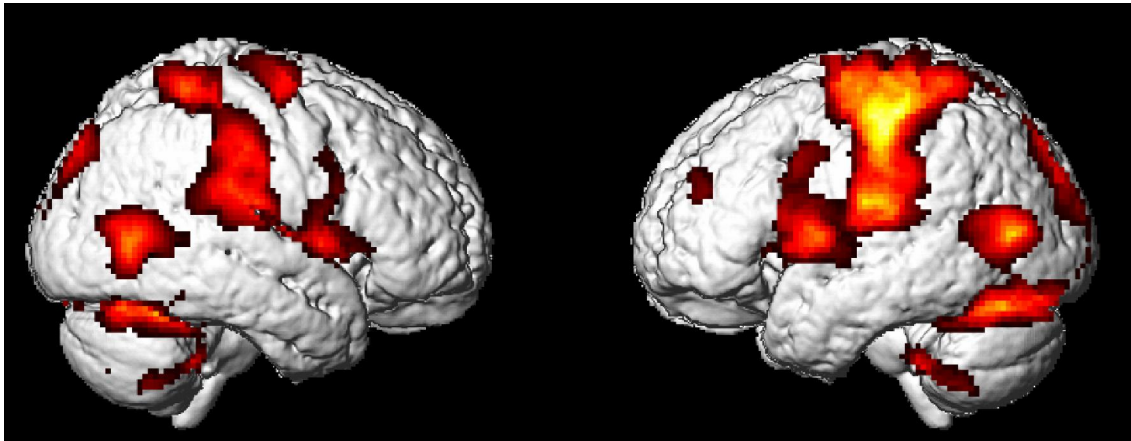


Figure 5.4. Regions of increased activation for 'action execution'.

5.3.3 Conjunction analysis

As shown in Figure 5.5, various regions showed overlapping activation related to both the execution and the observation of grasping actions. These regions were the left ventral premotor cortex together with bilateral activation within the dorsal premotor cortex, caudal cingulate motor area, the middle temporal gyrus and the parietal cortex. Subcortical regions included the left thalamus and the cerebellum bilaterally. These results confirm the pattern of overlaps for action execution and observation previously reported by Gazzola et al. (2007; Gazzola & Keysers, 2009).

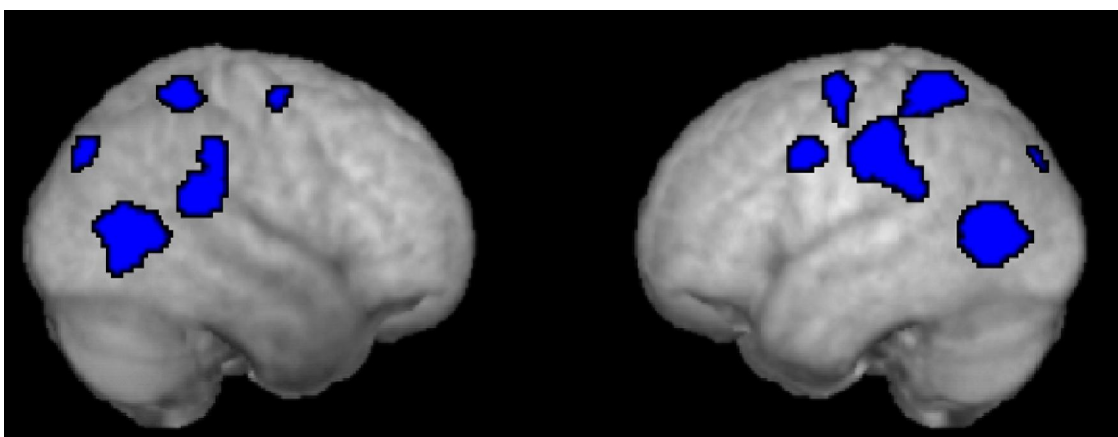


Figure 5.5 Regions of overlapping activity for both 'action observation' and 'action execution'.

5.4 Discussion

The results for the conjunction analysis revealed the existence of various regions of overlap in which activations related to both observation and execution of grasping actions were independently significant. The fact that areas showing possible ‘mirror’ type of activity exceed those considered as being part of the core ‘mirror’ system supports recent neuroimaging studies in both humans and monkeys (Gazzola et al., 2007; Gazzola & Keysers, 2009; Raos et al., 2007; Evangeliou, et al., 2009). In ‘human’ terms, Gazzola et al. (2007; Gazzola & Keysers, 2009) reported a pattern of activation within a network involving temporal, parietal, and frontal areas similar to ours. In ‘monkey’ terms, two studies (Raos et al., 2007; Evangeliou et al., 2009), by using the quantitative deoxyglucose method to map the activity pattern throughout the cortex of macaques, have found overlapping activity for the generation and the perception of hand actions within posterior parietal, somatosensory, motor and premotor cortices. Importantly, these latter results confirmed previous ‘human’ neuroimaging studies revealing activations for action execution and observation within the primary motor and somatosensory cortices, the dorsal and ventral premotor cortical areas together with medial cortical and the cingulated areas.

Altogether these and the present findings suggest that the resonant system responding to both action perception and action generation encompasses much more of the cortex than the mirror neuron concept would lead one to believe, suggesting the existence of a broader process possibly entailing mental simulation of action (Goldman et al., 2005; Raos et al., 2007; Gazzola et al., 2007; Gazzola & Keysers, 2009; Evangeliou et al., 2009).

6. The response of the human ventral premotor ‘mirror’ region to the type of observed agent ⁴

Abstract

Recent neuroimaging evidence in macaques (Nelissen et al., 2005) has shown that mirror area F5c is modulated by whether an observed action is performed by a person in full view or an isolated hand (i.e., type of agent manipulation). Although a human homologue region has been identified, whether in humans the neural processes involved in this capacity are modulated by the type of observed agent remains unknown. Here I used fMRI to investigate this issue, i.e. whether this ‘mirror’ region within the ventral premotor cortex, responds differentially depending on the type of agent. In contrast to monkeys, results from region of interest (ROI) analyses showed that this area responded in a similar fashion following the observation of either an isolated hand or an entire model acting.

⁴*Published.* Turella, L., Erb, M., Grodd, W., & Castiello, U. (2009). Visual features of an observed agent do not modulate human brain activity during action observation. *NeuroImage*, *46*, 844-53.

6.1 Introduction

Recent findings from an fMRI study investigating the neural underpinnings of action observation in monkeys add a further level of complexity regarding the visual requirements necessary to activate the ‘observation’ component of the mirror system (Nelissen et al., 2005). Monkeys observed video clips showing either a full view of a person or an isolated hand grasping objects and, as control condition, static single frames or scrambled videos. It was found that premotor area F5c (the area in which mirror neurons were first discovered) was active only when the monkey observed a human model presented in her entirety grasping an object, but it was not active when it observed a human hand detached from the body performing the task. In the other subregions of F5 (i.e., F5a, F5p) activation due to action observation was reported for both the model and the hand alone acting. These results seem to suggest that different types of agent alerts specific sectors of the premotor cortex and this occurs despite the goal for the two agents, both biological in nature and presumably showing similar kinematics, remains the same. Therefore it might well be that visual features of agents are as important as action goal for modulating action observation activity, at least within the core ‘mirror’ area F5c.

Here I capitalize on the above mentioned findings to investigate for the first time whether in humans, as happens in monkeys, the human homologue region of monkey F5c is differentially activated depending on the type of agent irrespective of action goal. This is a reasonable question to ask considering that a number of fMRI studies in humans have shown that action observation in humans evokes widespread frontal activation, including that of premotor area 6 and of prefrontal areas 44 and 45 (Gazzola et al., 2007). Therefore, I defined the homologue human ‘mirror’ region in ventral

premotor cortex and then I tested the effect of observing a grasping action performed either by a fully visible model or by a hand alone.

6.2 Materials and Methods

Please note that all methodological procedures were similar to those described in Chapter 5.

6.2.1 Participants

The participants were the same who participated in the experiment reported in Chapter 5.

6.2.2 Stimuli

Four different types of video clips served as stimuli (4 s duration, AVI format, Xvid codec compression, resolution 360x240, 25 frames per second). As shown in Figure 6.1 these video clips could represent: i) a human model grasping an object ('model grasping', see Figure 6.2A); ii) a static human model with the hand resting on the table in the proximity of the object ('model static', see Figure 6.2B); iii) a human hand grasping an object ('hand grasping', see Figure 6.2C); and iv) a static human hand resting on the table in the proximity of the object ('hand static', see Figure 6.2D). The 'hand' video clips were created from the 'model' video clips. Specifically, this was done by editing the video in which the body of the human model was entirely visible. This ensured that exactly the same movement was presented for all 'action' conditions, therefore eliminating possible differences in kinematics which may confound data interpretation. Two different models (a female and a male) performed the action for a total of 30 videos for each condition. The video clips depicted either the model or the

model's hand from the right side. Both the 'model' and the 'hand' videos were resized to 360x240 pixels in order to keep the same resolution. The resized videos were then compressed and their quality was the same in all conditions.

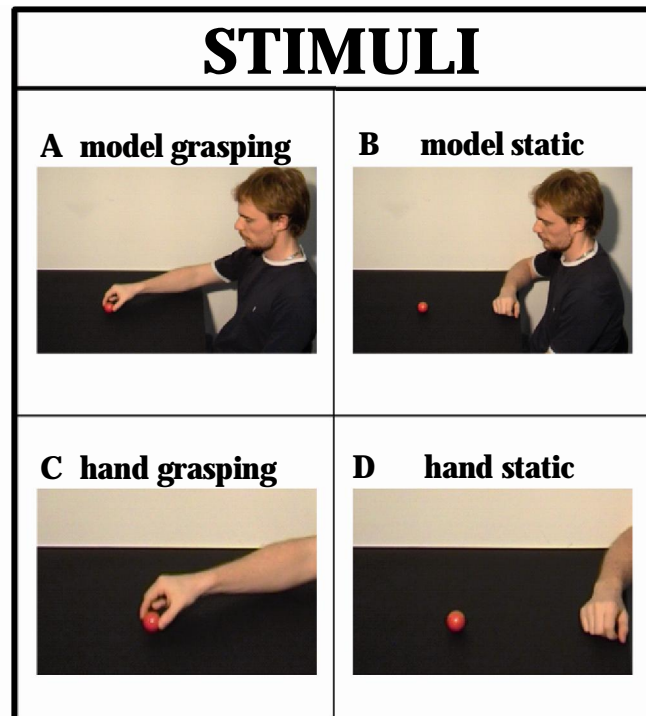


Figure 6.1. Stimuli. Frames extracted from the videos for each experimental conditions characterizing the 'observation' component of the experiment. From top left to bottom right the following conditions are represented: A) 'model grasping', B) 'model static', C) 'hand grasping' and D) 'hand static'.

6.2.3 Procedures and task

The procedures and task was the same as in Chapter 5. The subjects were instructed to observe carefully the video-clips.

6.2.4 Data Analysis

In order to make my analyses more consistent with the analyses performed in the study which inspired the present research (Nelissen et al., 2005), I performed a ROI analysis confined to one region, i.e. the ventral premotor cortex. This area was chosen as to compare the most probable functional homologue region between my study and

the Nelissen et al. (2005) investigation. Further, a recent meta-analysis on human fMRI data regarding action observation and execution supports my choice indicating that the area in the human brain consistently activated in both conditions is the left ventral premotor cortex (Chouinard & Paus, 2006). Because in humans it is more difficult than in monkeys to determine a precise localization for this ventral premotor ROI, I determined the ROI on the basis of functional properties. Specifically, the ROI was identified by means of the results obtained from the conjunction analysis defining regions activated during action observation and execution (see Chapter 5). The ROI analysis was performed on the mean percent signal change (PSC) extracted using Marsbar SPM Toolbox (Brett et al., 2002) from all the voxels within the selected region. PSC data were analysed by means of an ANOVA similar to that performed for the whole-brain ‘observation’ part. This analysis included two within-subjects factors, ‘type of observed task’ (grasping, static) and ‘type of view’ (model, hand).

6.3 Results

An ANOVA, conducted on the PSC for the entire region of overlap within the ventral premotor cortex, yielded only to a significant main effect of ‘type of observed task’ ($F_{1,15} = 89.4$, $p < 0.001$, see Figure 6.2A for a plot of the data and Figure 6.2B for a localization of the ROI). Both the main effect of model and the interaction between the two factors were not significant ($F_{1,15} = 1.36$, $p = 0.263$ and $F_{1,15} = 0.07$, $p = 0.800$, respectively). On the basis of these results I reasoned that testing the mean effect of an entire ROI (comprehending 99 resampled voxels with a resolution of 2x2x2 mm) might not be representative of the real effects occurring in this region. More subtle effects could be present in subareas of the ventral premotor ROI. Indeed, when performing a ROI analysis a strong assumption is made, that is that all the voxels have a

homogeneous pattern of activation. Normally this is not tested, but taken as true. Therefore, to remove this possible confound I performed another analysis to test the presence or lack of effect in all the voxels within the ventral premotor ROI. I extracted every single voxel within the ROI. Each voxel was considered as an independent ROI. Then 99 separate ANOVAs on the PSC for each sub-ROI were conducted. Because of the exploratory nature of this analysis, I used no correction for the number of tested regions. None of the 99 voxels showed a significant interaction between the two considered factors ($p > 0.05$ uncorrected). But, all voxels showed a main effect of type of ‘observed task’ (grasping vs static, $p < 0.001$ uncorrected). This indicates that the results obtained for the original ROI analysis were not biased by restricting the analysis only to the mean timecourse of the entire region.

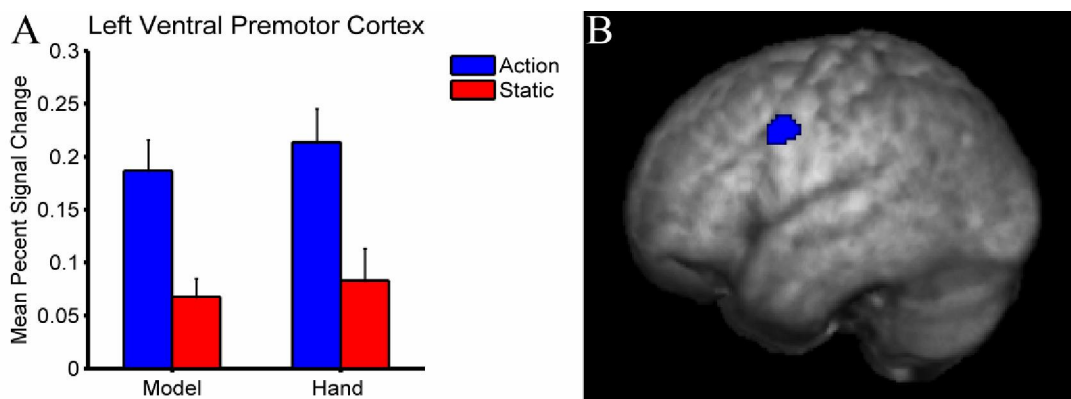


Figure 6.2. Human ‘mirror’ ventral premotor cortex. A) Mean percent signal change occurring for the four experimental conditions for the region of overlap within ventral premotor cortex extracted from all the voxels using Marsbar. B) Localization of the premotor ventral ROI (as extracted from the conjunction analysis) rendered on the mean anatomy of the group using MriCron software.

6.4 Discussion

A recent fMRI study in monkeys has defined subregions within area F5 and the adjacent prefrontal cortex responding to different properties of observed actions (Nelissen et al., 2005). Specifically area F5c responded only when the monkey observed a fully visible human model, but not when a hand alone was presented, whereas in other

subregions of F5 (i.e., F5a, F5p) activation due to action observation was similar for both a fully visible model and a hand alone grasping an object (Nelissen et al., 2005). Here I wanted to test whether a similar subdivision applies to humans.

Indeed on the basis of monkeys fMRI findings using a similar paradigm to mine (Nelissen et al., 2005) differences in action observation activity depending on type of view were expected, at least within premotor and other prefrontal areas. However, in our study the type of view had little impact at the level of action observation. I suspect that this might be ascribed to the fact that the processing of particular stimulus properties, which in principle should occur in homologue areas, might not be common to both species (Sereno & Tootell, 2005; Orban, Van Essen, & Vanduffel, 2004; Nakahara, Adachi, Osada, & Miyashita, 2007). In this perspective the conclusion would be that in humans the observation of a grasping hand alone (and an object) is sufficient to trigger significant differential activity (Morin & Grezes, 2008). Alternatively the lack of effects might be due to two methodological factors. The first stems from the difficulty in defining homologue areas between monkey and human even using the same approach (i.e., architecture, connections or function). The second refers to the nature of the presented stimuli. To elaborate, in the study by Nelissen and colleagues (2005) the presented stimuli greatly differ in terms of salience. For instance, the grasping hand for the ‘hand alone grasping’ stimuli was much more visible than the grasping hand for the ‘model grasping’ stimuli. Therefore it might not be a matter of having elicited different type of representations within the premotor cortices. Rather, of having triggered a differential level of activity for the ‘acting person’ stimulus in F5c. This is because it was more difficult to decode the aspect of the context which was more salient for the animal, that is the grasping hand. It might be reasonable to hypothesize that if the grasping hand was not easy to contextualize, then the presentation of such

stimuli may have produced significant BOLD signal increase with respect to when the grasping hand was easily coded ('hand alone grasping' condition). In this respect I took great care in preparing the stimuli for the present study, maintaining the proportions and kinematics for our stimuli similar. Importantly the videoclips for the hand alone condition were extracted from the videoclips in which the model was in full view. Therefore it is such consistency which might have allowed to reveal similar activations within the premotor cortex for the two types of stimuli. Such contention is supported, at least in humans, by a recent review examining activations in the premotor cortex during passive observation of actions (Morin & Grezes, 2008). The suggestion here is that it is the specificity of used stimuli which determines activity within specific areas of the prefrontal cortices.

7. General conclusions

7.1 Conclusive remarks

For humans, understanding the meaning of others' actions is a fundamental ability. It allows our species to survive in the complex environment which constitutes our society. In this respect, the discovery of mirror neurons has certainly helped to understand the neural mechanisms underlying this capacity. Although the initial research on the AOS focused on action observation and understanding, researchers from various domains have linked the notion of the human AOS system (or MN system) to a variety of behaviours such as imitation, empathy, speech processing, etc. The idea that the AOS might be implicated in all these processes is certainly appealing, but in my opinion, a bit premature when considering that the exact role and properties of the AOS during action observation is far from being fully understood. Therefore here I decided to step back and to further investigate the basic properties of the human AOS.

The first experimental study (Chapter 3) investigated the AOS in a population affected by a severe neurological disorder, i.e. relapsing remitting multiple sclerosis (RRMS) patients. The aim of the study was to test for the presence of a selective impairment within this network. The results showed a general, but unspecific, effect of BOLD over-activation in MS patients probably determined by the extensive demyelination caused by the disease and by the consequent repairing process carried out by our organism. This opens to the possibility that the 'spared' action observation system might be used for neurorehabilitation. Specifically, the ability of the neural system underlying action observation to re-enact stored motor representations has been utilized as a mean for rehabilitating motor control (action observation therapy; Ertelt et

al., 2007; Buccino, Solodkin, & Small, 2006). Therefore it might well be that the action observation therapy may help in bringing back to normal values brain activity related to action understanding in RRMS patients.

Then, in the second experimental study (Chapter 4), I focused on a more basic feature of the human AOS, the coding of not-object-related (intransitive) actions. This is a unique feature of the human AOS, because in non-human primates the AOS seems not to code for such types of actions. The central advance of this study is the demonstration that transitivity conveyed by previous knowledge regarding the presence/absence of a target-object modulates activity within the AOS. By manipulating this type of information it was revealed that the AOS is able to generate a representation of an observed action on the basis of the action goal, even when the most crucial part of the action (i.e., hand-object interaction) is not visible. A number of studies put forward the proposal that the human AOS responds to both transitive and intransitive actions (Buccino et al., 2001; Wheaton et al., 2004; Dinstein et al., 2007, 2008; Lui et al., 2008) suggesting that when a target-object is not present the AOS is still recruited. The present study investigates such issue using a controlled paradigm having the advantage of eliminating possible confounds. The presence of the partition allowed the introduction of two controls which were fundamental for the interpretation of the results. First, without the partition I would have made a direct comparison between fully visible object-related actions and pantomimed grasps which might not represent an optimal test for transitivity. The pattern of activity stemming from this contrast could have been ascribed to the presence of the object per se rather than to the representation of the action goal. To date, neurons responding to the simple observation of graspable objects (i.e., ‘canonical’ neurons; Murata et al., 1997; Murata, Gallese, Luppino, Kaseda, & Sakata, 2000; Raos et al., 2006; Rozzi et al., 2008) have been found within the very

same or nearby cortical regions in which cells responding to action observation were detected. Second, the introduction of the partition allowed me to use the same video-clips for the ‘grasping’ and ‘pantomime’ conditions, minimizing the potential confounds due to subtle differences in kinematics.

The present study add to the literature revealing that the AOS has the ability to discriminate and understand observed actions on the basis of the properties characterizing their goal (i.e. transitivity) rather than their perceived physical features. In this sense, I was able to find a modulation within the AOS with respect to the transitivity, suggesting that at least part of the system can be extremely selective in terms of processing the abstract properties of the goal characterizing the observed action.

The two final experimental Chapters (Chapters 5 and 6) of the present thesis were concerned with the definition/identification of the human ‘mirror’ regions and (if any) of what they code for. In general, the present results support the notion that in humans ‘mirror’ activity spreads across a number of areas which exceeds those classically thought to be part of the ‘mirror’ system (e.g., Raos et al., 2007; Gazzola et al., 2007; Gazzola and Keysers, 2009; Evangelidou et al., 2008). In details, it seems that activity within this network is triggered by both a model or a hand alone acting, and that is not modulated by the type of agent. In this respect, the present results highlight that it is the goal of an action which might be the most important determinant causing action observation activation. Further, a region of interest analysis (ROI) on the putative human region F5c indicate that, in contrast to monkeys (Nelissen et al., 2005), this region is activated in a similar fashion irrespective of whether the agent's entire body, or only the grasping hand, is seen.

Altogether these results are discussed in terms of the possibility that the AOS and the ‘mirror’ systems chiefly represent actions in terms of goals independently by contextual information (Ferrari et al., 2005; Gazzola et al., 2007). The idea is that what is represented in the premotor cortex is not bounded to the physical appearance of the agent, but it is a rather abstract representation centred on the goal of the action, independently of what is acting, a human, a robot, or even a tool. The present findings confirm and extend this notion by broadening the several dimensions within which action goals affect the response of the AOS and how such dimensions may vary across species. In humans, the observation of an effector alone (the hand) grasping a target-object is sufficient to activate the AOS. This aspect is particularly important because most of the human studies on the AOS have been conducted with movies zooming into the hand-part of the stimulus. If, as monkeys’ fMRI indicated (Nelissen et al., 2005), this were to cancel out key mirror areas, much of the human literature would have been challenged. The present data, however, show convincingly that this is not the case, at least in humans, and therefore enhance the validity of a large number of studies providing important and novel evidence within this flourishing field of research.

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Appendix A: information regarding magnetic resonance imaging sequences

Data acquisition information regarding the experiment reported in Chapter 3

All functional and structural images collected for the present study were acquired using a whole body 1.5T Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) equipped with a standard 8-channel head coil. Functional images were obtained with a standard single shot echo-planar (EPI) T2*-weighted sequence in order to measure blood oxygenation level-dependent (BOLD) contrast throughout the whole brain (TR = 3 s; TE = 50 ms; flip angle = 90°; 32 contiguous axial slices with a slice thickness of 3.5 mm/0.5 mm gap; FOV = 224 x 224 mm; matrix size = 64 x 64; in-plane resolution of 3.5 x 3.5 mm). 356 volumes were acquired in four scanning runs (89 volumes for each run). Immediately after the acquisition of the functional EPI volumes, a structural image of the brain was acquired for each participant with the following sequence: three-dimensional T1-weighted Fast Field Echo (FFE) sequences (TR = 25; TE = 4.6; flip angle = 30°; 120 contiguous axial slices with a slice thickness 1.2 mm, FOV = 250 x 250 mm, matrix size = 256 x 256, in-plane resolution of 0.98 x 0.98 mm).

Data acquisition information regarding the experiment reported in Chapter 4

Whole-brain data were acquired on a three Tesla Magnetom Trio Scanner (Siemens, Erlangen, Germany) equipped with a 12-channel head array RF coil. Functional images were obtained with an EPI T2*-weighted sequence in order to measure blood

oxygenation level-dependent (BOLD) contrast throughout the whole brain (TR = 2000 ms, TE = 33 ms, field of view 192x192 mm, matrix 64x64, in-plane resolution 3x3 mm, slice thickness = 3.5 mm, 0.7 mm gap, 32 slices). Scans were acquired for each participant in different scanning runs, three runs (462 volumes each) for the observation part of the experiment and one for the localiser (425 volumes). The first 5 volumes of every run were discarded due to initial instability in the signal of EPI images. High-resolution T1-weighted images were also acquired for each participant during the experimental session (MP-RAGE, TR = 2300 ms, TE = 3.03 ms, flip angle = 8°, field of view 224 x 256 mm, 1 mm isotropic voxels, 160 slices).

Data acquisition information regarding the experiment reported in Chapters 5 and 6

fMRI data were acquired on a 3T scanner (Siemens Magnetom Trio, Erlangen, Germany) equipped with a 12-channel head array RF coil. Functional images were obtained with a gradient echo-planar (EPI) T2*-weighted sequence in order to measure blood oxygenation level-dependent (BOLD) contrast throughout the whole brain (36 slices, 3 mm isotropic voxel size, 0.75 spacing, inplane resolution of 64 x 64 voxels, FOV = 192 x 192 mm, flip angle = 90°, TR=2500 ms, TE = 35 ms). The first 5 volumes of every run were discarded from the analysis due to initial instability in the signal of EPI images. Scans were acquired for each subject in four scanning runs, two runs (437 volumes each) for the observation part of the experiment and two runs (229 volumes each) for the execution part. In addition, high-resolution T1-weighted images (anatomical scans) were also acquired for each participant (MP-RAGE, 160 slices, in-plane resolution 224 x 256, 1 mm isotropic voxels, TR = 2300 ms, TE = 3.03 ms, flip angle = 8°).

Appendix B: supplementary materials of Chapter 3.

TABLE B1: Demographic, clinical and MRI parameters of both early RRMS patients and healthy controls.

	<i>MS patients</i>	<i>Healthy controls</i>
Female/male	8/7	7/8
Mean age in years	30.6 (19-44)	34 (24-54)
Mean disease duration in months	16.2 ± 9.2 (3-34)	n.a.
T2-WM-LV	2.4 ± 1.7 (0.8-5.3)	0
CLs number	3.1 ± 2.1 (0-7)	0
BPf (%)	83.1 ± 3.2 (81.1-86.7)	82.8 ± 3.4 (81.2-85.8)
GMf (%)	39.1 ± 1.6 (36.2-41.1)	40.2 ± 2.1 (38.2-42.3)
WMf(%)	44.0 ± 1.8 (41.0-46.8)	42.6 ± 2.0 (40.9-46.3)
EDSS	1.5 ± 0.6 (1-3)	n.a.
DMT:		
None	5	n.a.
Immunomodulatory	10	

Data are reported as mean, ± standard deviation, and range in brackets. T2-WM-LV = T2 white matter lesion volume; CLs = cortical lesions; BPf = brain parenchymal fraction; GMf = grey matter fraction; WMf = white matter fraction; EDSS = expanded disability status scale; DMT = disease modifying therapy; n.a. = not applicable.

TABLE B2: Regions activated for the main effect of group

Peak localization	x	y	z	tvalue
<i>Frontal cortex</i>				
Left Precentral gyrus	-50	-4	46	12.72
Left Paracentral lobule	-10	-28	70	11.28
Right Precentral gyrus	50	2	36	10.57
Right Superior medial gyrus	10	66	8	6.78
Left Superior frontal gyrus	-14	32	48	6.17
Left Superior frontal gyrus	-20	64	0	5.49
Right Superior frontal gyrus	18	46	38	4.36
<i>Temporal cortex</i>				
Middle temporal gyrus	58	-38	2	8.46
Hippocampus	28	2	-36	8.04
Hippocampus	18	-10	-12	6.96
Inferior temporal gyrus	-62	-56	-6	6.66
Insula	28	18	-12	5.32
Middle temporal gyrus	-66	-46	4	5.24
Superior temporal gyrus	-66	-26	12	4.87
Insula	-32	-6	12	3.98
Inferior temporal gyrus	58	-46	-12	10.53
<i>Visual cortex</i>				
Fusiform gyrus	-22	-86	-18	24.22
Middle occipital gyrus	-22	-94	4	16.65
<i>Cerebellum</i>				
Cerebellar cortex (crus II)	-38	-66	-46	5.03
Cerebellar vermis	-2	-62	-40	4.98
<i>Subcortical structures</i>				
Amygdala	-24	-6	-14	4.10

Stereotaxic coordinates in MNI space are reported in mm.

TABLE B3: Regions activated for the main effect of 'condition' (Hand grasping > Hand resting).

Peak localization	x	y	z	tvalue
<i>Frontal cortex</i>				
Left Middle frontal gyrus	-34	60	14	4.61
Right Inferior frontal gyrus	54	20	28	4.42
Right Inferior frontal gyrus	52	36	-8	4.23
Left Inferior frontal gyrus	-48	40	14	4.11
Left Precentral gyrus	-54	10	30	3.99
<i>Parietal cortex</i>				
Right Postcentral gyrus	50	-22	38	5.82
<i>Visual cortex</i>				
Right Inferior occipital gyrus	28	-90	-6	11.89
Left Middle occipital gyrus	-36	-90	0	11.06
Right Superior occipital gyrus	26	-80	32	4.6

Stereotaxic coordinates in MNI space are reported in mm.

TABLE B4: Regions activated for the main effect of 'condition' (Hand resting > Hand grasping).

Peak localization	x	y	z	tvalue
<i>Visual cortex</i>				
Right Calcarine gyrus	4	-86	-2	8.40

Stereotaxic coordinates in MNI space are reported in mm.

Appendix C: supplementary materials of Chapter 4.

TABLE C1: Regions activated for the localizer.

Peak localization	x	y	z	tvalue
<i>Frontal cortex</i>				
Left Precentral gyrus	-26	-12	52	4.95
Right Precentral gyrus	28	-12	54	4.08
Left Superior Frontal gyrus	-14	-8	72	3.77
<i>Parietal cortex</i>				
Left Superior Parietal lobule	-36	-46	60	7.3
Right Intraparietal cortex	42	-38	54	5.39
Right Supramarginal gyrus	60	-24	42	4.26
<i>Temporal cortex</i>				
Left Inferior Temporal gyrus	-40	-46	-18	4.89
Left Superior Temporal gyrus	-46	-44	14	3.71
<i>Occipital cortex</i>				
Left Middle Occipital gyrus	-50	-72	4	14.19
Right Superior Occipital gyrus	24	-80	36	5.88
<i>Cingulate cortex</i>				
Left Posterior Cingulate cortex	-14	-26	34	3.72
<i>Cerebellum</i>				
Left Cerebellum Crus II	-4	-78	-40	4.02
Left Cerebellum lobule VI	-24	-40	-40	3.9
<i>Subcortical structures</i>				
Right Thalamus	16	-22	8	4.66
Left Thalamus	16	-30	2	4.58

Stereotaxic coordinates in MNI space are reported in mm.

TABLE C2: Paired *t*-tests for percent signal change within regions of interest.

ROI localization	Tested contrast	<i>t</i>value	<i>P</i>value
<i>Parietal cortex</i>			
Right aIPS	grasping > pantomime	3.032	0.007
	object > no object	-1.023	0.320
Left aIPS	grasping > pantomime	2.466	0.024
	object > no object	-1.301	0.210
<i>Temporal cortex</i>			
Right pSTS	grasping > pantomime	3.312	0.004
	object > no object	-1.723	0.102

aIPS = anterior intraparietal sulcus, pSTS = posterior superior temporal sulcus.

Appendix D: supplementary materials of Chapters 5 and 6.

TABLE D1: Regions activated for action observation.

Peak localization	x	y	z	tvalue
<i>Frontal cortex</i>				
Left Precentral gyrus	-28	-12	56	8.57
Left Precentral gyrus	-54	4	38	6.68
Right Precentral gyrus	34	-6	56	5.59
<i>Parietal cortex</i>				
Left Postcentral gyrus	-34	-42	58	9.89
<i>Temporal cortex</i>				
Right Middle Temporal gyrus	44	-66	0	11.43
Left Middle Temporal gyrus	-40	-68	4	9.29
Right Superior Temporal gyrus	66	-36	18	8.07
<i>Occipital cortex</i>				
Superior Occipital cortex	-24	-84	34	6.44
Superior Occipital cortex	26	-80	34	6.31
<i>Cingulate cortex</i>				
Left Cingulate cortex	-14	-24	40	8.87
Right Cingulate cortex	14	-22	44	5.98
<i>Cerebellum</i>				
Cerebellum: lobule VI	-12	-70	-20	5.48
Cerebellum: lobule VII	10	-74	-40	5.43
Cerebellum: lobule VI	32	-52	-26	5.00
<i>Subcortical structures</i>				
Thalamus	-14	-22	8	5.71

Stereotaxic coordinates in MNI space are reported in mm.

TABLE D2: Regions activated for grasping execution.

Peak localization	x	y	z	tvalue
<i>Frontal cortex</i>				
Right Superior Frontal gyrus	28	-6	66	13.84
Middle Frontal gyrus	-34	42	26	6.21
<i>Parietal cortex</i>				
Left Postcentral gyrus	-42	-22	56	24.7
<i>Temporal cortex</i>				
Middle Temporal gyrus	-42	-74	6	18.4

Stereotaxic coordinates in MNI space are reported in mm.

