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NEW EVIDENCE OF FUNCTIONAL INTERACTIONS WITHIN THE HAND MOTOR SYSTEM

PhD thesis

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NEW EVIDENCE OF FUNCTIONAL INTERACTIONS WITHIN THE HAND MOTOR SYSTEM

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a Nonna Rosetta,
a chi ha creduto in me,
e a chi continuerà a farlo.

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Chapter 1

Introduction

The ability to manipulate objects to interact efficaciously with the surrounding world is one of the capabilities that allowed human beings to stand out from other species. The characteristics of the human hand, with its opposable thumb, as well as the creation of tools to facilitate everyday life, represented a turning point in the history of our evolution. From this perspective, there is no doubt that an impairment in controlling hand actions, e.g. following a brain lesion, has dramatic social consequences and a devastating impact on most of the everyday life activities, such as eating, driving, getting dressed, etc. Understanding the organization of the neural substrates underlying hand actions is not only a very fascinating scientific question per se, but also it can positively affect the development of rehabilitative programs for patients with motor deficits, tangibly affecting their quality of life.

Performing a hand movement toward an object requires the computation of an enormous amount of information, transforming incoming sensory inputs into an appropriate motor output. To perform a successful interaction with an object, a series of processes are necessary. The target needs to be visually perceived and its intrinsic properties (e.g. size, shape and orientation) extracted and integrated. The spatial location of the object needs to be detected, as well as the position of the hand/arm with respect to it. Other relevant features that are processed and integrated are the function of the object and the way it has to be grasped to be suitably used. These neural computations are already happening before the action is executed, during the planning phase of a movement. Finally, the intention of the acting subject plays a significant role in leading which type of interaction will occur with the target: i.e. what does the subject want to do

with the object? Does he want to move it, to use it, to throw it? All these different types of information are processed and possibly combined already at a neural level to produce the final motor output that allows a successful interaction with the environment.

In the past decades, hand actions have been widely explored both using qualitative (e.g. clinical observation of patients' behavior) and quantitative observations (e.g. neuroimaging investigation). The first insights about the neural correlates of hand movements came from neurophysiological studies in monkeys and patient studies in humans. These studies have been essential in describing the role of brain areas in processing information leading to hand motor outputs, and helped in defining a general model of the main functional paths subtending visual processing within the brain (Goodale and Milner, 1992).

The influential model by Milner & Goodale proposed the presence of two main visual routes: the ventral stream, where visual information is used to perceive the surrounding world ("Vision for Perception"), and the dorsal stream, where visual inputs are processed to guide actions ("Vision for Action"). These two streams were considered specialized and independent (Goodale et al., 1991).

However, recent evidence unveiled a much more complex and dynamic organization, showing the functional interplay between the ventral and dorsal streams during specific tasks, such as delayed actions and pantomimes (Cloutman, 2013; Milner, 2017). This novel perspective raised unanswered questions about the dynamics occurring between different areas constituting these two visual pathways. Additionally, the dorsal stream includes two main parieto-frontal routes involved in hand actions that constitute the so-called 'hand motor network' (Rizzolatti and Matelli, 2003). The areas of the two streams process different action information that leads to a motor output. In the past decades, different studies explored the role and the representational content

of the hand motor network, revealing a specialized but also integrated neural system, where information processed by different areas are exchanged and integrated.

Thanks to the advent of more sophisticated methodologies (i.e. multivariate analysis), not only low-level motor features of hand actions (e.g. grip, direction of movement, etc.) can be explored, but also the representations at a higher hierarchical level (e.g. action's goal) can be studied.

With this thesis, I will firstly provide a general review of the main literature on the hand motor system, considering both animal models and the human brain (Chapter 1). Secondly, I will introduce the unanswered scientific questions in the field that we have tackled in three experimental studies. They will be presented in the central chapters (Chapters 2, 3,4). Lastly, the discussion about the main results and their relevance with respect to the current knowledge of the hand motor system will be provided.

1.1 Hand motor network in non-human primates and human brain

Historically, neurophysiological studies on non-human primates (NHP) provided one of the first descriptions of the neural correlates of hand actions. Before the advent of human neuroimaging, NHP' neurophysiological studies already provided crucial insights into the structure and the function of the hand motor network. From the pioneering study of Mountcastle (Mountcastle et al., 1975) to recent multisite and multielectrode recordings (Schaffelhofer and Scherberger, 2016), single cell recordings are still providing invaluable information for understanding the neural correlates underlying hand actions. Only recently, with the application of decoding approaches to fMRI data (multivariate pattern analysis - MVPA) (Gallivan and Culham,

2015), researchers managed to describe similar encoding within the human brain (see paragraph 1.1.2.3).

As previously mentioned, the dorsal stream comprises two distinct parieto-frontal pathways that subtend hand movements (Rizzolatti and Matelli, 2003). These two routes are: (i) the dorsolateral pathway that starts in the extrastriate visual areas of the occipital lobe and continues through the parietal lobe (anterior intraparietal area (AIP), inferior parietal areas (PF and PFG)) arriving at the frontal regions (ventral premotor area, area F5); (ii) and the dorsomedial pathway that starts in the same occipital visual areas and runs through the medial portion of the parietal lobe (V6A), reaching the frontal regions of the brain (dorsal premotor cortex, area F2). This subdivision has been found to be true both in human and monkey's brain. Figure 1.1 represents the two main dorsal pathways of the hand motor system in the human brain.
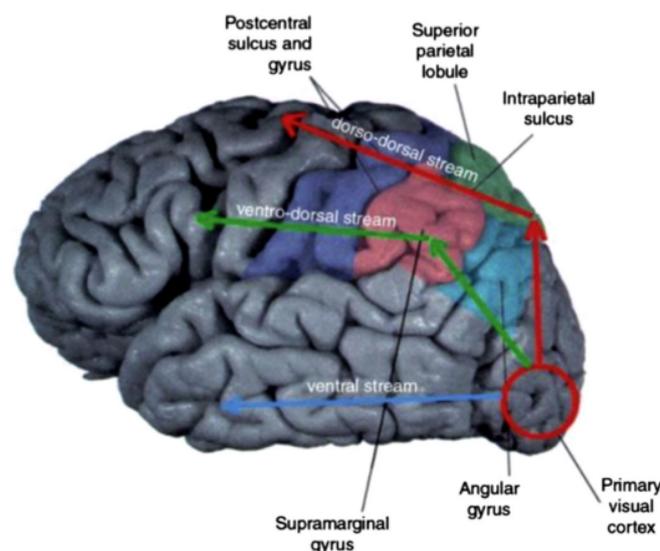
Figure 1.1: A classical subdivision of the hand motor network: Prehension, our capability to grab an object, has been traditionally decomposed into a reaching component, which consists in moving the arm toward the target, and a grasping component, in which the fingers of the hand are shaped to match the object size. Red arrows show the path of the dorsomedial pathway (from V1 to V6 and to the superior parietal lobule - SPL) traditionally associated with the reaching aspects of hand action. The green line shows the dorsolateral pathway (from V1 to the middle temporal - MT and intra-parietal lobule - IPL) traditionally associated with the grasping component of a prehension movement. Blue arrows show the ventral visual stream running through the temporal lobe. Adapted from Binkofski & Buxbaum (2013)

These two dorsal pathways have been traditionally described as separate and independent. They have been classically considered to be involved in prehension movement, which consists in the

capability to approach and grasp an object. This movement has been traditionally decomposed into a reaching component, which consists in moving the arm toward the target, and a grasping component, in which the fingers of the hand are shaped to match the object size. The dorsomedial pathway has been considered involved in the reaching component of the prehension movement, while the dorsolateral pathway in the grasping component (Rizzolatti and Matelli, 2003) (see Figure 1.2). Recent discoveries seem to challenge this view of two independent pathways, supporting a more integrated view based on the reciprocal interplay between these two streams (Nelissen and Vanduffel, 2011; Galletti and Fattori, 2017). However, this distinction is useful for an initial definition of the properties of the areas constituting the hand motor network.
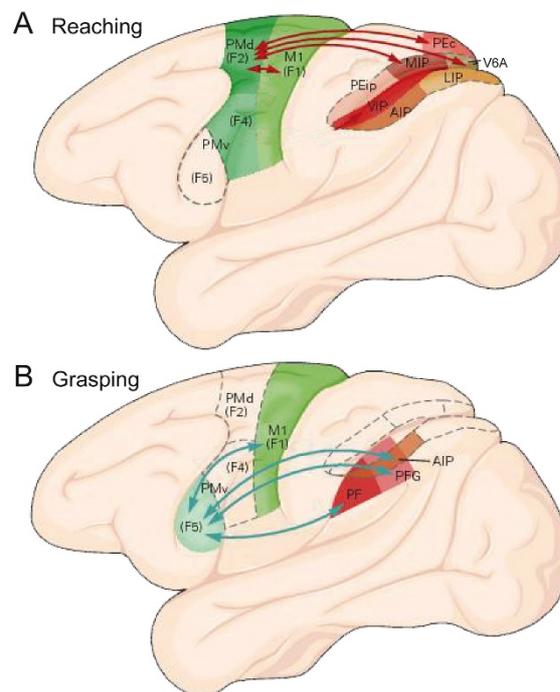
Figure 1.2: The dorsal pathway. A. Dorsomedial pathway: Dorsomedial/reaching (red) pathway including area V6A and F2, associated with reaching movements and **B. Dorsolateral pathway:** dorsolateral/grasping (blue) pathway including the anterior intraparietal area (AIP) and the (area PF and PFG) within the frontal lobe, and the ventral premotor cortex (area F5), associated with grasping movements. Adapted from Galletti and Fattori (2017).

In the following paragraphs, I will summarize the main functional characteristics of the areas constituting the monkey's hand motor network, and I will describe the homologies with respect to the human brain.

1.1.1 Action-related areas within the NHP brain

1.1.1.1 Neurophysiological studies

A series of monkey's studies explored the neural circuits involved in hand actions (for review see Rizzolatti et al., 2014; Janssen and Scherberger, 2015; Galletti and Fattori, 2017); here, I will focus on those studies relevant to frame the topic of this thesis.

In the late nineties, an increasing number of neurophysiological studies classified the neurons constituting some of the monkey's brain regions involved in hand actions and graspable object perception (see supplementary box 1). The main method used to investigate the properties of neurons in specific areas of the hand motor network is single-cell recording. With the insertion of a number of electrodes in a cytoarchitectonically and anatomically defined brain area, it is possible to register the firing rate of the neurons recorded during different experimental conditions. This method allows understanding which properties of the stimulus and/or of the action are preferentially processed.

For example, the properties of neurons within the lateral part of the dorsal stream, in particular within the intra-parietal sulcus (IPS) were investigated by training monkeys to fixate or to grasp different types of objects (Sakata et al., 1995; Murata et al., 2000). Within the anterior portion of IPS, the anterior intra-parietal area (AIP) has been described as the crucial hub involved in the transformation of sensorial inputs into motor commands. Within this region, the firing rates of the recorded neurons showed a preference during the observation of graspable objects and execution of grasping actions towards similar objects. Different neurons showed a preference for observing

and/or grasping objects with different shapes, sizes and orientations (Murata et al., 2000) (Figure 1.3). Visuomotor neurons, responding both for the visual observation of one object and for the grasping action performed over the same object, have been defined 'canonical' neurons, and have been proposed to be at the basis of the transformation of visual inputs into motor programs. Moreover, AIP activity is modulated by external cues triggering upcoming actions already during the planning phase of a delayed task (Baumann et al., 2009). Overall, AIP has been described as sensitive to the intrinsic features (i.e. size and shape) of the grasped objects, but also to the information extracted from the external context in which the action is performed.
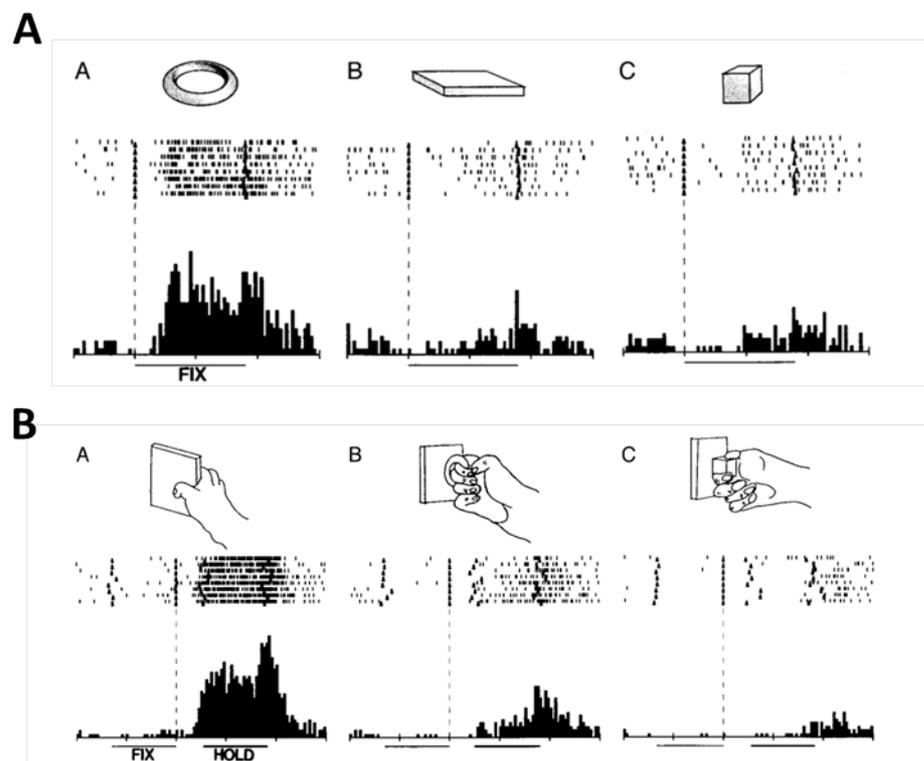
Figure 1.3: Object and grip representation in area AIP. **A.** Firing rate of visuo-dominant neurons. Objects were medium size (A. horizontal ring, B. horizontal plate, C. cube). Neurons showed strong preference for the horizontal ring. **B.** Firing rate of AIP visuomotor neurons. Objects were medium size (A. vertical plate, B. vertical ring, C. cube). Neurons showed strong preference for the vertical plate. Adapted from Murata et al. (2000).

Adjacent to AIP, on the IPL convexity, the inferior parietal area PFG is also involved in sensorimotor transformation for grasping movements and in the fine control of objects

manipulation. It has been shown that PFG activity in grasping actions can be modulated also during the planning phase of the action, and not only by the visual perception of the object (Bonini et al., 2011). Following studies demonstrated that PFG is also involved in high-level processing: the activity of half of PFG neurons is modulated by the final goal of the action (e.g. grasp-to-eat or grasp-to-place) and by the context in which the action is performed (e.g. external cues) (Bonini et al., 2012). Based on these findings, PFG has been described as a relevant region in action organization processes since both grip type information and high-level representations (i.e. goal of the action) are integrated here.

In the frontal lobe, other studies investigated the properties of the neurons within the ventral premotor area F5, finding similarities with AIP neurons in processing context-specific cues (Fluet et al., 2010) and grip type (Raos et al., 2006). This evidence suggested a pivotal role of F5 in selecting the most appropriate hand orientation and grip type.

In F5, two categories of visuomotor neurons with different peculiarities have been also described: (i) the 'canonical neurons', already found in AIP, which respond selectively to the visual presentation of 3D objects with specific size, shape and orientation, also when no motor response is required. This population seems to transform an object's features into potential motor acts (Jeannerod et al., 1995; Bonini et al., 2014);

(ii) the extensively debated 'mirror neurons', present also in PFG and in AIP, that fire also when the monkey observed the execution of a particular action performed by a different agent (Rizzolatti et al., 2014). For this characteristic, mirror neurons have been considered responsible for action understanding and action recognition.

All in all, F5 neurons seems to encode for the final goal of an action representing high-level information related to the aim of an action and not only based on the representation of the physical act itself (Borra et al., 2017).

The primary motor area (M1) represents the principal area involved in motor output, as it generates neural impulses that allow the contraction of peripheral muscles and the execution of movements. Recently, Schaffelhofer et al. (Schaffelhofer et al., 2015) showed that different hand configurations can be decoded in AIP, in F5 and in M1, both during the planning and the execution phase of a grasping action. The simultaneous recording within these three regions (Schaffelhofer and Scherberger, 2016) showed the cooperation of these nodes in creating the correct motor output, but each region maintained a certain degree of specialization. In general, AIP seems more related to processing visual information; M1 is dedicated to the execution of the prepared motor command; F5 functions as a hub where visual AIP-like information is stored temporarily and then transformed to motor information to be sent to M1, confirming the central role of F5 in visuomotor transformation.

Overall, the dorsolateral pathway seems to be included in a more extended lateral grasping system comprising additional regions within the fronto-parietal and temporal cortex with specific functional specialization (e.g. inferior frontal gyrus IFG, interior temporal IT). This complex temporo-parieto-frontal system supports the integration and exchange of different action-related information (e.g. perception of special relationships, storage of complex representation of actions). We focused on the main regions of the dorsolateral pathway, however, for a complete overview of all the involved areas please refer to Borra et al. (Borra et al., 2017).

The second route constituting the dorsal stream is the dorsomedial pathway, whose main nodes are: the parietal area V6A and the dorsal premotor cortex, area F2 (see Figure 2, Fattori et al., 2015; Galletti and Fattori, 2017).
V6A is a visuomotor area located in the superior and posterior part of the parietal lobule. It receives afferents from the more posterior visual area V6, and its activity is related to reaching properties, such as arm direction toward different spatial location (Marzocchi et al., 2008; Bosco

et al., 2010; Fattori et al., 2012, 2015; Breveglieri et al., 2016). However, V6A activity is not only involved in reaching, but it shows modulation to hand orientation and grip type both during the planning and the execution of a grasping movement (Fattori et al., 2009, 2010; Galletti and Fattori, 2017). This finding suggests the processing of grasp–related information also within the dorsomedial pathway. As in F5 and AIP, there are 'canonical neurons' also in V6A, particularly selective for shape, orientation and 3D features of the object even before the actual execution of a movement (Fattori et al., 2012).

In the dorsal premotor cortex, F2 neurons discriminate between different types of hand configurations (grip and hand orientation) during the execution of a movement (Raos et al., 2004). Together with AIP and F5, F2 contributes to process visually guided information sent from the dorsolateral areas and spatial inputs from posterior areas of the dorsomedial pathway (Raos et al., 2006).

These findings showed an involvement of dorsomedial and dorsolateral pathways in processing both reaching and grasping information, suggesting an integrated system of areas interacting with each other. It is more and more evident that the two dorsal pathways are not segregated and specialized in processing specific information. Different areas are involved in the same process and, according to the task, interact with each other to orchestrate the generation of a correct motor output (Galletti and Fattori, 2017).

Box 1: Motor, visuomotor and visual neurons in hand motor networks (following the classification of Sakata et al., 1995)

In 1995, Sakata. (Sakata et al., 1995) classified different categories of intra-parietal (IPS) neurons based on their functional properties. In their study, monkeys were trained to fixate or to grasp different types of objects both in the light and in the dark.

According to their neural response, neurons within the intraparietal sulcus (IPS) were classified in three different categories: i) 'Motor-dominant', if neurons showed maximum activity when grasping in the light and in the dark, but do not show activity in the fixation condition; ii) 'Visual-dominant', also subdivided in 'object-type', if neurons were fully activated by object fixation and object manipulation; and 'Non-object-type', if neurons were not activated during object fixation in the light nor during manipulation in the dark; iii) 'Visual and motor', subdivided in 'object-type' when showing selectivity for the object to grasp, particularly when grasping in the light compared to the dark condition, and discharging during the fixation of the preferred object; and 'Non-object-type', highly active during the manipulation of a specific object, more in the light than in the dark. This last group of neurons does not show activity during the fixation of the object.

Referring to this classification, and considering following studies investigating different brain regions, it is possible to describe the functional properties of the main areas of the monkey's prehension network based on this first classification.

SAKATA LIKE NEURONS CLASSIFICATION:

F5	AIP	PFG	V6A	F2
Somatotopically organized; represent specific hand (i.e. PG-WH grip), mouth movements and object features (i.e. orientation); respond to 3D objects fixation ('canonical neurons'); involved in distal movements and specific goal-directed actions	Sensorimotor transformation (i.e. size, shape, orientation, 3D features) for object-oriented actions (i.e. grasping, hand and fingers movements). Mirror-like properties of neurons	Sensorimotor transformation. Fine control of object manipulation. Integration hub for high (i.e. goal) and low (i.e. physical features) levels of action representations.	Represent reach and grasp movements. Its activity is modulated by hand orientation	Represent both reach and grasp movements planning and execution. Neurons are modulated by wrist orientation and type of grasp.
MOTOR NEURONS Active only during grasp execution	MOTOR DOMINANT NEURONS Active for both grasping in the light and grasping in the	MOTOR DOMINANT NEURONS Selectivity for hand grip	MOTOR DOMINANT NEURONS Active during reach-to-grasp execution both in	MOTOR NEURONS Active only during grasp execution

AIP	PFG	F5	V6A	F2
	dark. Silent during object fixation		the light and in the dark	
VISUO-MOTOR NEURONS Active during grasp execution and during the visual presentation of specific objects	VISUO-MOTOR NEURONS More active for grasping in the light, less active for grasping in the dark. Respond when the object is presented		VISUO-MOTOR NEURONS Active during reach-to.-grasp task both in the light and in the dark but with different strength	**VISUO-MOTOR NEURONS** Active during grasp execution and during the visual presentation of specific objects
	VISUAL DOMINANT NEURONS Active during grasping in the light and during object fixation. Not active when grasping in the dark		**VISUAL DOMINANT NEURONS** Active during the execution of reach-to-grasp task only in the light	VISUALLY MODULATED NEURONS They lose their grip and wrist orientation specificity when grasping in the dark
Anatomically connected with AIP, M1, S2 thalamus, cerebellum, prefrontal cortex F2	Anatomically connected with parietal F5, c-IPS, IPL, CIP, V6A S2, temporal MTG, TEa, TEm, ITC, frontal area 46 and 12, FEF	Anatomically connected with AIP and F5	Anatomically connected with parietal MIP, PEc, AIP, LIP, area46, S1, V6, frontal F2	Anatomically connected with F5, V6A, thalamus, M1
A lesion caused the incapacity to preshape the hand using visual feedback. Reaching preserved	A lesion caused the incapacity to correctly preshape the hand using visual feedback		A lesion caused misreaching and misgrasping, exaggerated fingers extension and erroneous wrist orientation	

References

AIP: (Sakata et al., 1995; Murata et al., 2000; Baumann et al., 2009); PFG: (Bonini et al., 2011, 2012); F5: (Jeannerod et al., 1995; Murata et al., 1997; Raos et al., 2006; Fluet et al., 2010; Bonini et al., 2014; Rizzolatti et al., 2014; Schaffelhofer et al., 2015; Schaffelhofer and Scherberger, 2016; Borra et al., 2017); V6A: (Marzocchi et al., 2008; Fattori et al., 2009, 2010, 2012, 2015; Bosco et al., 2010; Breveglieri et al., 2016); F2: (Raos et al., 2004).

1.1.1.2 Lesion perspective

The functional properties of the areas within the dorsal pathways have been confirmed by lesion studies. Different types of motor deficits have been described depending on the lesioned cortical site. With respect to the dorsolateral pathway, both AIP and F5 are involved in coding different types of grips to match with the object features. A temporary lesion (muscimol injection) in AIP leads to the incapacity of pre-shaping the hand using visual feedback (Gallese et al., 1994; Janssen and Scherberger, 2015); as well, a lesion in F5 leads to similar deficits (Fogassi, 2001). With respect to the dorsomedial pathway, surgical removal of V6A determines misreaching and erroneous grasping movements (Battaglini et al., 2002; Galletti et al., 2003). This result was one of the first suggesting a possible processing of grasping information within the dorsomedial pathway, which was later demonstrated using neurophysiological recording (Fattori et al., 2004, 2009, 2010).

1.1.1.3 Monkey's fMRI studies

Functional magnetic resonance imaging (fMRI) allows measuring metabolic changes in the brain during different experimental conditions (univariate approach). Recently, novel decoding methods permitted also to compare the neural pattern of activity subtending the representation of various experimental conditions (MVPA approach) (see paragraph 1.1.2.3). These two different approaches give different, but complementary, information about the function and organization of the hand motor network.

There are only a few fMRI studies investigating the neural correlates of hand actions in monkeys. The first investigation (Nelissen and Vanduffel, 2011) performed a univariate analysis and showed higher activity within AIP, PFG and F5 when comparing reaching-and-grasping (in the

dark) with reaching-only (Figure 1.4). These results supported the central role of the dorsolateral

pathway in the execution of grasping movements.
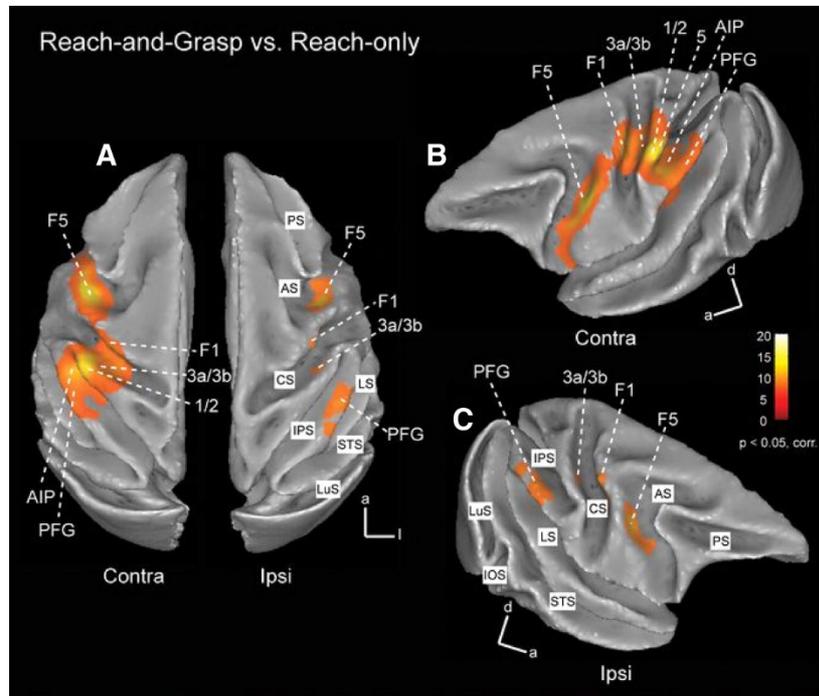
Figure 1.4. Monkey fMRI. Neural correlates of grasping: Activation for the univariate contrast [reach-and-grasp vs reach-only] activation map is overlaid on a 3D representation of a macaque brain. Adapted from Nelissen et al. (2011).

A second study (Nelissen et al., 2017) used MVPA to investigate how grasping is encoded

within the hand motor network. They found that AIP, PFG and F5 significantly decode grasping

related information. Moreover, significant decoding was also evident within the dorsomedial

pathway in V6A and F2. These MVPA results confirmed previous neurophysiological findings

showing the processing of similar information in both dorsal pathways, and supported the idea of

an integrated motor network, which relies on the interplay between dorsomedial and dorsolateral

areas.

1.1.1.4 Connectivity evidence

Different studies investigating the connectivity profiles within the hand motor network showed that dorsomedial and dorsolateral pathways do not only share similar functional properties, but they are also anatomically connected (Davare et al., 2011) (Figure 1.5).

Areas belonging to the same pathway are anatomically connected one with the other, and anatomical connections have been identified also between the two pathways. The presence of reciprocal anatomical connections between dorsomedial and dorsolateral streams support their possible functional interaction, as suggested by recent investigations showing similar encoding within the two streams (Galletti and Fattori, 2017).
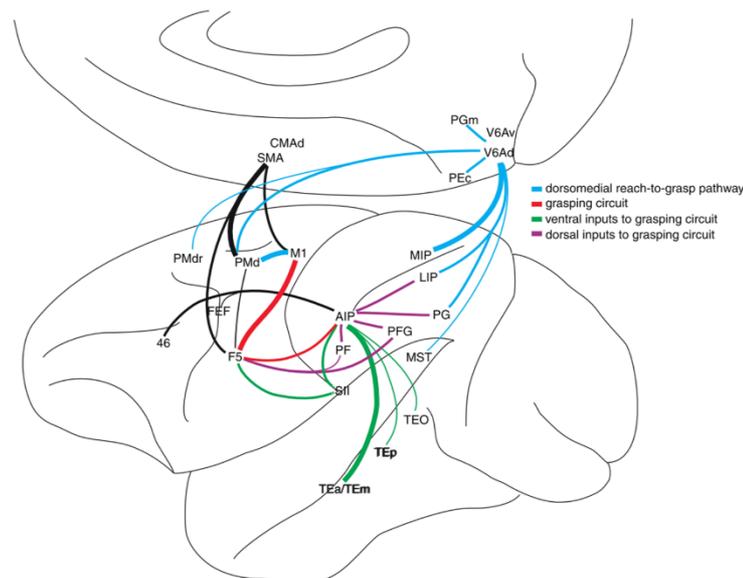
Figure 1.5. Anatomical connections between nodes of the monkey's hand motor network. Adapted from Davare et al. (2011).

Within the dorsolateral pathway, AIP is connected with visual areas in the occipital lobe (V6A), somatosensory and parietal areas (SII, F5, IPS, IPL), frontal areas (area 46) and also with regions of the temporal lobe (i.e. MTG) (Borra et al., 2008). AIP is also reciprocally and strongly connected with the premotor area F5. This connection between AIP and F5 supports the idea of a functional

circuit where AIP process object's features mainly relying on visual feedback, while F5 is more engaged in translating the inputs processed by AIP into motor-related information (Rizzolatti and Matelli, 2003; Raos et al., 2006; Janssen and Scherberger, 2015). F5 is also linked, among others, with the dorsal premotor cortex (F2), the somatosensory area (SII), the primary motor cortex (M1). Recently, Bonini et al. (Bonini et al., 2012) suggested to include to the AIP-F5 hand-grasping circuit also the intraparietal PFG area. This area is connected with AIP and F5, and the properties of its neurons are similar to those observed in F5.

Within the dorsomedial pathway, V6A is anatomically connected with the adjacent visual area V6, the premotor area F2, the dorsolateral AIP and with the primary somatosensory area (SI) (Gamberini et al., 2009). Area F2, in addition to the connections with V6A and F5, has direct projections to M1.

It is evident that there are strong connections among areas of the dorsolateral and dorsomedial pathways, as there are anatomical pathways linking their parietal and premotor nodes. This allows the transfer and the integration of different information between different cerebral nodes of the hand motor network through these specific anatomical pathways.

Anatomical connections have also been found between dorsal and ventral streams (Figure 1.5), linking temporal regions with areas of the dorsolateral pathway within the inferior parietal lobule (IPL). The dorsolateral pathway might represent a crucial hub where functional interactions between the dorsal and ventral streams occur (Davare et al., 2011). For example, Premereur et al. electrically stimulated different regions of monkey IPS (e.g. anterior and posterior AIP) and, using fMRI, described effective connectivity between IPS and the ventral stream (Premereur et al., 2015). Nonetheless, very few is known about the timing and the dynamic of these interactions.

In summary, connectivity studies showed the existence of structural pathways permitting the possible communication (i) within the two pathways of the dorsal stream and (ii) between the

dorsal and the ventral streams. The anatomical connections between these different brain pathways suggest the possible exchange of information between cortical regions, supporting a more integrated and dynamic view of the hand motor system (Galletti and Fattori, 2017), as opposite to the traditional one asserting segregated pathways with a modular organization (Goodale and Milner, 1992). However, what is encoded within these pathways and how information is transferred is still poorly understood and investigated. Starting to answer this question is the main aim behind the present thesis.

In the supplementary Box 2, I will introduce a specific type of action (tool actions) used to investigate the functional interaction between dorsal and ventral stream. This information will be useful later on to clarify the role of temporal areas in motor control.

Box 2: Learning to use a tool: Orchestrating complex hand actions in a monkey's brain

In the first lines of this introduction, we argued that the ability to manipulate the surrounding world and to create tools to facilitate our daily activities is one of the abilities that distinguishes humans from other species. In light of this ability, we define ourselves as *Homo faber*: a man who uses and makes tools (Iriki 2005). Nonetheless, the human species is not the only one able to use tools in order to achieve different goals. A very famous study on tool use in NHP is the one of Kohler (Kohler 1927). Here, a chimpanzee who wanted to eat bananas hanged on its cage ceiling, after some time of reasoning, piled some boxes and climbed them to reach the top of the cage and eat the fruit. This study showed the ability of chimpanzees to use objects within the environment as tools to accomplish a specific goal. Additional studies, however, showed that rarely monkeys spontaneously use tools (Tommasello, Call 1997), but they can be trained to do so. In 2009, Quallo et al. (Quallo et al., 2009) reported changes in grey and white matter of macaque monkeys after they were trained to use a rake to retrieve food. These changes were evident in the

superior temporal sulcus, second somatosensory area and intraparietal sulcus of the right hemisphere, and to a lesser extent also in the left hemisphere. These results show that both the dorsal and ventral streams are structurally modified when the brain 'learns' to perform tool actions.

When a monkey learns to use a tool, the neural representation of tool-related actions seems to be integrated into the existing hand motor network. In a single-cell recording study, after a training in which monkeys learnt to use simple and reverse pliers, Umiltà et al. showed that some neurons within F5 and to a less extent within M1 discharging for hand grasping actions, started to fire also when grasping with a tool (Umilta et al., 2008). The authors stated that the monkey motor system is based on a goal-centered functional organization, which allows primates to achieve a goal either with their hand or with a tool. Following studies on the human brain partially confirmed these findings, showing a common neural substrate recruited when using tools and when performing hand actions (see paragraph 1.1.2.4) (Lewis, 2006; Gallivan and Culham, 2015).

1.1.1.5 Summary

Overall, single-cell recordings, lesion, imaging and connectivity studies support the idea of an integrated network involved in hand actions. The two parieto-frontal routes constitute a functional hand motor network, where visual, sensory and spatial information is processed and integrated to produce meaningful motor outputs. Moreover, recent findings further suggested that the two dorsal pathways do not differ for the type of information they process (reaching vs. grasping), but rather differ for the level of action information represented within each pathway (e.g. online control of the action vs. processing of object-related information (Verhagen et al., 2008); action organization vs. online control of the action (Rizzolatti and Matelli, 2003)). Nevertheless, the representation of similar action-related information within the two dorsal

pathways suggests their functional interaction. Moreover, anatomical connections between dorsal and ventral pathways might be used to transfer action information. Based on this evidence the ventral stream might be considered as part of the hand motor network.

Based on their connectivity profiles, it is plausible to assume that these three pathways interact. However, the dynamics of these interactions are still poorly understood.

Understanding the functioning of hand motor network in monkey represents a useful starting point to explore the neural correlates subtending the same functions in the human brain. However, the approach adopted to investigate this issue in humans is radically different to the ones adopted in monkey studies. Indeed, human scientific investigation is performed mainly with non-invasive neurophysiological methods (i.e. transcranial magnetic stimulation - TMS), neuroimaging techniques (i.e. fMRI) and machine learning approaches. As a consequence, possible homologies between the two species are not always straightforward to draw.

1.1.2 *From monkey to human brain: Hand motor network within the Human brain*

Monkeys' studies have been useful to understand the human motor system. In humans, one of the first associations between movement production and specific brain regions comes from the observation of patients suffering from apraxia, i.e. motor impairments following brain lesions (Liepmann, 1905) (for supplementary information see Box 3). The advent of neuroimaging techniques (fMRI, positron emission tomography - PET, magneto encephalography -MEG), and of non-invasive neurostimulation (TMS) allowed to explore the neural substrate of hand actions and to investigate the causal involvement of specific brain areas in different experimental conditions/tasks. Following neurophysiological studies in NHP, two different dorsal pathways have been identified also in humans, constituting the homologous of the monkey's hand motor network. There is a general correspondence between the areas described in the monkey's brain

and those recruited in the human brain, giving a starting point for the interpretation of the results of human studies.

In the following sections, I will summarize the most relevant investigations for the present thesis about hand action processing in the human. A particular focus will be given to functional imaging (i.e. fMRI), transcranial magnetic stimulation (i.e. TMS) and connectivity studies.

Box 3: Lesion studies

In the second half of '800, medical doctors described the association between the location of a brain lesion and deficits in producing motor outputs, inferring the properties and the information processed by a cerebral area (Liepmann, 1905). One of the classical tests administered to clinical patients consists in imitating, pantomiming or performing the use of a familiar tool. Clinical studies of patients with focal brain lesions demonstrated that tool-manipulation and semantic knowledge about tools function are represented in a pool of anatomically distant brain areas called tool-network. Overall, patients with lesions in the motor centers of the tool-network (left parietal lobe) are affected by ideomotor apraxia. They preserve the knowledge of what they should do in order to manipulate a tool, but they are not able to activate the correct motor sequence to perform it correctly (they know what, but they do not know how). On the other hand, patients with lesions in the conceptual centers of the tool-network (left temporo-parieto-occipital areas) show conceptual apraxia: they do not know what to do with a given object; they do know how to use a tool. These observations support the functional segregation of dorsal and ventral streams, and highlighted the central role of the left hemisphere in processing tools information. Indeed, the big majority of apraxic patients showed a lesion in the left hemisphere (Johnson-Frey, 2004). Other recent evidence corroborated the left lateralization of the praxic system. Adopting lesion-mapping techniques different groups (Buxbaum et al., 2014; Hoeren et al., 2014) showed a

predominant involvement of the left hemisphere when evaluating the site of lesions associated with apraxia and/or tool use deficits. Interestingly, patients with left posterior temporal gyrus lesions (Buxbaum et al., 2014), showed deficits in tool related gestures performed with both right and left hand, supporting the role of the left hemisphere in controlling both hands when pantomiming. However, findings coming from often older clinical populations can be difficult to interpret due to the traumatic nature of the deficit and the comorbidity with other impairments. Only recently, thanks to imaging studies on healthy subjects, it has been possible to describe the neural basis of tool manipulation (Lewis, 2006; Gallivan et al., 2013a; Brandi et al., 2014; Valyear et al., 2017) (see paragraph 1.1.2.4) and new evidence suggested a functional interaction between dorsal and ventral stream, particularly when performing tool-related actions.

1.1.2.1 Neuroimaging studies of the human brain

Functional MR evolved enormously in the past 25 years. This methodology combines a good spatial resolution (standard voxel size: 3x3x3mm) with a reasonable temporal resolution in detecting physiological changes in the neural metabolism (BOLD peak: around 6 seconds after stimulus onset). These characteristics represent an acceptable compromise for studying non-invasively brain functioning. Nonetheless, investigating the neural correlates of hand movement with fMRI can be particularly difficult, due to the limited space within the scanner and the high risk of producing movement artifacts on the MR images. To overcome these limitations, different groups realized MR compatible setups to be able to present real objects to interact with within the scanner. In the first experiment of this kind, Jody Culham's group developed a so-called grasparatus (Figure 1.6) a rotating support with different compartment each one containing a different object to grasp (Culham et al., 2003).
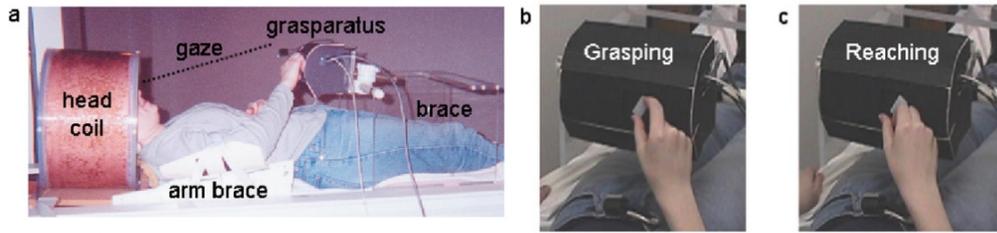
Figure 1.6: Experimental setup. A. MR setup: head of the subject tilted, grasparatus with target object placed on subject's pelvis. **B.** Grasping action. **C.** Reaching action. Adapted from Culham et al. (2003)

fMRI data can be analyzed in different ways. The first approach consists in univariate analysis, which is based on the comparison between neural activity evoked by a specific experimental condition with the baseline or on the comparison of different conditions. This approach showed that the execution of hand movement recruited a parieto-frontal motor network within the human brain similar to the one described with neurophysiological recordings in monkeys (Culham et al., 2003) (Figure 1.7a). Moreover, it demonstrated that grasping an object, compared to reaching it, induce greater activity within parietal areas, particularly within the anterior intraparietal sulcus (aIPS, the possible human homologous of monkey's AIP) and the superior parietal lobule (SPL) (see figure 1.7b). These findings suggested the possibility of establishing homologies between the monkey and human brains based on specific motor features, e.g. a similar selectivity for grasping.
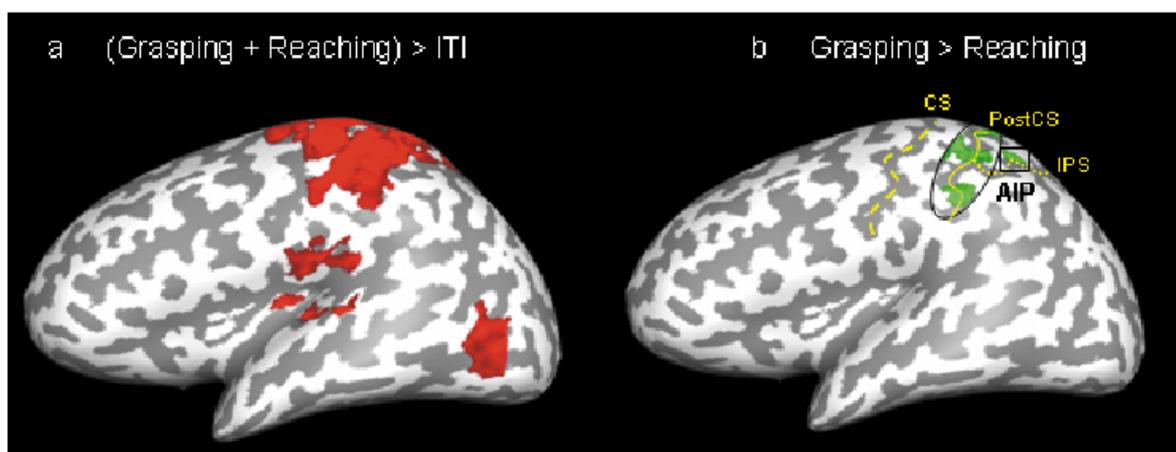
Figure 1.7: Univariate activation maps overlaid on 3d inflated brain template: A. Activity for the univariate contrast [grasping+reaching vs baseline]. **B.** Activity for the univariate contrast [grasping vs reaching]. Adapted from Culham et al. (2003).

A similar selectivity for aIPS function has been confirmed in a more recent study (Cavina-Pratesi et al., 2010). Moreover, the neural substrates of reaching compared to grasping were investigated and, for the first time, the dissociation between the two components was documented. This study showed that the cortical areas more involved in the reaching task are the superior parieto-occipital cortex (SPOC, the human homologous of monkey's V6A) and SPL. The study showed a possible integration of reaching and grasping information within the dorsal premotor cortex (PMd), putative homologous of the monkey area F2, the supplementary motor area (SMA), primary and secondary somatosensory cortices (SI-SII) and in M1. Overall, these first studies on the dorsal stream supported the specialization in processing specific information (grasping vs. reaching) within the two dorsal pathways; however, following investigations overstepped this description showing the processing of specific information both in the dorsomedial and in the dorsolateral pathway. For instance, hand orientation selectivity for grasping was demonstrated also using fMRI adaptation paradigm (fMRA), which consists in presenting the same stimulus repeatedly, leading to the reduction of BOLD response to the same type of process. This approach allows describing the selectivity to specific characteristics of a certain neural population. Monaco et al. showed that SPOC (part of the dorsomedial pathway) is engaged in processing wrist orientation for grasping an object (Monaco et al., 2011). The processing of grasp-related information (i.e. wrist orientation) and not only of reach-related information in the dorsomedial pathway, mirrors the finding of grasping response in monkey parietal area V6A and dorsal premotor area F2vr.

Overall, a series of univariate studies showed that there is a correspondence between monkeys and human hand motor network (Figure 1.8) (for reviews see (Culham and Valyear, 2006; Filimon, 2010; Turella and Lingnau, 2014)), showing a similar subdivision in dorsomedial and dorsolateral pathways. Moreover, monkey and human brains show strong similarities in

information processing within homologous areas (e.g. human PMv homologous to monkey's F5 in the dorsolateral pathway; human PMd homologous to monkey's F2 in the dorsomedial pathway).

Monkey fMRI studies (see paragraph 1.1.1.3) (Nelissen and Vanduffel, 2011; Nelissen et al., 2017) provided a clear demonstration that the neuroimaging investigations in human were looking at a similar network, reconciling decades of non-human primate neurophysiological evidence with human fMRI evidence.
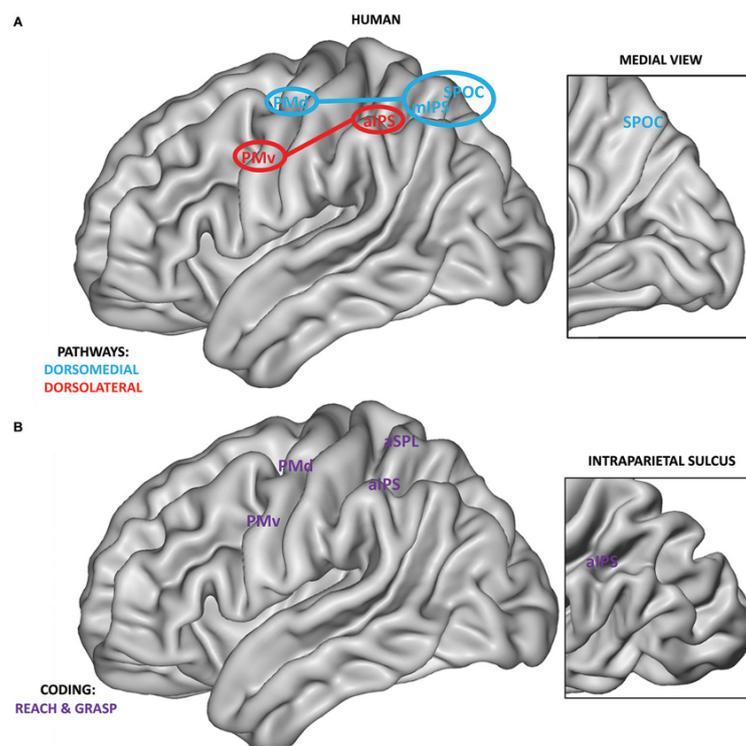
Figure 1.8: Anatomical location of human grasping regions. A. Visual representation of dorsomedial (blue) and dorsolateral (red) pathways. **B.** Areas coding both reach and grasp actions. Adapted from Turella & Lingnau (2014).

The merit of univariate fMRI studies is to identify which areas are recruited by a specific motor task and if there is a difference in the level of the activity elicited between two experimental conditions (Kriegeskorte and Bandettini, 2007a). However, fMRI is correlational in nature, so it cannot provide information about the casual role of a region in a determined process, particularly when the area is active for two or more different conditions or tasks. Other approaches can be used to investigate the causal relationship between specific brain areas in

different experimental conditions. The following paragraph will consider neurostimulation studies on humans used to describe the properties of specific brain areas and their involvement in specific motor tasks.

1.1.2.2 Neurostimulation studies

Neurostimulation techniques in human, and particularly transcranial magnetic stimulation (TMS), can be adopted as a complementary tool to test the specific causal role of the dorsomedial and dorsolateral networks in hand movements. TMS is used to transitorily alter the normal activity of a brain region by inducing an electrical current, which influences the activity of a population of neurons underlying the TMS coil. This approach can be used to test the causal role of a brain region in a specific cognitive or motor task (Miniussi et al., 2013).

TMS studies tried to assess the role of the areas within the hand motor network: for example, it was used to investigate the characteristics of the posterior parietal cortex (PPC), stimulating different regions of the intraparietal sulcus, the superior and the inferior parietal lobule (Desmurget et al., 1999; Vesia et al., 2008, 2010).

In one study (Desmurget et al., 1999), participants had to reach a target located in the periphery of the visual field, the spatial position of the target could change or be kept invariant during the task, and TMS pulse was delivered soon after the movement onset. This study demonstrated that PPC is involved in detecting and correcting hand trajectory errors that occur after a change in the location of the target. On the contrary, when the target was kept to a stable location, TMS did not affect the reaching performance. Overall, PPC has an active role in guiding reaching movements particularly in the online control and monitoring of hand actions.

Different studies explored grasping related properties of regions within the dorsolateral pathway (Tunik et al., 2005; Rice et al., 2006; Davare et al., 2012; Schettino et al., 2015). TMS stimulation of

aIPS (65ms after the visual perturbation of the orientation of the target) affected the adjustment of fingers aperture for grasping (Tunik et al., 2005). Given these results, the visuomotor transformations computed by aIPS occur in a defined time window outside which aIPS does not seem to be involved. The role of different parts of IPS (anterior: aIPS, middle: mIPS, caudal: cIPS), in both planning and execution phase, was investigated adopting a similar setup as the one used in the aforementioned study (Rice et al., 2006). The results showed that TMS stimulation disrupted grasping (execution phase) when aIPS, but not mIPS nor cIPS, was perturbed. This was true both with and without changes of the context, i.e. after the visual perturbation of the target (90° changing orientation). aIPS seems to have a role in updating the internal model of the action in relation to the features of the object (Rice et al., 2006); moreover, it allows correcting for possible errors in action execution when a sudden visual change (Tunik et al., 2005), but no haptic changes, in the properties of the target occurs (Schettino et al., 2015). Another aspect processed by aIPS concerns the amount of force needed to lift an object. This specific peculiarity has been investigated for the first time by Davare et al. (Davare et al., 2007), showing that left aIPS perturbation can alter the grip force scaling in a grasp to lift movement. More precisely the inactivation of both left and right aIPS leads to a suboptimal positioning of fingers on the object (grasping deficit), while the perturbation of left aIPS only, causes an excessive force scaling in relation to the object to lift. By changing the mass of the object, Dafotakis et al. (Dafotakis et al., 2008) confirmed the involvement of aIPS in selecting and correcting errors related to the selection of hand force scale, and showed the complementary role of PMv in predicting the right scale of grip force to use. In addition, PMv seems to exercise a compensatory function in response to haptic perturbations during grasping tasks (Schettino et al., 2015). As previously seen in imaging studies (Culham et al., 2003; Cavina-Pratesi et al., 2018), PMv and PMd are involved in grasping actions, but it was difficult to assess their specific functional role with univariate fMRI analysis.

TMS stimulation over these two regions was used to investigate the contribution of the premotor cortices in a grasp and lift task (Davare, 2006). The perturbation of left and right PMv altered the positioning of the fingers on the object to lift, impairing the grasping action, while left PMd perturbations caused an effect in the timing of the lifting phase. These findings confirmed the role of PMv in operating visuomotor transformation necessary to perform efficient precision grasping movement; and support the idea of a functional loop between PMv, PMd and aIPS as proposed from monkeys 'studies' (Raos et al., 2006). Moreover, the contribution of left aIPS in force scaling (Davare et al., 2007; Dafotakis et al., 2008), suggests an interaction between PMv and aIPS during precision grasping. This hypothesis was supported by recent findings, showing that the perturbation of aIPS influences the interaction between PMv and M1 in the planning phase of a grasping movement, as evident from electromyographic (EMG) signal modifications in the specific muscles recruited for the subsequent grasping task (Davare et al., 2010).

Overall, TMS studies showed that hand action information might be present at a specific time and in specific tasks (e.g. online control) within the dorsal pathways. These studies refined our understanding about hand motor system, showing how regions within the two pathways might process specific aspects of hand actions in a time-dependent manner. However, the interactions between the pathways of the dorsal streams are still poorly investigated. The role of different areas in specific motor tasks, the timing and location of exchange of information that can occur between the two dorsal pathways it is still unclear. To shed light on what is represented within a specific area, new analytical approaches can be adopted (e.g. multivariate pattern analysis, MVPA, of fMRI data). I am going to describe this approach in the following paragraph.

1.1.2.3 Multivariate Pattern Analysis of fMRI data

Recently, multivariate pattern analysis (MVPA) has been introduced to investigate the modifications in neural patterns produced by specific experimental conditions. In this analysis, we train a classifier to distinguish between the neural patterns produced by different experimental conditions (e.g. precision grip vs. whole hand prehension) and we then test if the same classifier can recognize to which category a new set of data belongs. MVPA allowed describing properties of the motor system that could not be defined with a univariate approach (Gallivan and Culham, 2015). The description of many of these properties were previously accessible only in monkey with single-cell-recording. Moreover, MVPA allows exploring, non-invasively, the representational content within brain areas during different phases of an action, comprising the planning phase of a movement and its execution. For example, recent studies investigated the basic properties within the parieto-frontal motor network such as grip selectivity or directional tuning (i.e. selectivity for movement towards specific directions/spatial positions). In different studies Gallivan et al. showed that it is possible to decode the direction (Gallivan et al., 2011a) and the type of action (Gallivan et al., 2011b) the subject is going to perform over an object (i.e. grasp top, grasp bottom or reach) even before the action is executed (planning phase). The regions circled in Figure 1.9, show a significant above chance decoding accuracy (i.e. how well the classifier can discriminate between experimental conditions) when comparing between grasp vs reach conditions during the planning phase of the action. Despite the reduced sample size (N=8), these results showed that areas of both dorsomedial (SPOC, SMA, PMd) and dorsolateral (aIPS, PMv) pathways stored the same type of information, i.e. grip type.
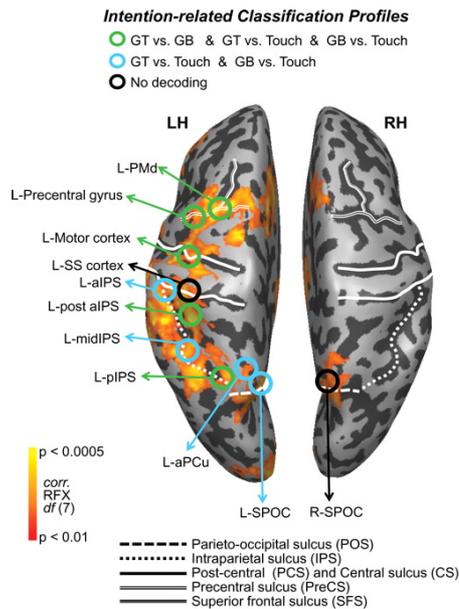
Figure 1.9: ROIs' decoding accuracy during planning phase. Activation map contrast [plan > object fixation]. Green circles represent ROIs decoding significantly for [grasping small object vs grasping large object], [grasping small vs reach] and [grasping large vs reach]; blue circles represent ROIs decoding for [grasping small vs reach] and [grasping large vs reach]; black circles show no decoding. Adapted from Gallivan et al. (2011b).

MVPA can be used to investigate 'abstract' representations of actions using the so-called cross decoding analysis. With the term *abstract,* we intend a representation that generalizes across specific motor feature (e.g. hand, direction, etc.). In the cross-decoding analysis, the classifier is trained to discriminate between two experimental conditions (e.g. saccade left vs saccade right) and then tested on a new set of data where the same pairwise comparison is tested, but one or more features are not present (e.g. reach left vs reach right). This is done to investigate whether the representation generalizes across the missing feature or if it is invariant from it (e.g. the neural representation of the direction irrespective to the type of movement performed, i.e. hand or saccade (Gallivan et al., 2011a)). In a recent paper (Gallivan et al., 2013c), participants had to grasp or to reach the same object either with their right or left hand. Cross-decoding MVPA analysis showed that the type of action (grasp or reach) is encoded within certain brain areas of the PPC (i.e. SPOC, IPS) and of the frontal lobe (PMd) (Gallivan et al., 2013c), independently from the hand used to perform them (see also (Turella et al., 2016)).
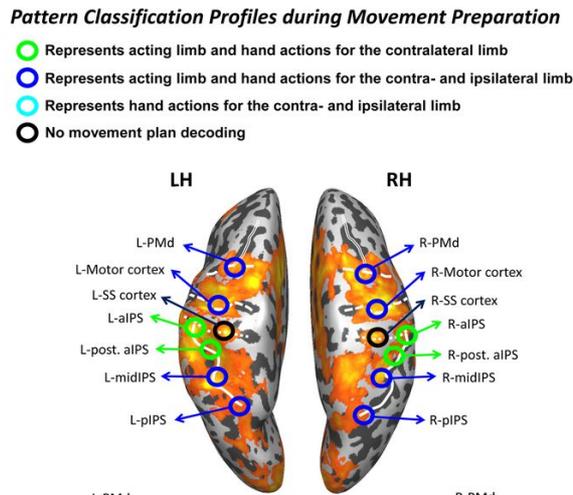
Figure 1.10: Planning phase, decoding of hand used and type of action planned. The activation map represents the contrast [execute>preview]. The considered ROIs are circled and the colors represent the general pattern of pairwise discriminations made during movement planning. Adapted from Gallivan et al. 2013b.

1.1.2.4 The neural representation of complex actions: tool use

As described for monkeys (see paragraph 1.1.1.5), tool-related actions rely on a subset of areas within the hand motor network (Lewis, 2006). Nevertheless, tool manipulation includes a series of additional cognitive processing compared to simple hand actions, such as the identification of the tool, understanding its function and visuo-spatial integration that allows moving toward the to-be-used tool. All these processes are performed by a dedicated tool-network, classically considered left-lateralized, that includes multiple areas located in the dorsomedial, dorsolateral, and in the temporal cortex (Lewis, 2006). The main areas involved in tool use are pMTG in the temporal lobe, SMG, pIPS and aIPS in the parietal lobe and PMd and PMv in the frontal lobe. The same network has been shown to be recruited both when using a real object and when pantomiming the use of the object (Hermsdörfer et al., 2007).

Recently, one MVPA study investigated what type of information is processed within the tool network areas. Gallivan et al. (Gallivan et al., 2013a) showed that in the posterior parietal and posterior temporal cortex, some areas encode actions performed with a tool (SMG, MTG) while some others encode hand-only action information (SPOC and the extra striate body area (EBA)

involved in processing body's parts) (Figure 1.11). These results are referring to a concrete level of representation, where low-level features (e.g. tool features, hand used) are represented. A higher level of encoding, that we could define a more abstract representation of the goal of the action, was described within the areas located in the frontal lobe, i.e. PMv, PMd, and in the parietal lobe, i.e. pIPS, in which the type of action (grasp vs reach) is represented independently from the effector used (tool or hand).
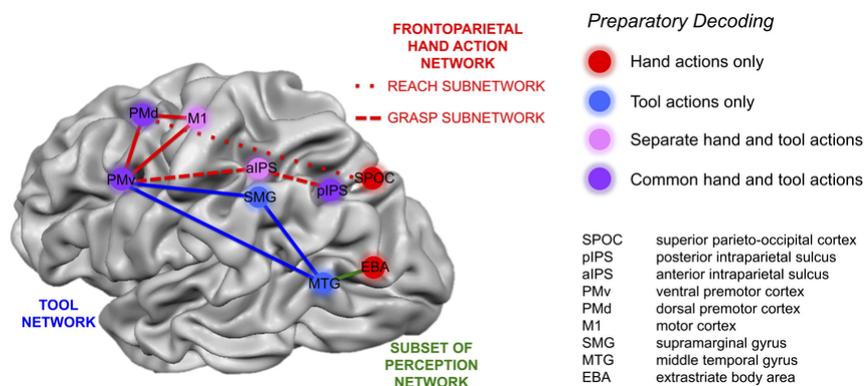
Figure 1.11: Areas involved in action plan decoding of hand and tool movements. SMG and MTG decode for actions performed with a tool (blue). SPOC and EBA decode for actions performed with the hand (red). M1 and aIPS decode actions performed with the hand or with a tool but maintaining the representation separate (pink). PMd, PMv and pIPS show a shared representation for an action performed either with the hand or with a tool (purple). Adapted from Gallivan et al. (2013a).

These studies revealed a complex organization of action encoding within the human brain revealing a different pattern of activity depending on the level of abstraction of the information processed (i.e. low-level concrete representation, high-level abstract representation). Still, we are only at the beginning of our understanding of how and where specific motor properties and abstract action representations are encoded within brain regions of the parieto-frontal motor networks and of the ventral stream. Overall, considering the involvement of both dorsal and ventral pathways in processing tool related information, tasks involving real or pantomimed tool-use, are more and more adopted to investigate not only the representational content of each area of the tool network, but also to explore the communication between dorsal and ventral streams.

Indeed, semantic information about the identity and the function of a tool are processed mainly within the ventral stream, and seems to be integrated and transformed within the dorsolateral areas in order to generate a suitable motor planning (van Polanen and Davare, 2015).

1.1.2.5 Connectivity analysis

Several evidence coming from anatomical studies (see paragraph 1.1.1.4), described a net of anatomically interconnected areas that constitute the hand motor network. The analysis of these connectivity profiles would help in (i) understanding whether these areas involved are also functionally connected and (ii) in describing where different information is exchanged and integrated. The approaches that can be used in human studies to investigate functional interactions between brain areas involve specific analyses of neuroimaging data alone (MVPA and connectivity) or combined with neurostimulation (TMS). They have been used to investigate both dorso-dorsal interactions and dorso-ventral communication.

MVPA was discussed within paragraph 1.1.2.3, where I described some of the studies that explored the encoding of different information within the hand motor network (Gallivan et al., 2011a, 2011b, 2013a). MVPA, as well as TMS combined with some other imaging techniques (e.g. fMRI), can be used to infer the functional communication between different cerebral areas. For instance, the communication between areas of the two dorsal pathways, has been investigated by Johnen et al. (Johnen et al., 2015) combining TMS and fMRI approaches. They showed that delivering TMS paired pulses on PMv and M1 with a specific short inter-pulse-interval could enhance the functional connectivity between PMv and M1 when grasping. A strengthening in the functional connectivity was also registered between PMv and AIP in the dorsolateral pathway. Similarly, Verhagen and colleagues adopted a TMS-EEG approach (Verhagen et al., 2013) to test the communication between the dorsolateral and dorsomedial pathways. They showed that TMS

delivered on aIPS produced an alteration in the dorsomedial circuit; the same was registered in the dorsolateral pathway when TMS was delivered on sPOS, but later in time compared to TMS over aIPS. Overall, these two studies showed a communication between the dorsal pathways.

Another approach to study the communication between areas is the dynamic causal modelling (DCM) analysis. DCM evaluates different possible dynamic solutions (models) occurring between well defined, preselected areas and uses the Bayesian statistic to evaluate which connectivity model better explains the univariate data collected during a specific task (Stephan et al., 2010). In a 2007 paper, Grol et al. used DCM to test the connectivity between the two dorsal pathways, and the modulatory effects (i.e. how a particular condition alters the communication between the considered cerebral nodes) caused by different object sizes when performing a grasping task (Grol et al., 2007). Their results showed a modulatory effect within the dorsolateral pathway (V3A, AIP, PMv) when grasping a small object and increase coupling between the nodes of the dorsomedial pathway (V3A, V6A, PMd) when grasping mainly a large object, but also when grasping a small one. The authors argued that dorsomedial and dorsolateral pathways to be differently recruited according to the amount of on-line control needed to perform different types of grasp. Indeed, grasping a small object induced a stronger modulation from the dorsolateral pathway (i.e. higher online control) compared to grasping a large object, which required less on-line control and that relies mainly on the dorsomedial pathway. Instead of considering the two dorsal pathways as separate and independent, it would be more appropriate to describe them as functionally interconnected and complementary, as involved in different aspects of both grasping and reaching tasks.

The presence of functional and anatomical connections between the dorsal streams, and the involvement of the ventral pathway in processing complex hand motor actions, such as tool manipulation, lead to hypothesize that a similar interaction might occur between the dorsal and

the ventral streams. Dorso-ventral interactions have been described, among others, by Janssen and Scherberger (Janssen and Scherberger, 2015), that proposed that the areas of the lateral fronto-parietal pathway could use and integrate the conceptual information about object identity coming from the temporal lobe. In particular, the temporal perceptual area LOtv seems to interact mainly with aIPS and with PMv in the dorsolateral pathway (Verhagen et al., 2008).

The presence of dorso-dorsal and dorso-ventral interactions reveal an organization of the hand motor network that is more articulate than a set of separate, independent and specialized neural pathways. However, the description of the dynamics occurring between the involved cerebral areas is still poorly understood.

1.1.3 Open questions and thesis rationale

The research evidence summarized in the previous sections suggested that both the dorsomedial and dorsolateral pathways process reaching and grasping information, which is necessary to produce complex hand actions. Moreover, it is clear that this type of information has to be integrated and transferred within the hand motor system by means of specific functional interactions. At the moment, two main issues are still poorly investigated: (i) what type of information is processed within the areas of the hand motor network and (ii) how the functional interactions between regions of the system support the performance of skilled hand actions. In the studies hereafter presented, we tried to address these unanswered questions within this field of research.

The first aim of this thesis is to understand the possible interplay between the two dorsal pathways. The first study (Chapter 2) adopted a combined TMS-fMRI approach and focused on understanding the interactions between the dorsomedial and dorsolateral pathways during a reach-and-grasp task. We adopted a delayed-grasping task, performed under different perceptual

conditions (eyes opened or closed), and we perturbed the activity of SPOC in the dorsomedial pathway of the left hemisphere by means of rTMS. We used univariate and multivariate analysis to investigate the modifications within areas functionally connected with the region stimulated with TMS. We found that when the normal activity of SPOC is altered, changes in encoding grasping action information occur within the dorsolateral pathway. This study showed a causal interaction between the dorsomedial and dorsolateral pathways of the hand motor network, which are traditionally considered to be specialized and independent.

The second aim of the thesis is to investigate the supportive role of the ventral stream in representing hand action information. In the second study (Chapter 3), we adopted fMRI to explore the possible communication between the dorsal and ventral stream, verifying the possible complementary and supportive role of the temporal cortex in motor control. To this aim, we adopted a delayed tool-pantomiming task known to recruit the ventral stream. The delayed design of the task allowed us to consider the planning phase of the movement together with the execution of the pantomime. With multivariate analysis, we explored where in the dorsal and in the ventral streams different abstract goals of an action, i.e. independent from the tool identity, are represented with respect to more concrete aspects, related to the identity of the tool considered in the pantomime. In addition, we investigated the possible functional interactions between temporal and fronto-parietal regions, showing an exchange of information between the two pathways both with MVPA and connectivity analysis (DCM). Overall, these results suggested that the hand motor system not only relies on the specialized-for-action dorsal network, but also on other brain regions, i.e. the temporal lobe areas.

In the third study of the thesis (Chapter 4), we combined data from Chapter 3 with a complementary fMRI session. The second session adopted the same experimental design and task used in Study 2, but different instruction modality and effector were considered. This approach

allowed focusing on understanding: (i) changes in the encoding of concrete and abstract representation based on task requirements (i.e. different instruction modality and effector) and (ii) the possible encoding of tool pantomimes' information also outside the dominant side of the brain, in homologous regions within the right hemisphere. Overall, we found task-dependent changes in the representational content of the considered areas both in the left and in the right hemisphere. These results provided novel insights into the neural correlates of tool pantomime, pointing towards the supportive role of the temporal cortices and of the right hemisphere when planning and pantomiming this type of action.

Finally, in Chapter 5 we considered the results of the three imaging studies from a broader perspective and contextualize these new findings within the current literature.

Chapter 2

Imaging functional interactions between fronto-parietal networks during action planning: a TMS-fMRI study [1]

2.1 Abstract

Prehension movement, i.e. reaching and grasping objects, is the hand action that allows our interactions with the surrounding world. Two fronto-parietal pathways subtend this ability: the dorsomedial and the dorsolateral. The former comprises the superior parieto-occipital cortex (SPOC) and the dorsal premotor area (PMd); the second includes the anterior intraparietal sulcus (aIPS), the supramarginal gyrus (SMG) together with the ventral premotor cortex (PMv). Both pathways are involved in planning hand actions, but little is known on how information is transferred between them. To this aim, we combined offline 1Hz repetitive Transcranial Magnetic Stimulation (rTMS) and functional Magnetic Resonance Imaging (fMRI). While lying in the MR scanner, participants performed a delayed prehension task towards an object, either with their eyes open or closed. The task was performed after offline repetitive TMS or after sham stimulation over SPOC, a crucial hub within the dorsomedial pathway.

During action planning, SPOC perturbation induced task-specific modifications within both pathways, but mainly within the dorsolateral one, in terms of modifications of BOLD response and in representational content. Our results support a task-related functional interplay between these two pathways and highlighted their complementary role in orchestrating prehension.

2.2 Introduction

The ability of our brain to transform incoming sensory information into complex motor programs is fundamental for interacting with the surrounding environment. Prehension, i.e. the capacity of reaching and grasping objects, is at the basis of these interactions and requires the transformation of visual information for planning an appropriate motor output. However, the neural substrates underlying these visuo-motor transformations in humans remain poorly understood.

Prehension relies on two cortical parieto-frontal routes: the dorsomedial and the dorsolateral pathways (Rizzolatti and Matelli, 2003; Culham and Valyear, 2006; Culham et al., 2006; Vesia and Crawford, 2012; Rizzolatti et al., 2014; Turella and Lingnau, 2014; Gallivan and Culham, 2015). Within the dorsomedial pathway, SPOC and the dorsal premotor cortex (PMd) play a pivotal role in the planning and online control of reaching. Within the dorsolateral pathway, regions of the inferior parietal lobule (IPL), the supramarginal gyrus (SMG) and the anterior intraparietal sulcus (aIPS) together with the ventral premotor cortex (PMv) compute visuomotor transformations of the intrinsic properties of an object (shape, size, orientation) into a suitable grasping pattern.

Traditionally, these two pathways were considered to be independent with these two different functional specializations (Rizzolatti and Matelli, 2003). Nevertheless, recent monkey studies challenged this classical view showing that the dorsomedial pathway performs object-related and grasping-related processing similarly to the dorsolateral network (Fattori et al., 2004, 2009, 2010, 2012, 2015; Nelissen et al., 2017). Human studies confirmed object- and grasping-related processing within the dorsomedial network adopting neuroimaging techniques (Gallivan et al., 2009, 2011b, 2013c; Fabbri et al., 2014; Turella et al., 2016) and transcranial magnetic stimulation (TMS, Vesia et al., 2017). In humans, most of these effects have been demonstrated also when planning actions.

Similar object- and grasping-related activity in both dorsomedial and dorsolateral pathways suggest a possible exchange of information. Nevertheless, whether these two pathways communicate during action planning is still largely unknown (for a review see Davare et al., 2011).

Here, we investigated the possible interplay between these two pathways adopting offline TMS-fMRI with the so-called *condition-and-map* approach (Siebner et al., 2009), which allows to describe task-related changes in fMRI signal in functionally connected, but distant, regions with respect to the cortical site stimulated with TMS. In our study, effective or sham offline 1-Hz repetitive TMS (rTMS) was applied to SPOC, a key parietal area of the dorsomedial pathway. After stimulation, we investigated changes in fMRI activity during a delayed prehension task, suitable to isolate the planning phase of an action.

SPOC was selected as it shows 'dorsomedial' specificity for reaching movements, but also object-related and grasping-related activity, typical of the dorsolateral pathway (for a review see Fattori et al., 2015). Furthermore, the putative homologous of SPOC, monkey area V6A, is anatomically connected with the dorsolateral pathway (Gamberini et al., 2009, 2015), supporting SPOC as a possible exchange node between the two pathways. Finally, recent data in monkey shows that activity in V6A is enhanced if grasp planning is performed with direct sight of the target object with respect to perform it without visual feedback (Breveglieri et al., 2017). In humans, preliminary fMRI data show similar higher activity within SPOC during action preparation if the to-be-grasped object is visible (Monaco et al., 2016).

We exploited this visual specialization of SPOC for designing our experimental task. Participants planned and performed reach-to-grasp movements towards a target object either with the eyes open (visually-guided) or closed (non-visually-guided). Overall, our experimental paradigm entailed a 2x2 design with factors: *stimulation* (sham vs effective rTMS) and *visual feedback* (visually-guided vs non-visually-guided).

Our hypothesis was that if the dorsomedial pathway is functionally connected to the dorsolateral one, then perturbation of SPOC should have task-specific effect on distant areas within the dorsolateral network. As a measure of this interaction, we compared TMS-induced modifications in response with univariate and multivariate pattern analysis (MVPA). To anticipate our results, we showed that SPOC perturbation induced modifications within the dorsolateral network, supporting the idea of task-related interactions between the two pathways during action planning.

2.3 Materials and Methods

2.3.1 Participants

17 right-handed participants (10 females, mean age 27.4, age range 18-43 years) took part in the experiment. The ethical committee for human research of the University of Trento approved the protocol of the study. All participants gave written informed consent for their participation and were reimbursed for their time. Three participants were excluded from the analysis: one subject was eliminated due to the excessive head movement during the experiment; the second was discarded because of the excessive number of errors and omitted responses through the runs; the third subject did not finish the experimental session. Overall, the analyses involved data of 14 volunteers.

2.3.2 Experimental protocol

Each experimental session consisted in several preparatory stages, following a setup similar to the one adopted in a recent TMS-fMRI study (Arfeller et al., 2013).
The main steps of the experimental protocols were the following.

- Participants' structural T1- weighted images, acquired in a previous session, were used for TMS neuronavigation. For each participant, we identified the location of the target area, SPOC, to be stimulated with the TMS.

- The individual motor threshold at rest (rMT) was measured. The rMT was used to set the intensity of the TMS pulse for the subsequent stimulation (100% of the rMT).

- Participants were trained during a practice session to familiarize with the delayed prehension task they had to perform in the MR scanner.

- The MR setup was prepared and adapted for each subject and a new T1- weighted image was acquired.

- Each participant underwent four experimental sessions comprising an alternation of offline stimulation (either sham or rTMS) and the acquisition of fMRI data while performing the prehension task.

2.3.3 Experimental task and setup

Participants performed a delayed prehension task, consisting in executing reach-to-grasp movements towards a plastic button with their right (dominant) hand (Figure 2.1A). The subject's head was tilted to allow the direct sight of the object. Neck and forearm were supported with pillows and foam pads. Participants' right hand was kept at rest on a MR compatible button box fastened on their chest with a Velcro belt, which allowed the measurement of the reaction times (RTs) during the experimental fMRI session. The target object was positioned on a transparent MR compatible support situated over the subject pelvis (Figure 2.1B). A fiber optic-based system within the object permitted to collect the time when the subject pressed the button and therefore to calculate the movement time (MT) of the action. Stimulus delivery and response collection

were controlled with the Presentation software (version 16, Neurobehavioural Systems, https://www.neurobs.com/).
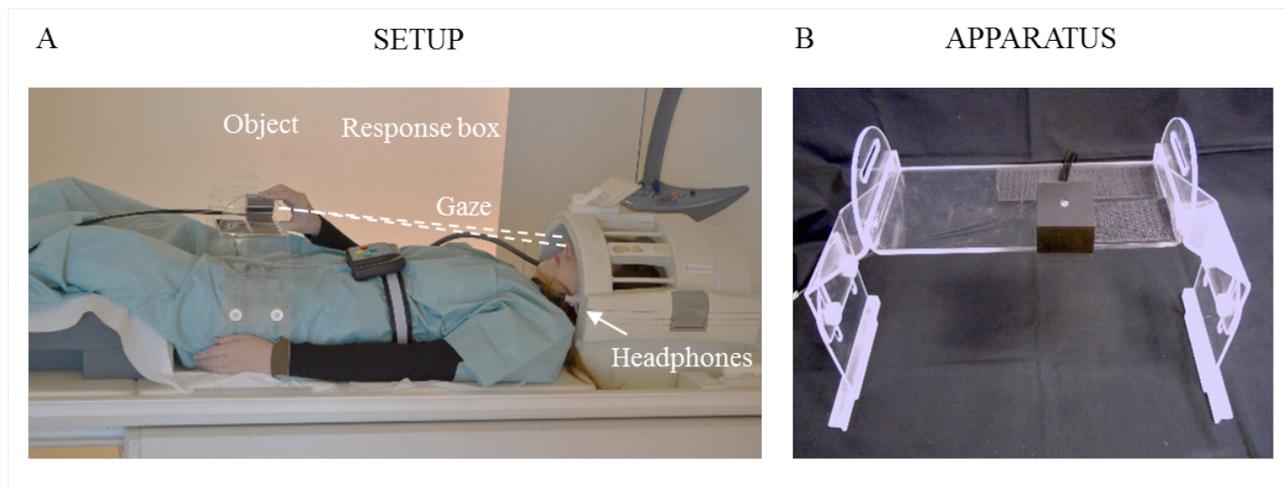
Figure 2.1. A. MR Setup. Participants were positioned in the MR scanner with their head tilted having direct sight of the object. Pillows and foam pads were positioned under the arm in order to make the position as comfortable as possible and to minimise movements. The button box was fastened on a Velcro belt on participant's abdomen. **B. MR-compatible object.** The target object consisted in a fiber-optic button fastened on a plexiglass support that could be grasped by the participant. The apparatus was positioned above participant's hips.

2.3.4 Experimental design and paradigm

We adopted a 2x2 factorial design (Figure. 2.2). The two considered factors were: (i) *stimulation*, either offline 1 Hz rTMS or sham stimulation, and (ii) *visual feedback*, planning and performing the action with eyes open (visual grasp condition) or with eyes closed (blind grasp condition).
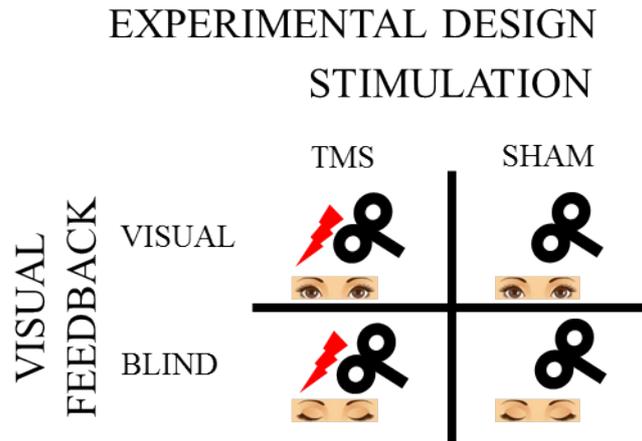
Figure 2.2. Experimental design. The 2x2 factorial design entailed two factors: stimulation (1 Hz rTMS or SHAM), and visual feedback (visual grasp or blind grasp).

The experimental timeline was the same for each participant, comprising an alternation of stimulation and fMRI data collection (Figure 2.3A). Every functional run was performed after stimulation, either 1 Hz rTMS or sham. Stimulation was performed in a separate area next to the magnet room. During TMS stimulation (duration: 20 minutes), the participant had to lay prone and still on a MR-compatible gurney. Immediately after the end of the stimulation, the subject was brought with the gurney in the MR room and rapidly positioned to start a functional run (average time from the last TMS pulse to the start of the MR sequence: 4.05 minutes). The sequence of rTMS and sham stimulation was counterbalanced across participants.

Functional data were acquired in four experimental runs (duration: 12 minutes and 20 seconds each), two after rTMS stimulation and two after sham stimulation (Figure 2.3A). Each run included 28 trials (14 per each condition, i.e. visual and blind grasp). The order of the runs was counterbalanced across participants.

After each functional run, participants waited for at least 5 minutes before starting with the subsequent stimulation session to avoid any carry over effect of the rTMS (Figure 2.3A). The fMRI

sessions were recorded with a video camera and controlled offline for possible errors in the performance.

The experimental paradigm consisted in a delayed prehension task (Figure 2.3B), which allowed dissociating the planning phase, the focus of this investigation, from the execution phase of the movement. Participants were instructed to keep their eyes closed through the experiment. After the first 20 seconds of baseline, a verbal instruction was presented to the subject via headphones (auditory cue). The instructed movement could be either 'visual grasp' or 'blind grasp'. In the first condition, participants had to open their eyes as soon as they heard the auditory cue and fixate the object; in the second condition, they had to maintain their eyes closed. After 10 seconds of delay, a verbal 'go' cue signalled to execute the planned action. Participants had to release the home key button and grasp the object (execution phase: 2.5 seconds). Another verbal instruction ('eyes closed') signalled to release the button, to return to the home key position and to close the eyes, if they were open. A baseline period (12.5 second) was presented between the trials (inter-trial interval, ITI).
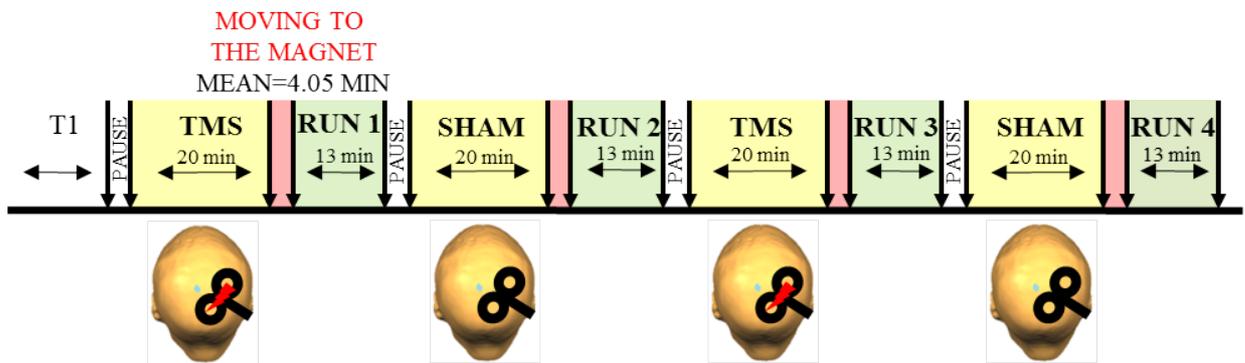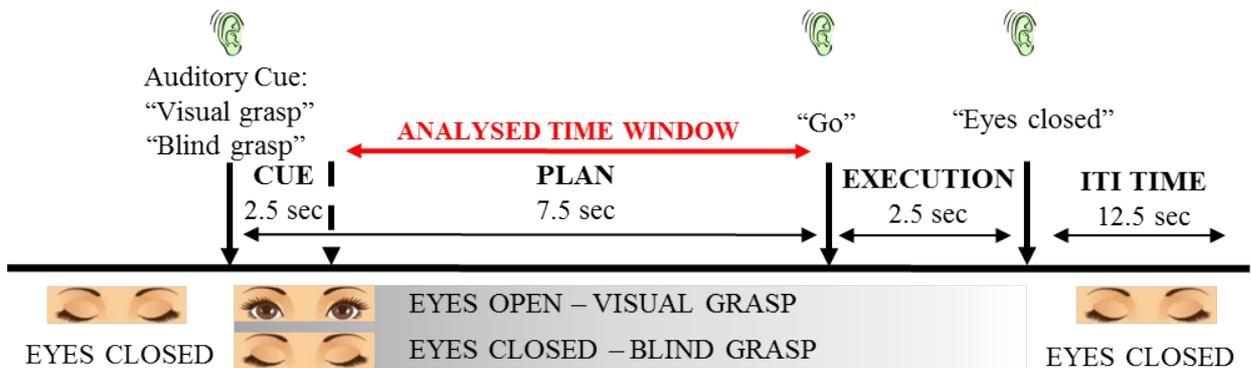
Figure 2.3. A. Experimental session timeline. Each participant underwent four functional runs. Every run was performed after 20 minutes of either offline 1 Hz repetitive TMS or SHAM stimulation over SPOC. The sequence of TMS and sham stimulation was counterbalanced across participants. Participants were moved inside the MR room on a non-magnetic gurney. The average time from the last TMS pulse to the start of the MR sequence was 4.05 minutes. **B. Timing of experimental trial.** The trial began with a verbal cue instructing the subject which action to perform (either 'visual grasp' or 'blind grasp'). The subject opened or kept the eyes closed according to the experimental condition. After a delay of 10 seconds, the subject was instructed with a verbal cue ('go') to perform the reach-to-grasp action (execution phase). After 2.5 seconds, another verbal cue ('Eyes closed') indicated the end of the trial. The participant waited for a new cue to start the following trial (ITI phase, duration 12.5 sec).

2.3.5 TMS Neuronavigation

Neuronavigation was performed adopting BrainVoyager QX (Brain Innovation BV, The Netherlands) and an ultrasound tracking system (Zebris Medical GmbH, Isny, Germany) to identify the correct location of the target area for TMS stimulation (Sack et al., 2008).

The stimulation site was SPOC within the left hemisphere, the possible human homologue of monkey's area V6A (Fattori et al., 2010, 2012, 2015). SPOC has been shown to be critically

involved in planning, online monitoring and executing of hand actions, adopting a variety of techniques, such as TMS (Vesia et al., 2010, 2013, 2017) and fMRI (Gallivan et al., 2009, 2011b, 2013c; Cavina-Pratesi et al., 2010; Monaco et al., 2011; Rossit et al., 2013). Furthermore, it has been shown to be engaged while observing graspable objects within reach of the contralateral hand, suggesting its possible role in extracting motor affordance from visual information in monkey (Breveglieri et al., 2015) and in human (Gallivan et al., 2009).

The neuronavigation procedure consisted in the co-registration of the anatomical image of each participant (collected in a previous session) with his\her physical head. A 3D reconstruction of the participant' scalp and brain surface was created. SPOC coordinates [TAL: -18 -68 37] were extracted from an independent fMRI study adopting a similar setup and paradigm (Monaco et al., 2016). Starting from these coordinates, we reconstructed the position of SPOC on the brain mesh of each participant based on individual neuroanatomical landmarks. The scalp location corresponding to SPOC stimulation was marked to be adopted throughout the whole experimental session.

2.3.6 TMS stimulation parameters

TMS pulses were delivered using a MagPro 3100 stimulator (MagVenture A/S, Denmark) and a figure-of-eight coil (MC-B70 MagVenture A7S, Denmark). The orientation of the coil, perpendicular to the midline and with the handle pointing medially, was maintained constant through the experiment and between participants.

For each subject we calculated the lowest stimulation intensity able to elicit a visible contraction of the relaxed fingers of the right hand at least 5 times out of ten pulses (rMT). We adopted 1 Hz-rTMS with 100% of the rMT intensity (Arfeller et al., 2013). SHAM stimulation was performed at the same intensity but rotating the coil of 90°, so that the side of the figure-of-eight coil touched

the participant scalp. Participants were not informed about the type of delivered stimulation. Offline stimulation was delivered for 20 minutes in both conditions.

2.3.7 Behavioural analysis

Behavioural performance during the execution phase was registered with the MR-compatible response box and with the target object. Offline control of video recorded of participant's performance showed a low number of errors (one participant made 1 errors, another made 2 errors). These trials were excluded from further behavioural and fMRI analysis.

We considered the reaction time (RT) as the time interval between the 'go' cue and the time point at which the hand was released from the button box. Movement times (MT) were defined as the time interval between the release of the MR-compatible response box and the press of the target fiber-optic button.

We collected behavioural data for 13 subjects out of 14 (for technical problem the responses for one participant were not registered). On the RT and MT data, we performed a repeated measure 2x2 ANOVA (factors: stimulation and visual feedback), followed by paired t tests for the comparisons of interest.

2.3.8 Magnetic Resonance (MR) Data Acquisition

MR data were acquired with a 4T Bruker MedSpec scanner using an 8-channel head coil. Functional images were acquired with a T2* echo-planar imaging (EPI) sequence. Before each functional run, we collected the point-spread function (PSF) of the subsequent acquired sequence to correct for possible distortions (Zaitsev et al., 2004). We acquired 35 slices tilted to be parallel with the ACPC line (TR 2.5 s; TE 33 ms; FOV: 192 × 192 mm; in-plane resolution 3x3; slice thickness 3 mm; gap size: 0.45 mm). Participants completed four runs of 296 volumes each. For each

participant, we acquired also a T1-weighted anatomical image (MP-RAGE, 176 axial slices, 1 mm isotropic voxels).

2.3.9 MR Data pre-processing

Data pre-processing and analysis were performed with BrainVoyager QX (version 2.8 software, Brain Innovation), MatLab (MathWorks) and the NeuroElf Toolbox (http://neuroelf.net/). The first five volumes of each run were discarded in order to avoid the saturation effect. The pre-processing of the fMRI data included slice timing correction, 3D motion correction and high-pass temporal filtering (three cycles per run). In the 3D motion correction, we aligned each volume to the first volume acquired of the first run. The functional data were then co-registered to the high-resolution anatomical image. Spatial smoothing was applied (8 mm) for univariate analysis only. All the data were transformed into the Talairach space to allow group level analyses.

2.3.10 Experimental Design and Statistical Analysis: Univariate approach

The focus of the study was to investigate task-specific interactions between two parieto-frontal pathways, the dorsolateral and the dorsomedial pathways, during action planning. To this aim, we modelled the data separating the task into three distinct parts: the presentation of the cue, the planning phase (delay between the cue and the go signal) and the execution phase (Figure 2B). A similar approach has been recently adopted to investigate the encoding of specific information during the planning phase of reaching actions (Chen et al., 2014; Cappadocia et al., 2016).

For each participant, a General Linear Model (GLM) was defined with a total of 12 predictors of interest, corresponding to the 4 conditions (visual grasp TMS, blind grasp TMS, visual grasp

sham, blind grasp sham) x 3 task phases (Cue, Plan, Execution). The predictors were created with boxcar functions convolved with hemodynamic response function (Boynton et al., 1996). The duration of the boxcar function was equivalent to the duration of the phase (Cue: 2.5 sec, Plan: 7.5 sec, Execution: 2.5 sec). In addition, we modelled movement parameters (3 rotations and 3 translations) and error trials if present, as predictors of non-interest. The estimated beta weight for each condition of interest was z-scored.

Random effect analysis (RFX) GLM analysis was conducted at the group level focusing on the planning phase of the task. In order to identify the brain areas underlying effects of interest, we performed t-contrasts considering the corresponding experimental conditions extracted from the design matrix.

2.3.11 Experimental Design and Statistical Analysis: MVPA

We adopted COSMoMVPA toolbox (Oosterhof et al., 2016) () to perform MVPA. For each participant, a GLM was estimated on non-smoothed data modelling every single trial for each condition separately. A total of 336 regressors of interest were considered, originating from the 4 conditions (visual grasp TMS, blind grasp TMS, visual grasp sham, blind grasp sham) x 3 time phases (Cue, Plan, Execution) x 28 repetitions (14 trials x 2 runs). In addition, we modelled movement parameters as predictors of no interest. As the present study investigated the possible interaction between two pathways of the dorsal stream during action planning, we focused on this phase also for the MVPA analysis.

A volume-based searchlight MVPA approach was adopted (Kriegeskorte and Bandettini, 2007; Oosterhof et al., 2011). The searchlight was defined as a sphere with a radius of 4 voxels using Linear Discriminant Analysis (LDA) as classifier and adopting as features the z-scored beta value estimated for the single trial of the condition of interest.

For each subject, we adopted a leave-two-trials-out cross-validation approach to estimate decoding accuracy. The classifier was trained on all the trials except two (one for each condition) and then tested on the remaining two. This procedure was repeated for each trial pairs (28 times). For subjects who made errors during the task, we randomly excluded error trials from the analysis selecting the same number of trials for the two conditions. MVPA was performed separately for the same pairwise comparison (planning visual grasp vs. planning blind grasp) after sham and after TMS stimulation, obtaining two decoding accuracy maps for each subject.

Then, we compared these two decoding accuracy maps performing a paired t test at a group level. The rationale behind this analysis was to test a sort of "multivariate" interaction. The idea was to investigate if TMS stimulation over SPOC affected the pattern of activity within functionally connected regions, when comparing planning visually guided and non-visually-guided actions, with respect to sham stimulation. If the comparison between the two maps is significantly different, then information content within these cerebral regions is affected by TMS stimulation in a task-specific way.

2.3.11 Testing univariate and "multivariate" interactions

The aim of the study was to identify the possible task-related interaction between the dorsomedial and the dorsolateral pathways during the planning phase of this task. To this aim, we performed two different analyses, a classical univariate RFX and a novel MVPA approach (described above). The statistical threshold for the two effects of interest was set at $p < 0.001$ uncorrected at the voxel level and $p < 0.05$ corrected for multiple comparisons adopting familywise error correction (FWE) as implemented in the SPM12 software (). This is the default setting of SPM software and this combination of cluster-forming threshold and cluster-correction procedure

ensures that the rate of false positives are appropriate effectively correcting for multiple comparisons when using parametric approach (Eklund et al., 2016; Flandin and Friston, 2016).

In addition, we provided a complementary analysis with a more liberal threshold to allow a better understanding of our multivariate effects (see below). For this analysis, t-maps were thresholded at $p<0.025$ at the voxel level and at $p<0.05$ corrected at the cluster level adopting familywise error correction (FWE) as implemented in SPM12.

Significant activation and decoding maps were overlaid onto a group-averaged surface mesh obtained with cortical based-alignment procedure implemented in Brain Voyager.

2.4 Results

2.4.1 Behavioural data (RTs & MTs)

We extracted RTs and MTs of the actions performed during the execution phase of each trial. Outliers were not considered. A 2x2 repeated measure ANOVA showed a main effect of Visual feedback, significant both for RT [$F_{(1, 12)}$ =31.81, $p<0.001$] and MT [$F_{(1, 12)}$ =68.65, $p<0.001$]. Neither the main effect of stimulation nor the interaction was significant for RT and MT ($p>0.05$) (Figure 2.4).
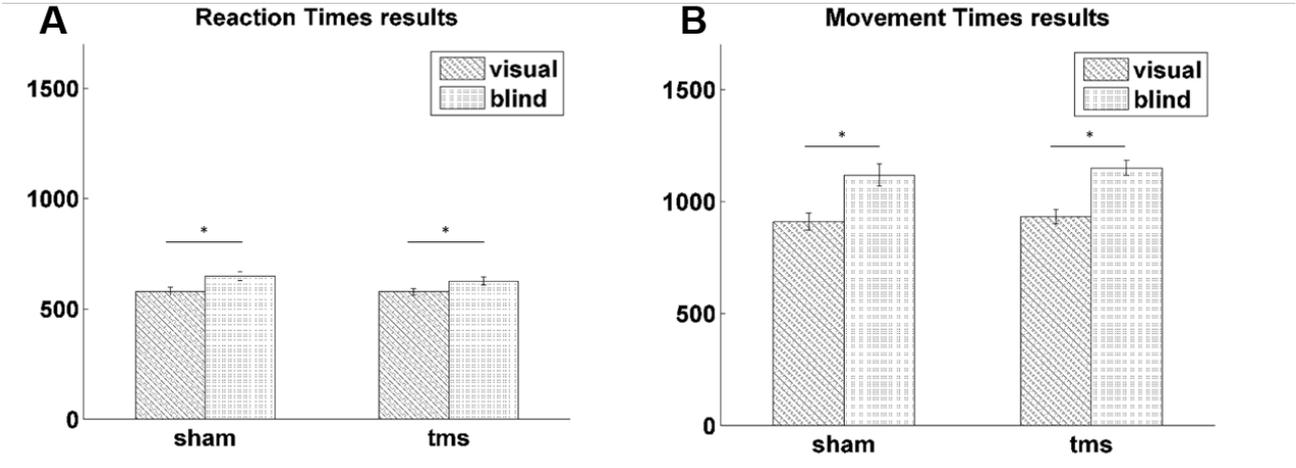
Figure 2.4. A. Reaction Times (RTs) results. Mean RTs for the the visual grasp condition (first column) and for the blind grasp condition (second column) are plotted separately for SHAM and TMS stimulation. **B. Movement Times (MTs) results.** Mean MTs for the the visual grasp condition (first column) and for the blind grasp condition (second column) are plotted separately for SHAM and TMS stimulation. Asterisks indicated significant difference between visual and blind conditions (* p<0.05).

2.4.2 Testing univariate and 'multivariate' interactions

The main aim of the study was to test for a possible task-related interplay between dorsomedial and dorsolateral pathways. This translates into demonstrating a possible significant interaction at univariate or multivariate level. At the selected threshold, there was no significant regions for the univariate interaction (visual grasp sham – blind grasp sham > visual grasp TMS – blind grasp TMS). The 'multivariate' interaction was significant (visual grasp sham – blind grasp sham > visual grasp TMS – blind grasp TMS) within a cluster located within the anterior part of the postcentral sulcus at the border with the postcentral gyrus (Figure 2.5).

MVPA DECODING INTERACTION

3.43   8
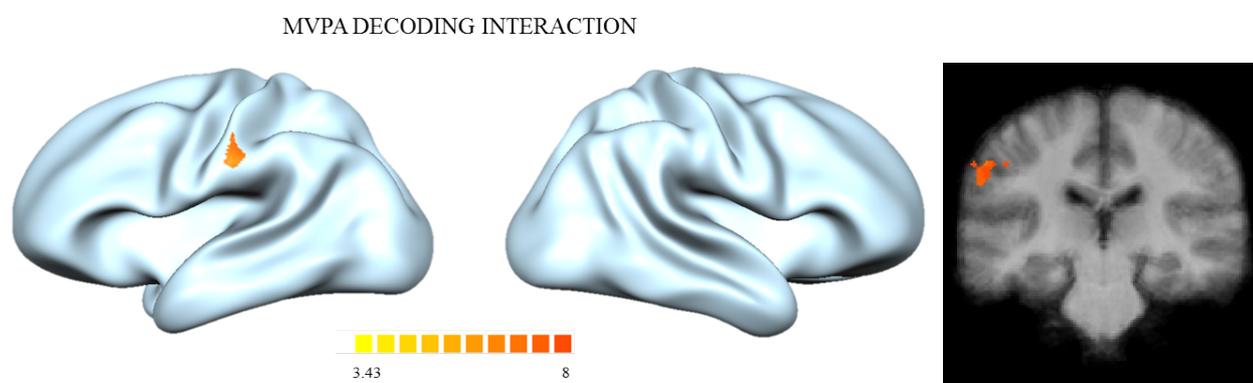
Figure 2.5. Multivariate decoding interaction. The figure depicts the significant cluster for the 'multivariate' interaction (p<0.001 uncorrected voxelwise and p<0.05 FWE-corrected at the cluster level).

This result showed that TMS stimulation over SPOC can have a significant task-specific effect within a distant region of the dorsolateral pathway during action planning. This region showed a significant reduction of decoding when comparing visual grasp and blind grasp following rTMS with respect to sham stimulation. Nevertheless, the exact nature of this multivariate effect is not

clear. This modification might be due to subtle univariate effects which might not be identified by our univariate approach adopting a conservative correction, as MVPA approach might be more sensitive to TMS-induced modifications.

2.4.3 Exploratory univariate analysis

To better characterise our multivariate effects, we performed additional exploratory analyses with less stringent voxel-wise threshold to investigate the univariate interaction. To have a clearer picture of this effect, we first computed the two simple main effects comparing the visual grasp with the blind grasp condition separately for sham and TMS (see Figure 2.6 A, B).
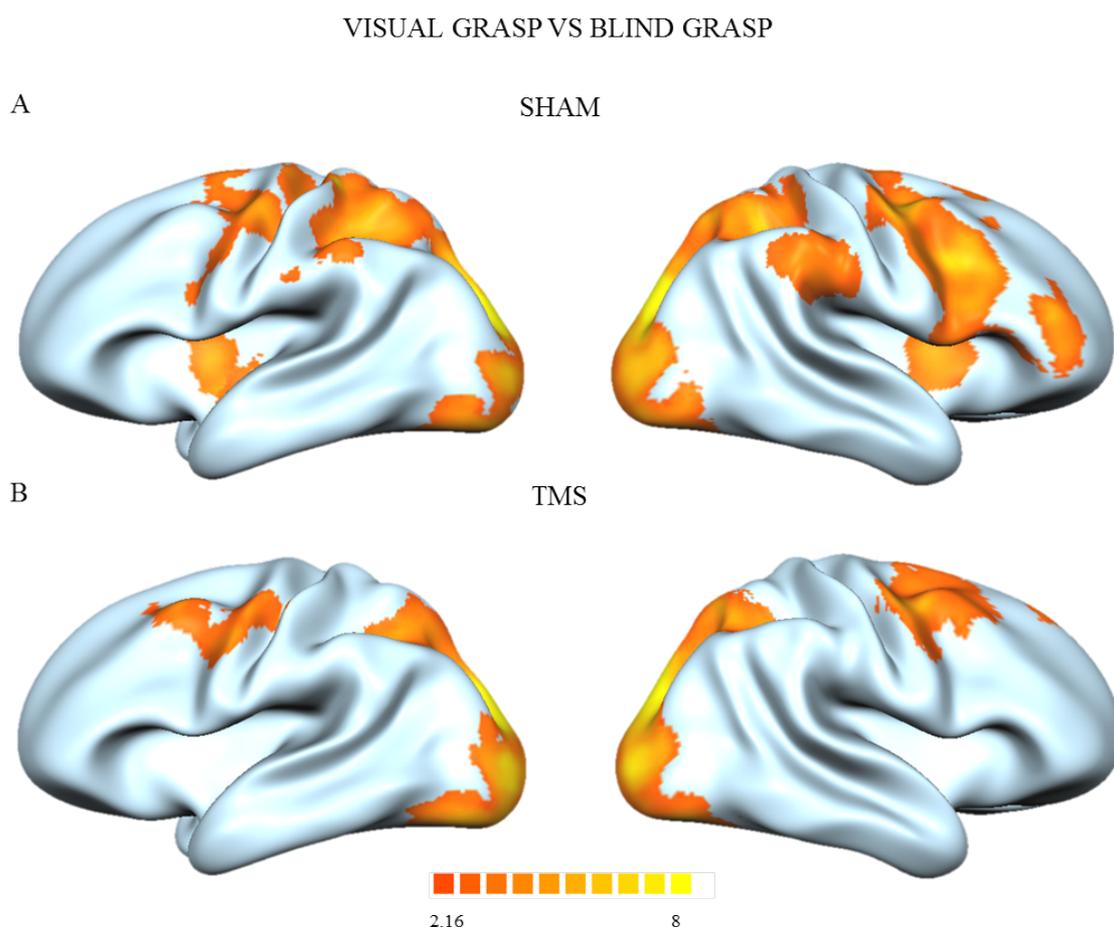
Figure 2.6. A. Simple main effect of visual feedback for sham conditions during planning phase. The regions recruited were defined with the univariate contrast: visual grasp vs blind grasp sham. **B. Simple main effect of visual feedback for TMS conditions during planning phase.** The regions recruited were defined with the univariate contrast: visual grasp TMS vs blind grasp TMS. The statistical threshold for both comparisons was set at p<0.025 uncorrected voxelwise and p<0.05 FWE-corrected at the cluster level.

A similar pattern of activation was found for the two comparisons of interest, but the contrast between the two conditions following TMS showed significant activation within a smaller set of cortical regions. To statistically test this possibility, we computed the positive interaction between the two factors (visual grasp sham − blind grasp sham > visual grasp TMS − blind grasp TMS). This contrast of interest permitted to identify cortical regions showing task-related modifications induced by TMS stimulation during the planning phase of the prehension task (Figure 2.7). The interaction was significant bilaterally within SPL, aIPS, the insular cortex, the parietal operculum and the superior temporal gyrus. Within the right hemisphere there was a significant interaction within the IFG and the ventrolateral prefrontal cortex. No significant effect was found for the negative interaction.

INTERACTION

2.16   8
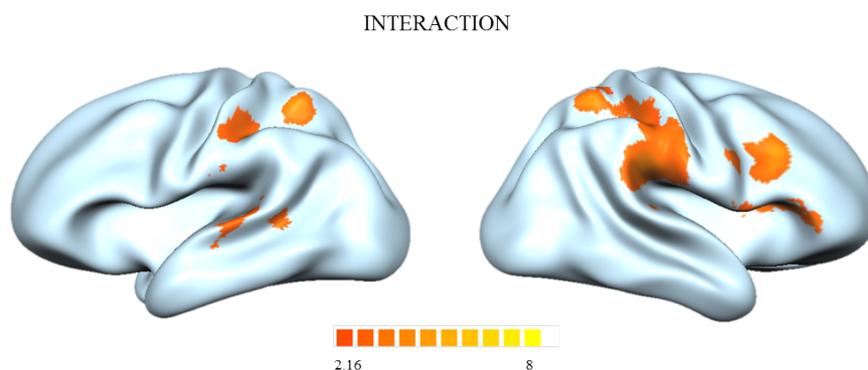
Figure 2.7. Stimulation by visual feedback interaction during planning phase. The regions recruited were defined with the univariate contrast: (visual grasp sham vs blind grasp sham) − (visual grasp TMS vs blind grasp TMS). The statistical threshold for both comparisons was set at p<0.025 uncorrected voxelwise and p<0.05 FWE-corrected at the cluster level.

Then, we tested again the multivariate interaction at a lower statistical threshold (Figure 2.8). Significant reduction of decoding accuracy following SPOC stimulation was evident within brain regions mainly located within the dorsolateral pathway, such as bilateral SMG, left aIPS and ventral part of PMv. Within the left hemisphere, there were also significant reduction in decoding within the anterior temporal pole and the insular cortex. Differences in decoding were also present within the contralateral dorsomedial pathway within SPL and the posterior intraparietal

sulcus. There was no significant increase in decoding in any brain regions following SPOC stimulation.

MULTIVARIATE INTERACTION
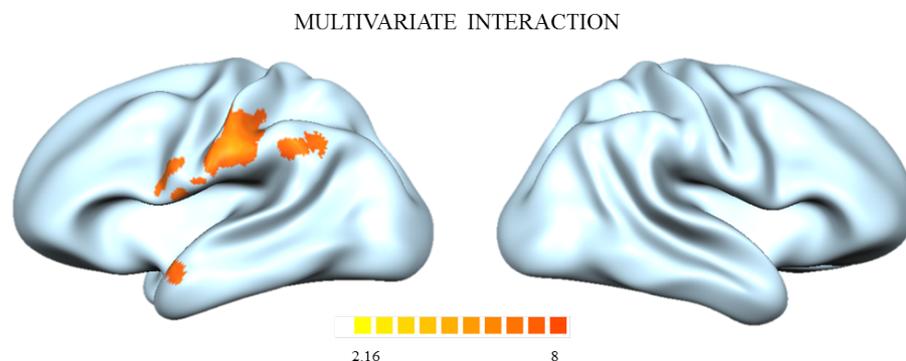
Figure 2.8. Multivariate interaction. The statistical threshold for both comparisons was set at p<0.025 uncorrected voxelwise and p<0.05 FWE-corrected at the cluster level.

To further understand the relationship between univariate and multivariate modifications induced by TMS stimulation, we performed a conjunction between these two effects (Figure 2.9). This analysis was performed to highlight modifications common to both types of analyses, which were partially overlapping with the area which we identified adopting the conservative threshold (Figure 2.10). This suggests that the effect we described using MVPA is at least in part determined by subtle univariate effects. Moreover, these results point towards a possible dissociation between the effects highlighted using MVPA and univariate approach as many regions were showing either one or the other effect.

A

B
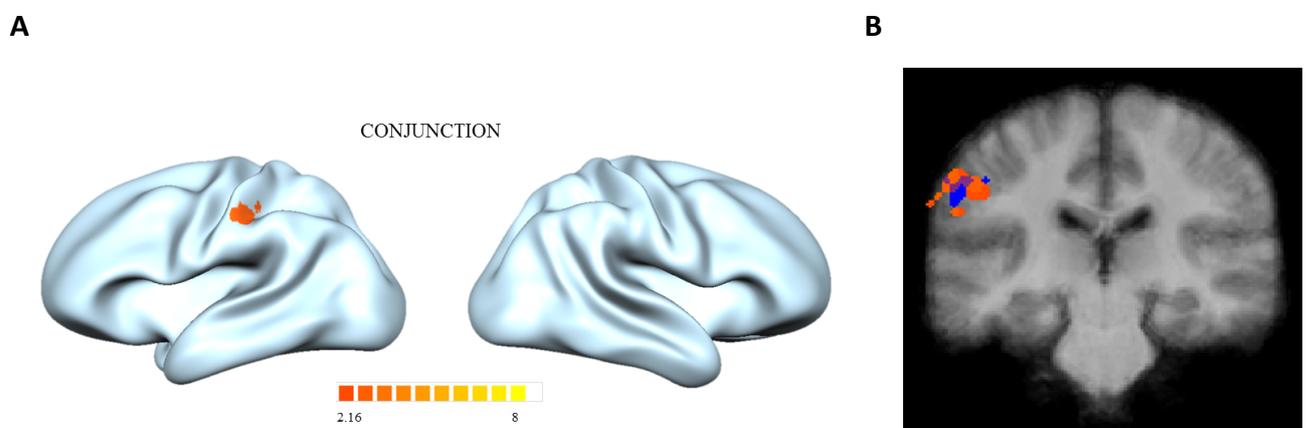
CONJUNCTION

Figure 2.9. A. Conjunction. Conjunction between univariate interaction map and multivariate interaction map. **B. Overlap conjunction and MVPA interaction.** between conjunction (orange) and significant reduction in decoding evident in the multivariate interaction using the conservative threshold, at p<0.001 and FWE correction (blue).

2.5 Discussion

Previous human neuroimaging (Gallivan et al., 2011b, 2013c; Chen et al., 2014; Cappadocia et al., 2016; Turella et al., 2016) and TMS investigations (Koch et al., 2008, 2010; Koch and Rothwell, 2009; Vesia et al., 2010, 2013) show that planning of hand actions jointly recruits the dorsomedial and the dorsolateral pathways. The present study directly demonstrated interactions *between* these two fronto-parietal pathways during the planning phase of prehension by means of offline TMS-fMRI approach. Offline rTMS perturbation of SPOC - within the dorsomedial pathway – caused modifications, - evident as a decrease in decoding - within a region of the dorsolateral pathway (multivariate analysis). Moreover, exploratory analysis showed that univariate and multivariate modifications were only partially overlapping, suggesting a possible differential sensitivity of these analyses to TMS-perturbation.

These results indicate that dorsolateral and dorsomedial pathways share information during action planning, and therefore both concur in orchestrating appropriate prehension movements. We discuss the implications of such interaction between dorsolateral and dorsomedial pathways for the organizational principles of parieto-frontal circuits.

2.5.1 Evidence of interplay between dorsomedial and dorsolateral pathways during action planning

Combining TMS with brain mapping techniques allows the identification of specific networks of interconnected areas starting from a seeding point where stimulation is applied (Siebner et al., 2009; Miniussi et al., 2013). In our exploratory univariate interaction, we identified TMS-induced BOLD changes within both dorsolateral and dorsomedial pathways. During action planning, a perturbation within the dorsomedial pathway 'propagated' into both pathways. MVPA analysis

seemed more sensitive and allowed exploring further the interaction between the two dorsal pathways from the point of view of the representational content.

These findings resonate with recent studies combining neuroimaging and neurostimulation showing that, during prehension, TMS-perturbation of one pathway can induce changes within the other pathway measured as modifications in fMRI connectivity profiles (Johnen et al., 2015) and EEG power (Verhagen et al., 2013).

Johnen et al. (2015) showed a complementary effect to the one described in our study. A perturbation within the dorsolateral pathway - offline paired-pulse TMS protocol applied between PMv and M1 - modified the connectivity profiles not only, as expected, within the stimulated pathway, but also within the dorsomedial one. These modifications were evident during the execution of visually guided prehension. Here, we show that a similar effect could be described even during action planning, i.e. before the performance of any movement, supporting the interaction between these pathways along the entire temporal evolution of the action.

Our results supported and expanded also the findings of a TMS-EEG study (Verhagen et al., 2013), investigating the functional organization of the two dorsal pathways during prehension. Their results supported the hypothesis of a serial recruitment of the two pathways, with the dorsomedial recruitment being dependent on the dorsolateral one. Moreover, they suggested a compensatory effect of the dorsolateral pathway, with a particular involvement of EEG sensors located over the dorsolateral pathway, when TMS stimulation was delivered over the posterior SPL, on a more dorsal site with respect to our site of stimulation. Even if it is difficult to directly relate EEG and fMRI findings, our results showed similar modifications within regions of the dorsolateral pathway after dorsomedial perturbation both in terms of BOLD signal and representational content, supporting task-specific communication and direct exchange of information between the two parieto-frontal pathways.

What are the regions in the dorsolateral pathway that share functional connections with SPOC? The first striking result is that interconnected regions were found bilaterally, even if the task was unimanual and TMS was applied over the left hemisphere. MVPA provided complementary results task- and TMS-specific changes mainly within the left dorsolateral pathway, i.e. unilateral to the stimulated SPOC. More in detail, the dorsolateral regions showing up in both analyses (though with only limited overlap) were the aIPS, the SMG, the PMv and the adjacent caudal IFG. Several regions, such as left PMv and left IPL, showed a modification only at the level of MVPA that was not evident at the level of the univariate interaction. Only one region within the left hemisphere, showed both type of modifications, suggesting a direct link between changes in BOLD signal and reduction in decoding.

2.5.2 One extended hand motor network

Our data support a model of the hand motor network that goes beyond the strict anatomical distinction between dorsolateral and dorsomedial pathways and the dichotomy between reaching and grasping. We propose that, at least for prehension behaviour, information is widely shared between the two pathways. The functional distinction between the two pathways needs to be looked at a different level of information processing.

Our data complemented and supported a recent fMRI connectivity study (Hutchison and Gallivan, 2018). This study started to look at task-related modulations of dorsal and ventral stream regions during different motor tasks, including prehension, showing that the relative contribution of the same region within the network is flexible and task-specific. If, as in our case, one hub of the network is perturbed, the role of the others will be necessarily modified and reorganised. However, further research is needed to investigate the 'normal' connectivity profiles within these pathways to explore how task requirements influence their interactions.

2.5.3 TMS-induced modifications: Univariate and MVPA evidence

This study is the first investigation applying an MVPA approach to analyse offline TMS-fMRI data during the planning of hand actions. Our results showed possible association and dissociation between univariate and multivariate modifications within specific brain regions, which might highlight TMS-induced modulatory effects with different physiological properties.

MVPA might be more sensitive in revealing TMS-induced effects with respect to univariate approach as it relies on patterns of activation rather than on metabolic BOLD difference. As a consequence, MVPA is much less sensitive to subjects' variability and can highlight significant modifications which are not visible at univariate level. Subject's variability might be determined by the type of adopted TMS protocol or by the particular subject-specific brain response to the stimulation. Nevertheless, the exact nature of TMS-induced modifications is still open to different types of interpretation, so additional research is needed to fully understand the meaning of this reduction in decoding.

Even if we are still at the beginning of our understanding of these modifications, it is clear that MVPA might unveil potential modifications that are not evident using univariate analysis alone, helping in defining subtle changes in informational content even in the absence of (significant) metabolic alterations. MVPA should be considered as an additional powerful tool to reveal compensatory and reorganizational phenomena together with univariate and connectivity analysis (Hartwigsen et al., 2015).

2.5.4 Conclusions

Our combined TMS-fMRI approach showed widespread task-specific interactions between two fronto-parietal motor circuits classically thought to be segregated. Cross-talk between dorsomedial and dorsolateral pathways occurred even before an action is performed - during its

planning - and this interplay is likely to be a specific feature of complex hand movements. This interplay might not only provide crucial insights into the functional organization of the 'normal' hand motor network in the healthy brain, but it could also be possibly exploited as a possible compensatory mechanism after brain damage.

Chapter 3

Intention encoding and functional interactions within the tool network

3.1 Abstract

Tools manipulation allows us to perform flexible interactions with the physical world, e.g. different goals can be fulfilled using the same tool or the same goal can be achieved with different tools. We aimed to explore what type of information about the goal of an action is encoded within the regions of the tool network. We adopted a delayed pantomime-task in the MR, asking participants to perform either a grasp-to-move or a grasp-to-use pantomime with two different tools (scissors and axe). These tools share the possibility to achieve the same final goal (being moved or adopted to cut). We used multivariate pattern analysis (MVPA) to analyze the pattern of activity during the planning and the execution of the pantomime and described two distinct level of representational content about the goal of a tool pantomime (concrete - different goals performed with the same tool; abstract - different goals irrespective of the tool used). We found differences in the encoding of abstract goal representation during the planning and execution phase, suggesting an exchange of information between posterior (i.e. parietal and temporal) and anterior (i.e. premotor) areas of the network. Interactions between the recruited ventral and dorsal pathways were further explored with connectivity analysis that highlighted a strengthening in the task-specific coupling between temporal and frontal regions.

3.2 Introduction

In everyday life, we manipulate a variety of tools to achieve different purposes. We can use the same tool to perform specific actions with different goals, e.g. we could either grasp scissors to move them to a different location or to cut a string. At the same time, we can achieve the same final goal with different tools, e.g. we could cut the string with a scissors, a knife or a scalpel. Our brain represents information about tool actions not only during their actual execution, but even before, as motor intentions. Nevertheless, the neural correlates and functional interactions subtending tool actions are still poorly understood.

The likely candidate to encode this type of action-related information is the so-called 'tool' network (see for review Johnson-Frey, 2004; Lewis, 2006; Valyear et al., 2017). Neuroimaging studies identified this left lateralized system recruited while observing tool images (Chao et al., 1999; Chao and Martin, 2000) or while performing real or pantomimed tool actions (Rumiati et al., 2004; Johnson-Frey et al., 2005; Króliczak and Frey, 2009; Gallivan et al., 2013a; Brandi et al., 2014). This network comprises regions of the ventral stream, like the posterior middle temporal gyrus (pMTG), and of the parieto-frontal motor networks, including the supramarginal gyrus (SMG) and the anterior intraparietal sulcus (aIPS) within the inferior parietal lobe (IPL), the superior parietal lobe (SPL), the superior parieto-occipital cortex (SPOC) and the ventral and dorsal premotor cortices (PMv, PMd).

Recently, several fMRI studies adopting MVPA have started the description of different encoding of intention-related information within this network. Gallivan et al. (2013) showed encoding of intention-related information during the planning of real tool actions. Both temporal (pMTG) and parieto-frontal regions (SMG, aIPS, PMv, PMd) within the network represented specific planned actions (grasping or reaching with a plier). Moreover, connectivity analysis

showed that real tool actions involved functional interactions between ventral stream regions and parieto-frontal motor pathways (Hutchison and Gallivan, 2018).

Prompted by these results on real tool actions, we aimed at further characterizing the encoding and the functional interactions within this network underlying tool pantomimes. To the best of our knowledge, no study has yet investigated the encoding of intention-related information and the functional interactions for this specific type of actions.

In our study, we adopted MVPA and connectivity analyses of fMRI data to investigate: a) which regions within the tool network host intention- and action-related encoding and b) how information is transferred between ventral stream and parieto-frontal regions during the execution of tool use pantomime.

To address the first question, we started with defining two different levels of representation for intended and executed actions. We investigated 'concrete' representations by decoding different pantomimes (grasp-to-move or grasp-to-use) performed with a specific tool (scissors or axe). Then, we focused on decoding 'abstract' goal information, defined as information about the final goal of the pantomime irrespective of the adopted tool.

To anticipate our results, MVPA showed both types of encoding within the network and that abstract goal information was represented differently during the two phases of the task (planning and execution). This type of information was decoded significantly already in the planning phase within temporal (pMTG) and parietal regions (aIPS, SPOC), whereas it was significant within premotor regions (PMv) only during pantomime execution. The temporal difference in encoding suggested a transfer of information between ventral stream and/or parietal regions towards premotor cortex.

The second aim of the study was to test the functional interactions subtending pantomime of tool use. Classical neuropsychological (summarised in Johnson-Frey, 2004; Valyear et al., 2017)

and recent patient studies (Buxbaum et al., 2014; Hoeren et al., 2014) indicated two crucial sites within the tool network which if lesioned, caused impairments in this task: posterior lateral temporo-occipital cortex (LOTC), comprising pMTG, and the IPL. Our connectivity analysis showed that the normal performance of tool use pantomime is possible through a specific interplay between the ventral stream and the frontal nodes of the tool network.

3.3 Materials and Methods

3.3.1 Participants

Seventeen right-handed participants (7 females and 10 males, mean age 28.35, age range 24-44 years) took part in the experiment. All participants gave written informed consent for their participation in the study and were reimbursed for their time. The ethical committee for human research of the University of Trento approved the protocol of the study which was prepared in accordance with the Declaration of Helsinki.

3.3.2 Experimental task and design

Participants were requested to perform a delayed pantomiming task within the MR scanner. Pantomimes did not involve any interaction with a real tool and were executed moving the forearm only, without the involvement of the arm and the shoulder. The movement included an initial grasping component followed either by a pantomime of the use of a tool ("grasp-to-use" condition) or by a pantomime consisting in moving the tool laterally ("grasp-to-move" condition). Participants had to pretend to use two different tools for the pantomimes, either scissors or an axe.

These experimental conditions were embedded in a 2x2 factorial design, including as experimental factors: a) the final goal of the pantomime (either 'grasp-to-move' or 'grasp-to-use') and b) the type of tool (either scissors or axe).

We selected these two tools as we can exploit their functional properties to characterise "concrete" action representation and "abstract" goal encoding with MVPA. The two tools differ in terms of the kinematics of their two associated pantomimes, but they can be used to achieve the same final goal, as they can be moved or can be used to cut.

3.3.3 Experimental session and trial

Each experimental session consisted of eight functional runs (duration: 7 minutes each). After an initial baseline period (duration: 16 seconds), each run included 16 trials (4 repetitions for every condition). After the last trial ended, an additional baseline period was presented (duration: 20 seconds).

The structure of each experimental trial was the following (Figure 3.1). A verbal cue (duration: 1 second) was delivered to the subject via headphones signalling the action to be performed. The verbal cues corresponded to the experimental conditions ('use scissors', 'move scissors', 'use axe' or 'move axe'). Following each cue, participants pantomimed the corresponding grasp-to-use or grasp-to-move action.

After the verbal cue, the participant had to wait for 9 seconds (planning phase) until an auditory signal ('beep') indicated to execute the planned action. Participants had to release the home key button and had to perform the instructed pantomime with the right hand (execution phase). After 2.5 seconds, another auditory instruction ('beep') signalled to return to the home key position. A baseline period (11.5 seconds) was presented between the trials (inter-trial interval, ITI).
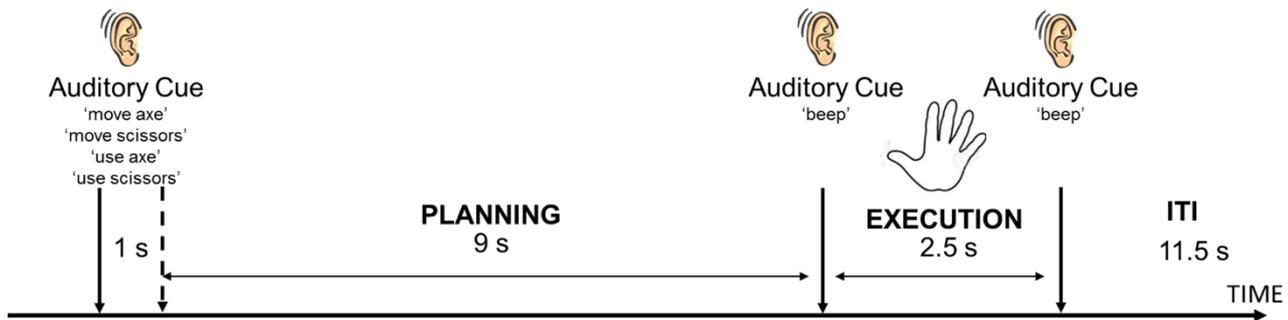
Figure 3.1. Timeline of experimental trial. The trial started with a verbal cue instructing the subject about the type of action to pantomime (duration 1 second). After 9 seconds of delay (Planning), the subject was instructed with an auditory cue ('beep') to perform the pantomime (Execution) with the right hand. After 2.5 seconds, another auditory cue ('beep') indicated the end of the trial. The participant waited for a new cue to start the following trial (ITI phase, duration 11.5 seconds).

Participants performed the task with the head in a 'standard position' within the MR coil. Throughout the entire experimental session, they had to look at a fixation cross projected on a screen through a mirror placed on the head coil. The right hand was kept at rest on an MR-compatible button box fastened on their chest with a Velcro belt. The button box allowed the recording of the reaction times (RTs) during the fMRI session. Stimulus delivery and response collection were controlled with the Presentation software (version 16, Neurobehavioural Systems, https://www.neurobs.com/).

Before the MR session, participants were trained to correctly perform the pantomimes. The experimenter explained how to pantomime the grasp-to-use and grasp-to-move for both the scissors and axe. They were requested to perform the pantomime, as if they were using the real tool. The participants practised the task outside the MR scanner under the supervision of the experimenter to ensure the understanding of the timings and of the to-be-performed pantomimes.

Within the MR room, participants were also trained to perform the pantomime without moving the upper arm and the shoulder while lying on the scanner bed. We asked them to pretend to grasp the object from their abdomen and to perform the pantomime without excessive

emphasis to avoid abrupt movements within the MR scanner. In addition to these initial training, the fMRI session was also recorded with an MR-compatible video camera and the performance of the participants checked offline for possible errors.

3.3.4 Tool Localizer

After the main experimental session, participants underwent a functional localizer to identify the areas of the tool network (adapted from Gallivan et al., 2013a). A single functional run was collected for each participant. The localizer consisted in alternating blocks (duration: 16 seconds each) presenting 18 images of tools or 18 images of scrambled tools (6 blocks per condition). A blank image with a fixation dot was presented at the beginning and the end of the functional localizer (duration: 20 seconds each). Participants had to perform a one-back task, pressing a button when the same tool or scrambled image was presented consecutively.

3.3.5 MR Data Acquisition

All the MR data were acquired with a 4T Bruker MedSpec scanner using an 8-channel head coil. T1-weighted anatomical scan (MP-RAGE, 176 axial slices, 1 mm isotropic voxels) images were acquired at the beginning of every session for each participant. The BOLD functional images were acquired with a T2* echo-planar imaging (EPI) sequence. Before each functional run, we collected the point-spread function (PSF) of the subsequently acquired sequence to correct for possible distortions (Zaitsev et al., 2004). We acquired 28 slices tilted to be parallel with the ACPC line (TR 2 s; TE 33 ms; FOV: 64 × 64 mm; in-plane resolution 3x3; slice thickness 3 mm; gap size: 0.45 mm). For the main experiment, participants completed eight runs of 210 volumes each (duration 7 minutes). After the last functional run, we collected one functional 'tool' localizer for each subject

(116 volumes, duration 3 minutes and 52 seconds, same acquisition parameters as in the runs of the main experiment).

3.3.6 Behavioural analysis

Offline analysis of the video-recorded fMRI sessions showed that participants could perform well the task. We removed trials in which participants: a) did not perform the correct pantomime, b) did not perform any action and/or c) released the button before the auditory signal. These trials were considered errors and were removed in the following behavioural and fMRI analyses. The total number of errors was eight. Six participants performed one error, and a single participant performed two errors.

We extracted RTs defined as the time interval between the first 'beep' cue and the time when participants lifted their hand from the button box to perform the pantomime. RTs above two standard deviations from the mean were considered as outliers and removed. Then, a repeated measure ANOVA was performed on the RTs. Reaction times were collected for sixteen subjects, due to technical problems with the button box, the RTs for one participants were not registered.

3.3.7 MR Data pre-processing

Data pre-processing and analysis were performed with MatLab (MathWorks) and the SPM12 toolbox (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The first five volumes of each run were discarded to avoid the saturation effect. Functional data were realigned to the first volume acquired, and slice-timing correction was performed. Then, the T1-weighted anatomical image was co-registered with the realigned functional mean EPI image. Normalization of the anatomical image was performed adopting the unified segmentation approach implemented in SPM12. The resulting normalization parameters were applied to the functional images (resampling voxel size at

3x3x3 mm). Spatial smoothing was applied to functional data using 8mm FWHM Gaussian kernel for univariate analysis only. High-pass filter (128 seconds) was also applied to the time series.

3.3.8 Tool localizer: univariate analysis

For each participant, a General Linear Model (GLM) analysis was estimated for the tool localizer. The predictors of interest consisted in the two categories of the presented images: tools and scrambled tools. The predictors were created with boxcar functions convolved with hemodynamic response function (HRF). The duration of the boxcar function was equivalent to the duration of the block (16 seconds). In addition, we modelled movement parameters (3 rotations and 3 translations) as predictors of non-interest. As we were interested in localizing activity related to tool observation, we computed the t contrast at the group level between the two conditions of interest: tools vs scrambled tools. The resulting activation map was used to independently select the regions of interest (ROIs) within the tool network. We adopted a cluster-forming threshold of $p<0.001$ uncorrected at the voxel level and then adopted Family Wise Error Rate (FWE) at the cluster level to control for multiple comparisons ($p<0.05$ corrected).

3.3.9 ROIs selection for MVPA

The first aim of the study was to characterize different levels of intention and action encoding within the tool network with MVPA. To this aim, we selected eight ROIs in the left hemisphere based on previous investigations on hand actions (for review see Culham and Valyear, 2006; Culham et al., 2006; Vesia and Crawford, 2012; Turella and Lingnau, 2014; Gallivan and Culham, 2015) and on the 'tool' network (see for review Johnson-Frey, 2004; Lewis, 2006; Valyear et al., 2017). We adopted the 'tool' localizer to identify the ROIs independently from the main experiment (see previous section).

The selected ROIs included:

- the left dorsal premotor cortex (L-PMd), at the junction between the precentral sulcus and the superior frontal sulcus (Valyear et al., 2012; Gallivan et al., 2013a);

- the left ventral premotor cortex (L-PMv), located within the precentral gyrus (Gallivan et al., 2011b, 2013a; Valyear et al., 2012);

- the left superior parietal lobule (L-SPL), located posteriorly to the postcentral sulcus and superiorly to the intraparietal sulcus (Lewis, 2006);

- the left superior-parieto-occipital cortex (L-SPOC) in the superior end of the parieto-occipital sulcus (Monaco et al., 2011; Gallivan et al., 2013a);

- the left anterior intraparietal sulcus (L-aIPS) located in the junction between intraparietal sulcus and post central sulcus (Culham et al., 2003; Grefkes and Fink, 2005; Valyear et al., 2007; Valyear and Culham, 2010; Gallivan et al., 2013a);

- the left supra-marginal gyrus (L-SMG), lateral to the segment of IPS and posterior to the lateral sulcus (Lewis, 2006; Gallivan et al., 2013a);

- the left posterior middle temporal gyrus (L-pMTG) within the posterior portion of the ventral stream (Valyear et al., 2012; Gallivan et al., 2013a);

- the left primary motor area (M1), identified in the 'hand knob' along the anterior part of the central sulcus (Yousry et al., 1997), has been localized adopting a univariate contrast from the main experiment (execution vs baseline, see below).

For each ROI, we started from the activation at the group level obtained from univariate analysis of the tool localizer. Within a radius of 8 mm from the group peaks, we defined subject-specific ROIs extracting individual peaks from the activation map of each specific participant. ROIs were created as spheres with a radius of 12 mm centred on the subject-specific peaks. Table 3.1

contains the coordinates of the peaks at group level together with subject-specific peaks and their

standard deviations.

MVPA ROIs COORDINATES

ROIs name	Group level peak coordinates			Mean single subject coordinates + standard deviation		
	x	y	z	x	y	z
Left pMTG	-51	-55	5	-49 +/- 3	-55.4 +/- 4.2	5.6 +/- 4.1
Left SMG	-57	-38	34	-50.5 +/- 4	-25.4 +/- 3.4	36.9 +/- 4.4
Left PMv	-42	2	44	-43.3 +/- 3.2	2 +/- 3.9	42.1 +/- 3.9
Left aIPS	-39	-37	44	-39.3 +/- 4.4	-36 +/- 4.1	43.6 +/- 4.1
Left PMd	-27	-7	62	-28 +/- 4.1	-4.1 +/- 5.2	57.5 +/- 3.6
Left SPOC	-24	-76	29	-25.4 +/- 2.9	-75.1 +/- 4.4	30.1 +/- 4.2
Left SPL	-30	-52	56	-29 +/- 3.1	-50.9 +/- 3	55.6 +/- 5
Left M1	-36	-28	59	-33.8 +/- 2.9	-24.8 +/- 2.2	58 +/- 3.9

Table 3.1: MVPA ROIs coordinates. The table reports the coordinates for each ROI at group level, the mean of the coordinates extracted from all the subjects, and the standard deviation for the three coordinates (X, Y, Z) of each ROI. These ROIs were extracted from the independent localizer (observing images of tools vs. scrambled). The last row of the table shows the same information for M1, identified with a different contrast (execution vs. baseline) Coordinates are reported in MNI space.

3.3.10 Multivariate pattern analysis (MVPA)

For MVPA, we defined a GLM considering separately the three parts of the task: the cue, the

planning phase and the execution phase (Figure 3.1). A similar approach has been recently

adopted to investigate the neural correlates of the planning of hand actions (Chen et al., 2014;

Cappadocia et al., 2016).

For each participant, we estimated a GLM on non-smoothed data modelling every single trial for each experimental condition. A total of 384 regressors of interest were considered, originating from the 4 experimental conditions (move axe, move scissors, use axe, use scissors) x 3 time phases (cue, planning, execution) x 4 repetitions per run x 8 runs. In addition, we modelled movement parameters (3 rotations and 3 translations), and errors, if present, as predictors of no interest.

COSMoMVPA toolbox (Oosterhof et al., 2016) () was adopted to perform MVPA analysis. We performed a ROI-based MVPA analysis (Kriegeskorte and Bandettini, 2007; Oosterhof et al., 2011) adopting the Linear Discriminant Analysis (LDA) as classifier. We considered subject-specific ROIs defined with the independent tool localizer (see previous section).

Decoding accuracy was estimated with a leave-one-run-out cross-validation approach (summarised in Figure 3.2). For each participant, the classifier was trained on single trials of seven runs and then tested on the trials of the remaining run, considering all the possible combinations. We excluded the error trials from the analysis by randomly selecting the same number of trials for each condition. This procedure ensured having the same number of trials for each condition when training and testing the classifier.

For each ROIs, we tested two pairwise comparisons separately for intended (planning phase) and performed actions (execution phase). The aim was to investigate the encoding of: a) 'concrete' action information, comparing different pantomime (grasp-to-move vs. grasp-to-use) performed with the same tool (see blue box in Figure 3.2) and b) 'abstract' action information, representing the same final goal irrespective of the adopted tool (see red box in Figure 3.2).

To test for 'concrete' representation, we trained and tested the classifier on data for the two different actions (grasp-to-move vs grasp-to-use), separately for each tool (scissors and axe); then, we calculated the mean of the decoding accuracy for these two decoding accuracy maps in every

voxel (see blue box in Figure 3.2). This subject-specific average decoding accuracy was then tested

at the group level.
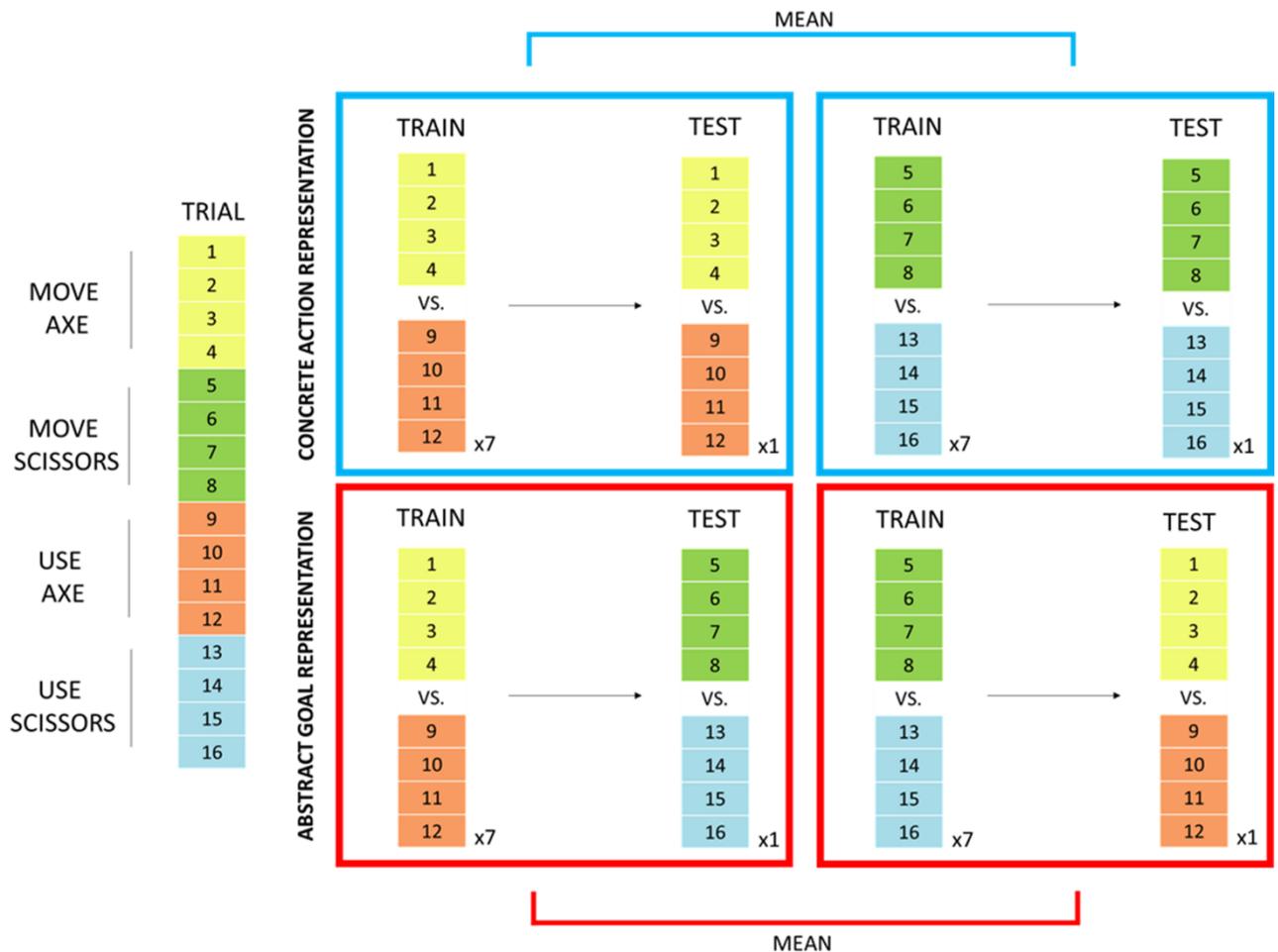
Figure 3.2. MVPA decoding and cross-validation. Each experimental run comprised 16 trials, 4 for each experimental condition (Left part of the Figure). Decoding accuracy was estimated with a leave-one-run-out cross-validation approach**,** by training the classifier on single trials of seven runs and then testing the classifier on the trials of the remaining run. To test for concrete action representation (blue box), we performed an MVPA analysis, comparing trials for the grasp-to-move and grasp-to-use conditions. We trained the classifier on data from seven runs and tested the classifier on the data from the remaining run on the same tool. We then averaged the decoding accuracy values for both tools (upper part of the blue box). For the abstract goal representation (red box), we adopted cross-decoding. We trained the classifier on the pairwise comparison between grasp-to-move vs grasp-to-use for one tool (e.g. axe) using data from seven runs and then tested the classifier for the same comparison on the other tool (e.g. scissors) using data from the remaining run. Then, we performed the same analysis but switching the trials adopted for training (e.g. scissors) and testing (e.g. axe) the classifier. Finally, we averaged the decoding accuracy values for the two combinations (lower part of red box).

In analogy with previous fMRI studies (Gallivan et al., 2013a, 2013b, 2013c; Ariani et al., 2015;

Tucciarelli et al., 2015; Turella et al., 2016), we adopted cross-decoding to test for 'abstract' action

representation, i.e. the representation of the final goal irrespective of the adopted tool (Figure

3.2). Here, we trained the classifier on the pairwise comparison between pantomimes for one tool

and then tested the classifier on the same comparison but for the other tool (see red box in Figure 3.2). This was performed in both directions and then we calculated the mean of the two decoding maps.

For all the comparisons, we tested decoding accuracy at the group level against chance (50 %) with a one-sample t-test (one-tailed) and corrected for multiple comparisons (across all ROIs and comparisons) applying a False Discovery Rate correction (FDR, q<0.05, (Benjamini and Yekutieli, 2001).

3.3.11 Connectivity analysis: Rationale

The second aim of the study was to investigate how the interplay between cortical regions within the network orchestrates successful pantomime of tool use. At the moment, this is still scarcely explored and strongly debated (Buxbaum et al., 2014; Hoeren et al., 2014). MVPA can provide evidence about the possible interactions within the network by comparing how information is encoded during the two phases of the task within each ROIs. Nevertheless, this definition is only indirect and not sufficient to establish the direction of information exchange within the network. Complementary information might come from connectivity analysis.

To address this issue, we focused on the pathways that are considered crucial in executing tool use pantomime. Recent frameworks proposed that both the ventral and the dorsal stream are involved in hand action (Binkofski and Buxbaum, 2013; Brandi et al., 2014; Gallivan and Culham, 2015; van Polanen and Davare, 2015) and should play specific roles in tool-related processing (Binkofski and Buxbaum, 2013; Gallivan et al., 2013a; Brandi et al., 2014).

Two pathways within the dorsal stream, the dorsomedial and the dorsolateral one, have been involved in processing action-related information (Rizzolatti and Matelli, 2003; Binkofski and Buxbaum, 2013; Turella and Lingnau, 2014). The dorsomedial pathway connects the SPL and SPOC

with PMd. This pathway is thought to be particularly relevant for online movement control (Rizzolatti and Matelli, 2003; Binkofski and Buxbaum, 2013; Fattori et al., 2015). The dorsolateral pathway connects the IPL - comprising SMG and aIPS - with PMv. This pathway seems to host representations of learned skilled actions acquired through experience (Binkofski and Buxbaum, 2013; Niessen et al., 2014).

The ventral stream have also been recently involved in hand action processing (Gallivan and Culham, 2015). Tool-selective regions within the pMTG are connected with the IPL within the dorsolateral pathway (Ramayya et al., 2010). Furthermore, temporal cortices - comprising pMTG - are also connected to the ventro-lateral frontal cortex, comprising the inferior frontal gyrus (IFG) (Vry et al., 2015). The ventral stream has been associated with the conceptual aspects of tool-use (Binkofski and Buxbaum, 2013).

Recent lesion-mapping studies supported a causal role of left ventral stream and dorsolateral pathway in producing pantomime of tool-use. Buxbaum et al. showed a specific impairment for pantomime of tool use associated with damage to the left posterior lateral temporo-occipital cortex (LOTC), comprising pMTG, in chronic stroke patients (Buxbaum et al., 2014). Another group study in acute stroke patients supported and extended these findings (Hoeren et al., 2014). A selective impairment during the pantomime of tool use was associated with damage within the left IPL (within the dorsolateral pathway), the LOTC and in white matter fibers (extreme capsule) connecting the temporal cortex with the frontal cortex. In the same study, lesions within the SPL were associated more strongly to deficits in imitation for meaningless hand and finger postures (Hoeren et al., 2014).

Based on these studies (Buxbaum et al., 2014; Hoeren et al., 2014), we focused our connectivity analysis on the dorsolateral pathway and the ventral stream, as they have been

shown to play a causal role in performing pantomime of tool use. Nevertheless, if and how these two pathways communicate to accomplish this task in the healthy brain is unknown.

3.3.12 Connectivity analysis: Anatomo-functional constrains for Dynamic Causal Modelling (DCM)

The aim of this analysis was to investigate the connectivity profiles between the ventral stream and dorso-lateral pathways during the execution of tool use pantomime. The idea was to investigate (i) how information is transferred between the temporal, parietal and frontal nodes of the tool network and (ii) which is the direction of this interplay. To test the interactions between these two pathways, we performed a Dynamic Causal Modeling (DCM) analysis.

DCM has been shown to be suitable for understanding functional interactions during motor tasks in the healthy and damaged motor system (Grefkes and Fink, 2014), but also for investigating semantic information and manipulation knowledge about tool (Kleineberg et al., 2018).

DCM allows estimating and making inferences about the coupling occurring between different brain regions influenced by specific experimental manipulations (Friston et al., 2003). The exchange of information does not necessarily reflect a direct anatomical connection between two considered regions, but the effect of the experimental conditions on a specific area. In the DCM approach, the model space explaining the interaction between brain regions has to be defined a priori and it needs to embed the best balance between accuracy and complexity of a reliable explanatory model (Stephan et al., 2010).

Given the previous anatomo-functional considerations, we focused on three ROIs within the left hemisphere in our connectivity analysis (see Figure 3.3 for spatial location and table 3.2 for peaks coordinates): aIPS, pMTG, PMv. Previous studies showed the dorsolateral areas PMv and aIPS to be involved in hand motor actions (Brandi et al., 2014) and to encode specific information

about hand and tool actions (Gallivan et al., 2013a), including tool pantomimes (Chen et al., 2016, 2017). Univariate analysis showed that aIPS in particular is activated both for real and pantomimed tool actions. aIPS seems to be crucial during the execution of tool pantomime, as it is the only brain region more strongly activated during pantomime execution (Hermsdörfer et al., 2007).

Within the ventral stream, pMTG is well known to play a pivotal role in processing semantic knowledge necessary to perform complex hand actions (Johnson-Frey, 2004; Lewis, 2006; Buxbaum et al., 2014; Hoeren et al., 2014; Martin et al., 2015; Chen et al., 2016; Milner, 2017). The role of these three regions and their location within the brain make them suitable nodes to investigate possible functional interaction between the dorsolateral pathway and the ventral stream.

DCM approach requires investigating the simplest models as possible (Stephan et al., 2010) to avoid over fitting of the data,. We selected three regions as they allowed defining the simplest and more plausible anatomical model to test the functional interactions between the two streams. DCM uses univariate signal changes over time to define how the coupling between the considered areas is affected during the overall task (intrinsic-fixed connectivity), and how a specific experimental condition can affect the functional communication between nodes (modulatory effect).

3.3.13 Connectivity analysis: DCM implementation

The raw functional data underwent a standard preprocessing analysis including realignment, slice timing, normalization and smoothing. Data and trial onsets of each run were then concatenated and merged in a single dataset used to estimate and test different models of connectivity.

The ROIs (12mm) for each of the three considered regions were obtained for each participant at individual level. The same 8mm masks created on the peaks extracted from the localizer for the MVPA analysis were used to identify the univariate individual peaks (Table 3.2) from the concatenated GLM considering the contrast [execution vs baseline]. Time series of the VOI were extracted for supra-threshold voxels as the first eigenvariate within a sphere of 12 mm radius around the individual maxima at a threshold of $p < 0.05$ (uncorrected).

We assumed that the auditory instructions of the task entered and perturbed the activity of the considered network in pMTG (acoustic input). Based on anatomical connectivity studies on non-human primates (Davare et al., 2011), we tested the intrinsic connectivity including forward and backward connections between all the considered nodes (Figure 3.3). Anatomical connections between aIPS and PMv are direct, whereas between the ventral stream and PMv are mediated through the IFG. The communication between pMTG and aIPS could be direct, however there are no evidence in the human brain confirming this hypothesis. Nevertheless, functional connectivity studies showed a strong coupling of pMTG with aIPS and PMv within the left hemisphere (Bracci et al., 2012; Hutchison et al., 2014).
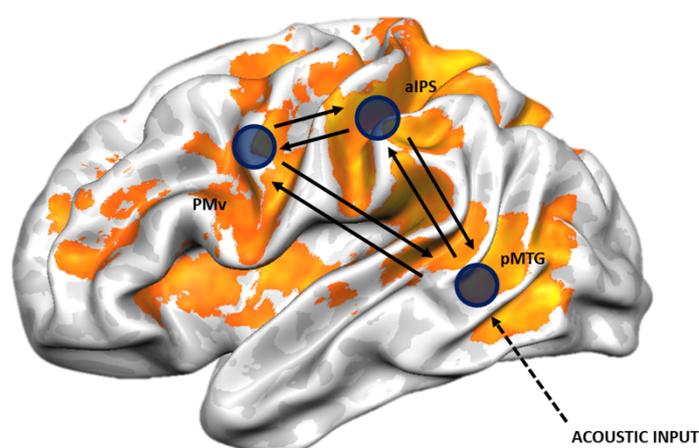
Figure 3.3: ROIs location and intrinsic connections considered in DCM analysis. Visual representation of the single peaks coordinates for each node. The map shows the conjunction between the univariate contrast [execution vs. baseline] and the t maps of the multivariate conjunction between 'abstract' and 'concrete' aspects of the action during the execution phase. The black arrows represent the intrinsic connections between the nodes considered in the DCM analysis. The information (AUDITORY INPUT) enter into the system from MTG, which is reciprocally connected with aIPS, and PMv. The two dorsal nodes aIPS and PMV are also reciprocally connected as found in non-human primates' studies.

DCM ROIs COORDINATES

ROIs name	Group level peak coordinates			Mean single subject coordinates		
	x	y	z	x	y	z
Left pMTG	-54	-46	8	-51.03 +/- 3.05	-54.92 +/- 4.23	5.17 +/- 4.38
Left aIPS	-42	-40	47	-39.9 +/- 3.03	-36.35 +/- 3.36	48.21 +/- 4.11
Left PMv	-48	5	44	-48.31 +/- 4.16	4.82 +/- 3.49	41.05 +/- 3.87

Table 3.2: **Coordinates for regions adopted in the DCM analysis.** The group level peaks were identified from the conjunction between the univariate contrast [execution vs. baseline]. A mask (radius 8 mm) with centre the group coordinates was created. Single subject peak coordinates were extracted from the univariate concatenated GLM [execution vs. baseline] within the created mask. A VOI (radius 12mm) was then created with centre the individual peak. Peak positions are reported in MNI space.

Our aim was to understand how the information related to the execution of our delayed-tool pantomiming task is transferred through the three selected regions. We investigated the modulatory effect of the 'grasp-to-use' task on the coupling between these cortical areas, as damage to regions within the ventral stream and the dorsolateral pathway lead to impairment in this task (Buxbaum et al., 2014; Hoeren et al., 2014).

We defined fifteen different models considering all the meaningful patterns of modulatory effect on the connections within the considered network (see Figure 3.4). We included models considering coupling from the ventral to the dorsolateral areas, as well as a communication from the dorsal to the ventral stream, or a mix of the two.

We adopted a random-effect Bayesian model selection (BMS) to identify the model that better explains the given data in terms of posterior probability percentage compared to the other tested models (winning model). Then, we extracted for each subject the parameters of the winning model and averaged them across subjects; finally, we statistically tested the model parameters values at group levels for the intrinsic and for the modulatory connections with a one-

sample t test corrected for multiple comparisons with an FDR (q<0.05, Benjamini and Yekutieli, 2001)).
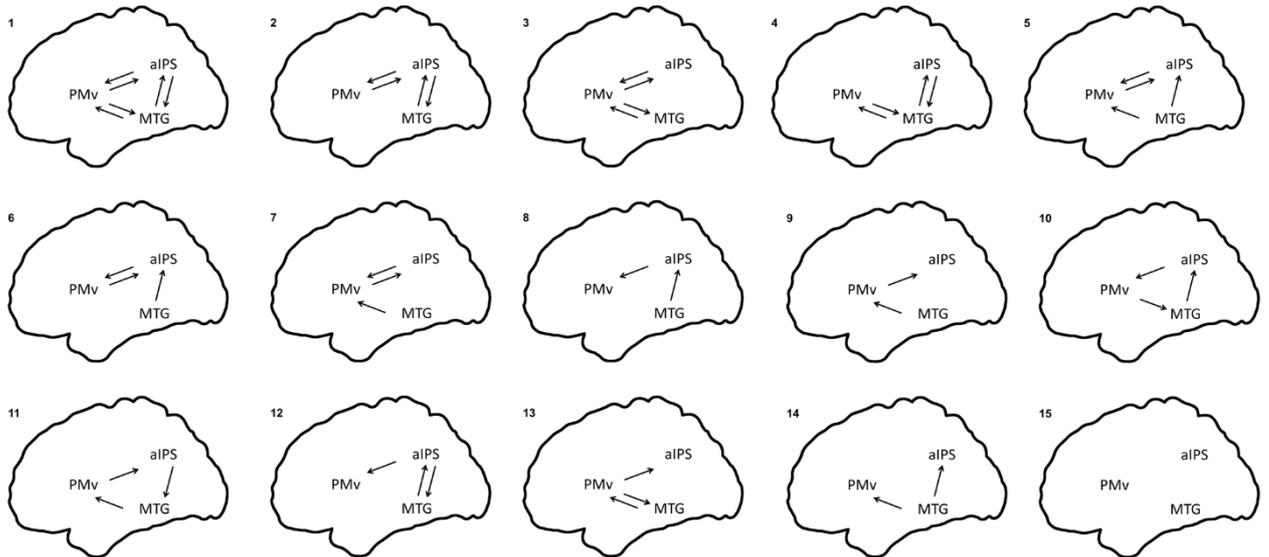
Figure 3.4: Models tested for the modulatory effects. We tested the modulatory effect of the 'grasp-to-use' task considering all the possible meaningful combinations of forward and backward modulatory connections between the considered nodes. A total of fifteen models were considered and tested. The connections considered in each model are schematically represented.

3.4 Results

3.4.1 Behavioural results

We performed a 2x2 repeated measure ANOVA (factors: tool type and action goal) on the RTs of 16 participants. The main effect for tool type [$F_{(1,15)}$= 0.905; p= 0.356], action goal [$F_{(1,15)}$= 1.155; p= 0.299] and the interaction between the two factors were not significant [$F_{(1,15)}$= 0.064; p= 0.804].

3.4.2 Univariate analysis: tool localizer

The independent localizer was designed to identify the ROIs for MVPA within the cortical regions recruited during the observation of tool (contrast: images of tools vs scrambled images). The univariate contrast showed widespread activations within areas of the temporal lobe

(comprising pMTG), of the parietal lobe (comprising SMG, aIPS, SPL, SPOC) and of the frontal cortex (comprising PMd, PMv and IFG). All the ROIs, except for M1, were defined using this localizer. The peak coordinates of M1 were extracted from the univariate contrast (execution vs baseline) performed on the data of the main experiment. Figure 3.5 shows the location of the selected ROIs within the tool network depicted on a three-dimensional template of the left hemisphere. The univariate contrast map of the independent localizer was overlaid on the image of the left hemisphere.
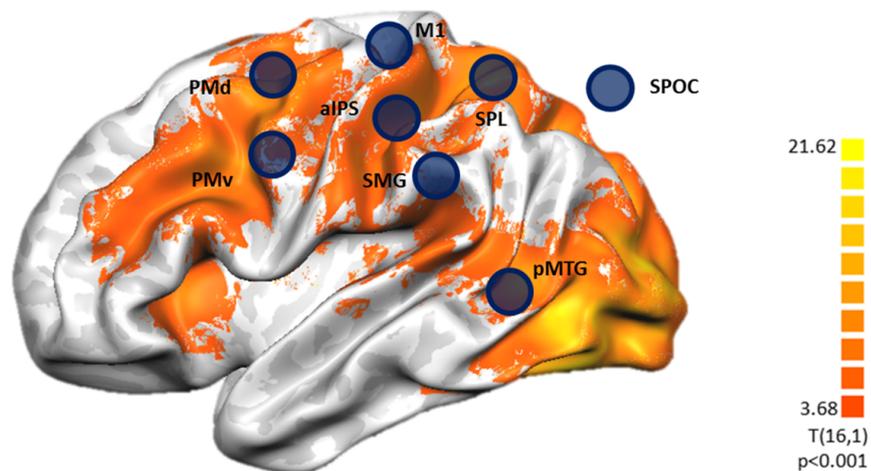
Figure 3.5: Univariate activation for the tool localizer and position of ROIs. The activation map for the independent localizer (contrast tool images vs. scrambled images) was overlaid on an anatomical 3D template of the left hemisphere. The statistical map threshold was set at p<0.001 uncorrected at voxel level and p<0.05 FWE corrected at cluster level. The location of the regions of interest (ROIs) considered in the study. The ROIs include the posterior middle temporal gyrus (pMTG), the supramarginal gyrus (SMG), the anterior intraparietal sulcus (aIPS), the superior parietal lobule (SPL), the superior parieto-occipital cortex (SPOC), the dorsal premotor cortex (PMd) and the ventral premotor cortex (PMv). The primary motor area (M1) has been identified from the univariate contrast (execution vs. baseline) performed on the data of the main experiment.

3.4.3 ROI based MVPA results

MVPA analysis was performed separately for the planning and the execution phases, investigating two different action representations within the tool network. Decoding accuracy was extracted within subject-specific ROIs and the average was computed at the group level for each

tested pairwise comparison. Figure 3.6B schematically summarised the same results presenting separately the two different phases of the task.
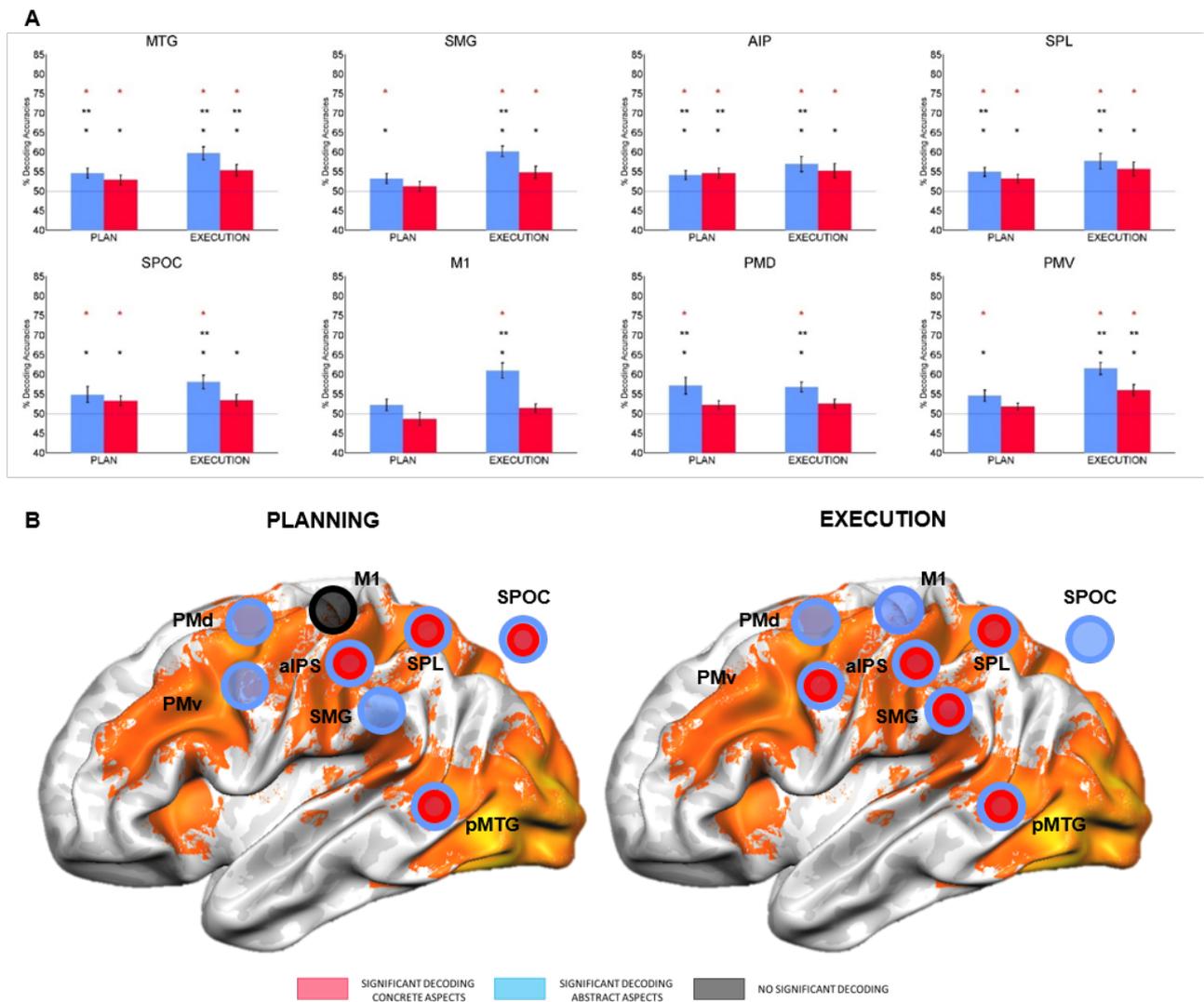
Figure 3.6: A. Decoding results for ROI-based MVPA. The bar graphs show the decoding accuracy for 'concrete' goal encoding (blue) and for 'abstract' goal encoding (red), for the planning phase (left) and in the execution phase (right). Significant decoding is indicated with asterisks (p<0.05 *; p<0.005 **; FDR q<0.05 red star). **B. Summary of MVPA ROI analysis.** Schematic representation of the results divided in planning and execution phase: in blue, the ROIs with a significant decoding (FDR q<0.05) for concrete aspects of the action, in red the ROIs with a significant decoding for abstract information related to the goal of the action. In black areas with no significant decoding for none of the considered conditions.

ROI-based MVPA showed different decoding results for concrete and abstract representations during the two phases of the task. During planning, we found that decoding of the intention to perform a specific concrete action is significant in all the ROIs, except for M1. The representation of specific concrete hand actions within posterior parietal and premotor regions is in line with

neuroimaging studies on object-directed movements in human (Gallivan et al., 2011b, 2013a, 2013c) and neurophysiological investigations in monkey (Fattori et al., 2015; Janssen and Scherberger, 2015). Our study extended these finding showing that even planned pantomimed actions can be decoded from the very same regions.

Already during the planning phase, preparatory information regarding abstract goal could be decoded in pMTG, aIPS, SPL, and SPOC (Figure 3.6B). This result confirmed the role of posterior parietal areas in processing intention-related information for subsequent hand movements (Andersen and Buneo, 2002). SPL has been described as involved in the online transformation of proprioceptive information into a system of coordinates able to lead a motor action (Barany et al., 2014). Similarly, SPOC is involved in the online control of an action and in the transformation of object related information into possible motor program (Monaco et al., 2011). A significant encoding of abstract information might be related to the maintenance of a representation of intended action adopted for subsequent online monitoring within both SPL and SPOC. If in the concrete condition the control of the action could be related to low level features as kinematic, the encoding of abstract information could represent a higher level of control related to the goal of the action. Left aIPS is not only involved in the execution of hand action (Rice et al., 2006), it is known to integrate objects features to generate motor programs (Buxbaum et al., 2005), our results support its involvement also in storing and retrieving engrams about possible hand movement. The posterior part of MTG is known to be an important hub for processing semantically related information about the identity and function of tools and encoding information about upcoming movements (Lingnau and Downing, 2015).

It is interesting that abstract goal representation involves both the parietal and the ventral streams. This result suggests that the ventral stream is involved in storing programs of potential actions also independently from the characteristics of the tool.

Recent studies found differences in encoding action-related information within the tool network during the execution of the task (Chen et al., 2016, 2017). Different types of pantomime of object-oriented actions can be decoded in the temporal lobe (fusiform gyrus) and in the IPL, possibly comprising anterior intraparietal sulcus (aIPS). The left intraparietal lobule (IPL) seems to be involved in processing action information, independently from the performed task (identification vs pantomime), from the stimulus modality instructing the movement and from the adopted tool (Chen et al., 2017).

During the execution phase of our task, concrete action information was represented in all the considered ROIs. Interestingly, we found a change in the encoding of abstract goal information in the dorsolateral regions PMv and SMG (see Figure 28B), whereas pMTG, aIPS and SPL kept their representational content stable across the two phases of the task. The temporal difference in goal encoding between the planning and execution phase suggested that abstract action-related information reach frontal regions later in time compared to the posterior nodes of the network. These results suggest a transfer of information during the execution of the pantomime between functionally connected areas of the tool network.

The areas that are likely to transfer abstract goal information to the premotor cortex (PMv) are the temporal pMTG and/or the parietal aIPS (directly or through SMG) as they host the representation of abstract goal information already during the planning phase of the task. IPL and pMTG might retrieve the knowledge related to the function of a specific tool as established in lesion studies (Binkofski and Buxbaum, 2013; Buxbaum et al., 2014) and transfer this information through functionally connected areas (Lingnau and Downing, 2015).

Connectivity analysis can provide direct evidence on the information flow between the temporal and fronto-parietal nodes of the tool network, defining the interaction between pMTG and the dorsolateral regions, aIPS and PMv. Complementary to our MVPA results, where changes

in the representational content have been highlighted within dorsolateral areas, connectivity analysis might allow to indirectly infer the possible transfer of task-specific information within the network.

3.4.4 DCM results

The second aim of the study was to investigate the connectivity profiles between the two main networks involved in the execution of tool use pantomime: the dorsolateral pathway and ventral stream (Vry et al., 2015). To this purpose, we defined connectivity models considering three main nodes of the tool network (pMTG, aIPS and PMv).

Our DCM analysis tested how the connectivity between the three nodes is overall affected by the task (intrinsic connectivity). Within the intrinsic connections, we tested both forward and backward projections (Figure 3.7A).
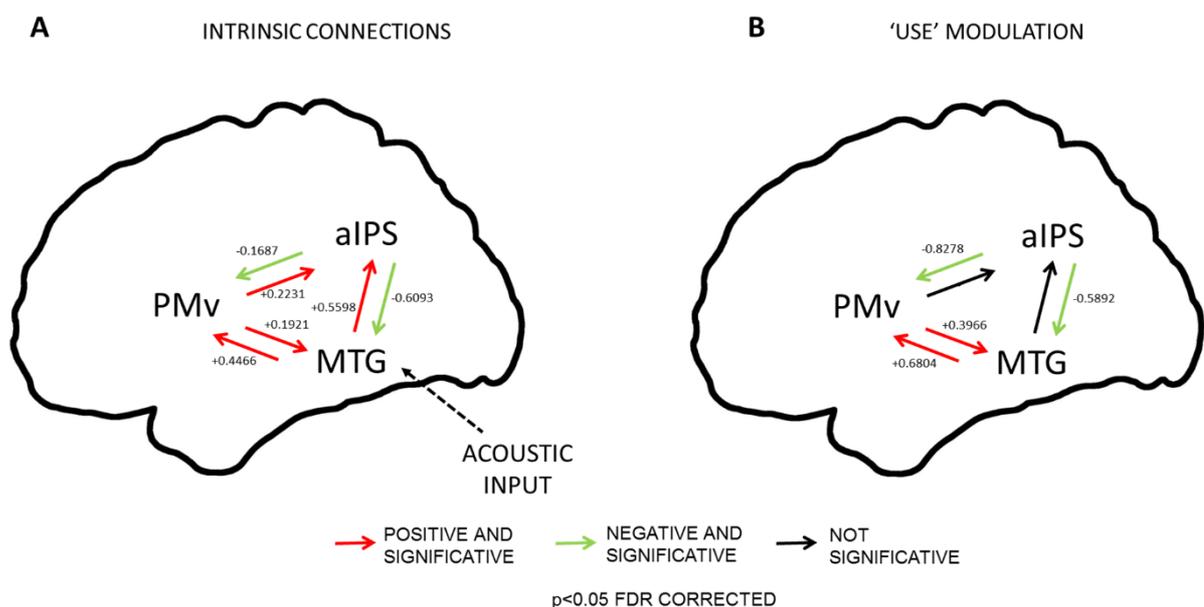
Figure 3.7: A. Intrinsic connections. The red arrows indicate the positive and significant connections between two nodes; the green arrows indicate the negative and significant connections between two nodes (p<0.05 FDR corrected). In the intrinsic connectivity is reciprocally highlighted between MTG and PMv and unidirectionally highlighted from MTG to aIPS and from PMv to aIPS. A reduced connectivity can be found between aIPS and the two functionally connected nodes **B. Modulatory connections within the winning model**. The 'use' condition positively modulates the reciprocal connections between MTG and PMV. The communication between aIPS and the other two nodes is instead reduced when pantomiming the use of a tool.

We have found that the communication between pMTG and PMv during the execution of the task is enhanced in both directions. The same is true for the connections between pMTG and aIPS and between PMv and aIPS. On the other hand, the connections starting from aIPS to the other two nodes is significantly reduced during the execution of the task. These results defined a significant coupling between the ventral and the dorsolateral pathway during the execution of our pantomiming task.

Secondly, we examined how the communication between the two considered pathways is affected by the use condition (modulatory effect). The results obtained considering the modulatory effect are quite interesting (Figure 3.7B). aIPS showed a decrease in coupling with the other two nodes during tool pantomime, whereas there was an enhanced bidirectional interplay between the ventral stream and PMv when pantomiming the use of a tool. The random-effect Bayesian selection (BMS) showed, out of fifteen tested models, that the one that better explains our data with an exceedance probability of 79%, is the model including all the forward and backward connections between the considered nodes (see Figure 3.7B and Table 3.3).

EXEEDANCE PROBABILITIES

Single model	Exceedance probability
Model 1	0.7903
Model 2	0.0166
Model 3	0.0260
Model 4	0.0028
Model 5	0.0242
Model 6	0.0060
Model 7	0.0206
Model 8	0.0041
Model 9	0.0045
Model 10	0.0034
Model 11	0.0039
Model 12	0.0024
Model 13	0.0049
Model 14	0.0036
Model 15	0.0867

Table 3.3: Exceedance probabilities derived from the comparisons of single models. Adopting a BMS approach, between all the tested models, Model 1 has a probability of 79% to explain the data. For this reason, compared to the exceedance probability of the other entire tested model, Model 1 is the winning model of our analysis.

Four out of six connections were significant as shown in Figure 2.9B. Interestingly, we found an increased reciprocal connectivity between pMTG and PMv, while there was a significant reduction in the strength of the connectivity between aIPS and PMv and between aIPS and pMTG. Overall, the modulatory effect of the use condition strengthened the communication between the ventral and the dorsolateral pathway, but only through the connections between temporal and frontal regions. These results supported a recent study (Vry et al., 2015) combining fMRI and tractography. Based on these fMRI activation patterns, tractography identified a ventral pathway,

connecting temporal cortex to prefrontal regions, as the main cortical anatomical route underlying object-directed pantomime. Our results extended this finding providing direct evidence for a bidirectional functional interplay between temporal and frontal regions during tool use pantomime.

Even if it is difficult to directly compare MVPA and DCM results, as these two methods capture different aspects of fMRI signal, our results are in line with previous studies showing a functional communication between the two streams (Mahon et al., 2007; Valyear and Culham, 2010). Considering the presence of connections between dorsolateral and ventral streams in monkeys (Borra and Luppino, 2017) and also in humans (Cloutman, 2013), this communication was described at two different levels. Our MVPA results showed a possible transfer of abstract goal information from the ventral stream and/or the parietal cortex to PMv, whereas DCM showed a task-specific bidirectional functional interplay between the temporal and frontal cortex.

3.4.5 Limitations for interpreting MVPA and DCM analysis

We acknowledged the presence of few limitations in our study. First, the encoding of intention- and action-related information within the ventral stream might be caused by the specific selection of pantomime, which seemed to engage specifically the ventral pathway (Vry et al., 2015). Second, the adoption of a delayed pantomiming task might have also caused the recruitment of the ventral stream. It has been suggested that the ventral stream might be particularly involved in task requiring a delayed action and/or memory-based movements (Milner and Goodale, 2008, 2012). Third, we cannot exclude that the directionality of the flow of information found with our connectivity analysis might be at least partially determined by the adoption of a non-visually-guided task and by delivery of the go signal in the auditory modality. It is possible that a visually guided task might engage less the ventral stream. Nevertheless, a recent

study adopting visually-guided tool and hand actions (Gallivan et al., 2013a) showed significant intention-related decoding within the ventral stream and functional interactions between temporal and fronto-parietal regions (Hutchison and Gallivan, 2018).

Even considering these limitations, our analyses showed a modification of encoding and of task-specific functional interactions within the ventral and the dorsolateral pathway, suggesting a communication between these two pathways.

3.5 Discussion

Our study had a twofold aim: 1) to investigate how different intention-related information is encoded within the tool network and 2) to understand its functional interactions during the pantomime of tool use.

First, we showed that the tool network hosted two different levels of representation of planned and executed pantomimes: concrete and abstract. Our results showed a widespread representation for concrete action within the tool network and significant encoding for abstract goal information within the posterior parietal and temporal nodes during the planning phase. We have also found that the abstract representation within some of the selected regions changes at different time windows (planning and execution). The temporal discrepancy in decoding accuracy during the two phases of the task suggested a possible exchange of information between the ventral stream and/or the parietal regions, with the premotor cortex.

Second, our connectivity analysis showed an increased bidirectional coupling between the ventral pMTG and the dorsolateral PMv during the execution of tool use pantomime. The 'grasp-to-use' task modulation highlights the communication between temporal and frontal areas bypassing the parietal nodes of the dorsal stream.

We will discuss our findings in light of recent advances on the description of the neural underpinnings underlying tool pantomimes.

3.5.1 Concrete and abstract representations within the tool network

A recent study adopted MVPA to distinguish between grasping and reaching actions performed either with a real tool or with hands (Gallivan et al., 2013a). They showed that the intention to perform specific hand actions and real-tool actions are represented separately within the fronto-parietal networks and within the ventral stream. In the present study we complemented these results considering the encoding of two levels of action goal information. We have found that the concrete action representation was encoded within the same areas of the tool network that are involved in both real-tool actions and in tool pantomime tasks. In Gallivan's study, specific hand actions, similar to our concrete level, could be significantly decoded only within SPOC and the posterior temporal area EBA, whereas specific real tool actions could be decoded only within SMG and pMTG. Overall, this study supported the idea of a specialization of the ventral stream for real tool actions. Nevertheless, we found significant decoding for specific tool pantomime (during planning and execution) within areas described as specialized for real-tool actions only (SMG and pMTG) even if our task did not involve real tools.

Our results suggest that pantomimes represent a specific action category that, as tool actions, requires the retrieval of semantic information and memory-based experiences about tool-use from the ventral stream to be correctly performed.

A similar consideration can be made to the representation of abstract action information within the posterior nodes of the temporal lobe (pMTG) and the posterior-parietal areas of the motor network (SPL, SPOC, aIPS). These areas showed a significant encoding of abstract goal information during the planning phase as well as during the execution of the pantomime.

The demonstration of encoding of abstract information expands previous finding (Gallivan et al., 2013a; Chen et al., 2016) showing that the goal of pantomimes of tool action is represented within pMTG already before the execution of the task regardless of the adopted tool. This seems to suggest that even if tool pantomimes are hand actions, they engage the temporal pMTG as real tools do.

So far different studies adopting a univariate approach, described a stable organization of the hand motor network showing the recruitment of the same areas both for real-tool action and tool pantomimes (Lewis, 2006; Hermsdörfer et al., 2007; Brandi et al., 2014; Valyear et al., 2017). Our results are in line with these findings showing a recruitment of the network when pantomiming a tool action. On the other hand, we have found that the abstract representation of the goal of an action has a different spatial organization when considering the planning and the execution phase. In these two time points, some areas of the tool network changed their abstract representation (SMG, PMv, SPOC), while some other maintained a stable representation of the information about the final goal of the action (pMTG, aIPS, SPL). The posterior areas of the temporal and parietal lobes fulfill different roles in tool action organization and integrate visual, auditory and proprioceptive stimuli. pMTG is implicated in processing semantic lexical knowledge, which is essential in understanding action words and associating tool names with their properties (Johnson-Frey et al., 2005). Within the parietal lobe, IPL is involved in representing action knowledge (Mahon et al., 2007; Binkofski and Buxbaum, 2013; Chen et al., 2017) and is influenced by the final goal of an action (Fogassi et al., 2005; Hamilton and Grafton, 2006). Recently, it has been shown that IPL is particularly sensitive to the use of a tool irrespectively from the task adopted (pantomime - identification) and from the stimulus modality used (written words - visual pictures) (Chen et al., 2017). Along with these results, we confirmed the role of IPL in processing

high-level representation of the action, showing that aIPS/IPL host a representation of abstract goal information already when preparing for the action.

All in all, our findings are corroborated by studies on non-human primates (Fogassi et al., 2005; Bonini et al., 2010, 2011) and on human (Hamilton and Grafton, 2006) showing the role of the dorsolateral parieto-frontal pathways (connecting SMG and aIPS with PMv) in processing complex hand action information. Moreover, our results expand these findings confirming the involvement of the ventral stream (pMTG) in action planning and execution (Lingnau and Downing, 2015).

Our study showed that the representational content of the tool network flexibly changes according to the timing phase considered, highlighting a not univocal representation of the action-related information within the tool network.

3.5.2 Functional communication between ventral and dorsal streams

Recent investigations supported the possible interaction between dorsal and ventral stream during hand actions (van Polanen and Davare, 2015; Milner, 2017). Nevertheless, the dynamics of these interactions are still poorly understood (Cloutman, 2013). Our MVPA analysis showed differences in encoding abstract goal information between planning and execution showing a flexible representation of action goal information within the tool network. This is probably one of the most interesting finding of this study, as it suggests a transfer of information from the posterior nodes of the network, where the encoding of abstract goal information is stable through time (pMTG, aIPS), to the more anterior one (PMv). There are two possible alternatives for the information to reach the frontal cortex: in one case, information could enter from pMTG and then flow through aIPS and from there to PMv; or it could enter from pMTG and be sent to the premotor cortex directly (PMv). Our connectivity analysis helped in shading light upon the

dynamic of the interaction. DCM helped defining the directionality of the communication between the considered areas and highlighted a reciprocal exchange of information between the three considered nodes occurring during the execution phase of the task.

Analyzing the specific effect of our grasp-to-use condition (modulatory effect), we showed that pantomiming the use of a tool selectively enhances the functional coupling between the ventral pMTG and the dorsal PMv. With our study, we have been able to highlight the functional communication between the dorsal and ventral pathways. Moreover, we described the direction of this communication, which seems to process from the ventral to the dorsal stream. This result is in accordance with what recently stated by Milner about the communication between visual streams (Milner, 2017). Based on previous studies, he asserted that a ventro-dorsal interaction could allow the performance of meaningful actions by conveying visual and semantic information. Overall, as already showed in clinical studies (Buxbaum et al., 2014; Hoeren et al., 2014), we found that the temporal lobe is crucial in supporting hand and tool actions. Moreover, with this study we contribute to the increasing number of evidence showing that these two visual pathways are not segregated and independent, but share task-related information.

3.5.3 Conclusion

Our study demonstrated that different levels of action and intention-related information are encoded within the tool network. The network entails a distributed representation of concrete aspects of the actions that is maintained in time, whereas abstract goal information is transferred from posterior to anterior regions during the unfolding of the movement. This exchange of information might be possible thanks to the functional interaction between ventral and dorsal streams. Connectivity analysis confirmed a bidirectional interplay between temporal and frontal regions, evident as a task-related modification in connectivity profiles. Overall, the tool network

has a dynamic structure, which maintains and flexibly transfers different types of action

information in time and across regions.

Chapter 4

Task-dependent action encoding within and outside the 'classical' tool network

4.1 Abstract

Performing a tool action or pantomime recruits a dedicated left lateralized tool network including temporal and fronto-parietal areas. Recently we observed that the representational contents of the tool network changes according to the considered experimental time window (planning/execution). However, we were not able to define if the patterns of activity described were also task dependent. To this aim, we asked 17 right-handed participants to perform two separate session of a delayed- pantomiming task while in the MR scanner. We investigated which brain regions within the tool network represent: different goals achieved with the same tool (concrete representation) and the same goal achieved with different tools (abstract representation). In session A, the task was performed with the right (dominant) hand, and the instructions were delivered using auditory modality. In session B, participants used the left hand following visual instructions.

As several clinical studies reported deficits in tool use even after a lesion in the right hemisphere, we included in the MVPA the regions of the right hemisphere corresponding to those considered in the left hemisphere as part of the 'classical' tool network. Our aim was to investigate the possible encoding of different action-related information, i.e. concrete and abstract goal representations, within the classically defined 'tool' network and in the corresponding regions of

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the right hemisphere.

Our results showed tool action-related encoding in both in the left hemisphere and in the right hemisphere in both the sessions. Furthermore, we demonstrated changes in the encoding of abstract goal information between the two experimental sessions showing a flexible representation of abstract action goal information within the considered regions depending on task demands (adopted hand and instruction modality).

4.2 Introduction

The neural correlates of our daily interactions with tools have been identified in a dedicated left-lateralized cortical network (Johnson-Frey et al., 2005; Lewis, 2006; Gallivan and Culham, 2015; Valyear et al., 2017). fMRI studies adopting a univariate approach, showed a similar recruitment of the tool network irrespective of the adopted hand, either left or right (Johnson-Frey et al., 2005; Króliczak and Frey, 2009; Brandi et al., 2014; Kroliczak et al., 2016), and of the performed task (real tool manipulation (Brandi et al., 2014), pantomimes (Johnson-Frey et al., 2005)). This overlap in fMRI activation was interpreted as evidence for the system to represent the same type of action-related information across different tasks and/or effectors (Lewis, 2006). This interpretation relied on the assumption that the recruitment of the same cortical region can be interpreted as subtending common neural processing. However, the univariate approach provided only indirect evidence of possible common processing, as it cannot test the informational content represented within a cortical region, which is only possible with MVPA (Kriegeskorte and Bandettini, 2007b). To this aim, our study adopted a multivariate approach (MVPA) to test if the regions within and outside the tool network host the same representational content or if it changes depending on task demands.

We focused on the planning phase, the time window preceding execution where the intention of the subject to act is generated (information about the execution phase can be found in the supplementary materials). We asked participants to perform the same delayed pantomiming task in the MR scanner in two independent sessions (A and B) adopting different effectors (right vs left hand) and following different instruction modality (auditory vs visual). We focused on investigating the neural underpinnings of the goal of an action. Our experimental conditions allowed us to distinguish between two different levels of action goal representation. Indeed, we can manipulate differently the same tool to achieve different purposes (i.e. grab a mug from its handle to drink or grab a mug from the top to move it), as we can achieve a specific final goal using different tools (bring food to the mouth with a fork or with chopsticks). We defined the first level (i.e. the possibility to achieve different goals with the same tool) as a 'concrete' representation of the action, and the second level (i.e. the possibility to achieve the same final goal irrespective from the considered tool) as an 'abstract' representation.

We performed a ROIs-based analysis considering the regions of the 'classical' tool network within the left hemisphere, including areas of the ventral (pMTG) and of the dorsal streams (SMG, aIPS, PMd, PMv, SPL, SPOC) (Johnson-Frey, 2004; Lewis, 2006; Valyear et al., 2017). The selection of the ROIs in the left hemisphere refers to the results of clinical studies investigating the correlation between lesioned brain regions and motor deficits (e.g. apraxia), showing the predominant involvement of the left hemisphere in supporting such ability (Choi et al., 2001; Manuel et al., 2013). Nevertheless, based on patient studies reporting deficits in tool actions following lesions in the right hemisphere (Marchetti and Della Sala, 1997; Salazar-López et al., 2016) and on fMRI univariate studies reporting right activity when performing a tool-related task (Johnson-Frey et al., 2005; Brandi et al., 2014), it is plausible to assume that the right hemisphere plays a role in supporting tool actions. At the moment, the role of the right hemisphere in tool actions is still

unknown. For these reasons, we included in the analyses the same regions contralateral to the classical tool network and investigated the role of the right hemisphere in processing the action's goal in a tool-pantomiming task.

4.3 Material and Methods

4.3.1 Participants

In this study, we consider the same participants recruited to perform the study described in chapter 3 of this thesis. Results for Session A have been already partially reported in the previous Chapter. The experimental sample consisted of 17 right-handed participants (7 females and 10 males, mean age 28.35, age range 24-44 years) that performed the two MR sessions in different days and a counterbalanced order.

The ethical committee for human research of the University of Trento approved the protocol of the study. All participants gave written informed consent for their participation in the study and were reimbursed for their time.

4.3.2 Experimental design and setup

The two experimental sessions shared the same design and differed for: i) the modality in which instructions were delivered (auditory in session A and visual in session B) and ii) the effector used to act (right hand in the auditory session and left hand in the visual session).

Participants were requested to perform a delayed pantomiming task within the MR scanner. Pantomimes did not involve any interaction with a real tool and were executed moving the forearm only, without the involvement of the arm and the shoulder. The movement included an

initial grasping component followed either by a pantomime of the use of a tool ('grasp-to-use' condition) or by a pantomime consisting in moving the tool laterally ('grasp-to-move' condition). Participants pretended to use two different tools for the pantomimes either a pair of scissors or an axe.

These conditions were embedded in a 2x2 factorial design, including as experimental factors: a) the final goal of the pantomime (either 'grasp-to-move' or 'grasp-to-use') and b) the type of tool (either scissors or an axe).

4.3.3 Experimental sessions and trials

The two experimental sessions were identical in terms of the number of experimental runs participants had to complete. Each experimental session consisted of eight functional runs (duration: 7 minutes each). After an initial baseline period (duration: 16 seconds), each run included 16 trials (4 repetitions for every condition). After the last trial ended, an additional baseline period was presented (duration: 20 seconds).

The structure of each experimental trial was the following. In the auditory session (Session A, Figure 4.1A), a verbal cue (duration: 1 second) was delivered to the subject via headphones signalling the action to be performed. The verbal cues corresponded to the experimental conditions 'use scissors', 'move scissors', 'use axe' or 'move axe'.

After the verbal cue, followed 9 seconds of delay (planning phase), after that a sound ('beep') indicated to execute the planned action (execution phase). Participants had to release the home key button performing the instructed pantomime with the right hand (execution phase). After 2.5 seconds, another auditory instruction ('beep') signalled to return to the home key position. A baseline period (11.5 seconds) was presented between the trials (inter-trial interval, ITI).

In the experimental visual session (Session B, Figure 4.1B), at the beginning of each trial, a visual cue was presented. The cue was a black-and-white picture either of scissors or an axe. Each coloured frame (yellow, blue, green or red) surrounding the presented image, was associated with a specific action. Each colour represented a specific action goal and a specific tool (e.g. yellow = move axe, blue = use axe, red = move scissors, green = use scissors). The association colour-action/tool was learnt during a training session performed outside the scanner before the experiment and was pseudo-randomized between subjects. The timing and the sequence of each trial are the same as the one described in the auditory session with the only difference that in the visual session, the execution of the pantomime was performed with the left hand.
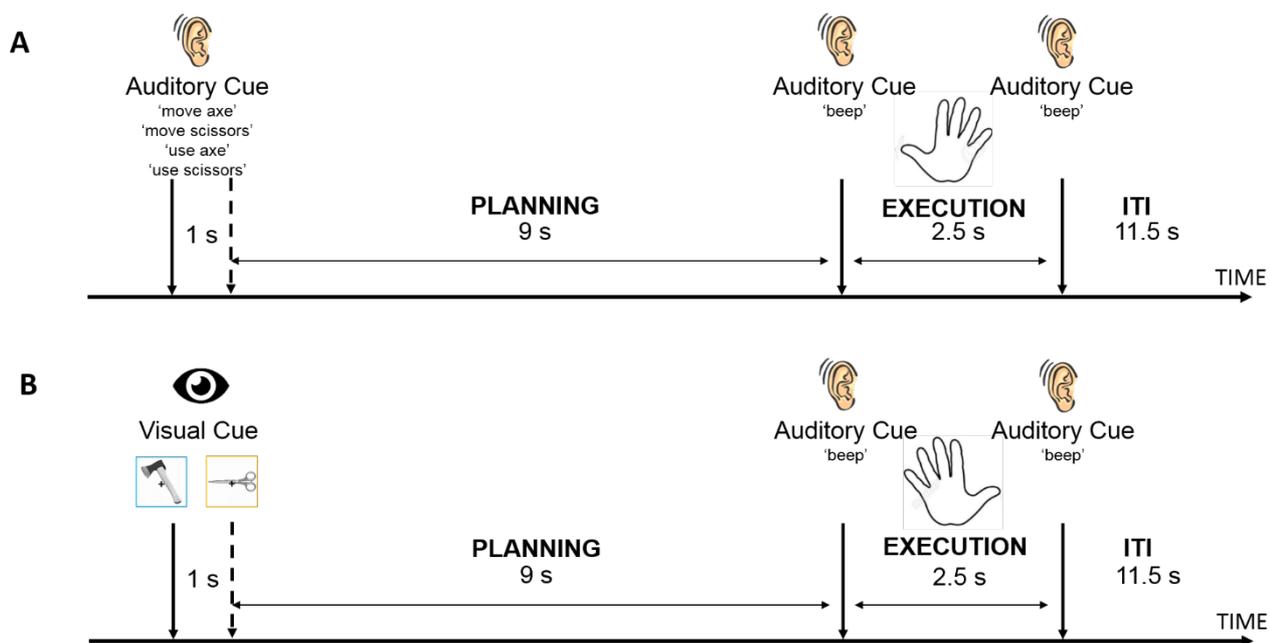
Figure 4.1 A. Timeline of experimental trial for Session A. The trial started with an auditory cue instructing the subject about the type of action to pantomime (duration 1 second). After 9 seconds of delay (Planning), the subject was instructed with another auditory cue ('beep') to perform the pantomime (Execution) with the right hand. After 2.5 seconds, another auditory cue ('beep') indicated the end of the trial. The participant waited for a new cue to start the following trial (ITI phase, duration 11.5 seconds).
B. Timeline of experimental trial for Session B. The timeline of the visual session is the same as one of the auditory session. It starts with the visual presentation of one of the tool-pictures surrounded by a colour (instruction's duration 1 second). After 9 seconds delay where participants had to keep in mind the instruction seen (planning phase duration 9 seconds), an auditory signal indicated to release the button box and to perform the requested pantomime (execution duration 2.5 seconds); another auditory cue informed the participants to stop pantomiming and to go back to the rest position. Between each trial occurred 11.5 seconds window (inter-trial-interval ITI), which allowed the BOLD signal to go back to the baseline.

Throughout the entire experimental session, participants had to maintain the gaze on a fixation-cross projected on a screen through a mirror placed on the head coil. The hand used in the task was kept at rest on an MR-compatible button box fastened on their chest with a Velcro belt. The button box allowed the measurement of the reaction times (RTs) during the fMRI session. Stimulus delivery and response collection were controlled with the Presentation software (version 16, Neurobehavioural Systems, https://www.neurobs.com/).

Before each MR session, participants were trained to perform the instructed pantomimes correctly and familiarized with the task. Subjects were requested to perform the pantomime as if they were using a real tool. The same training was performed within the MR room while lying on the scanner bed. In order to monitor the execution of the task, each fMRI session was recorded with an MR-compatible video camera and the performance was checked offline for possible errors.

4.3.4 Tool Localizer

At the end of the first main experimental session, a single functional run was collected for each participant to identify the dorsal and ventral areas involved in tool observation (adapted from Gallivan et al., 2013a). The localizer consisted of 12 blocks (6 blocks per condition) where 18 images of tools or 18 scrambled patterns of the same images were presented alternately (duration: 16 seconds each). At the beginning and the end of the run, participants had to fixate a fixation dot on a white background (duration: 20 seconds each). In order to keep the attention high, participants were requested to perform a 1-back task, pressing a button every time they saw a consecutively repeated image or scrambled pattern.

4.3.5 MR Data Acquisition

A 4T Bruker MedSpec scanner and a 8-channel head coil were used to acquire T1-weighted anatomical scan (MP-RAGE, 176 axial slices, 1 mm isotropic voxels) and a T2* echo-planar imaging (EPI) for each of our functional runs (TR 2 s; TE 33 ms; FOV: 64 × 64 mm; in-plane resolution 3x3; slice thickness 3 mm; gap size: 0.45 mm). A total of 28 slices tilted to be parallel with the ACPC line were acquired for each subject in each run. Before each sequence, we acquired a point-spread function (PSF) to correct for possible distortions (Zaitsev et al., 2004). For each session, participants completed eight runs of 210 volumes each (duration 7 minutes). At the end of the first experimental session, each participant underwent a functional 'tool' localizer (116 volumes, duration 3 minutes and 52 seconds, same acquisition parameters as the runs in the main experiment).

4.4 Analyses

4.4.1 Behavioural analysis

We collected reaction times for 16 subjects. Due to technical problems with the button box, the RTs for one participant in session A and one participant in session B were not registered.

Each recorded video was analysed offline, and errors were taken into account and removed from the analyses. Overall, the performance accuracy was very high with only 8 errors in session A and 2 errors in session B. All the trials where participants did not perform the correct pantomime, did not perform any action and/or released the button before the auditory cue were considered as errors.

The time interval between the first auditory cue ('beep') and the time registered from the button box when participants lifted their hand to perform the pantomime was considered as reaction time (RT) and was extracted for each trial of every run performed.

We removed RTs of pantomimes performed too slowly (more than two standard deviations from the mean) from the behavioural analysis. On the RTs extracted we computed a repeated measure ANOVA using SPSS (IBM SPSS Statistics, Version 21).

4.4.2. MR data pre-processing

fMRI data were pre-processed and analysed using the Statistical Parametric Mapping software package (SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/)) and MatLab (MathWorks). We removed the first five volumes of each run to avoid the saturation effect, and we realigned the functional data to the first volume acquired to correct for inter-scan movements; then the slice-timing correction was performed. The t1-weighted anatomical image was co-registered with the realigned functional mean EPI image. Data were spatially normalized to the Montreal Neurological Institute (MNI) template adopting the unified segmentation approach implemented in SPM12 (voxels were resampled at 3x3x3 mm). Lastly, functional data were smoothed using an FWHM Gaussian kernel of 8mm and filtered with high-pass temporal filtering (128s).

4.4.3. Univariate analysis

For each independent experimental session, a General Linear Model (GLM) was estimated for each participant. We defined 12 predictors of interest, containing parameters of the 4 experimental conditions (move axe, move scissors, use axe, use scissors) x 3 time phases (Plan,

Execution, instructions). The BOLD response was modelled using a boxcar function convolved with hemodynamic response function (HRF). The duration of the boxcar function was equivalent to the duration of the phase specified (instruction: 1 sec, Plan: 9 sec, Execution: 2.5 sec). Head movement parameters and error trials, if present, were modelled in the design matrix as predictors of non-interest.

All statistical analyses were performed using a p values at $p < 0.05$ for single comparisons. To control for multiple comparisons, we used cluster-level correction with $p < 0.05$ (TFCE corrected, see (Smith and Nichols, 2009).

4.4.5. ROIs selection

We selected the regions constituting the tool network within the left hemisphere, as described in chapter 2.

Besides, we identified homologous regions within the right hemisphere to test the possible role of these regions in encoding tool pantomime. Several clinical studies showed that a lesion in the right hemisphere produces deficits in tool use and pantomimes (Marchetti and Della Sala, 1997; Salazar-López et al., 2016). Within the non-dominant hemisphere, we defined the regions of interest by defining the contralateral peaks of each area on the tool localizer map and repeating the same procedures performed to select the ROIs in the left hemisphere.

We defined the group coordinates of each ROI starting from the reference coordinated provided by the literature (Gallivan et al., 2013a) and then moving to the nearest maximum peak of the localizer activation map. We then defined subject-specific ROIs extracting, for each participant, individual peaks within a radius of 8 mm from the group peaks. ROIs were created as spheres with a radius of 12 mm centered on the subject-specific peaks. The considered ROIs included seven areas within the fronto-parietal motor network and one area within the temporal lobe in each

hemisphere. Anterior to the precentral sulcus we identified the dorsal premotor area (PMd) (Valyear et al., 2012; Gallivan et al., 2013a) and the ventral premotor area (PMv) (Gallivan et al., 2011b, 2013a; Valyear et al., 2012). The superior parietal lobule (SPL), was situated posteriorly to the postcentral sulcus and superiorly to the intraparietal sulcus (Lewis, 2006). The superior-parieto-occipital cortex (SPOC) can be found medially in the superior end of the parieto-occipital sulcus (Monaco et al., 2011; Gallivan et al., 2013a). The anterior intraparietal area (aIPS) was located in the junction between intraparietal sulcus and postcentral sulcus (Culham et al., 2003; Valyear et al., 2007; Valyear and Culham, 2010; Gallivan et al., 2013a). The supra-marginal gyrus (SMG), is lateral to the segment of IPS and posterior to the lateral sulcus (Lewis, 2006; Gallivan et al., 2013a). The posterior middle temporal gyrus (pMTG) can be found within the posterior portion of the ventral stream (Valyear et al., 2012; Gallivan et al., 2013a). The primary motor cortices (left and right M1) were identified in the 'hand knob' along the anterior part of the central sulcus (Gallivan et al., 2013a). The peak of M1 was identified on a univariate contrast of the main experiment (execution vs baseline), one for each session.

Table 4.1 contains the coordinates of the peaks at group level extracted from the independent localizer together with the mean of subject-specific peaks and their standard deviations, both for the left and the right hemisphere.

ROIs name	Group level peak coordinates			Mean single subject coordinates			Standard deviation mean coordinates		
Independent localizer									
	x	y	z	x	y	z	x	y	z
LEFT MTG	-51	-55	5	-49	-55.4	5.6	3.0	4.2	4.1
RIGHT MTG	45	-67	-1	44.4	-66.3	-3.5	2	3.6	4.5

LEFT SMG	-57	-38	34	-50.5	-25.4	36.9	4.0	3.4	4.4
RIGHT SMG	63	-37	41	55.2	-34.8	34.4	2.7	3.3	5.8
LEFT PMv	-42	2	44	-43.3	2	42.1	3.2	3.9	3.9
RIGHT PMv	42	2	44	43.5	3.5	41.9	3.9	2.6	4.8
LEFT aIPS	-39	-37	44	-39.3	-36	43.6	4.4	4.1	4.1
RIGHT aIPS	45	-34	47	46.2	-34.1	45.4	2.9	3.5	3.9
LEFT PMd	-27	-7	62	-28	-4.1	57.5	4.1	5.2	3.6
RIGHT PMd	33	-1	56	33.4	-0.4	54.6	6	3.2	3.5
LEFT SPOC	-24	-76	29	-25.4	-75.1	30.1	2.9	4.4	4.2
RIGHT SPOC	27	-73	32	27.9	-73	32.3	2.7	5.4	2.8
LEFT SPL	-30	-52	56	-29	-50.9	55.6	3.1	3	5
RIGHT SPL	36	-49	59	32.9	-49.8	57.9	2.8	3.3	3.1
Univariate contrast [execution vs. baseline]									
LEFT M1	-36	-28	59	-33.8	-24.8	58	2.9	2.2	3.9
RIGHT M1	30	-22	65	32.3	-22.2	63.5	2.8	3.1	3.3

Table 4.1: **ROIs Coordinates for both sessions.** The table reports the coordinates extracted from the independent localizer (observing images of tools vs scrambled), in both left and right hemisphere, of each ROI at the group level; the mean of the individual coordinates extracted from all the subjects; the standard deviation for XYZ of each ROI. The last two rows of the table show M1 coordinates, coordinates identified with a univariate contrast of the main experimental sessions (execution vs baseline). Coordinates are reported in MNI space.

3.4.4. Multivariate pattern analysis (MVPA)

We used CoSMoMVPA toolbox (Oosterhof et al., 2016) (http://www.cosmomvpa.org/) to perform decoding analysis. We defined a GLM estimated on non-smoothed data for each participant modelling single trials for each experimental condition. A total of 384 regressors of interest were considered, originating from the 4 experimental conditions (move axe, move

scissors, use axe, use scissors) x 3 time periods (Instruction, Plan, Execution) x 8 runs x 4 repetitions per run. Head movement parameters were included as predictors of no interest.

We performed ROI-based MVPA analysis on the areas of the tool network in the left hemisphere and on the same contralateral areas in the right hemisphere. We also performed a whole brain searchlight MVPA to check for possible significant brain regions not included in the ROI analysis (Kriegeskorte and Bandettini, 2007; Oosterhof et al., 2011). The results concerning the searchlight analysis are provided as supplementary materials (see 4.8 supplementary material paragraph).

The radius of the ROIs and of the searchlight measured 4 voxels. The classifier used in the MVPA analysis is the Linear Discriminant Analysis (LDA).

We estimated the decoding accuracy with a leave-one-run-out cross-validation approach repeated for each tested pairwise comparison. For each participant, the classifier was trained on the trials of seven runs and then tested on the remaining one. This procedure was repeated for all the possible runs combination. We removed errors from the analysis randomly selecting the same number of trials for the two conditions, to maintain the same number of trials for each condition in the training and in the testing set of the classifier.

For each session, we tested two different levels of action representation:

- *Concrete action representation*, distinguishing between different types of pantomime (to use vs to move) performed with the same tool [move vs use mean between tools]. To explore the concrete representation, we run separately the pairwise comparisons between [move axe vs use axe] and [move scissors vs use scissors]. We then calculated the mean of the decoding accuracy for these two pairwise comparisons [move vs use mean between tools].

- *Abstract goal encoding*, distinguishing between different pantomime goals (to use vs to move) irrespective of the adopted tool [move vs use across tools]. In analogy with previous fMRI studies

(Gallivan et al., 2013a, 2013b, 2013c; Ariani et al., 2015; Tucciarelli et al., 2015; Turella et al., 2016), we adopted cross-decoding to test for the abstract representation of action goal irrespective of the adopted tool. We trained the classifier on the comparison [move axe vs use axe] and tested it on the comparison [move scissors vs use scissors] and vice versa. With cross decoding, we can understand if a region represents the final goal of a pantomime irrespective of the tool used to achieve it.

In the following paragraph, the results concerning the planning phase are reported. Results for the execution phase can be found in the supplementary materials section.

4.5 Results

4.5.1. Behavioural results

We performed a 2x2 repeated measure ANOVA (factors: tool type and action goal) on the RTs of 16 participants in each session. The main effect for tool type [session A: $F_{(1,15)}$= 0.905; p= 0.356; session B: $F_{(1,15)}$= 0.285; p= 0.601], action goal [session A: $F_{(1,15)}$= 1.155; p= 0.299; session B: $F_{(1,15)}$= 0.462; p= 0.507] and the interaction between the two factors [session A: $F_{(1,15)}$= 0.064; p= 0.804; session B: $F_{(1,15)}$= 0.848 ; p= 0.372] were not significant.

4.5.2 Univariate analysis

4.5.2.1 Tool localizer

The localizer was designed to identify the cortical regions recruited during tool observation (contrast: tool images vs scrambled images). As expected, the univariate analysis highlighted areas in the temporal lobe (pMTG), in the parietal lobe (SMG, aIPS, SPL, SPOC) and the premotor areas (PMd and PMv). Starting from group level local peaks coordinates, we created a mask for each ROI

of the left and the right hemisphere. Within each mask, an ROI was then created centered on the individual peak of each subject. Left M1 coordinates were extracted from the univariate contrast (execution vs baseline) performed on the data of the main experiment of the first session (see the previous chapter); equally, the right M1 coordinates were extracted from the univariate contrast (execution vs baseline) of the second session.

4.5.3 ROI based MVPA analysis

We are going to present the results of the ROIs based analysis considering the regions within the 'classical' tool network, in the left hemisphere, and then the results of the equivalent contralateral regions within the right hemisphere.

4.5.3.1 Representational content within the 'classic' tool network

ROI-based MVPA showed different results for concrete and abstract representations within the left hemisphere.

Planning phase - Concrete representation.

In the planning phase of session A (Figure 4.3A), all the regions of the left hemisphere encoded for concrete action information with the exception of M1, in which the encoding did not pass the statistical threshold. In session B (Figure 4.3B), decoding of concrete action information is significant in all the regions of the left dorsal hand motor network and in the considered regions of the left ventral stream. The representation of actions information within posterior parietal and premotor regions in the left hemisphere is in line with neuroimaging studies in humans (Gallivan et al., 2011b; Monaco et al., 2011) and neurophysiological investigations in monkeys (Jeannerod et al., 1995; Sakata et al., 1995; Galletti and Fattori, 2017).

Planning phase - Abstract representation.

Looking at the representation of abstract goal information during the planning phase, we have found different scenarios between the two experimental sessions. Overall, the encoding of abstract goal information within the 'classical' tool network is stable in pMTG and SPL in the two experimental sessions. In session A, also aIPS represents abstract goal information above chance level (FDR corrected); while in session B a significant abstract encoding was found in the left premotor cortices (L-PMD, L-PMV).
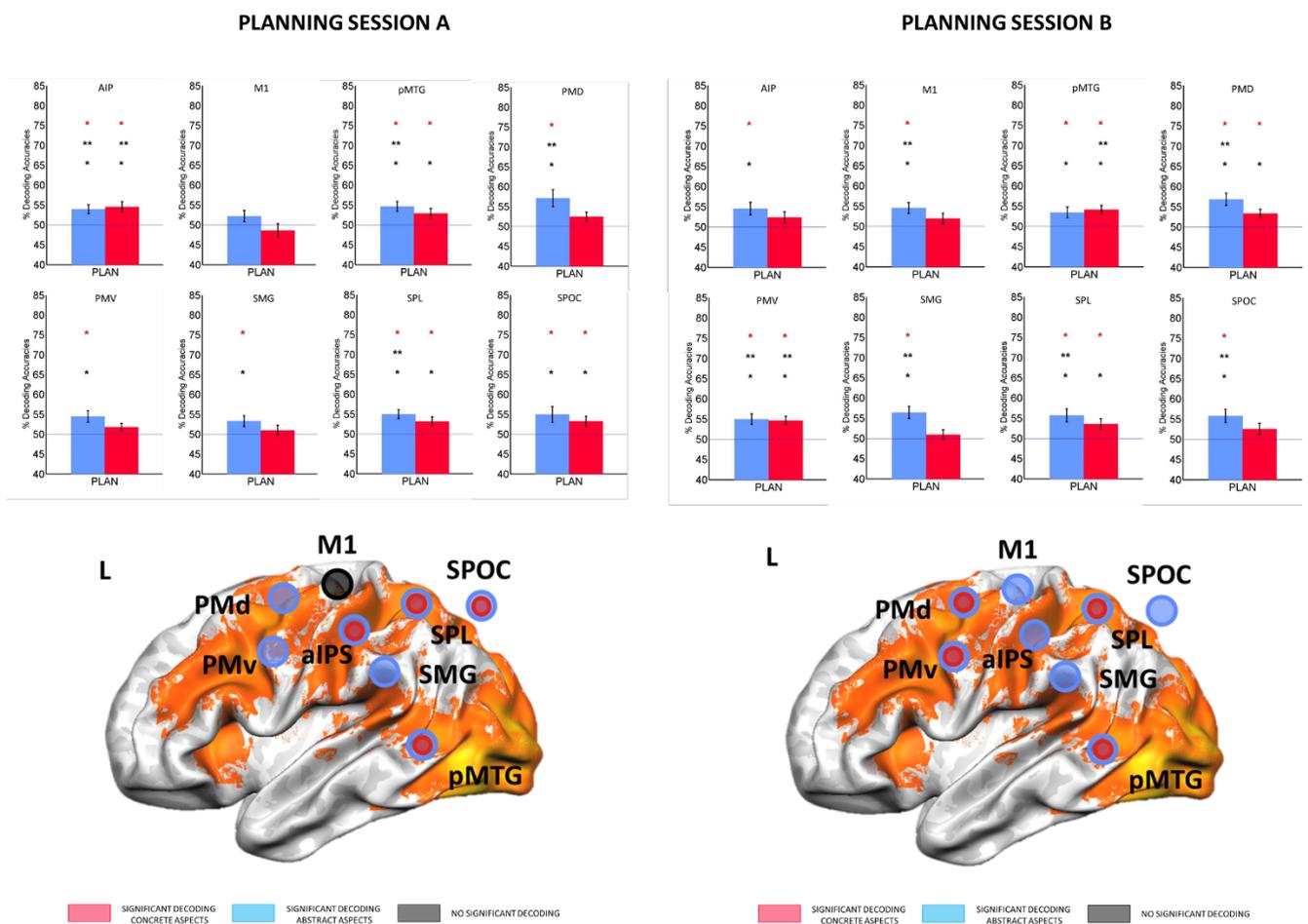
Figure 4.3: Results for ROIs within the left hemisphere. Planning phase: the MVPA results concerning the planning phase are represented in bar graphs showing the percentage of decoding accuracy extracted within each ROI of the 'classic' tool network (upper part. In blue the concrete encoding; in red the abstract encoding). Significant decoding is represented with asterisks: p<0.05 *; p<0.005 **; FDR q<0.05 red star. In the lower part of the figure, there is a schematic representation of the same FDR corrected results (in blue the ROIs with significant decoding for concrete information; in red the ROIs encoding for abstract goal information; in black areas with no significant decoding). **A. Session A.** All the considered regions, except for M1, encode for the concrete representation of the goal; only the posterior nodes pMTG, aIPS, SPL and SPOC encode for the abstract representation of the goal already during the planning phase. **B. Session B.** In session B, all the considered regions encode concrete information, but differently from session A, PMv, PMd, SPL and pMTG encode abstract goal information (FDR corrected).

4.5.3.1 Representational content 'outside' the tool network. The role of the right hemisphere in intention/action encoding

One of the aims of the study was to investigate if concrete and abstract information about the goal of an action can also be represented outside the tool network. We included in the analysis areas of the right hemisphere homologous to the regions of the 'classical' tool network.

Planning phase - Concrete representation.

Exception for M1 in session A, SMG in session B and PMV both in session A and session B, all the selected ROIs encode above the chance level (FDR corrected) concrete information about tool-action pantomimes (Figure 4.4). These findings show that the right side of the brain, together with the left hemisphere, host a concrete representation of the goal of the intended action already when planning a movement and independently from the characteristics of the task.

Planning phase - Abstract representation.

The encoding of abstract goal information is significant in the right SPOC both in session A and in session B (FDR corrected). In session B, also pMTG, SMG, SPL and aIPS encode abstract goal information (Figure 4.4).

Different studies tried to describe the organization of the network supporting hand motor action (Goodale and Milner, 1992; Rizzolatti and Matelli, 2003; Milner and Goodale, 2012; Binkofski and Buxbaum, 2013); nevertheless, the representational content within the involved areas is still not completely understood. Based on previous univariate findings we would expect the same regions to encode the same abstract goal information irrespectively from the hand and the instruction modality adopted, but this is not the case.
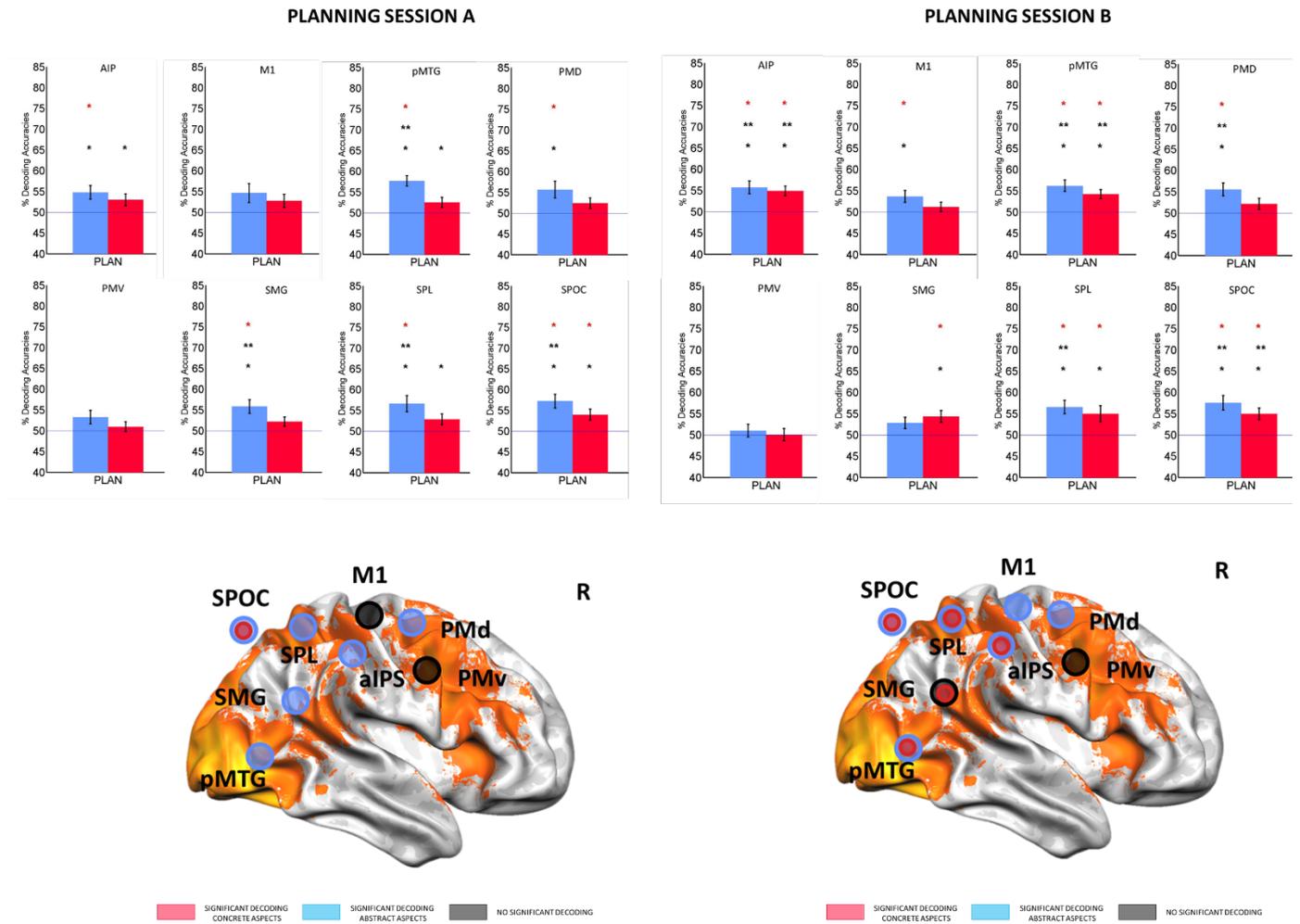
Figure 4.4: Results for ROIs within the right hemisphere. Planning phase: : the results of each ROIs within the right hemisphere are represented both in bar graphs (In blue the concrete encoding; in red the abstract encoding. p<0.05 *; p<0.005 **; FDR q<0.05 red star); and schematically showing the location of each ROI (blue ROIs: significant decoding for concrete information; red ROIs: encoding for abstract goal information; black ROIs: no significant decoding). **A. Session A.** The ROIs encoding for concrete information are pMTG, SMG, SPOC, aIPS, SPL, PMd. Only SPOC host also an abstract representation of the goal of the action. **B. Session B.** In session B, the areas encoding for concrete information are pMTG, aIPS, SPL, SPOC, M1, PMd. Abstract goal information is represented in pMTG, SMG, aIPS, SPL and SPOC.

Overall, we showed clear differences in goal encoding between the two sessions in both hemispheres (see Figure 4.3 and 4.4), suggesting a task-dependent representation of this information. Nevertheless, we found univariate activation differences between the two sessions in the areas recruited (see supplementary materials), which might partially explain our MVPA results.

4.6 Discussion

In the present study, two main issues have risen: (i) we found that the representational content of the considered areas can change according to the task requirements (i.e. adopted effector and instruction modality). Moreover, (ii) we showed how abstract information about the goal of an action is represented within the considered regions of the tool network but also outside of it, in the right hemisphere.

We will now discuss the main findings of our study, focusing mainly on the differences in encoding abstract goal information and on the role of the right hemisphere, providing hints to better understand the organization of the hand motor network in a tool-pantomiming task.

4.6.1 A flexible representation of action goal information

Previous studies adopting univariate approach showed similar recruitment of the tool network irrespective of effector and/or task requirements (Johnson-Frey et al., 2005; Brandi et al., 2014). However, as already discussed in the introduction, this overlap is not a guarantee of a shared representation of action information within these regions. We showed that, even if there are areas commonly recruited during the planning phase of our two experimental sessions (see univariate results in appendix 6.1.1), the spatial distribution of the representational content about the abstract goal of an action within the tool network changes in a task dependent way. Our MVPA analysis highlighted that in some areas (i.e. left SPL, left pMTG, right SPOC) the information about the goal of an action is maintained stable through time and tasks, while some other flexibly change their content. This confirms that the tool network is a flexible and dynamic system, in which the transfer of information happens not only between functional pathways within the same hemisphere (see previous chapter), but also across hemispheres. Our finding extended the

proposal by Galletti and Fattori (Galletti and Fattori, 2017). The authors suggested a dorsal network in which the involved areas are not specialized for processing a single functional property. Alternatively, they hypothesise an interconnected network where several functional aspects of a motor action can take place relying on the same areas. We provided evidence for a flexible interaction not only within the pathways of the dorsal stream but also between the ventral and the dorsal stream expanding this previous hypothesis.

We hypothesise that the different distribution of abstract goal information during session B compared to session A, might be related to the visual modality adopted to deliver task's instructions and/or to the use of the left hand. The non-dominant hand might partially explain the greater involvement of the right hemisphere in decoding abstract goal information; nevertheless, the two tasks recruited similarly the right hemisphere (see univariate results in appendix 6.1.1). Besides, it is plausible that visual and auditory instructions engage different neural resources, so encoding within the left and right hemispheres cannot be explained by common linguistic processing in the two sessions. As a consequence, this difference in goal encoding might be interpreted in two different ways: (i) a behaviourally relevant information transfer between the two hemispheres, particularly evident for left hand pantomime, or (ii) as an epiphenomenon with no causal role in behaviour. As most fMRI studies reported, univariate and multivariate effects focused on the 'classical' regions of the tool network within left hemisphere, thus it is impossible to interpret our results with respect to the current neuroimaging literature. Indirect evidence for an interpretation of our results come from pantomime deficits in split-brain patients (Lausberg et al., 2003; Frey et al., 2005). Lausberger et al. tested patients (one group with complete callosotomy, one group with partial callosotomy) and controls in two tasks: pantomiming tool use and performing real-tool manipulation (Lausberg et al., 2003). All the groups performed normally in the real-tool manipulation, both with the right and the left hand. Split-brain patients displayed

apraxic errors only when performing the pantomime task with their left hand. The same specific deficit in tool pantomime performed with the left hand was described in another study on split-brain patients (Frey et al., 2005). The deficit was present irrespective of the type of adopted cue, i.e. verbal instruction, line drawings or visual presentation of a real tool (Frey et al., 2005). The authors argued shared information in both the hemispheres and left hemisphere dominance for tool-use skills. Nevertheless, the right hemisphere seems to be able of accessing and controlling information necessary to perform tool pantomimes, as these patients were still able to perform pantomimes with the left hand, even if to a more limited degree.

4.6.2 The right hemisphere in tool pantomime

From a classical perspective, the tool network is left lateralized in right-handed and left-handed subjects (Lewis, 2006). Besides, the pivotal role of the left hemisphere in supporting tool actions has been confirmed by clinical studies (Manuel et al., 2013; Buxbaum et al., 2014; Hoeren et al., 2014). Our ROI-based MVPA analysis confirmed the involvement of the left (dominant) hemisphere in encoding tool-action information. At the same time, we showed that concrete and abstract encoding of tool pantomime was also evident within regions of the right hemisphere, outside the classical tool network. What is the possible functional role of this encoding within the non-dominant hemisphere?

As previously described, the recruitment of contralateral areas during tool use has been reported in several studies adopting univariate analysis (Johnson-Frey et al., 2005; Lewis, 2006; Króliczak and Frey, 2009; Brandi et al., 2014), but most studies stressed the stronger lateralization of the tool network in the left hemisphere. For example, a neuroimaging study on healthy participants (Brandi et al., 2014), showed a left lateralization index in using real tools with both left and right hand but showed also activity for planning and real tool use in the right hemisphere. Also Kroliczak

et al. showed a strong recruitment of the right hemisphere in healthy subjects when pantomiming reaching and grasping actions over a simple-shape object compared to real reaching and grasping (Króliczak et al., 2007); and reported bilateral activity in the PPC when pantomiming tool use, either in the planning and in the execution phase (Króliczak and Frey, 2009). Nevertheless, the role of the right hemisphere in tool-actions has been poorly considered, and most of the studies reporting an involvement of the right hemisphere in tool actions, hypothesised it to be an epiphenomenal activation (Buxbaum et al., 2014). Opposite to this interpretation, other studies reported apraxic deficit after injuries in the right hemisphere, suggesting a causal role of the right hemisphere in tool related actions (Marchetti and Della Sala, 1997; Raymer et al., 1999).

Overall, considering our findings, neuroimaging and lesion results, three possible scenarios about the role of the right hemisphere can be outlined.

A first scenario could depict the right hemisphere as not involved in processing tool use information relevant to the performance of the motor task. Its recruitment might be the result of other collateral cognitive processing occurring during motor tasks (e.g. attention). Nevertheless, split-brain patients showed that the right hemisphere can guide real tool action manipulations (Lausberg et al., 2003; Frey et al., 2005), making this hypothesis unlikely.

A second scenario has already been proposed in the past by Marchetti and Della Sala (Marchetti and Della Sala, 1997). To justify praxic deficits after a lesion in the right hemisphere, the authors suggested that, as seldom happen for language function, some individuals might have the praxic system in the right hemisphere compared to the majority of the population. Our participants were all right-handed and, given the rare occurrence of a right lateralization of praxis in the right hemisphere, it is unlikely that a possible sampling bias causes the effect at the group level. Thus, also this scenario seems unlikely.

A third scenario suggests that in normal conditions, the left hemisphere is more strongly engaged during tool-use and/or the left hemisphere might more easily compensate for lesion in the right hemisphere damage. A strong dissociation between the two hemispheres is difficult to support as patient with right hemisphere lesion have difficulties in tool pantomime (Lausberg et al., 2003; Manuel et al., 2013) and in real tool use (Salazar-López et al., 2016) with respect to controls. Nevertheless, Voxel Lesion Symptoms Mapping studies failed to clearly define right brain regions associated with specific impairments, as it was possible in the left hemisphere (Salazar-López et al., 2016). This might suggest that tool-action information within the right hemisphere is represented in a more widespread way within the nodes of the network and/or recruited only for specific task requirements. The difference between the organizations of action information within the two hemispheres would lead to different behavioural impairments. For example, a lesion to specific nodes within the left hemisphere lead to strong tool-related impairment, whereas the same lesion in the right hemisphere would lead to a milder or no impairment. It would be interesting to understand if, after an injury to the left praxic system, some areas of the right hemisphere might become salient and compensate for possible deficits. A recent clinical study (Martin et al., 2016) might support this interpretation. The authors evaluated the relationship between behavioural performance in neuropsychological tests for apraxia and fMRI BOLD signal during an action observation task. They found that the performance of left-hemisphere-strokes patients, with preserved tool-related and imitative abilities, positively correlates with activity within the right premotor areas and right inferior frontal gyrus together with other left regions (i.e. left dorsolateral prefrontal cortex, left anterior temporal areas).

Overall, our MVPA results support this last scenario and suggest further investigations about the role of the right hemisphere in tool-use. The potential compensatory role of the right hemisphere in processing action related information should be addressed in future lesion-mapping

investigations in patients and TMS in healthy individuals (e.g. (McDowell et al., 2018)) . These approaches could provide a direct test of our hypothesis for the differential role of the left and right hemisphere in tool-use. Moreover, understanding the role of the right hemisphere in supporting tool actions might also represent a new track to develop rehabilitative programmes for motor-impaired patients.

4.6.4 Conclusions

To conclude, our findings showed flexibility in the encoding of goal information during action planning and suggested a functional interaction between ipsilateral pathways and across hemispheres. In line with the literature, we remarked the central role of the left hemisphere in processing tool action information, but we also highlighted the task-dependent involvement of the right hemisphere. These last findings suggest a possible parallel/complementary role of the contralateral hemisphere in processing the tool action information, supporting the idea that these regions should be considered as part of a larger and integrated tool network.

Chapter 5

General discussion

5.1 Thesis summary

In a 2018 keynote talk, professor Miniussi exemplified the organization of the brain comparing it to a big-city metro line. Metro line is constituted by several stations, displaced all over the area they cover. Each station is connected with other stations, some distances are longer while some others are shorter. Within the line, some central stations are more relevant than others, as several trains stopover or crisscross allowing reaching different location easily. As such, the brain can be compared to a metro line: each cerebral area represents a station, which is connected with other areas by fibers of white matter. Instead of passengers, electrical impulses travel from one area to the other changing the excitability of specific areas conveying information. The cerebral equivalents of central stations of a metro line are areas called hubs, which represent important junctions where different information can be exchanged, integrated and sent to other regions. I find this analogy simple enough to define the general functioning of the main brain networks, but at the same time complex enough to give an idea of the variety of the functional dynamics occurring between cerebral areas.

We could summarize the processes that led to the current knowledge about the organization of the brain networks subtending hand motor actions following this analogy. In the beginning, research focused on understanding the possible role of each specific node involved in hand motor actions. This is equivalent to looking at the metro map to see which touristic attraction you can visit when you are in a specific station. Without considering the context in which the areas are

embedded, the first research line on hand actions started describing the functional properties of single station/region adopting single-cell recording in NHP (Murata et al., 1997; Raos et al., 2006; Brochier and Umiltà, 2007; Fattori et al., 2009). Only later, researchers focused on the general structure and interactions that might occur between areas: which line color do I have to take and where should I change train to get to my final destination? Researchers described different pathways spread through the brain in which different areas are involved in processing information that leads to the specific final goals (e.g. ventral stream = vision for perception; dorsal stream = vision for action). Following the metro-line analogy, each colored line would represent a pathway; each one includes a series of stations that need to be passed through before reaching the last stop. Similarly, within the brain, different pathways subtending different functions (e.g. reaching and grasping) have been found and described (e.g. dorsomedial and dorsolateral pathways).

One of the first descriptions of the organization of the brain pathways included two different and specialized visual routes: the ventral stream, which elaborates features and identity of objects; and the dorsal stream, which uses visual information to create a motor program to interact with the surrounding environment (Goodale and Milner, 1992). Following investigations described two separate pathways involved in processing motor information, running through the dorsal route: the dorsomedial, specialized in processing reaching movements, and the dorsolateral, involved in grasping actions (Jeannerod et al., 1995; for a review see Binkofski F. and Buxbaum L., 2013). Recent findings suggested a less strict functional specialization of these cerebral pathways; however, these pioneering studies had the merit of delineating the organization of the brain and as considered here, of the hand motor system. A new research perspective described the areas involved in the hand motor actions as integrated and functionally interconnected, constituting a dynamic and complex system that cooperates to properly orchestrate a meaningful motor output (Cloutman, 2013; Verhagen et al., 2013; Milner, 2017; Nelissen et al., 2017). Thanks to

connectivity analyses and with the advent of analytic tools such as multivariate pattern analysis, it was possible to deepen the investigation regarding the organization of the networks involved in hand actions. Indeed, MVPA approach allowed describing the neural pattern subtending different action-related information (Gallivan et al., 2011b; Chen et al., 2016) even when no brain activity is detected with a univariate approach. Besides, connectivity analysis allows investigating the direction and the strength of the flow of information transferred through functionally connected areas (Grol et al., 2007). Nevertheless, these types of approaches have not been extensively adopted yet to investigate the hand motor system. As a consequence, what type of information is elaborated within the areas of this network/system and the dynamics occurring during their functional interaction are still poorly understood. Considering the present scenario, in the present thesis, we focused on understanding two main unresolved issues within the field: (i) what type of information is represented and (ii) how the information is transferred within the areas of the hand motor system. First, we investigated the functional connectivity between the two pathways of the dorsal stream occurring during the planning of hand actions (Chapter 2); then we focused on investigating the interplay between ventral and dorsal stream during a tool-pantomiming task (Chapter 3); finally, we explored the representational content of the dorsal and ventral stream considering regions in both hemispheres (Chapter 4). In the next sections, we will discuss the main findings of these studies referring to the current literature, providing novel insights on the functional organization of the hand motor system.

5.2 The dynamic organization of the hand motor network

The analogy of the hand motor system as a complex and well-organized metro-line, in which anatomically distant areas share and integrate information to produce a meaningful motor output, is a recent concept. I started this thesis aware that the traditional rigid functional

subdivision of the main visual pathways of the brain seen as distinct and independent (i.e. ventral stream = vision for perception, dorsal stream = vision for action) (Goodale and Milner, 1992) was reductive. In the past few years, it started to become clear that there were interactions within the regions of the dorsal stream and that the ventral stream could contribute with task-specific information to the performance of hand actions. However, the dynamics happening within the hand motor network were not clear.

Starting from these recent findings, we focused on understanding the more flexible aspects of the hand motor system organization and found evidence supporting this perspective.

Hereafter, we are going to summarise the main findings of each study and we are going to consider the implications of each result contextualizing them in light of the current literature.

Study 1: main findings

With the first study (Chapter 2), we tested the possible interaction between the two main pathways within the dorsal stream. We perturbed the activity of the superior parieto-occipital cortex (SPOC) with repetitive offline TMS stimulation and measured the metabolic cerebral consumption with fMRI. In the scanner, participants had to perform a simple delayed-reach-and-grasp task either keeping the eyes opened or closed. We analysed the functional data at a univariate level and using a multivariate approach (MVPA) we focused on the planning phase of the task. Considering separately the sham stimulation (sham) and the real stimulation (rTMS), we compared activity for grasping an object under visual feedback (visual condition) with grasping the same simple-shape object with no visual feedback (blind condition). Critically, the univariate analysis did not reveal any significant difference between the sham and the TMS condition in the activity of the dorsal areas; however, MVPA highlighted a significant change in the encoding of the grasp condition within the IPL. Overall, MVPA is much less sensitive to subjects' variability and can

highlight significant modifications which are not visible at the univariate level. We interpreted this result as a proof of functional connectivity between the dorsal pathways, as we found changes in the encoding of the dorsolateral pathway when real TMS stimulation was delivered over the dorsomedial area SPOC.

Implications of Study 1: Dorso-dorsal interactions

It is not a new concept that a clear-cut functional specialization of the dorsal motor networks (i.e. dorsomedial = reaching, dorsolateral = grasping) was a limited description of the complexity of the neural computations happening in the dorsal stream. NHP investigations (Fattori et al., 2004, 2009, 2010, 2012, 2015; Nelissen et al., 2017) and human studies (Monaco et al., 2011; Fabbri et al., 2014; Galletti and Fattori, 2017; Vesia et al., 2017), revealed that the dorsomedial pathway performs object-related and grasping-related processing similarly to the dorsolateral pathway, giving evidence against a net specialization of the two dorsal pathways. A different description proposed that the dorsomedial route is more involved in the online control of the action, while the dorsolateral is more involved in the organization of the action (Rizzolatti and Matelli, 2003). In support to this perspective, Grol et al. showed that fine and precise movements, such as precision grip, seem to rely more on the dorsolateral pathway compared to a whole-hand grasping, suggesting a specific role of the dorsolateral pathway in performing fine movements (Grol et al., 2007).

Recently, a TMS-EEG study suggested that similar information is processed by the two dorsal pathways, but following a hierarchical organization (Verhagen et al., 2013). This study showed a time and task-dependent exchange of information from the dorsolateral to the dorsomedial pathway when planning and performing grasping actions towards tilted objects, which was selectively perturbed by TMS stimulation.

Together with evidence supporting a functional interplay between the dorsal pathways, anatomical NHP investigations described several connections between the two routes that could serve as anatomical routes for functional interactions (Davare et al., 2011). Nevertheless, additional investigations are required to understand what type of functions such anatomical connections might subserve. We corroborated the hypothesis of a functional interaction between the two pathways showing a complementary transfer of information, i.e. from the dorsomedial to the dorsolateral. In our study, the dorsolateral pathway modifies its encoding in response to the transitory disruption of the dorsomedial pathway. Moreover, this modification should be subtended by a direct functional link between SPOC (region perturbed) and IPL (the region that changed its encoding in response to SPOC's perturbation).

Previous investigations similarly proposed the anterior portion of IPL as a crucial hub for visuo-motor transformation (van Polanen and Davare, 2015). Van Polanen and Davare (2015) hypothesise aIPL to be a good candidate to integrate information coming from different regions due to its anatomical connections with different brain areas and due to its multimodal nature, processing spatial, pictorial and haptic information. Our study showed that a functional reorganization occurs within IPL in response to the transitory perturbation of SPOC, confirming a functional transfer of information between distant areas of the dorsal stream.

Our results increase the amount of evidence highlighting the dynamic interactions within the pathways of the dorsal stream while performing motor tasks with different requirements. Moreover, we provided new evidence showing functional communication between areas of the dorsal stream even before the execution of the task, during the planning phase of the movement. Overall, our investigation supports a flexible organization of the dorsal stream involving a dynamic network of interconnected areas (Galletti and Fattori, 2017), rather than the rigid traditional model considering two distinct and independent pathway.

Study 2: main findings

In the second study (Chapter 3), we investigated the organization of the hand motor system in a more complex task, i.e. pantomime of tool actions. We focused on understanding where the information about the goal of an action is represented and how the dorsal and ventral stream could interact during a complex motor task. We adopted tool pantomimes, as they are known to recruit the tool network, which comprises a subset of regions of the hand motor system including both the dorsal and the ventral streams. To answer our first question (i.e. encoding of action goal information), we tested two hierarchically distinct levels of goal representation: a concrete representation, distinguishing between different goals for one specific tool at a time; and an abstract representation, where the final goal of the action is defined irrespectively from the considered tool. MVPA analysis allowed us to show that the concrete representation of the goal is encoded in all the regions of the tool network, both in the planning and in the execution phase. On the other hand, within the same brain regions, the abstract goal representation (i.e. final goal of the action irrespectively from the tool) is encoded differently in the planning and in the execution phase of the task. This last result suggested a transfer of abstract goal information from the posterior nodes (pMTG, aIPS) to anterior areas (PMv) of the tool network.

Our second aim was to investigate the functional connectivity between dorsal and ventral streams. We did so by performing a dynamic causal modelling analysis (DCM) considering the execution phase of the task, when the transfer of information occurred. DCM analysis highlighted an increased functional communication between the posterior node of the temporal lobe (pMTG) and the ventral premotor cortex.

Implications of Study 2: Flexible encoding within the tool network

The first description of the involvement of defined cerebral areas in specific cognitive functions came from clinical studies. For example, a focal lesion occurring either in the ventral or in the dorsal pathway could produce specific and dissociable perceptual or motor deficits, confirming the dorso-ventral functional independence. The first model proposed by Goodale and Milner (Goodale and Milner, 1992) was in line with these clinical observations. The dorsal stream was described as dedicated to the processing of visual information to produce motor output, and the ventral stream was specialized in processing visual inputs to perceive the surrounding world.

In the past years, however, this dichotomy has been questioned. Specific tasks, e.g. those involving tool use, demonstrated the common recruitment of ventral and dorsal streams. In the second study (Chapter 3), we wanted to understand what type of information is encoded within the tool network, and to what extent dorsal and ventral streams share the same type of representation. We found that hierarchically distinct levels of action goal representations (i.e. concrete and abstract levels) are encoded differently within the regions of the tool network according to the considered time window (i.e. planning and execution phase). Similarly to our study, other groups have studied the representational content of specific hand action areas using MVPA, providing information about the role of each area in the tool network (Gallivan et al., 2011b, 2013a, 2013b, 2013c; Gallivan and Culham, 2015; Chen et al., 2016). Gallivan et al. (Gallivan et al., 2013a) described the encoding of actions performed with the hand (i.e. reaching and grasping with the hand) and actions performed with a real tool (i.e. reaching and grasping with a tool). They found that the pMTG in the ventral stream is specialized in decoding upcoming actions performed with a tool but not performed with the hand. In contrast, our results showed encoding within pMTG for action goal information already in the planning phase of a pantomiming task, which by definition is performed with hands. To explain this difference in results, we argued that pantomimes might

represent a hybrid category of actions, which is more similar to actions performed with a tool compared to actions performed with the hand. Indeed, it has been proved that pantomimes of tool use rely on the same areas recruited when performing real tool actions (Lewis, 2006; Valyear et al., 2017). Overall, even if a well-defined network of areas subtends the hand action, the involved regions flexibly process information about the upcoming movement, both in the planning and in the execution phase of the action, in a time-dependent manner.

Implications of Study 2: Ventro-dorsal connectivity

Different studies investigating the neural substrates of tool-oriented actions have shown that the ventral stream is not functionally independent from the dorsal stream and vice versa. As demonstrated by Creem and Proffitt in a nice behavioural task (Creem and Proffitt, 2001), the semantic information of the ventral stream and the visuo-spatial information coming from the dorsal stream needs to be integrated to perform a correct tool manipulation. The authors used a dual-task paradigm including a spatial or semantic task performed while grasping different tools by their handle. The results showed interference in grasping the tool's handle and performing the semantic task, but no inference when grasping and performing the spatial task. As suggested by Cloutman (Cloutman, 2013), these results showed that the dorsal stream can process visuo-motor information independently from the ventral stream. However, when the cognitive load increases, the support of the ventral stream is essential. In addition, it has been demonstrated that the functional exchange between the two pathways is bidirectional. This has been proved by showing that the dorsal stream supports the semantic processes computed within the ventral stream, facilitating the identification of graspable objects (i.e. tools) in a priming task (Almeida et al., 2010). How and where these functional interactions take place is still a matter of debate. aIPS has been hypothesised to represent a crucial hub where dorso-ventral interaction can occur (Verhagen

et al., 2008; van Polanen and Davare, 2015), also in light of evidence showing functional connectivity between aIPS and the temporal area for tools LOTC (Bracci et al., 2012; van Polanen and Davare, 2015). As a consequence, we would expect to find coupling between aIPS and the other nodes within the frontal and temporal regions of the network. Nevertheless, our connectivity analysis showed a bidirectional coupling between the temporal area pMTG and the ventral premotor area (PMv) during the grasp-to-use task, but not with aIPS. The results obtained with the DCM analysis are complex to explain. The experimental design we adopted might partially account for the strengthening of the connectivity between pMTG and PMv, and for the reduced coupling with aIPS when performing a tool use pantomime. Online visuo-motor transformations are known to be processed by aIPS (Goodale, 2014). However, our task included a delayed design to allow a distinction between planning (delayed phase) and execution. After 9 second delay, the information about the pantomime to perform might have already reached the prefrontal region (PMv) and be somehow maintained and reactivated in working memory during the execution of the task. The time dependencies of aIPS have been proved by a TMS study investigating the causal relationship of the posterior parietal cortices in a reach-to-grasp task (Tunik et al., 2005). It showed that the activity of aIPS is disrupted only when the stimulation is delivered 65 ms after object perturbation. Other TMS intervals over aIPS did not affect the performance in the task. This evidence might justify why in our connectivity analysis we did not find a strengthening of the connections with aIPS. However, additional investigations are needed to address this issue, possibly adopting methodologies with a better temporal resolution.

In a recent review, Gallivan and Culham (Gallivan and Culham, 2015) explained the involvement of the ventral stream in processing information about upcoming hand motor actions, suggesting a predictive role of the ventral stream. The temporal areas might receive feedbacks about a planned action form the parieto-frontal network, and predict its visual consequences. Our connectivity

analysis showed a bidirectional flow of information between the ventral pMTG to the dorsal PMv when pantomiming the use of a tool. This bidirectional interaction might suggest that during the execution of a pantomime the motor program stored in memory can be compared to the action performed. In general, these results revealed a direct involvement of temporal areas in transferring action information. To corroborate our findings, another imaging study reported the involvement of the ventral stream in processing and integrating hand action information, such as the weight of an object to lift (Gallivan et al., 2014).

Overall, we suggest an active and complementary role of the ventral stream in supporting hand motor actions. Our findings are in line with those asserting a functional interaction between the two visual streams. In the classification proposed by Cloutman (Cloutman, 2013), our contribution is arranged between the 'feedback' account and the 'continuous cross-talk' account. These two accounts respectively hypothesise: the presence of multiple connection between the dorsal and ventral pathways that constitute several feedback loops, which allow monitoring and updating the processing occurring along the hand motor system ('feedback' account); and the possibility of recurrent and direct exchange of information between dorsal and ventral streams ('continuous cross-talk' account).

Study 3: main findings

In the third study (Chapter 4), we wanted to understand if the representation of the action goal within the tool network is stable or if it changes according to the task requirements within and outside the regions of the classical left lateralized tool network. To answer this question, we considered the results of study 2 and we collected data of a second experimental session. The experimental design was identical in the two sessions, but we changed the effector used to perform the pantomime (right/left) and the modality in which the task instructions were delivered

(auditory/visual). We performed the same MVPA analyses as in study 2 and found changes in the encoding of abstract goal information between the two experimental sessions within the same regions of interest. Moreover, we found a significant encoding of concrete and abstract information also in the contralateral regions within the right hemisphere.

Overall, our results showed that the tool network flexibly processes abstract action goal information depending on the hand used to perform the pantomime and the modality used to deliver instructions. The encoding within the right hemisphere suggested a possible role of the non-dominant hemisphere in supporting tool-pantomimes.

Implications of Study 3: Flexible encoding within and outside the classical tool network

In the third study, we found a different pattern of activity between session A and session B. Even though the task performed in the two sessions is the same, the encoding of action goal information varied in a task-dependent manner. These results, together with those of the second study, showed a flexible representation of action goal information that changes according to the time window and the task requirements. As previously discussed, several studies investigated the representational content within the classical tool network, but none of them focused on describing the representational content of the regions of the right hemisphere.

The role of the right hemisphere in tool actions has been poorly considered, even though several studies reported activity of the contralateral right hemisphere during tool action tasks (Johnson-frey et al., 2003; Johnson-Frey et al., 2005; Króliczak et al., 2007; Króliczak and Frey, 2009; Brandi et al., 2014; Martin et al., 2016). Moreover, few clinical studies showed tool deficits after a right cerebral lesion (Marchetti and Della Sala, 1997; Raymer et al., 1999; Salazar-López et al., 2016).

In our last study (Chapter 4), we found encoding for the goal of a pantomimed action also in the right hemisphere. Moreover, we demonstrated different pattern of goal encoding depending on the task performed. Given the involvement of areas outside the classical tool network, the role of such areas in supporting hand actions remains to be clarified. Voxel-based lesion mapping studies showed a clear correlation between the impairment in the performance of tool tasks and areas within the left hemisphere (pMTG, IPL), but failed in finding a similar strong correlation with right hemisphere areas (Manuel et al., 2013; Buxbaum et al., 2014; Hoeren et al., 2014; Salazar-López et al., 2016). Damages to the right hemisphere seem to cause less focal impairments compared to lesions of the left hemisphere, in which specific regions can be identified as responsible for specific deficits. These results might suggest the presence of central hubs, possibly within the left hemisphere, where action information is processed and then transferred to different contralateral cerebral areas. Our findings suggest that from these hubs, action information is spread differently through the involved areas depending on task requirements. An alternative and complementary hypothesis is that the left hemisphere is more strongly involved in praxis with respect to the right hemisphere and that interactions between the pathways of the two hemispheres are evident only in specific tasks (e.g. pantomime with left hand). TMS studies would be helpful in understanding if the involvement of the right hemisphere is an epiphenomenon, or if it could become vicarious after damage of dominant left regions, hypothesis already suggested by Buxbaum et al. in a recent study (Buxbaum et al., 2014). Understanding this aspect would have a massive impact on the design of rehabilitative programmes for people with motor deficits.

5.3 Future directions

Overall, our studies contributed in describing the organization of the hand motor network, considered as a functionally interconnected system in which, to different degrees, various areas located in three main cerebral pathways (ventral stream, dorsolateral and dorsomedial pathways) communicate to build a meaningful motor output. We adopted several methodologies (TMS, fMRI) and analysis approaches (univariate, MVPA, DCM), to investigate respectively the involvement of defined areas in specific motor tasks, their representational content and their connectivity profiles. Nevertheless, our results confirmed the complex organization of the hand motor network and raised new questions that need to be better explored.

First, there is the increasing need of studies describing more precisely which information is represented within the regions of the hand motor system, as well as delineating the connectivity profiles allowing the functional interaction between areas. For example, we have found the involvement of pMTG in the ventral stream during the planning phase of our task and found a functional communication with premotor areas during the pantomime. It is not clear why the ventral stream should be involved in the planning phase, when the intention to act is built. It has been hypothesised that the ventral stream could use this type of information to anticipate the output of a motor action to guide it (Gallivan and Culham, 2015).

Second, as mentioned in the previous paragraph, moving the research focus to other regions outside the classical tool network by investigating the functional reorganization of different regions after a traumatic event, might lead to building new rehabilitative strategies that could accelerate and/or improve the chance to recover from a motor deficit. We have found that contralateral regions within the right hemisphere show a representational content almost equal to the one found in the left hemisphere. We are not able to tell if these areas could be vicarious to the activity of those in the left hemisphere or if their role is just complementary to the one of the

dominant hemisphere. This information might be tested and explored in healthy participants using a combined TMS-fMRI approach as we did in study 1. Moreover, it could be also investigated in patients showing lesions in the left hemisphere without specifically associated motor deficits, to see whether other regions can substitute the injured ones. Answering these unresolved questions would have a direct impact on the planning of rehabilitative programs. If the right hemisphere proved to have a functional role in orchestrating hand motor actions, it would be possible to hypothesise rehabilitative interventions (e.g. cognitive trials, TMS stimulation) aimed to reinforce the connectivity between right hemisphere regions and spared left areas, bypassing the lesioned ones. Moreover, a better understanding of the functional role of the nodes of the hand motor system could lead to the development of more sophisticated devices using neuroprosthetics (e.g. robotic hands). Indeed, it has already been shown that in patients with peripheral lesions (e.g. paresis) it is possible to train a machine learning algorithm to discriminate different patterns of neural activity within a specific regions associated with different hand actions and to move a prosthetic hand accordingly (Hochberg et al., 2012; Aflalo et al., 2015).

5.4 Conclusion

In conclusion, our studies expanded the knowledge about the motor network, investigating the representational content of different brain areas, and bringing evidence about a functional interaction between areas of the same pathway, across pathways and across hemispheres. Moreover, we showed flexibility in encoding action related information depending on the requirements of the task performed. These results support a new description of the organization of the hand motor system, which seems not to be hyper-specialized and segregated. Nowadays, it is evident that the hand motor network is a dynamic system involving dorsomedial, dorsolateral

and ventral pathways that cooperate in processing various levels of action information and build

meaningful motor outputs that allow us to successfully interact with the surrounding world.

Chapter 6

Appendix

6.1 Supplementary materials: chapter 4

6.1.1 Main univariate contrasts

The analysis of the main univariate contrast of session A and session B allow us to understand better the multivariate results. In figure 6.1A, the overlap between the activation maps showing the main effect of planning (TFCE corrected (Smith and Nichols, 2009)) in session A and in session B, shows a strong recruitment of the hemisphere that is ipsilateral to the hand used in the session (i.e. left hand- left hemisphere, right hand-right hemisphere). Moreover, the overlap between the activation maps of the execution phase (figure 6.2B), is almost indentical between the two sessions. Looking at these univariate results seems that several regions (PMd, SPL, pMTG) of the tool network and also the equivalent areas in the right hemisphere, are recruited independently from the considered hand, as previously described in the literature (Johnson-Frey et al., 2005; Króliczak and Frey, 2009; Brandi et al., 2014; Kroliczak et al., 2016).
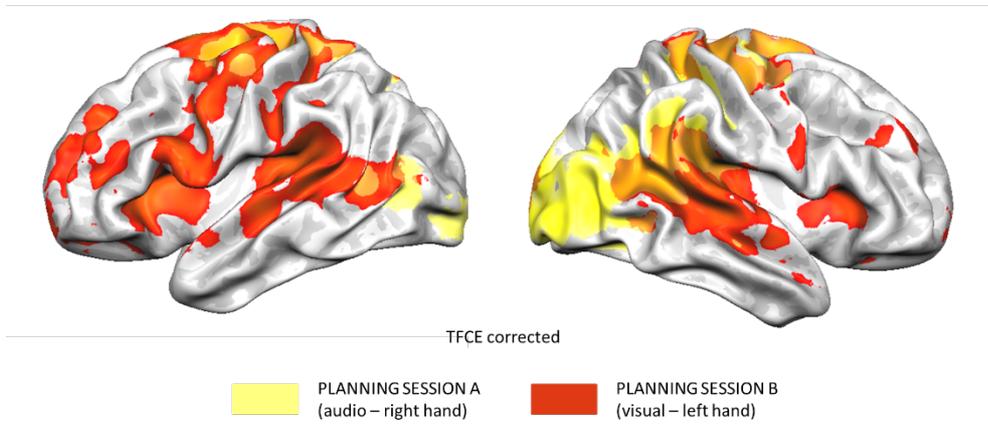
PLANNING
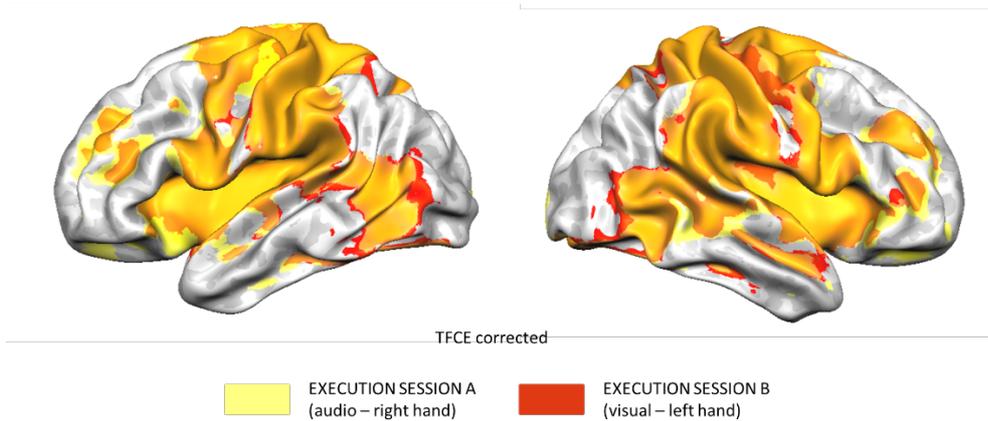
EXECUTION

Figure 6.1: A. Overlap main effect planning. Visualization of the overlap between the two unvariate contrast [planning vs baseline] (tfce corrected) of session A (yellow) and session B (red). **B. Overlap main effect execution.** Visualization of the overlap between the two unvariate contrast [execution vs baseline] (tfce corrected) of session A (yellow) and session B (red).

6.1.2 Interpretation

Some might argue that a difference in decoding between the two experimental sessions might

be due to a different univariate recruitment related to the hand used (right vs. left) instead of a

task-dependent change in the representation of the goal of the action (move vs. use). If this is the

case, we would expect to find greater activity in the left hemisphere when considering the right

hand (session A) and greater activity in the right hemisphere when considering the left hand (session B).

However, the recruitment of different areas under different experimental conditions does not imply that the information represented within each region is the same. Recent investigations adopting MVPA analysis started to describe which information about tool action is represented within the regions of the tool network, finding different pattern of activity for action performed with the hand or with a tool (Gallivan et al., 2013a). Other studies (Chen et al., 2016, 2017) investigated the encoding of high-level order concepts like the knowledge of the function of a tool (cutting, opening a bottle) and the properties of the action performed (pliers movement, twist).

With this study we wanted to contribute to a better understanding of the processing occurring within the tool network, in particular concerning the representation of different action goals, and we wanted to shed light on the extremely wide and generalized role of the left hemisphere in processing tool action information (for review see Lewis, 2006).

Overall, considering our univariate reults, we can assert that a hypothetical difference in goal encoding in the two experimental sessions (MVPA) cannot be ascribed to a different recruitment of the areas at a univariate level.

6.1.3 Execution phase results: left hemisphere ROIs

As for the planning phase, we analysed the results concerning the execution phase of our task, considering the encoding for concrete goal information and the encoding for abstract goal information.

Execution phase - Concrete representation.

During the execution phase, the encoding of concrete and abstract representation changes compared to the planning phase (Figure 6.2). Our MVPA results showed that all the considered

regions in the left hemisphere, both in session A and in session B, encode significantly (FDR corrected) for a concrete representation of the action goal.

Execution phase - Abstract representation.

On the other hand, abstract information is represented differently between the two sessions. In session A, abstract goal information is encoded in SPL and in all the regions of the left dorsolateral and of the left ventral pathway (Figure 6.2A). In session B, the abstract representation of the goal of the action is encoded in all the considered regions (Figure 6.2B).
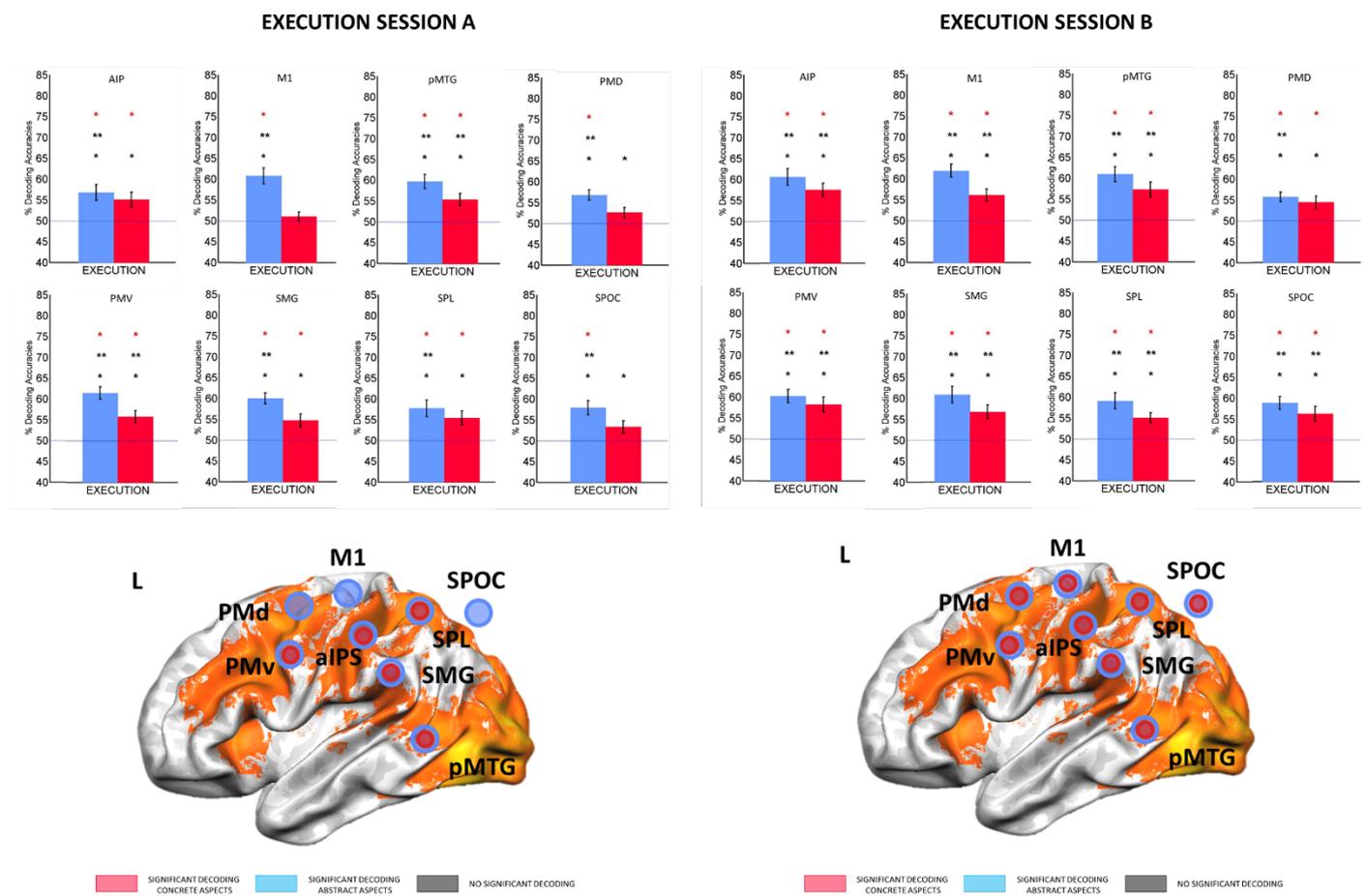
Figure 6.2: Left ROI results execution phase: execution phase results are here represented with bar graphs and (blue bar = concrete representation ; red bar = abstract goal representation) in a schematic visualization showing in blue the ROIs encoding for concrete information of the goal of the action and in red ROIs encoding abstract goal information (FDR corrected). **A. session A:** all the regions of the tool network host a concrete representation of the action goal. All the regions of the dorsolateral pathway (PMv, aIPS SMG), the temporal pMTG and the dorsomedial SPL represent, during the execution of the pantomime, abstract information about the goal of the action. **B. session B ROI results:** In session B, all the considered regions encode both for the concrete and for the abstract representation.

6.1.4 Execution phase results: right hemisphere ROIs

Execution phase - Concrete representation.

During the execution phase (Figure 6.3 of both the experimental sessions, concrete information is encoded in all the considered right regions (Figure 6.3), exactly as in the left tool network.

Execution phase - Abstract representation.

Similarly to the planning phase, abstract goal information is represented differently from one session to the other. In session A, only pMTG, SPOC and PMd host an abstract representation (Figure 6.3A); in session B, all the considered regions, except for PMd, significantly encode abstract information of the action goal (FDR corrected) (Figure 6.3B).
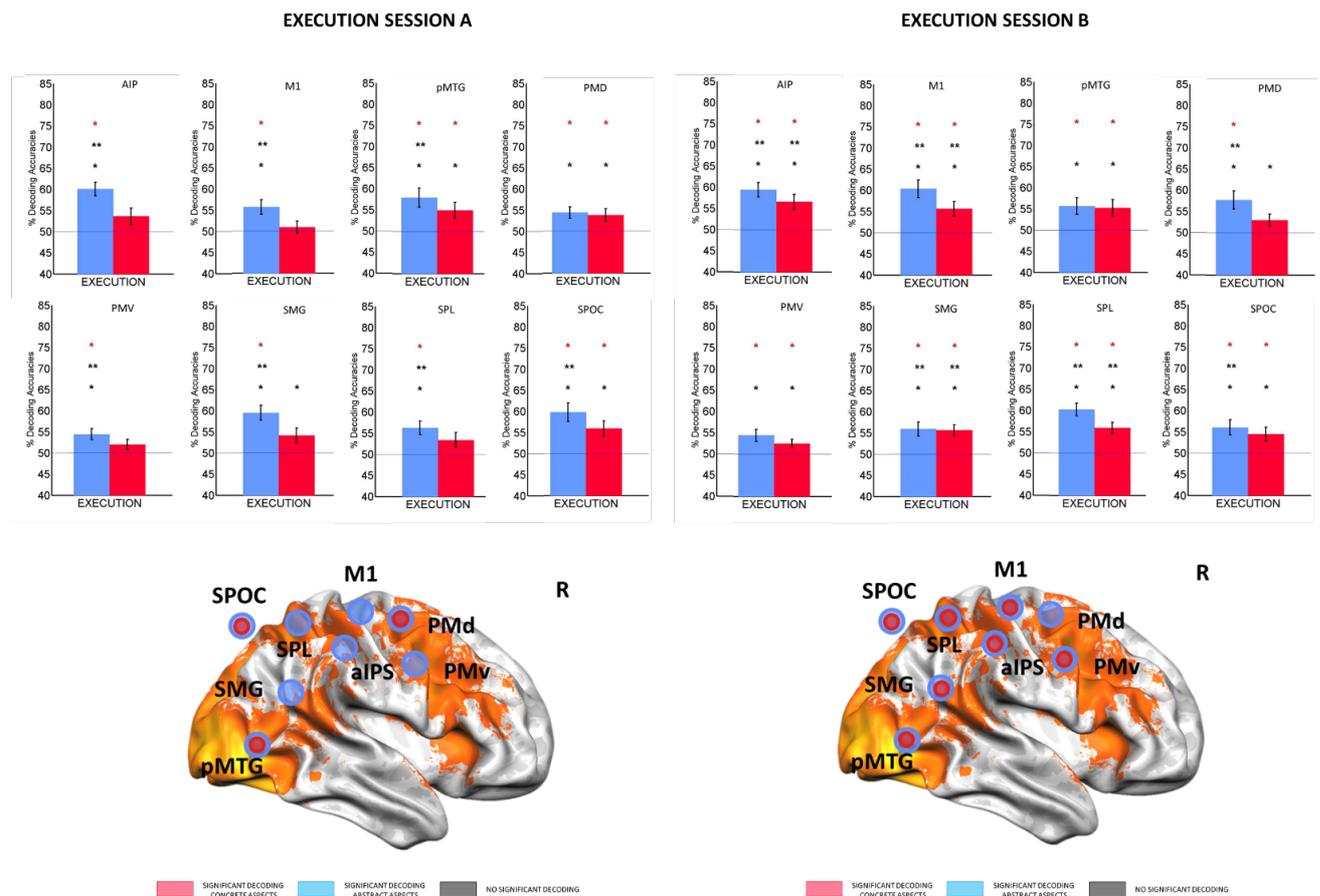
Figure 6.3: Right ROI results execution phase: the results of each ROIs within the right hemisphere are represented both in bar graphs (In blue the concrete encoding; in red the abstract encoding. p<0.05 *; p<0.005 **; FDR q<0.05 red star); and schematically showing the location of each ROI (blue ROIs: significant decoding for concrete information; red ROIs: encoding for abstract goal information; black ROIs: no significant decoding). **A. session A:** During the execution of the pantomime all, the regions encode concrete information; only pMTG, SPOC and PMd encode also abstract goal information **B. session B:** during the execution phase of session B, all the regions encoded concrete information; as well abstract goal information was represented in all the considered regions, except in PMd.

6.1.5 Searchlight analysis

In addition to the ROI based MVPA, we performed a whole brain searchlight analysis to localize areas outside the tool network that could encode tool-action pantomimes. We performed the same MVPA comparisons implemented in the ROIs analysis, looking at the concrete and abstract representation of the goals of our tool-action pantomime (see appendix).

In figure 6.4 are shown the conjunctions of the searchlight of all the searchlight comparisons performed (concrete and abstract representations) on session A and on session B, considering planning phase (6.4A) and execution separately (6.4B).

A

B
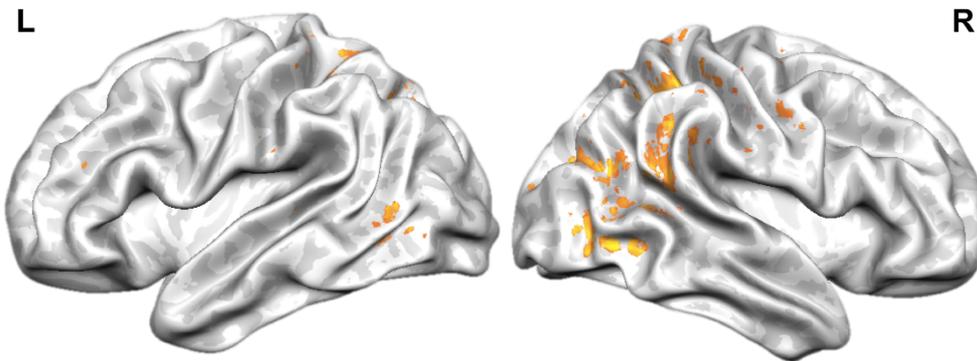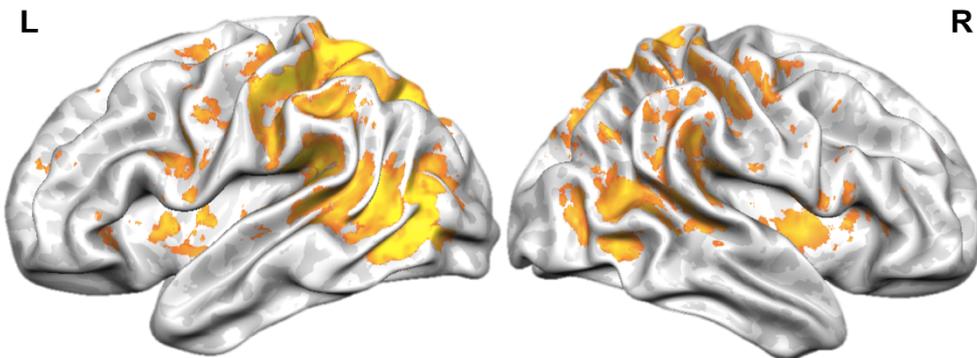
Figure 6.4 A. Planning phase conjunction map of searchlights. Conjunction of concrete and abstract information of both session A and session B. The map shows common encoding in the left pMTG and left SPL, and a wider conjunction in the posterior part of the right hemisphere. **B. Execution phase conjunction map of searchlights**. Conjunction of concrete and abstract comparisons of both session A and session B. The areas, whose encoding is in common between the four considered maps include areas both in the left and in the right hemisphere.

The visual representation of this conjunction, allow us to identify the areas that share the same pattern of activity involved in tool action pantomimes irrespectively of the task characteristics.

Figure 6.5A shows the conjunctions of four decoding accuracy maps regarding the planning phase of the task: concrete representation session A + abstract representation session A + concrete representation session B + abstract representation session B. The areas significantly encoding in all the considered searchlight MVPA comparisons, are in the left hemisphere pMTG and SPL; and in the right hemisphere pMTG and SMG. Comparing the left and the right hemisphere we can notice how there are more overlapping areas between the four conjunct maps in the right hemisphere respect to the left hemisphere.

Figure 6.5B shows the conjunctions of the four comparisons regarding the execution phase of the task. In this time window, there is encoding in all the considered maps in most of the posterior part of the left hemisphere (pMTG, SPL, IPS, insula) and in most of the regions of the right hemisphere (pMTG, SPL, IPS, insula).
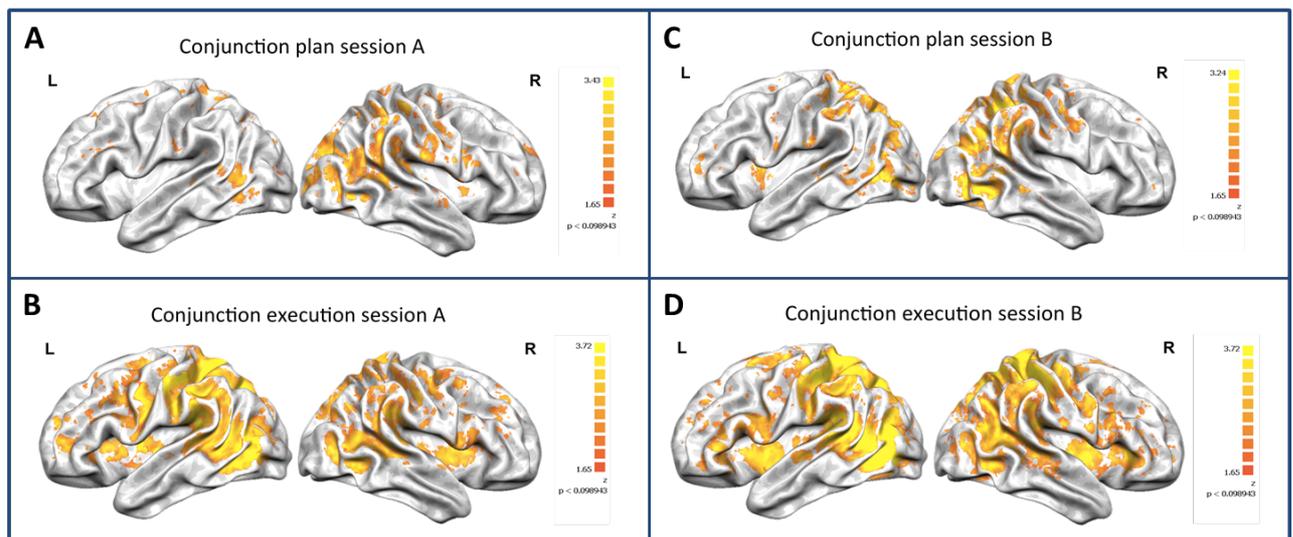
6.5 Searchlight conjunction maps. A. Conjunction between concrete and abstract representation searchlight maps of session A planning phase. **B.** Conjunction between concrete and abstract representation searchlight maps of session A execution phase. **C.** Conjunction between concrete and abstract representation searchlight maps of session B planning phase. **D.** Conjunction between concrete and abstract representation searchlight maps of session B execution phase.

The searchlight analysis confirmed the results coming from the ROI-based approach we adopted, showing an extensive involvement of homologous areas of the right hemisphere in decoding different action goals (refer to conjunction map).

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