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Rule-dependent and stimulus-dependent visuomotor mappings of other's actions

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PART I: INTRODUCTION

Chapter 1 - The dorsal and the ventral visuomotor streams

The empirical evidence that we collected in my work on automatic and voluntary responses to action observation indicates that the two well-known visual streams may be considered as the initial portion of two visuomotor pathways that ultimately share the task of transforming visual information into appropriate movements. In the present literature review I will present the anatomical and physiological evidence in non-human and human primates of two separate pathways that link the visual cortex to the motor cortex.

The output signals coming from the visual areas are transmitted by two fasciculi: the inferior longitudinal fasciculus (ILF), that following a ventral pathway, arrives into the inferotemporal region; the superior longitudinal fasciculus (SLF), which fibres, following a dorsal pathway, terminate in the posterior regions of the parietal lobe. Ungerleider and Mishkin (1982) hypothesized that these two pathways are involved into two different information processing (Figure 1). The authors demonstrated, because of the nature of the deficits resulting from these regions, that on one hand, the ventral pathway is involved in the perception and recognition of the objects to determine what we are seeing, on the other hand, the dorsal pathway should be involved in the spatial perception, to determine where is the object, and to

analyse the spatial configuration of different objects in a scene. Another related but different view is described by Goodale and Milner (1992, Milner and Goodale 1993), who demonstrated that the functional distinction between these two pathways involves information processing for action vs. perception, both of them process spatial information about object form, but for different aims.

Boussaoud and colleagues (1995) described these two pathways as a Vision-for-action (VFA) versus a Vision-for-perception (VFP), there systems can be dissociated behaviourally and neurophysiologically but not anatomically (Lebedev and Wise 2002). Both the areas involved in the dorsal and in the ventral stream play a role in the visually guided movement and in visual perception. The presence of visuomotor signals in higher order cortical areas, such as the prefrontal cortex, could be explained in two ways: one explanation is that the visuomotor information play a role in perception but the perception consists in one's action; the other reason could be that the dissociation between VFA and VFP underlie a specialized information-processing in the higher brain areas. The prefrontal cortex probably close the sensory-motor cycle of interactions and links the individual with its environment, integrating cognitive representations of perception and of action in goal-directed behaviour (Fuster 2001).

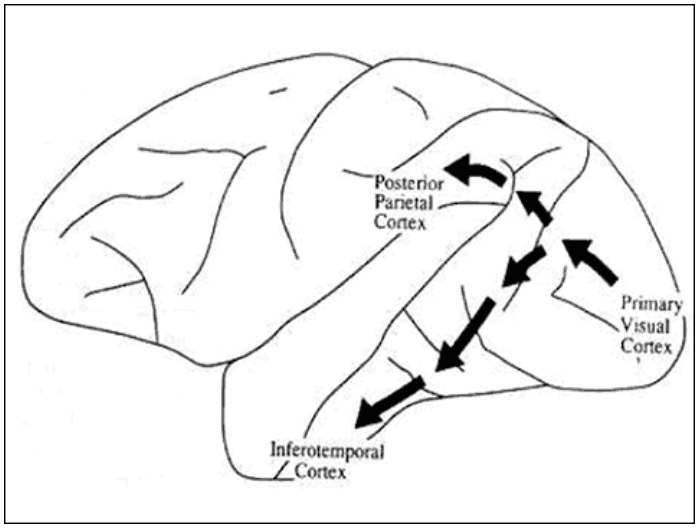

Figure 1. Schematic representation of the anatomical organization of the dorsal and the ventral streams described by Ungerleider and Mishkin (1982).

1.1 Anatomy of the frontal motor areas

The classical descriptions of the motor cortex gave to the motor system a peripheral and merely executive function. The simiunculus described by Woosley (Woolsey et al. 1952, Woolsey 1958) and the homunculus described by Penfield (Penfield and Rasmussen 1950) divided the motor cortex into two areas: the primary motor cortex (MI) and the supplementary motor area (SMA, sometimes called MII). In MI were represented the fine movements and it included BA4 and most of the lateral part of BA6; while in SMA were represented the gross movements and it coincided with the mesial portion of BA6. However, it wasn't clear the link between the cytoarchitectonic map and the cognitive function of these areas. Matelli and colleagues (1985, 1991, Petrides and Pandya 1997) divided the motor cortex into many different regions, showing that MI coincides with BA4 and from now on it

will be called F1 (F stays for Frontal agranular cortex), while BA6 appears to be divided into three regions (mesial, dorsal and ventral), that are divided into two portions (rostral and caudal). The mesial region is composed by areas F3 (SMA) and F6 (pre-SMA): in particular in F3 are represented the whole set of body movements, in F6 could be evoked slow and complex movements of the arm. The dorsal region is divided in area F2 (proper dorsal premotor cortex, PMd) and in the area F7 (pre-PMd): in particular F2 presents a somatotopic organization in which the leg is represented dorsally and the arm is represented centrally), on the contrary area F7 is poorly excitable and its functional properties are not clear. Finally the ventral region (ventral premotor cortex, PMv) is divided into F4 and F5: they are both electrically excitable, in F4 there is the representation of the movements of arm, neck and face, while in F5 they are represented the movements of hand and mouth (Luppino and Rizzolatti 2000).

However to understand the motor system it is important to understand the connections along the brain that allow us to perform a movement. These connections could be: endogenous, exogenous or descendent. Endogenous connections, that rely on the mesial cortical system, and descendent connections, which project to the sub-cortical regions and the spinal cord, are beyond the scope of the present review. In the next paragraphs they will be described the exogenous connections, which are mediated by two distinct pathways: a dorsal visuo-motor stream and a ventral visuo-motor stream.

1.2 Anatomy of the dorsal visuomotor stream

The areas of the frontal agranular cortex receive afferent connections from the prefrontal lobe, the cingulate cortex and the parietal lobe (the primary somatosensory cortex, SI and the posterior parietal lobe). The role of the prefrontal lobe will be discussed later in a proper paragraph. The cingulate cortex is usually involved in the processing of motivational and affective information related to the action intention, influencing the action.

The connections with the posterior parietal lobe are well described in the primates (Rizzolatti and Matelli 2003): it is divided by a sulcus (intraparietal sulcus, IPL) into two principal regions, both of them are divided into different segregated regions that elaborate different aspects of the sensory information: the superior parietal lobe (SPL; areas: PE, PEc, Peip, MIP, V6A) and the inferior parietal lobe (IPL; areas: PF, PFG, PG, AIP, LIP, VIP) (Figure 2).

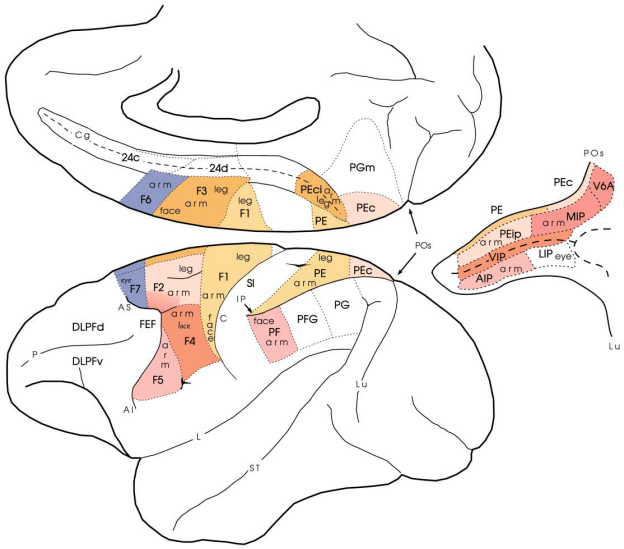

Figure 2. Mesial and lateral view of the macaque brain showing the areas of the agranular frontal cortex described by Matelli et al. (1985, 1991) and the areas of the posterior parietal lobe described by Pandya and Seltzer (1982).

It is possible to identify multiple connections, between the premotor and parietal areas, that allow the visual and sensorimotor integration (Figure 3).

The circuit F5-AIP seems to be the anatomical correlate for the visuo-motor transformation necessary for grasping movement. In F5 there are neurons that are activated during movements related to grasping and manipulation of objects that are interesting for the monkey. Some of these neurons are more excitable for a specific kind of grip (precision grip, grasp with two fingers, grasp with the whole hand). These neurons are selective for the grip but not for the hand, the spike is indeed observed for both the right hand and the left hand. Thus, these neurons code the goal of the movement (Rizzolatti et al. 1988). The 20% of the motor neurons in F5 respond also to visual stimuli, suggesting that F5 is involved in the visuo-motor transformation necessary to grasp an object. In the area AIP the neurons can be

divided into three categories: motor dominant neurons, visuo-motor neurons and visual dominant neurons. These neurons have features similar to the ones in F5, but there are more visual neurons in AIP than in F5. This could be due to the fact that AIP has many connections with the occipital lobe and thus could suggest the core function of this area for the integration of the visual information in the coding of the visuo-spatial features of an object. Rizzolatti and colleagues (1997) proposed a model for the grasping circuit, in which AIP codes multiple structural descriptions of a three-dimensional object, providing F5 with different kinds of affordances for that object. Then F5 decides the correct affordance for that object given the context and the goal of the action. This model predicts that the AIP neurons have a short-term memory for the affordable objects. The motor discharge (corollary discharge) that is observed in AIP could be the neuronal correlate of this function. Thus the feed-forward circuit AIP -> F5 -> AIP could have the function of keeping in memory the best representation of the object for a specific kind of grasping, even if the object is not visible (Murata et al. 1996).

However to make a successful grasping it is necessary to reach with the arm the correct spatial position of the object. The frontal area involved in this visuo-motor transformation is F4. Most of the neurons in F4 have sensorial properties and they are subdivided into two categories: pure somatosensory neurons and bimodal neurons. The first ones respond to tactile stimuli and they have receptive fields on the face, on the chest, on the arms and on the hands. The bimodal neurons respond both to tactile and to visual stimuli and the visual receptive field is anchored to the tactile receptive field. The parietal area VIP is strongly connected to F4 and it has

neurons that have similar properties to the ones in F4, even if in VIP there are many neurons that are purely visual. Thus the circuit F4 <--> VIP codes the reaching movements.

It is important to mention that in the end of last century, Rizzolatti and colleagues (di Pellegrino et al. 1992) discovered in the area F5 some neurons, called *mirror neurons*, that have a surprising property: they discharge not only when the animal performs a specific grasp but also when the animal see another individual doing the same goal-directed action. For this reason, the function of these neurons is suppose to be action understanding (Rizzolatti et al. 2009), but the debate about the function of this class of neurons is still open.

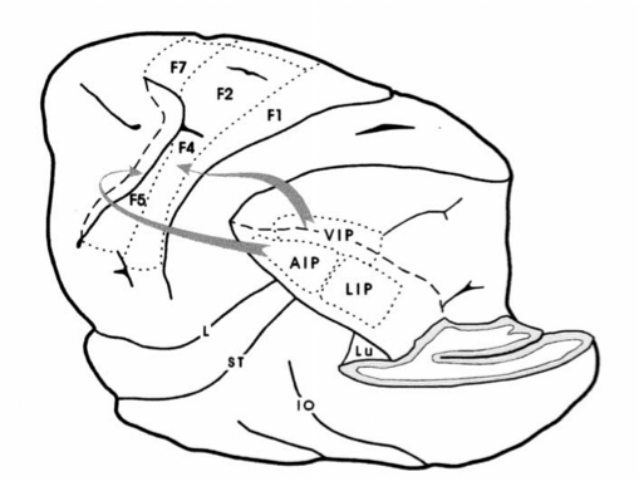

Figure 3. Parietal projections from the areas in the intraparietal sulcus to the frontal areas (Rizzolatti et al. 1998, Rizzolatti and Luppino, 2001).

1.3 Anatomy of the ventral visuomotor stream

The connections between the visual regions and the inferior temporal cortex are described by Baizer and colleagues (1991). The authors injected six macaques in the lateral surface of cytoarchitectonic areas TE and TEO. They described a circuit that projects from prestriate cortex (areas V4, V4t, DP, the foveal representation of V2 and the central field representations of areas V3d and V3v) and passes through the temporal lobe areas, as the fundus of superior temporal area (FST), the middle temporal area (MT), the area TG at the temporal pole, the areas TF and TH on the parahippocampal gyrus and the presubiculum. In particular TE receives projections from the primary visual cortex, V1 through the ventral visual pathway (V1-V2-V4-TEO-TE). Moreover there are jumping projections, such as the one from V2 to TEO and the one from V4 to the posterior part of TE (Tanaka 1996). The more ventral areas in the inferior temporal gyrus, such as TE3, TE2, TE1, TEa and TEm, are unimodal visual areas. On the contrary the areas in the anterior and dorsal part of the superior temporal sulcus, such as TPO, IPa and IPg, are specialized for the analysis of moving visual stimuli (Rolls 2000) (Figure 4). Nonetheless these neurons have properties that are in line with the role of the temporal lobe, as object recognition. Neurons in V4 discharge for many visual features such as color, spatial frequency, orientation, width and length, all of them are relevant for object perception. The selectivity for shape of these neurons is described also by Ito and colleagues (1995), who describe that the images of objects are stored in a size-specific manner in the long-term memory. The image processing, both size-dependent and –independent,

likely occurs in anterior IT. These data corroborate the thesis that the ventral visual stream is involved in the object perception. The inferotemporal cortex (IT) has other projections, outside the visual cortex, such as the perirhinal cortex, the prefrontal cortex, the amygdala and the striatum of the basal ganglia.

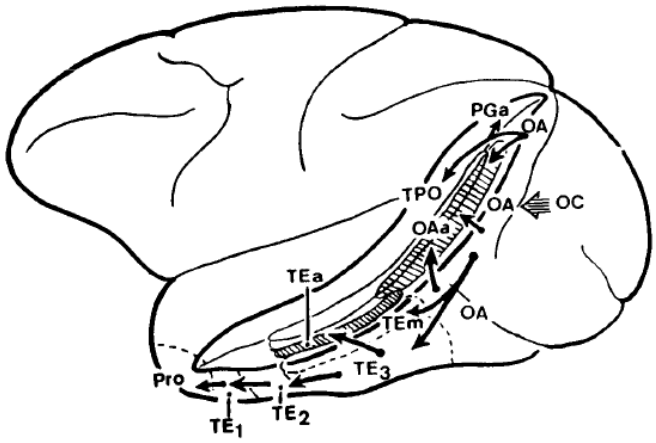

Figure 4. Lateral view of the macaque brain showing the different architectonic areas (Rolls 2000).

1.4 Pathways linking the posterior parietal cortex and the inferotemporal cortex with the frontal cortex

The principal pathway that links the parietal cortex with the frontal cortex is the superior longitudinal fasciculus (SLF) that can be divided into three fascicles: SLF I, SLF II and SLF III (Petrides and Pandya 2002). SLF I originates from the superior parietal region and the medial parietal cortex and terminate in the supplementary motor area, the dorsal area 6 and area 8Ad. These connections are

reciprocal. The superior parietal lobule codes the location of body parts in a body-centred coordinate system, thus this pathway could have a core role in the higher aspects of motor behaviour that require the location of the body parts in the space. SLF II originates from the caudal part of the inferior parietal lobule (PG) and the occipitoparietal region (Opt) and the fibers terminate in the posterior dorsolateral frontal lobe (dorsal area 6, area 8 and mid-dorsolateral areas 9/46 and 46). Areas PG and Opt play a crucial role in visual and oculomotor aspects of spatial function, thus this connection with the prefrontal cortex could play a role in the perception of visual space. The fibers that are directed back to the parietal region, from the prefrontal cortex, could underlie a function of regulating the focus of attention within different parts of the space. Finally SLF III originates from the rostral portion of the inferior parietal lobule (area 40 or PF) and from the parietal opercular region and it terminates in ventral premotor area 6, area 44, the frontal opercular region and the ventral part of area 9/46. Neurons in the rostral portion of the inferior parietal lobule have complex somatosensory responses related to face and arm, so these fibers give to the ventral premotor region and area 44, higher-order somatosensory input. These frontal regions are known to be specific for goal-directed actions and to code the action in abstract terms; in these areas there are indeed the mirror neurons that may be necessary for action imitation. Moreover the fibers that link the rostral inferior parietal lobule and ventral area 9/46 could be important in the monitoring of orofacial and hand actions.

For what concerns the pathway that links the occipital lobe with the inferotemporal cortex and the frontal cortex, there is a stream that originates from

the ventrolateral occipital region and passes through different areas in the rostral part of the inferotemporal region. These fibers lie between the optic radiations and subjacent to the temporal cortex. These fibers form the inferior longitudinal fasciculus (ILF) and they descend ventrally and forward terminating in the inferotemporal cortical region and in the parahippocampal gyrus. The rostral part of the inferotemporal cortical region is linked to the prefrontal cortex via the uncinate fasciculus (UF). This stream provides information about the nature of the objects and what concerns object perception and identification.

To conclude it is important to understand that these pathways provide the prefrontal cortex with sensory-specific and multimodal information, that allow this anterior region to modulate information processing in the posterior ones. In particular with the presence of bidirectional connections it is possible for the prefrontal cortex to play a role in the selection of the correct action on the basis of conditional rules, comparing somato-spatial, visuo-spatial and audio-spatial information (Petrides 1987).

1.5 Dorsal and ventral stream: evidences in humans

The evidences in humans of a dual route model have been proposed by Milner and Goodale (1998). In their work, they described a patient (DF) who had broad occipito-temporal lesions with substantially preserved visual functions (such as visual acuity), but an impossibility to discriminate among geometrical shapes (visual agnosia). DF showed dissociation between the incapability to discriminate

among shapes and the preserved skill of acting with the objects; she was indeed able to use objects of daily life. Milner and Goodale proposed that the fundamental difference between the two streams isn't the type of percept (space vs object), but how the higher order cortical areas elaborate this information. An opposite dissociation was seen in patients with optic ataxia: these patients have a bilateral occipito-parietal lesion. They were impaired in grasping objects or places fingers on the edge of irregularly shaped objects, but they had no difficulties describing these same objects or discriminating different objects from each other (Goodale et al. 1994). However this model cannot explain the unilateral neglect, which is due to a parietal lesion and which drives patients to fail to attend or respond to objects located on the side of space contralateral to their lesion. This could mean that the dorsal stream is not involved only in the movement control, but also in the spatial representation. For this reason it was proposed another subdivision of the dorsal stream into a dorso-dorsal stream, which is involved in the organization of motor activity (e.g. on-line control of the action), and a dorso-ventral stream, which is involved in multiple perceptive-motor functions (Figure 5).

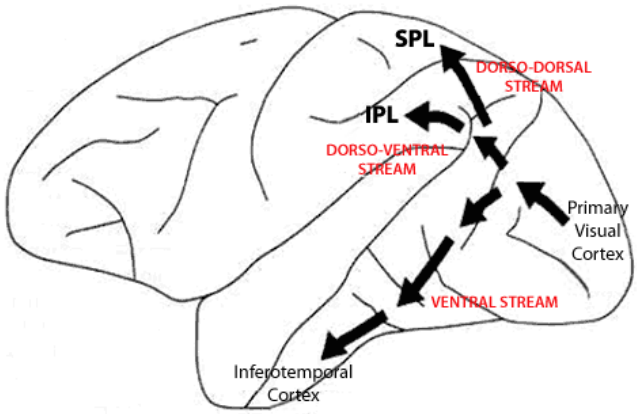

Figure 5. The three visual streams in a monkey brain: the ventral stream and the two subdivisions of dorsal stream: dorso-dorsal and dorso-ventral streams.

The presence of a dual route could explain the reason why in everyday behavioural situations the individuals respond to environmental stimuli with motor act different from those pre-programmed in automatic visuo-motor associations (motor mirroring).

In the domain of motor mirroring, the interaction between automatic and voluntary behavior is poorly defined. To address this question Catmur and colleagues (2007, 2011) described the so-called associative sequence-learning hypothesis, based on the rational that the mirror neurons acquired their mirror properties by means of stimulus-response learning. Given this hypothesis, it should be possible to change the tuning of this particular pool of neurons, after a counter-imitative training. In their works they demonstrated that after a brief counter-imitative training the mirror neurons become counter-mirror neurons, during an action-observation task. However these experiments present a strong spatial bias due

to the position of the participant's hand and the stimuli presented, which could invalidate the results.

We propose an alternative possibility, based on the dual-route model. Accordingly, a brief sensorimotor experience is not capable of reconfiguring in the short term the visuomotor associations that commonly produces imitative tendencies. On the contrary, the capacity of producing non-imitative behavior, at least in the short term, is supported by a neural route separate from that of automatic mirroring. A brief counter-imitative training would recruit this second route, leaving functionally unaltered the mirror route. One hallmark of a dual route to action is the occurrence of response competition between automatic tendencies and rule-dependent responses. In particular, such competition can become evident as a biphasic pattern of motor output, with an initial tendency toward the automatic response (between 100 and 300 ms from visual stimuli, varying according to the sensory modality) and a later implementation of the arbitrary rule.

This biphasic pattern can be demonstrated as a covert motor program, by means of any high-temporal resolution physiological measures of motor output such as electromyography (EMG), electroencephalography, dynamometry or motor evoked potentials (MEPs) to transcranial magnetic stimulation (TMS). Alternatively, it becomes overt in the form of the so-called partial errors (Coles et al. 1995) that are produced in conflictual stimulus-response conditions.

1.6 Stimulus-response compatibility effects

In choice-reaction tasks, responses are faster and more accurate when stimuli are mapped to spatially compatible responses than when they are mapped to spatially incompatible responses. The difference in response times and accuracy for the compatible and incompatible mappings is termed the stimulus-response compatibility (SRC) effect.

The SRC effect was paradigmatically described by Simon and his associates (e.g., Craft and Simon 1970; Simon 1969; Simon 1970; Simon and Rudell 1967), using a paradigm that is similar to the Stroop paradigm to investigate the effect of conflicting cues on information processing. In a typical task, subjects might press a left- or right-hand key, depending on the colour of a stimulus light, which appears on the right or left side of a display panel. Results indicate that the location of the light provides an irrelevant directional cue that interferes with processing the relevant symbolic cue, which is the colour of the light. In other words, reactions are faster on trials in which the location of the stimulus and response correspond than on trials in which they do not correspond.

There were many attempts to explain this phenomenon: one theory is the so-called Dimensional Overlap Processing model (Kornblum et al., 1990; Kornblum and Lee, 1995; Zhang et al., 1999). The rational behind this model is that if a stimulus set and a response set share features in common (e.g. spatial location), then the elements of the stimulus set will activate automatically the corresponding responses in the response set. The model hypothesizes a second route, a voluntary

one originating as well from the stimulus encoding stage, which matches the stimulus element with the response required for the task to be performed. The voluntary route matches the correct response through different strategies: when there is a clear overlap between stimulus and response an identity rule can be applied by the voluntary route, as well as a counter rule, while another strategy could be the search through a list of visuo-motor matching, but this is usually slower. Once the identification stage has assigned the correct response, the responses picked up from the automatic and the voluntary routes are compared: if they coincide, then the response is executed, otherwise, the response activated from the automatic route is aborted and the response from the voluntary route is instantiated and executed (Figure 6).

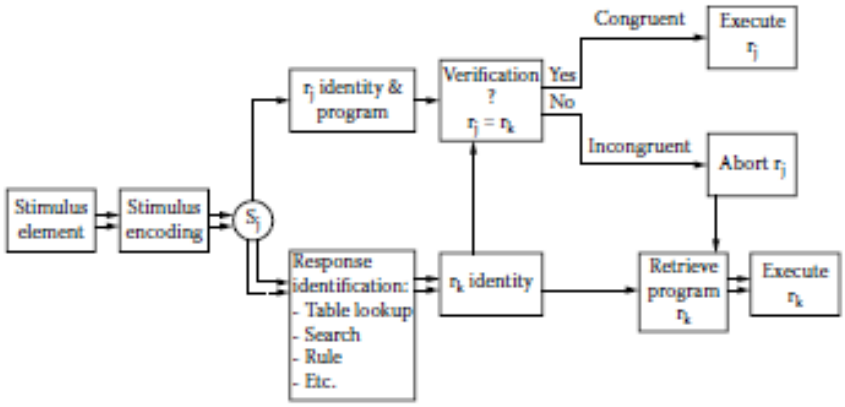

Figure 6. The Dimensional Overlap Processing model (Kornblum, Hasbroucq, and Osman, 1990).

Another model, which attempt to explain Simon effects has been described by Zorzi and Umiltà (Zorzi and Umiltà, 1995). The assumptions behind this

connectionist model is on one hand, that Simon effect arises from a conflict between discrepant stimulus and response spatial codes and on the other hand, that the spatial code of the stimulus, though task irrelevant, is formed automatically. The model considers the stimuli as represented by two different independent set of nodes (stimulus feature, representing the color and stimulus location features representing the position), both connected to the response nodes (response location). The stimulus location nodes have fixed excitatory connections to the response nodes, representing a long-term association between the two poles of the model (pole of the response, pole of the stimulus presentation), whereas the stimulus feature connections with the response nodes are determined by short term connections determined by the task requirements. When the stimulus is presented the activation of the stimulus location node excites the corresponding response node and inhibits the other. The activation of the long-term connection is very fast but decays also fast over time, so that the weighted sum of the input reaching the response nodes favours the non-correct response at the early stages of the trial, while soon after it favours the correct response through the short-term links. The response is executed by the system when the activation of one of the response nodes exceeds an activation threshold (Figure 7).

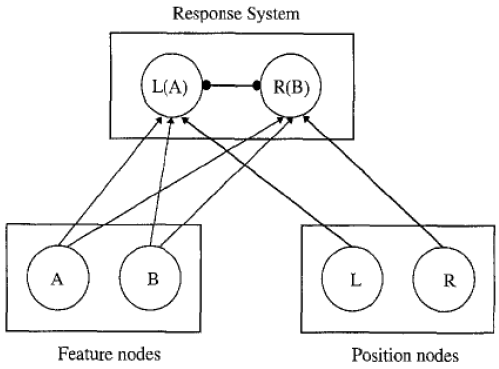

Figure 7. The connectionist model of Zorzi and Umiltà (Zorzi and Umiltà, 1995).

These models highlight the fact that response selection implies, in SRC tasks, the activation of two response codes. In a task in which participants select a motor response between two alternatives, it is not clear how the brain selects the correct action to be performed, and in particular how the alternatives are managed by the system. Several models have been proposed in which the alternatives are not inhibited, but only the correct response is activated, models exploiting lateral inhibition have been hypothesized in which the more activated alternative inhibits the others or a feed-forward inhibition were the source of activation of the response is also the source of the inhibition for the alternative response (Tsetsos et al., 2012).

At the level of the motor system, considering motor evoked potentials recorded from both hands as markers of the implementation of the two alternative responses, some evidence exists that both responses are facilitated at the early stages of a trial, but subsequently, close to the reaction time, one prevails on the other, which becomes more and more inhibited (Tandonnet et al., 2011). A similar

argument has been brought in favour of inhibitory processes arising in a Simon task. The RT distributions as the delta plot analysis show that in the Simon task participants response is captured by the stimulus feature non relevant for the task but overlapping with the response. This capture however is especially present when participants provide early responses, while in slower responses this conflict diminishes. This effect however could be due to the deactivation of the alternative response or to the active inhibition of the same. Van Campen and colleagues (van Campen et al. 2014), studied the time course of two response markers, the MEPs and the silent period, the latter considered the result of inhibitory GABA interneurons in motor cortex. They found that participants' responses were early captured by the location of the stimulus, as shown by MEPs on the non-correct effector but this tendency was suppressed soon after, as indicated by the increasing duration of the silent period on non-correct muscle in conflict trials (where stimulus and response were spatially incompatible). According to the Dual Process Activation-Suppression model (Van Campen et al., 2013) the selection of the appropriate response is underpinned by pre-SMA while the inhibition of the right inferior frontal cortex suppresses the inappropriate responses (Ridderinkhof et al., 2011).

This alternation between early responses (stimulus-dependent) and late responses (rule-dependent) seems to be pervasive to all domains of visuomotor behavior. It has been observed during visual search tasks (van Zoest and Donk 2006), during object-directed hand movements (Goslin et al. 2012) and during spatially oriented hand movements (Michelet et al. 2010). Depending on the

visuomotor tasks, the processes responsible for the early and the late responses have received the most disparate nomenclatures. It has been referred to as stimulus-driven, bottom-up, automatic, exogenous, or the what system. Conversely, the process responsible for the late response has been defined as goal-driven, top-down, voluntary, executive, or the how system (Theeuwes et al. 1998; Passingham and Toni 2001; Siebold et al. 2011; McBride, Boy, et al. 2012; McBride, Sumner, et al. 2012; Barchiesi and Cattaneo 2013).

One recent work has demonstrated a similar biphasic pattern in the domain of motor responses to others' actions (Barchiesi and Cattaneo 2013). The authors asked participants to observe a hand performing an action and to implement a counter-imitative rule, that is, to perform an action opposite to, and mutually incompatible with, the observed one. As an aftereffect of learning such counter-imitative training, participants exhibited an early (250 ms from video onset) pattern that was essentially imitative of the observed movement and a late (>300 ms from stimulus onset) pattern that was compatible with the arbitrary counter-imitative rule. These data only showed the aftereffect of the stimulus-response conflict and were potentially biased by a spatial compatibility effect.

To better understand the problem of action selection (decision making) and action specification (movement planning) Cisek proposed the Affordance Competition hypothesis (Cisek, 2007, Cisek and Kalaska, 2010). According to this hypothesis there are different systems that compete for the motor output instead of serial information processing stages (Figure 8).

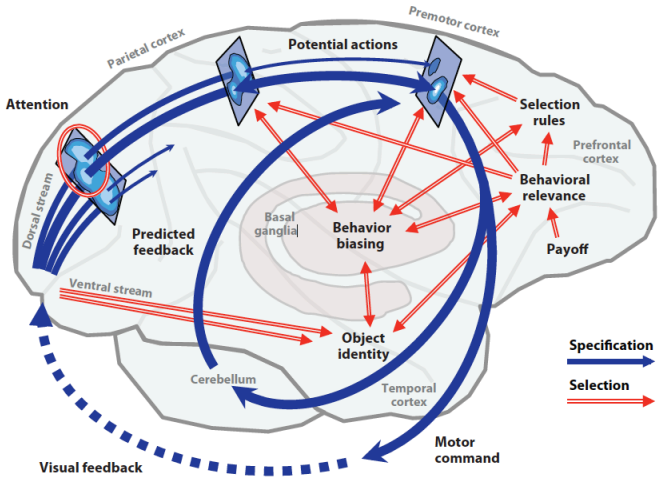

Figure 8. The Affordance Competition hypothesis (Cisek, 2007)

The action specification may involve the dorsal visual stream and the areas that are interconnected with it, such as the posterior parietal and the caudal frontal cortices. These circuits convert information about objects in sensory coordinates into the parameters of actions. Each area, along the way, can represent information about every potential action and possible movement toward a specific object. The same brain regions that represent these information, ultimately guide the execution of those actions. Since multiple action cannot be performed at the same time, there is a competition among them, probably through mutual inhibition and differential selection in corticostriatal circuits. If this competition exists in frontoparietal circuits, then other factors can influence the output, such as punishment, reward, risks and other components which can contribute to the good choice. Also other cortical and subcortical structures can contribute to chose the correct motor response. In the meanwhile the prefrontal areas receive information pertinent to

action selection, such as object identity from the temporal areas (Tanaka et al. 1991) and subjective value from the orbitofrontal regions (Schultz et al. 2000; Wallis 2007). In the end the interaction with the environment involves continuous and simultaneous processes of sensorimotor control and action selection that allows the individual to perform the best action for a defined context.

PART II: EXPERIMENTAL WORK

Chapter 2 - Techniques

2.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is the non-invasive method of choice for studying the causal relevance of a cortical area in the human brain. TMS interacts directly in a non-invasive way with cortical processes by passing a brief and strong current by means of a stimulation coil, which induces a perpendicular magnetic field that penetrates the scalp. This magnetic field induces a week transient current at the site of stimulation (Bestmann, 2008).

TMS is a very useful technique to investigate motor preparation in the motor cortex using a single pulse paradigm. A single impulse is given by means of TMS to elicit a motor evoked potential (MEP), which amplitude is considered an index of motor excitability. The first demonstration of motor resonance effect in humans has been provided by Fadiga and colleagues (Fadiga et al. 1995), by means of TMS single pulse. The rational behind the choice of this paradigm is that if the action observed is automatically represented in the brain, then the active representation of the observed action in participants' own motor system should activate the same muscles they are observing to do the movement. The authors demonstrated that when the participants were seeing a grasping movement, the MEPs, related to the

same muscle involved in the grasp, had greater amplitude compared to the vision of meaningless arm movements.

Other authors replicated these data, for example Alaerts and colleagues (Alaerts et al., 2009) found an increase in MEP's amplitude of the muscle involved in the flection of the wrist when the participants were seeing the same movement from an egocentric perspective. Urgesi and colleagues (Urgesi et al., 2006) tested their participants with the palm of the hand positioned up or down while they were seeing index or little finger abduction with the palm up or down. They demonstrated that a motor facilitation of MEPs in those muscles involved in the action they are observing irrespectively for the posture of the observed hand.

Cattaneo and colleagues (Cattaneo et al., 2009) explored the issue about an involvement of the mirror system in goal coding. In this study the experimenter performed in front of the participants actions that could or could not involve a goal-directed movement: grasp an object or non goal-directed actions. These movements were performed using two kinds of pliers: regular pliers, that needed the extension of the hand to be opened; and inverse pliers, that needed the flection of the hand in order to be opened. The authors found an interaction between the type of pliers and the context (goal-directed versus non goal-directed actions), such as, when the action performed was non goal-directed, the motor facilitation was observed in accordance with the actual movement performed by the researcher; while if the action was goal-directed (grasping) the motor facilitation was independent from the type of pliers.

Many studies showed the importance of using TMS to investigate the brain functions in vivo in a non-invasive way. Single pulse TMS is a very useful instrument to have a picture of the excitability of the motor cortex in a precise moment during action observation. Another TMS paradigm is the repetitive stimulation (rTMS) and in particular the low-frequency (1-Hz) stimulation that interfere with a specific brain target area causing in that region a virtual lesion, in other words it changes the cortical excitability in a specific brain portion in order to study functional connectivity.

Avenanti and colleagues (Avenanti et al., 2007) used this paradigm to investigate the contribution of premotor, motor and sensory regions, recruited during action observation, in mapping different types of observed actions onto the corticospinal system. The authors demonstrated a double dissociation that suggested an active involvement of the ventral premotor cortex and the somatosensory cortex, in simulating efferent and afferent components of observed actions.

2.2 Condition-and-map approach

An interesting paradigm that is very useful to investigate the changeability of functional brain networks is the so-called condition-and-map approach (Siebner et al., 2009). This approach combines offline rTMS with neuroimaging techniques, such as functional magnetic resonance imaging (fMRI). Neuroimaging techniques have a great potential to map temporo-spatial patterns of functional reorganization

that are induced in the human brain by rTMS. Among other possibilities, neuroimaging after rTMS conditioning can map the lasting functional impact of rTMS on task-related neuronal activity at a systems level. Neuroimaging should start as quickly as possible after rTMS to ensure that short-lasting aftereffects of rTMS a re-captured, indeed the effect of rTMS decades after a pair amount of time in respect of the stimulation time. One way of detecting the conditioning effects of rTMS on regional neuronal activity is to compare task-related activation before and after rTMS. However, any change in activation may simply be a time effect caused by the fact that the experimental task has been repeatedly performed in the MRI scanner. To dissociate temporal order effects from the effects that are causally related to rTMS, the experimental design should include a control session in which subjects perform the same experimental task but without effective rTMS, the so-called sham condition. Ideally, sham rTMS should be matched to real rTMS in terms of auditory and somatosensory stimulation but without effective transcranial stimulation of the cortex. A specific change in the pattern of task-related activation after real but not after sham rTMS would indicate a true reorganization in response to rTMS conditioning. This approach allows also a demonstration of functional and anatomical orthodromic neural connections from the targeted area to the remote neural structures in a specific task.

Low-frequency TMS is conventionally thought to inhibit the behaviour associated with the stimulated cortex. It is therefore intuitive to expect the Blood Oxygen Level-Dependent (BOLD) correlate of 1-Hz rTMS to be a relative decrease of activity. However, in the literature there is not a clear relationship between

BOLD signal and 1-Hz rTMS (Ward et al., 2010; Verstynen and Ivry, 2011; van der Werf et al., 2010; Tracy et al., 2010; Siebner et al., 1998; Siebner et al., 2003; Rounis et al., 2005; O'Shea et al., 2007; Nowak et al., 2008; Lee et al., 2003; Havrankova et al., 2010; Grefkes et al., 2009; Fox et al., 1997; Conchou et al., 2009; Chouinard et al., 2003; Bohning et al., 1999). All these studies present a different pattern of activation or deactivation, it can be noticed that absence of local effects of TMS in spite of the presence of remote effects at distant cortical sites has been found in four studies. More importantly, a prevalent increase in BOLD signal rather than a decrease in areas distant to the stimulated one was reported in nine out of 15 works (Table 1). It seems therefore that it is not to be expected that the polarity of the hemodynamic effects match TMS

In our recent study (Artfeller et al., 2013) we used the same approach in order to perturb the neural activity of the posterior superior temporal sulcus (pSTS) cortex on the whole action observation system. We found an higher increase of BOLD signal in response to observation of praxic movements compared to non-praxic ones, in a network of areas represented by lateral occipital-temporal cortex (LOTC), anterior intraparietal sulcus (AIP) and ventral premotor cortex (PMv). Thank to this powerful approach we could reveal the existence of a serial network of cortical areas passing from the pSTS, which respond to the observation of praxic behaviour of the distal upper limb.

Imaging method (reference)	Target	Intensity	No. of stimuli	Haemodynamic effects		Measurement interval (min)	Behavioural measure
				Local	Remote		
PET (Fox et al. 1997)	Left motor	120 % RMT	1,800	Increase	Mainly increase	10	None
fMRI (Bohning et al. 1999)	Left motor	80–90–100–110 % RMT	18	Increase	Increase	0.5	None
PET (Chouinard et al. 2003)	Left motor	90 % RMT	900	Increase	Mainly increase	30	MEP recordings
	Left premotor	90 % RMT	900	No effect	Mainly increase	30	
PET (Lee et al. 2003)	Left motor	90 % RMT	1,800	Increase	Mainly increase	60	Motor task
PET (Siebner et al. 2003)	Left premotor	90 % RMT	1,800	Decrease	Decrease	60	Motor task
PET (Rounis et al. 2005)	Left motor	90 % RMT	1,800	Increase	Mainly increase	60	Motor task
fMRI (O'Shea et al. 2007)	Left premotor	90 % AMT	900	No effect[a]	Mainly increase	15	Motor task
	Left somatosensory	90 % AMT	900	No effect[a]	No effect	15	
fMRI (Nowak et al. 2008)	Motor	100 % RMT	1,000	Decrease	Decrease	14	Motor task
PET (Conchou et al. 2009)	Right motor	90 % RMT	1,200	No effect[a]	Increase	40	Motor task
fMRI (Grefkes et al. 2009)	Motor	100 % RMT	600	ND	n.d.	12.5	Motor task
fMRI (Havrankova et al. 2010)	Left somatosensory	90 % RMT	1,800	Increase	Mainly increase	6	Motor task
fMRI (Tracy et al. 2010)	TPJ	90 % RMT	960	Decrease	Mainly decrease	>10	Auditory task
fMRI (van der Werf et al. 2010)	Left DLPFC	90 % RMT	1,200	No effect	Decrease	–	Resting state
fMRI (Ward et al. 2010)	Left premotor	90 % RMT	1,800	Decrease	Decrease	22	Visuomotor task
fMRI (Verstynen and Ivry 2011)	Left premotor	115 % RMT	1,200	Decrease	Decrease	32	Motor task

Table 1. Summary of findings of previous studies which used condition-and-map approach (Artfeller et al., 2013)

Chapter 3 - Experiment 1

3.1 Aim of the study

In the first work we decided to test in the former experiment the automatic-voluntary conflict in motor mirroring during the actual implementation of a conflicting visuomotor task with non-spatially defined stimuli. In a second experiment, we explored with repetitive TMS (rTMS) the role of the putative human anterior intraparietal area (phAIP) and of the dorsolateral prefrontal cortex (dlPFC) in generating automatic and rule-dependent responses. The reason for choosing the phAIP is that, in models of the action observation network, related to hand movements, this is the node that receives visual information from the occipito-temporal cortex and relays it to the premotor cortex (Cattaneo et al. 2010; Arfeller et al. 2013). For a review, see Cattaneo and Rizzolatti (2009). It is therefore an obligatory passage upstream of the motor cortex in the stimulus-dependent circuit. The dlPFC was chosen as a target area because it is a core node of the prefrontal executive system that selects different possible motor responses according to arbitrarily defined visuomotor rules (Passingham and Toni 2001; Toni, Ramnani, et al. 2001; Bunge et al. 2002, 2003). We preferred not to target the premotor cortex in the present study because it is a potential point of convergence of the voluntary and the automatic systems (Boussaoud et al. 1995).

3.2 Methods and materials

EXPERIMENT 1

Participants and experimental design

Twenty-one healthy participants (5 males, mean age 22 years) took part in the first experiment. Thirty healthy participants (9 males, mean age 22 years, one left handed) took part in the second experiment and were divided in two equal groups of 15 volunteers who received rTMS over the dlPFC, (prefrontal group, 3 males, mean age 23 years) or over the phAIP (parietal group, 6 males, mean age 20 years). None of the participants took part in more than one experiment. The experiments were approved by the Ethical Committee of the University of Trento and were conducted in compliance with the revised Helsinki declaration (World Medical Association General Assembly 2008). All participants gave written informed consent to the experiment and were screened for contraindication to TMS (Rossi et al. 2009).

We tested all participants during a session of counter-imitative training as defined in (Catmur et al. 2007) and in (Barchiesi and Cattaneo 2012). They were required to move their little finger when they saw an index finger moving and vice-versa. In every trial participants saw the biological stimulus and then waited for single pulse TMS (spTMS) over the motor cortex, which served as go-signal to perform the response. SpTMS was delivered at different time intervals (0-300 ms) from the onset of the stimulus (inter-stimulus intervals – ISIs). Motor evoked potentials (MEPs) to spTMS were recorded and were the source of the main

dependent variable after data processing. In the first experiment 6 ISIs (0, 100, 150, 200, 250 and 300 ms) were used. The experimental setup and trial structure are shown in Figure 9.

Trial Structure

Stimuli were presented using E-Prime 2.0 software on a 22 inches monitor with a refresh rate of 60 Hz. Trials were 5-s long and started with a white fixation cross on a black background for 1000 ms. Participants were then shown a still frame depicting the dorsum of a hand oriented horizontally but with fingers pointing either leftward or rightward for 1000 ms which was followed by another still frame of the same hand that was touching an object with the index finger or with the little finger for 1500 ms. The quick succession of the 2 images produced an apparent motion of the finger. Trials with leftward or rightward orientation of the fingers were randomly intermixed in the same block. The whole set of visual stimuli is shown in Figure 10. In the following frame, the participants were given a feedback related to the performance of their manual response. If they responded within 1500 ms, they were then presented with a green number correspondent to their reaction time in case of a correct response, or with the word Wrong in case of an incorrect response. If no response was given within 1500 ms, they were presented with the phrase No Response. The feedback frame lasted for 500 ms and was followed by a black background until the total trial duration (5 s) was reached. It should be noted that the orientation of the observed hands was orthogonal to that of the subject (which was

pointing anteriorly) in order to avoid any spatial mapping of the motor response on the cue movement.

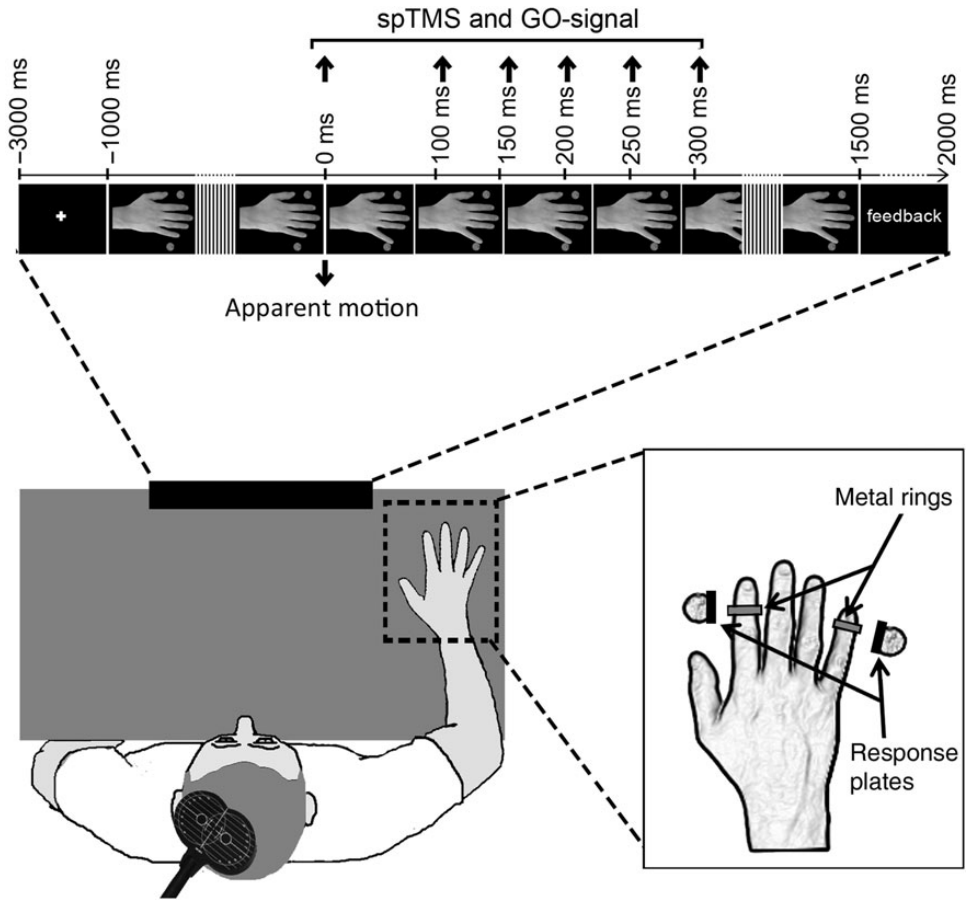

Figure 9. Experimental setup. The upper panel indicates the trial structure. The time of change between the first and the second frame of the stimulus hand is defined as t=0 ms and referred to as stimulus onset. Participants were stimulated over the primary motor cortex with spTMS at 6 different time intervals from stimulus onset. TMS was the GO-signal that prompted participants to produce the rule-dependent response (i.e. moving the finger other than the one moving in the video stimuli). The time of contact between the finger and the response plates (schematized in the lower right panel) was logged as response time.

Task and Data Logging

Participants were seated in a dimly lit room with the head positioned on a chinrest at 0.6 m from the presentation screen. Their right arm was positioned orthogonally to the body, and their right hand was positioned on a custom-made response box. The participants were instructed to wait for the TMS pulse as their GO-signal and then to move sideways the finger opposite to the one that they saw in the video, as fast as they could until it reached the side plates of a custom-made response box (schematized in Fig. 9). This was constituted by 2 metal plates placed close to the index and to the little finger; these plates were connected to the ground pins of the computer's parallel port. Two adhesive rings were tightened on participants' index and little fingers; on the side of each ring a piece of metal was connected to the input pins of the computer's parallel port. When one of the metal pieces, on the rings, touched the nearest plate, a response was detected and the corresponding response time (RT) was logged.

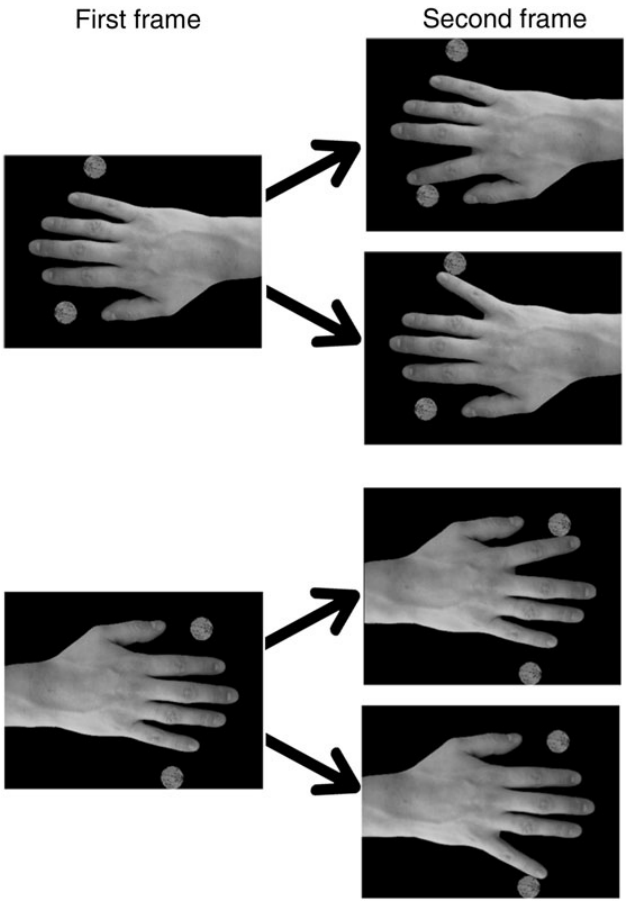

Figure 10. Visual stimuli. The succession of the 2 frames generating apparent motion is shown. All hands were presented horizontally (orthogonal to the participant's right hand) and were randomly oriented leftward or rightward. The second frame represented either an index finger movement or a little finger movement.

Single-Pulse TMS

SpTMS was delivered with a biphasic Magstim Rapid (Magstim, Dyfed, UK) stimulator connected to a custom-made figure-of-eight coil with outer winding diameter of 50 mm. The coil was positioned with the handle pointing backward at 45° from the midline over the optimum scalp location where MEPs, with the maximal amplitude, could be obtained from the first dorsal interosseus (1DI) and abductor digiti minimi (ADM) muscles with the minimum stimulus intensity. Then

the resting motor threshold (RMTh) for the 1DI muscle was determined. RMTh is defined as the minimum intensity at which TMS produces MEP amplitude of at least 50 μV in 5 of 10 trials (Rossini et al. 1994).

The stimulation intensity was then set at 120% of the RMTh. In the present experiment the MEPs evoked by the same TMS pulse were recorded simultaneously from the 2 separate muscles. This experimental approach requires the motor threshold for both muscles to be as near as possible. However, it is common experience that this is not achievable in all participants. We tackled this problem by using 1 target muscle (the 1DI) and then excluding participants in which the stimulation at 120% of the RMTh evoked responses, in the 2 muscles, with amplitudes that differed by more than 1 mV. Three participants were excluded from the analysis for this reason. The final population analysed was therefore of 18 participants. SpTMS (which served as GOsignal) was applied at 6 different ISIs, corresponding to 0, 100, 150, 200, 250, and 300 ms. A total of 576 trials were presented in each experiment, corresponding to 96 repetitions per each of the 6 ISIs.

Electromyographic Recordings and Processing of MEPs

The EMG signal from the subjects' right hand was collected by means of 2 pairs of surface Ag/AgCl electrodes positioned on the skin overlying the belly and tendon of the 1DI and of the ADM muscles. The EMG signal from the 2 muscles was collected by 2 analog amplifier channels (CED 1902 unit: Cambridge Electronic Design, UK), amplified 1000 times and digitized (4 KHz sampling rate) by means of a CED power 1401 analog-to-digital converter, controlled by the Signal

software (CED 1902 unit: Cambridge Electronic Design, UK). Recordings were digitally band-pass filtered between 20 and 2 KHz with a notch filter at 50 Hz. The data extracted from each of the 2 recorded EMG channels were 1) the peak-peak amplitude of MEPs, which was used to produce the main experimental variable, and 2) the maximum and minimum values of spontaneous activity in the 120-ms preceding the MEP, which was used to check for voluntary muscular activity. Trials with maximum–minimum activity exceeding 50 μV on any of the 2 EMG channels were discarded, in order to avoid analysing trials with anticipation of responses with respect to the GO-signal. After trimming the data, the MEPs were normalized within each muscle by simply dividing the amplitudes of single MEPs by the grand average of MEPs from that same muscle from the whole experiment. At this point, within each trial, the 2 MEPs recorded from the 2 muscles were classified as congruent MEP and incongruent MEP. The congruent MEP was the one recorded from the muscle corresponding to the prime mover of the observed act (i.e., the 1DI muscle when observing index finger abduction and the ADM muscle when observing little finger abduction). Vice versa, the incongruent MEP was the one recorded from the muscle not involved in the production of the observed act. The pool of congruent MEPs was therefore composed by half of MEPs form the ID1 muscle and half of MEPs form the ADM muscle and the same was true for the pool of incongruent MEPs. Congruent and incongruent MEPs were averaged separately within each ISI. The variations of their relative amplitudes indicated either an imitative response if the congruent MEPs were larger than the incongruent MEPs, or a counter-imitative response whether congruent MEPs were smaller than the incongruent MEPs.

Data Analysis

Any trial with no response, error responses or with EMG activity detected in the 2 target muscles prior to the TMS pulse was discarded from further analysis. All the data were grouped according to several experimental variables, namely they were characterized by ISI, that is, the interval between the cue and TMS (6 levels: 0, 100, 150, 200, 250, 300 ms), MOVEMENT, that is, the movement that was shown in the cue pictures, (2 levels: index or little finger movements) and ORIENTATION, that is, the side to which the fingers were pointing in the cue stimuli (2 levels: right or left). Finally, in the MEPs' analysis, a further factor was added, CONGRUENCE, indicating whether the MEP was congruent or incongruent with the observed movement (2 levels: congruent or incongruent). A first analysis was carried out on RTs, defined as the time of contact of the finger with the touch-sensitive target, which were averaged within each experimental condition and entered an ANOVA with ISI, MOVEMENT, and ORIENTATION as within-subjects factors. Raw MEPs from single trials were first normalized dividing the amplitude of the potentials by the mean value of potentials within that experimental condition. This procedure was carried out to equalize the amplitudes of the MEPs from the 2 muscles (1DI and ADM). Normalized MEPs were analysed by means of an ANOVA with ISI, MOVEMENT, ORIENTATION, and CONGRUENCE as within-subjects factors. In order to account for possible violations of the sphericity assumptions, all within-subjects effects of the ANOVAs were corrected by means of the Geisser–Greenhouse lower-bound adjustment (Geisser and Greenhouse 1958; Keselman and Rogan 1980; Berkovits et al. 2000) as calculated by the

STATISTICA 6.0 (StatSoft, Inc.) software package. All post hoc comparisons were carried out with Bonferroni-corrected t-tests where appropriate.

EXPERIMENT 2

Participants and Experimental Design

Thirty healthy participants (9 males, mean age 22 years, 1 left handed) took part in the experiment and were divided in 2 equal groups of 15 volunteers who received 1-Hz rTMS over the left dlPFC, (prefrontal group, 3 males, mean age 23 years) or over the left phAIP (parietal group, 6 males, mean age 20 years). None of the participants took part in more than 1 experiment. All participants gave written informed consent to the experiment and were screened for contraindication to TMS (Rossi et al. 2009).

The general experimental design was to test participants with spTMS on the motor cortex in trials structured identically to those of Experiment 1, but after offline modulation with 1-Hz rTMS of cortical regions distant from the motor cortex. The experiment's assumption was that any selective effect on motor cortex excitability, by neuromodulation of a remote cortical site, indicates that the stimulated area is involved in the visuomotor circuit underlying the experimental task. The task, the trial structure, the spTMS parameters, as well as the EMG recordings were identical to those of Experiment 1. Only the number of ISIs was reduced to 2 time-points, 150 and 300 ms from the onset of the cue movement, which were the ISIs of interest according to the results of Experiment 1. Also the number of repetitions per condition was reduced to 32 per time interval,

corresponding to 64 trials per each of the 3 blocks. This choice was dictated by the need to shorten the experimental blocks within the time-window of the aftereffects of the 10-min train of rTMS. The aftereffects of 1-Hz rTMS are expected to be about as long as the duration of the train of stimulation itself (Chen et al. 1997, 2003).

In both the parietal and the prefrontal groups, each participant underwent 3 consecutive experimental blocks. The first one was performed in baseline conditions, without rTMS (no-rTMS block). In the remaining 2 blocks, offline 1-Hz rTMS was first applied to the cortical target (left dlPFC or the left phAIP) and was followed by the experimental task with spTMS. In 1 of these 2 blocks (low-intensity rTMS block), rTMS was delivered at low intensity and in the other one (high-intensity rTMS block) it was delivered at high intensity. The order of the no-rTMS block was fixed (first of the series), while the order of the low-intensity rTMS and the high-intensity rTMS blocks was balanced between participants. Both the no-TMS and the low-intensity rTMS blocks were assumed to be control conditions for the main experimental intervention, that is, the high-intensity stimulation. The low-intensity rTMS condition represents robust baseline measure because it accounts for all TMS effects that are not related to cortical stimulation, such as noise, cutaneous sensations, pain, weight, and position of the TMS coil. According to data obtained on the motor cortex, (Fitzgerald et al. 2006), and on the temporal cortex (Arfeller et al. 2013), intensities lower than 80% of RMTh should be ineffective. However, there are no available data in literature on how low should TMS intensity be, in

order not to produce efficient stimulation of the parietal or prefrontal cortices with 1-Hz frequency.

Therefore, the ineffectiveness of low-intensity TMS can only be inferred post hoc, by comparing it to a block with no stimulation at all. As a result, in the present experiment, we needed to adopt 2 control conditions in order to highlight only the effects of TMS due to cortical stimulation. Repetitive TMS, Target Definition, and Neuronavigation Repetitive TMS was delivered offline prior to MEP collection with a biphasic Magstim Rapid (Magstim, Dyfed, UK) stimulator connected to a figure-of-eight coil with outer winding diameter of 70 mm. The intensity of high-intensity rTMS was set to 100% of the RMTh, and the intensity of the low-intensity rTMS was set to 40% of the RMTh. Stimuli were delivered at 1 Hz for 10 min. The Talairach coordinates of the left dlPFC target were identified averaging the results of 5 previous imaging studies investigating the learning of arbitrary visuomotor associations (Toni and Passingham 1999; Schluter et al. 2001; Toni, Rushworth, et al. 2001; Bunge et al. 2002, 2003) and corresponded to [x = −52, y = 32, and z = 20] for dlPFC. For the left phAIP position, we used the coordinates indicated in 2 recent large meta-analyses of imaging studies on action observation (Caspers et al. 2010; Molenberghs et al. 2012), corresponding to [x = −42, y = −46, and z = 57].

The left dlPFC and left phAIP sites on the subject's scalp were automatically identified using the SoftTaxic Evolution Navigator system (E.M.S., Bologna, Italy) that can operate in the absence of radiological images on the basis of digitized fiducial points on the skull which are related to standard cerebral anatomy.

Therefore, although individual magnetic resonance images were not available, Talairach coordinates of cortical sites, underlying coil locations, were automatically estimated for the participants by the navigator system, on the basis of an MRI constructed stereotaxic template.

Data Analysis

The participant exclusion criteria were the same described in Experiment 1 and 2 participants were excluded from the analysis; thus, the final population resulted in 28 participants divided in 2 groups of 14 participants each. Any trial with no response, error responses or with EMG activity detected in the 2 target muscles prior to the TMS pulse was discarded from further analysis. The data were grouped according to the between-subjects factor TARGET (2 levels: prefrontal and parietal) and the within-subjects factors BLOCK (3 levels: no-rTMS, low-intensity rTMS, and high-intensity rTMS), ISI (2 levels: 150 and 300 ms), MOVEMENT (2 levels: index or little finger movements), ORIENTATION (2 levels: right or left) and, limitedly to MEP analysis, CONGRUENCE (2 levels: congruent or incongruent). The RTs were analysed by means of a TARGET*BLOCK*ISI*MOVEMENT*ORIENTATION ANOVA. As in Experiment 1, raw MEPs from single trials were first normalized by dividing the amplitude of the single potentials by the grand average of the MEPs from that same muscle. This procedure was done separately for the 3 blocks. The normalized MEPs were then analysed by means of a TARGET*BLOCK*ISI*MOVEMENT*ORIENTATION*CONGRUENCE

ANOVA. In order to account for possible violations of the sphericity assumptions, all within-subjects effects of the ANOVAs were corrected by means of the Geisser–Greenhouse lower-bound adjustment (Geisser and Greenhouse 1958; Keselman and Rogan 1980; Berkovits et al. 2000) as calculated by the STATISTICA 6.0 (StatSoft, Inc.) software package. All post hoc comparisons were carried out with Bonferroni-corrected t-tests where appropriate.

3.3 Results

EXPERIMENT 1

None of the participants reported any adverse side effect from TMS. Two percent of trials were discarded because of pre-TMS EMG activity. Errors accounted for 4% of all trials. The analysis of RTs indicated that subjects were faster when responding with their index finger (main effect of MOVEMENT ($F_{1,17}= 28.8$, $P = 0.00005$)), as well as when they were responding to hands oriented leftward rather than rightward (main effect of ORIENTATION ($F_{1,17}= 30.6$, $P = 0.00004$)). Participants responded increasingly faster with increasing time between stimulus presentation and the GO-signal (main effect of ISI ($F_{5,85} = 94.2$, $P < 0.000001$)), which is illustrated in Figure 11. This main effect was investigated by comparing with multiple t-tests the RTs from consecutive ISIs in order to assess the advantage of increasing stimulus-dependent processing time. The critical P-value was Bonferroni-corrected for 5 multiple comparisons to 0.01. Significant differences were found between all pairs of consecutive ISIs (all $P < 0.0005$) except

for the last one (P = 0.06) (see asterisks in Fig. 11), indicating that the advantage of increasing ISI reached a floor level at 250–300 ms between stimulus onset and the GO-signal. Finally, a MOVEMENT*ORIENTATION interaction (F1,17 =12.4, P = 0.003) was found indicating that the advantage in responding to the leftward-oriented stimuli was present only when responses were given with the index finger. The mean values of RTs for each cell of the full factorial design are presented in Table 1.

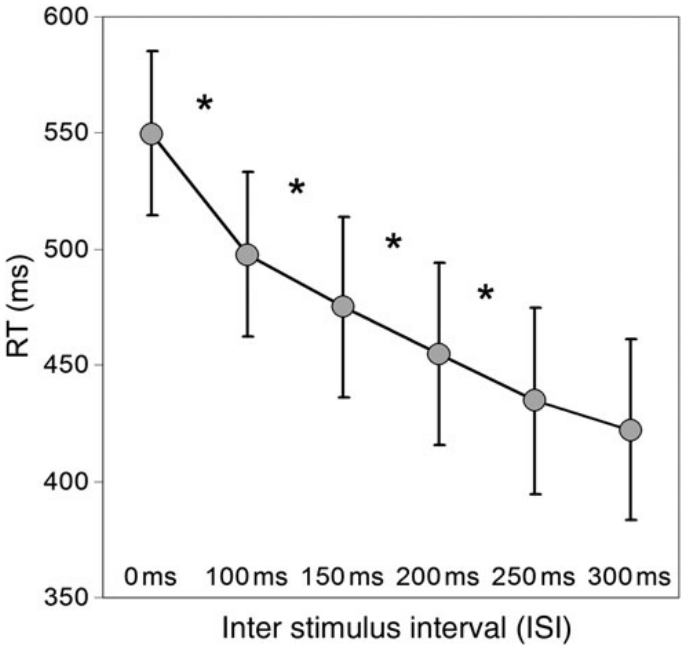

Figure 11. Experiment 1. Mean response times given for each of the 6 ISIs. The P levels of the pairwise t-tests comparing data from consecutive ISIs are presented. Note that all comparisons were significant aside from the one between the 250- and the 300-ms ISIs. Error bars indicate 95% CI of the mean.

The results of the ANOVA performed on normalized MEP amplitudes yielded a main effect of CONGRUENCE (F1,17 = 5.1, P = 0.038), an interaction of ORIENTATION*CONGRUENCE (F1,17 = 5.2, P = 0.036) and an interaction of

ORIENTATION* MOVEMENT*CONGRUENCE ($F_{1,17} = 4.6$, $P = 0.047$). These effects were not further investigated because, given our a priori experimental hypothesis, the results of interest were only those highlighting changes in the relation between congruent and incongruent MEPs at different ISIs, that is, accordingly, the only significant interaction of interest that was found is an ISI*CONGRUENCE interaction ($F_{1,17} = 7.2$, $P = 0.016$) (see Fig. 12 and Table 2 for details).

Observed movement	Index finger movement		Little finger movement	
	Leftward	Rightward	Leftward	Rightward
Observed hand orientation (ms)				
ISI 0	582.9 (38)	585.1 (42)	493.0 (41)	538.4 (35)
ISI 100	536.5 (37)	526.3 (44)	437.7 (40)	490.3 (38)
ISI 150	509.7 (39)	512.5 (49)	420.6 (41)	457.7 (41)
ISI 200	497.0 (49)	495.5 (47)	402.7 (35)	423.7 (41)
ISI 250	483.1 (52)	477.4 (42)	381.1 (38)	397.5 (44)
ISI 300	457.4 (43)	461.0 (47)	377.6 (38)	392.9 (45)

Table 1. Mean response times (ms) from Experiment 1 (95% CI).

Recorded muscle	Congruent muscle		Incongruent muscle	
	Leftward	Rightward	Leftward	Rightward
Observed hand orientation (ms)				
ISI 0	0.912 (0.065)	0.908 (0.073)	0.905 (0.07)	0.942 (0.063)
ISI 100	0.939 (0.056)	1.018 (0.072)	0.96 (0.07)	0.99 (0.078)
ISI 150	1.009 (0.064)	1.037 (0.061)	0.921 (0.089)	0.916 (0.056)
ISI 200	1.034 (0.04)	0.957 (0.044)	1.053 (0.045)	0.992 (0.058)
ISI 250	1.053 (0.082)	0.965 (0.057)	1.066 (0.067)	1.15 (0.159)
ISI 300	1.041 (0.093)	0.987 (0.082)	1.135 (0.144)	1.164 (0.111)

Table 2. Mean values of normalized MEP amplitudes from Experiment 1 (95% CI).

Post hoc comparisons were made between congruent and incongruent MEP amplitudes within each ISI and the critical P level was adjusted for 6 multiple

comparisons to P = 0.008. At the ISI of 150 ms, the congruent MEPs were significantly larger (P = 0.005) than the incongruent ones. Conversely, at the ISI of 300 ms, the incongruent MEPs were significantly larger (P = 0.003) than the congruent ones. The remaining 4 comparisons were not significant (all P's > 0.04). In summary, the main finding of Experiment 1 was that the reciprocal pattern of congruent and incongruent MEPs indicated a mirror response at 150 ms from stimulus onset and a counter-mirror response at 300 ms from stimulus onset.

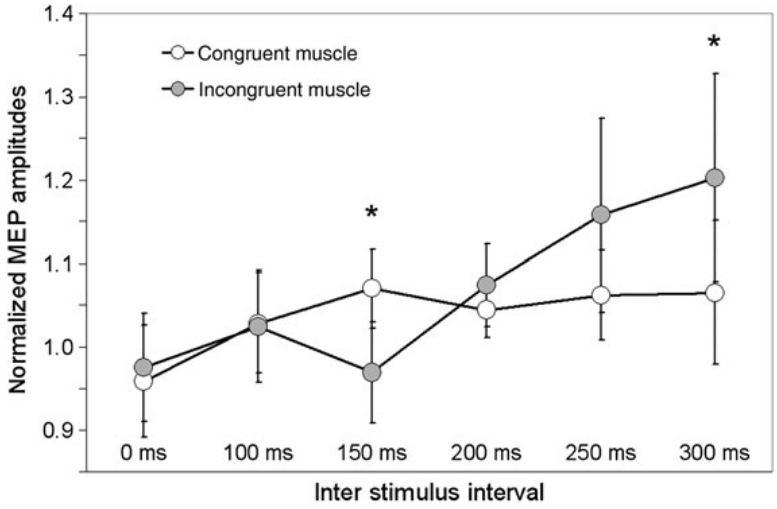

Figure 12. Experiment 1. Mean values of normalized congruent and incongruent MEP amplitudes. Error bars indicate 95% CI of the mean. Asterisks denote significant t-tests comparing congruent and incongruent MEPs within the same ISI (critical P level corrected for multiple comparisons to 0.008).

EXPERIMENT 2

None of the participants reported any adverse side effect from TMS. Three percent of trials were discarded because of pre-TMS EMG activity. Errors accounted for 4% of all trials. The ANOVA on RTs showed that participants responded faster at the ISI of 300 ms than at 150 ms as shown by the main effect of

the ISI factor ($F_{1,26} = 174.40$, $P < 0.00001$). A main effect of ORIENTATION ($F_{1,26} = 7.9641$, $P = 0.009$) showed that participants were faster in response to leftward-oriented hands. Unlike in Experiment 1, no main effect of MOVEMENT was found ($P = 0.25$) but, as in Experiment 1 a significant MOVEMENT*ORIENTATION interaction ($F_{1,26} = 6.83$, $P = 0.015$) was found indicating that an advantage of responding with the index finger was indeed present but only in response to hands oriented rightward. Importantly, no effects of the BLOCK factor on RTs were found. Experiment 2 was planned to highlight differential effects of effective and non-effective rTMS on the difference between congruent and incongruent MEPs at the 2 different ISIs in the 2 groups. Therefore, the a priori defined effect of interest was any interaction involving all the TARGET, BLOCK, ISI, and CONGRUENCE. Effects that did not fall within the focus of interest were a main effect of CONGRUENCE ($F_{1,26} = 5.5670$, $P = 0.02609$), a main effect of ISI ($F_{1,26} = 48.763$, $P < 0.00001$), and a main effect of ORIENTATION ($F_{1,26} = 8.58$, $P = 0.007$). Additionally, we found an interaction of ISI*CONGRUENCE ($F_{1,26} = 44.67$, $P < 0.00001$), an ISI*CONGRUENCE*TARGET interaction ($F_{1,26} = 7.53$, $P = 0.01$), a CONGRUENCE*BLOCK interaction ($F_{2,52} = 4.73$, $P = 0.03$), and a CONGRUENCE* MOVEMENT*ORIENTATION interaction ($F_{1,26} = 6.17$, $P= 0.02$). The only effect of interest that emerged from the ANOVA was a TARGET*BLOCK*ISI*CONGRUENCE interaction ($F_{2,52} = 10.73$, $P = 0.0029$), which is illustrated in Figure 13 and detailed in Table 3. The interaction was further explored by 2 TARGET*BLOCK*CONGRUENCE ANOVAs, 1 for each of the 2

ISIs, which both resulted in significant TARGET*BLOCK*CONGRUENCE interactions ((F2,52 = 4.41, P = 0.045) for the 150-ms ISI and (F2,52 = 5.69, P = 0.024) for the 300-ms ISI). The data from the 150-ms ISI were further analysed by means of 2 BLOCK*CONGRUENCE ANOVAs, separately for each of the 2 groups, which yielded only a main effect of CONGRUENCE (F1,13= 21.43, P = 0.0005) in the prefrontal group and a main effect of CONGRUENCE (F1,13 = 30.90, P = 0.00009) in the parietal group. The main effects were due in both groups to congruent MEPs being larger than incongruent ones. It is important to note that the present results strongly replicate the finding of a congruency effect at 150 ms that is reported in Experiment 1. However, to fully demonstrate that the results of Experiment 2 replicate those of Experiment 1, we also compared congruent and incongruent trials in the no-rTMS condition of the 150 ISI conditions in the parietal group, since, in these conditions, we found a significant BLOCK*CONGRUENCE interaction. The comparison yielded a significant result showing that congruent MEPs were larger than incongruent MEPs (P = 0.019).

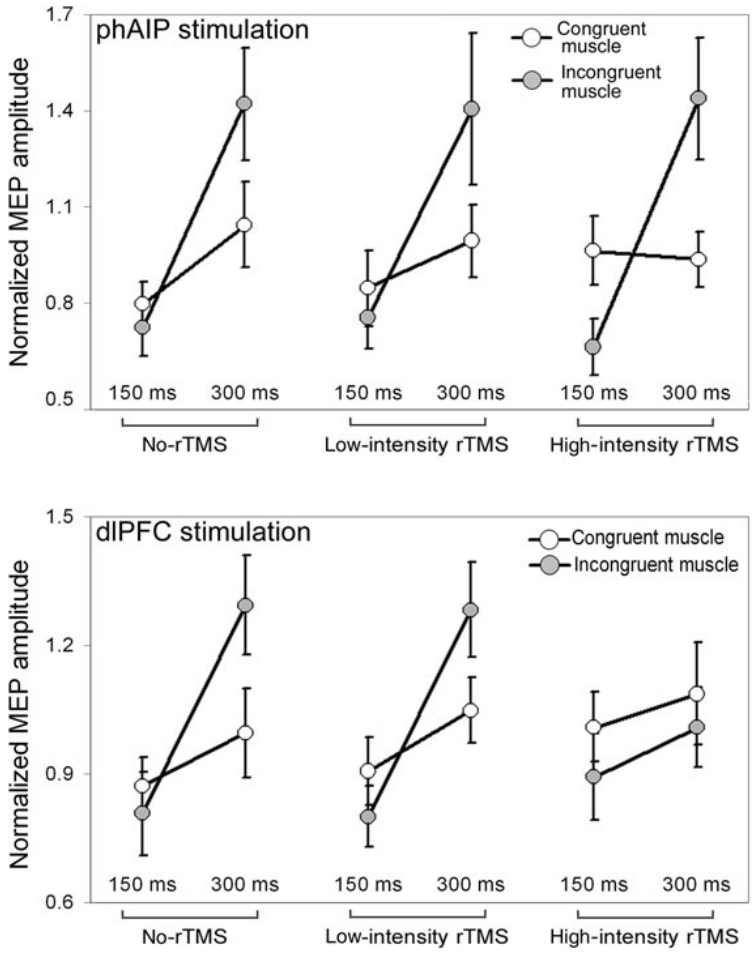

Figure 13. Experiment 2. Mean values of normalized congruent and incongruent MEP amplitudes collected at the 2 ISIs of 150 and 300 ms and represented separately for the 3 experimental blocks. The data from the phAIP group are shown in the upper panel and those from the dlPFC group are shown in the lower panel. Error bars indicate 95% CI of the mean.

More interestingly in the light of interpreting the complex interactions, only in the parietal group a BLOCK*CONGRUENCE interaction ($F_{2,26}$= 9.04, P = 0.01) was found. This interaction was finally explored by comparing separately, for each MEP type (congruent or incongruent), the mean values of normalized MEP amplitudes between each of the 3 blocks, corresponding to a total number of 6 multiple comparisons. The critical P level was therefore adjusted to P = 0.008. The

results showed that congruent MEPs in the high-intensity rTMS block were significantly larger than those in the no-rTMS block (P = 0.002) and than those in the low-intensity rTMS block (P = 0.005). No significant differences were found between blocks in the incongruent MEPs (all P's < 0.16).

Target	Block	Congruent muscle		Incongruent muscle	
		150 ms	300 ms	150 ms	300 ms
phAIP	No-rTMS	0.801 (0.07)	1.047 (0.133)	0.725 (0.086)	1.426 (0.177)
	Low-intensity rTMS	0.847 (0.12)	0.994 (0.114)	0.752 (0.096)	1.411 (0.24)
	High-intensity rTMS	0.964 (0.108)	0.937 (0.085)	0.664 (0.088)	1.439 (0.191)
dlPFC	No-rTMS	0.873 (0.068)	0.996 (0.104)	0.808 (0.097)	1.293 (0.116)
	Low-intensity rTMS	0.907 (0.078)	1.05 (0.077)	0.801 (0.07)	1.283 (0.111)
	High-intensity rTMS	1.009 (0.082)	1.087 (0.12)	0.892 (0.1)	1.009 (0.093)

Table 3. Mean values of normalized MEP amplitudes from Experiment 2 (95% CI).

Also, the data from the 300-ms ISI were analysed by means of 2 BLOCK*CONGRUENCE ANOVAs, separately for each of the 2 groups, which yielded a main effect of CONGRUENCE in both the parietal group ($F_{1,13} = 12.75$, P = 0.003) and in the prefrontal group ($F_{1,13} = 13.451$, P = 0.003). Also at this ISI, these findings represent a replica of the data from Experiment 1, namely of the counter-imitative tendency was observed at the 300-ms ISI. However, similarly to the 150-ms ISI condition, the finding of a significant BLOCK*CONGRUENCE interaction required that we perform a direct comparison between the congruent and the incongruent MEPs in the prefrontal group for the no-rTMS block. A paired-

samples t-test indicated a significant difference (P = 0.022) between the 2 conditions, with congruent MEPs more ample than incongruent MEPs.

Interestingly, only in the prefrontal group a BLOCK* CONGRUENCE ($F_{2,26} = 8.95$, P = 0.01) interaction was found. This interaction was explored within the prefrontal group by comparing separately for each MEP type (congruent or incongruent) the mean values of normalized MEP amplitudes between each of the 3 blocks, corresponding to a total number of 6 multiple comparisons. The critical P level was therefore adjusted to P = 0.008. Congruent MEPs showed no difference between blocks (all P's > 0.23). On the contrary, incongruent MEPs in the high-intensity rTMS block resulted to be significantly smaller than those in the low-intensity rTMS block (P = 0.003) and from those in the no-rTMS block (P = 0.005).

In summary, the main result of Experiment 2 indicated that (A) high-intensity rTMS applied to the prefrontal cortex produced effects only at the 300-ms ISI and that these effects consisted in a lesser increase of incongruent MEPs and (B) high-intensity rTMS applied to the parietal cortex produced effects limited to the 150-ms ISI and these consisted in a more marked increase in amplitude of congruent MEPs. Additionally, it should be noted that the results validated the original experimental assumption that low-intensity rTMS was equivalent to the no-rTMS condition.

3.4 Discussion

We show here that during a simple social interaction, such as producing a rule-dependent motor response to the movements of another individual, 2 distinct processes occur in the motor system of the participant, in the critical period between the onset of the cue movement and completion of the task. The earliest phenomenon that can be read in the motor output of the participant appears around 150 ms from the onset of the cue movement (Fig. 12). It is a visuomotor mapping that specifically imitates of the observed motor act and does not depend on the arbitrary rule to be implemented. Two basic properties of this early response appear to be particularly relevant to identify it as a pure mirror response. First, the response is specific for the observed effector's identity, because it mirrors equally the cue-movements of the little finger or the index finger. Second, the early mirror effect is independent from the spatial relations between the participant's effector and the observed one because (Fig. 10) visual stimuli were orthogonal to the participant's hand and randomly oriented leftward or rightward. Furthermore, this early phenomenon can be defined as automatic, if the pivotal property that distinguishes automatic from controlled processes is that an automatic process is triggered without the actor's intending to do so and cannot be stopped even when the actor intends to and it is in that actor's best interests to do so (Kornblum et al. 1990).

The second phenomenon becomes evident around 300 ms from the onset of the cue-movement and is specifically following the rule of the arbitrary visuomotor task. As for the early mirror response, also this executive response is body part

specific and devoid of stimulus-response spatial features. This component reflects therefore the output of the executive processes that transform the visual cue into the rule-dependent response. We propose that the early mirror response is produced by a stimulus-dependent process and the later one is produced by a rule-dependent goal-driven process. The results of the first experiment of the present work validate and extend the results of our previous work (Barchiesi and Cattaneo 2013) showing biphasic responses to others' actions in conflictual stimulus-response behavior. The latency of the early response observed in Barchiesi and Cattaneo (2013) was of 250 ms from stimulus onset, while, in the present work, it was confined at 150 ms from movement onset. Though the biphasic pattern is preserved in the 2 experiments, the difference in timing of the early response is due, in our view, to 2 separate phenomena. First, the mirror response is highlighted earlier in the present experiment because it is recorded during movement preparation compared with the passive viewing condition of Barchiesi and Cattaneo (2013), thus decreasing the excitability threshold of the motor system. Second, one possible explanation why the mirror response is no longer seen at 250 ms could be that the task stresses the speed of response and the counter-mirror effect is anticipated and therefore it overrides the mirror response at an earlier time than during passive observation. Also indirect evidence from the electroencephalographic literature indicates that body-related visual information accesses the visuomotor system well before 200 ms from stimulus onset (van Schie et al. 2008; Bortoletto et al. 2011).

We performed a second experiment to explore the anatomical substrates of this model. With 1-Hz rTMS, we produced neuromodulation in 2 cortical regions

that are related to stimulus-dependent mirror responses, that is, the phAIP, and rule-dependent control of arbitrary visuomotor associations, that is, the dlPFC.

The phAIP is robustly activated by action observation (Molenberghs et al. 2012) and the dlPFC is crucial in implementing visuomotor rules (Passingham et al. 2000). The results showed a clear-cut double dissociation of the effects of rTMS on early and late motor responses. As expected, interference with the dlPFC only reduced the size of the late response (Fig. 13) and rTMS over the phAIP produced effects exclusively on the early response consisting in an increase of the mirror phenomenon.

These findings favor our experimental hypothesis that the 2 sensorimotor phenomena, the early mirror and the late executive response, are mediated by 2 different neural systems. The early responses are conveyed along the dorsal visual stream and are unequivocally stimulus-dependent responses. The late responses are mediated by the prefrontal cortex and are rule-dependent responses. The present double dissociation also provides information on the feeding of visual information to the prefrontal cortex. The fact that targeting phAIP did not influence the late response as well as the early one indicates that the prefrontal cortex can gain visual information on the cue-movement by pathways other than the ones producing automatic mirror responses. The present interpretation of the data by no means excludes the possibility of a cross-talk between stimulus-dependent and rule-dependent processes, which are known to influence each other at several levels of response processing. This datum actually fits well with models proposed in nonhuman primates (Boussaoud et al. 1995; Lebedev and Wise 2002) and in

humans (Passingham and Toni 2001; Toni, Rushworth, et al. 2001; Vry et al. 2012) describing 2 routes to action that rely on the dorsal and the ventral visual streams. An additional dissociated effect of rTMS over the 2 target areas is that parietal stimulation produced an effect on the representation of the congruent movement, that is, on the movement that was seen in the visual stimulus and, on the contrary, prefrontal stimulation produced an effect limited to the incongruent movement, that is, to the movement that was NOT seen in the visual stimulus but that had to be implemented in the counter-mirror task. These result further supports the association of the parietal cortex with true mirror responses, related to the observed movement, and of the prefrontal cortex with the counter-mirror response, based on an arbitrary motor mapping. The present experiment was not designed to test motor efficiency in counter-imitative tasks and this is possibly the reason why we did not find in Experiment 2 an effect of rTMS on RTs. In particular, the presence of a delay between the cue and the response which was necessary to apply TMS during the trials may have reduced potential effects of rTMS on motor performance.

It is interesting to note that the remote effects of rTMS on the 2 targets were of different polarity, that is, a reduction of the specific physiological response pattern in the case of prefrontal rTMS and an increase in the physiological pattern in the case of parietal rTMS. When investigating the effects of 1-Hz rTMS on distant cortical areas, it is difficult to make a priori assumptions on what the net effect will be on the remote region.

This is clearly observed when coupling 1-Hz rTMS with a whole brain measure such as fMRI. As reviewed in Arfeller et al. (2013), the hemodynamic

effects of 1-Hz rTMS on regions distant from the stimulated one are strictly task-dependent and can be, unpredictably, inhibitory or excitatory. Also in the specific field of brain responses to action observation, remote effects of 1-Hz rTMS on the mirror neuron circuit can be facilitatory after rTMS of the posterior superior temporal sulcus (Arfeller et al. 2013; Avenanti et al. 2013) or inhibitory after stimulation of the premotor cortex (Avenanti et al. 2007). The occurrence of facilitatory remote effects of 1-Hz rTMS is generally attributed to compensatory increase of the activity within a network in face of reduced functioning of the stimulated target (Avenanti et al. 2013). More generally, the traditional view of the effects of 1-Hz rTMS being considered inhibitory is no longer supported. The current view is that rTMS has effects which are not predictable a priori on the basis of the sole stimulation frequency, but that can be excitatory or inhibitory according to different experimental conditions (Silvanto et al. 2008) as reviewed in (Silvanto and Pascual-Leone 2008; Miniussi et al. 2013). Another possible explanation for the facilitatory effect of phAIP stimulation is that AIP is not involved in mirroring, but rather in the prevention of mirroring, for example, by mapping the observed stimulus onto the nonmirror response required by the task and, therefore, the increase in mirroring takes place because disruption of AIP releases the automatic mirror response, as proposed by Corbetta and Shulman (2002). In summary, the 3 possible hypotheses accounting for the facilitatory effects of phAIP stimulation include 1) phAIP has been disrupted and compensatory overactivity is observed in the mirror system; 2) rTMS produced facilitation of physiological phAIP activity rather than inhibition; 3) phAIP is disrupted by rTMS but it is located in parallel to

the pathway conveying automatic action mirroring which it actively inhibits. It is worth noting that all 3 hypotheses are consistent with a separate genesis of the early and the late motor responses, thus validating at least in part our initial hypothesis.

Chapter 4 – Experiment 2

4.1 Aim of the study

The results of the previous experiment suggested the presence of two parallel pathways that compete for the motor output when producing motor responses to others' actions. A further question is where these two pathways converge. In this respect several neuroanatomical models are possible and plausible, as illustrated in figure 14.

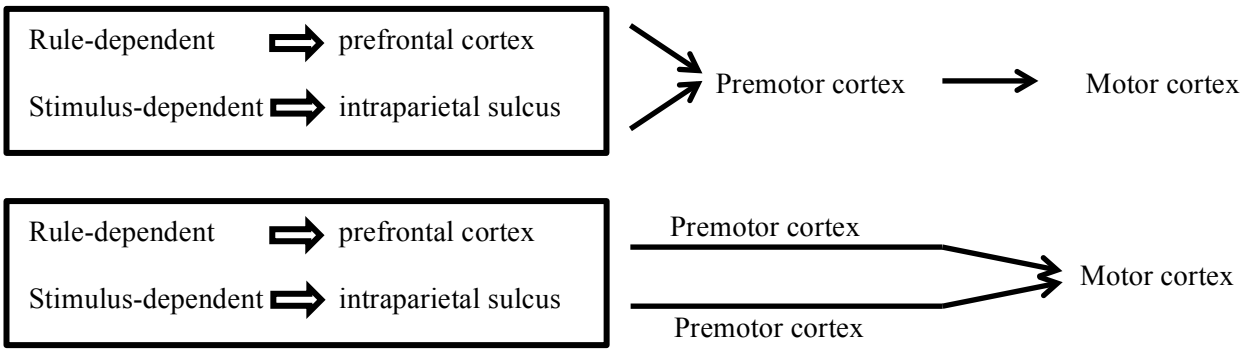

Figure 14. This image illustrates the different possible sites of interaction between the stimulus-dependent automatic pathway and the rule-dependent executive one.

To understand whether the two pathways converge or are segregated until they reach the motor cortex, a previous step is needed. We demonstrated that the rule-dependent executive system passes through the lateral prefrontal cortex; in this study we investigated whether, during a counter-imitative task, there is a further step between the prefrontal cortex and the motor cortex.

We designed an experiment with a condition and map approach (Siebner et al. 2009) where we combined low-frequency rTMS and functional Magnetic Resonance Imaging (fMRI).

We performed a first experiment with a group of participants to localize the target area that would have been stimulated in the second experiment. The participants were challenged with two tasks: an imitative and a counter-imitative task in the fMRI. The contrast between the counter-imitative and imitative task allowed us to locate in the left lateral prefrontal cortex a spot close to the one used in our previous work (Ubaldi et al. 2013) for the second experiment. A second group of participants performed the main experiment and they were stimulated using low-frequency rTMS over that target area with real stimulation or sham stimulation. Following they were carried into the scanner to perform one of the two tasks mentioned before. These two steps were repeated four times for the two stimulations and the two tasks. This approach highlights the connectivity between the target area and the other regions that are functional connected with the former. Our prediction was to find an interaction between stimulations and tasks in the premotor cortex that is likely a nodal area involved in solving a competition between different types of responses.

4.2 Methods and materials

Participants

A first group of eight healthy adult volunteers (6 females; mean age: 27.3 years; range: 19-32) participated in the fMRI experiment. None of these participants had taken part in the rTMS-fMRI experiment.

A second group of sixteen healthy adult volunteers (11 females; mean age: 25,5 years; range: 20-33) participated in the rTMS-fMRI experiment. All participants were right-handed (except one in the second experiment) with normal or corrected-to-normal vision, and no history of neurological or psychiatric disease. The experiments were approved by the Ethical Committee of the University of Trento and were conducted in compliance with the revised Helsinki declaration (World Medical Association General Assembly, 2008). All participants gave written informed consent to the experiment and were screened for contraindication to TMS (Rossi et al., 2009).

Visual stimuli

The stimuli used in rTMS-fMRI experiment were the same as those used in the fMRI experiment. Stimuli (480 × 480 pixels) were presented on a 17-inch LCD monitor (DELL 1908FP-BLK in a dimly lit room. Stimuli were presented using E-Prime 2.0 software (Psychology Software Tools).

Participants were shown a still frame depicting the dorsum of a hand oriented vertically over a button box with two buttons for 500 ms which was

followed by another still frame of the same hand that was pressing a button with the index finger or the middle finger for 800 ms. The quick succession of the two images produced an apparent motion of the finger. In the following frame they were given a feedback related to their performance. The feedback frame lasted for 700 ms, thus whether they had responded within the 1500 ms they were presented with the word Correct in green in case of a correct response, or with the word Wrong in red in case of an incorrect response. If no response was given within 1500 ms they were presented with the phrase No Response (Figure 15). It should be noted that the orientation of the observed hands was orthogonal to that of the subject (which was positioned horizontally over the belly) in order to avoid any spatial mapping of the stimulus response association.

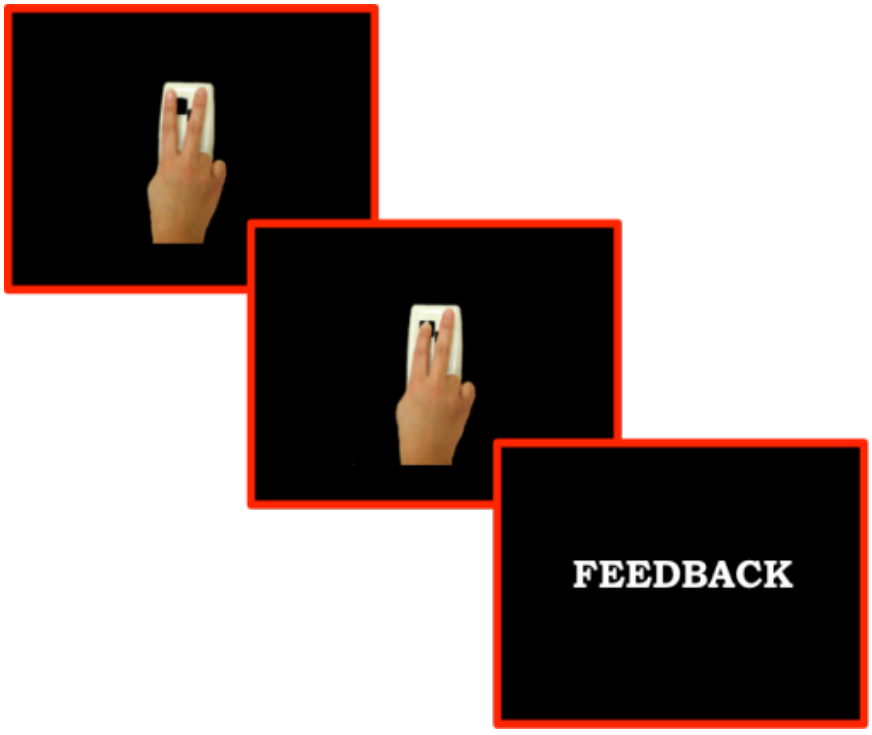

Figure 15. Trial structure. The succession of the 2 frames generating apparent motion is shown. All hands were presented vertically (orthogonal to the participant's right hand). The second frame represented either an index finger pressing or a middle finger pressing. The third frame was the feedback about the participant's performance.

General design

Both the groups of participants performed the same fMRI experiment in which they were challenged with two types of tasks: an imitative and a counter-imitative task in a counter-balanced blocked design. Participants lay down in the scanner with their right elbow at 90 degree and the hand positioned over the belly where a button box was positioned. The participants were instructed to respond as fast and accurately as possible: in the imitative task by pressing the button with the same finger they saw in the video and in the counter-imitative task, by pressing the button with the opposite finger, in other terms if they saw an index finger pressing the button, they have to press the button with the middle finger and vice versa. Each run was divided into six mini-blocks with a fixation cross in between. In each run they were 240 trials.

The first group of participants performed six runs in the fMRI: three imitative and three counter-imitative in a counterbalanced order.

The second group of participants performed a combined rTMS and fMRI experiment. They started with a fifteen minutes stimulation with the repetitive TMS while they lay down on a stretcher, then they were quickly carried in the scanner to perform the task for nine minutes. They performed these two steps four times: two after effective rTMS and two after sham rTMS. The sham stimulation was performed using a sham coil. For each participant, the order of the runs (imitative/counter-imitative) was randomized among the types of stimulations and the types of tasks. The average time that was between the stimulation and the

beginning of the task was three minutes and a half, so the effectiveness of the stimulation was preserved.

fMRI data acquisition

Functional and structural data were collected with a Bruker BioSpin MedSpec 4-T scanner (Bruker BioSpin GmbH, Rheinstetten, Germany), while participants lay in the scanner and viewed the visual stimuli through a mirror system. Data collection was conducted at the Center for Mind/Brain Sciences (CIMeC), University of Trento using a USA Instruments 8-channel phased-array head coil.

Functional images were acquired using echo planar (EPI) T2*-weighted scans. Acquisition parameters were: repetition time (TR) of 2 s, an echo time (TE) of 33 ms, a flip angle (FA) of 73°, a field of view (FoV) of 192 mm, and a matrix size of 64 × 64. Each functional acquisition consisted of 28 axial slices (which covered the whole cerebral cortex) with a thickness of 3 mm and gap of 33% (1 mm).

Structural images with high-resolution (1 × 1 × 1 mm3) T1-weighted MPRAGE sequence were performed (sagittal slice orientation, centric phase encoding, image matrix = 256 × 224 [Read × Phase], field of view = 256 × 224 mm [Read × Phase], 176 slices with 1-mm thickness, GRAPPA acquisition with acceleration factor = 2, duration = 5.36 min, repetition time = 2700, echo time = 4.18, TI = 1020 ms, 7° flip angle).

rTMS methods

For correct placement of the TMS coil, structural MRI images (MP-RAGE sequence with 1 × 1 × 1 mm resolution) were acquired for all participants. The position of the rTMS coil was individuated by means of the participant's reconstructed head, and the location of TMS-stimulation was marked on the reconstructed surface of each individual's brain. We used the BrainVoyager Neuronavigator system (Brain Innovation BV, The Netherlands, version 2.1) to locate the target area.

We used the group-average Talairach coordinates [-43 35 6] to stimulate the second group. Talairach coordinates were transformed back into each subject's native space for correct neuronavigation. The repetitive TMS pulses were applied with a 75-mm figure-of eight coil (MC-B65) and a MagPro × 100 stimulator (MagVenture A/S, Denmark). The stimulation intensity during the experiment was set at 100% of the individual resting motor threshold, measured as the intensity that elicited five visible hand movements out of 10 stimulations. This resulted in an rTMS intensity that ranged between 42% and 54% of the maximum stimulator intensity.

fMRI data analysis

Data were analysed using BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands). After discarding the first 4 volumes of each run, all images were slice-time corrected, motion corrected, and low-frequency drifts were removed with a temporal high-pass filter (cut-off of 0.006 Hz). All data were spatially smoothed (8

mm Gaussian kernel) and transformed into Talairach space, which included resampling to 3 × 3 × 3 mm voxels.

For each participant and each run, general linear models were created to model the conditions in the experiment. All trials were included in the analyses.

In the first group of participants, the contrast of counter-imitative task greater than imitative task was used to define the location where we would have stimulated with rTMS the second group of participants.

We run a random effect analysis and we locate the grater activity ($p<0,01$) in the left lateral prefrontal cortex.

4.3 Results

Localizer

The target area that would have been stimulated in the main experiment was defined based on the localizer. Running the contrast: counter-imitation greater than imitation we found a significant spot (beta values are shown in figure 16) in the left lateral prefrontal cortex (Talairach coordinates [-44, 36, 7], $p_{uncorrected}<0,009$) (see figure 17) comparable to the one stimulated in our previous study (Ubaldi et al., 2013).

We run a t-test on the reaction times and we found a significant difference ($p<0.00001$) between the two tasks, in particular the reaction times related to the imitative task were significantly faster (mean= 343 ms, SD= 34

ms) compared with the ones in the counter-imitative task (mean= 413, SD= 42 ms) (Figure 18).

Main experiment (rTMS-fMRI)

We run a whole brain random effects analysis. To see whether the TMS has a local effect on the stimulated area, we tested the main effect of TMS. The results showed a decreasing in BOLD signal ($t(15)$, $p_{uncorrected}<0.001$) in the medial projection of the target area (talairach coordinates [-27, 39, 0]) and in a more anterior portion of the frontal lobe (talairach coordinates [-37, 55, 5]) as circled in figure 19. A strong decrease in the BOLD signal is to be noted in the posterior portion of the right insula (talairach coordinates [45, -12, 5]). Moreover the results showed an increasing in the BOLD signal in the EBA area (talairach coordinates [44, -72, -11]).

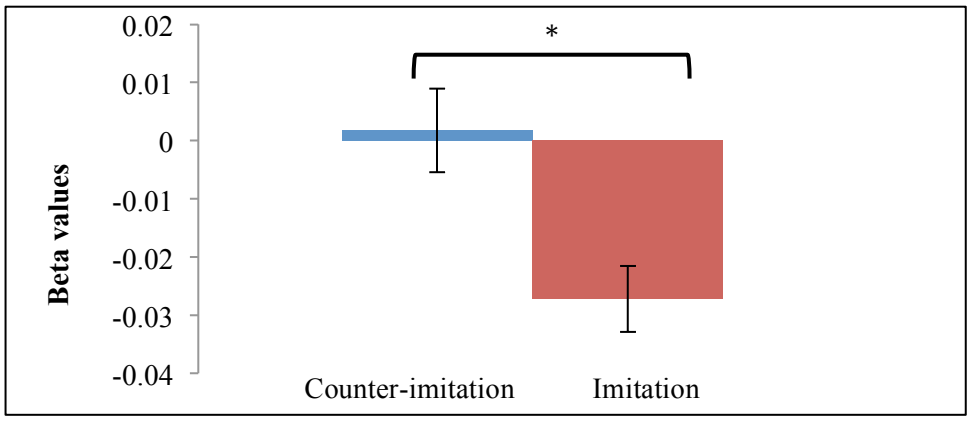

Figure 16. The data represent the beta-weights obtained from the target location ($p_{uncorrected}$<0,009).

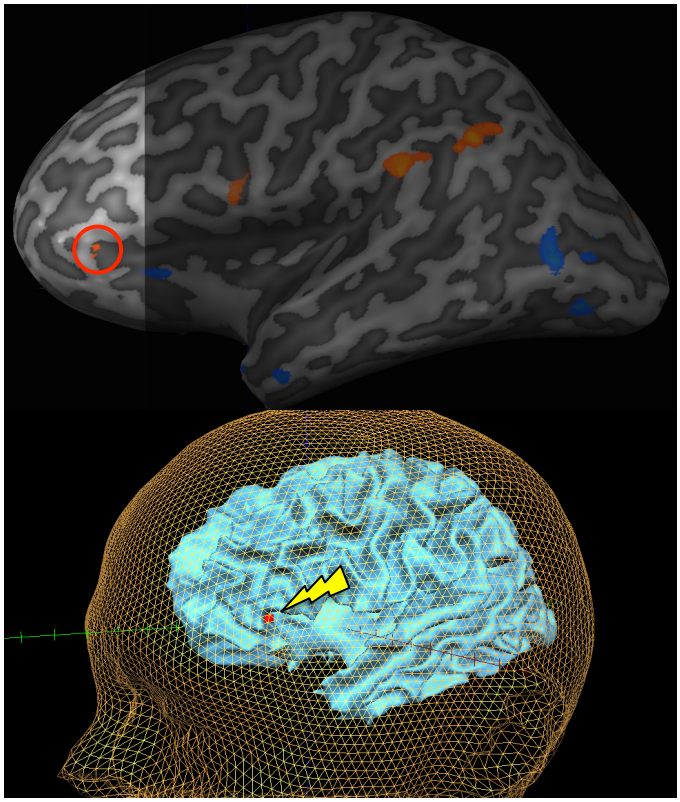

Figure 17. This image shows the spot located on the left lateral prefrontal cortex that would have been stimulated in the main experiment. The neuronavigation system allowed us to locate the correct spot over the participant scalp.

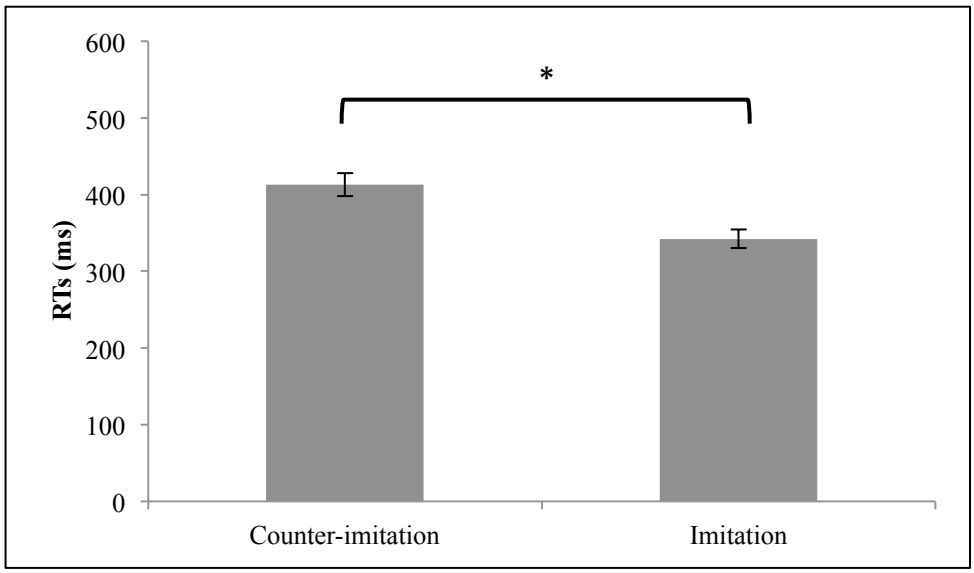

Figure 18. Reaction times (milliseconds) in the localizer showed a significant difference ($p<0.00001$) between the counter-imitation and imitation tasks.

Our main interest was to find the regions of interaction between the type of stimulation (TMS factor) and the kind of task (TASK factor). Thus we performed the interaction in the whole brain with a random effect analysis. As hypothesized we found a significant interaction TMS*TASK ($F(1,15)$, $p_{uncorrected}<0.001$) in the left dorsal premotor cortex (PMd) (talairach coordinates [-29, 23, 49]). Moreover we found an interaction in the portion of the supramarginal gyrus belonging to the BA 40 (talairach coordinates [-59, -18, 13]. It is to be noted the bilateral interaction in the posterior part of superior temporal sulcus (pSTS) (talairach coordinates [-52, -44, 9], [50, -43, 14]) and also the interaction in the right posterior portion of the insula (talairach coordinates [36, -27, 16]) (Figure 20) The beta values are shown in figure 21.

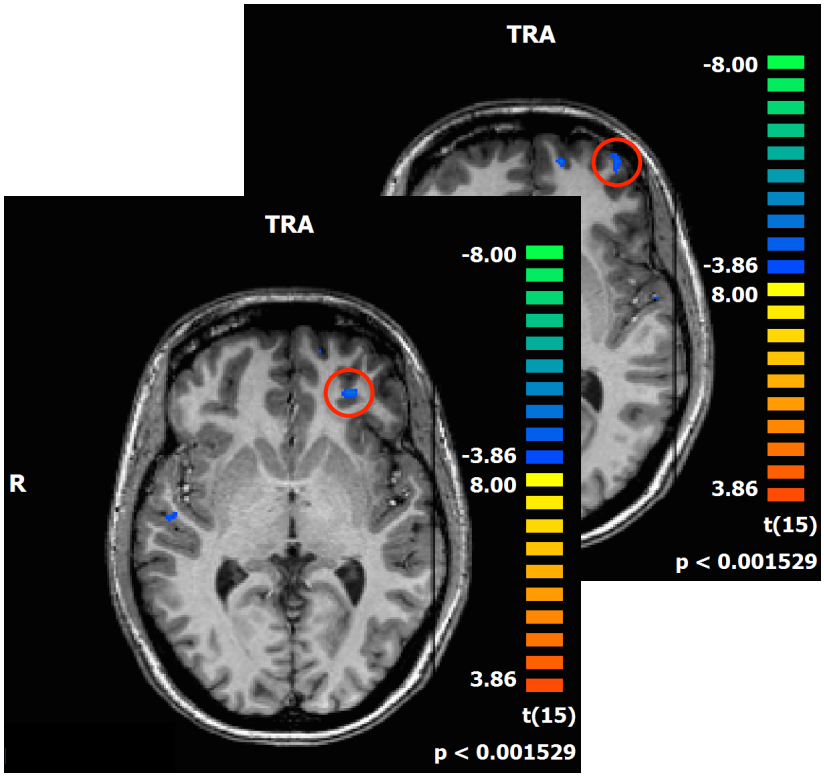

Figure 19. In this images it is shown the decreasing in the BOLD signal (t(15), puncorrected<0.001) in the projection of the stimulated area and in a more anterior portion of the frontal lobe.

Reaction times were analysed in a 2x2 repeated-measures ANOVA, with TMS (real TMS, sham TMS) and TASK (counter-imitation, imitation) as factors.

We failed to find a significant interaction between TMS and TASK ($F(1,15)= 0.14$, $p<0.71$), instead we found a very significant effect of the TASK ($F(1,15)=107,5$, $p<0.000$) as shown in figure 22.

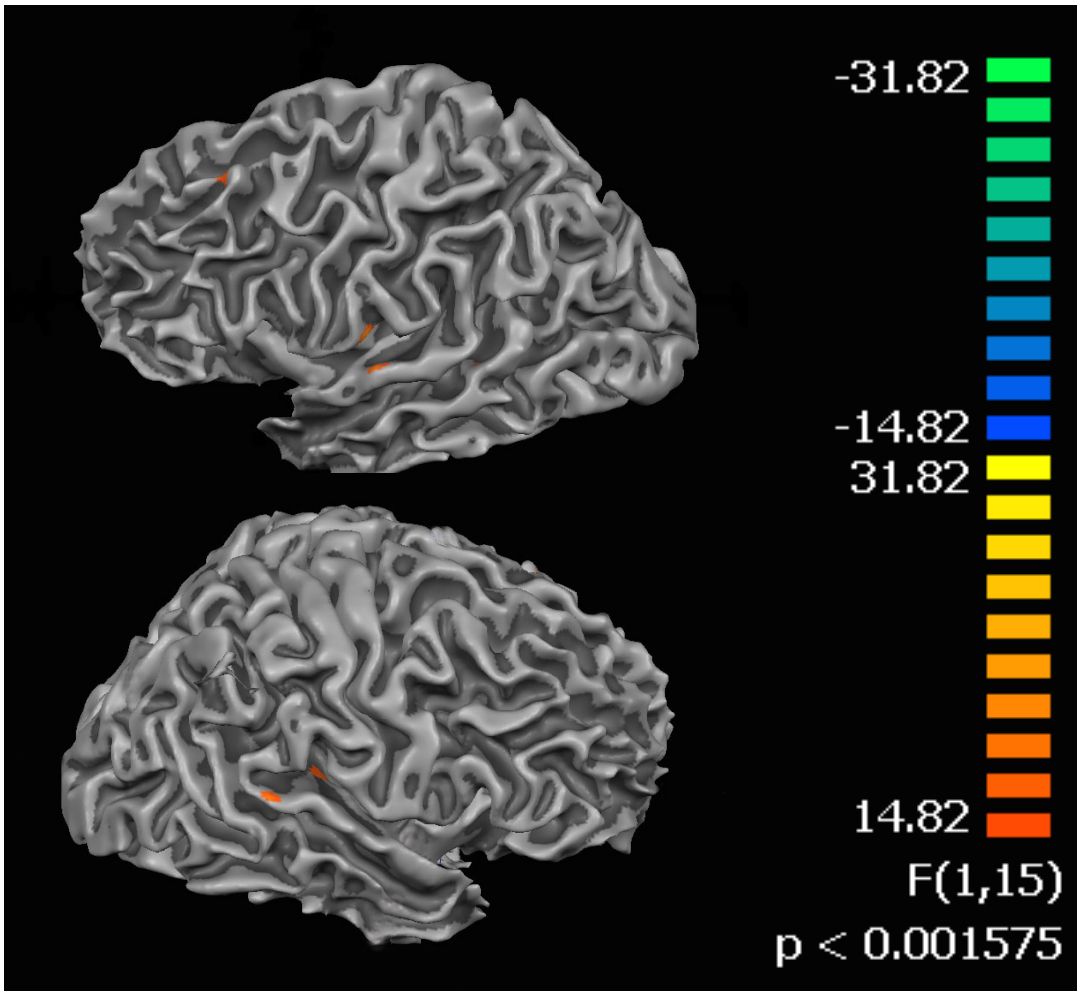

Figure 20. This image shows the spots of interaction. In the upper part is shown the left hemisphere with the areas PMd, the supramarginal gyrus and pSTS. In the lower part is shown the right hemisphere with the activations of pSTS and the posterior portion of the insular cortex.

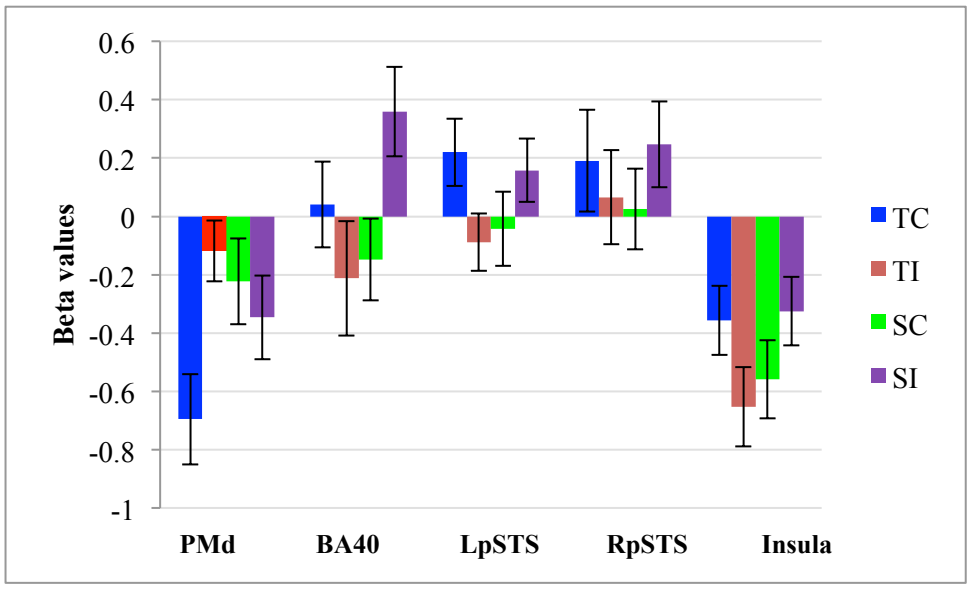

Figure 21. The data represent the beta-weights obtained from the areas that resulted significant in the interaction. ($p_{uncorrected}$<0,001). TC is real TMS before counter-imitation task, TI is real TMS before imitation task, SC and SI are respectively counter-imitation and imitation tasks in sham condition.

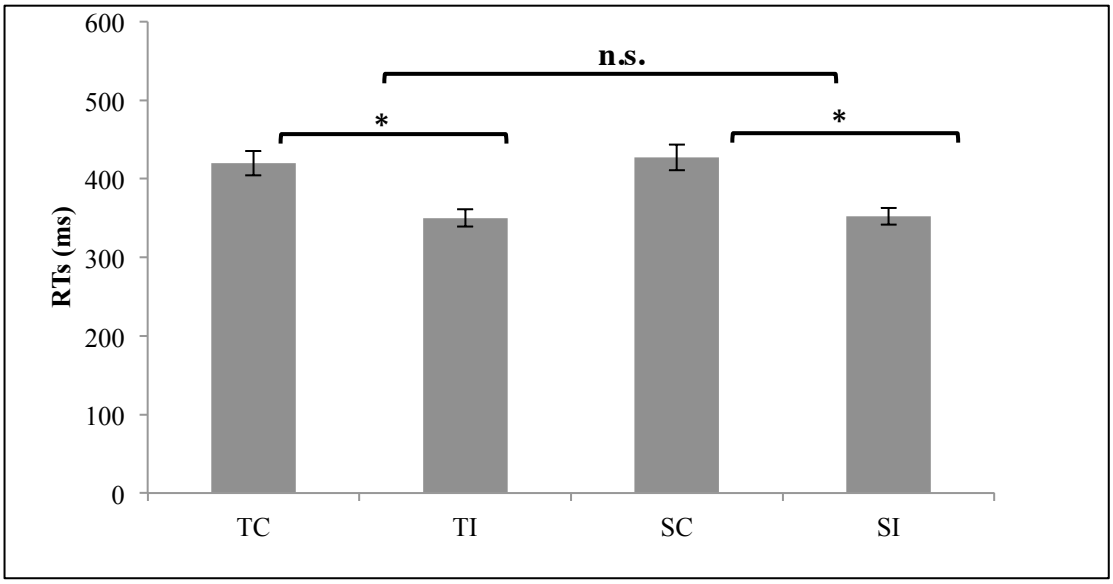

Figure 22. Reaction times (milliseconds) in the main experiment showed a significant difference (F(1,15)=107,5, p<0.000) between the counter-imitation and imitation tasks in both real TMS and sham TMS conditions. We did not find any significant interaction. TC is real TMS before counter-imitation task, TI is real TMS before imitation task, SC and SI are respectively counter-imitation and imitation tasks in sham condition.

4.4 Discussion

In the present study we replicate the results of the previous study (Ubaldi et al., 2013) in which we demonstrated the central importance of the lateral prefrontal cortex in the rule-dependent visuo-motor interactions. Indeed in the localizer, using a simple task with different instructions (rule-dependent or stimulus-dependent), we found a spot in the prefrontal cortex comparable to the one stimulated in the previous study. Moreover the slowing down of the reaction times in the counterimitative condition of both the experiments demonstrated the engaging of a non-imitative slower system that allows a person to perform correctly an arbitrary rule-dependent task, while a different faster imitative system is engaged automatically.

In the main experiment we tried to locate a nodal area where the two pathways, stimulus-dependent parieto-premotor (Rizzolatti & Sinigaglia, 2010) and the rule-dependent temporo-prefrontal systems, interact. We found, as hypothesised, a spot of interaction in the dorsal premotor cortex which represents the following step in the chain of neural connections that ultimately lead to the production of counter-imitative behavior.

Moreover we found other areas that are active in the interaction such as the supramarginal gyrus (rostral BA 40), which subregions are the homologue to the macaque area PF that is part of the mirror neuron system and active in humans during imitation. The supramarginal gyrus is also involved in the observation of tool use (Rizzolatti & Sinigaglia, 2010; Peeters et al., 2009).

It is interesting to notice the strong activation of the posterior portion of the insula and in particular the deactivation of it as one of the main effects of TMS. Since this portion of the insular cortex as a fundamental role in human pain (Segerdahl et al., 2015), this could indicate a rebound activity after the trains of real TMS, which are slightly more painful than the sham stimulation.

The absence of a significant interaction in the reaction times demonstrated that the TMS did not influence the performance of the participants, but the performance differed only in respect to the different kind of tasks.

Chapter 5 – Conclusions

My thesis aims at demonstrating the existence of two parallel networks that are involved in stimulus-dependent and rule-dependent visuomotor associations.

In the first experiment (Chapter 3) we demonstrated a biphasic time-course of motor cortical excitability during a counter-imitative task: a stimulus-dependent automatic simulation at 150 ms from the stimulus onset and a rule-dependent voluntary motor preparation at 300 ms. Moreover we identified two regions involved in this biphasic pattern by means of the repetitive transcranial magnetic stimulation (rTMS). The double-dissociation between the effects of offline rTMS to the lateral prefrontal and parietal cortices, on the early and late components, allowed us to hypothesize the presence of two different anatomo-functional pathways: a parieto-(premotor) network mediating early stimulus-dependent responses and a (temporo)-prefrontal network producing late rule-dependent responses.

It is worth putting these data in the perspective of current knowledge of stimulus-dependent and rule-dependent processes controlling the production of actions in other domains, where a biphasic time-course of visuomotor behaviour has been described. In the case of object affordance, when implementing a visuomotor rule, which conflicts with the affordance of an object, an early automatic stimulus-dependent response mapping occurs, between 100 and 200 ms from stimulus onset (Goslin et al. 2012). In this context also partial errors are frequently produced (McBride, Sumner, et al. 2012), consisting in an early tendency to produce an automatic motor response, later substituted by the rule-dependent response, which

further suggest the occurrence of a fast automatic affordance-driven response and a subsequent slower task-driven motor response. In tasks in which spatial stimulus-response conflicts are present (Simon task), automatic stimulus-dependent responses measured with EMG appear around 200 ms and disappear around 300 ms from stimulus onset (Burle et al. 2002; Hasbroucq et al. 2009). A recent study investigated the time-course of MEP modulation during the performance of an Eriksen flanker task and found for incongruent trials, a time-course of MEP amplitude changes strikingly similar to the ones presented here (Michelet et al. 2010), with stimulus-dependent responses appearing between 160 and 240 ms after stimulus onset. Also in the case of visual search tasks, it was found that independent stimulus-dependent and goal-driven mechanisms coexist but with different timing. Saccades produced in an early time-window between 150 and 250 ms are more frequently directed toward a distracter rather than toward the arbitrary target (Muller and Rabbitt 1989; van Zoest and Donk 2006; Siebold et al. 2011).

These data, taken together with our findings, indicate that the relative timing between early stimulus-dependent and late goal-driven responses is robustly preserved across domains in the visual modality, with small task-dependent variations in the absolute timing of the early responses.

Thus, the first experiment adds important information on the mechanisms by which the brain is both tuned to produce imitative responses in a fast automatic way but is also capable of overriding them by means of a parallel, more flexible, visuomotor coupling that follows arbitrary visuomotor associations. The fast imitative tendencies seemingly persist also during the online performance of a

counter-imitative task. The two processes access the motor output by two partially independent neural substrates.

We took a step forward in the second experiment (Chapter 4) where we investigated, by means of the combined rTMS and fMRI techniques, the functional connectivity in the rule-dependent executive pathway. We used the condition-and-map approach to highlight the brain regions where this pathway passes through to give a counter-imitative response. We hypothesised that this region could be the premotor cortex, and probably the dorsal part of it. Our data showed that exactly in the dorsal premotor cortex there is an interaction between the TMS stimulations and the tasks.

Taken together these results allowed us to add a new piece in the puzzle of the rule-dependent visuomotor pathway as illustrated in Figure 23. The further step will be to use the same paradigm to test the stimulus-dependent automatic pathway and finally to test where these two systems converge either in the premotor cortex or in the motor cortex.

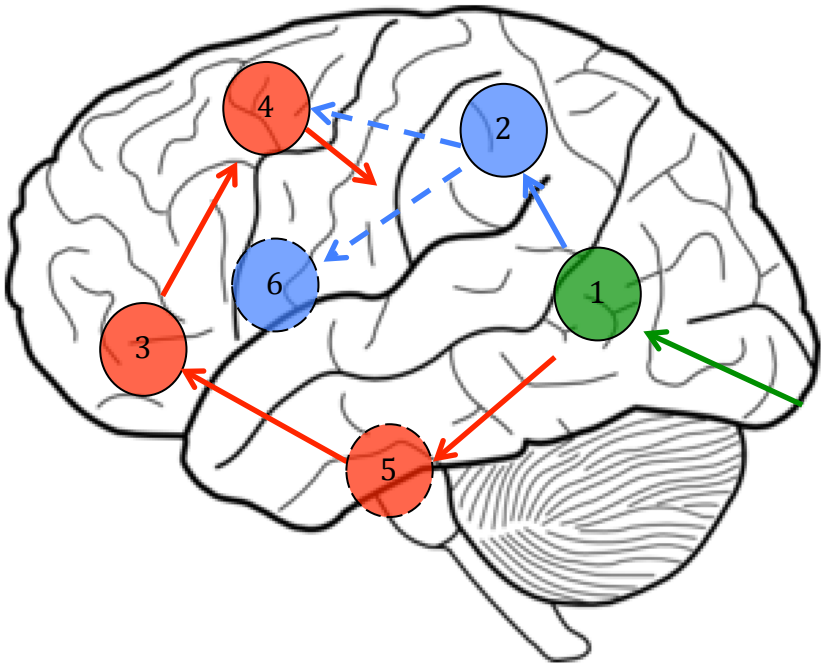

Figure 23. The figure illustrates the two parallel pathways hypothesised in this thesis and the areas that we identified to be involved in these systems (in solid lines): the posterior part of the superior temporal sulcus (1), the lateral prefrontal cortex (3), the anterior intraparietal sulcus (2) and the dorsal premotor cortex (4). In dashed lines and circles the two hypothesised further steps of the stimulus-dependent automatic way from the anterior intraparietal sulcus to ventral premotor cortex (6) or to the dorsal premotor cortex. It is likely that the rule-dependent pathway passes through another step in the temporal sulcus (5).

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