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THE ROLE OF ATTENTION IN MOTOR RESONANCE

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ABSTRACT

Motor pathways are activated not only during the obvious task of producing voluntary movement, but also during observation of actions performed by others. Execution and observation share a common pattern of activation, so that a subliminal "motor resonance" (MR) response is evoked, in primary motor cortex and spinal circuits, which reflects the specific motor program encoding the observed actions. There is growing evidence that MR is mediated by a parieto-frontal neural network, called the “Action Observation Network” (AON). While often described as an automatic and cognitively unmediated response, there is some experimental evidence suggesting that MR can in fact be modulated by cognitive processes. In this thesis the role of attention during the observation of a grasping action in both central and peripheral vision was investigated in three different studies.

In the first study, the level of attention that 56 subjects allocated to the observation of a cyclic flexion-extension hand movement was manipulated in four different experimental conditions. MR was measured as the excitability modulation of spinal motoneurones innervating a wrist flexor muscle (flexor carpi radialis), utilizing the H-reflex technique. In the first experiment (*explicit observation*) 14 subjects were asked to pay attention exclusively to the cyclic oscillatory movement of a hand. In the second experiment (*semi-implicit observation*) the attention of 14 different subjects was partly diverted from the hand movement, since they needed to monitor hand position in order to perform a parallel task. In the third experiment (*implicit observation*) 14 different subjects had to complete yet a different parallel task for which the hand movement was totally irrelevant. The modulation of H-reflex amplitude, i.e. of the MR response, in these experimental conditions was compared to a baseline condition, in which 14 new subjects observed the cyclic oscillatory movement of a mechanical device, which does not evoke any motor resonant response. Results show that attention manipulation in both second and third

experiments dramatically decreased the amplitude of the MR response, while not affecting its muscular and temporal specificity. These results support the hypothesis that MR response is not a fully automatic process, but can be modulated by top-down influences, such as selective attention.

In the second study, MR was investigated during observation of actions viewed in the peripheral field, where vision is far less accurate and where they don't automatically receive the same level of attention as in central vision. The excitability modulation of motor pathways was recorded, this time utilizing the Transcranial Magnetic Stimulation (TMS) technique, in 40 subjects who were asked to pay attention to a central fixation point on a screen while a hand grasping action was shown at 10° in their peripheral field of vision. TMS was selected for these experiments because it allows recording from more than one muscle simultaneously. Half of the subjects observed a video clip of a "natural" motor sequence (a hand grasping a red ball), and the other half observed an "impossible" version of the same grasping (a ball being grasped by flexing fingers towards the back of the hand). The presentation of an impossible movement allows us to define, through a questionnaire, to what extent subjects are able to perceive the kinematic aspects of the observed action. Motor-evoked potentials (MEPs) were elicited in the right OP and ADM muscles by stimulation of the left primary motor cortex, at different delays during the observed actions. Results show, first of all, that actions in near peripheral vision are effective in eliciting MR, but that the MR response is rough and inaccurate, compared to the response to the same actions viewed in central vision, because it does not reflect the motor program encoding the observed action. It is reasonable to hypothesize that due to their limited kinematic accuracy, these subliminal motor responses may provide information about the general aspects of observed actions, rather than specific motor information regarding the spatial and temporal activation of muscles during the observed action.

Finally, in the third study the role of attention in shaping MR responses was investigated by manipulating the attention of 64 subjects during the observation of the same peripheral grasping action used in the second study. MEPs were recorded in OP and ADM muscles of their resting right hand, at different delays during the observation of the video. In the first experiment, 29 subjects were asked to maintain their gaze on a fixation point but to pay (covert) attention to the video shown in periphery. The results of the experiment show a motor facilitation only during the observation of the natural grasping and only in the OP muscle. In order to explain the different results for the observation of natural and impossible movements two more experiments were carried out. Specifically, in order to test the hypothesis that selective attention could have played a role in focusing motor facilitation only on the OP muscle, in the second experiment 16 subjects were asked to observe the video of the natural grasping in central vision, while explicitly focusing their attention only on the thumb of the moving hand. Results show a strong effect of selective attention even in central vision since motor facilitation appears only in OP muscle. Finally, in the third experiment the hypothesis that the lack of modulation recorded during the observation of the impossible movement might be due to the high cognitive demand of perceptual decoding was examined. To this aim, the impossible condition of experiment 1 was replicated, but in this case 19 subjects were allowed to see the exact kinematics of the impossible movement before the beginning of the experiment. The idea being that previous knowledge of the impossible action would decrease uncertainty and complexity in the decoding process, freeing resources needed for the development of MR during observation in peripheral vision. Results show that observation of the impossible grasping action in covert attention condition, after previous familiarization, restores normal facilitation also in the ADM muscle. Altogether these findings suggest that a minimal level of attention is sufficient for the development of MR responses with correct temporal and muscular specificity, but that the gain of the process can be modulated by selective attention. Moreover, these data indicate that responses can be completely inhibited when the decoding of the action requires demanding inferential processes, suggesting that MR is not a completely automatic, bottom-up process, but requires the deployment of sufficient attentive resources supporting cognitive processing of visuo-motor transformation.

CHAPTER 1

MOTOR RESONANT RESPONSE BETWEEN TOP-DOWN AND BOTTOM-UP PROCESSES

1.1 **The motor resonance response**

Motor activity is usually described as a serial process in which an idea (the representation of a movement), often following a visual percept (an apple on a table), is converted by the motor system in a series of motor acts aimed at interacting with the environment (take the apple and eat it). This view has traditionally led to thinking of the motor system as a mere executor and the perceptual system as a mere representer of the external world. This vision has been dramatically challenged in the last 25 years it by different scientific evidences showing that perception and action (with their neural substrates) are closely linked and that this link is particularly evident when the object of the perceptual process is a particular kind of stimulus: actions performed by others. In 1890 James wrote “[..] every mental representation of a movement awakens to some degree the actual movement which is its object[..]” meaning that every time we are experiencing an external (e.g. perceived) or internal (e.g. imagined) action, the mental representation of the corresponding movement is triggered.

More recently this principle has been reclaimed in order to interpret the activation of a specific population of motor neurons in the monkey brain, discovered 25 years ago by Giuseppe di Pellegrino and colleagues: the so-called mirror neurons. In their experiment, utilizing a single unit recording technique, they observed that when a monkey passively observed an experimenter grasping a piece of food, the same neurons in the ventral premotor cortex (F5) fired as when the monkey performed that movement (Di Pellegrino et al., 1992; for a review see Casile, 2013). In other words such visuomotor neurons are active both when the monkey performs goal-directed hand actions and when it observes other individuals performing similar actions (Rizzolatti et al. 1996). Paralleling in neural terms the ideomotor principle proposed by James, Rizzolatti and Craighero (2004) described this motor mirroring in this way: “Each time an individual sees an action done by another individual, neurons that represent that action are activated in the motor cortex. This automatically induced motor representation of the observed action corresponds to what is spontaneously generated during active action[..]”. Neurons with the same characteristics as those in area F5 were also found in the inferior parietal lobule (IPL) of monkeys (Gallese, 2002; for a review see Casile, 2013). Due the fact that these areas are part of the parietofrontal network that organizes action execution, it has been suggested that the function of such activation could be to map the observed action onto the observer’s motor repertoire, to be understood from an inner point of view: we could understand the meaning of the action that we observe because we “replicate” the same action in our motor system (Rizzolatti et al., 2014). The revolutionary idea behind this interpretation is that areas, that until then had been described as merely executive because their activity leads to movement, were the same utilized (though not exclusively) in the perception of the movement of others. For example, although the first evidences showed that in F5 and IPL there are neurons which fire both during action execution and action perception, it was not clear whether the neurons activated during action observation were the same which send the motor command toward spinal paths. Recently this question was addressed in non-human primates (Vigneswaran et al. 2013; Kraskov et al. 2009, 2014) in single unit recordings showing that pyramidal tract neurons in F5 area directly involved in activation of hand muscles during grasping action show significant modulation of their activity while monkeys observe a grasping carried out by an experimenter. Direct evidence of the existence of mirror neurons in humans is still missing; however, there are many experiments showing that the observation of actions subliminally (i.e. without overt movement) activates the motor system, presumably resulting from the activation of a similar mechanism. Most authors refer to this process using the term "*motor resonance*", which highlights the attuning process of the neural activity in the observer's motor systems to that of the mover, and to the neural network underlying the process using the term Action Observation Network (AON). Both terms remain neutral as far as function and homology with non-human primates, and are adopted also in this thesis. A series of experimental data available in the literature about the human motor resonant response will be described below.

1.1.1 *Behavioural evidence: the automatic imitation effect*

One of the most cited behavioral evidence of a motor facilitation during action observation is the *automatic imitation* effect (for a review see Heyes et al., 2011): different experiments on imitation suggest that observed gestures automatically activate motor representations normally involved in the execution of those actions (Stürmer et al. 2000; Brass et al., 2000; Brass et al., 2001; Craighero et al. 2002; Kilner et al. 2003; Vogt et al. 2003). In this kind of experiments participants typically have to perform a standard movement when a trigger-movement is shown on a screen. Usually they are faster and/or more accurate when the trigger-movement is congruent with the standard movement (e.g. the same movement) rather than incongruent (e.g. a different movement). Interestingly, some studies show that such *automatic imitation effect (*or *visuomotor priming)* is also recorded when the observed movement is irrelevant to complete a different task. For example Stürmer et al. (2000) asked subjects to open or close their hand when the color of an observed hand changed, irrespective of whether the observed hand was opening or closing; crucially, when the movement of the observed hand was congruent with the requested movement, subjects were faster compared to the incongruent condition, i.e. attending to color in order to make a decision to move did not seem to interfere with the automatic imitation facilitation. In another study, Brass et al. (2000) examined the automatic imitation effect utilizing a paradigm with lifting movements of the index and middle fingers (Fig. 1.1).

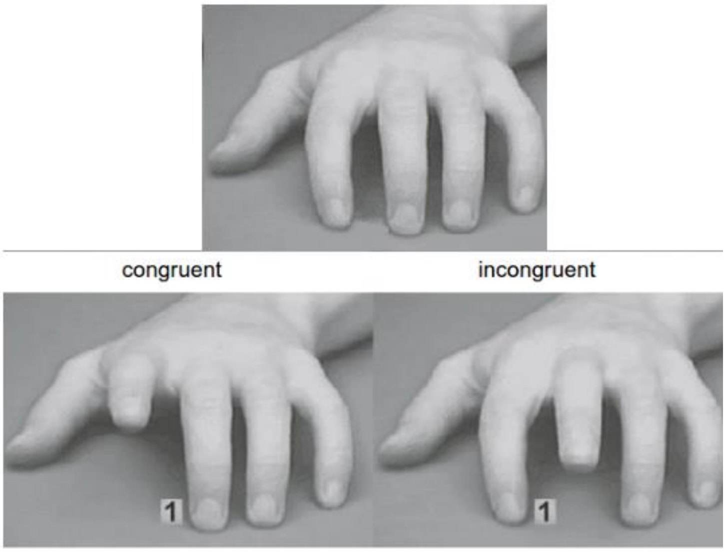

Fig. 1.1 Experimental stimuli used in Brass et al. 2000. Participants first saw a hand (upper panel) with fingers bent downward and each fingertip resting on a table. Shortly afterwards, a number (1 or 2) appeared between the fingers, indicating to participants that they were required to lift their index finger (number 1) or their middle finger (number 2). Appearance of the number coincided with lifting of either the index or the middle finger of the stimulus hand.

In each trial, participants first saw a hand with fingers bent downward and each fingertip resting on a table. Shortly afterwards, a number (1 or 2) appeared between the fingers, indicating to participants that they were required to lift their index finger (number 1) or their middle finger (number 2). In baseline trials, the stimulus hand did not move when the number was presented. In all other trials, the appearance of the number coincided with lifting of either the index or the middle finger of the stimulus hand. Results showed that responses were faster when the stimulus action was the same as in the correct response (compatible trials, e.g. index finger stimulus and index finger response) than in baseline trials, and responses in baseline trials were faster than when the stimulus action differed from the correct response (incompatible trials, e.g. middle finger stimulus and index finger response). This suggested that "movement observation exerts an automatic influence on movement execution" (Brass et al., 2000) and that this automatic influence both facilitates responding in compatible trials and interferes with responding in incompatible trials. The automatic imitation effect is thought to be mediated by the AON, which would sustain the subliminal activation of the motor pathways encoding the observed action (motor resonance), facilitating the repetition of the observed movement and, conversely, interfering with the execution of movements different from the observed one (Kilner et al., 2003; Press, Bird, Flach, & Heyes, 2005; van Schie, van Waterschoot, & Bekkering, 2008;Catmur, Walsh, & Heyes, 2009).

1.1.2 *Neurophysiological evidences: the action observation network*

Evidence of motor system activity during action observation in humans comes from a series of neuroimaging studies using functional magnetic resonance (fMRI), positron emission tomography (PET), and Magnetoencephalography (MEG) showing that the cortical areas active during action observation overlap with those that are active during execution of the same class of actions. These studies have contributed to the in-depth description of the areas forming the parietofrontal network called AON (Fig. 1.2), which includes the inferior frontal gyrus (Iacoboni et al., 1999; Kilner et al., 2009), the ventral and dorsal premotor cortex (Buccino et al., 2004; Gazzola et al., 2007), the inferior parietal lobe (Grèzes et al., 2003; Aziz-Zadeh et al., 2006), the anterior intraparietal sulcus (Shmuelof & Zohary, 2006 ; Dinstein et al., 2007), and the superior temporal sulcus (Gazzola et al., 2006). These data indicate that whenever a mental representation of an action is evoked by the perception of another acting agent, specific areas of the brain system normally subserving active movement is consistently activated. Several hypotheses have been proposed about the role of this fronto-parietal network during observation of actions. To date the most commonly accepted suggest that the AON could play a role in facilitating action understanding and empathic processes, allowing subjects to accesses the experience of others directly through an internal simulation of their actions (Rizzolatti et al., 2005; Iacoboni et al., 2005; Gallese et al., 2005), however the experimental evidence supporting this ambitious hypothesis is still rather scant.

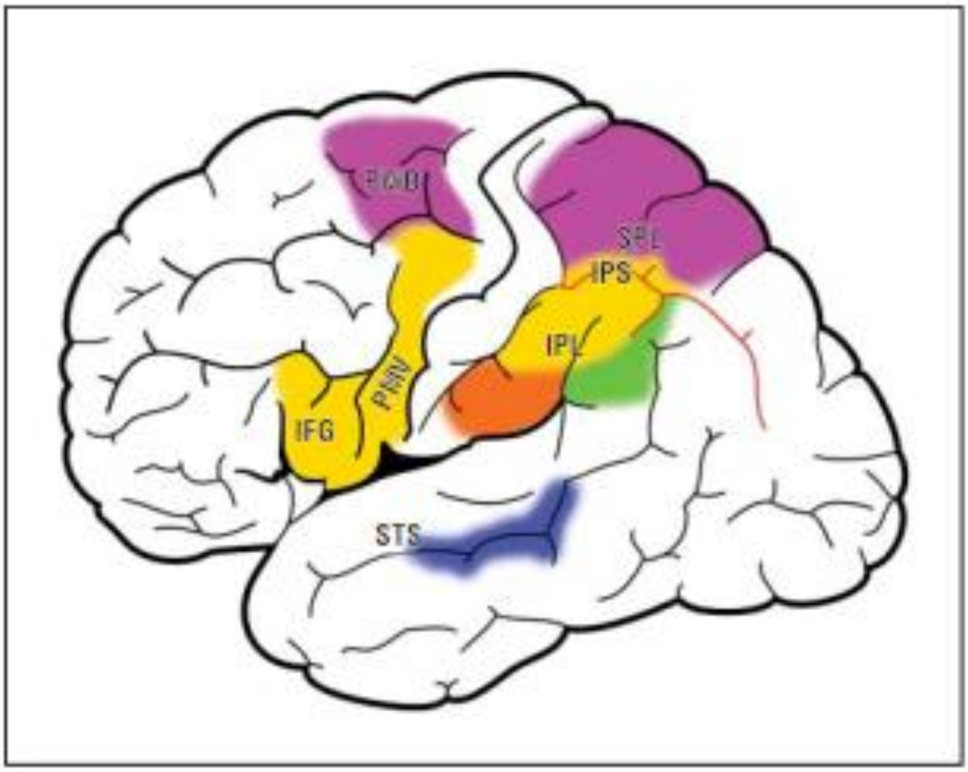

Fig. 1.2 Cortical areas related to perietofrontal mirror system responding to different types of motor acts (from Cattaneo & Rizzolatti, 2009). Yellow indicates transitive distal movements; purple reaching movement; orange, tool use; green, intransitive movement; blue portion of the superior temporal sulcus (STS) responding to observation of upper-limb movements. IFG indicates Inferior frontal gyrus; IPL, inferior parietal lobule; IPS, intraparietal sulcus: PMD, dorsal premotor cortex; PMV, ventral premotor cortex; and SPL, superior parietal lobule.

As can be seen from Figure 1.2, primary motor cortex is usually not described as part of the AON in neuroimaging studies, probably because its activation is rather weak, consistently with the lack of overt movement during action observation tasks. However, the fact that M1 is activated, though subliminally (without overt motor output), has been largely demonstrated. For example, in the Electro Encephalogram (EEG) the μ-rhythm, is suppressed during the observation of action (Muthukumaraswamy & Johnson, 2004; for a review see Pineda 2005); this is an oscillation with dominant frequencies in the 8 – 13 Hz and 15 – 25 Hz bands localized on sensorimotor cortex when the individual is at rest, which results suppressed during voluntary movement (Hari, 1997). The suppression of μ-rhythm during action observation is considered to reflect event-related desynchronization of the EEG induced by an activation of sensorimotor areas and is minimally affected by visual stimulation. Consistently Caetano & coll. (2007), by monitoring oscillatory MEG activity, demonstrate that cortical oscillatory activity at 20 Hz, typically originating in the primary motor cortex (M1) during movement, (Salmelin R, 1994; Gastaut H, 1952), shows an increase in amplitude irrespective of whether the same movement is performed, observed, or heard. Although these evidences suggest that primary motor areas, naturally active during action execution, are also affected by action observation, they do not allow to define which neural pool of M1 (innervating specific muscular groups) are more facilitated during action observation. In other words, they do not allow to conclude whether during action observation the observer's motor system is coding a specific motor program or is aspecifically facilitated.

An important question about the nature of motor resonance is to what extent the activation of the motor system during action observation is "replicating" (on-line) the observed movement, following the same muscular and temporal constraints as during the execution of the same action. To answer this question the use of more direct electrophysiological techniques, such as transcranial magnetic stimulation (TMS) or the H-reflex (see Appendix), can be very useful because they can measure the corticospinal (CS) excitability of motor pathways innervating specific muscles in a precise manner and with a temporal resolution adequate to the fast time-course of motor processes. For example, TMS used in conjunction with electromyography (EMG) can assess changes in corticospinal excitability in real time, monitoring which muscular groups (i.e. which neural pools of M1) are facilitated at specific moments during the observed action. Specifically, by stimulating the primary motor cortex (M1) with TMS it is possible to produce motor evoked potentials (MEPs) in the muscles controlled by the stimulated area (for example the portion of M1 which controls fingers of the hand); the amplitude of these MEPs (typically recorded transcutaneously using EMG) gives an index of the level of excitability of the stimulated portion of M1 and of the motor pathways controlled by it, with a timescale in the range of milliseconds. If action observation activates the AON, and in particular premotor areas, these in turn should increase excitability of M1 by virtue of the their strong anatomical and physiological connections (Cerri et al., 2003; Dum & Strick, 2005; Schmidlin et al., 2008; Boros et al., 2008), leading to larger MEPs. The first experiment in which this hypothesis was tested was a TMS study by Fadiga & coll. (1995), who found larger MEPs in muscles involved in grasping when participants viewed a grasping action, compared to when they saw a static object or dimming light. Since that experiment, many research groups have replicated similar results and added important new information using the same technique (for a review see Naish et al. 2014). Six years later Gangitano & coll. (2001) showed the temporal coupling between the changes in CS excitability and the dynamics of the observed action. They found that the amplitude of MEPs induced by TMS in the first dorsal interosseous (FDI), a muscle typically active during finger aperture, increased with the increase of finger aperture in the observed grasping action (Fig. 1.3).

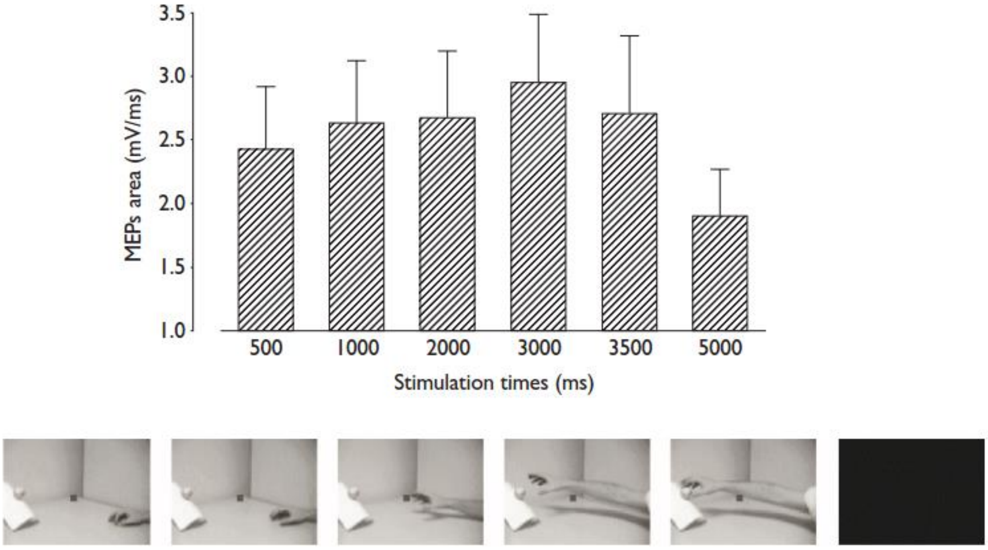

Fig. 1.3 Values of MEPs of FDI muscle collected by Gangitano & coll. (2001) at the different time-points of the observed action.

Specifically, MEP amplitude in the FDI was mainly facilitated at the point in time at which the observed hands were maximally opened, i.e., the point at which the FDI would be expected to be most active if the observer had done the same action. Using the H-reflex technique for assessment of CS excitability Borroni et al. (2005, 2008) showed that modulation of corticospinal excitability during action observation is tightly coupled to the entire progression of the observed movement. Specifically, they demonstrated that the excitability of motorneurones innervating wrist flexor muscles (FCR) was sinusoidally modulated by the observation of a sinusoidal flexion-extension movement of one hand (Fig.1.4) a phase relation between excitability modulation and observed movement was the same as between motor command and real movement during execution. All the above evidences support the idea that the motor resonant response is organized in accordance with the basic principles guiding motor areas during active motor programming

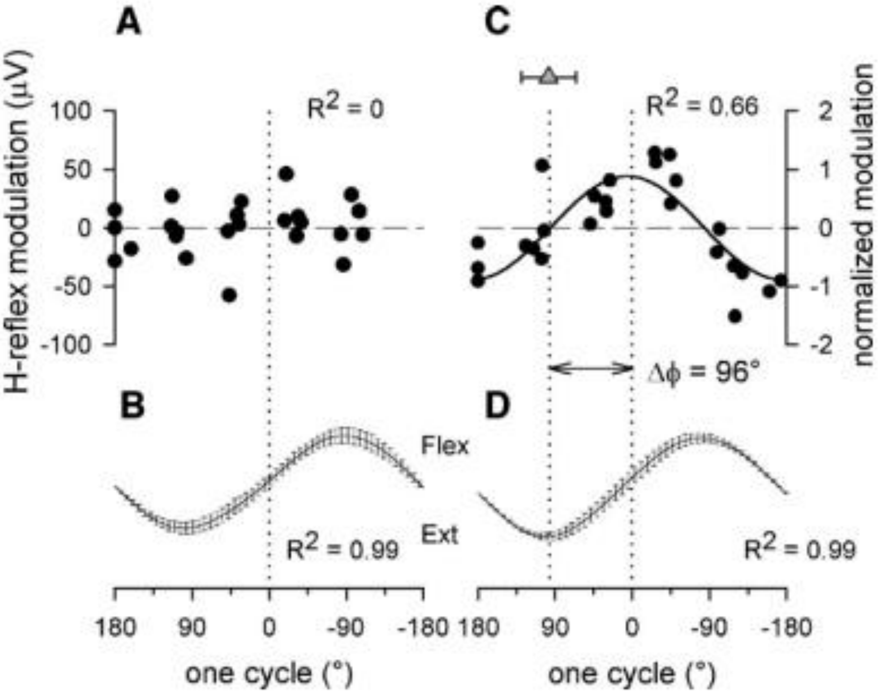

Fig. 1.4. H-reflex modulation recorded in FCR muscle by Borroni et al. (2005) during observation of one cycle of a hand flexion-extension movement. (A) H-reflex modulation (filled circles) in 5 subjects recorded at 5 delays during observation of the empty metal platform, could not be fitted by any sinusoidal function. (B) The movement of the empty platform. (C) H-reflex modulation (filled circles) in 5 subjects, recorded at 5 delays during observation of hand oscillations, instead was well fitted with a sine-wave function with the same period as of the movement of the observed hand (D).

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Consistently with the idea that the motor resonant response produces a faithful replica of the motor program encoding the observed movement, several other studies have shown that observation of a grasping action produces MEP facilitation of the same muscular groups and with the same time course as in the observed grasping (Gangitano et al., 2004; Press et al. 2011; Sartori et al. 2012; Cavallo et al. 2012, 2013; Mc Cabe et al. 2014). Borroni e coll. (2011), by using TMS/EMG combination, showed that the excitability modulation of the motor pathways to the opponent of the thumb (OP) and the abductor of the little finger (ADM) muscles during observation of a grasping reflected the activation of these muscles during the execution of the observed movement (Fig. 1.5): the OP was facilitated during the observation of fingers closing around the ball, corresponding in the natural movement to the thumb closing phase of the grasping action, while the ADM was facilitated during finger opening and extension, corresponding to the opening phase of the action.

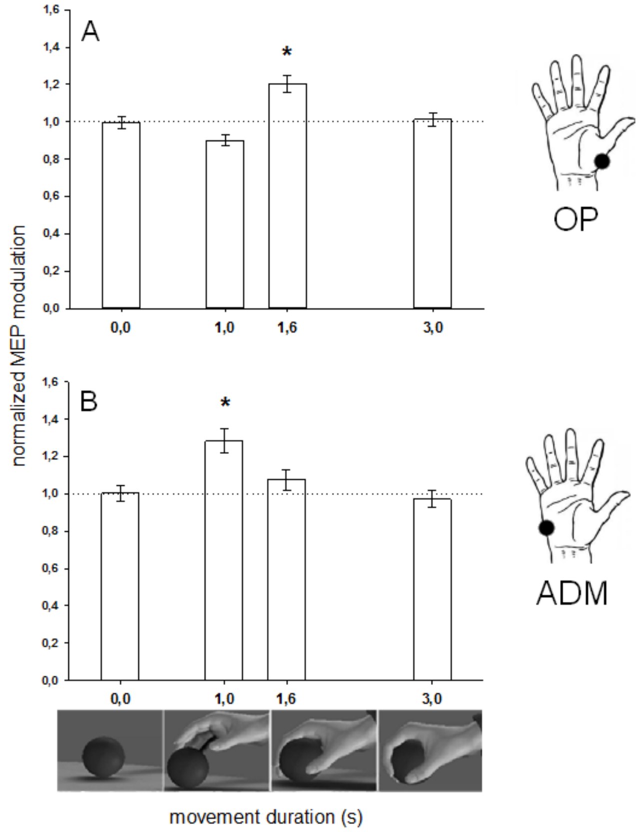

Fig. 1.5. MEP amplitude variations in the OP and ADM muscles (vertical bars, means ± SE) recorded at four selected delays (0, 1, 1.6 and 3 s) during the observation of a natural grasping action. The small figures on the bottom are the video frames illustrating positions of the hand at the four delays. OP MEPs (A) were significantly facilitated during the Grasping phase with respect to all other phases. ADM MEPs (B) were significantly facilitated in the Opening phase with respect to all other phases (from Borroni et al. 2011).

All these behavioral and neurophysiological evidences show clearly that our motor system is not indifferent to observation of others' movement. Moreover the activation of the primary motor cortex in a temporal/muscular specific manner, i.e., movement observation leads to highly muscle-specific subliminal replica of the observed action in M1. This supports the hypothesis that M1 also plays a functional role within the AON, by representing the observed movement in intrinsic, muscle-specific coordinates (Kilner & Frith, 2007). Although the function of such process is not fully-understood, some authors speculate that by encoding the kinematic aspects of an observed action, the specific subliminal activation of the primary motor cortex (M1) could facilitate its repetition as can be useful, for instance, during imitation for motor learning (Iacoboni et al., 1999; Mattar & Gribble 2005;Vogt et al. 2007)

1.2 **Effect of attentional processes in motor resonant response**

The motor resonant response is a robust and reproducible phenomenon which happens when subjects observe an action without any assignment to imitate or act during or after the action observation task. For this reason some authors suggest that this motor simulation occurs automatically and unconsciously (Gallese, 2001; Rizzolatti & Craighero, 2004), and this would suggest that it is not subject to interference from other concurrent cognitive processes. However, an important "limitation" of virtually all experimental studies, with respect to this suggestion, is that subjects are explicitly asked to pay full attention to the action they are observing. So in fact it is not known whether the motor resonant response would develop as automatically if subjects were not paying attention to it. Furthermore, these are visual and attentive conditions that don't exemplify typical natural situations of action observation: in daily life, people are often exposed to simultaneous actions, which cannot all be equally relevant or interesting to them or all viewed in central vision, and thus will not receive the same amount of their attentive resources. For this reason understanding whether and how attention and location of the observed action interact in producing motor resonant responses is a fundamental step in order to understand the nature of the process.

1.2.1 *Role of attention in visual perception*

In visual perception, ignoring irrelevant information which falls in our visual field allow us to attend to relevant information in a specific location: this process is named *visuospatial attention* and allows us to prioritize some regions in our visual field, at the expense of others. In the visuospatial attention literature the primary hypothesis for the existence of such a selective mechanism proposes that it is mainly due to energy limitations characterizing brain functioning. We can't pay attention to everything that appears in our visual field because our brain does not have enough metabolic power to process all these stimuli at the same time (Lennie, 2003). This view is suggested by a series of behavioral and neurophysiological studies.

For example, when attention is distributed over different locations of the visual field rather than being focused on only one site, the more regions are attended at the same time, the lower is the perceptual efficiency for each region (Castiello & Umiltà, 1990, 1992; Eriksen, 1990). These evidences are consistent with recent experiments studying the effects of distractors on primary visual tasks: in situations of low perceptual load (i.e. in which performing correctly the primary task requires a relative low attentive effort), attention resources 'spill over' to the perception of task-irrelevant distractors which then exert a distracting effect on the primary task. On the contrary, increasing the attentive demands necessary to execute the primary task, reduces distractor interference effects (Lavie, 2005). Neuroimaging studies show that neural activity in visual cortex associated with the perception of irrelevant distractors is lowered by the increase of the attentive load necessary to perform a primary task (Rees, 1997; Beck & Kastner, 2005,2007). Schwartz and coll. (2005) in a fMRI study assessing the activity of occipital areas during a visual task, asked subjects to monitor a central continuous rapid successive presentation of "T" symbols shown in a fixed central location with different orientations (upright or upside-down) and different colors in random order (Fig. 1.6). The low-load task required a response any time a specific color T appears, irrespective of its orientation; the high-load task (i.e. a more attention demanding condition) required a response to any upright yellow

T and upside-down green T. The central symbol stream (primary task) was sometimes accompanied by peripheral flickering checkerboard (distractors).

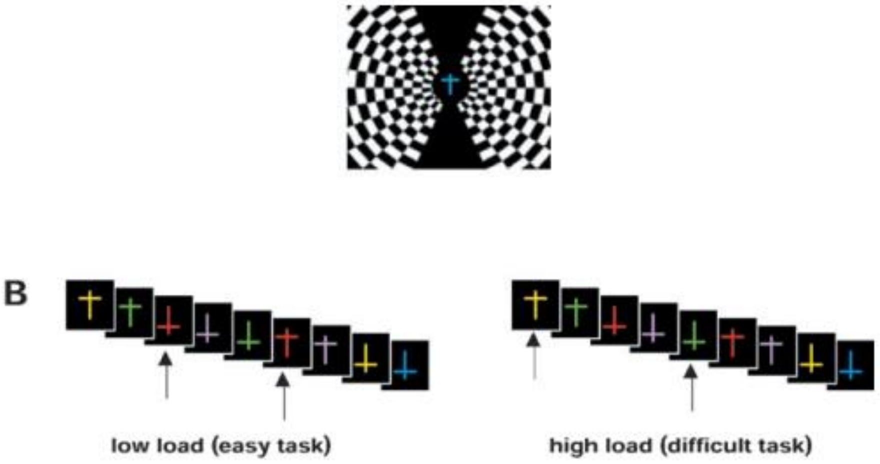

Fig. 1.6. Experimental paradigm used by Schwatz et al. (2005). The upper panel shows the screen showed to the subjects, with a “T” symbol to be fixated at the center and a flickering checkerboard in the peripheral field. The lower panel show the two different load condition: in the low load condition subjects had to identify all the red T; in the high load condition subjects had to identify all upright yellow T and upside-down green T.

The study found that increasing the attentive load of the primary task reduced the activity of visual areas related with the coding of peripheral visual stimuli (flickering checkerboard). These results are consistent with a limited capacity model of attentive process in which stimuli compete for limited resources (Treisman, 1960; Kinchla, 1992; for a review see Beck & Kastner, 2009).

Despite the fact that visuospatial attention is usually associated to gaze (*overt attention* in central vision), we can attend to an area of our visual field without actually directing our gaze toward it (*covert attention* in peripheral vision). For example we can look at somebody’s face while we are talking to him/her while paying attention to the facial expression of somebody else beside him/her. The importance of covert attention is evident in those daily situations in which monitoring the environment is necessary to our actions (objects searching, driving, crossing the street, playing sports etc…). Similarly to the experimental paradigms studying the role of distractor in a primary task described above, investigating covert attention necessitates that observers' eyes remain fixated at one location, while peripheral stimuli are shown in the peripheral field. However in this case subjects are asked to pay attention to peripheral targets, in order to study the effect of covert attention on the perception of peripheral stimuli. Numerous experimental evidences demonstrate that covert attention can be deployed through two different systems. S*ustained* (or endogenous) covert attention allows selecting information voluntarily at a given location in the peripheral field, while *transient* (or exogenous) covert attention is an involuntary system which automatically orients our attentive resources towards peripheral stimuli which occur suddenly or have particular salience. These two kinds of attentive systems are characterized by different temporal constraints: whereas it is possible to sustain endogenous attention at a given location for as long as is needed to perform a task, deployment of transient attention rises and decays quickly in about 100 ms (Hein, Rolke, & Ulrich, 2006; Ling & Carrasco, 2006). The existence of covert attention is demonstrated by different studies showing that when subjects are asked to perform a perceptual discrimination of peripheral stimuli their performance increases when they pay covert attention to the peripheral location at which the stimulus appears compared to when they don't. These evidences show that both endogenous and exogenous covert attentions improve visual performance (Carrasco, 2011; for reviews see Anton-Erxleben & Carrasco, 2013) in detection, discrimination and localization tasks, increasing contrast sensitivity and spatial resolution of peripheral stimuli. Interestingly, the increase of visual performance for attended areas matches with neurophysiological evidences that attention increases the blood oxygenation level-dependent (BOLD) responses in early visual areas which represent the attended spatial location (Li, 2009). Taken together these results show that both covert and overt attention enhance the neural metabolic efficiency of visual areas in order to code more accurately a stimulus inside the attended location.

1.2.2 *Role of attention in motor resonance response*

Different recent evidences are starting to suggest that motor resonant response can be affected by manipulation of visuospatial attention (Bach 2007; Chong et al

2009,2008). Bach et al. (2007) used a visuomotor priming task (see above Par.1.1.1.) in order to evaluate whether spatial attention has a role in motor facilitation of the hand or of the foot during action observation tasks. Interestingly, a priming effect was found only when the participants' spatial attention was directed toward the corresponding limb in the displayed image (Fig. 1.7), with faster foot responses when they payed attention to the sector of the image which contains the model's foot and faster hand responses when they pay attention to the image sector containing the model's hand, compared with conditions in which they were focused on the head sector. This study suggests that visuospatial attention is necessary to facilitate motor pathways to a given limb during observation of its movement: the motor system is able to resonate with a limb only when it is located in an attended area. Although in this experiment observed limbs facilitates the correspondent action only when they fall in the observer's attended section of the visual field (where the subjects are looking), what remains to be determined is whether selective mechanisms are capable of suppressing the visuomotor transformation of observed actions even when those actions appear at a spatially attended location (see the second experiment in Chapter 4).

Fig. 1.7. Examples of images presented to the subjects by Bach et al. 2007: subjects had to attend the colored dot while responding by the hand or the foot. The participants were instructed to press a foot key if the target was of one color, and a finger key if the target was of the other color.

Chong & coll. (2009) investigated this aspect using another attentive manipulation to study the effect of attention on visuomotor priming. Using a Go/NoGo paradigm they instructed subjects to respond to a go-signal by making only one of two prefixed movement (a precision or a whole hand grip). The 'Go' signal corresponded to the visual presentation of one of the two hand grip configurations (whole hand or precision) and alternated between blocks. Thus, in separate blocks, participants responded only to the precision grip or only to the whole-hand grip presented in the display (see Fig.1.8). Consistently with the typical visuomotor priming effect, when subjects were requested to respond with a whole hand grasping were faster when the Go signal was a whole hand grasping compared to the condition in which the Go signal was a precision grip. Conversely when subjects were requested to respond with a precision grip were faster when the Go signal was a precision grip. Interestingly, in another experimental condition, replicating the same experiment, participants had to respond to a different go-signal, that is to the color of a diamond superimposed on the same visual hand stimuli. Thus participants responded, in separate blocks, only to the red diamond or only to blue diamond (see Fig.1.8): the only difference between the two experiments was that in the "colored diamond" condition the type of observed grip was irrelevant to perform the task. While in the first experiment subjects had to pay attention to the type of observed grip in order to respond, in the second experiment the grip –though clearly visible- was totally useless and subjects had to pay close attention to the diamond color. The results show that visuomotor priming happens only when subjects attend to the details of the grip (and not to the color of the diamond), suggesting that visuomotor priming can be cancelled if attentive resources are diverted from the action, even when it falls in central vision.

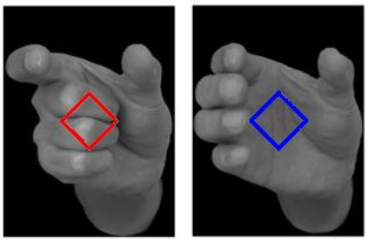

Fig. 1.8. The two different images showed to the subjects in Chong et al. 2009. They had to respond performing a whole hand or a precision (two fingers) grip. In the first condition they had to pay attention to the configuration of the hand; in the second condition they had to pay attention to the color of the diamond (from Chong et al. 2009).

To date evidences showing the effect of cognitive processes on AON are heterogeneous. PET studies for example (Decety et al., 1997; Grèzes, Costes, &

Decety, 1998, 1999) have shown that the activity of action observation areas depends on cognitive strategies used by the observers. Grèzes and coll. (1999) showed participants different kinds of hand gestures and asked half of them to observe these actions with no specific purpose, and the other half to observe the same movements with the intention to imitate them later. Results showed that areas that included the Inferior Parietal Lobule (IPL) and premotor cortices showed a greater activation when they had to imitate them, relative to the purposeless observation. In recent MEG study by Muthukumaraswamy and Singh (2008) subjects were asked to observe the same sequence of fingers to thumb flexions passively, without any purpose, or actively, either to imitate later or to sum the numbers corresponding to each moved digits (index finger = 1, middle finger = 2, ring finger = 3, and little finger = 4). The study found a beta desynchronization in the primary sensorimotor cortex in the imitation and math conditions compared with passive observation condition. Interestingly these data suggest that activation of AON recorded during action observation is enhanced when attention is particularly focused toward motor aspects of the action (e.g. focusing on which digit of the hand is moving). Perry et al. (2010) asked subjects to judge the intention, the emotion or the gender of a point-light displays of the same human biological motion in order to assess whether the EEG μ–rhythm suppression, a measure of AON activity (see Par. 1.1.2), depends on which aspect of a point-light display participants were asked to attend. They found a greater μ-suppression during intention assessment condition compared with condition in which subjects had to judge the emotion or the gender of the point-light moving silhouette. Similarly, Schuch et al. (2010) found that during observation of a reaching movement, i.e a hand reaching in different ways a cup with a colored symbol above it, a μ-suppression occurred when subjects had to attend the kind of reaching relative to when they had to attend the color of the symbol on the cup. These evidences show clearly that when subjects are observing a movement, the neural signal associated with activation of sensorimotor cortex can be modulated by cognitive manipulation. This suggests that sensorimotor response associated to AON activity during action observation is not a fully automatic and cognitive unmediated mechanism but depends on cognitive strategies used by the observers.

Woodruff and colleagues (2013) used a dual task paradigm to test the hypothesis that AON activity during action observation could be inhibited asking subjects to do a concurrent distraction task: in the first condition subjects had to normally focus their attention on the video of an index to thumb tapping action, in the second one subjects had to perform an imagery task (i.e. rotate letters) during the observation of the same action; in the third condition they had to perform a word-list generation task (i.e think of as many words as possible that began with either the letter K or R and verbally report the number of words generated at the end of the block). Results showed that in the latter condition (the more demanding secondary task) a μ-rhythm enhancement rather than suppression was recorded. This suggest that that μ-rhythm suppression could be totally eliminated if not enough cognitive resources remain available to code the observed action. Using a similar dual task approach, but different methodology (fMRI), Chong and coll. (2008) carried out a study to address the role of attention in AON activity. In this study subjects had to attend a central diamond and to judge the relative sizes of gaps that appeared on two sides of the diamond (Fig.1.9). The size difference between the two opposing gaps could be large, i.e. easy to detect (low attentional load) or small (high attentional load). Crucially a sequence of a grasping action was shown behind the diamond so that both diamond and grasping fall in central vision at the same time. Together with the gap discrimination task, subjects were asked to identify at the same time the type of grip showed in background (two-finger versus whole-hand). Interestingly fMRI results showed that activity of the frontal node of the AON (IFG) was reduced in the high load compared with the low load condition, suggesting that the coding of action by this area was inhibited by the concurrent non-motor task. Consistently, behavioral results showed that subjects were worse at discriminating the background stimulus (type of grasping) when they concurrently performed the high load relative to the low load gap discrimination task. These results suggest that the activity of a cortical area typically included in the AON is susceptible to be modulated by attention and specifically that a decrease in attention decreases the ability of the system to decode the observed action (Chong et al., 2008).

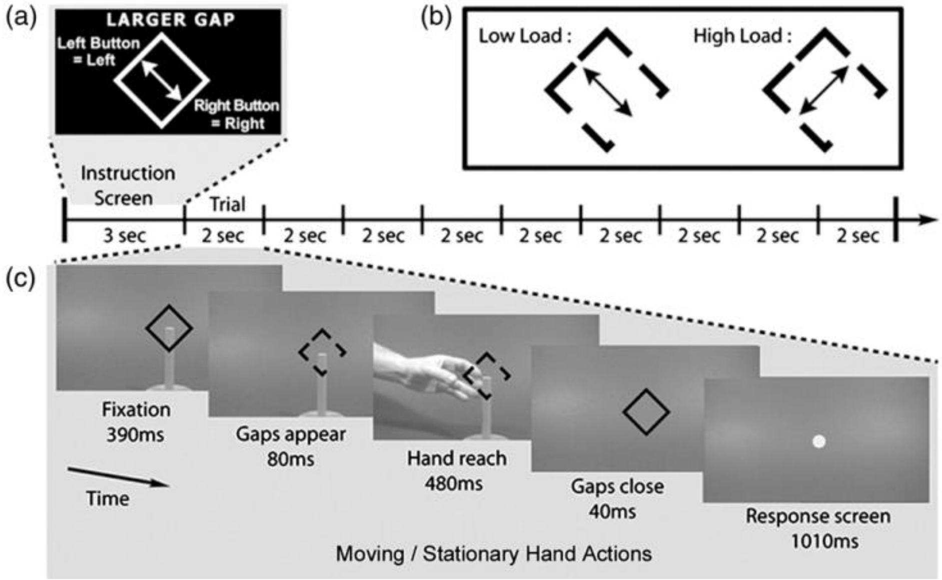

Fig. 1.9 Experimental paradigm used by Chong et al. (2008). (a) Participants had to attend the two opposite sides of a central diamond frame and to judge which gaps was larger (b) The relative difference could be large (low attentional load) or small (high attentional load). (c) During the gap estimation task a grasping action was shown behind the diamond and the subjects were also asked to identify the type of grip in background (whole hand or precision grip).

Taken altogether these studies describe a modulation of AON activity when action observation is performed with concurrent cognitive manipulation. Specifically, they show that the activation of the AON can be reduced by subtracting attentive resources from the coding of the observed action. However, as pointed out before BOLD, EEG, MEG, or PET cannot allow to describe accurately which pools of cortical motor neurons are activated during action observation or to correlate neural level of activity with quantifiable aspects of the motor resonance response. In Chapter 2 a series of experiments, in which the H-reflex technique is used to provide a more direct and quantitative measurement of the motor resonant response in condition of attentive manipulation, will be presented.

As already pointed out in most experimental studies on motor resonance subjects are put in visual settings that don’t exemplify the typical attentive conditions of daily life. In fact during common social interaction eye fixation is more frequently directed towards the face, and in particular the eyes (Birmingham et al., 2008), i.e. the actors’ limbs usually fall for the most of the time of the interaction in the observers’ peripheral field. In these conditions most actions we see in our daily experience are visually inaccurate because of the lower spatial sampling in the peripheral retina and of the reduced cortical representation of the peripheral visual field (Hubel and Wiesel 1968; Besharse & Bok, 2011). Despite this concern, experimental data in humans directly testing the possibility that actions located in the peripheral field of vision may evoke motor resonant responses are still lacking. In a study addressing the allocation of gaze in macaque monkeys observing actions performed by others, Maranesi & coll. (2013) showed that almost half of the recorded mirror neurons in area F5 (the homologue of the rostral part of the ventral premotor cortex in humans) are "gaze independent". In fact they fire during the observation of a grasping action (a man grasping a ball) irrespectively of whether the monkey is looking directly (in central vision) at the hand or at the face of the actor, implying that these neurons are activated by movement in the monkey's peripheral field (at locations >9° from the fixation point). Sartori & coll. (2009), in a study addressing whether social environment also affects the online control of action in humans, showed that a socially meaningful gesture, performed by a human agent in the peripheral visual field of experimental subjects, can perturb the trajectory of their executed grasping, indicating that actions seen in periphery can also affect the motor system of the observer; however, a more direct evidence is necessary to confirm the nature of the behavioral responses and to understand the physiological mechanism behind them. Specifically, three issues about the effect of actions seen in peripheral vision on the AON must be addressed. The first and foremost is understanding whether the observation of action in periphery indeed produces a motor resonant response and, if this is the case, how specific is the response. A second aspect to be understood is what is the role of attention in the process: since cortical magnification selectively boosts central vision (Wassle et al. 1990) and in peripheral vision visual acuity and phase discrimination are naturally decreased (Shapiro et al. 2011), the accuracy of visual information in peripheral action observation is expected to be much lower than in central observation. We could thus hypothesize that moving from central to peripheral vision, the degradation of the precision of motor resonance corresponds to a progressive loss of its replicative function, in favor of the progressive formation of a more contextual representation of the observed action rather than the precise representation of the action *per se*. These issues will be addressed in Chapter 3 and 4 with 2 studies which analyze whether and how the location of action in observers' visual field (peripheral or central vision) and the deployment of attentive resources interact with the development of the motor resonant response.

CHAPTER 2

THE EFFECT ON MOTOR RESONANT RESPONSE OF DIVERTING ATTENTION FROM OBSERVED ACTION

2.1 **INTRODUCTION**

Research in the field of motor control has revealed that, contrary to traditional views depicting the motor system as merely responsible for execution of movement conceived and planned elsewhere in the brain, it is actually involved in several cognitive processes. Evidence, ranging from the predictive nature of motor programming, to motor imagery, action perception and motor learning all contribute to the theoretical notion that cognitive processes are an intrinsic feature of the motor system (Epstein 1980; di Pellegrino et al., 1992; Jeannerod 1994; Wolpert et al. 1995; Decety et al.1988; Georgopoulos 2000; Gallese 2009; Blakemore & Sirigu 2013). Relevant to the present paper, many studies have demonstrated the important role of brain regions, associated with motor functions, in action perception. For example, the fronto-parietal action observation network (AON) has been found to increase activity when an observer views actions performed by others (Avenanti et al., 2013; for reviews see Rizzolatti & Sinigaglia 2010). Based on the fact that experimental observers are not in any way aware or in control of the activation of their motor pathways during the action observation tasks, it has usually been implicitly assumed that the AON is automatically recruited, without intervention of top-down control (Wilson & Knoblich, 2005; Bien et al., 2009).

Behavioral studies have also suggested that recruitment of the AON is an automatic process, showing priming and interference effects on movement execution induced by movement observation (Kilner et al 2003; Costantini et al. 2008). Such priming effects indicate that the effect of observation cannot be avoided even when irrelevant for the task at hand (Brass et al., 2000; Stürmer et al 2000). The distinction between automatic and controlled processes has been an important conceptual tool in the field of attention. Automatic processes are generally thought to be inflexible processes that are triggered involuntarily. Controlled processes, on the other hand, while being subject to the influence of automatic processes, require resources and voluntary cognitive control. Although automatic processes can affect controlled processes, the opposite, by definition, does not occur. Since automatic process require little to no resources (according to traditional views) they occur regardless of the current available resources (Cohen, et al., 1990; Bargh, 1992; Chong et al. 2009).

However, the question of automatic vs controlled activation of the AON need not be posed in mutually exclusive terms; similar to other perceptual processes (e.g. vision), the AON could be automatically recruited by the adequate stimulus in a bottom-up manner, and may be subject to modulation, due to available resources and deployment of top-down influences, such as attention (Kastner & Ungerleider 2000; Knudsen 2007). Usually, in action observation experiments, subjects are always overtly instructed to observe the actions shown or performed in front of them, so that their attention is always focused by default on the actions they are observing; often the experimental design includes procedures to control that attention is indeed maintained on the action observation task. Few studies have investigated the contribution of attention during action observation tasks (Bach et al., 2007; Chong et al. 2009; Gowen et al., 2010) or described the role of attention in shaping the activation of motor pathways when viewing actions performed by others, as measured by different electrophysiological and neuroimaging techniques (EEG: Woodruff et al., 2013; Perry et al., 2010; MEG: Muthukumaraswamy et al., 2008; fMRI: Chong et al., 2008). These studies have described a decrease in neural activity when action observation is disturbed by another cognitive load and have therefore concluded that the activation of the AON can be modulated by attention. But with the experimental techniques utilized it is impossible to interpret the results quantitatively. In other words, is the observed lower level of neural activity, measured by BOLD, EEG or MEG, a residual portion of the motor resonant response, which has been reduced by the decrease of cognitive resources available? Could this indicate that resonating with observed actions is automatic, but greatly enhanced by the deployment of attention? In order to answer these questions a more direct approach is necessary, adequate for quantifying the role of attention in modulating the motor resonant response.

To resolve the above questions, in the present study we utilize the H-reflex technique which provides a more direct and quantitative measurement of the motor resonant response, i.e. the activation of motor circuits in primary motor cortex (M1), generating a subliminal motor program that codes the observed action and descending to modulate the excitability of spinal motorneurons (Borroni et al. 2005; 2011). It is perhaps surprising to find that observation of others' actions, in absence of actual movement in the observer, is an effective stimulus in modulating the excitability of spinal motorneurons, given that they obviously do not receive direct visual input and are typically thought of as immediately concerned with the execution of movement. However recent elegant studies in the macaque monkey have described the activity of corticospinal mirror neurons, descending primary motor (F1) cortex, with either monosynaptic or interneuronal connections to spinal motorneurons innervating hand muscles (Vigneswaran et al. 2013; Kraskov et al. 2009, 2014); in addition, experimental evidence from our lab has demonstrated motor resonant responses in hand spinal circuits in humans (Borroni et al. 2005) driven by input from M1 (Borroni et al. 2008a,b). The evidence from these studies implies that primary motor cortex pyramidal neurons actively fire in response to action observation, but that their synaptic modulation of spinal motorneurons remains subliminal for movement execution. In the absence of actual movement, measuring variations in excitability of spinal motorneurons with the H-reflex technique amounts to measuring the activity of premotor and motor cortical input to the spinal cord, i.e. the result of the activation of these cortical areas by action observation. The advantage of the technique is that, while having the same high temporal resolution of other neurophysiological techniques, such as for example TMS, it samples the activity of motor circuits without magnetic (or electrical) stimulation of the cortex, providing an independent measurement of the same cortical phenomena while avoiding even minimal interference with cortical processing.

Specifically, the observation of a flexion-extension movement of the wrist will be utilized to describe the excitability modulation induced in the observer’s spinal and cortical motor pathways of a wrist flexor muscle (flexor carpi radialis, FCR). This is a simple intransitive movement (without a concrete goal, as instead object grasping): although motor resonant responses have been classically described in monkeys for goal directed actions, intransitive movements have also been shown to be effective stimuli for human observers (Iacoboni et al., 1999; Brass et al., 2000; Maeda et al., 2002; Catmur et al., 2007; Gallese 2014; Cross & Iacoboni 2014). Studies on primary motor cortex (M1) and spinal circuits have shown that the pattern of subliminal facilitation elicited in observers’ motor pathways reveals the activation of the same muscular groups that would be used to perform the observed action and that such activation is time-locked to the time course of the observed action (Fadiga et al., 1995; Gangitano et al., 2001, 2004; Borroni et al., 2005; Montagna et al., 2005; Cavallo et al., 2012; Sartori et al., 2012; McCabe et al., 2014). During the observation of a flexion-extension hand movement, the motorneuronal pool activating the FCR muscle show maximal facilitation during flexion and minimum during extension; moreover, motorneurons controlling antagonist muscles (flexor and extensor carpi radialis) are modulated in phase opposition, reflecting their natural reciprocal activation during execution of hand oscillations (Borroni et al., 2005).

Critically, since the observed flexion-extension movement has a sinusoidal time course, we can utilize the same mathematical function to fit both observed wrist oscillation and resonance effects on the observer’s wrist motor circuits and to generate a continuous parallel representation of the two events. With this tool, we can explore the role of attention in the development of the motor resonant response with different experiments in which the attention of subjects is diverted from the direct observation of the action and directed to different tasks (visual and cognitive), while they observe the same hand movement. We hypothesize that if attention is necessary for either the complete or partial development of the motor resonant response, the different attentive tasks will interfere with the H-reflex modulation, affecting it to different quantifiable degrees depending on their relative appropriation of cognitive resources.

2.2 **MATERIALS AND METHODS**

A total of 42 right-handed healthy adult volunteers (27 females, average age 28, range 19-40) participated in 3 experiments. The experimental protocol was approved by the local ethics committee and written informed consent was obtained from each subject in compliance with the rules of the declaration of Helsinky.

Experimental rational. The excitability modulation of cortical and spinal motorneurons was monitored as a measure of the modulation of the subliminal motor command induced in cortical motor areas by movement observation and descending to spinal circuits (Borroni et al. 2005). The role of attention in motor resonance was explored with different experiments in which the attention of subjects observing a hand movement was directed to different attentional tasks and thus diverted from the direct observation of the hand movement. The experimental conditions of the study were: in E*xpt.1* (n=14), the *semi-implicit observation* of a hand movement, in E*xpt.2* (n=14), the *implicit observation* of a hand movement, and in *Expt.3* (n=14), a control experiment, the observation of the movement of a non-biological object (a metal platform). Present results are statistically compared to results of a previously published experiment, from here on called *Baseline Expt,* in which motor resonant responses were recorded while the attention of 14 subjects was just directed to the observation of the hand movement (*explicit observation* of a hand movement; Borroni et al., 2008a). In all present and past experiments the hand movement was identical: a 1Hz cyclic hand flexion-extension performed by the experimenter in front of the subjects. The platform movement was also performed at the same frequency and with the same kinematics (see below).

2.2.1 *Experimental protocol.*

Subjects were sitting in an armchair with prone hands resting on lateral supports and were instructed not to move during the experimental trials. The amplitude of H-reflexes was measured in a flexor muscle of the right hand (flexor carpi radialis, FCR) of subjects viewing a cyclic oscillation of the right hand resting on a moving platform, performed in front of them by one of the experimenters (mover). Oscillation movements of the hand were executed in groups of 10 cycles, paced to the rhythm of 1 Hz by a metronome heard only by the mover wearing headphones.

In *Expt.1* and *2* a small LED light was fixed on the dorsal surface of the second phalanx of the middle finger of the mover's hand. In each trial of 10 hand oscillation cycles (10s trial) both frequency and number of LED activations varied randomly; the maximal on/off frequency was 2 Hz, so that each LED activation could be clearly separated perceptually. Therefore, during each 10s trial the LED could turn on from a minimum of 1 time to a maximum of 20 times, both frequency and number varying unpredictably. Hand movement and LED activation were synchronized at the beginning and proceeded independently, so that during the 10s period the LED could turn on at any time during the oscillation cycle. A beeping sound signaled both the beginning and the end of each 10s trial. In both experiments subjects received immediate feedback on the accuracy of their performance (at the end of each 10s trial), and made very few errors (see Results).

Expt.1: subjects were instructed to report whether, when the LED light turned on for the last time in each 10s trial, the moving hand upon which it rested was flexed upward or downward, or was in the intermediate, horizontal position. The task required constant attention because subjects did not know when the last LED would turn on. The subjects' attention was only partly diverted from the hand movement since they needed to monitor hand position in order to give the correct answer. The fact that the hand was moving was never mentioned in the instructions, but was relevant to the subjects' answer (semi-implicit movement observation).

Expt.2: subjects observed the same light regime as in *Expt.1*, but were instructed to count and report how many times during each 10s trial the LED light turned on. Because of the unpredictability of the LED activation, the task required constant attention. The fact that the hand was moving was never mentioned in the instructions and was irrelevant to the subjects' answer (implicit movement observation).

In Expt.3 the metal platform upon which the experimenter's hand normally rested in *Expt.1* and *Expt.2* was oscillating alone, without a hand on it. It was connected by a long rod attached to its pivot to the hand of a mover hidden behind a screen, so as to produce an oscillating movement with the same kinematic characteristics as that observed during the flexion-extension of the mover's hand. Subjects were instructed to observe the platform. Based on previous results showing that observation of a metal platform does not elicit a motor resonant response (Borroni et al. 2005), the goal of this experiment was to establish a baseline against which to evaluate the results of the other experiments.

2.2.2 *Data acquisition and analysis.*

Data acquisition and analysis have been previously described (Borroni et al. 2005). Briefly, H-reflexes were evoked in the FCR muscle by electrical stimulation of the median nerve at the elbow (square pulse, 0.8ms duration) and recorded with external bipolar electrodes placed on the muscle belly. Signals were amplified, filtered (10-1000 Hz) and A/D converted (5kHz sampling rate). All signals (H-reflex and movement traces) were recorded and stored for later analysis. Peak to peak amplitude of the FCR H-reflex at rest was maintained between 5 and 15% of the maximal direct motor response (Mmax). In order to exclude the possibility of voluntary or involuntary mimic activity in the observing subjects, the background EMG was monitored in the FCR and in another wrist muscle *(Extensor Carpi Radialis)*, throughout the whole movement observation.

To describe the specific temporal relation between the modulation of excitability in the FCR muscle and the observed movement, H-reflexes were recorded at 5 different points in time during the hand flexion-extension cycle (0, 200, 400, 600, 800 ms) corresponding to 5 different hand angular positions dividing the 1s oscillation cycle in five equal parts. For each subject a total of 100 presentations were obtained, grouped in 4 blocks of 25 trials, and subjects were instructed that they could rest at the end of each block. In order to synchronize the physiological responses in observers and the observed hand movement, hand position was used as a triggering signal for stimulus delivery and data acquisition: the position of the metal platform upon which the mover’s hand rested was continuously recorded with a Spectrol 534 1kΩ potentiometer coaxial with the pivot of the platform, and digitized at 250Hz. When, during the third hand oscillation the platform reached a pre-selected position in the cycle, a trigger signal was released to activate the stimulator to elicit H-reflexes in the FCR muscle of observers and data acquisition, at one of the 5 different delays. Therefore, H-reflex samples were always taken during the third of 10 hand oscillation cycles. Delays were selected automatically by the acquisition program in semi-random order, i.e. completing a cluster of all 5 delays before starting the new random selection again.

For each subject, averages of observed hand movements were calculated and fitted by a four-parameter (period, offset, amplitude and phase) sinewave function. Despite being paced by a metronome, the mover's hand cycle period varied among trials by about 5% of its average value: thus normalization was necessary to bring movement records from different trials back to unity (1 s). To maintain the temporal correlation between the time courses of observed movement and response modulation in observers, the same normalization was performed on the 5 delays at which the H-reflexes were recorded and average reflex values obtained at the same delay were assigned to their corresponding normalized delay. In order to minimize sources of variability of H-reflex amplitude over time (each experiment lasted 30-60 min) and thus independent of the experimental manipulation, the deviation (in μV) from the mean of the 5 responses recorded in each cluster was calculated for each delay, in each observer. This last value was then averaged with those obtained at the same delay in the other clusters. Average data points from all different subjects were plotted together and fitted with a common two parameter (amplitude and phase since, after normalization, period = 1 and offset = 0 for all) sinewave function. Significance of all sinewave regressions was ascertained with a standard analysis of variance.

Differences in amplitude of the motor resonant responses in the three hand-movement experimental conditions (*explicit, semi-implicit and implicit observation*) were evaluated by comparing with a repeated measure, one-way ANOVA and a post-hoc Tukey test, the single subject H-reflex modulation amplitude parameter derived from the sinewave function fitting each subject's average data points.

A circular-linear correlation analysis (Zar 1999) was utilized to compare the time-course of the H-reflex responses in the three hand-movement experimental conditions (*explicit, semi-implicit and implicit observation*) and in the baseline condition (*observation of metal platform),* and subsequently a one-way ANOVA was performed on the R coefficients obtained in the correlation analysis to which a Fischer transformation was applied to obtain a normal distribution.

For all statistical tests, significance level was set at $p<0.05$. Data were acquired and recorded using a custom program in LabView10 and stored for later analysis;

statistical analysis was conducted using SPSS software (SPSS Inc, Chicago, USA) except for the circular-linear correlation for which CircStat for Matlab toolbox (Barens, 2009) was utilized.

2.3 **RESULTS**

To determine the role of attention in motor resonance, first it is necessary to establish whether a motor resonant response is evoked in each of the four experimental conditions, i.e. whether in all conditions the time course of FCR H-reflex amplitude modulation is significantly correlated with the time course of the observed movement. Figure 2.1 (adapted from Borroni et al. 2008) shows that when subjects are explicitly instructed to observe the hand moving, their motor resonant response has the same time course as the observed movement: H-reflexes recorded in the right FCR muscle of right-handed observers are modulated during observation of the mover's hand flexion-extension movement, with increasing excitability developing in the flexor motoneuronal pool during the flexing phase of the observed movement. In panel (A) the cumulative plot of average data points from all subjects, aligned after time normalization, can be fitted with a common sinewave function (black curve, $R^2 = 0.42$, $p<0.0001$), with the same period as that fitting the average movement (panel B).

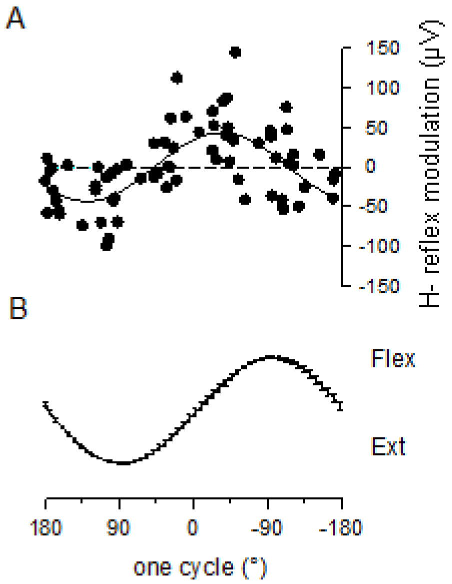

Fig. 2.1 Explicit observation condition. (A) H-reflexes modulation recorded in the right FCR muscle of right-handed observers, during observation of one cycle of a flexion-extension movement of the right hand (B) performed by a different subject are significantly modulated ($p<0.001$), showing that increasing excitability of the flexor motoneuronal pool develops during the flexing phase of the observed movement. In panel A the cumulative plot of the average data points from all subjects, aligned after time normalization, are fitted with a common sinewave function (black curve), with the same period as that fitting the average movement (panel B). Flex = downward direction of the moving hand.

In the semi-implicit movement observation condition (*Expt.1*, Fig.2.2), when subjects are instructed to report the hand position corresponding to the last light activation, the motor resonant response remains linked to the same time course as that of the observed movement, though there is a dramatic reduction in amplitude modulation of H-reflexes compared to the explicit movement observation condition; note the reduced scale of the ordinate compared to Fig. 2.1. In panel (A) the cumulative plot of average data points from all subjects, aligned after time normalization, are fitted with a common sinewave function (solid black line, $R^2 = 0.19$, $p<0.001$), with the same period as that fitting the average movement (panel B). The task required constant attention and subjects made very few errors (number of errors across all subjects: average = 3.1 errors/block of 25 trials; mode = 3; min = 1, max = 8).

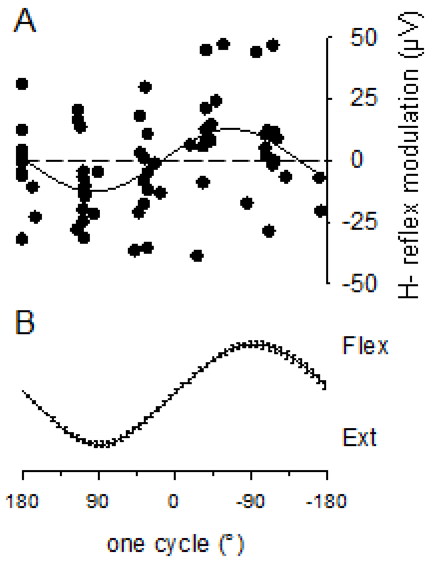

Fig. 2.2. Semi-implicit observation condition. (A) H-reflex modulation recorded in the right FCR muscle of right-handed observers, during observation of one cycle of a flexion-extension movement (B) of the mover's right hand, when observers are explicitly instructed to report the hand position of the mover corresponding to light activation. H-reflexes are slightly, but significantly, modulated ($p<0.003$) showing maximal excitability during the flexing phase of the observed movement. In panel (A) the cumulative plot of the average data points from all subjects, aligned after time normalization, is fitted with a common sinewave function (black curve), with the same period as that fitting the average movement. Flex = downward direction of the moving hand.

In the implicit movement observation condition (*Expt.2*, Fig.2.3), when subjects are instructed to count light activations on the moving hand, the motor resonant response also shows the same time course as the observed movement, while also suffering a dramatic reduction in amplitude compared to the explicit movement observation condition; note the reduced scale of the ordinate compared to Fig.2.1. In panel (A) the cumulative plot of average data points from all subjects, aligned after time normalization, are fitted with a common sinewave function (solid black line, $R^2 = 0.17$, $p<0.002$), with the same period as that fitting the average movement (panel B). This task also required constant attention and subjects made very few errors (number of errors across all subjects: average = 2.7 errors/block of 25 trials; mode = 2; min = 0, max = 9).

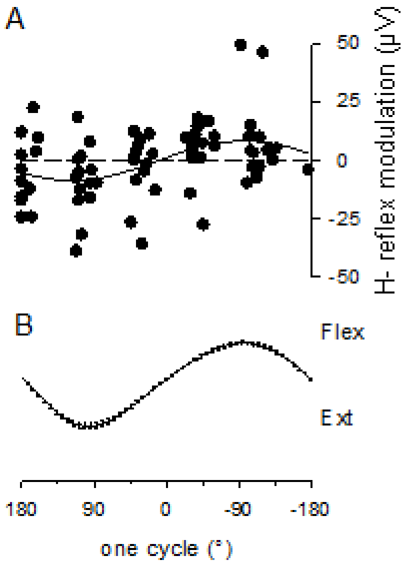

Fig. 2.3. Implicit observation condition. (A) H-reflex modulation recorded in the right FCR muscle of right-handed observers, during observation of one cycle of a flexion-extension movement (B) of the mover's right hand when subjects are instructed to count light activations on the moving hand. H-reflexes are slightly, but significantly, modulated ($p<0.002$) showing maximal excitability during the flexing phase of the observed movement. In panel (A) the cumulative plot of the average data points from all subjects, aligned after time normalization, are fitted with a common sinewave function (black curve), with the same period as that fitting the average movement. Flex = downward direction of the moving hand.

When subjects are instructed to observe a moving platform, the motor resonant response does not develop at all (E*xpt.*3, Fig. 2.4). The amplitude of FCR H-reflexes in this condition reflects random variations rather than being modulated with the time course of the observed movement of the metal platform (B). In panel (A) the cumulative plot of the average data points from all subjects, aligned after time normalization, could not be fitted by a sinewave function with the same period of the observed movement (and in fact with any sinewave function). This experiment replicates previous results (Borroni et al. 2005) showing that in order to induce a motor resonant response the oscillating movement must be executed by a hand, while a simple mechanical device is ineffective, and provides a baseline reference for comparison with responses in the other experimental conditions.

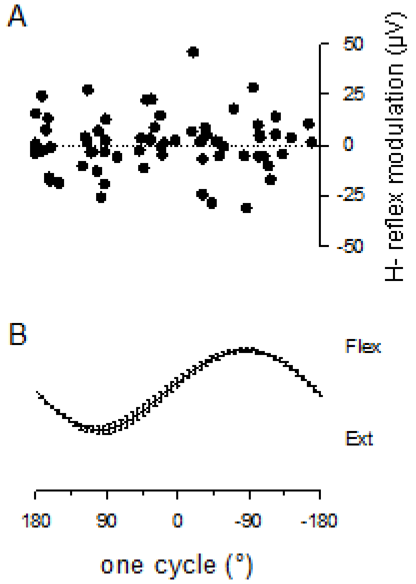

Fig. 2.4. Baseline observation condition. (A) H-reflexes modulation recorded in the right FCR muscle of right-handed observers are not modulated during observation of one cycle of a sinusoidal movement of a metal platform (B). In panel (A) the cumulative plot of the average data points from all subjects, aligned after time normalization, could not be fitted by any sinewave. Flex = downward direction of the moving hand.

To quantify the effect of diverting attention from the action observation task on the development of the motor resonant response, the amplitude of H-reflex modulation in the explicit, semi-implicit and implicit observation conditions was compared (Fig. 2.5A). The reflex modulation amplitude parameter was derived from the sinewave function fitting each subject’s average data points. It was not possible to include in this analysis data from *Expt.3* (observation of metal platform condition), since most single subject responses in this condition could not be fitted with a sinewave function in the first place. Figure 2.5A shows that while H-reflex amplitude modulation was different in the experimental conditions (one-way ANOVA, $F_{2,39} = 19.566$, $p < .0001$), it was significantly larger in the explicit observation condition, compared to the other cognitive task conditions (Tukey post-hoc for both comparisons p < .0001) which were not different from each other (p = 0.84).

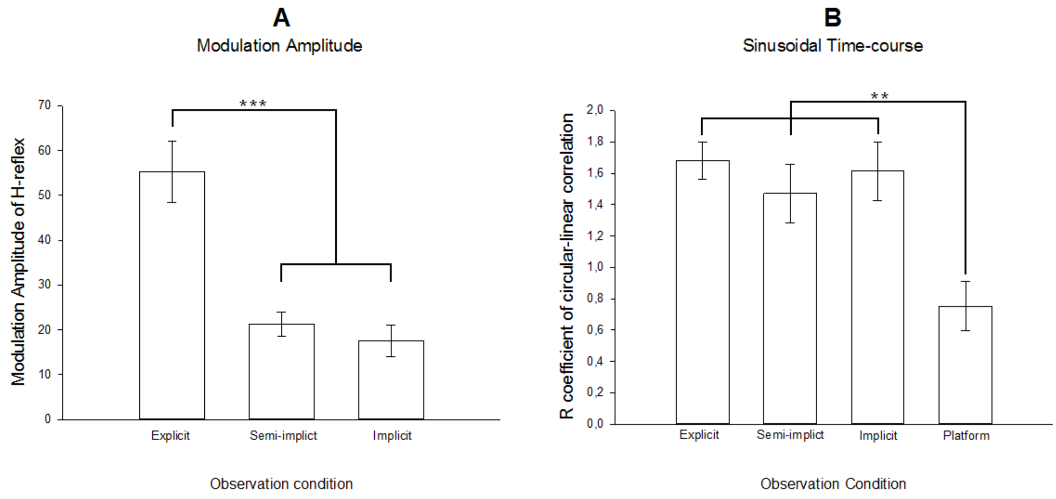

Fig.2.5. Panel A: Effect of diverting attention from the action observation task on the development of the motor resonant response. H-reflex modulation amplitude values (vertical bars, means ± SE) were derived from the sinewave function fitting each subject's average data points. H-reflex amplitude modulation was significantly larger (***) in the explicit observation condition, compared to the other cognitive task conditions (p < .0001) which were not different from each other (p = 0.84). Panel B: coefficient of circular-linear correlation (vertical bars, means ± SE) between the angular position of the oscillating hand (or platform) and H-reflex modulation. The correlation coefficient is significantly smaller (**) in the platform condition compared to the hand condition. The correlation coefficient in the platform condition is not significant (circular-linear correlation: R=0.07, p=0.873), indicating that H-reflexes in this condition are not related to the observed cyclic movement.

Finally, the result of all 4 experiments were analysed using a circular-linear correlation in order to obtain an estimate of how much the position of the observed hand predicts the amplitude of H-reflex evoked in the FCR muscle. In this comparison, *Expt.3* provides an important baseline control. In fact in this condition the sinusoidal time course of reflex modulation linked to the time course of the observed movement is absent (circular-linear correlation: R=0.07, p=0.873), while it remains measurable in the other experimental conditions, including those in which there is a dramatic decrease in amplitude, when attention is diverted from movement (explicit observation R=0.64, p=0.006; semi-implicit observation R=0.40, p=0.004; implicit observation R=0.42, p=0.002). Fig.2.5B shows that the correlation coefficients of the circular-linear analysis are not different in the explicit (*Baseline Expt*), semi-implicit (*Expt.1*) and implicit (*Expt.2*) observation conditions, but significantly different in the platform observation (*Expt.3*) condition (one-way ANOVA, $F_{3,52}$ = 6,753, p<0.001; Tukey post-hoc, platform vs explicit p<.001, vs semi-implicit p<.0016, vs implicit p<.003), with Bonferroni correction (significance level p<0.008).

2.4 **DISCUSSION**

The aim of this study was to explore the role of attention in the development of the motor resonant responses, i.e. whether the resonant activation of cortical and spinal motor circuits during action observation is sensitive to the degree of attentional deployment or whether it is a constant response, consistent with the notion that the AON is automatically triggered. The H-reflex technique was utilized to quantify the amplitude of the resonant response and the accuracy of the subliminal motor program in terms of muscle and time specificity (the facilitation of spinal motor neurons in the right muscle and at the right moment during the time course of the observed action), with three different experiments: 1) explicit observation (*Baseline Expt*, data from Borroni et al. 2005, Fig.2.1), in which subjects were asked to pay attention exclusively to the cyclic oscillatory movement of a hand; 2) semi-implicit observation (*Expt.1*, Fig.2.2), in which subjects had to attend - albeit implicitly- to the same movement in order to complete a different task requiring deployment of attentional resources; and 3) implicit observation (*Expt.2*, Fig.2.3), in which the same movement of the hand was totally irrelevant to the completion of the task, so that subjects could ignore the movement itself while attending their cognitive assignment. These experiments were compared to a baseline experiment (*Expt.3*, Fig.2.4), in which subjects observed the cyclic oscillatory movement of a mechanical device which did not evoke any motor resonant response. Results show that the attention manipulation dramatically decreased the amplitude of the motor resonant response, but did not affect its muscular and temporal specificity, i.e. the essence of the subliminal resonant motor program.

When a cognitive task was imposed parallel to the observation of the hand movement as in *Expt.1* and *Expt.2*, i.e. when subjects were distracted from paying explicit and focused attention to the movement in the implicit and semi-implicit conditions, the amplitude of the motor resonant response was profoundly affected and was subject to a dramatic decrease compared with the explicit observation condition (Fig.2.5A); in other words the number of spinal motorneurons recruited by a descending command during the observation of the movement was dramatically reduced by manipulating attention. This result is consistent with a gain modulation of the neural response due to attention. Gain modulation is a well described finding in sensory systems, when considering the role of attention on, e.g., sensory evoked responses (Maunsell and Treue 2006; Reynold and Heeger 2009). The other important result of the study is that a residual resonant response could still be recorded when attention was diverted from the action observation task, during either implicit and semi-implicit conditions, since the correlation between the time course of FCR H-reflex amplitude modulation and the time course of the observed movement remained highly significant (Fig.5B). It is important to recall that such a time-locked motor response was not recorded when subjects were observing the metal platform oscillating without the hand with the same sinusoidal rhythm. Instead, the specificity of the time course of the H-reflex modulation during implicit and semi-implicit observation was not different from that recorded in the explicit observation condition, in which subjects were asked to pay attention exclusively to the moving hand. As discussed above, the amplitude of the H-reflex modulation was significantly decreased, and in fact it was reduced to a similar level as during observation of the moving metal platform. Importantly, in the moving platform condition the response lacks any temporal correlation with the observed oscillation, and in fact any kind of temporal structure, and therefore is the result of random amplitude variations rather than a modulation induced by the observation task.

The fact that the muscular and temporal specificity of the motor resonant response is maintained when subjects are asked to perform a different secondary task, supports the hypothesis that the subliminal recruitment of spinal motorneurons during action observation is automatic. This result is consistent with behavioral evidence also showing that the activation of the motor system during action observation is automatic: different experiments on imitation suggest that observed gestures automatically activate motor representations normally involved in the execution of those actions (; Brass et al., 2000; Brass et al., 2001; Craighero et al. 2002; Vogt, et al. 2003; Kilner et al. 2003; Heyes et al., 2005;). In this kind of experiments, participants typically have to perform a standard movement (e.g. to lower a finger) when a trigger-movement is shown on a screen. Usually they are faster and/or more accurate when the trigger-movement is congruent (e.g. lowering a finger) rather than incongruent (e.g. lifting a finger). While these experiments don't include a secondary task besides observing the movement, further studies show that the automatic imitation effect is also recorded when the observed movement is irrelevant to complete a different task. For example Stürmer et al. (2000) asked subjects to open or close their hand when the color of an observed hand changed, irrespective of whether the observed hand was opening or closing; crucially, when the movement of the observed hand was congruent with the requested movement, subjects were faster compared to the incongruent condition, i.e. attending to color in order to make a decision to move did not seem to interfere with the automatic imitation facilitation.

It has been suggested that in this kind of experiments the automatic imitation effect is maintained because the attentive resources required to process the observed action are not exhausted by the secondary task (Chong et al. 2009). Consistently with the "perceptual load model" (Lavie 1994; 2005), the processing of the movement during action observation, should be inhibited only when the cognitive demand of another task is too high. The observation of movement (distractor task) can be excluded from perception when the level of perceptual load in processing task-relevant stimuli (light) is sufficiently high to exhaust perceptual capacity. Similarly, we can hypothesize that in both the implicit and semi-implicit conditions of the present study the perceptual load is low, and a sufficient amount of attentive resources is captured by motion also when this is irrelevant to execute correctly the task. Such an automatic processing of the observed action seems to be sufficient to modulate corticospinal excitability in a way that reproduces accurately the corresponding subliminal motor program (Fig. 2.5B).

When considering the dramatic decrease in amplitude of the resonant response, again behavioral experiments on automatic imitation come to our help, showing that indeed “automatic” imitation of actions requires attention (Bach et al. 2007) and that if attention is so strongly diverted from the action that no cognitive resources remain available to process it, the automatic imitation effect disappears (Chong et al. 2008, 2009). In the present study we made no attempt at subtracting all attentive resources from the action observation, but rather aimed at varying the degree of voluntary allocation of attention. In fact in the semi-implicit condition, in which the correct execution of the task depended in part on hand position, the observed movement should have been at least partly relevant. We expected that in this experimental condition more attention should have been directed to the hand movement itself, and therefore that the motor resonant response would have been larger than in the implicit condition. Instead, the decrease in amplitude modulation was the same in the two conditions, suggesting that there is a cognitive component to motor resonance influencing the recruitment of motor circuits and that, irrespective of the specific attentive task, the same amount of cognitive resources was subtracted from the observation of the action. In support of this interpretation both tasks appear to be just as difficult, since subjects make a very similar number of errors. Further experiments using the H-reflex technique and more challenging cognitive manipulations are necessary to verify whether it is indeed possible to subtract all attentive resources from the observation of actions, and cancel the physiological (motor resonant) response completely or whether motion is such a powerful exogenous cue that it always captures attention.

Interestingly the residual amplitude modulation observed in the present study is consistent with fMRI studies showing a residual activity in the AON when a secondary task or cognitive manipulation is imposed on subjects during action observation (Chong et al. 2008) although it is not possible to say whether decrease in BOLD signal has a functional correspondence in the modulation of the motor system during action observation. Our study suggests that this is the case, that when subjects are asked to perform a secondary attentional task the reduction in attention devoted to the observation of an action results in a reduction of the gain of neural processes leading to the subliminal activation of motor circuits.

The modulation of H-reflex during action observation tasks can be considered the result of the modulation of activity mainly in M1 (Borroni et al. 2008). M1 is usually not described as part of the AON in neuroimaging studies, probably because its activation is too weak to be measured as a BOLD response, consistently with the lack of overt movement. But other techniques (MEG, EEG, TMS and electrophysiology) have revealed its activation during action observation tasks (Hari et al. 1998; Nishitani & Hari 2000; Cochin et al. 1999; Fadiga et al. 1995; Gangitano et al., 2001; Press et al., 2011; Cavallo et al., 2012, 2013; Sartori et al., 2012; McCabe et al., 2014; Dushanova and Donoghue 2010; Vigneswaran et al. 2013; Kraskov et al. 2014) indicating that M1 should be considered the last node of this cortical network (Kilner 2003). During action observation, visual information travels from the occipital cortex, reaches EBA and STS where a visual description of the relevant action (Carr et al., 2003; Miall, 2003) is represented and then through the fronto-parietal network formed by the inferior parietal (BA40) and premotor (BA6) areas (Rizzolatti et al. 1996; Grèzes et al. 2001; Cabinio et al. 2010) where visuo-motor information is shaped, which then continues to the primary motor cortex to organize the motor resonant response (Borroni et al., 2008).

To explain the reduction in amplitude of the modulation of motor pathways in *Expt.1* and *Expt.2* we hypothesize that M1 must receive less input from the rest of the AON (primarily from premotor cortex), resulting in a reduced activation of spinal motoneurons and thus in a decreased amplitude modulation of the FCR H-reflex. We can speculate that selective processes modulate the activity at an early phase of the information processing: many studies show that the activity of visual cortex can be modulated by top-down influences (e.g selective attention) by medial prefrontal and parietal areas (Kastner 2000; Beck & Kastner 2009). From there all the following processing stages of observed action would be influenced, resulting in the corresponding modulatory effect on motor cortex activity during action observation. In alternative, or in parallel, a prefrontal and parietal input could exert a later modulatory activity directly on premotor areas (Rizzolatti & Luppino 2001), and thus regulate the excitability of primary motor cortex during action observation. For instance in the fMRI study of Chong and colleagues (2008) in the high cognitive load condition, when the attentive resources available to process the observed action are significantly reduced compared with the low cognitive load condition, only the activity of a frontal AON node (near the Inferior Frontal Gyrus) decreases, whereas no differences between high and low perceptual load are recorded in parietal areas of the AON.

Finally, an important role of attention on motor control is also described when a secondary task or cognitive manipulation is imposed on subjects executing - not observing - a movement. For example if subjects are distracted from their actions, it is more likely that they will make mistakes or perform the action more slowly (Passingham 1996). Consistently, Johansen et al. (2002) showed that reducing attention to finger movement by asking subjects to perform a concurrent counting task is associated with decreased BOLD signal in motor cortical regions, compared to the signal evoked by performing the movement without distraction. This decrease in BOLD signal in motor cortex induced by lowered attentional resources could indicate a similar mechanism of influence of attentive processes on motor pathways during either execution and observation of action.

Taken together, our results show that as long as enough attention is devoted to the observation of others' actions, and thus enough gain is maintained in the perceptual/motor system, a motor resonant response is produced which is as accurate, in term of muscular selection and time specificity, as when it receives full attention. Traditionally automatic processes are described as generated involuntarily and without conscious effort, they do not draw on general cognitive resources and do not require attention for their execution (Posner, 1978; Bargh, 1992). On the contrary voluntary processes are usually described as conscious and requiring attention. In the case of action observation and the deriving motor resonant response, such a contrasting definition appears too simplistic. More useful is the distinction by Kahneman and Treisman (1984) of at least three types of automatic processes – those that are 'strongly,' 'partially,' or 'occasionally' automatic – depending on the amount of attention required for their completion. We think that motor resonance should be described as a "partially automatic mechanism": as our data suggest, the AON seems to be automatically and correctly activated by the intrinsic salience of movement (of a hand), but its level of activity, i.e. the gain of the circuit, can be modulated by top-down influences, such as selective attention or the available resources. Generally, action observation studies have been carried out in conditions in which subjects were allowed or even required to observe the action with all, or sufficient, attentive resources. However, from a more naturalistic point of view, this is not the most common circumstance; on the contrary, in our daily life we are exposed to several simultaneous actions, performed by different people, with different meaning and consequences. Not all actions are necessarily as relevant or interesting to the observer, and we expect future studies to confirm and expand our results, showing that the activation of motor system during action observation is not an “all or nothing” event, but can vary presumably according with goals and needs of the observer.

CHAPTER 3

THE EFFECT ON MOTOR RESONANT RESPONSE OF LOCATING THE OBSERVED ACTION IN PERIPHERAL VISION[1]

3.1 INTRODUCTION

Being able to perceive what others are doing is important for social beings such as humans and requires a number of different sensory and cognitive abilities. The perception of other people’s behaviour, which encompasses both the goal of observed actions and the single movements necessary to reach that goal (Gallese 2014), occupies a special status in the nervous system. We are perceptually tuned to the kinematic aspects of the movement of others around us through the integration of different incoming information from both central and peripheral vision (Giese & Poggio, 2003; Thornton et al., 1998; Verfaillie, 2000). An impressive body of literature in the past twenty years has revealed a possible role played by cortical motor areas in action perception (Schuz-Bosbach & Prinz, 2007; Rizzolatti & Sinigaglia 2010; Avenanti et al., 2013), i.e. the subliminal activation of an action observation network (AON) when viewing others’ actions. In analogy with their respective functions during action execution, it has been proposed that the pattern of activity in ventral premotor and parietal cortices corresponds to the neural representation of the goal (Gallese et al., 1996; Fogassi et al., 2005; Hamilton & Grafton, 2006; Kilner et al., 2004) and that the primary motor cortex (M1) encodes

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[1] The data presented in this chapter has been published (see appendix II)

the specific motor program required to reproduce the observed action (Fadiga et al., 1995; Rizzolatti et al., 2001; Montagna et al., 2005).

Specifically, studies with transcranial magnetic stimulation (TMS) have shown that observation of a hand grasping an object elicits a motor resonant response, i.e. a pattern of motor evoked potentials (MEP) facilitation of the same muscular groups and with the same time-course as in the observed grasping of that object (Gangitano et al., 2001, 2004; Borroni et al., 2011; Press et al., 2011; Cavallo et al., 2012, 2013; Sartori et al., 2012; McCabe et al., 2014). Thus, by encoding the kinematic aspects of an observed action, the specific subliminal activation of the primary motor cortex (M1) facilitates its repetition as can be useful, for instance, during imitation for motor learning (Iacoboni et al. 1999; Mattar & Gribble 2005; Vogt et al. 2007). In motor learning neural resources must be dedicated to the acquisition of precise kinematic information about the single movements to be learned, while simultaneously contextualizing this fine scale in the larger scale of the entire action. For example as the pupil observes the fine finger movements of his/her violin teacher, he/she must also record the position of the wrists, arms, shoulders, neck, trunk, and so on, which remain in the periphery of the main finger action. Since cortical magnification selectively boosts central vision (Wassle et al., 1990) and in peripheral vision visual acuity and phase discrimination are naturally decreased (Hubel & Weisel 1968; Shapiro et al., 2011), the accuracy of visual information in peripheral action observation is expected to be much lower than in central observation. We can thus hypothesize that moving from central to peripheral vision, the degradation of the precision of motor resonance corresponds to a progressive loss of its replicative function, in favor of the progressive formation of a more contextual representation of the observed action. Similarly, the subliminal motor response recorded when observed actions fall in the peripheral field could have the more generic function of allowing monitoring biological movements in the environment, and to facilitate prediction and/or interaction between the observer and others. Indeed, Sartori et al. (2009) have shown that a socially meaningful gesture, performed by a human agent peripherally to the execution of a grasping action, can perturb the trajectory of the executed grasping, suggesting the possibility of a motor resonant effect in peripheral vision.

The possibility that actions located in the peripheral field of vision may evoke motor resonant responses in observers' motor pathways has not been investigated in human subjects and only indirectly in other primates. In a study addressing the allocation of gaze in macaque monkeys observing actions performed by others, Maranesi et al. (2013) showed that almost half of the recorded mirror neurons was "gaze-independent", i.e. that F5 neurons code the observed interaction between the agent's hand and the target irrespectively of whether the monkey is looking directly at it, implying that these neurons are activated by movement in the monkey's peripheral field of vision (at locations >9° from the fixation point).

In the present study we examine the time course of the excitability modulation of M1, utilizing motor potentials evoked by TMS at different delays during the observation of two actions composed of three phases (reaching, grasping and lifting a ball) viewed by subjects in their near peripheral field. By studying the entire time-course of the observed action we can discriminate fine but critical differences in the subliminal motor response that would not be evident with a single-time sampling during the action observation task and might lead to the wrong conclusions. A previous study by our group (Borroni et al. 2011) has shown that during observation of the same two actions in central vision the different patterns of MEP amplitude modulation the Abductor Digiti Minimi (ADM) and Opponens Pollicis (OP) muscles reproduce the subliminal motor programs consistent with the specific activation of these muscles during the execution of the *grasping phase* of the observed actions, i.e. during hand opening and closing respectively. The different patterns of MEP amplitude modulation recorded during observation of the different actions, with the same grasping goal, demonstrated that the resonant responses reproduces the kinematic aspects, not the goal of the entire action. These results will be directly compared to the results of the present study.

We hypothesize that the grasping actions observed in peripheral vision are effective in modulating the excitability of motor pathways, but we expect that the modulation pattern will have a lower kinematic specificity than in central vision (i.e. the facilitation of ADM and OP MEPs will not reflect the precise timing of the activation of these muscles during hand opening and closing). However, while the neural resources for perception of biological motion are concentrated in the central region of the visual field (foveal and parafoveal 0-5°; Ikeda et al., 2005; Brown et al., 2005; King et al., 2010), peripheral vision, less encumbered by high spatial frequency visual information, may be sufficient to discriminate the general aspects of a visual scene, such as its direction and overall gist (Gibson et al., 2005; Thompson et al., 2007; Gurnsey et al., 2008; Larson & Loschky 2009), and may thus actually be advantageous in the recognition of the goal of an observed action. Therefore, we explore the possibility that, even in the absence of precise kinematic visual information, motor resonance in peripheral vision might reflect the goal of the observed action. In this case the prediction is that the facilitation of ADM and OP MEPs will not reflect the precise timing of the activation of these muscles during hand opening and closing, not provided by vision, but will still be strictly limited to the *grasping phase* of the reaching-grasping-lifting action.

3.2 **MATERIALS AND METHODS**

Experiments were carried out on 40 healthy adult volunteers (23 females, average age 24.1 ±5.3), who were fully informed about the experimental procedures and signed a written consent. Experimentation was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Milan. All subjects had normal or corrected-to-normal vision, no history of neurological disorders or contraindication to TMS. All were right handed according to the standard Edinburgh Handedness Inventory (Oldfield, 1971).

3.2.1 *Experimental Paradigm*

In order to verify the presence and the quality of motor resonant response in peripheral vision we replicated the experimental paradigm utilized in a previous study from our group (Borroni et al., 2011) in which actions were shown in central vision, so that data from the two studies could be directly compared in the same ANOVA analysis. None of the subjects of the first study participated in the present study. Motor-evoked potentials (MEPs) were used to measure the excitability modulation of cortical and spinal motorneurons during observation of a grasping action performed by an avatar’s right hand with two different movements (see below). MEPs were evoked by single-pulse TMS of the hand area in the left M1 of volunteers and were recorded simultaneously from the right Opponens Pollicis (OP) and Abductor Digiti Minimi (ADM), two muscles normally utilized during a grasping action for finger closing and opening respectively. Half of the subjects observed a 5s video clip of a “natural” hand motor sequence (fingers flexing towards the palm of the hand), showing an avatar grasping a red ball positioned on a table, and the other half observed an identical video clip, except just for the frames in which the avatar grasps the ball using an “impossible” hand motor sequence (fingers flexing towards the back of the hand) (Fig.3.1).

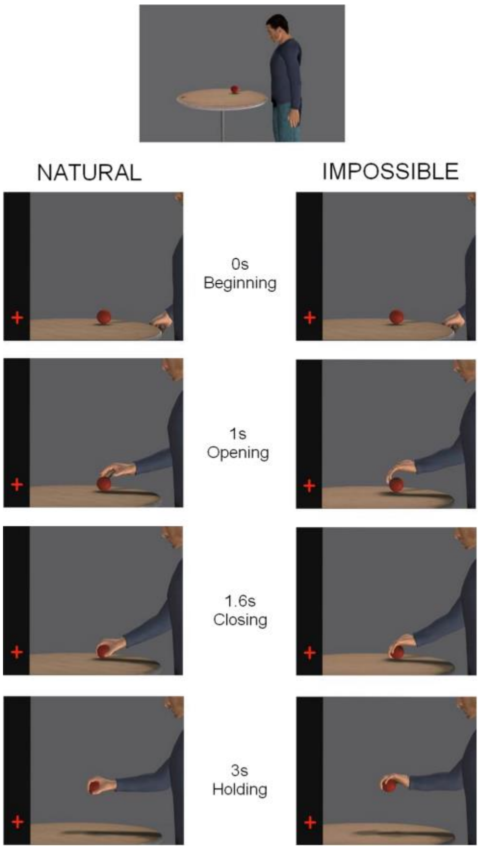

Fig. 3.1. Frames of the videos corresponding to the four experimental delays. The four vertical images in each column are frames of the video clip showing the avatar’s hand positions at each of the four TMS stimulation delays in the natural (left column) and impossible (right column) conditions. 0 s = d1, baseline, static delay in which hand is not interacting with the ball; 1 s = d2, opening, interactive delay of maximal finger aperture during the grasping action; 1.6 s = d3, closing, interactive delay in which the avatar’s fingers grasp the ball; and 3 s = d4, holding, interactive delay in which the avatar’s hand lowers the ball on the table after having lifted it. Subjects were asked to fixate a red cross on the left side of the screen while the action was shown in the right near periphery (10°). The upper middle image shows the entire avatar’s body, presented to the subjects at the beginning of the video.

The presentation of an impossible movement allows us to define, through a questionnaire, to what extent the subjects could be able to perceive the kinematic aspects of the observed action. We chose a between-subject experimental design with the natural and impossible actions observed by two separate groups of subjects to avoid influencing the observation of either actions with previous experience.

3.2.2 *Experimental Protocol*

The experimental protocol was identical to the one utilized in Borroni et al., (2011) except for one critical variable, i.e. that the grasping action was observed in peripheral vision instead of in central vision. Subjects were sitting in an armchair with prone hands resting on lateral supports and were asked not to move during the experimental trials. They watched a video on a 17” high-resolution computer screen placed at eye level at a distance of 1m. Subjects were instructed to fixate a red cross (4 cm in size) on the left side of the screen, while the video with the action appeared on the right side of the screen. The centre of the ball, focus of the grasping action, was placed at 17cm to the right of the fixating point, i.e. at 10° eccentricity on the horizontal plane with respect to central vision of subjects located at 1m distance from the screen. Eye position was continuously monitored during video presentation with electro-oculogram recordings obtained with self-adhesive monopolar surface electrodes placed laterally to each eye, to verify that subjects maintained their gaze on the fixation point. The room was quiet and lights were dimmed to minimize acoustic and visual distractions. Before the first trial, a short introductory video was shown, zooming in on a male avatar standing near a table where a red ball was resting; this scene was shown in central vision in order to familiarize subjects with the context of the action. Subsequently, during the experimental trials, a second video was shown in peripheral vision, consisting of a close-up of the avatar’s hand grasping the ball (Fig.3.1). This video started with the right hand moving from its resting position along the avatar’s body, to the ball. Then, in the natural grasping video, the hand opened with a finger extension and grasped the ball with a normal “palmar” finger flexion, while in the impossible grasping video the hand was supinated while opening with finger extension and grasped the ball with an abnormal “dorsal” finger flexion; after a brief lifting phase the sequences were concluded (see videos in Supporting Information).

MEPs were recorded with self-adhesive bipolar surface electrodes over each muscle belly. Electromyographic signals were amplified, filtered (100Hz to 1kHz) and digitally converted (sampling rate 5kHz). The head of subjects was restrained by a comfortable pillow wrapping around the neck and supported by a fixed head rest. A mechanical arm held a figure-of-eight-shaped coil connected to a magnetic stimulator (Magstim 200, Magstim Company Limited, Whitland, Wales, UK; maximal power 2.2 T). The coil was positioned and fixed on the left M1 so as to activate both selected muscles, and the stimulator output was set at about 110% of the motor threshold of the less excitable muscle (defined as the intensity giving 3 MEP responses out of 6 stimuli). The excitability time-course was explored at four relevant randomized delays from the onset of the video: d1) 0s = Baseline, static delay in which hand is not interacting with the ball; d2) 1s = Opening, interactive delay of maximal finger aperture during the grasping action; d3) 1.6s = Closing, interactive delay in which the avatar’s fingers grasp the ball and d4) 3s = Holding, interactive delay in which the avatar’s hand lowers the ball on the table after having lifted it.

For each subject, a total of 40 presentations were obtained, so that overall k=10 replications of MEP responses were recorded at each of the 4 delays (with the exception of three subjects with k=5,8,9 replications respectively). Presentations were grouped in 2 blocks of 20 trials, and subjects were instructed that they could rest at the end of the first block. Within each block of 20 trials, MEPs were evoked and recorded 5 times at each specific delay, chosen in a semi-random order (completing a set of 4 delays before starting the next set) by the data acquisition program. In order to do this, at the very first frame of the video a synchronizing signal was fed into the computer, which triggered both TMS stimulator and acquisition program at one of the selected delays. Presentations were spaced by 8s dark screen intervals (resulting in inter-stimulus intervals lasting a minimum of 10s). To exclude the possibility of voluntary or involuntary mimic activity of the observer, the background electromyographic activity was monitored in the muscles throughout the whole video presentation.

3.2.3 *Data analysis*

In each subject MEP responses for each muscle were measured as peak-to-peak amplitude; MEP values in all 4 delays were normalized to the average of values in d1 (Baseline, time = 0s), i.e. within each subject, the mean of MEPs recorded at delay 0 was computed, and then each MEP of that subject, at each delay (included delay 0), was divided by this computed mean. Average MEP values for the Baseline delay are shown in Table 1. Note that the experimental protocol (except for placement of the video in peripheral vision) and MEP normalization are identical to that of the Borroni et al., (2011) study regarding central vision, allowing us to directly compare the results of the two experiments: i) peripheral vision: 40 subjects with 40 MEPs for each muscle (k=10 replications for each delay except for three subjects as reported above); ii) central vision: 20 subjects with 100 MEPs for each muscle, i.e. k=25 replications for each delay, except for seven subjects with k=20 replications each).

Data were analyzed by a linear mixed model (McCulloch and Searle, 2001) with k replication for each of four *delay* (d1, d2, d3 and d4) in turn nested within two level of *muscle* (OP and ADM) as repeated measures within each subject; whereas *movement* (natural and impossible) and *vision* (peripheral and central) were set as between-subject factors. Post-hoc test with Bonferroni correction for multiple comparisons were performed. For all statistical tests, significance level was set at $p<0.05$. Data were acquired and recorded using LabView10 and stored for later analysis; statistical analysis was conducted using SPSS software (version 20, SPSS Inc, Chicago, USA).

Post-experimental questionnaire

In order to investigate the conscious perception of actions observed in peripheral vision, at the end of the experiments all subjects answered a questionnaire asking them to describe with words what they had seen and then to physically repeat it as accurately as possible.

3.3 **RESULTS**

Because of their direct relevance in the analysis of the present data, results of a previous study in which subjects observed the same videos in central vision (Borroni et al., 2011) are briefly outlined here. The excitability modulation of the motor pathways to the OP and ADM muscles during observation of the natural (palmar finger flexion) and impossible (dorsal finger flexion) grasping action reflected a pattern consistent with the specific activation of these muscles during the execution of each observed movement: the thumb opponent was facilitated during the observation of fingers closing around the ball in the natural movement, corresponding to the thumb closing phase of the grasping action, and not during observation of the impossible movement, when the thumb is always extended. The little finger abductor was facilitated during finger opening and extension, corresponding to the opening phase in the natural action and both opening and closing phases in the impossible grasping action (Fig. 3.2), when this finger is always extended.

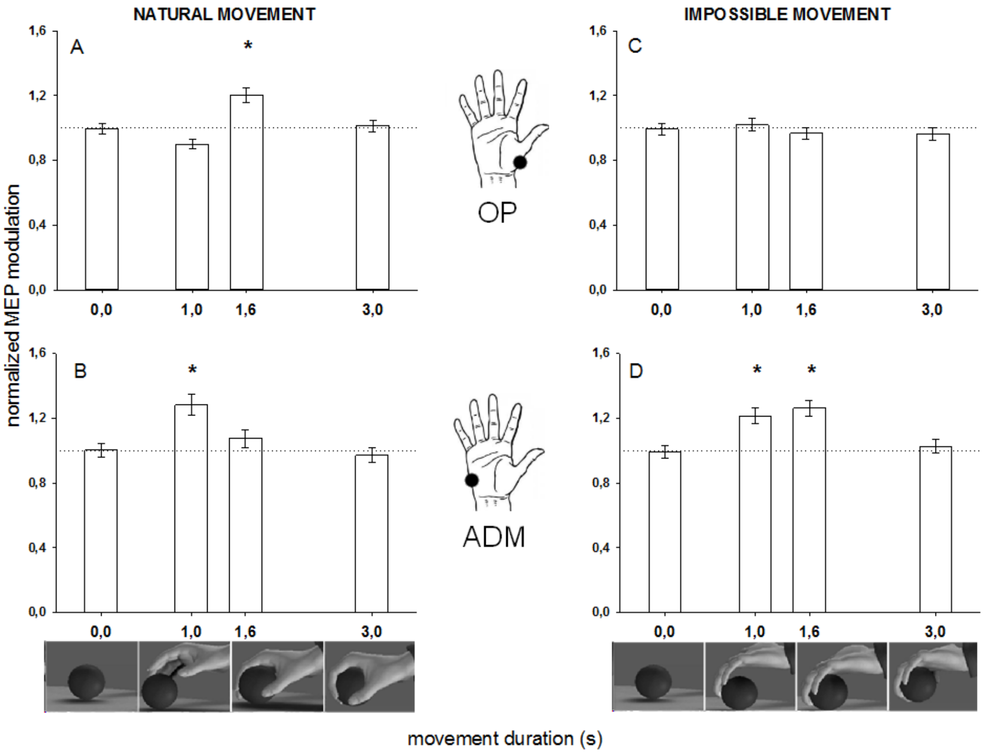

Fig.3.2 MEP modulation during action observation in central vision. Modified from Borroni et al., 2011. MEP amplitude variations in OP and ADM muscles (vertical gray bars, means ± S.E.) recorded at four selected delays (0, 1, 1.6, and 3s) during the observation of the avatar's natural or impossible grasping action, in central vision; OP MEPs in the natural action (panel A) were significantly facilitated during the Grasping phase (1.6s) with respect to all other phases. OP MEPs in the impossible action (panel C) were not modulated. ADM MEPs in the natural action (panel B) were significantly facilitated in the Opening phase (1.0s) with respect to all other phases. In the impossible action (panel D) ADM MEPs in the Opening and Grasping phases were not different from each other, but significantly facilitated with respect to the other phases. On the bottom, video frames of the Grasping phase of both natural and impossible actions are reported at their corresponding delay.

3.3.1 *Modulation of Motor Evoked Potentials in peripheral vision*

Observation of the natural or impossible grasping actions in near peripheral vision (present study) resulted in a modulation of the excitability of M1 very different from the modulation measured during observation of the same actions in central vision (Borroni et al., 2011; Fig.3.2 vs. Fig.3.3).

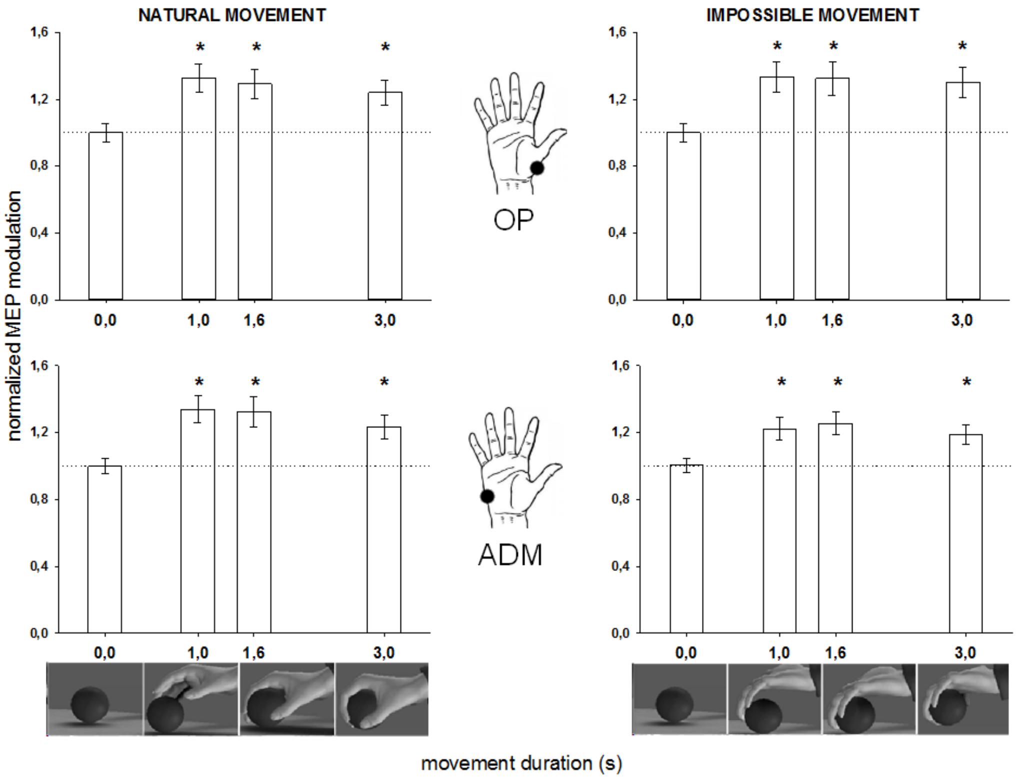

Fig.3.3 MEP modulation during action observation in peripheral vision. MEP amplitude variations in OP and ADM muscles (vertical bars, means ± SE) recorded at four selected delays (d1=0s, d2=1s, d3=1.6s and d4=3s) during the observation in peripheral vision of the avatar's natural or impossible grasping action. Small figures on the bottom are the video frames illustrating positions of the hand at the four delays. OP and ADM MEPs were significantly facilitated during the delays 1, 1.6 and 3s (interactive phases of the grasping) with respect to delay 0s (static phase) in both natural and impossible movements

Subjects' eye position was continuously monitored during video presentation to verify that subjects maintained their gaze on the fixation point (see Methods). In order to investigate such difference, normalized OP and ADM MEP modulation data from both studies were analyzed by linear mixed model, with replications of each delay, *delay* and *muscle* as repeated measured within subjects and *movement* and *vision* as between-subject factors (see Methods). Results revealed a significant main effect of the *vision* factor ($F_{(1,5046.9)} = 63.94$, $p<0.001$) indicating that, considering all the delays together in both muscles and in both movements, MEP modulation was different between peripheral and central vision. A significant main effect for the *delay* factor ($F_{(3,2860.4)} = 29.64$, $p<0.001$) and a significant *delay/vision* interaction

($F_{(3,2819.5)} = 10.11$, $p<0.001$) also emerged suggesting that, considering both muscles in both movements, MEP modulation was different in the delays between the two vision conditions. Most importantly, the four-way interaction between *muscle, delay, vision* and *movement* was significant ($F_{(25,1414.9)} = 3.46$, $p<0.001$) indicating that MEP modulation patterns are different when all four factors are considered. Then multiple comparisons between the same delays in the different vision conditions were performed for each movement and each muscle. For the natural movement, MEPs in the OP muscle were significantly smaller in central than in peripheral vision in d2 ($p<0.001$) and in d4 ($p<0.035$); and MEPs in the ADM muscle in d3 ($p<0.039$) and in d4 ($p<0.027$). For the impossible movement, OP MEPs were significantly smaller in central than in peripheral vision in d2 ($p<0.001$), in d3 ($p<0.025$), and in d4 ($p<0.028$); and ADM MEPs only in d4 ($p<0.050$). These results confirm all expected differences in MEP modulation between peripheral and central vision in the different experimental conditions, showing that MEP modulation was much more specific in central vision to the times of actual activation of muscles during the observed action, while in peripheral vision activation was more generalized because MEPs were facilitated at all dynamic delays of the observed action (see below). These results also deliver an unexpected result, namely the facilitation of OP MEPs during the peripheral observation of the impossible grasping action. In this condition in fact the thumb is always hyper-extended and in the central vision experiments OP MEPs were never facilitated (Fig.3.2 vs. Fig.3.3).

During peripheral observation, differently from central observation, of both natural and impossible grasping actions, the excitability of the primary motor cortex and of the corticospinal projections to the OP and ADM muscles were facilitated in a rough and inaccurate manner, involving equally both muscles and all “interactive” delays (d2, d3, and d4), in which the hand was interacting with the ball to be grasped. In fact, multiple comparisons between different delays revealed a significant difference only between baseline (d1) and all other delays (d2, d3 and d4) (Natural grasping condition, OP: d1 vs d2 $p<0.001$, d1 vs d3 $p<0.033$, d1 vs d4 $p<0.029$; ADM: d1 vs d2 $p<0.001$, d1 vs d3 $p<0.021$, d1 vs d4 $p<0.039$. Impossible grasping condition, OP: d1 vs d2 $p<0.001$, d1 vs d3 $p<0.042$, d1 vs d4 $p<0.037$; ADM: d1 vs d2 $p<0.049$, d1 vs d3 $p<0.026$, d1 vs d4 $p<0.047$). MEP facilitation in OP and ADM is not consistent with the activation of these muscles during the actual execution of either observed actions, because the observed MEP modulation is the same in the two different muscles (a flexor and an extensor) and in all the different active phases of the action, rather than reflecting either a grasping pattern or a hand hyper-extension pattern.

3.3.2 *Post-experimental questionnaire*

The presentation of an impossible movement allows us to define to what extent the subjects could be able to perceive the kinematic aspects of the observed action. The answers to the post-experimental questionnaire indicate that most subjects described either natural or impossible actions observed in peripheral vision as a natural grasping action: only 1 of the 21 subjects observing the natural movement and 6 of the 19 subjects observing the impossible movement reported uncertainty about the grasping goal and suggested vague actions, such as bouncing or stroking the object. Interestingly, concerning kinematics detection, as many subjects observing the natural movement as observing the impossible movement (5) were not able to define accurately some details of the observed movement, for example the orientation in space of the hand approaching the object, confirming the intrinsic inaccuracy of peripheral vision. In fact, none of the subjects observing the impossible grasping detected its bizarre kinematics, i.e. described it as it was or, when requested to simulate the movement they had seen, actually tried to perform an impossible grasping, indicating that such an unfamiliar movement could not be recognized given the limited visual information available.

3.4 **DISCUSSION**

The results of the study support our first hypothesis, showing that observation of grasping actions in near peripheral vision was effective in eliciting a modulation of the excitability of primary motor cortex areas projecting to hand muscles normally involved in grasping, and that the reduction of visual resolution resulted in a dramatic decrease of the kinematic specificity of motor resonant responses compared to central vision. MEP facilitation recorded in the OP and ADM muscles was inaccurate in terms of muscle selection and timing of their activation during the observation of all the different phases of the two actions: it was virtually identical at all interactive delays (d2, d3, and d4), irrespective of the muscle (flexor or extensor) and of the type of observed movement (natural or impossible). Strictly speaking, the recorded responses could be viewed as different from proper motor resonant responses, because MEP modulation is not consistent with the motor program corresponding to the observed grasping, in which ADM and OP MEPs are facilitated differently and at different times during hand opening and closing respectively. Importantly, single responses were rather consistent across all subjects of the study and with the averaged response pattern. This is a relevant point because the absence of a grasping pattern in the averaged responses could otherwise be seen as the result of a de-synchronization of responses in single subjects due to the visual uncertainty of peripheral vision, which could have caused a temporal shift between perfectly good grasping motor resonant responses of different subjects, blurring the effect in the common pattern. But this was not the case. In contrast, most single subject responses of the central observation study (Borroni et al., 2011) reflected a clear grasping pattern of MEP facilitation.

The unexpected facilitation of OP MEPs during the observation of the impossible grasping action in peripheral vision, compared with the absence of facilitation in central vision, also deserves a comment. In fact, the lack of visual details in peripheral observation appears to have lead subjects into a perceptual error (as also supported by subjects' answers in the post-experimental questionnaire), in which the supine hand performing an impossible movement was seen as a prone hand performing a normal movement (while the absence of modulation in OP MEPs during central observation was consistent with the clearly visible thumb, which in the impossible movement is never flexed). With fewer details available in peripheral vision, the motor simulation underlying the resonant response relied more heavily on internally generated information, rather than being totally externally guided (Chambon et al. 2011; Vogt et al., 2013). In other words, because the natural grasp is a more familiar, canonical (Palmer et al., 1981) condition for the hand, subjects tended to see the little finger extending to grasp the ball in the forefront of the video as if it were the thumb, and to resonate according to their internal representation of what a hand is and how it normally moves. As a consequence, the OP motor resonant response during observation of the impossible grasping is identical to that evoked by observation of the normal grasping.

Data from the present experiment did not confirm the second hypothesis of the study, namely the idea that the visual information available during peripheral action observation, though reduced compared to central observation, could be utilized to evoke a resonant response encoding the gist of the visual scene, i.e. the goal of the observed action. If that had been the case, the expected pattern of MEP modulation should have encoded only the actual *grasping* action, i.e. the opening and closing phases of the hand movement (d2 and d3), while results show that MEPs in both muscles are facilitated at all interactive delays (d2, d3 and d4). With the lower kinematic accuracy imposed by peripheral vision it would not have been reasonable to expect the same accurate pattern of ADM and OP MEP facilitation recorded in central vision, because in the observed video hand opening and closing are separated only by 600ms and subjects are probably not seeing the moving hand clearly enough for this fine temporal resolution. However MEP facilitation should still have shown some attempt at reflecting the grasping action, with the activation of both muscles and in only in both *grasping* delays, reflecting the compromise between decreased visual accuracy and goal encoding. Instead MEPs are facilitated also at the last delay, which occurs at the very end of the video, a long time (1400ms) after the grasping action is concluded. This suggests that the responses evoked by peripheral action observation reflect a rough and inaccurate activation of motor circuits, rather than a true resonant copy of the motor program encoding the observed action. It is however worth noting that the modulation of the primary motor cortex is still linked to the observation of the hand interacting with the ball, given that there is a significant facilitation of the three interactive delays compared with the first static delay, when the hand is not directly interacting with the ball yet.

During action observation visual information from the occipital cortex reaches inferior parietal (BA40) and ventral premotor (BA6) areas, forming the well described parieto-frontal human action-observation network (Rizzolatti et al., 1996; Grèzes et al., 2001; Cabinio et al., 2010), where it has been suggested that an embodied motor representation of the observed action is generated, with its more abstract goal (Gallese 2007). From the ventral premotor (vPM) cortex, motor information continues to the primary motor cortex where it shapes a motor resonant response (Borroni et al., 2008). Many different studies have shown evidence supporting the hypothesis that the pattern of activation of premotor and parietal circuits during action observation encodes the goal of the observed action (Gallese et al., 1996; Kilner et al., 2004; Fogassi et al., 2005; Hamilton & Grafton, 2006; Rizzolatti & Sinigaglia, 2010) and this interpretation was inspired by the role that these areas play in action execution. Therefore, reflecting the goal of an observed action could be seen as a logical function of M1, which receives visuo-motor information from the premotor cortex. However, encoding the kinematic aspects of an observed action is much more consistent with the actual motor functions of M1 which, by virtue of its low position in the motor hierarchy, is typically involved in aspects of movement more immediately concerned with choice of muscular synergies, temporization of muscle activation, and force production, rather than its intention or ideation. Thus, it is precisely because M1 controls kinematic aspects of movement during active performance, that during action observation we expect it to encode information at this same level, embodied through the mirroring of the specific muscular and temporal details of the movements comprising the observed action. In this context, the activation of M1 during action observation could play a critical role in facilitating its repetition during imitation and motor learning (Iacoboni et al., 1999; Rizzolatti et al., 2001; Mattar & Gribble, 2005; Vogt et al., 2007). In the present experiment, during action observation in peripheral vision the visual information that reaches the parieto-frontal network lacks the high frequency spatial resolution of kinematics that is only available in central vision, and does not evoke a resonant response useful for goal-coding in M1. However, the visual information *per se* contains enough information to allow most observers to recognize the goal of the observed action (see post-experimental questionnaire). Whether this recognition is actually realized through an embodied simulation in the premotor-parietal action observation network, or as a result of different cognitive processes in other, associative cortical areas (where the uncertain visual information could be combined with an internal model of grasping based on previous experience, Vogt et al., 2013), cannot be resolved here. Our data show that even assuming that an abstract motor representation of the observed grasping action and its goal had indeed been created in the action observation network, the corresponding information for generating a grasping motor program was not transferred to M1. Instead, in M1 “what you see is what you get”: consistently with the poor kinematic detail available from peripheral vision, the pattern of excitability modulation in this cortical area encodes only very roughly the activation of hand muscles involved in grasping.

Several studies have shown that the neural resources for perception of biological motion are concentrated in the central region of the visual field (Brown et al., 2005; Larson & Loschky, 2009; King et al., 2010;) and that peripheral vision contains less precise spatial and temporal phase information than central vision (Azzopardi & Cowey, 1996), increasing the uncertainty of biological motion perception (Ikeda et al., 2005; Shapiro et al., 2011). On the other hand, the low spatial frequency information available in peripheral vision may actually be advantageous in the recognition of the gist of a visual scene (Larson & Loschky 2009), rather than in the recognition of specific objects, which requires higher spatial frequency resolution. In our study, the majority of subjects had in fact correctly recognized the goal of the action seen in peripheral vision, i.e. a hand grasping a red object (even when the grasping was done in an impossible way), but many were uncertain about the exact identity of the object being grasped (about one third reported seeing an apple instead of a ball). In this context it is difficult to argue for a functional role of M1 resonant responses in peripheral vision, since the ambiguous kinematic information they provide appears to be of limited use in facilitating the precise repetition of observed actions, as would be necessary, for example, during imitation for motor learning. However, during any kind motor learning two parallel strategies are necessary. On the one hand, one must allocate resources to the acquisition of precise information regarding specific details of the movements composing the action to be learned, so as to be able to replicate them, while simultaneously contextualizing the fine scale of the single movements in the larger scale of the entire action. This view is consistent with the results of the present experiments and the complementary roles of central and peripheral vision responsible, respectively, for analyzing the details of objects and scenes vs. scanning the environment for changing conditions and initiating quick responses (Johansson, 1977; Palmer & Rosa, 2006). In this more ecological perspective when an action appears in peripheral vision, evokes a rough and inaccurate subliminal activation of motor circuits, the natural responses might be to either shift one's gaze so as to observe the action in central vision (Yarbus, 1967; Wilson & Knoblich 2005), where a more accurate motor resonant response can be generated, or to keep it in periphery if it is complementary to a different action that is already engaging the resonant action observation network.

CHAPTER 4

MOTOR RESONANT RESPONSE IN CENTRAL AND PERIPHERAL VISION: THE ROLE OF ATTENTION AND COGNITIVE LOAD

4.1 INTRODUCTION

Observation of others' actions evokes a motor resonant response, i.e. a subliminal activation involving many different levels in the observer's motor pathways, from parietal to premotor and motor cortices all the way to the spinal cord (Rizzolatti & Sinigaglia 2010; Rizzolatti & Craighero 2005, Borroni et al.2008, 2005). In primary motor cortex (M1) this activation reflects the motor program encoding the observed actions and is characterized by a high level of muscular and temporal specificity. Specifically, studies with transcranial magnetic stimulation (TMS) have shown that observation of a hand grasping an object elicits a motor resonant response, i.e., a pattern of motor-evoked potential (MEP) facilitation of the same muscular groups and with the same time course as in the observed grasping of that object (Gangitano et al. 2001; Press et al. 2011; Cavallo et al. 2012, 2013; Sartori et al. 2012; McCabe et al. 2014, for a review see Naish et al. 2014). A previous study from our group showed that when subjects observed the same grasping action performed with either a natural movement ("palmar" finger flexion) or with an impossible movement ("dorsal" finger flexion) MEPs in the Opponent Pollicis (OP) and Abductor Digit Minimi (ADM) muscles were facilitated in a pattern consistent with the activation of these muscles during the execution of each observed movement (Borroni et al. 2011). Results from all the above studies suggest that motor resonance in M1encodes movement kinematics of the observed actions.

Motor resonance is a well-established phenomenon and various hypotheses and criticisms about its function have been proposed (Rizzolatti and Craighero, 2004; Kilner and Frith 2007; Hickok 2009). However, physiological studies focused on the cognitive and perceptual conditions under which MR happens are few and far apart. For example, usually in action observation studies subjects are allowed or even required to pay full attention to the observed action, which is always viewed in central vision. However, from a more naturalistic point of view, this is not the most common circumstance; on the contrary, in daily life people are often exposed to several simultaneous actions, which cannot all be in central vision or equally relevant or interesting to them, and thus will not receive the same amount of their attentive resources. In the present paper we set out to analyze the interaction between location in observers' field of view and deployment of their attentive resources, during the observation of actions performed by others.

Evidence from a few behavioral and neuroimaging studies shows that even in central vision the activation of the motor system during action observation is not an "all or nothing" event, occurring every time an action falls in the visual field of an observer, but that it can vary in scale and shape according to attentive resources available to the observer (Bach et al. 2007; Muthukumaraswamy et al., 2008; Chong et al., 2008; 2009; Perry et al., 2010; Woodruff et al., 2013; cfr. Chapter 2). For example, behavioral experiments on the automatic imitation effect – i.e. the automatic activation in the observer of motor representations normally involved in the execution of the observed action - show that in reality such process is not so automatic, but requires attention (Bach et al. 2007). Moreover Chong et al. (2009) showed that when coding of an observed action is hindered by adding a demanding perceptual secondary task, the automatic imitation effect disappears. Consistently, in the study presented in Chapter 2, in which the resonant response was quantified in a more direct manner by H-reflex technique, a decrease of amplitude of CS excitability of motor pathways necessary to execute the observed action was found when attention is partially diverted from it.

These studies clearly show that the activity of the motor system during action observation in central vision depends on the load of concurrent perceptual and cognitive activities and consequently on the availability of the attentive resources needed to process the observed action. In peripheral vision the task is further complicated by the intrinsic reduction of visual acuity and of allocation of attentive resources. Although it is well known that peripheral vison plays a fundamental role in the recognition of general aspects of a scene and it allows also the recognition of biological motion (Gibson et al. 2005; Thompson et al. 2007; Gurnsey et al. 2008; Larson and Loschky 2009), the possibility that actions located in the peripheral field may evoke motor resonant responses has become subject to investigation only recently (Sartori et al. 2013, see Chapter 3). The study presented in Chapter 3, showed that observation of a grasping action in peripheral vision elicits a generalized pattern of activation in M1, much less specifically organized than the precise modulation recorded during observation in central vision (i.e. the facilitation of ADM and OP MEPs doesn't reflect the precise timing of the activation of these muscles during hand opening and closing). In that study, we have chosen to keep the analysis of visual and attentive influences on motor resonance separate; therefore the experimental approach and results of the study were focused on the contribution of lower visual acuity in the peripheral field to the development of resonant responses. In the present study the experimental focus was shifted to the contribution of attention, since actions viewed in peripheral vision, in addition to being subject to less accurate vision, intrinsically receive less attention with respect to those viewed in central vision, where attentive resources are automatically deployed (Larson and Loschky 2009).

In order to clarify the role of attention we first converted the experiment of action observation in peripheral vision (cfr. Chapter 3) in a covert attention paradigm (Posner, 1980), in which subjects had to pay endogenous attention to the grasping actions showed in peripheral field (Experiment 1). Interestingly, our results showed two different results for the observation of the natural and impossible movements. In order to explain this difference we carried out two other experiments in which we evaluated the role of the selective attention (Experiment 2) and cognitive load (Experiment 3) in the generation of motor resonance responses.

4.2 **GENERAL METHODS**

A total of 64 healthy adult volunteers (34 females, average age 23 ± 1,2) took part in the study, after approval by the local Ethics Committee and written informed consent of each subject. 29 subjects took part in Experiment 1, others 16 in the Experiment 2, 19 in the Experiment 3. All subjects had normal or corrected-to-normal vision, no history of neurological disorders or contraindication to TMS. All were right handed according to the standard Edinburgh Handedness Inventory (Oldfield, 1971).

Different groups of subjects observed a natural or an impossible grasping. The natural movement video clip was identical to the impossible one, except just for the frames in which the avatar grasps the ball using an impossible sequence (see Chapter 3). Both the videos started with the right hand of the avatar moving from its resting position, along the avatar's body, to the ball.

In all the experiments motor-evoked potentials (MEPs) were used to measure the excitability modulation of cortical and spinal motorneurons during observation of the grasping action. All the subjects, sitting in a comfortable armchair with prone hands resting on lateral supports, were asked not to move and to observe the video on a 17" high-resolution computer screen placed at eye level at a distance of 1m. MEPs were evoked by single-pulse TMS of the hand area in the left M1 of right-handed volunteers and were recorded simultaneously from the right Opponens Pollicis (OP) and Abductor Digiti Minimi (ADM), two muscles normally utilized during the grasping action for finger closing and opening respectively. MEPs were elicited at different delays during the observed action (0s=beginning, 1s=hand opening, 1.6s=hand closing; 3s=ball lifting) by single-pulse transcranial magnetic stimulation (TMS) of the hand area in the left M1 and recorded from the right OP and ADM muscles. MEPs were recorded with self-adhesive bipolar surface electrodes over each muscle belly. Electromyographic signals were amplified, filtered (10Hz to 1kHz) and digitally converted (sampling rate 5kHz). The head of subjects was restrained by a comfortable pillow wrapping around the neck and supported by a fixed head rest. A mechanical arm held a figure-of-eight-shaped coil connected to a magnetic stimulator (Magstim 200, Magstim Company Limited, Whitland, Wales, UK; maximal power 2.2 T). The coil was positioned and fixed on the left M1 so as to activate both selected muscles, and the stimulator output was set at about 110% of the motor threshold of the less excitable muscle (defined as the intensity giving 3 MEP responses out of 6 stimuli). The excitability time-course was explored at four relevant randomized delays from the onset of the close-up part of the video: d1) 0s = Baseline, avatar's hand just beginning to move; d2) 1s = Opening phase, moment of maximal finger aperture during the grasping action; d3) 1.6s = Grasping phase, moment in which the avatar's fingers grasp the ball and d4) 3s = Holding and lifting phase, moment in which the avatar's hand lowers the ball on the table after having lifted it.

For each subject a total of 40 presentations were obtained, so that overall 10 MEP responses were recorded at each of the 4 delays (0, 1.0, 1.6 and 3.0 s). Presentations were grouped in 2 blocks of 20 trials, and subjects were instructed that they could rest at the end of each block. Within each block of 20 trials, MEPs were evoked and recorded 5 times at each specific delay, chosen in a semi-random order (completing a set of 4 delays before starting the next set) by the data acquisition program. In order to do this, at the very first frame of the close-up video a synchronizing signal in the video was fed into the computer, which triggered both TMS stimulator and acquisition program at one of the selected delays. Presentations were spaced by 8s dark screen intervals (resulting in inter-stimulus intervals lasting a minimum of 10s). To exclude the possibility of voluntary or involuntary mimic activity of the observer, the background electromyographic activity was monitored in the muscles throughout the whole video presentation.

4.2.1 *Data analysis*

In each subject MEP responses for each muscle were measured as peak-to-peak amplitude; MEP values in all 4 delays were normalized to the average of values in the first delay (Baseline, time = 0s, avatar's hand just beginning to move) and then normalized responses were averaged across all subjects. Data were analyzed with repeated-measures ANOVA and t-test post-hoc multiple comparisons. For all statistical tests significance level was set at $p<0.05$, Bonferroni corrected to $p<0.008$ for post-hoc analysis of differences between delays. Data were acquired and recorded using LabView10 and stored for later analysis; statistical analysis was conducted using SPSS software (SPSS Inc, Chicago, USA).

4.3 **EXPERIMENT 1. COVERT ATTENTION**

The aim of Experiment 1 was to understand the role of endogenous attention in motor resonant responses during observation of action in peripheral vision. Utilizing the same paradigm of a previous experiment of Leonetti et coll. 2015 modified into a covert attention paradigm (Posner, 1980), data from the two studies could be directly compared in the same statistical model: while in the original experiment subjects were asked to focus their attention on a central fixation point while grasping action was presented in their peripheral field of vision (passive peripheral vision), in the present experiment subjects were instructed to actively pay close (covert) attention to the content of the video shown in peripheral vision, but without shifting their gaze from the same central fixation point (Fig.4.1).

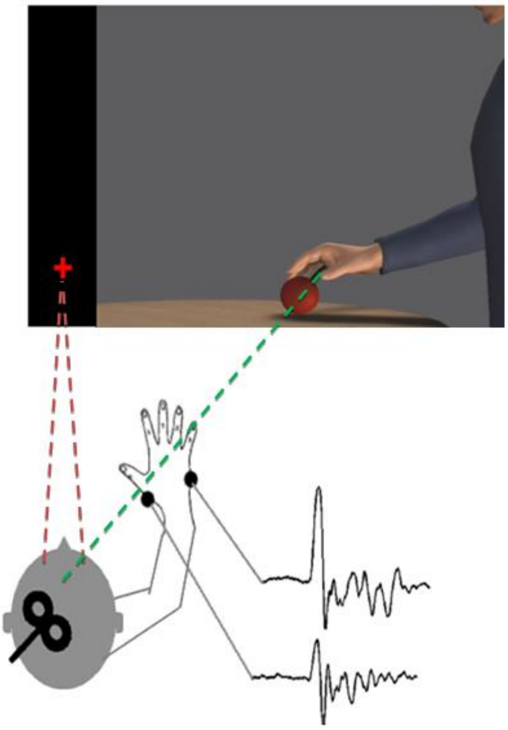

Fig 4.1. Experimental setting. Subjects were asked to look at the cross (dashed red line) but to pay close attention to the movement showed in peripheral vision (dashed green line), while CS excitability was recorded from OP and ADM muscles.

Since covert attention improves performance in peripheral vision (Hein et al., 2006;Yeshurun et al., 2008), we hypothesized that the observation of actions viewed in the peripheral field and covertly attended would improve visual perception, thus also improving the specificity of the resonant response, perhaps even restoring the accuracy measured in central vision (Borroni et al. 2011). In passive peripheral vision MEP facilitation recorded in the OP and ADM muscles (see Chapter 3) was found to be inaccurate in terms of muscle selection and timing of activation during the observation of the different phases of the grasping action: it was virtually identical at all interactive delays (d2, d3, and d4), irrespective of the muscle (flexor or extensor). This MEP modulation is not consistent with the motor program corresponding to the observed grasping, in which ADM and OP MEPs are facilitated differently and at different times during hand opening and closing respectively (Borroni et al. 2011).

While we expect that focusing attention on the action improves the specificity of MEP facilitation, the possibility exists that it may not compensate completely the limits of peripheral vision. Therefore we hypothesize that the results of Experiment 1 will show either 1. a total recovery of resonant response specificity in OP and ADM or 2. the facilitation of the two muscles only during the most salient grasping phase of action (opening and closing phases) instead of generally during all interactive delays (opening, closing and lifting) as found in Leonetti et al. 2015.

4.3.1 *Experimental paradigm*

In Experiment 1 subjects (n=14) observed a 5s video clip of a natural motor sequence showing an avatar grasping a small red ball positioned on a table, while the excitability modulation of cortical and spinal motorneurons controlling OP and ADM muscles was measured (for details see General Methods). Different subjects (n=15) observed an identical video clip, except just for the frames in which the avatar grasps the ball using an impossible sequence (cfr. Chapter 3). Both videos started with the right hand of the avatar moving from its resting position, along the avatar’s body, to the ball. Subjects were instructed to pay close (covert) attention to the action showed at 10° horizontally in their peripheral vision, while maintaining their gaze on a fixation point (red cross) in the left lower corner of the computer screen (Fig.4.1). In order to confirm that subjects' gaze did not move from the fixation point, eye movements were monitored by electro-oculography (EOG) throughout the whole video presentation. After the experiment, we also investigated whether subjects had recognized the movement by asking them to describe and reproduce what they had seen.

4.3.2 *Results*

Observation of the natural and impossible grasping actions in peripheral vision with covert attention condition resulted in a modulation of the excitability of the primary motor cortex very different from the modulation measured during observation of the same actions in passive peripheral vision (Fig. 4.2). Normalized OP and ADM MEP modulation data were analyzed by means of a four way repeated measure ANOVA, with delay (d1, d2, d3 and d4) and muscle (OP and ADM) as within-subject factors and movement (natural and impossible) and attention (passive and covert attention) as between-subject factors.

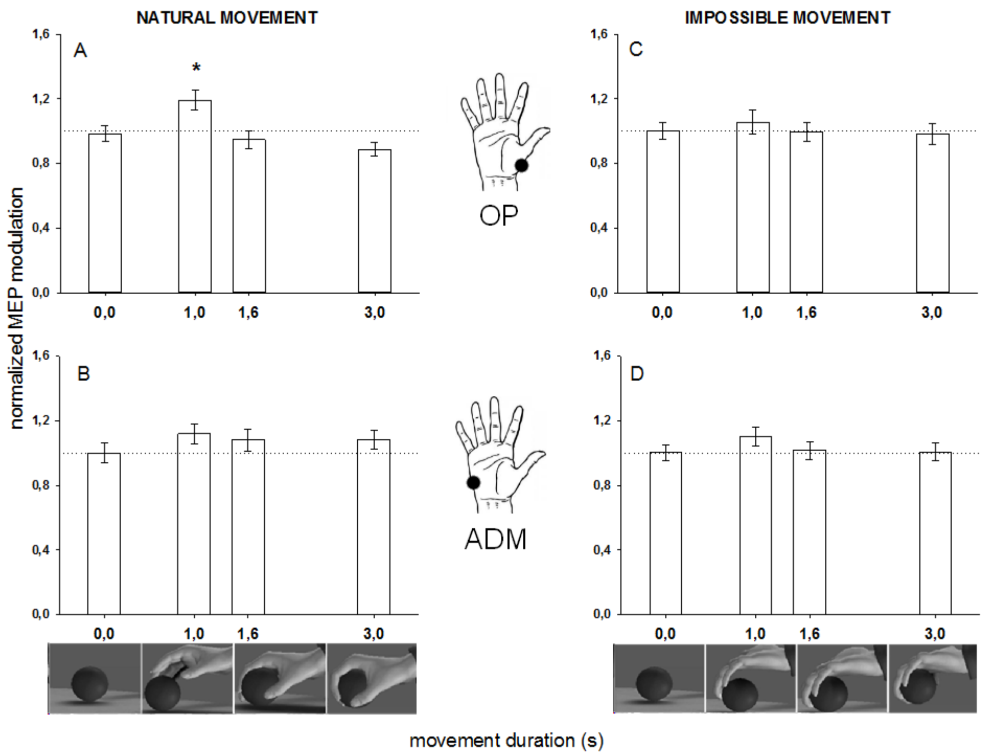

Fig. 4.2. MEP modulation during action observation in covert attention condition. MEP amplitude variations in OP and ADM muscles (vertical bars, means ± SE) recorded at four selected delays (d1=0s, d2=1s, d3=1.6s and d4=3s) during the observation in peripheral vision of the avatar's natural or impossible grasping action. Small figures on the bottom are the video frames illustrating positions of the hand at the four delays. OP MEPs were significantly facilitated (*) during the delays 1 (initiation phase of the grasping) only in the natural movement.

Results revealed a significant main effect of the attention factor ($F_{(1,673)}$=28.284, p<.001) indicating that, considering all the delays together in both muscles and in both movements, MEP modulation was different between the passive and covert attention conditions. A significant main effect for the delay factor ($F_{(3,673)}$=13.704, p<.001) and a significant delay/attention interaction ($F_{(3,673)}$=7.705, p<.001) also emerged, suggesting that considering both muscles in both movements, MEP modulation was different at the four delays between the two attention conditions. Differently from the passive condition (see Chapter 3), during observation of the grasping in covert attention condition, the excitability of M1 and of the corticospinal projections results facilitated only during the vision of natural grasping in the OP muscle, i.e. only in d2 (hand opening delay) compared with all other delays (t-test p<0.008 Bonferroni corrected).

This is a puzzling result: while, as expected MEP facilitation in the OP muscle is sharpened to a single delay by attention, in analogy to the facilitation of this muscle during observation of the same grasping action in central vision (Borroni et al. 2011), it occurs at the “wrong” time (d2 instead of d3). Also puzzling is the total absence of any facilitation in either muscle during observation of the impossible grasping.

In order to investigate the conscious perception of the actions observed in the two videos, at the end of the experiments all subjects were asked to describe with words what they had seen and then to physically repeat it. Subjects observing the natural movement referred seeing a hand grasping action and imitated accurately the grasping action. Subjects observing the impossible action reported seeing an unusual grasping movement (for example the hand approaching the object with a strange orientation in space) and two of them reported correctly the impossible grasping movement.

4.3.3 *Discussion*

With Experiment 1 we tested the hypothesis that deploying (covert) attention to the action observed in peripheral vision to the observation of a grasping action in peripheral vision would improve the accuracy of the evoked motor resonant response, possibly even restoring the accuracy measured in central vision (Borroni et al. 2011). The results of this simple experiment are rather complex and support the above hypothesis only partially.

First of all, the accuracy of the motor facilitation response in the OP muscle was indeed improved by attention, since it is now recorded only in one of the central delays, corresponding to the initiation of the grasping action (Fig.4.2), rather than being generalized to all dynamic delays (see Chapter 3). The fact that addition of endogenous attention to the action observed in peripheral vision results in a more specific MR resonance appears consistent with evidences showing that covert sustained attention improves performance in texture segmentation and contrast sensitivity tasks at peripheral location (Ling & Carrasco 2006; Yeshurun et al., 2008) and temporal order judgment (Hein et al. 2006). However, OP acilitation occurs at the “wrong” delay (d2, Fig.4.2). In fact during observation of the same grasping in central vision OP MEPs are normally facilitated (consistent with the utilization of this muscle in grasping execution) in d3 (hand closing around the ball), while d2 corresponds to the observation of the hand opening phase, during which the thumb opponent is not active (Borroni et al. 2011). The fact that OP responses significantly occur too early (instead of being equally spread over the two central delays, as would be expected if it happened by chance) points to a systematic "error". This error in timing appears to be an anticipation of the resonant response, probably due to the low visual resolution of peripheral vision and driven by the onset of the hand grasping movement. A parallel result is that motor resonance in the ADM muscle is greatly reduced, so that even though there appears to be some facilitation at d2, it never reaches the level of significance even with 15 subjects.

We interpret the above results as the consequence of the attentive process selecting only the most salient component of the perceptual stimulus, i.e the thumb, in a situation of difficult perception, and therefore producing a facilitation only in the correspondent muscle (OP). To our knowledge the possibility that selective attention could focus motor facilitation in a single muscular group has not yet been investigated. Bach et al. (2007) used a visuomotor priming task in order to evaluate whether spatial attention has a role in motor facilitation of the hand or the foot during action observation task. Interestingly a priming effect was found only when participants' spatial attention was directed toward the corresponding limb in the displayed image, with faster foot responses when they payed attention to the leg and faster hand responses when they pay attention to the hand compared with conditions in which they are focused on the head of the observed model. However there are no studies (not even in central vision) investigating in a more detailed scale (muscular selection) and directly (measuring the activity of motor cortex) the role of selective attention in MR response. Experiment 2 (see below) was designed to test the hypothesis that selective attention can focus motor resonant response on a specific muscular group, isolating the resonant response to the specific motor pathway.

Secondly, no significant MEP modulation was recorded in either muscle, during the observation of the impossible movement (Fig.4.2), while again in the passive peripheral vision condition both OP or ADM were facilitated at all dynamic delays (Leonetti et al. 2015). We explain these data as the result of the high cognitive load introduced by the decoding process of the impossible movement: as subjects pay (covert) attention to the peripheral scene the oddity of the impossible grasping action becomes more evident (than when observing without attention, or simply observing the natural movement), forcing a more complex inferential process in order to decode the intricacy of the movement. This is also confirmed by the results of the post experimental questionnaire: while all subjects observing the natural grasping reported without hesitation having seen a hand grasping a ball, half of the subjects observing the impossible movement reported having seen a hand grasping, but also having difficulties in identifying the exact manner in which it is performed. Moreover, results of the questionnaire of the passive peripheral vision study (Chapter 3) indicated that when subjects are not instructed to pay attention, they tend to be less sure about the goal of the observed action (several subjects referred having seen actions different from a grasping, such as bouncing or stroking). Instead, in the present experiment no subjects had doubt about its goal (i.e a grasping), despite explicated doubts about movement kinematics, denoting a stronger voluntary effort to understand the action. These results suggest that subjects who observed the impossible movement in the covert attention condition were performing a more demanding perceptual task, which subtracted cognitive resources to the motor resonance process. In other words, the cognitive effort necessary to decode the kinematics of the impossible movement did not leave sufficient neural resources for a proper motor resonance response. This is consistent with studies showing that when the coding of an observed action is hindered by the adding of a secondary task so that no cognitive resources remain available to process the observed action, the motor facilitation effect decreases (Chong et al, 2008, 2009). In order to verify if the present results are explained by a cognitive load account we carried out the Experiment 3 (see below), in which we manipulated the load of the perceptual processing of the impossible movement.

4.4 **EXPERIMENT 2. SELECTIVE OVERT ATTENTION**

The main result of the observation of the natural movement in the covert attention condition is that motor facilitation was recorded only in the OP muscle, and we interpreted this result as the consequence of an automatic allocation of selective attention to the most salient effector (the thumb). In Experiment 2 we explore this, examining whether and to what extent selective attention can influence the resonant facilitation of the individual muscles during observation of an action, even in central vision. The hypothesis tested in this experiment is that selective attention focused on the movement of the thumb evokes a motor resonant response only in the OP muscle, even though the whole hand grasping movement is observed.

4.4.1 *Experimental paradigm*

Subjects (n=16) were asked to observe, in central vision, the natural grasping video clip, and to pay close attention to the movement of the thumb. MEPs were recorded in the OP and ADM muscles (see General Methods).

4.4.2 *Results*

Observation of natural grasping with selective attention focused only on the thumb, evoked an exclusive modulation of MEPs in the OP muscle (Fig.4.3).

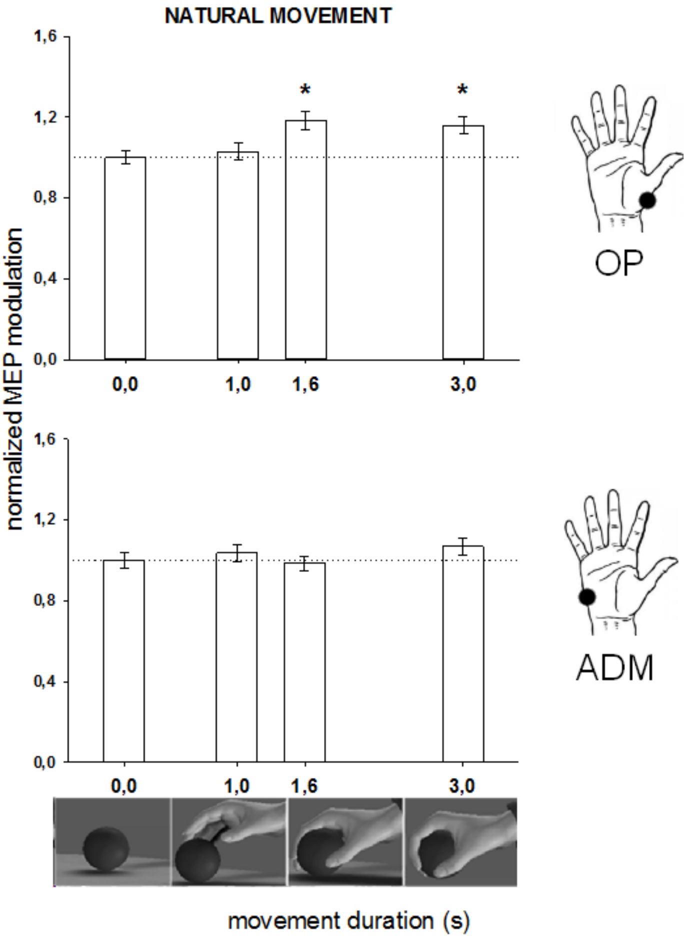

Fig. 4.3. MEP modulation during grasping action observation in central vision when subjects are asked to focus their attention on the thumb. MEP amplitude variations in OP and ADM muscles (vertical bars, means ± SE) recorded at four selected delays (d1=0s, d2=1s, d3=1.6s and d4=3s) during the observation in central vision of the natural grasping action. Small figures on the bottom are the video frames illustrating positions of the hand at the four delays. MEPs were significantly facilitated only in OP muscle during the delays 3 and 4 (*; grasping and holding phases of the action).

Thus the pattern of excitability in of the primary motor cortex resulted very different from the modulation measured when attention is focused on the whole hand (Borroni et al. 2011); data from this experiment were directly compared to data from the latter study. Normalized OP MEP modulation data were analyzed by means of a three-way repeated-measures ANOVA, with delay (d1, d2, d3, d4) and muscle (OP, ADM) as within-subject factors and focus (whole hand, thumb) as between-subject factor. A significant main effect was found for the delay factor ($F_{(3,1368)}$ =5.176,

$p<.001$) and a significant delay/focus interaction ($F_{(3,2559)}=7.278$, $p<0.01$) also emerged suggesting that, considering both muscles together, MEP modulation was different at the four delays between the two focus conditions. Both a muscle/delay and a muscle/delay/focus interactions were significant ($F_{(3,456)}=17.778$, $p<.001$ and $F_{(3,456)}=5.710$, $p<.001$), further indicating that MEP amplitude recorded from the two muscles were modulated differently in the two focus conditions. Multiple comparisons performed between different delays in the same muscle revealed that excitability of primary motor cortex and corticospinal projections results facilitated only for the OP muscle during the observation of delay 3 and delay 4 (d1 vs d3, $p<0.001$; d1 vs d4, $p<0.01$; Fig.4.3). In addition, multiple comparisons between the same delays in the two different focus conditions were performed for each muscle. In the OP muscle a significant difference between "whole hand" and "thumb" condition was found only for delay 4 ($p<.008$), while in ADM muscle a significant difference was found for delay 2 ($p<.008$). Results of Experiment 2, when subjects' attention is overtly focused only on the thumb in central vision, are therefore twofold: 1. MEP modulation in OP muscle is facilitated as expected in the hand closing delay (d3), but surprisingly also in the holding and lifting delay (d4). 2. MEP modulation in ADM muscle is absent.

4.4.3 *Discussion*

Results of Experiment 2 confirm the hypothesis that selective attention can isolate single digit (thumb) and evoke a motor resonant response in the relative individual muscle (OP), even when the action of the whole hand is directly observed. They also support our interpretation of the absent ADM modulation in Experiment1. We interpreted those data as the result of the automatic selection by covert attention of the thumb as the most salient effector in a grasping action, so that a motor resonant response was evoked only in the OP muscle, causing the ADM to be ignored. By instructing subjects to focus their attention on the thumb, the salience of this effector was experimentally manipulated and other resonant responses, in the ADM, were eliminated (Fig.4.3). These results provide physiological evidence in support of the behavioral data already discussed in Chapter 1 (Bach et al 2007) that show that visuospatial attention plays a role in selecting the facilitated limb (an hand or a foot) during action observation tasks. Moreover, they extend the experimental evidence to include an effect of selective attention at a smaller scale, i.e. of the individual components of the limb, such as the muscles of the thumb while observing the movement of the whole hand. Interestingly, if compared with results of MR response recorded during the observation of the same movement under natural observation conditions (i.e. in central vision without explicit attentional manipulation, see Borroni et. al 2015). Finally, the unexpected result that facilitation of OP MEPs is also recorded in the holding and lifting phase (d4, Fig.4.3) of the observed action suggests that the instruction to focus one’s attention on the thumb ends up manipulating also the duration of the motor resonant response, maintaining the facilitation as long as the muscle is active in the observed action. In contrast, the spontaneous resonant response during observation without specific instructions is limited to the dynamic phases of grasping (hand opening and closing; Borroni et al. 2011 and Fig.3.2).

4.5 **EXPERIMENT 3. COGNITIVE LOAD**

In Experiment 3 we examined whether by manipulating the level of cognitive load during observation in peripheral vision of the impossible movement we could replicate one of the results of Experiment 1, i.e. the fact that in the covert attention condition no significant MEP modulation was recorded in either OP and ADM muscle during the observation of the impossible movement. We interpreted these results according to a cognitive load account: subjects observing the impossible movement in peripheral vision with instruction to pay covert attention were performing a more demanding perceptual task (with respect to observing the natural movement), in which the effort of decoding the kinematics of the odd movement was subtracting cognitive resources to the motor resonance process. In Experiment 3 the cognitive load necessary for decoding the impossible grasping was lowered by allowing subjects to watch the video several times before the experiment. We reasoned that if subjects already knew the content of the video and the details of the unfamiliar movement, their cognitive effort when seeing it in peripheral vision would have been considerably reduced, thus freeing resources for motor resonance.

4.5.1 *Experimental paradigm*

In Experiment 3, subjects (n=19) watched the 5s video clip of the impossible grasping movement for 10 times in central vision-in order to familiarize themselves with the odd motor sequence; after that they were asked to observe the same video in peripheral vision in the covert attention condition, identical to Experiment 1. Knowing the exact kinematics of the impossible movement before the peripheral presentation allows subjects to observe the stimulus without uncertainty about its nature. The hypothesis of the experiment is that this manipulation, by lowering the load of perpetual processing, should free cognitive resources for the motor resonant process and restore normal responses in the ADM muscle (Borroni et al. 2011 and inset Fig.5). MEPs were recorded in the OP and ADM muscles (see General Methods). In order to confirm that subjects' gaze did not move from the fixation point, eye movements were monitored by electro-oculography (EOG) throughout the whole video presentation.

4.5.2 *Results*

Observation of the impossible grasping action in covert attention condition (as in Experiment 1), after lowering the cognitive load thanks to previous familiarization with the odd movement, restored normal facilitation of the ADM muscle (Fig.4.4).

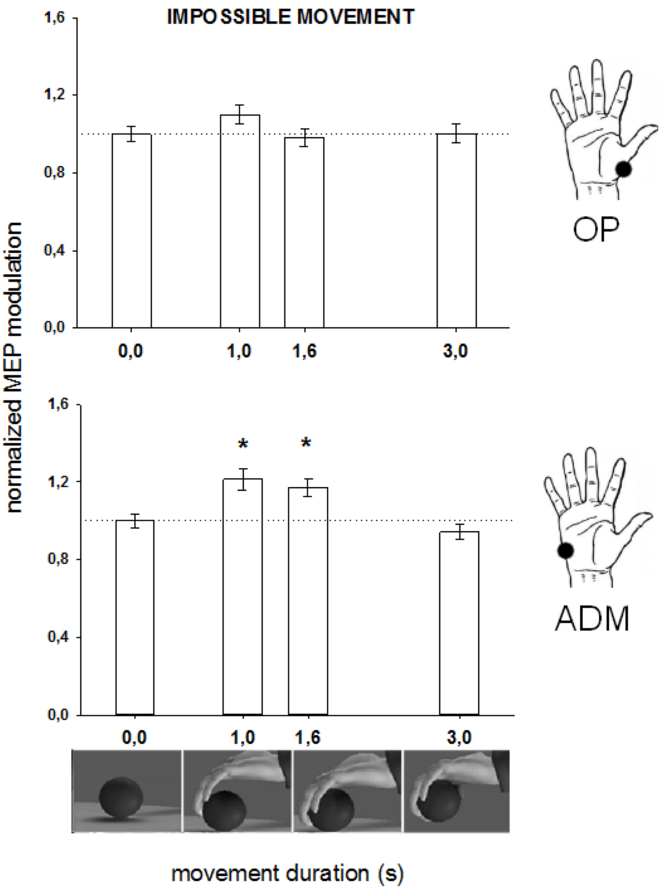

Fig.4.4. MEP modulation during action observation in covert attention condition with lowered cognitive load. MEP amplitude variations in OP and ADM muscles (vertical bars, means ± SE) recorded at four selected delays (d1=0s, d2=1s, d3=1.6s and d4=3s) during the observation in peripheral vision of the impossible grasping action. Small figures on the bottom are the video frames illustrating positions of the hand at the four delays. ADM MEPs were significantly facilitated during the central delays 2 and 3 (*; opening and grasping phases).

In order to verify whether a MEP modulation occurs when covert attention was deployed on a well-known impossible movement in peripheral vision (low cognitive load), normalized MEP modulation data were analyzed by means of a two-way repeated-measures ANOVA, with delay (d1,d2,d3,d4) and muscle (OP-ADM) as within-subject factors. A significant main effect was found for the delay factor and a significant delay/muscle interaction also emerged, indicating that MEP amplitude was modulated differently in the two different muscles. Multiple comparisons performed between different delays in the same muscle revealed that excitability of primary motor cortex and corticospinal projections results facilitated only for the

ADM muscle, during the observation of delay 2 and delay 3 (d1 vs d2, $p<0.001$; d1 vs d3, $p<0.003$). This result shows that, differently from the observation of an impossible movement in covert attention condition (high cognitive load; Experiment 1, Fig.4.2), reducing the cognitive load during the same task restores the portion of the response (ADM muscle) that was inhibited in the high load cognition.

4.5.3 *Discussion*

Results of Experiment 3 show that when sufficient cognitive resources are available the expected pattern of MEP facilitation is recorded in the ADM muscle even in the covert attention condition. This pattern of MEP modulation (i.e the facilitation of ADM muscle only in the two central phases and a lack of MEP modulation in OP) is expected based on the result of observation of the impossible movement in central vision (Borroni et al. 2011, Fig.3.2) and in fact the two patterns are virtually identical (Fig. 4.4). This result confirms our hypothesis that decreasing the cognitive effort necessary to decode the impossible movement ought to improve the quality of the motor resonant process and restore responses. Moreover it confirms our interpretation of the results of Experiment 1 that the low level of facilitation recorded in the ADM muscle during the peripheral observation of the impossible movement is actually hindered by deployment of covert attention, because in this case it subtracts cognitive resources from the motor resonance process.

FINAL CONCLUSIONS

Since James' ideomotor theory in the 19th century, numerous experimental evidences have shown that action observation and action execution are strictly linked; in particular, from the discovery of mirror neurons in macaque monkeys 25 years ago, many experiments have corroborated the original findings and given ideomotor theory a strong neurophysiological foundation. The recording, in non-human primates, of a population of neurons in motor areas which fire similarly during both execution and observation of the same action (mirror neurons), demonstrated that acting and seeing an action can share a common neural representational code. An important amount of behavioral and neurophysiological evidences have suggested that a similar mechanism is present also in human cortical motor areas forming the action observation network (AON), or putative human mirror system: when we observe somebody doing an action, our motor system is subliminally activated in a pattern consistent with the pattern encoding the motor program necessary to execute the same action (motor resonance) (Chapter 1).

Because in most studies in which motor resonant responses are recorded subjects are asked to just observe the action, without imitating or moving, many researchers have described this process as implicit (observers are not aware of the subliminal modulation in their motor pathways) and automatic (activated each time an action falls in our visual field, irrespective of other concurrent cognitive processes). However recent experiments show that behavioral responses (automatic imitation effect) and neural activity (EEG μ-rhythm suppression), typically associated with the activation of AON, seem to be dramatically modulated by the manipulation of attention or by cognitive strategies adopted by the observer. Some of these evidences clearly show that the effect of action observation on the motor system can be modulated by attentional manipulation, but fail to quantify precisely the effect of such modulation on observer's motor cortical activity . In other words, what remains to be determined is to what extent the motor resonance response, i.e. the subliminal activation of the same motor program encoding the observed action, is actually affected by the manipulation of subjects' attention. A parallel unanswered question is whether and how the motor resonant system encodes actions that appear in the peripheral field of view. In fact, in daily life most actions are seen in peripheral vision, where vision is far less accurate and where they don't automatically receive the same level of attention as in central vision. The aim of this thesis is to address these questions, assessing the consequences of manipulating attention and visual field, as well as their interaction, in the genesis of motor resonance responses measured as corticospinal excitability changes during action observation tasks.

In order to clarify the role of attentive processes, in the first study (Chapter 2) we assessed the specificity of motor resonant response in subjects who were asked to observe a cyclic flexion-extension movement of the hand, in 3 different attentive conditions: if attention plays a role in modulating the motor resonant response, we should obtain different levels of motor facilitation in the different experimental conditions. Results show that attention manipulation dramatically affects the gain of motor resonant responses while not affecting its muscular and temporal specificity: the amplitude of the H-reflex evoked in the flexor muscles of the wrist involved in the observed oscillating movement was significantly decreased when attention was partially diverted from the observed action, but maintained the correct cyclic time-course irrespective of the attentive manipulation.

This evidence is consistent with previous EEG experiments in which the diversion of attentive resources from the observed movement is associated with a lower suppression of μ-rhythm compared with conditions in which attention remains focused. Similarly in different experiments, using a dual task paradigm, a decrease of BOLD activity was found in the frontal node of AON when attention was heavily diverted from the observed action. Together with these evidences the results of the first study support the hypothesis that the motor resonant response is not an "all or nothing" event, which occurs every time an action falls in our visual field but, similarly to other visual processes, it can be modulated by top-down influences, such as selective attention.

In the second study (Chapter 3), the possibility that a motor resonant response is evoked by actions presented in the visual peripheral field was examined: excitability modulation of motor pathways which control hand muscles was measured in response to grasping actions viewed in near peripheral vision. Results, confirm the hypothesis that actions observed in peripheral vision are effective in modulating motor pathways excitability, but that such response is rough and inaccurate compared to central vision and does not reflect the motor program encoding the observed action. Consistently with the general notion that the decreased visual acuity in periphery is sufficient to discriminate only general aspects of movement, these data would suggest that due to limited kinematic accuracy, the subliminal motor responses recorded during peripheral observation of actions may code general aspects of observed actions (presence or absence of a limb movement), rather than their specific execution (how this movement is made).

Even though the results of the second study can be explained by the poor visual resolution of the peripheral field, it must be kept in mind that the lack of attention, (attention is automatically deployed with gaze), could also play a role in this process. This aspect has been examined in the third study (Chapter 4), in which excitability of motor pathways controlling the hand was assessed as in study 2, but in this case subjects had to explicitly focus their attention on the peripheral movement, without shifting their gaze (covert attention). Results of the first experiment of this study show that when peripheral actions are observed in condition of covert attention, the specificity of motor resonant responses is improved, but only in the most salient motor component of the grasping action (in the case of grasping, the movement of the thumb). The possibility that attentive processes can focus motor resonant response on only one effector was then tested in the second experiment of the third study, in which it was demonstrated that when subjects are asked to focus their attention on the thumb of the moving hand (in central vision), a motor facilitation is recorded only in the thumb opponent muscle, and not in the abductor of the little finger, as normally occurs during observation of the grasping action without the specific focalization of attention. These results are consistent with behavioral experiments showing that spatial attention plays a crucial role in selecting the facilitated limb during action observation tasks. The present results clarify the extent to which selective attention can affect and modulate the motor system during action observation: they suggest that visuospatial attention could play a direct role in selecting specific corticospinal neuronal populations innervating specific muscular groups.

A further interesting outcome of this third study is that addition of attention to the observation of a grasping action in peripheral vision, doesn't necessary always improve the specificity of motor resonant response. In the case of the peripheral observation of an impossible movement, paying covert attention seems to actually inhibit the motor resonance response. Since the impossible movement viewed in periphery is a difficult action to decode, this suggests that cognitive (inferential) visual decoding processes can interfere with the motor resonance process i.e. it appears to compete with a "non-mirror" processing of action. The proposed interpretation is that when the decoding of an action requires more inferential processing because of perceptual or contextual constraints, this cognitive activity comes into conflict with, and inhibits, mirror processing.

Altogether these findings tell us that if, on the one hand, a minimal level of attention is sufficient for a motor resonant response to develop a high level of temporal and muscular specificity when the action to be mirrored is simple and predictable, the process can also be completely inhibited when the decoding of the action requires demanding inferential processing. Thus we can conclude that the activity of the AON can be modulated by top-down influences, such as selective attention. Moreover these data suggest that attentive processes seems to play an important role in the selection of different kinds of visual computation active during the decoding of actions. This interpretation suggests the possibility for a dual action representation mechanism (mirror and inferential) in which the default mechanism of action decoding mediated by motor simulation in the AON (mirror), in conditions of perceptual complexity is substituted by an inference-based mechanism; the switch between the two mechanisms appears to be mainly mediated by attentive processes.

Finally, the function of the motor resonance process in primary motor cortex and the role of attention in this function must be briefly considered. While experimental evidence in support of a specific functional role for the activation of corticospinal pathways during action observation is still lacking, a reasonable and conservative hypothesis is that by subliminally replicating the motor program necessary to perform an observed action, the AON facilitates imitation and motor learning. Thus we can also hypothesize that a motor resonant response of higher quality (in terms of gain and temporal/muscular specificity) should allow better, faster, more accurate motor learning. Future studies should examine in more detail how the “quality” of motor resonant response during action observation is modulated by top-down processes (attention *in primis*), and whether and how this modulation can in fact affect motor learning processes.

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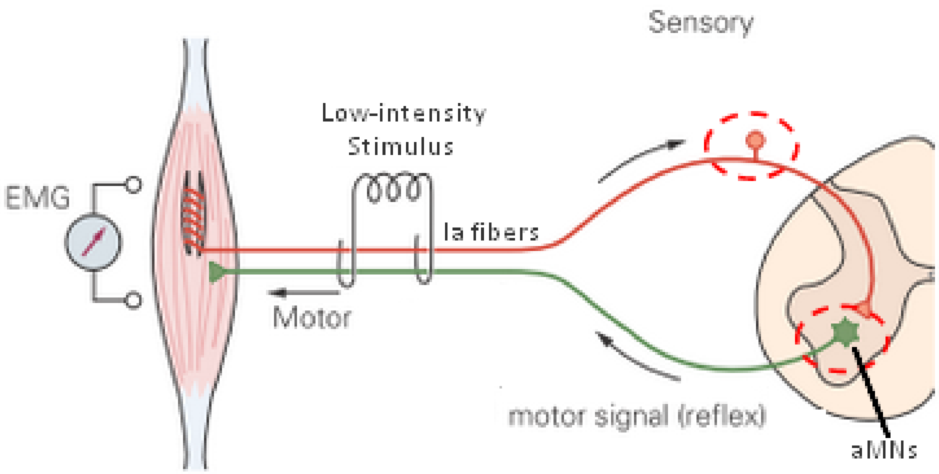
APPENDIX I

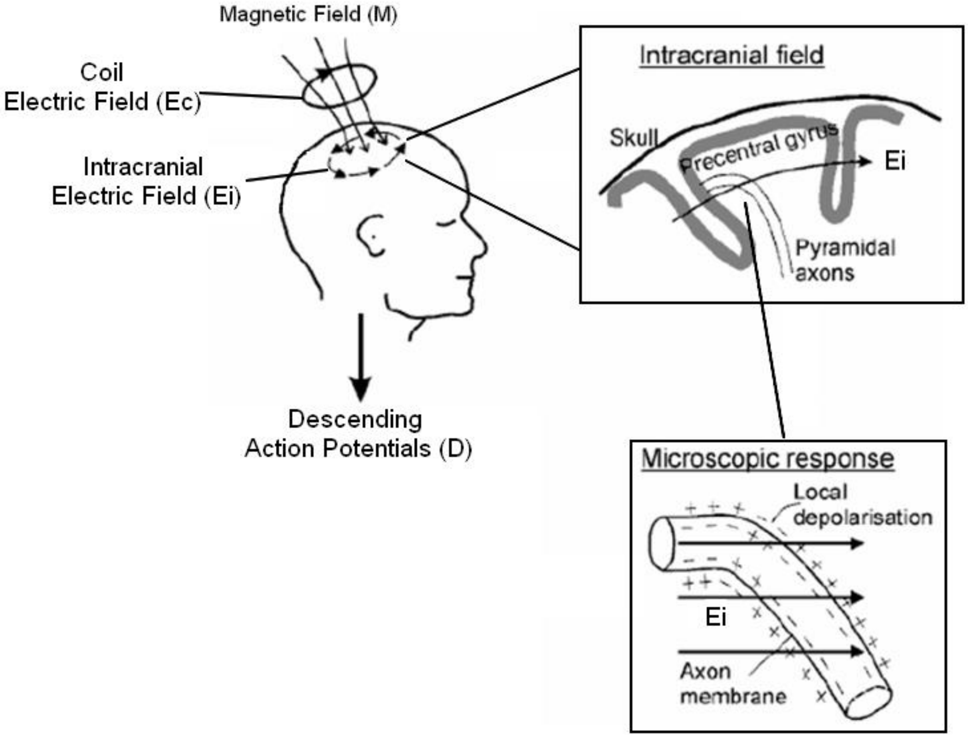
H-reflex

Eliciting an H-reflex in human subjects requires the cutaneous electrical stimulation of a mixed nerve. The technique exploits the anatomical arrangement of the stretch reflex, in which Ia afferent fibers from muscle spindles activate motorneurons to the same muscle monosynaptically, and is useful to monitor non-invasively the excitability of spinal motorneurons. For example, in the experiment presented in Chapter 2, H-reflexes were evoked in the flexor muscle of the right wrist (flexor carpi radialis, FCR), utilizing an electrical square pulse applied to the median nerve at the elbow. Due to the fact that Ia afferent fibers (red line in the figure) arising from muscle spindles have a greater diameter of the other afferent fibers in a mixed nerve, and thus a lower threshold, they are selectively activated by low-intensity stimuli. Activation of the Ia afferents results in action potentials that propagate towards the spinal cord. If the activity in the Ia afferents is sufficient to cause depolarization of the presynaptic Ia terminals, excitatory postsynaptic potentials (EPSPs) are elicited in motoneurons (αMNs, in green in the figure). If then these EPSPs are able to depolarize the αMNs to threshold (depending on the state of the MN membrane potential and on the size of the EPSPs), action potentials are generated causing a contraction in the FCR muscle, i.e an H-reflex. H-reflexes are therefore electromyografic recordings of the electrical activity preceding muscular contraction. The amplitude of the H-reflex evoked in a muscle depends on the number of motor units recruited by a given electrical stimulus in the pool of αMNs innervating that muscle. Crucially for the experiments in Chapter 2 if, due to the activation of motor cortex during action observation, corticospinal fibers depolarize near threshold a number of αMNs, these are more easily recruited by the same electrical impulse and the amplitude of the H-reflex increases. In this manner the amplitude of the H-reflex can be used as an indirect measure of corticospinal excitability.



Transcranial Magnetic Stimulation (TMS)

Using electromagnetic induction TMS allows for noninvasive stimulation of the motor cortex by simply placing a magnetic field generator (usually a coil of metal wire) next to subject’s head (over the precentral gyrus). A fast and strong electrical current is discharged into the coil (Ec in the picture), generating a magnetic field (M in the picture) up to 2.2T orthogonally oriented to the plane of the coil. Such magnetic field passes through the bone of the skull and produces, in turn, an electric field (Ei in the picture), once again orthogonal to the direction of the magnetic field. Cortical motorneurons located in the most intense part of the induced electric field will be depolarized leading, through a number of corticocortical synapses, to the firing of action potentials in axons descending to the spinal cord (D in the picture). Similarly to H-reflexes, MEPs are electromyografic recordings of the electrical activity preceding muscular contraction, and the amplitude of MEPs evoked in a muscle depends on the number of motor units recruited by a given TMS stimulus in the pool of cortical (and consequently spinal) motorneurons innervating that muscle. For example in the experiments presented in Chapters 3 and 4 TMS was used to stimulate the hand area in the left primary motor cortex, while MEPs were recorded in the right opponens pollicis and abductor digiti minimi muscles. Crucially for the above experiments if, due to the activation of motor cortex during action observation, a number of cortical motorneurons are depolarized near threshold, these are more easily recruited by the same magnetic impulse and the amplitude of the MEP increases. In this manner MEP amplitude can be used as an indirect measure of corticospinal excitability.



APPENDIX II

Scientific Production

Published journal articles relative to the present thesis:

Leonetti, A., **Puglisi, G**., Siugzdaite, R., Ferrari, C., Cerri, G., Borroni, P. (2015). What you see is what you get: motor resonance in peripheral vision. *Experimental Brain Research*, *233*(10), 3013–3022. http://doi.org/10.1007/s00221-015-4371-0

Journal articles in preparation relative to the present thesis:

Puglisi, G., Leonetti, A., Landau, A., Cerri, G., Borroni, P. Diverting attention from action observation decreases the gain of motor resonant response. (data presented in chapter 2).

Puglisi, G., Leonetti, A., Cerri, G., Borroni, P. Motor resonance in central and peripheral vision: the role of attention and perceptual load. (data presented in chapter 4)

Poster presentation relative to the present thesis:

Puglisi, G., Leonetti, A., Cerri, G., Borroni, P. (2015). Motor resonance response in central and peripheral vision: the role of attention. Annual Workshop in Concepts, Actions and Objects, Rovereto.

Puglisi, G., Leonetti, A., Siugzdaite, R., Cerri, G., Borroni, P. (2014). Attention shapes motor resonant responses during action observation. 21st Annual meeting of Cognitive Neuroscience Society, Boston.

Cabinio, M., **Puglisi, G.,** Leonetti, A., Falini, G., Cerri, G., Borroni, P. (2013). Fronto-parietal activation during observation of impossible grasping actions : an fMRI study. Annual Workshop in Concepts, Actions and Objects, Rovereto.

Leonetti, A., Siugzdaite, R., **Puglisi, G.,** Cerri, G. Borroni, P. (2013). Motor resonance in peripheral vision and the role of covert attention. Annual Workshop in Concepts, Actions and Objects, Rovereto.

Siugzdaite, R., Leonetti, A., **Puglisi, G.**, Cerri, G., Borroni, P. (2012). What you see is what you get: motor resonance in peripheral vision. Mirror Neurons: new frontiers 20 years after their discovery, Erice

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