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Modulation of social context on the activity of motor and mirror neurons in the ventral premotor cortex (F5c) of Macaque monkey

Coordinatore:
Chiar.mo Prof. Vittorio Gallese

Tutor:
Chiar.mo Prof. Pier Francesco Ferrari

Dottoranda: Giulia Toschi

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ABSTRACT

Several studies show that both motor neurons' and mirror neurons' (MN) discharge in the monkey ventral premotor cortex (F5) and in the inferior parietal lobule (IPL) is modulated by the action's goal and by the context in which the action has been executed (Fogassi et al., 2005; Bonini et al., 2010). In this Ph.D thesis, I investigated if biological information, such as the presence of a container, and social cues, such as social interactions with another experimenter or with the observer, can differently affect the activity of motor and mirror neurons in F5 convexity during a visual and motor task. Moreover, I also investigated if the *passive* or *active* observation of an action could have a different effect on the visual discharge of mirror neurons. During *passive* observation the monkey is required to simply observe an action without any interaction with the observed agent. Conversely, during *active* observation the monkey is required to observe an action in order to plan a subsequent action coordinated with the observed agent. We carried out electrophysiological recordings in the ventral premotor cortex of two macaques (Macaca mulatta), with a sixteen channels multielectrode system. The neural activity was recorded in the visual task (*VT*) and in the motor task (*MT*). The visual task was characterized by 4 conditions in which the monkey observed an experimenter grasping an object/piece of food followed by one out of four possible action goals: 1. *Container* (grasping to place into a container), 2. *Hand* (grasping to place into another experimenter's hand), 3. *Monkey* (grasping to give to the monkey), 4. *Mouth* (grasping to eat). The motor task was characterized by 3 conditions *Container*, *Hand* and *Mouth* of the *VT*, but in this case, the monkey performed the grasping action.

We found that, both motor and mirror neurons could discriminate the same motor act according to the final goal of the action and that, both during the motor and the visual tasks, motor neurons' and MNs' discharge is modulated by the social context, in particular, by the social goal of the action (condition *Hand* and *Monkey* for the visual task, and condition *Hand* for the motor task). Our results seem to support the idea that motor and mirror neurons could have a key role not only in the encoding of other's intentions, but also in the encoding of others' intention in relation to social goals. This would allow the

individual to create an internal representation of the action performed by others, that integrates different aspects not only of the biological context but also of the social context.

Moreover, unexpectedly, we found that a number of MNs which prefers the *Monkey* condition in the *VT* is virtually absent. It's also interesting to note that, in this condition, the MNs spiking activity, during the observation of the action, tends to decrease in relation with the increase of the time occurring from the experimenter's hand contact with the target object to the monkey's hand release from its handle in order to grasp the object offered by the experimenter. These results, together with those obtained from the Local Field Potential (LFP) analysis, suggest that the active action observation has an inhibitory effect on the MNs visual discharge in F5. The mechanism, by which this attenuation would occur, could be attributed to the events related to the observation of the action performed by the experimenter in the *Monkey* condition. In fact, in this phase, the monkey has to prepare the action but, at the same time, it has to maintain suppressed the action until the experimenter starts his movement toward the monkey. During this phase it is possible that the motor neurons (and probably also some F5 mirror neurons) can induce an interference or inhibitory effect on the visual discharge of mirror neurons, activated during the observation of the grasping action. These results seem to contrast previous hypothesis about the functional role of mirror neurons, which postulate that the activity of mirror neurons during the action observation reflects a motor preparation or an action anticipation (Kilner et al., 2004; Fontana et al., 2012).

1. GENERAL INTRODUCTION

1.1 The organization and the motor control of the voluntary movement

In the Fifties, Penfield and Rasmussen (1951), on human, and Woolsey (1952) on monkey, described, in the frontal lobe, the existence of a complete representation of body movements in two distinguishable motor area. In the monkey motor cortex, it was possible to define a primary motor area (M1) and a supplementary motor area (SMA). The former area, located before the central sulcus, on the lateral surface of the frontal lobe, corresponded to the area 4 and in part of the area 6 of Broadmann classification. Its stimulation evoked discrete motor responses of the contralateral part of the body, so that it was considered as the principal component of the motor control. The supplementary motor area, area 6 of the Broadmann classification, located on the mesial surface of the hemisphere, evoked a variety of synergistic and bilateral movements and was involved in more complex functions such as, for example, the control of posture. Subsequently, different studies proposed the principle that the different areas of the brain corresponded to different cognitive and motor functions. That is, sensory, perceptual and motor phenomena, as different psychological functions, had to be divided into clearly distinct cortical areas. The coding of the different sensations (visual, somatosensory, acoustic, olfactory and gustatory) was attributed to the primary sensory areas, while the processing of their perception was attributed to the association areas (especially the parietal and temporal cortices). From these areas, the information could then be transmitted to the frontal areas, where the motor cortex, in the posterior part of the frontal lobe, could control the execution of the responses in relation to these stimuli. In this way, the brain network was described as a mono-directional model, according to which the information would proceed from a cortex “who knows” and interpreter the sensory information to a cortex “that makes” and control the movement. Subsequently, Mountcastle (1975) and Hyvarinen (1981) demonstrated that the posterior parietal areas could play a key role in the motor control and other cytoarchitectonic (Barbas and Pandyas 1987; Matelli et al., 1997) and biochemical studies (Matelli et al., 1985) showed that the motor system was constituted by different frontal and parietal areas with more complex functions. So that, the sensory and motor information could be coded by specific frontal-parietal circuits, which work in parallel and which are involved in sensorimotor transformation; further experiments demonstrated that these circuits could also be important for other complex cognitive functions such as space perception (Anderson et al., 1996; Rizzolatti et al., 1997), object coding and action understanding (Rizzolatti & Matelli 1998; Gallese et al., 2002; Fogassi et al., 2005; Fogassi & Luppino, 2005).

1.2 Functional Anatomy of frontal motor cortex

The frontal lobe of primates, located before the central sulcus, is composed by two main areas. The first one, the prefrontal cortex (PFC), is the most rostral and granular area and is involved in several cognitive functions in relation to the movement, such as the temporal planning of the actions, executive control and the organization of the motor events. The second area, located in the more caudal part of the frontal lobe and characterized by the almost total absence of the fourth cortical layer (agranular cortex), is constituted by the motor (M1) and premotor cortices (PMC), identified respectively with the 4 and 6 Broadmann area, with evident motor functions.

Several studies have revealed how the 4 and 6 Broadmann areas can be distinguished, both anatomically and functionally, in at least seven sub-areas denominated from F1 to F7 (Figure 1), which contain the representations of the body movements and which are strongly connected to each other and with the rest of the cerebral cortex (Matelli et al., 1985; Barbas e Pandya, 1987; Matelli et al., 1991; Petrides e Pandya, 1994). The F1 area or M1, located ventrally to the central sulcus, substantially corresponds to the 4 Broadmann area, while the other six areas (F2, F3, F4, F5, F6, F7) are a subdivision of the 6 Broadmann area or PMC. Moreover, the premotor cortex could be divided into three main areas: the dorsal area (F2 and F7), ventral area (F4 and F5) and mesial area (F3 and F6), each of them present a rostral and a caudal portion.

Several neurophysiological studies investigated the distinct anatomical sub-areas of the premotor and motor cortices, mostly focusing on area F4, F5 and F1 (Gentilucci et al., 1988; Rizzolatti et al. 1988; Fogassi et al., 1996; di Pellegrino et al., 1992; Ferrari et al., 2003; Raos et al., 2003; Maranesi et al., 2012). *Maranesi* et al. (2012) performed, in the ventral motor cortex, different extracellular electrophysiological recordings coupled to intracortical microstimulations. Based on the distribution of the functional properties in the investigated region (hand, arm and mouth motor properties, somatosensory properties and visual properties), *Maranesi* and colleagues individuated a further functional border within areas F4 and F1, by subdividing both of these areas into a dorsal and a ventral sectors. Concerning area F4, they called these sectors F4d (dorsal) and F4v (ventral); concerning F1, as they did not study the whole area but only its ventral part (F1v), they divided it in two subdivisions, F1vd (dorsal) and F1vv (ventral). The dorso-ventral border could also extend rostrally through area F5 to the arcuate sulcus. However, because of the low number of penetrations available in this cortical sector, they referred to the rostral investigated region as to the F5c described by *Belmalih* et al. (2009) (this sector will be described in detail in the following paragraph). In line with previous works (Gentilucci et al., 1988; 1989; Fogassi et al., 1996; Lemon et al., 1986; Alexander and Crutcher 1990; Umiltà et al., 2001), they found that F4d and F1vd appear to represent a functional cluster where the neuronal activity was related to forelimb and mouth goal directed motor acts and the control of goal directed arm movements toward different space sectors. Moreover, the functional similarity between F4d and F1vd was also supported by the distribution of the visual and somatosensory properties, such as responses to visual stimuli moved within specific fields of the peripersonal space. Compared with the dorsal sectors of areas F4 and F1, the F4v and F1vv neuronal activity was mainly related to simple movements of the tongue and the jaw rather than to goal directed motor acts. In these ventral sectors, visual responses were nearly absent, whereas somatosensory responses were widely represented. In particular, somatosensory responses were more frequently found following passive stimulations of inner mouth structures (tongue, teeth, palate) rather than of the hairy and glabrous skin of the lips and the face. Finally, the functional properties of F5c differed in several aspects from those of both F4 and F1: the great majority of sites in area F5c showed selective responses during goal directed motor acts, particularly hand and mouth. In lines with the previous studies (Murata et al., 1997), *Maranesi* and colleagues showed that F5c neuronal activity is strongly affected by the type of grip (precision or whole hand grip), while F4d neuronal responses are largely independent from the hand shape required for grasping.

Regarding the visual properties, F5c is characterized by the presence of visual responsive neurons, the activities of which are mainly related to the observation of others' motor acts: mirror responses (di Pellegrino et al., 1992; Gallese et al., 1996). Taken together these findings support a more direct involvement of F4d-F1vd and F4v-F1vv in motor control processes related to hand/arm motor acts and mouth movements within working spaces, and high level pragmatic and perceptuo-cognitive roles for the convexity of area F5.

Furthermore, the posterior areas of the PMC (F2, F3, F4 and F5) are directly connected with F1 and they receive afferents from the parietal lobe, which is why they are called *parietal-dependent* areas (Luppino and Rizzolatti, 2000; Rizzolatti and Luppino, 2001). The anterior premotor areas (F6 and F7) do not project to F1 and they are not connected to the parietal cortex, but they receive their primary afferents from the posterior motor areas and the PFC, so that they are called *prefronto-dependent* areas (Matelli et al., 1986; Luppino et al., 1993; Rizzolatti and Luppino, 2001). From anatomical data, it is know that these two circuits may play different roles in the motor control: the *parietal-dependent* areas directly project to the spinal cord, being part of the cortico-spinal tract, while the *prefronto-dependent* areas project to the brain stem. It follows that, the *parieto-dependent* areas appear to be involved in sensory-motor mechanisms aimed at transforming sensory information arising from the parietal areas in appropriate movements for a specific context. These mechanisms operate in parallel in different parieto-frontal circuits. On the contrary, the *prefronto-dependent* areas, which receive few sensory information, seem mainly responsible for the control of different functions, executed on the basis of information received from the prefrontal cortex. Moreover, these areas could control, only indirectly, the movement through their descending projections or their connections with other premotor areas (He et al., 1993; Rizzolatti, Luppino 2001).

It is also interesting to note that the nervous fibers, originating from F1, reach both the IX lamina of the spinal cord where the motor neurons are located and the intermediate region, while the corticospinal projections from the other premotor areas (F2, F3 , F4, F5) arrive predominantly at the intermediate region (Porter and Lemon, 1993). This different anatomical organization has a probable functional value: the projections of F2, F3, F4 and F5 would activate preformed spinal circuits defining the overall framework of the movement. Those of F1, however, reaching directly the motor neurons, would be responsible for the fine control of the movement (Gentilucci et al. 1988; 1989; Luppino et al, 1991; Raos et al., 2003; Coudé et al., 2005).

Finally, the parietal-frontal connections would play a key role in transmitting sensorimotor information needed for the execution and control of the movement (Fogassi et al., 1992; 1996; Gallese et al., 1994; Rizzolatti et al., 1997; Matelli and Luppino, 2000; Matelli and Luppino, 2001) and in the recognition and understanding of the action of others (Gallese et al., 1996; Fadiga et al., 2000; Rizzolatti and Craighero, 2004). The connections between the prefrontal cortex and the anterior motor areas (especially with the area F6) may, instead, provide more complex, motivational, mnemonic information which are related to the context (Tanji, 2001; Hoshi and Tanji, 2004). In this way, the anterior areas of PM could be seen as a "prefronto-dependent" system aimed at controlling "when" and "under what circumstances" the activity of the posterior areas should result in the effective execution of an action (Rizzolatti and Luppino, 2001).

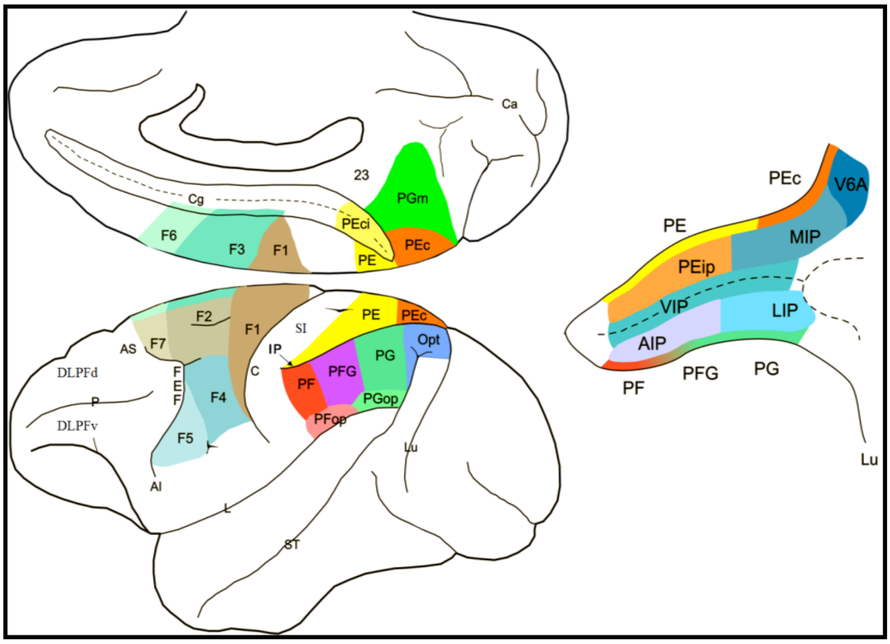

Figure 1 Medial and lateral view of the macaque brain. The different subareas of the motor cortex, posterior parietal cortex and cingulate cortex are indicated in different colors. The subares of the posterior parietal cortex are indicated with the letter P followed by one or more letters following the von Economo convention, modified by Pandya, Seltzer, (1982). The areas in the intraparietal sulcus are illustrated in a two-dimensional view on the right part of the figure: AIP, anterior intraparietal area, LIP lateral intraparietal area, MIP medial intraparietal area, PEIp intraparietal area PE, VIP ventral intraparietal area. AI: inferior arcuate sulcus; AS superior arcuate sulcus; C: central sulcus; Cg: cingulate gyrus; DLPFd dorsal area of dorsolateral prefrontal cortex; DLPFv ventral dorsolateral prefrontal cortex; L: lateral sulcus; P: principal sulcus; Pos: parieto-occipital sulcus; SI: primary somatosensory cortex; ST: superior temporal sulcus (Luppino, Rizzolatti 2000).

1.3 Anatomy and Functional Properties of F5 area

Area F5 is located in the rostro-ventral portion of the 6 Broadmann area. In front of F4, F5 extends, rostrally, until the limits of the prefrontal cortex, close to the region of the arcuate sulcus, where the Frontal Eye Fields (FEF) are located on the front bank of the lower arcuate sulcus. On the basis of cytoarchitectonic and immunohistochemical studies, the F5 area has been classically divided into two main districts: the first (F5c), placed on the cortical convexity, and the second (F5ab) located in the posterior bank of the lower arcuate sulcus (Matelli et al., 1985).

More recent studies, with innovative cytoarchitectonic and neurochemical techniques, show a new revision of the organization of the F5 (Luppino and Rizzolatti, 2000), according to which, on the posterior bank of the lower arcuate sulcus, where F5ab is located, exist, actually, two different types of area with different cytoarchitectonic and neurochemestry properties: caudal sector and rostral sector (Figure 2). The former includes the posterior part of F5 (F5p) and is located in the posterior bank of the inferior arcuate sulcus and presents a columnar organization, with a clear division of the fifth layer in two sublaminaes. The immunopositive to calbindin (CB) cells are concentrated in the second layer and on the top of the third layer. The latter sector includes the anterior part of F5 (F5a) and is located on the bottom of the inferior arcuate sulcus, which extends, rostrally, towards the prefrontal cortex. This sector presents a dense and homogeneous fifth layer and a large concentration of immunopositive to CB cells in the in third layer.

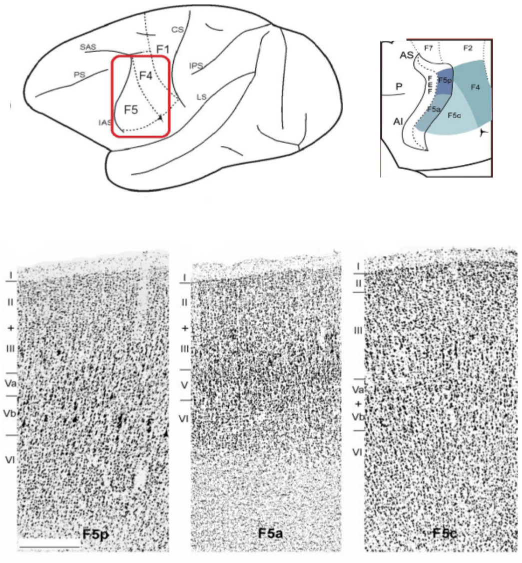

Figure 2 Organization of the ventral premotor cortex (F5). (**A**): Macaque brain, the red rectangle indicates the ventral premotor areas. (**B**): partitions of area F5 in subareas F5a, F5p, and F5c. (**C**, **D**, **E**): cytoarchitectonic structure of F5p, F5a, F5c respectively.

In the rostral portion of the ventral premotor cortex (F5v), electrical microstimulation and single cell neuron studies showed the existence of motor neurons and visual-motor neurons that are active during the execution of goal directed motor acts, such as grab, hold, handling or break (Rizzolatti et al., 1987; Gentilucci et al., 1988).

Motor acts are characterized by sequences of simple movements, performed smoothly, coordinated and mutually reinforcing and aimed at the attainment of a goal. For example, the act of grabbing an object requires a bending of the thumb and the index or whole hand in relation to the object's size. In F5, if we record the activity of typical grasping neurons, that are involved in the encoding of the grasping action, during the act of scratching, their firing rate will be almost absent, because, despite the same effectors are involved both in the grasping and in the scratching actions, the goals are very different. For this reason, several scholars suggested that F5 motor neurons encode the movement in terms of the goal of the action.

The visual-motor neurons in F5 have been divided into two classes: canonical neurons and mirror neurons, located in different areas of F5 (the canonical neurons in the bank of the inferior arcuate sulcus (area F5p), whereas the mirror neurons in the adjacent convexity (area F5c)) (Rizzolatti & Luppino, 2001). However, a recent electrophysiological study performed in area F5 using a multielectrode probes to simultaneously record single-neuron activity from multiple sites located at different cortical depths, approximately covering all the cortex layers (Bonini et al., 2014), shows that canonical and mirror neurons are often present in the same cortical sites. Although mirror neurons have been described to be slightly more concentrated in the first 2 mm of the cortex, they are also present in the deepest investigated sites (up to 4 mm), intermingled with canonical neurons.

Mirror neurons are those neurons that become active both when an individual performs a motor action and when he or she observes a similar action made by another individual. Moreover, they code mostly for distal actions, as grasping with the hand or mouth, holding and tearing, and they show a selectivity for the way in which the action is carried out (precision hold, the whole hand) (Gallese et al., 1996 Rizzolatti et al., 1996; Ferrari et al., 2003). The canonical neurons discharge during the objects presentation and are characterized by a close congruence between the type of grip and the visual characteristics of the object presented (Murata et al., 1997; Raos et al., 2003). For example, if a neuron discharges during the grasping of a particular type of object, for example a small object, its visual discharge will be congruent with the presentation in the visual field of a small object. This suggests that, in F5, objects are represented in relation to the motor interaction that an individual can have with them.

The area F5 is, therefore, constituted by a vocabulary of goal directed motor acts, in which the words are the neuronal populations that encode a particular motor act or one of its aspect, the final goal of the action or the way in which this action should be performed. In addition, the coexistence of visual and motor responses in the same neuron would demonstrate the ability to evoke a motor act even when the latter does not have to be actively performed.

1.4 Mirror Neurons System

As mentioned above, mirror neurons are those neurons that become active both when an individual performs a motor action and when he or she observes a similar action made by another individual. Then, the mirror system would be able to activate, in the observer, the same motor representation that would be generated when he or she performs the same action (di Pellegrino et al., 1992; Rizzolatti et al., 1996, Ferrari et al., 2003; 2005). The activation of the canonical neurons would be able to provide a visual representation of the physical and geometric characteristics of the object on which the individual can, if necessary, program an action. The activation of mirror neurons, instead, would provide the observer with a real comprehension of the goal of the observed action, for example grasp an object or eat a piece of food. (Gallese et al., 1996; Fogassi et al., 2005).

There are several stimuli that could modulate the activation of mirror neurons. For example, the valence of the stimulus, such as a piece of food or a metallic cube, could evoke different responses with different intensity (Caggiano et al., 2012). In this study, the visual responses of most mirror neurons were stronger if the observed motor act was associated with the most relished reward, although a minority of mirror neurons seemed to prefer objects not followed by rewards or, alternatively, by less-relished rewards. Taken together, these results suggest that the discharge of F5 mirror neurons is influenced by the information on the value that the object targeted of the motor act has for the observer and this information may help the observer to interpret the action meaning and to read the intention of an observed agent.

In addition, the mirror neurons, similar to the F5 motor neurons, may show a preference for certain types of action (grab, hold, handling), for action made with tools (Ferrari et al, 2005) or for the direction of the movement (left or right) (Gallese et al, 1996, Rizzolatti et al., 1996). An other important property of mirror neurons is the congruence they show between the effective observed and the effective executed action. This congruence may be very strict such that only the observation of an action virtually identical to that motorically coded by a given neuron may activate it. On the basis of these properties, it was suggested that F5 mirror neurons are part of a neural system matching the visual description of an action with its execution and that this observation/execution matching system plays a role in action understanding (Rizzolatti et al., 2001).

A recent study (Bonini et al., 2014) shows the presence, in area F5, of a third class of neurons that has both mirror properties and canonical properties: the canonical-mirror neurons. In particular, this study demonstrates that the activation of the canonical and canonical-mirror neurons to the presentation of objects is mostly evident when the stimulus is presented in a peripersonal space respect of the observer. In contrast, mirror and canonical-mirror neurons are activated during the action observation when the action takes place both in a peripersonal and extrapersonal space respect of the observer. These results are in agreement with the study of *Caggiano* (2009), in which the recorded mirror neurons present a different activation depending on whether the observed action is performed in a peripersonal or extrapersonal space respect to the monkey. These results, therefore, demonstrate that, in the individual, the mirror system is able to represent the space, in which the action is performed, not as metric distance, but rather as any motor preparation for a possible and consequent interaction. In fact, performing a motor act in the peripersonal rather than extrapersonal space to the observer, could lead to the activation of a motor plan aimed at a subsequent and immediate interaction between the agent and the observer.

Moreover, another study performed in F5 shows the presence of mirror neurons that are also activated during the observation of an “inhibited” action (inaction) (Bonini et al., 2014). These neurons are defined inaction neurons. In this study, through different sound cues, the monkey is aware of when the experimenter will perform or not (inaction) a grasping of metallic cube. In addition, it was trained to perform or inhibit the same motor act according to the same sound cues used in the observation task. The results show the activation of the inaction neurons both during the observation of the inhibited action by the experimenter and when the monkey itself has to inhibit the execution of the motor act. Not only, most of these neurons is also activated when, on the contrary, the action is performed or by the agent (visual task) or by the animal itself (motor task). Since these neurons show the same pattern of activation during the observation and execution of actions and inaction, probably, as mirror neurons, they could generate an internal representation of the motor act both when the action is performed and when it is deliberately withheld. In this way, the mirror neurons would become crucial to understanding and encode not only the motor act that we or others are going to perform, but also the motor act that we or others are going to retain or inhibit.

Some responses to the observation of goal directed motor acts have been localized, also, in neurons in the superior temporal sulcus (STS), but such responses would not seem exactly “mirror responses” as they do not become active during the execution of the movement itself (Perrett et al., 1989). It was suggested that the superior temporal sulcus could serve as a "source" of visual information to F5, however, there is no evidence of direct connections with this area (Matelli et al., 1986). On the other hand, the inferior parietal lobule (IPL), in particular the PFG area and the anterior intraparietal area (AIP) seem to be connected with the area F5 (Petrides & Pandya, 1984; Matelli & Luppino, 1997) and STS (Rizzolatti et al., 1998; Rozzi et al., 2006; Borra et al., 2008). So that, the visual information could be coded in the STS and sent to F5 through two separate circuits: one, STS (higher bank) could send the information to PFG which in turn refers that information to F5c and the second, STS (front bank) to F5a, through AIP. These two circuits are called temporo-parieto-frontal circuits (Nelissen et al., 2011). The F5a and F5c areas show different responses: F5a is also activated by the presence of only the effectors of the body (hand or forearm) that perform the action, while F5c could be activated only when there is the whole individual figure who performs the action (Nelissen et al., 2005). Indeed, in PFG the motor responses, while performing grasping actions, and its direct connection with the spinal cord would suggest a more complex role of the parietal lobe than a simple visual integration site from STS to F5. Also the AIP area shows a more complex activation, such as during the performance of manual grasping actions and observation of the same (Rizzolatti and Sinigaglia, 2010).

Finally, other brain areas show mirror responses, in particular neurons of the dorsal premotor area (PMd) could be activated during the execution of actions, such as moving a cursor on a monitor and during the observation of the same movement. These neurons are also activated by the only presence of the final goal of the action, so they don't need the vision of an effector that interacts with the object (Cisek and Kalaska, 2005).

Moreover, close to these complex circuits, there are other inhibitor and modulator circuits aimed at preventing to evoke a spontaneous gestural imitation of the observed action. *Kraskov* and colleagues (2009, 2013) demonstrate the presence, in area F5 and M1, of neurons with mirror-like properties that project through the pyramidal tract directly or indirectly to the motor neurons of the spinal cord (PTNs). This subgroup of PTNs is called "suppression PTNs". These neurons show a clear inhibitory response when the monkey observes the action and an activation during the execution of the same action. These inhibitory PTNs may play their inhibitory effect or through feedforward inhibitory circuits of cervical propriospinal systems or through cortico-cortical circuits that suppress the outputs directly from the primary motor cortex. In this way they could inhibit any spontaneous imitative behavior. The impact on the spinal motor system of mirror neuron PTN activity in F5 versus M1 outputs is likely to be fundamentally different. M1 contributes 50% of the descending corticospinal projection from the frontal lobe, terminates heavily in the lower cervical cord and includes direct corticomotoneuronal projections influencing digit muscles. In contrast, area F5 contributes only 4% to the frontal lobe corticospinal projection and terminates mainly in the upper cervical cord.

Moreover, Kraskov and colleagues found, in both area, also the presence of "facilitation PTNs": they increased their activity during both observation and execution; however these neurons are less active for observation than execution. During execution, facilitation PTNs were around three times as active compared with the observation. During observation, discharge of M1 and F5 facilitation PTNs was attenuated and even reversed in suppression mirror PTNs: taken together, these results demonstrate that some F5 motor neurons and mainly M1 neurons connected with the spinal interneurons and involved in hand and digit muscles, could be strongly disfacilitated during observation (Krascov et al., 2009, 2013).

1.5 Action and Intention Understanding

Motor acts, such as grasping, are often chained to other motor acts within specific sequences aimed at a final goal. Action could be defined as a sequence of motor acts aimed at a specific final goal. For example, grasping a piece of food involves the activation of subsequent different motor acts (reaching, grasping, lifting) and can be aimed at bringing it to the mouth or at placing it in a container. Studies carried out in area F5 and the inferior parietal lobule (IPL) show that the activation of specific motor neurons, during the execution of a goal directed motor act, and mirror neurons, during both the observation and the execution of a goal directed motor act (grasping to place or grasping to eat), is modulated by the final goal of the action (eating or placing) (Bonini et al, 2010; Fogassi et al, 2005). In these studies, the monkey was trained to observe two motor acts performed by the experimenter: grasping a piece of food and bring it to the mouth and grasping an object and place it into a container. Not only, it was trained to execute the same actions as well. The results show that most of motor and mirror neurons in both area IPL and F5 code the same act (grasping) in a different way according to the final goal of the action in which the act is embedded (both in visual and motor tasks) (Figure3 and Figure 4). The ability of the motor and mirror neurons to encode the same action according to the final goal, probably, derives from the presence of external instructive cues, as the context (container) or the type of object (piece of food or metallic object).

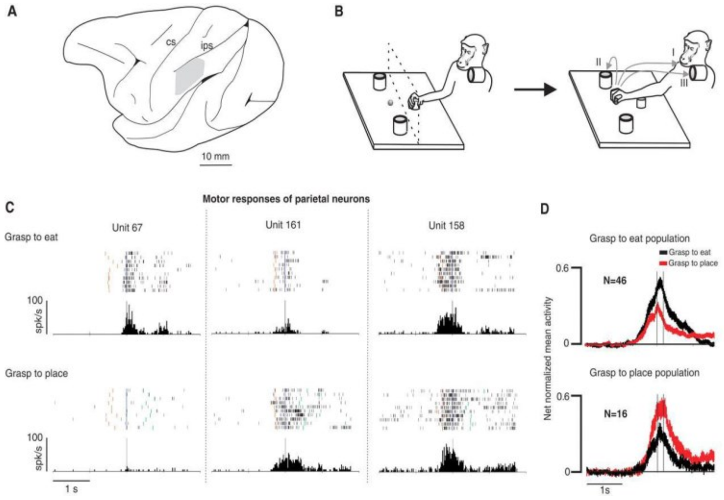

Figure 3 **A**. Macaque brain: the grey part indicates the IPL area of the recordings. **B**. Motor task. **C**. Examples of three motor responses of neurons in IPL during the motor task. **D**. Two motor neuron populations according to the final goal of the action. (Fogassi et al., 2005)

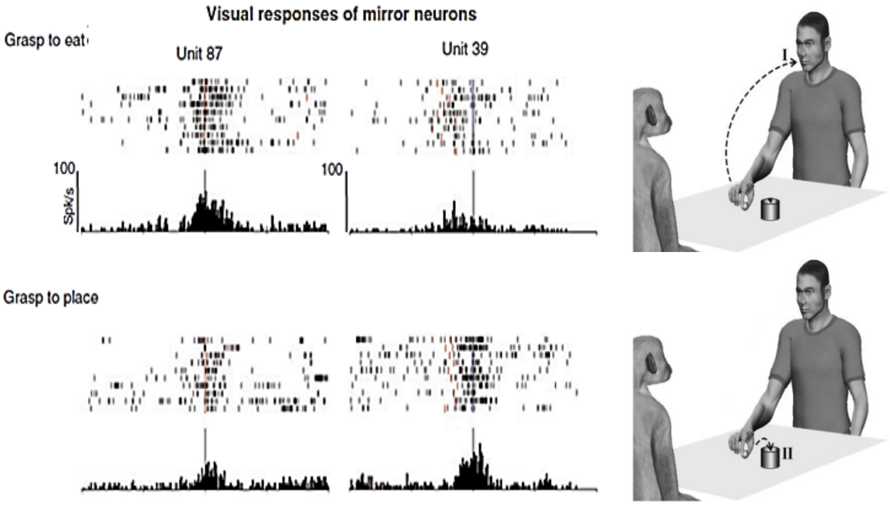

Figure 4 Example of visual responses of mirror neurons in IPL during the visual task. (Fogassi et al., 2005).

In addition, it has been proposed that in IPL actions are coded by neuronal chains, each of which leading to a specific action goal, that corresponds to the motor intention of the acting agent (Fogassi et al., 2005). The activation of a neurons population in a node of the chain could facilitate the activation of the next population in the sequence, so that, when the first motor act is activated, the next one is already pre-activated. *Chersi* et al. (2011) modeled the IPL region as a specific network, in which its structure is motivated by anatomo-functional evidence suggesting the organization of neural circuits into assemblies of cortical neurons, that possess strong excitatory and inhibitory interconnectivity both locally and, less strongly, between different cortical area. In this model, neurons tend to form local assemblies (pools) that respond in a similar way to incoming stimuli: different pools form the different neuronal chains. The transmission of the activity along the chains during a motor action starts when the specific intention pool is activated in the prefrontal cortex by sensory and contextual information, then the activity is transmitted from PFC to the first pool of a selected chain in IPL. In the moment in which neurons of the first pool begin to fire, this activity is transmitted to the connected premotor and primary motor cortices, which in turn start the first motor act of the action (Chersi et al. 2011). In IPL, the chains composed by mirror neurons are activated during action observation in the same way as during the action execution (Chersi et al. 2011). The visual input causes the firing of mirror neurons for a specific motor act and their activity immediately propagates to a second population of mirror neurons coding the subsequent motor acts; moreover during the observation of a motor sequence executed by another individual, information about, for example, hand configuration and motion, originating from STS reaches specific pools in the chains. Elements composing the chains are activated step by step in relation to the execution of each motor act by the observed agent. On the contrary of motor execution, in the action observation, the output of the pools directed to PMC is not transmitted to the primary motor cortex: an inhibitory mechanism blocks the propagation of motor commands after premotor areas and no overt motor output is produced (Chersi et al., 2011).

Taken together, all these results show that IPL and F5 neurons, in addition to recognizing the goal of the observed motor act, discriminate identical motor acts according to the action in which these acts are embedded. Since the discriminated motor act is part of a chain leading to the final goal of the action, this neuronal property allows the observer to predict the goal of the observed action and thus to read the intention of the acting individual. Depending on which motor chain is activated, the observer is going to have an internal representation of what the agent is going to do. What is more complex is to specify how the selection of a particular chain occurs (Fogassi et al., 2005). It has been proposed that this selection mechanism is localized in the ventral prefrontal cortex, because of its role in integrating and processing these factors (Tanji & Hoshi, 2008) and of its connections with IPL (Rozzi et al., 2006). These connections could constitute the anatomical pathway through which prefrontal cortex, on the basis of contextual information and previous experience, could recruit specific chains (Chersi et al., 2011). So that, external information, as the context and the type of the object, may play a crucial role. For example, if an object is edible (type of object), then the most probably action will be grasping to eat, rather than move it. However, the presence of a container will probably remind the action “grasping to place into a container”. These two types of information, clearly, interact with each other to give the best possible performance (Fogassi et al., 2005). So that, the action observation would induce in the observer a "incarnate" simulation mechanism that would constitute an implicit form of action understanding itself (Gallese et al., 2004; Ferrari et al. 2012).

Moreover, the observed action representation would be generated within the premotor area, even in the absence of the entire vision of the action itself. For example, hiding the end of the action could still elicit the activation of F5 mirror neurons. (Umiltà et al., 2001). Not only, also audio-visual information may generate the activation of specific mirror neurons (Kohler et al., 2002). These neurons discharge at the sound of the action (breaking a peanut, tear paper etc.), even if the action is not carried out in the visual field of the monkey. They are also selective for a specific type of motor action, showing congruence between the execution and the "hearing" of the motor act. Therefore, the representation of the evoked motor act still remains the same even changing the nature of sensory information (Keysers et al., 2003).

In the present Ph.D thesis I will consider and extend these aspects of motor control and goal coding, by investigating more in depth the possible role of the social context in modulating the activity of motor and MNs during the execution and observation of different action sequences leading to different goals.

More specifically in Study 1 I investigated the modulation of the activity of motor neurons in ventral premotor cortex (F5c) during the execution of a precision grip in three conditions which only differ in term of their final goal, that is either social (placing in the experimenter's hand) or non-social (placing in a container or bringing to the mouth for eating).

In Study 2, I will consider the modulation of social context, as in the previous study, but on the visual discharge of mirror neurons in F5, during the observation of a grasping action performed by the experimenter in four conditions, which only differ by their final goal, that is either social (social interactions between a second experimenter or with the observer) or non-social (placing in the container or bringing to the mouth to eat). Specifically, in this study, I also explored the different modulation of the social context on the activity of MNs during *active* or *passive* observation of grasping acts aimed at different social or non-social goals.

The thesis is therefore articulated into two different experiments that will be described in the following sections.

2. STUDY 1: Grasping neurons in the ventral premotor cortex of macaques are modulated by social goals.

2.1 INTRODUCTION AND AIMS

It is now well established that F5 motor neurons code the goal of the motor acts (Rizzolatti et al., 2009). When planning and executing an action, such as “grasping and eating a piece of food”, an individual must have an overarching goal, for instance “eating”, that leads to the selection of an appropriate sequence of motor acts. Distinct motor acts like reaching, grasping, and biting are each endowed with a specific motor goal. For instance, “grasping” imply the motor goal of taking possession of an object. The coding of different types of motor acts relies on specific parietofrontal circuits (Bonini et al., 2011; Fogassi et al., 2005). For instance, reaching depends on the PG/VIP-F4 circuit, while grasping hinges on the AIP-F5 circuit (Bonini et al., 2010; Borra et al., 2008; Nelissen et al., 2011; Rizzolatti et al., 1998; Rizzolatti and Luppino, 2001; Rozzi et al., 2006). The ventral premotor area F5 is a core region for coding the goal of hand motor acts such as grasping, tearing, and holding (Rizzolatti et al., 1998). The neurons of F5 have the crucial property of coding goal independently of the sequence of movements or, in some cases, of the effector used to achieve it (Rizzolatti et al., 1988; Umiltà et al., 2008). This property of F5 neurons shows that they can display a high level of motor abstraction, thus suggesting that their functions could be considered wider than previously thought, encompassing from motor control to higher order socio-cognitive functions (Rizzolatti et al. 2002).

Studies carried out in area F5 and the inferior parietal lobule (IPL) showed that the activation of specific neurons, during the execution of a goal directed motor act (grasping to place or grasping to eat), is modulated by the final goal of the action (eating or placing) (Bonini et al., 2010; Fogassi et al., 2005). In these studies, the monkey was trained to execute two motor acts: grasping a piece of food and bringing it to the mouth and grasping an object and placing it into a container (and also to observe the experimenter performing these two motor acts). The results show that a large proportion of motor neurons in F5 and area IPL code the same act (grasping) in a different way according to the final goal of the action in which the act is embedded. The ability of the motor system to encode the same action according to the final goal, probably, derives from the presence of contextual cues provided by the container in the grasp-to-place condition (i.e. the presence of a container inside of which the grasped object was subsequently placed) or by the type of object to grasp - a piece of food – in the grasp-to-eat condition.

The experiments cited above have used inanimate cues (i.e. objects) to instruct the monkey about the goal of the action, however, in normal daily life, animals and humans do not just move independently from others' actions, but are often required to perform actions directed to others or to perform complementary parts of an action made by others (Sebanz et al., 2006). The motor system, therefore, must express a high degree of versatility in order to activate similar motor programs that are aimed at different goals (placing an object into a container or placing an object into the hand of another individual).

In the present study we aimed at investigating the role of the social context in modulating the activity of motor neurons in the monkey ventral premotor cortex (area F5). Here we devised a simple motor task in which the monkey is grasping an object with a precision grip in three conditions which only differ in term of their final goal, that is, a subsequent motor act that is either social (placing in the experimenter's hand or "hand" condition) or non-social (placing in a container or bringing to the mouth for eating, "container" or "mouth" condition). We hypothesize that different populations of neurons, similarly to previously described (Bonini et al. 2011-2010), may emerge with differential discharge during the grasping motor act, depending on the final goal of the action. We also expect that in the social context, some neurons could be preferentially modulated during the first phase of the sequence (i.e. in conjunction with the first grasping act).

2.2 EXPERIMENTAL PROCEDURES

2.2.1 Animals and surgical procedures

Two captive-born and individually housed adult female rhesus macaques (*Macaca mulatta*) served as subjects (M1 and M2). The animal handling, as well as surgical and experimental procedures, complied with the European guidelines (86/609/EEC 2003/65/EC Directives and 2010/63/EU) and Italian laws in force on the care and use of laboratory animals, and were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012) and authorized by the Italian Health Ministry (D.M. 294/2012-C, 11/12/2012). The monkeys were housed and handled in strict accordance with the recommendations of the Weatherall Report about good animal practice. The well-being and health conditions of the monkeys were constantly monitored by the institutional veterinary doctor of the University of Parma. A titanium head post (Crist Instrument, Hagerstown, MD, USA) was surgically implanted on the skull using titanium screws. A cilux recording chamber (18X18 mm, Alpha-Omega, Germany) was stereotaxically implanted and secured with dental cement. For both procedures, each animal was deeply anaesthetized with ketamine hydrochloride (5 mg/kg i.m.) and medetomidine hydrochloride (0.1 mg/kg i.m.) and its heart rate, temperature and respiration were carefully monitored and kept within physiological range. Pain medication was routinely given after surgery: (Dexamethasone, 2mg/kg, every 12 hours, from 1 day before to 3 days after surgery; Ketoprofen, 5mg/kg, every 12 hours for 3 days following surgery)

2.2.2 Monkey task

The task is illustrated in *Figure 5*. The monkey was seated facing a table (60X60 cm) onto which a metallic cube was placed along the monkey body midline, at 13 cm from monkey's hand starting position. The monkey had to reach and grasp the object and then place it in a small container located 10 cm to the left of the grasping location. At the beginning of each trial the monkey had to keep the right hand on a handle attached to the table for at least 1000 ms (Figure 5A-I), after which, a transparent barrier was removed to give the "go" signal and the monkey grasped the object (Figure 5A-

II) and placed it in the container (Figure 5A-III). A juice reward (and a solid food reward) was delivered after 500-1000 ms, if the monkey correctly executed the trial (Figure 5A-IV). The task was run in one or more blocks of trials and reached a minimum total of 12 trials per condition. Any trial in which the grasping action was not properly executed was aborted and no reward was delivered. Note that the reward consisted in juice (0.14 ml) and a piece of apple of about 1cm^3 for each trial and was the same for all three conditions (See Experimental conditions below). Such reward schedule has been set because in the *Mouth* condition (see below) the monkey had to grasp a piece of food and bring it to the mouth for eating. Therefore, in the other two conditions we decided to introduce the same type of reward to avoid possible neuronal differential activity induced by the type of reward.

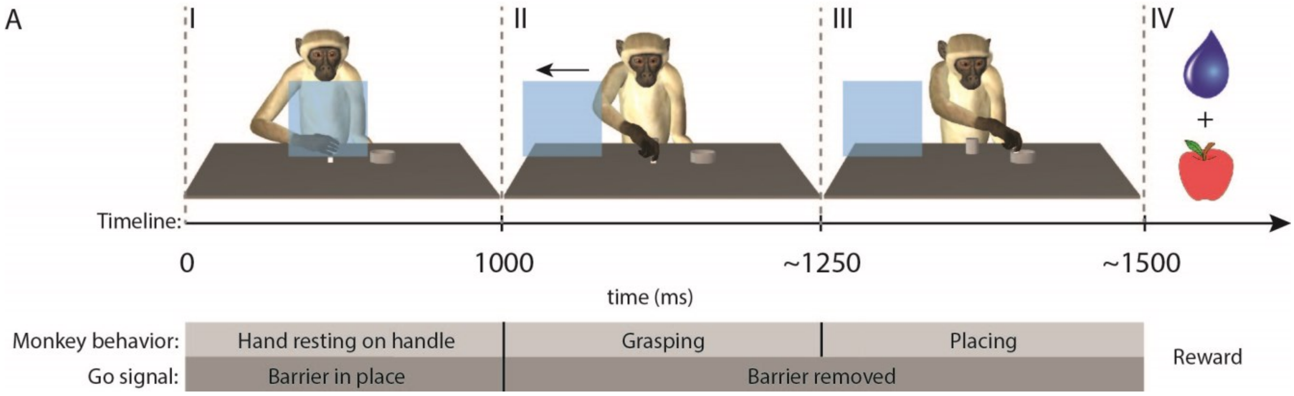

Figure 5. Task and conditions. A). Task illustration. A-I) The monkey has his right hand at rest on the starting handle for at least 1000ms (baseline). A transparent barrier is in place between the monkey and the target object. A-II) The barrier is removed (go signal). The monkey is reaching and grasping the target object. A-III) The monkey places the object in the container (or either place in the hand of the experimenter or eat, according to the experimental condition tested). A-IV) A liquid and a solid reward is delivered if the task is successfully executed.

2.2.3 Experimental conditions

The experimental conditions are illustrated in *Figure 6*. Three experimental conditions were designed. In the first part of the task, the monkey was required to do the same thing: releasing the handle and grasping an object or a piece of food with a precision grip. The experimental conditions differed in the second part of the task were the monkey had to: 6B-I) place the object in a container (*Container* condition); 6B-II) place the object in the hand of the experimenter (*Hand* condition); 6B-III) bring the object (a food morsel) to the mouth and eat it (*Mouth* condition). Since the grasped object is a metallic cube in the *Container* and *Hand* conditions, but a cubic piece of apple in the *Mouth* condition, we had to balance the reward value across all conditions. Therefore, in addition to the juice reward delivered in all conditions, the same pieces of apple used in the *Mouth* condition were given to the monkey after the correct completion of the trials in the *Container* or *Hand* conditions.

Figure 6 B) Experimental conditions. B-I) *Container*: Grasping to place in a container; B-II) *Hand*: Grasping to place in the hand of an experimenter, and B-III).*Mouth*: Grasping to eat.

2.2.4 Data recording

Neuronal activity was recorded with a linear multi-site electrode (16-channel, 250 micrometers spacing; U-probe, Plexon inc., Dallas, TX, USA) and digitalized at 40kHz using the Omniplex 16-channel recording system (Plexon). Task control (including handle holding, hand-object contact detection and reward delivery) was computer-controlled through a customized Labview© program. Contact-detection panels (Crist Instrument, Hagertown, MD, USA) were used to record the exact moment in which the monkey's hand released the handle and touched the target. This latter event was used to align the neuronal activity recorded for every trial in all conditions. We collected usable motor neuron data in 15 sessions from Monkey 1 and in 17 sessions from Monkey 2. Neuronal activity was recorded from area F5 of the ventral premotor cortex (see below for details). During the insertion of the electrode shaft in the brain we ensured that the topmost electrode was positioned under the dura, but remained outside of the cortex, so that it could be used as a reference channel. Of the 15 available recording channels, two were dedicated to eye movement control for another task (see Study 2). The remaining 13 channels were used for single-unit recording. The multi-electrode was lowered in the brain through the intact dura and the general properties of the neurons were tested. When motor properties for grasping were found, we waited about one hour to allow the neural activity to stabilize and then started to record single units using the behavioral protocol described above.

2.2.5 Preliminary testing of neuronal activity

Before proceeding with the neural testing using our behavioral protocol, single- and multiunit activity were systematically tested for visuomotor properties to identify recording sites in area F5 endowed with MN activity (Rozzi et al., 2008; see Maranesi et al., 2012). Briefly, we required the monkey to grasp food items in various conditions (i.e. with eyes closed, or without flexing the wrist, elbow or shoulder) enabling us to disentangle neuronal activity related to visual stimulation, reaching or grasping objects. Also, to exclude the possible presence of mouth-related responses, we tested any neural activity changes related to the delivery of small pieces of food directly into the mouth while the monkey’s eyes were closed. Finally, visual properties were studied by presenting the monkey with 3D-objects (e.g. food items and solids) of different shape, size and orientation, moved in various space locations, directions and distances from the monkey, as well as different manual actions performed by the experimenter.

2.2.6 Movement kinematics analysis

The kinematic parameters of reaching and grasping movements were recorded in separate sessions. The action was filmed from a lateral view encompassing the hand on the starting handle and object to grasp using a 50 Hz temporal resolution camera (Canon EOS-7D). A black marker was placed on the wrist of the monkey in order to track the hand excursion in the videos. The hand position in a XY axis was measured using an open source video analysis software (Tracker). We recorded 20 trials for each condition and compared the reaching/grasping duration, the velocity and the movement amplitude between the three conditions, using a one-way ANOVA.

2.2.7 Data analysis

Neuronal spikes waveforms were classified offline into units using a commercial spike-sorting software (OfflineSorter, Plexon). For each of the 13 electrodes, a mean of 2.7 units were isolated. The neural response selectivity was determined for each neuron comparing the spike frequency for each trial across two epochs (*Baseline* and *Grasping*, described below) and 3 conditions including *Container, Hand* and *Mouth.* The statistical analysis consisted in a 2X3 repeated measures ANOVA (factors: epochs and conditions) followed by a Newman-Keuls *post hoc* test, all with a 0.05 alpha level. The two epochs had a 350 ms duration and were precisely calculated with reference to the digital events and the contact with the object to grasp, and also by an offline kinematic analysis recorded in a separate recording session (see above *Movement kinematics analysis*). The *Baseline* epoch corresponded to a time period in which the monkey remained still and was leaning his hand on the starting handle. This epoch lasted from 1000 to 650 ms before the monkey release of the handle. The *Grasping* epoch was defined as starting 100 ms before the release of the handle (when his hand started to move as determined by the kinematic analysis) and ended when the monkey had grasped the object or the piece of food and started to lift it (as indicated by the "off" signal of the contact detection panel occurring 100 ms after the contact with the object). The *Grasping* epoch had a duration of 350 ms and was defined such as to encompass the whole reaching-grasping phase without including the placing phase of the action (expected to be kinematically different between the conditions). The moment when the monkeys' hand touched the object (indicated by the "on" signal on the contact detection panel) was used to align the trials.

Moreover, we also performed an electromyographic recording (EMG) to test differences in the monkey hand muscle movements in all conditions of the action execution task. The EMG recordings were carried out in different sessions with respect to those performed for the acquisition of the neuronal activity. The EMG electrode was located on the *flexor digitorum superficialis* muscle and the signal was filtered (0.5-500 Hz) and amplified through the Plexon recording system. For each motor condition, the results were averaged to analyze the muscular activity (µV) during the *Grasping* epoch.

The population analysis was carried out by constructing a spike density function for each neuron by averaging the spike frequency for each 20 ms bins across trials. The baseline activity (mean spike activity during the *Baseline* epoch) was subtracted from that of each bin. The data were then normalized by dividing the spike frequency within each bin by the maximum absolute value found across all bins. The result is a discharge rate ranging between 1 and -1 for each neuron. The spike density function was smoothed using a Gaussian kernel of 30 ms using the following formula:

$$\frac{e^{-\frac{t^2}{2\sigma^2}}}{\sqrt{2\pi}\sigma}$$

where σ corresponds to the width (30 ms) of the smoothing function and t the n$^{\text{th}}$ successive bin in the data vector.

The population curve was computed as the algebraic sum of the spike frequency for each bin of each neuron. The statistical differences between populations of neurons were calculated based on the firing rate during the *Grasping* epoch. One way repeated-measure ANOVAs were calculated, followed by Newman-Keuls post hoc procedures.

We also determined the latency with which the goal-modulated neurons were discriminating between the conditions. We compared the spiking frequency in the preferred vs non-preferred conditions of the neuron by performing a Wilcoxon rank sum test in a 100-ms-width window that was shifted by 20 ms. The discrimination threshold time was established as the first time bin of consecutive three where significant differences where found ($p<0.05$).

2.3 RESULTS

2.3.1 Goal-modulated neurons

The activity of 1499 neurons was recorded from area F5 of two monkeys. A total of 444 single units from area F5 in the right hemisphere of two monkeys (monkey M1, 153 units; monkey M2, 291 units) were revealed to be "hand grasping neurons" as tested with the task illustrated in Figure 5 and statistically confirmed with the 2X3 ANOVA described in the *Data analysis* section above. The neurons were collected from 32 electrode penetrations and 13 different cortical depths. We found that 193 neurons (43% of the recorded motor neurons) had a preference for one or two of the conditions (Figure 7). We hereafter refer to these neurons as *goal-modulated*. The majority of these goal-modulated neurons (62%, 120/193), had a preference for only one of the conditions. Three examples of goal-modulated neurons having a preference for one condition are presented in *Figure 8*. The first neuron (Container-goal-modulated neuron; **A**) had a preference for the grasping condition that is followed by placing the object in a container. The second neuron (Hand-goal-modulated neuron; **B**) had a preference for the grasping condition that is followed by placing the object in the hand of an experimenter. The third neuron (Mouth-goal-modulated neuron; **C**) had a preference for the grasping condition that is followed by eating the piece of food.

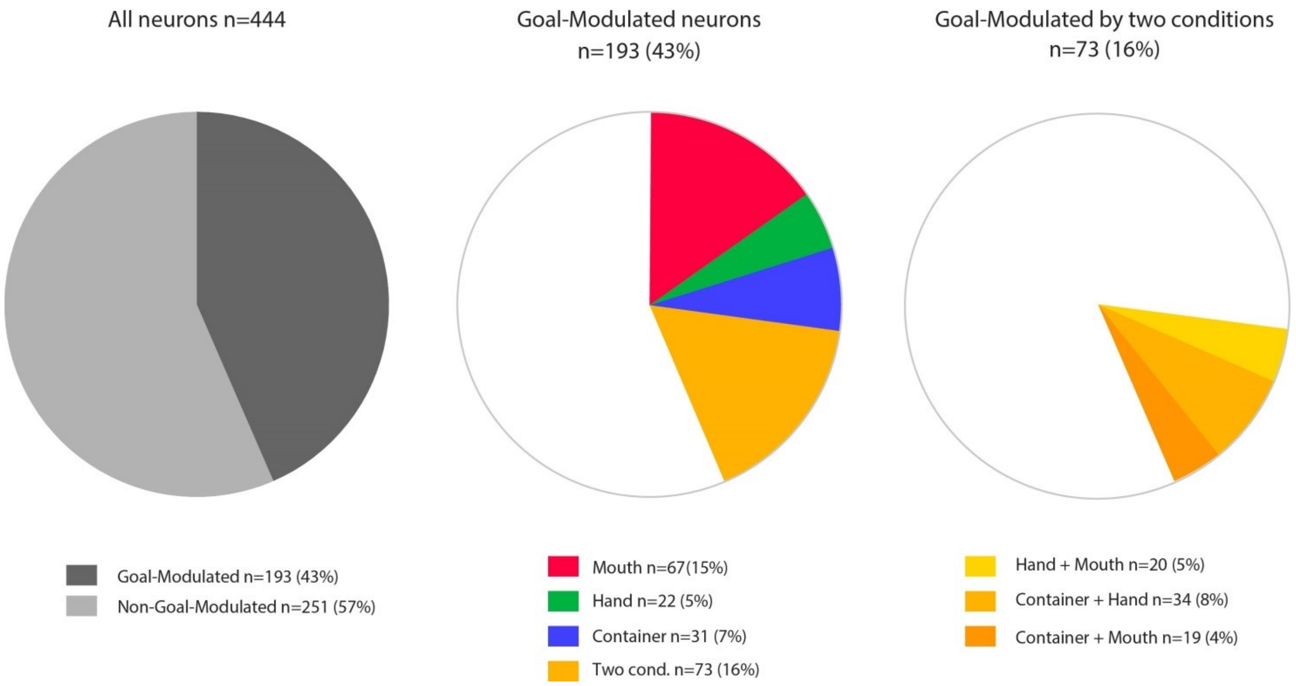

Figure 7. Proportion of goal-related preferences. Left) Overall proportion of F5 motor neurons that are goal-modulated. Forty-three percent (n=193) of the neurons had a significant preferential modulation for one or two of the three contexts, i.e., either when the goal was placing in a container, placing in the hand or eating. Center) Type of goal-modulation. Right) Type of goal modulation for neurons having two preferences.

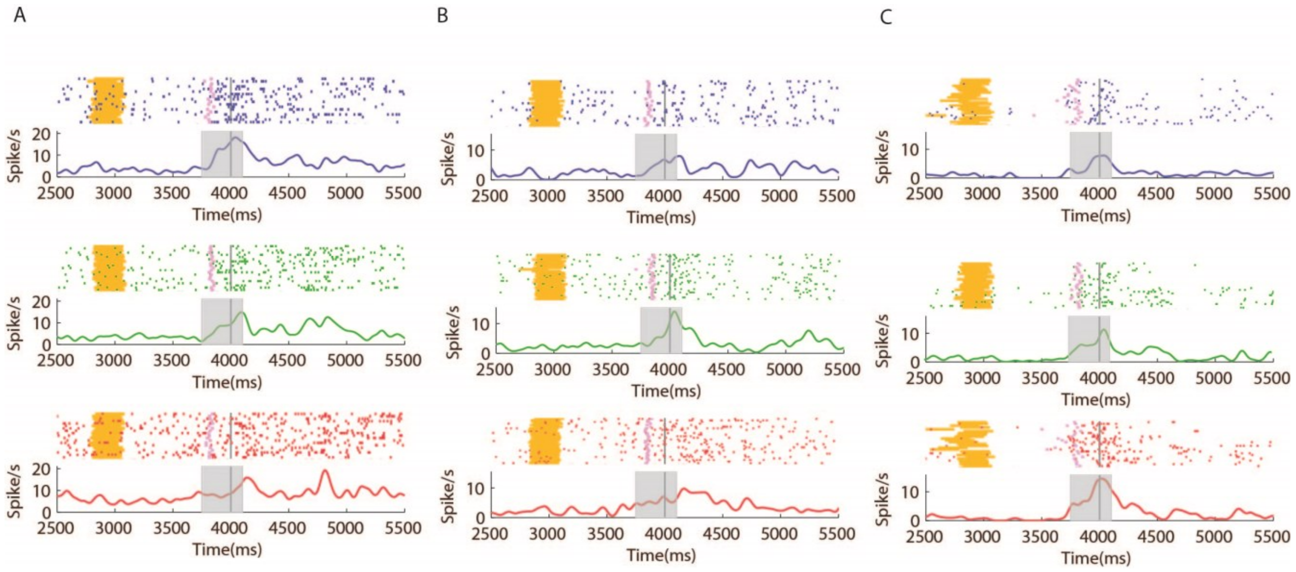

Figure 8. Examples of goal-modulated neurons. A, Unit having a preference for the *Container* condition (blue). B, Unit having a preference for the *Hand* condition (green). C) Unit having a preference for the *Mouth* condition (red). The gray shaded areas represents the grasping epoch (250 before to 100 ms after the grasping). The yellow mark represents the baseline epoch for each trial. The pink mark denotes the handle release. The grey vertical line indicates the hand object touch.

In order to test any significant monkey hand muscle movements during the action execution task, we performed EMG recordings in different sessions with respect to those performed for the acquisition of the neuronal activity. *Figure 9* shows the muscular activity recorded during the action execution task for all conditions. The graphic represents the muscular activity (μV) in relation to the time (seconds, s). As it can be noted, the muscular activity of the monkey in correspondence of the grasping action event (t=0) shows no significant differences between conditions (p>0.05, N.S.).

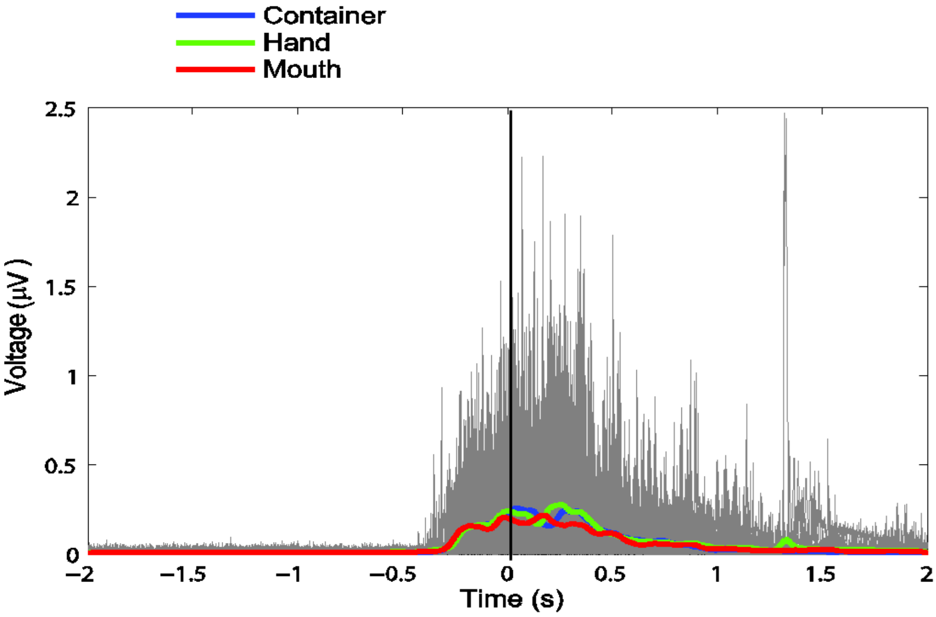

Figure 9 Muscular activity (rectified signal) during the action execution task in the three conditions. *x* axis: Time, *y* axis: Voltage. The black line indicates the agent's grasping action for all conditions.

2.3.2 Population analysis

We first examined the overall discharge of all goal- and non-goal-modulated neurons, in order to investigate whether the pattern of response across the three conditions was similar. The results are shown in the population analysis illustrated in *Figure 10*, where the *Goal-Modulated* neurons (**A-B**) and the Non-Goal-modulated neurons (**C-D**) are shown. For both populations, the *Mouth* condition yield a stronger discharge. Significant differences between the conditions among the Goal-Modulated neurons were found (Figure 10**A-B**); one-way repeated-measure ANOVA; $F_{(2,240)} = 9.21$; $p<0.01$). *Post hoc* comparisons showed that neurons tested in *Mouth* condition are firing significantly more than in the other conditions (Mouth vs Container, p<0.01; Mouth vs Hand, p<0.01). Likewise, for the Non-Goal-Modulated neurons, significant differences between conditions were found (Figure 10**C-D)**; $F_{(2,188)} = 10.69$; $p<0.01$). *Post hoc* comparisons showed that neurons tested in *Mouth* condition are firing significantly more than in the other conditions (Mouth vs Container, p<0.01; Mouth vs Hand, p<0.01). The tendency for a stronger discharge in the *Mouth* condition is therefore not only a characteristic of goal-modulated neurons but is also present among the Non-Goal-modulated. Such results could be due to the fact that the action of “grasping-to-eat” is a natural behavior that is already present in the repertoire of the monkey, unlike the “grasping-to-place” behavior. Figure 11 shows the population analysis for each of the *Goal-Modulated* neurons that are modulated by a one goal. For each Goal-Modulated neurons population the discharge in the preferred condition was significantly stronger (Figure 11**A-C**; *Container-Goal-Modulated* neurons; $F_{(2,29)} = 30.89$; $p<0.01$; *Post hoc*, *Container* vs *Hand*, p<0.01; *Container* vs *Mouth*, p<0.01; Figure 11**D-F**; *Hand-Goal-Modulated* neurons; $F_{(2,20)} = 22.00$; $p<0.01$; *Post hoc*, Hand vs Container, p<0.01; *Hand* vs *Mouth*, p<0.01; Figure 11**G-I**; *Mouth-Goal-Modulated* neurons; $F_{(2,65)} = 238.46$; $p<0.01$; *Post hoc*, *Mouth* vs *Container*, p<0.01; *Mouth* vs *Hand*, p<0.01)).

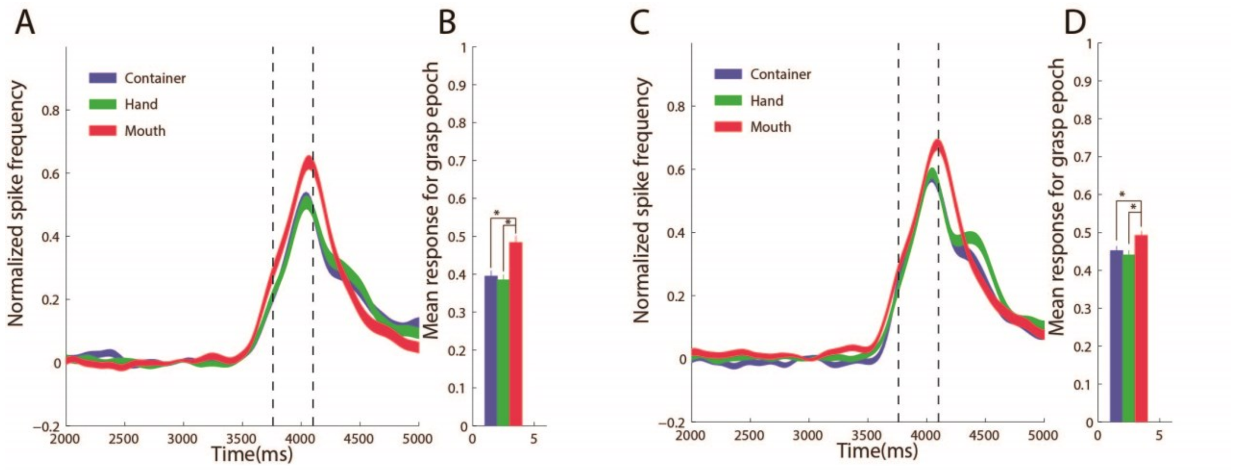

Figure 10 Analysis of goal-modulated and non-modulated motor neuron population in relation to condition. A) Neuronal response of goal-modulated neurons during grasping in the three conditions. Normalized activity of a population of 189 excitatory neurons. The two vertical dashed lines indicate the beginning and the end of the grasping epoch (350 ms). The thickness of the curves represents ± 1 SE. B) Mean normalized neuronal discharge during the grasping epoch of the goal-modulated neurons. One-way ANOVA (*p<0.01). C) Neuronal response of non-modulated neurons during grasping in the three conditions of 241 excitatory neurons. Same conventions as in A. D) Mean normalized neuronal discharge during the grasping epoch of the non-modulated neurons. Same conventions as in B.

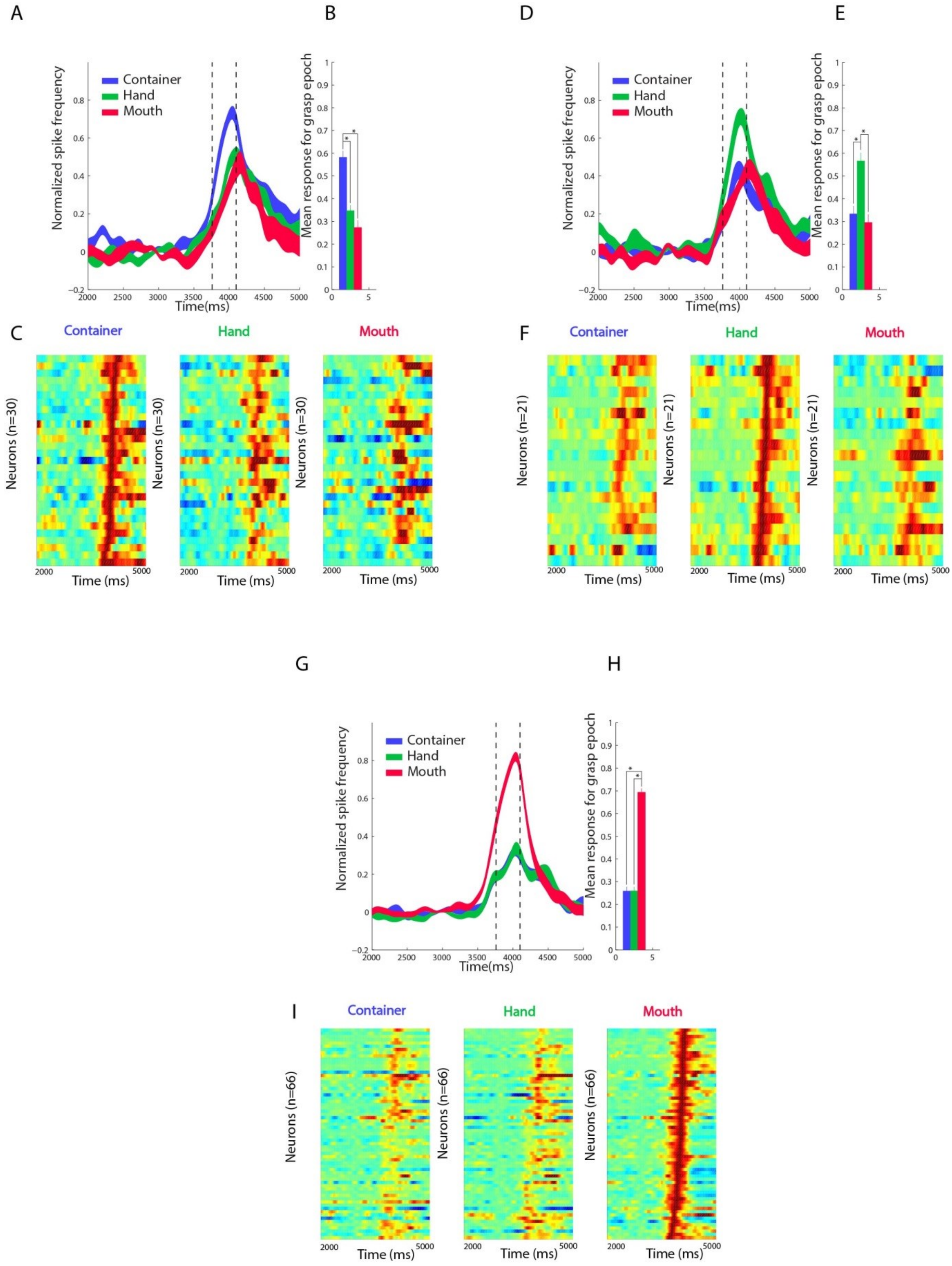

Figure 11. Goal-modulated neurons. A, D, G Neuronal response of goal-modulated neurons during grasping in each condition (Container, A; Hand, D; Mouth, G) Same conventions as in Figure 10. C, F, I: Normalized spiking activity of each neuron included in the population analysis. For each population, neurons are sorted from early-to-late peak neuronal activity for the preferred condition (bottom to top).

2.3.3 Goal-modulation and discrimination latency

To better characterize the Goal-modulated neurons, we analyzed the time at which they began to discriminate the final goal for their preferred condition (*Container*, *Hand* or *Mouth*). The analysis of the discharge latency is presented in *Figure 12*. There are significant differences between the discrimination time latency of the three types *of Goal-Modulated* neurons (one-way ANOVA, $F_{(2,114)}=8.97$, $p<0.001$). *Post hoc* Neuman–Keuls tests showed that the mean *Mouth* (7.29±0.69) discrimination time latency is significantly lower than for *Container* (10.23±0.90; $p<0.05$) as well as for *Hand* (12.52 ± 0.99; $p<0.001$). In contrast, we verified the timing of the peak discharge and found no significant difference between the three types of *Goal-Modulated* neurons (*Container*: 12.40±0.75, *Hand*: 13.67±0.96, *Mouth*: 13.08±0.58) (one-way ANOVA, $F_{(2,114)}=0.50$, n.s.).

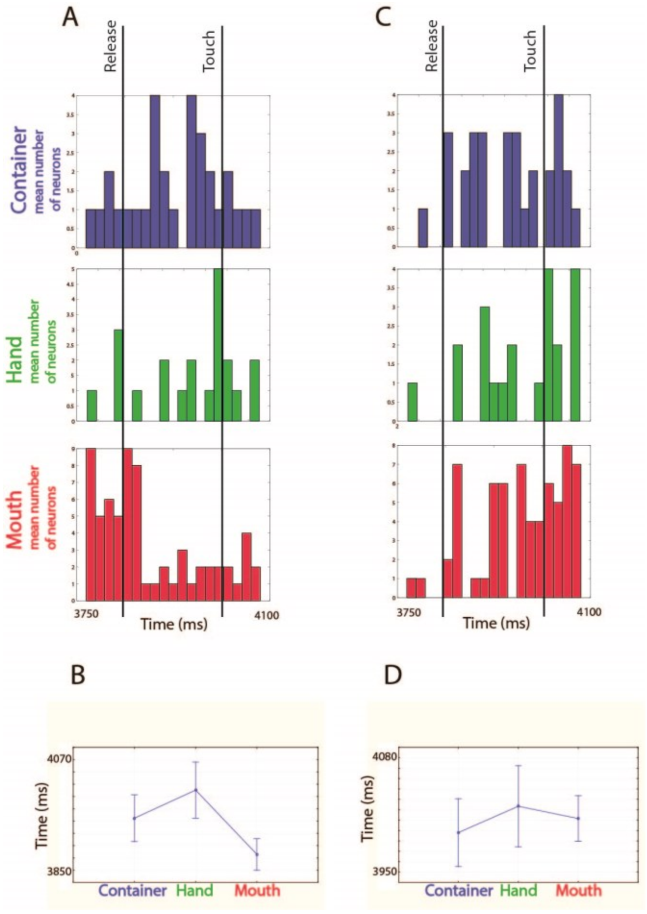

Figure 12 Discrimination latency and time of peak discharge. A. Distribution of neuronal discrimination latency for each condition within the Grasping epoch. B. Mean discrimination latency. C. Distribution of neuronal discharge peak activity for each condition within the Grasping epoch. D. Mean neuronal peak discharge.

2.3.4 Movement kinematics

Since the scope of the present experiment was to compare neuronal discharge between similar grasping actions that were differing only by the subsequent action in which they were embedded, it was important to verify that the kinematic parameters of the reaching-grasping actions were the same across the three conditions. We found that there was no differences in movement excursion (in height or in length) during the reaching phase between the three conditions. However, we found that reaching was slower in the *Mouth* condition (*Container*: 787.94±19.02 mm/s; *Hand*:747.87±15.72 mm/s, *Mouth*:706.47±6.88 mm/s; F=7.59, $p < 0.005$), probably indicating a deceleration of the reaching movement in order to grasp the cubic food morsel that is a different material than the metallic cube used in the *Container* and *Hand* condition. This difference is however unlikely to have had an impact on the proportion of *Goal-Modulated* neurons found in this experiment. Indeed, we found that the “grasping to eat” trials were also slower during the recording of neurons preferring the other conditions (*Container* or *Hand* condition), ruling out that the grasping velocity was a determinant factor in neuronal preference. We verified if the overall neuronal firing was linked to the duration of the reaching phase in all Goal-Modulated neurons. We found no significant correlation between the duration of the reaching phase (from handle release to object touch) and the neuronal discharge across all the trials, for neither of the conditions (*Container*: r=-0.013, n.s.; *Hand*: r=-0.037, n.s.; *Mouth*: r=-0.046, n.s.).

2.4 DISCUSSION

The main findings of the present study show that “social goals” are coded in the motor system of macaques. We found that among a population of “hand grasping motor neurons” in F5 having a preference for the final goal of an action, some were coding goals implying a social interactions between two individuals. In fact, a number of neurons (5% of all neurons, and 11% of the goal-modulated population) had a preference for the condition *placing in the hand of the experimenter*, a condition involving a social interaction. These results extend previous findings (Bonini et al., 2010; Fogassi et al., 2005) showing that hand grasping motor neurons can code the same grasping act differently according to the goal of the action in which the coded act is embedded (Bonini et al., 2010; Fogassi et al., 2005). Our results show that different aspects of the context are capable to modulate the neuronal activity. Some of these contextual cues, such as the presence of a container or the type of object to be grasped, are important elements that are probably taken into account by the monkey in order to allow itself to establish what can be the final goal of the action. Such information clearly modulates the activity of motor and MNs in F5 and IPL. In line with this, these findings describe a new property of the motor neurons in F5, namely the neuronal modulation due to the upcoming interaction with another individual. In the present experiment, this interaction consists in placing an object in the hand of the experimenter. The type of motor act sequence required to accomplish such goal was not different from “placing in a container” (*Container* condition), nevertheless the differential activity of some grasping neurons in F5 demonstrates that such difference is indeed relevant for the monkey and is represented in the ventral premotor cortex. The overall proportion of neurons, that discharged differently during grasping when this act (grasping) was embedded into actions aimed at different goals, from the present study (43%) is comparable with the proportion found in a previous study (38%, Bonini et al., 2010). The novelty of the present study is that almost half of the goal-modulated F5 neurons have a preference for the social condition alone (*Hand*) or in combination with another condition (*Hand* + *Mouth* and *Hand* + *Container*). It is interesting to note that in the population of neurons modulated by two conditions, the *Container* + *Hand* is the most represented, suggesting that the conditions *Container* and *Hand* have similarities in different aspects, such as the kinematics of the placing phase or the space location where the object was placed. It is therefore possible that the activation of similar motor programs involving similar goals (placing objects) could result in comparable neuronal modulations, independently from the contextual features provided by the environment.

The influence of social cues on the motor system has been shown in many contexts in human studies (Chinellato et al., 2015; Dolk et al., 2014; Sartori et al., 2011; Sebanz et al., 2006). For instance, in a task where participant were required to performed a grasp while observing an actor doing a grasping with an interactive purpose (Chinellato et al., 2015), it was found that participants' grasping movements were delayed and trajectories deviated indicating that socially relevant stimuli are acknowledged by the motor system.

Social information is also crucial for non-human primates (Emery, 2000; Klein et al., 2009; Nummenmaa and Calder, 2009). The importance of such social information for macaque monkeys has been shown is a behavioral study (Deaner et al., 2005). In that study, male monkey observers are required to look at various pictures, among which images of dominant male monkeys or of female's hindquarters. It was found that monkeys accepted to renounce to a certain amount of juice reward (i.e. monkeys were willing "to pay") to view the socially relevant images. Although the importance of social information to monkeys is widely recognized, to our knowledge, no studies have yet investigated the impact of social cues or social interactions on the monkey's premotor cortex. Socially relevant cues, like gaze direction, have been showed to directly impact the discharge of grasping mirror neurons in area F5 (Coudé et al., 2016). In that study, the visual discharge of some neurons (mirror neurons) preferred a gaze direction either congruent or incongruent with the location of the grasped object. One study investigated the impact of social context on parietal neurons (Fujii et al., 2007). In that experiment, parietal activity resulted to be strongly tuned to the use of the arm contralateral to the recorded hemisphere when two monkeys were sitting side-by-side and could reach for and grasp food without interacting. However, when the food was put in a shared space and social conflict could emerge, the neurons developed different combinations of preferences to self and other motion (Fujii et al., 2007).

These results showed that some neurons are coding social space within the parietofrontal circuits involved in the action of grasping.

2.4.1 Mouth condition preference

The neuron preferring the *Mouth* condition have a stronger response in their preferred condition when compared to the neurons preferring *Container* or *Hand* condition. Also, the discharge in the *Mouth* condition is also stronger in the non-modulated neuron population. This could be due to the fact that the *Mouth* condition is a more natural one and correspond to a behavior that is already present in the repertoire of the monkey. Neurons preferring "Grasping to eat" were found to represent the majority of goals-modulated neurons in previous studies (Bonini et al., 2010; Fogassi et al., 2005). In the monkey, the representation of actions in the parietal and frontal lobe is based on chains of motor acts (Chersi et al, 2011; Fogassi et al. 2005). These chains are formed by action-constrained neurons that code specific motor acts (e.g., grasping) according to the final goal of the action in which the motor act is embedded. When an individual intends to grasp food to eat it, a motor chain starting with "grasping" neurons and ending with "bringing-to-the-mouth" neurons is selected at the very outset of the action, whereas if the same individual intends to grasp the food for placing it, a different chain is selected (Chersi et al, 2011; Fogassi et al. 2005). Furthermore, long-train intracortical microstimulation of this premotor region elicits hand-to-mouth movements similar to the actions naturally performed by the monkeys (Graziano et al., 2005, 2002), so that the action goal relatedness of some F5 neurons shown in this study could constitute a possible mechanism underlying the organization of natural actions in this region. The strength of the neuronal firing in the *Mouth* condition, consisting in a natural and very motorically familiar action, probably reflect this organization and is also supported by the overlapping representation of hand and mouth in F5 (Rizzolatti et al. 1988; Maranesi et al 2012).

2.4.2 Latency of goal discrimination

A large proportion of the goal-modulated neurons recorded in the present study are characterized by an early selectivity. Most of these neurons present a differential activity before the hand-contact with the object (78%). This means that these neurons discriminate when the hand approaches the object and the finger aperture “preshapes” itself to fit the physical characteristics of the object features. The kinematic parameters of grasping in human and monkeys have been shown to involves the activation of the motor processes before the actual grasping (Jeannerod et al., 1995; Roy et al., 2000). Neurophysiological studies on the grip selectivity of F5 motor and visuomotor neurons showed that the selectivity for a certain grip type appeared already in an early stage of the grasping task to be performed, and it progressively increased as the grasping execution approached (Murata et al., 1997; Raos, 2005; Umilta et al., 2007). The present data suggest that, beyond coding grip type and being influenced by the goal of the action, some F5 neurons are modulated by the social context in which the grasping action is made. The goal discrimination occurs especially early in the *Mouth* condition and this is probably linked to the fact that such behavior is part of the motor repertoire of the monkey, as mentioned above. Overall, it is not surprising to also find early goal-discrimination in the interactive condition. Indeed, the reactive processes of adjusting own movement are probably not sufficient for the fine tuning of on-line interpersonal coordination. In real life, co-agents cannot simply react to a partner’s behavior but need to predict it for a successful interaction (Knoblich and Jordan, 2003).

2.4.3 Possible effects of reward delay and mouth cortical representation

Although the total reward quantity (liquid and solid) is the same in all conditions, the temporal proximity of the solid reward in the mouth condition is a possible factor that could have had an impact on the neuronal discharge reported between the conditions. In the *Mout*h condition, the solid reward delivery was temporally close to the grasping action (the monkey brought the reward to the mouth and ate it right after having grasped it). The monkey received a juice reward afterward. In *Container* and *Hand* conditions, the reward sequence was inverted: the monkey received juice after the placing action had been successfully completed, and then received the solid reward. However, the time lapse between the two rewards were short (about 1 second) and spurious factors, such as motivation and reward expectancy can be reasonably ruled out. In addition, we found neurons preferring each of the three conditions, so the reward expectancy is unlikely responsible for the overall neuronal preference pattern found in this study. Also, this increased activity in *Mouth* condition could also be linked to a further motor preparation of the mouth. However, we can rule out this hypothesis since all the recorded neurons have been selected for their specificity to grasping actions executed with the hand.

2.4.4 Experimental conditions limitations

The present experiment was a first step in investigating the influence of social interactions of PMv neuronal response. Thus, in order to avoid any visual stimulation (experimenter hand movement), the interaction between monkey and experimenter was kept minimal. The experimenter remained passive with the hand open, palm up, in the same position where the container is in the *Container* condition. The monkey was required to place in the hand and therefore, its action was stereotypical and did not require an actual coordination with the experimenter. In a task requiring more elaborate interactions, the pattern of neuronal preference could be more complex and could be more specific for the type of interactions. It could also potentially unveil preference patterns more specific to particular motor acts of the motor sequence embedded in the interaction. Still, even with the limited interactions allowed by our experimental protocol, we could find neurons preferring the social interaction condition. The use of more elaborate interactions in future experiments could potentially reveal even stronger preference with richer specificity patterns. Among possible new conditions, it would be interesting to test a condition with the presence of both the container and the hand of the experimenter. Such condition could help disentangle the role of the social cues in itself from the decision of the monkey to interact.

3. STUDY 2: Mirror neurons in the ventral premotor cortex of macaques are modulated by social goals.

3.1 INTRODUCTION

3.1.1 The Importance of Social Context to understand the intention of others

In the study by Fogassi et al. (2005) the idea that action sequences were composed by a series of motor acts aimed at specific goals was investigated through single cell recording in the convexity of the inferior parietal lobule. Both motor and mirror neurons in IPL were investigated in two tasks: a) a motor task b) a visual task. In the motor task the monkey was instructed to grasp an object with a precision grip and to bring it to the mouth for eating or to place it into a container. Thus, the first motor act was the same in the two sequences but the final goal (eating or placing) was different. Similarly, during the visual task, the monkey had to observe the same action performed by an experimenter. Two main findings emerged: 1. IPL neurons discharged in association with a specific motor act: grasping act; 2. most of IPL neurons code the same act (grasping) in a different way according to the final goal of the action in which the act is embedded. Similar findings have been also obtained in the ventral premotor cortex (F5) (Bonini et al., 2010). The ability of motor and mirror neurons in IPL and F5 to encode the same action according to the final goal, probably, derives from the presence of external instructive cues, such as the context (container) or the type of object (piece of food or metallic object). According to the chain model proposed by Fogassi et al. (2005) and Chersi et al. (2011), visual inputs related to the context could contribute to the activation of specific chains of neurons, encoding for the grasping motor act, that, in turn, could pre-activate those neuronal chains that specify the scope of the action (for example, grasping to eat), allowing the observer to predict the final goal, or the motor intention, of the action performed by the observed agent (Chersi et al. 2011).

Thus, these findings demonstrate that motor neurons and mirror neurons in F5 and IPL are modulated by different aspects of the context. This would allow the individual to create an internal representation of the action performed by others, that integrates different aspects not only of the biological context but also of the social context. This could allow a better understanding of the action and intention of others. Previous studies investigated the role of the context in the modulation of MNs activity. Despite the hypothesized role of MNs in social cognition, it is surprising, that previous studies did not explore the possible role of the social context in modulating MNs activity. In fact, some aspects of the social environment may play an important role in the construction of this representation in order to better decipher the behavior of other in relation to social goals. Among these aspects, gaze direction could have a central role because it is closely associated with the attentional orientation which anticipate the actions of an individual. Gaze direction has been studied both in humans and in non-human primates (Emery et al., 2000; Ferrari et al., 2000; 2008). About this issue, *Coude* and colleagues (2016) investigated the activity of hand mirror neurons in PMv of the monkey during the observation of grasping actions in which the observed agent shift his/her head/gaze in a direction congruent or incongruent with that of reaching and grasping. The results of this study show not only the activation of gaze-sensitive mirror neurons in F5, but also that they are distributed in two categories, showing preference for congruent or incongruent gaze direction. According to the authors, neurons of the former category are expected, because from the early stages of postnatal development, gaze behavior is naturally coordinated with arm direction during the execution of a reaching/grasping task (Johansson et al., 2001; Crawford et al., 2004). The importance of this coordination is also confirmed by a series of behavioral studies on eye-hand integration in humans, showing that hand reaching toward objects is affected when gaze is diverted from the target (Helms et al., 1991; Henriques et al., 1998, 2003; Terao et al., 2002). Regarding the incongruent responses, they could be the results of prolonged training of the monkey, in which the mirror neurons became attuned with the two set of visual stimuli, which were equally presented. Moreover, the results show also a significant number of mirror neurons that have a preference for the direction of grasping action, even in the absence of gaze preference, but the gaze preference will be only relevant if grasping is performed in the neuron’s preferred direction (Coude et al., 2016). Taken together, the results suggest that these two stimuli (gaze direction and space in which the action is performed) could be critical to support the observer's capacity to correctly decode motor intentions during the observation of others' action.

Furthermore, other areas, specifically in the temporal cortex, appear to encode for the gaze direction of others. For example, the superior temporal sulcus presents neurons that are activated during the observation of the head directional movements (Jellema and Perrett, 2003). Similar neurons were found also in the intraparietal sulcus (LIP area). Differently from the STS neurons, neurons discovered in the LIP area show, also, an activity related to eye movement. In fact, these neurons are activated by both the orientation of the head and the eyes in a particular direction, and during the observation of an individual who directs the head in the same direction (Shepherd et al., 2009).

All of these studies mentioned above, included, as paradigm, a "passive observation" of the action performed by another individual, in which the observer simply observed the action without any interactions, behavioral choices or cooperation with the other individuals.

Indeed, the others' behavior and choices, in an ecological and social context, provide important information to which individuals need to pay attention and to prepare any appropriate interactive responses. In fact, the social context, in which the individuals live, requires not only passive behaviors, in which the single individual performs its own actions without any consequences for the others, but more often active situations in which individuals are forced to cooperate and interact with others in order to preserve their social interpersonal relations. In fact, other areas of the cortex, in addition to those described above, seem to be involved in the encoding of others' actions in interactive situations. For example, *Yoshida* and colleagues (2011) found, in the medial frontal cortex of the macaque (MFC), neurons that were known as "partner-type" because they tend to increase their activity during the observation of the action performed by the partner. In this study, two monkeys (actor and observer) sat face to face and took turns making a choice for a reward. The actor was required to choose one of the two color target buttons: the actor's correct choice yielded a liquid reward to both monkeys, whereas neither monkeys were rewarded if the actor made wrong choice. Moreover some of these "partner-type" neurons shows a modulated activity depending on whether the action, performed by the partner, was correct or not, even although their activity was not a representation of hierarchical relationship. So that, these partner-type neurons appear to be involved in more complex aspects of the encoding of the others' action, for example the value of the same action. *Yoshida* and colleagues suggest that, in complex social environments, the behavior of others provides a rich supply of fictive information, such as fictive outcomes (rewards or punishments not directly experienced but observed) and fictive actions (not directly executed but observed). Humans and other animals have the capability of learning from fictive information, such as learning from others and learning about others (Frith C. and Frith U., 2010; Hayden et al., 2009). Therefore, the MFC contains neurons encoding fictive rewards and could encode fictive actions and plays a critical role in social learning (Yoshida et al., 2011).

Also the orbitofrontal cortex (OFC) is involved in the coding of some aspects of the others' action, in particular, the value of a reward. In fact, an fMRI study by *Sescousse* of 2010, has shown that humans reveal a reinforcement-specific representation in different areas of the OFC: specifically, the ventral lateral part of the OFC is implicated in the development of monetary rewards (secondary reinforcement), while the dorsal lateral part appears to be involved in the processing of erotic stimuli (primary reinforcement). Other brain structures seem to be involved in the encoding of the subjective value of a reward, in particular the ventral part of the striatum, the anterior insula, the anterior cingulate cortex (ACC) and mid-brain (Sescousse et al., 2010).

All these mechanisms are useful to make decisions between different alternatives and in relation to different social contexts in which there is the need to operate a cost-benefit assessment. In social situations, we are aware of our actions and their consequences but we also consider those of others, especially those with whom we might interact (Fehr and Fischbacher, 2003). We also estimate the internal states of others, perhaps by simulation, which in turn shapes our future actions (Gallese et al., 2004; Chang et al., 2013). Social situations can drive observational learning and can influence neural computations that, ultimately, result in cooperation, altruism or spite (Rilling et al., 2002; Chang et al., 2013). Neuronal circuits involved in learning-reinforcement and decision-making are crucial for normal social interactions. Critical nodes include the ACC, OFC and subcortical areas (Rudebeck et al., 2006; Tsujimoto et al., 2009). Neuroimaging studies in human report activation of some of these areas by both giving rewards and receiving rewards, and lesions to some of these areas result in impaired social decision making (Chang et al., 2013). For example, *Azzi* et al. (2011) recorded single OFC neurons in two monkeys who were trained on a simple visual discrimination task in which they could earn rewards for themselves and for two passive monkey partners physically present in the testing room. Two main blocks of trials were run: in the "non social" block, only the active monkey earned reward, in the form of a small, medium, or large drop of water, while in the "social block" both the monkey and a pre-designated partner earned a medium-size reward. It has been proposed that one characteristic of the OFC is its capacity to encode reward value in a common neural currency (Padoa Schioppa and Cay, 2011). This capacity is a useful mechanism in decision making when one needs to choose between disparate alternatives, like different kinds of food, and between alternatives that are complex and require combining several positively and negatively weighted attributes, as in most real-life situations. In the study by Azzi and colleagues, when the monkey expected to acquire a given reward size for itself but also for the partner, the monkeys' motivation, as measured through its correct response rate, was reduced to a level corresponding to a smaller reward size (Azzi et al., 2011). Moreover, the results of this study demonstrate that the monkeys' lower motivation to work for joint rewards is encoded through reduced activity in one class of neurons, the firing rate of which is a direct function of reward value, and through enhanced activity in another class of neurons, the firing rate of which is an inverse function of reward value. A further analysis indicates that OFC neurons may also track social preference by showing enhanced activity when joint rewards are obtained with the session's preferred partner. In general, prosocial and altruistic behaviors appear to be related mostly to the social rank of the individuals or to the type of relationships in the group. Primates live in highly social environments, where prosocial behaviors promote social bonds and cohesion and contribute to group member's fitness (Ballesta and Duhamel 2015). In order to act in accordance with their motivation for social interaction, macaques need to be able to predict the consequences of their behavior on future social bonds (Ballesta and Duhamel 2015).

These finding argues for a motivational-value representation in the OFC that integrates the influence of social context on goal-directed actions. Such mechanisms would be well suited to play a role in evaluation processes taking place during social comparison, and in emotions such as regret, envy, or gloating, which influence our decision making (Shamay-Tsoory et al., 2004; Bault et al., 2008).

3.1.2 During social interactions planning an action may interfere with the internal motor representation generated during observation of others' actions

Humans beings spend most of their time interacting with others. Several studies, performed both in human and primates, have described different aspects, effects and theoretical development on how people represent their own and other person's action (Gallese et al., 1996, Fogassi et al., 2005; Bonini et al., 2010), but there is still a considerable lack of understanding of the precise cognitive mechanisms governing interactive performance. Part of this gap is probably due to the fact that several paradigms have typically relied on single individuals passively observing or imitating other individuals. In contrast, in interaction contexts, individuals are often required to perform complementary action of an observed action, complementing, in this way, each other's movement in a balanced manner rather than acting in the same manner (Sartori et al., 2013a, 2013b). When an individual observes an action performed by another individual, an internal motor representation of the observed action is activated in order to facilitate the others' intention understanding to be able to be coordinated with others (Fogassi et al., 2005; Bonini et al., 2010). But, when an individual observes an action performed by another individual and knows that this action is aimed to interact with him, two internal motor representations are activated, one concerning the observed action, the other concerning the motor plan of the action which is going to be execute. Since these two representations are activated at the same time, it is possible that they could come in conflict with each other. In other words, observing an interactive gesture automatically generates an internal representations of the required movement to interact. However, this internal representation could be in conflict with the internal representation associated with the observed action when they are both active at the same time (Sartori et al., 2013a). It has been show that observing the movements of others can facilitate or interfere with concurrent movement execution, depending on observed and executed movement congruency (Stűrmer et al., 2000; Brass et al., 2001; Castiello 2002). That is, observing a movement primes the execution of that movement, thereby interfering with the execution of another movement (motor priming). Kinematic results on studies performed in human, during the observation and execution of congruent stimuli (non interactive situations) and incongruent or complementary stimuli (interactive situations), demonstrate a general delay in the grasping and reaching components and an increased trajectory deviation when an object is grasped with the intention to interact with the agent (Chinellato et al., 2015). These effects could be due to the interference between the motor representation of the observed action and the motor representation associated to the complementary movement which is going to be performed. In this way, during action observation, the specific networks subserving that particular movement are already tuned for the action, but observing congruent stimuli can have measurable interference effects on simultaneously executed actions, depending on the context (Sartori et al., 2011, 2012, 2013a, 2013b).

3.1.3 Brain to Brain Coupling

As described above, several studies in neuroscience have mainly focused on the recordings of brain activity of a single individual performing a task, partly because of the difficulty of designing an experimental model that is able to investigate simultaneously more individuals in a social context and under ecological conditions compatibly with the electrophysiological techniques required. Typically, neurophysiological experiments tend to isolate humans or animals from their natural environments by placing them in a sealed room where interactions occur solely with a computerized program (Hasson et al., 2012).

However, our minds are molded in an interpersonal space, so that it is necessary to move from a "single-brain" approach to a "multi-brain approach". Often, the processes, taking place inside the brain of a single individual, are coupled to those that occur in the brain of another individual, through a complex network of signals emitted into the environment. Typically, in order to better control all the variables, the individuals are separated from the social context and they have to interact only with computerized programs. This has, however, prevented from studying some important aspects of our social life, such as cooperation, which is one essential aspect for the study of social cognition. Many nonverbal abilities require the collaboration of more individuals and it’s difficult to study them in an isolated individual. For example, learning by observation allows individuals to acquire information and to learn new skills using others’ negative and positive experience (Isbaine et al., 2015). The ability to learn from others is present in many animal species including birds, fish and mammals (Meunier et al., 2007). For example, it has been shown that monkeys increase their capacity of learning and improve their performance after the observation of a conspecific or of a human individual who performed an associative visuomotor task (Isbaine et al., 2015; Subiaul et al., 2004). In addition, they learn more from the mistakes made by the investigator, rather than by his successes (Isbaine et al., 2015). This was one of the first studies that showed how learning by observation can occur even in very strict conditions, such as, for monkeys, being seated on a primate chair without the possibility to move the head and using an abstract associative visuomotor task. The theoretical tenet of the *brain-to-brain coupling* is that the sensory system of an individual can be coupled to the motor system of another individual. Many joint behaviors, such as mating, group cohesion and predator avoidance, depend on accurate production and perception of social signals, and accordingly, movements must be tightly coordinated with others. As a result, the development of these behaviors is strongly influenced by interactions with other group members. Developmental processes ultimately result in coupling between the sensory system of one individual with the signals produced by the motor system of another individual. Individuals are not passive receivers of information, but active subjects that can modulate their responses through the eyes, facial expressions and the body. Coordination and interactions between individuals depends on accurate production and perception of social cues, and this may depend in turn by the cohesion of the group and the interaction with the same.

Further, coupled systems can generate complex behaviors that otherwise can not be performed in isolation. Many human actions, such as playing basketball or operating a sail-boat, require tight spatiotemporal coordination across team members. During joint actions, people become implicitly coupled at motor, perceptual and cognitive levels (Knoblich et al., 2011). Also the choices an individual makes are often influenced by the decision of others. Individuals playing a strategy game keep track not only of the actions of the opponent, but also of how opponents are influenced in response to their own actions (Hampton et al., 2009).

In conclusion, some aspects of the environment are determined by the physical environment. Other aspects, however, are determined by a group of individuals, who together establish a shared set of rules that shape and constrain the perception and actions of each member of the group (Hasson et al., 2012).

2.2 AIMS OF THE STUDY

Electrophysiological studies, performed on monkeys, show that mirror neurons of the ventral premotor cortex and the inferior parietal lobule are modulated by the final goal of the action (Fogassi et al 2005; Bonini et al 2009, 2010), and that several contextual cues (i.e. type of object grasped, or a container where the grasped object was then placed) play a key role in modulating such discharge (Fogassi et al., 2005). Such modulation has been proposed to reflect the capacity of the monkey to predict the final goal of a sequence of actions. Such property is adaptive as it allows the observer to predict the ongoing action, and therefore to understand the motor intention of the observed agent. From a biological perspective, the survival in a social group is inherently related to the ability to understand and predict the others' behavior and this happens, often, on the basis of cues provided by other individuals and by the type of interaction occurring between group members (Pelpherey et al., 2003; 2004). A recent study on the activity of F5 mirror neurons has demonstrated that also social cues, such as the gaze direction of the observed agent, modulate the visual discharge of MNs (Coudé et al. 2016). It is therefore possible that several aspects of the social context could modulate the discharge of mirror neurons, even though this has not been thoroughly investigated. In addition, MNs activity has never been investigated within more ecologically-relevant situations in which the monkey, following the observation of an action performed by the experimenter, has to interact with him, thus performing a complimentary action.

Kinematic studies in humans have shown that the observation of movements performed by another individual produces motor facilitation, but it could also produce motor interference if the observer has to perform actions that are different from those observed (Kritikos and Castiello 2000; Brass et al., 2001; Edwards and Castiello 2002; Castiello et al., 2002; Sturmer et al. 2000a, 200b). In addition, it has been shown that, under interacting conditions, where the observer has to prepare an action that is complementary to that of the observed agent, but different in terms of kinematics and general patterns, cortical motor activity, recorded though TMS and EMG, could be somehow affected. More specifically, several studies showed that under such circumstances there is an effect of interference of the complementary action preparation on the cortical motor excitability of the observer during the observation phase (Sartori et al. 2011; Sartori et al. 2012; Sartori et al. 2013a, 2013b). One limitation of these studies was that actions were observed on a video and therefore it is difficult to understand what could have been the motor patterns when the observer was interacting with a live actor. Authors explained such interference effect with possible competing motor programs activated during the observation phase (due to the observing action) and the preparation phase (generated by the observer) (Sartori et al. 2013). Despite these studies clearly demonstrate that under more interactive conditions the motor system might differently recruit neural circuits in order not only to decode the observing actions but to simultaneously prepare and coordinate an action with the observed agent, the underlying neurophysiological mechanisms remain still unexplored.

In the current thesis, I therefore explored at neurophysiological level, the different modulation of the social context on the activity of MNs during *active* or *passive* observation of grasping acts aimed at different social or non-social goals. For example, if the final goal of grasping an object performed by an individual is to give it to the observer, the observer, on the basis of visual information of the context and previous interaction with the individual, must prepare a motor program complementary to that of the observed agent, in order to extend the arm and receive the object, with a sequence of actions that must be well coordinated with the observed individual. Conversely, under situations where such action is not aimed at an interaction with the observed agent, it is possible that MNs may have a different modulation. In other words, the *passive* action observation, which does not require any motor planning, may have a different impact from the *active* action observation, on neuronal populations of F5 related to the encoding of the others' action.

The purpose of this study is, therefore, 1) to investigate the role of contextual stimuli, either social (e.g. the presence of an experimenter interacting with another individual) or non-social (a container where the grasped object had to be placed in), can modulate the visual mirror neurons discharge in the ventral premotor cortex, during the observation of goal directed actions; 2) to investigate the modulation of the activity of the mirror neurons during the *passive* or *active* action observation. From a theoretical perspective, this is a key issue in MNs research because one of the criticism about the hypothesis that mirror neurons are involved in the action understanding is that their visual discharge could reflect a motor preparation or anticipation of the action that the monkey will perform (Fontana et al., 2012; Kilner et al., 2004). And thus, the mirror neurons' visual discharge could be interpreted as a "product" of a motor activity, because the monkey, anticipating the action goals, could potentially prepare an action that is in relation to the observed one.

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Animals and surgical procedures

Two female rhesus macaques (*Macaca mulatta*) served as subjects (M1 and M2) for this experiment. Before training the monkeys to perform the action observation task described below, they were habituated to be seated on a primate chair and to interact with the agent. After training was completed, a titanium head post (Crist Instrument, Hagerstown, MD, USA) was surgically implanted on the skull using titanium screws. After about one month, a cilux recording chamber (18X18 mm, Alpha-Omega, Germany) was stereotaxically implanted and secured with dental cement. For both procedures, each animal was deeply anaesthetized with ketamine hydrochloride (5 mg/kg i.m.) and medetomidine hydrochloride (0.1 mg/kg i.m.) and its heart rate, temperature and respiration were carefully monitored and kept within physiological range. Pain medication was routinely given after surgery (Fogassi et al., 1996; Rozzi et al., 2006).

The animal handling, as well as surgical and experimental procedures, complied with the European guidelines (86/609/EEC 2003/65/EC Directives and 2010/63/EU) and Italian laws in force on the care and use of laboratory animals, and were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012) and authorized by the Italian Health Ministry (D.M. 294/2012-C, 11/12/2012). The well-being and health conditions of the monkeys were constantly monitored by the institutional veterinary doctor of the University of Parma.

3.3.2 Behavioral procedures: Action observation task

In the action observation task, the experimental set up consisted of a table (60X60 cm), the monkey was seated at one end of the table, facing the experimenter (hereafter called *agent*) sitting at the other end of the table with his right hand resting on a central platform, used as a starting point. The monkey had to gently hold a handle (leaning its hand on it) throughout the whole experiment, except for the condition in which it had to interact with the agent (see below *Monkey* condition). The grasping target (either the food or the object, see *Experimental conditions* below) was located in front of the agent, at about 15 cm from the starting platform. The monkey was trained to maintain its gaze in a Fixation Window (22°X22°), centred on the target, while holding the handle. The experimental setup, the timeline of events, and the task are illustrated in *Figure 13*. A task trial started if the monkey was leaning its hand on the handle. A red laser and a sound instructed the monkey to fixate the window for 1500 ms. Time=0 in *Figure* 13 indicates the beginning of the fixation; while the monkey was fixating, the agent was cued to grasp the target (500 ms after the laser was turned on). At the end of the fixation, the agent placed the object in a container or gave it to another individual or eat the piece of food, depending on the condition (see details in the *Experimental conditions* described below). A juice reward was delivered after a fix time after the agent's hand touch of the target object in all conditions, if the monkey correctly fixated the window for the established period of time. The monkey was required to hold the handle throughout the entire trial to get the reward. The release of the handle automatically aborted the trial, except for the *Monkey* condition where the monkey was trained to release the handle in order to grasp the object that was given by the experimenter (see details in the *Experimental conditions* described below).

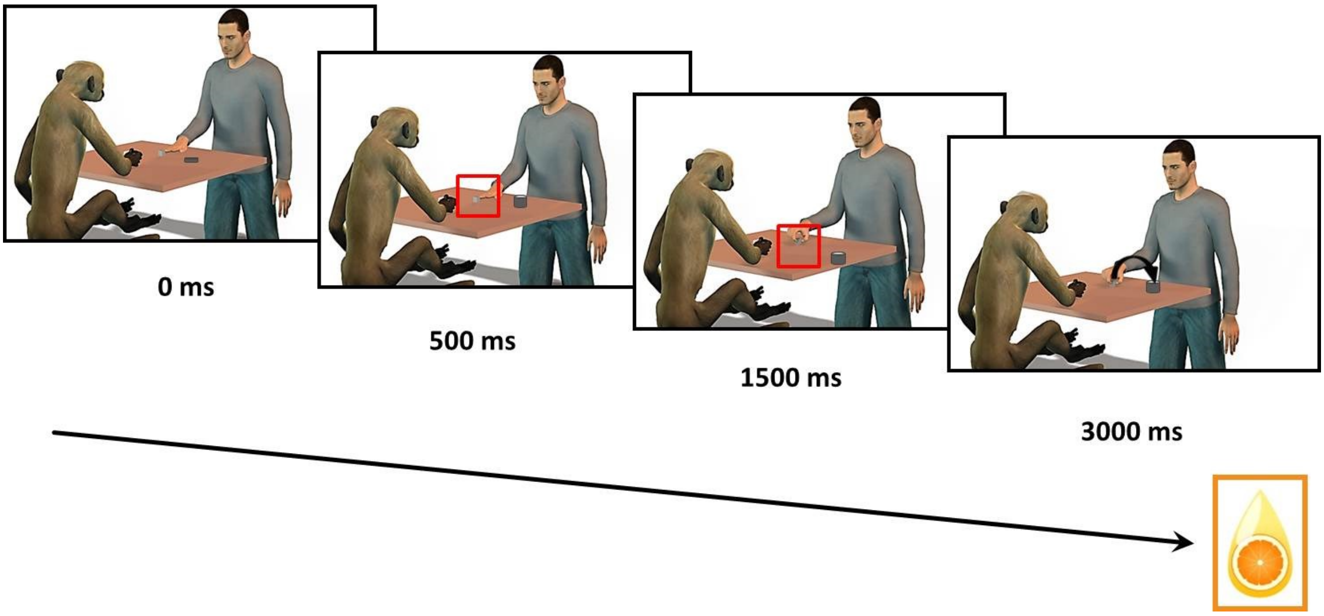

Figure 13 Time course and set up of the Visual task: example of *Container* condition trial.

3.3.3 Experimental conditions

The experimental conditions are illustrated in *Figure 14*. We tested two monkeys in four experimental conditions in which they observed the agent's behaviour, which consisted in a grasping act performed with the right hand. The conditions were: *Container* condition – the agent grasped the object and placed it into a container (diameter: 6 cm) located about 20 cm on the left of the grasping platform (grasp-to-place); *Hand* condition – the agent grasped the object and placed it in the right hand of a second agent who stood next to the first one (grasp-to-give in the hand). The hand of the second agent was maintained wide open and it was still directed rightwards, towards the first agent; *Monkey* condition – the agent grasped the object, lifted it for approximately one second and then gave it to the monkey (grasp-to-give to the monkey), who detached the hand from the handle and grasped the object offered by the agent with the right hand. Soon after the object was grasped, the monkey placed the object into a container located 10 cm left of the handle; *Mouth* condition – the agent grasped a piece of food, which had the same shape and dimension of the metallic object, and brought it to his mouth to eat it (grasp-to-eat). The *Hand* condition and *Monkey* condition represented the two social conditions we introduced to better analyse the contribution and the role of the social context on the MNs activation.

The food and the object had the same size and shape (a cube of 1 cm). Note that these four conditions consisted in the same motor act (i.e. grasping) but it was aimed at different final goals. The contextual cues (the presence of a container next to the object to be grasped in the *Container* condition, or next to the monkey handle in the *Monkey* condition, the presence of a second agent, the piece of food to be grasped in the *Mouth* condition) were probably the key cues that could be used by the monkeys in order to understand what was the final goal of the observed action sequence. Previous studies have already discussed the importance of contextual cues to modulate MNs response and to support the capacity of predicting the final goal of the action sequence (Fogassi et al. 2005; Bonini et al., 2010). In the *Monkey* condition, the contextual cues consisted in the mere presence of the object to grasp and the presence of the container (next to the monkey), where the object had to be placed by the monkey.

In all but one condition, the monkey was required to simply observe the action. Indeed, during one condition, the *Monkey* condition, the monkey was allowed to release the handle, take the object from the hand of the agent and interact with him. Note that the monkey was allowed to release the handle only when the agent was starting the forward movement, and thus entering the reaching space of the monkey in order to coordinate with the monkey the release (agent)-grasp (monkey) action., otherwise the trial was aborted. Additionally, it should be taken into account that, in this condition, the agent, after the grasping action, could decide arbitrarily when to start the forward movement toward the monkey, so that the movement latency with which the monkey released the handle is variable from trial to trial and dependent from the agent's motor behaviour.

Note that before the beginning of the trial in all conditions, the agent had to set the contextual cues according to which condition would have been carried out. This allowed the monkey to learn the final goal of the action beforehand (before the trial even started).

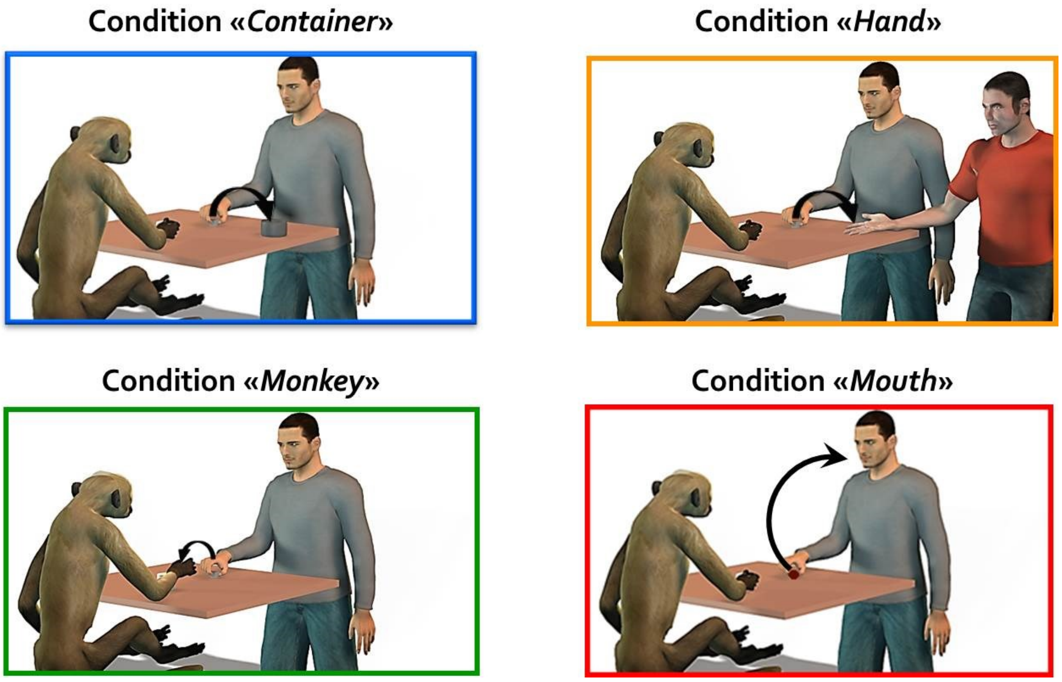

Figure 14 The four conditions of the Visual task: *Container*: grasping to place. *Hand*: grasping to give in the hand of a second agent. *Monkey*: grasping to give to the monkey. *Mouth*: grasping to eat.

3.3.4 Data recording

Neuronal activity was recorded with a linear multi-site electrode (16-channel, 250 µm spacing; U-probe, Plexon inc., Dallas, TX, USA) and digitalized at 40kHz using the Omniplex 16-channel recording system (Plexon). Eye movements were monitored throughout the experiment using a customized eye tracker (University of Tubingen) equipped with a CCD camera (Ganz, Commack, NY, USA, model ZC-F11CH4). The left pupil was lit with infrared LEDs, allowing the eye position (sampled at 50Hz) to be tracked by the CCD camera. The X and Y coordinates of the eye position were fed to the Omniplex recording system. The visual task (including window size, fixation duration, reward delivery, etc.) was computer-controlled through a customized Labview© program. Contact-detection panels (Crist Instrument, Hagertown, MD, USA) were used to record the exact moment in which the agent's hand touched the target. This event was used to align the neuronal activity recorded for each trial in all conditions.

The recording sessions were performed on well-trained monkeys having reached about 95% correct trials criterion for the fixation in the task described above. We collected usable MN data in 12 sessions recorded over a period of 5 months from Monkey 1 and in 11 sessions recorded over 4 months from Monkey 2. During the insertion of the electrode shaft in the brain, we ensured that the topmost electrode was positioned under the dura, but remained outside of the cortex, so that it could be used as a reference channel. Of the 15 available recording channels, two were dedicated to eye movements (X and Y coordinates). The remaining 13 channels were used for single-unit recording. The multi-electrode was lowered in the brain through the intact dura and the general properties of the neurons were tested. When putative mirror properties for grasping were found, we waited about one hour to allow the neural activity to stabilize and then started to record single units using the behavioral protocol described above. For each recording session, to test also the presence of a motor discharge of the MNs, we also performed a Motor task, described in Study 1, in which the monkeys had to grasp either the object or a piece of food.

3.3.5 Preliminary testing of neuronal activity

Before proceeding with the neural testing using our behavioral protocol, single- and multiunit activity were systematically tested for visuomotor properties to identify recording sites endowed with MN activity (Rozzi et al., 2008; Maranesi et al., 2012). Briefly, we required the monkey to grasp food items in various conditions (i.e. with eyes closed, or without flexing the wrist, elbow or shoulder) enabling us to disentangle neuronal activity related to visual stimulation, reaching or grasping objects. Also, to exclude the possible presence of mouth-related responses, we tested any neural activity changes related to the delivery of small pieces of food directly into the mouth while the monkey's eyes were closed. Finally, visual properties were studied by presenting the monkey with 3D-objects (e.g. food items and solids) of different shape, size and orientation, moved in various space locations, directions and distances from the monkey, as well as different manual actions performed by the experimenter.

3.3.6 Data analysis

Neuronal spikes waveforms were classified offline into units using a commercial spike-sorting software (OfflineSorter, Plexon). For each of the 13 electrodes, a mean of 2.7 units were isolated. The neural response selectivity was determined for each neuron comparing the spike frequency for each trial across two epochs (*Baseline* and *Grasping*, described below) and 4 conditions including *Container* condition, *Hand* condition, *Monkey* condition and *Mouth* condition. The statistical analysis consisted in a 2X4 repeated measures *ANOVA* (factors: epochs and conditions) followed by a Newman-Keuls *post hoc* test, all with a 0.05 alpha level. The two epochs had a 600 ms duration and were precisely calculated with reference to the digital events. The *Baseline* epoch corresponded to a time period in which the monkey remained still and was leaning his hand on the handle. This epoch lasted from 1000 to 400 ms before the beginning of the fixation signaled by the red laser being turned on. The *Grasping* epoch started 300 ms before the agent's hand contact with the object and finished 300 ms after that same event.

Moreover, we performed an electromyographic recording (EMG) to test whether any monkey hand muscle movements during the action observation task or if any preparatory movements occurred during the *Monkey* condition. The EMG recordings were carried out in different sessions with respect to those performed for the acquisition of the neuronal activity. The EMG electrode was located on the *flexor digitorum superficialis* muscle and the signal was filtered (0.5-500 Hz) and amplified through Plexon recording system. For each visual condition, the results were averaged to analyze the muscular activity (µV) in the *Grasping* epoch.

3.3.7 Population analysis

The population analysis was performed by constructing a spike-density function for each neuron by averaging the spike frequency for each 20 ms bins across trials. The baseline activity (mean spike activity during the baseline epoch) was subtracted from that of each bin. The data were then normalized by dividing the spike frequency within each bin by the maximum absolute value found across all bins. The result is a discharge rate ranging between 1 and 1 for each neuron. The spike density function was smoothed using a Gaussian kernel of 30 ms. The population curve was computed as the algebraic sum of the spike frequency for each bin of each neuron. The statistical differences between populations of neurons were calculate based on the firing rate during the *Grasping* epoch. One-way repeated-measure *ANOVA*s were calculated, followed by Newman-Keuls *post hoc* procedures.

3.3.8 Monkey movement latency vs neuronal discharge

To better investigate the relationship between the neuronal discharge and the monkey movement latency in the *Monkey* condition (namely the only condition in which the monkey knows it has to observe the action while preparing to interact with the agent), a time/frequency correlation analysis was carried out. The correlation was performed on a single monkey and it was calculated between two parameters for each neuron and for each trial. One parameter (the monkey movement latency) consisted in the time difference between the agent's hand contact with the object and the monkey's hand release from the handle. The second parameter (the neuronal discharge) was the spike frequency calculated in the *Grasping* epoch. Both linear and non-linear correlations were calculated. Then a Spearman correlation analysis was carried out.

3.3.9 Local Field Potential

In order to further investigate the relationship between neuronal activity and monkey movement latency, we analyzed the Local Field Potential (LFP). LFP analyses were performed considering the signal of all the recording sites with mirror properties and considering only the *Monkey* condition trials executed. Raw LFP data were filtered (1-500 Hz, Butterworth forward-reverse) and cut into trials ranging from 2s before to 2s after the alignment event, that is the agent's hand contact with the object. We then calculated the power in the frequency range of 1 to 120 Hz, considering a 500 ms sliding windows with 50 ms steps. The signal was then normalized with respect to the baseline (the first 500 ms from the beginning of the trial) and averaged for all the correct trials.

To better characterize the relationship between monkey movement latencies and LFPs, we also made separate analysis of short and long movement latencies. Since our data didn't follow a normal distribution, we separated the short and long latencies using the median latency as a threshold (T= 0.89 s). While the LFP high gamma activity could be considered as a measure of neuronal activity of the neuronal population near the electrode (Ray et al., 2008), conversely, the beta desynchronization activity could reflect several neuronal phenomena such as a steady-state isometric contractions, sensory-motor binding and motor planning (Sanes and Donoghue 1993; Baker et al., 1997; Donoghue et al., 1998; Kilavik et al., 2012; Waldert et al., 2015) . For these reasons, two statistical analyses (t-test for independent variables) were performed considering the mean power in the high gamma (60-120 Hz) band and beta (15-30 Hz) band, both for short and long movement latency within the *Grasping* epoch (-300 to +300 with respect to grasping touch). The results were calculated as Event Related Desynchronization (ERD) and Event Related Synchronization (ERS).

3.4 RESULTS

We recorded the activity of 131 neurons from PMv of two monkeys during the action observation task, in 41 recording sessions. Twenty-three of the 41 penetrations have mirror neurons properties. Eighty-four neurons of the 131 total analysed neurons discharge both when the animals performed grasping movements and when they observed the same action performed by the agent. We hereafter refer to these neurons as “mirror neurons” (MNs). The remaining 47 neurons show other properties, such as purely motor, visual, peripersonal and tactile properties or they are mouth grasping mirror neurons.

Figure 15 represents the images regarding the location of the recording chambers and the functional properties of the penetrations we performed in M1 (on the left) and in M2 (on the right). Note that, the reconstruction of the hypothetical position of the recording chamber is based on the cerebral sulci positions from the MRI images and on the functional properties analysed through clinical testing. The location of the penetrations positions in coordinates (anterior-posterior, medio-lateral) are based on their functional properties and depth. In this figure, *Panel A* and *Panel B* represent MRI pictures and graphic images of the left hemispheres of the monkeys in which the cerebral sulci and the hypothetical position of the recording chambers (in PMv cortex) are indicated. *Panel C* shows the visual and motor properties of the recording sessions we performed. In both monkeys, most of the penetrations shows the motor properties related to the hand and the mouth movements. In general, the neurons show a specific activity for goal directed movements, such as the grasping of objects. In some penetration, (more lateral), the properties correlate with movements of the hand (for example, reaching-grasping and object manipulation) and mouth (for example, mouth opening or chewing). Some penetrations (more anterior and within the Frontal Eye Field) have different properties from those typical of F5, such as eye movements (saccades or eye movement for biological motion) and have not been portrait in the figures. In both figures, *Panel D* represents the 23 penetrations we recorded in both monkeys with mirror neurons properties. All of them were recorded in the hand motor area: the mirror properties we found are correlated with the observation of actions correlated with hand movements and not with mouth movements.

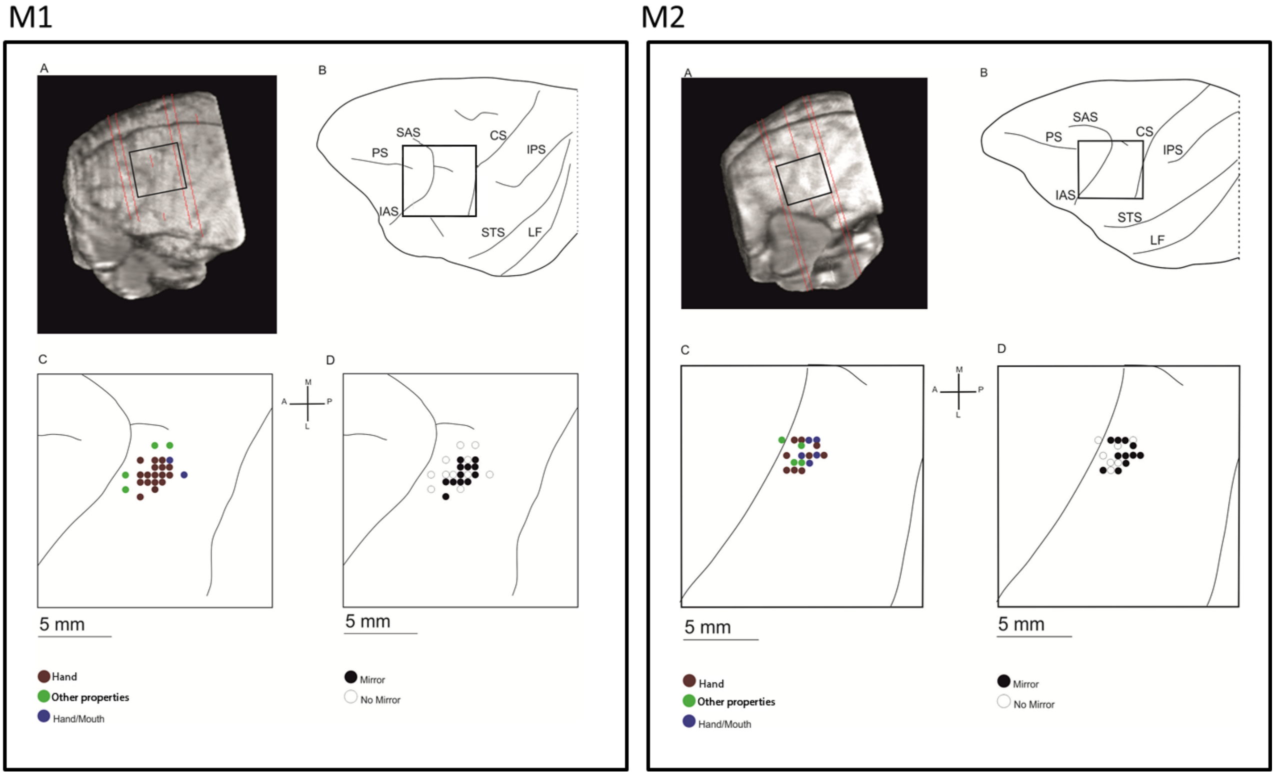

Figure 15 For both **M1** and **M2**: **A.** MRI of the left hemisphere. Red lines indicates the region of interest through which it was possible to collocate the recording chamber. In black is represented the recording chamber. **B**. Graphic images of the left hemispheres of the monkeys in which the principal sulci are approximately indicated. The black rectangle indicates the position of the recording chamber. CS: central sulcus; SAS: superior arcuate sulcus; IAS: inferior arcuate sulcus; PS: principal sulcus; STS: superior temporal sulcus; LF; lateral fissure. **C**. Distribution of the penetrations. The coloured circles represent the penetrations coordinates with different visual and motor properties. Brown: hand properties; blue: hand/mouth properties; green: other properties. **D.** Penetrations with mirror properties. Black: mirror neurons properties. White circles: no mirror properties.

3.4.1 Social Context Effect

The main scope of this present work is to investigate if contextual information, such as the presence of a container, and social cues, such as social interactions with another experimenter or direct interaction with the observed agent, can differently affect the activity of mirror neurons in F5 convexity during an action-observation task. We found that the majority of the 84 MNs (76 neurons) had a significant preference for at least one of the four conditions of the action observation task. In particular, 33 neurons had a preference for two conditions or more, but the 43 remaining neurons showed a significant preferential modulation for only one specific condition. *Figure 16* summarizes the properties of these MNs. Among the MNs preferring a single condition, a higher number showed a preference for the non-social conditions: n= 16 (37,2%) for *Mouth* condition and n= 14 (32,6%) for *Container* condition. We also found a consistent number of neurons which 'preferred' *Hand* condition (n= 12, 27,9%). Surprising, we found only one MN with higher visual discharge for *Monkey* condition (2,3%) compared to the other conditions, that is the only one condition in which the monkey observed and interacted with the agent. A further analysis was carried out in order to define the preferred conditions between the 33 mirror neurons modulated by two conditions or more. The results are summarized in Table 1: 11 MNs (33,3%) preferred both *Container* and *Hand* conditions, 1 MN (3,03%) preferred *Container* and *Monkey* conditions, 4 MNs (12,12%) preferred *Container* and *Mouth* conditions, 6 MNs (18,2%) preferred *Container*, *Hand* and *Mouth* conditions, 8 MNs (24,3%) preferred *Container*, *Hand* and *Monkey* conditions, 2 MNs (6,06%) preferred *Hand* and *Monkey* conditions, 1 MN (3,03%) preferred *Hand*, *Monkey* and *Mouth* condition.

Figures 17 shows two examples of mirror neurons. On the left, a neuron preferring one condition, *Mouth*, and on the right, a neuron with a preference for three conditions (*Container*, *Hand* and *Monkey*). The graphics represent the spike frequency (spk/s) during the trial time course (second,s) of the four conditions. They are aligned on the event t=0 that represent the grasping of the object performed by the agent (hand-object contact) and in grey the *Baseline* and *Grasping* epochs are illustrated.

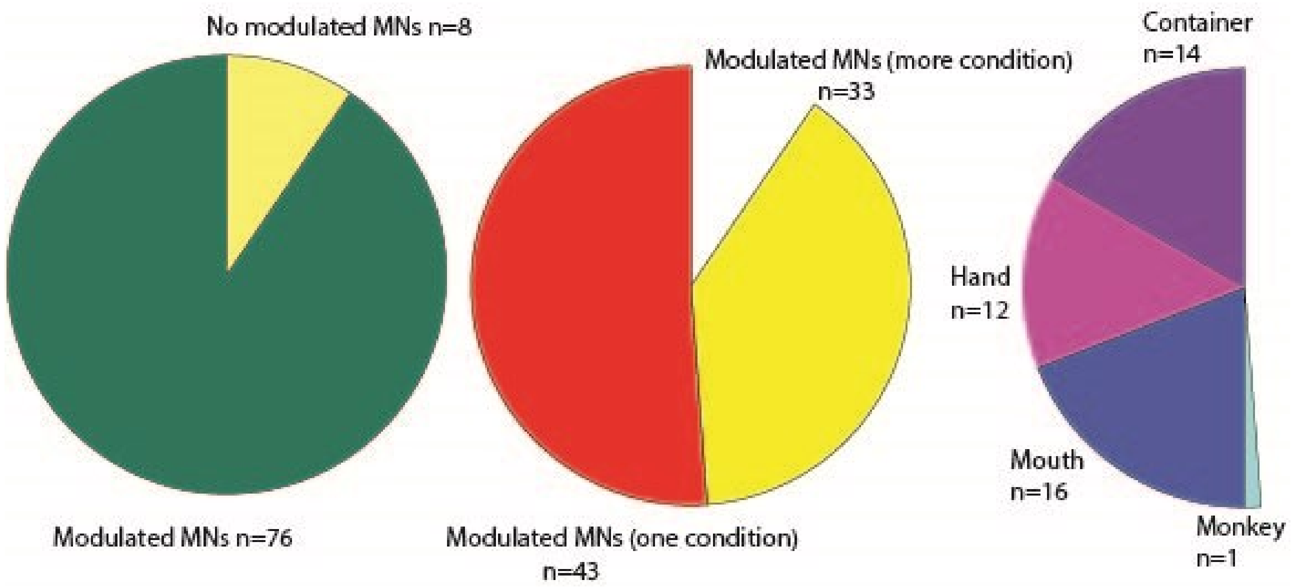

Figure 16 Mirror neurons of F5 results recorded during the visual task.

<table>
<tr><th colspan="7">MNs modulated by more conditions

33 (43,4%)</th><th>MNs modulated by one condition 43 (56,6%)</th></tr>
<tr><td><i>C/H</i>
11
(33,3%)</td><td><i>C/M</i>
1
(3,03%)</td><td><i>C/Mou</i>
4
(12,12%)</td><td><i>C/H/Mou</i>
6
(18,2%)</td><td><i>C/H/M</i>
8
(24,3%)</td><td><i>H/M</i>
2
(6,06%)</td><td><i>H/M/Mou</i>
1
(3,03%)</td><td><i>C</i>= 14 (32,6%)
<i>H</i>= 12 (27,9 %)
<i>Mou</i>= 16(37,2%)
<i>M</i>= 1 (2,3%)</td></tr>
<tr><td colspan="8">Total of MNs modulated

76 (100%)</td></tr>
</table>

Table 1 Number of mirror neurons (MNs) modulated by more condition of the visual task. C: *Container*; H: *Hand*; M: *Monkey*; Mou: *Mouth.*

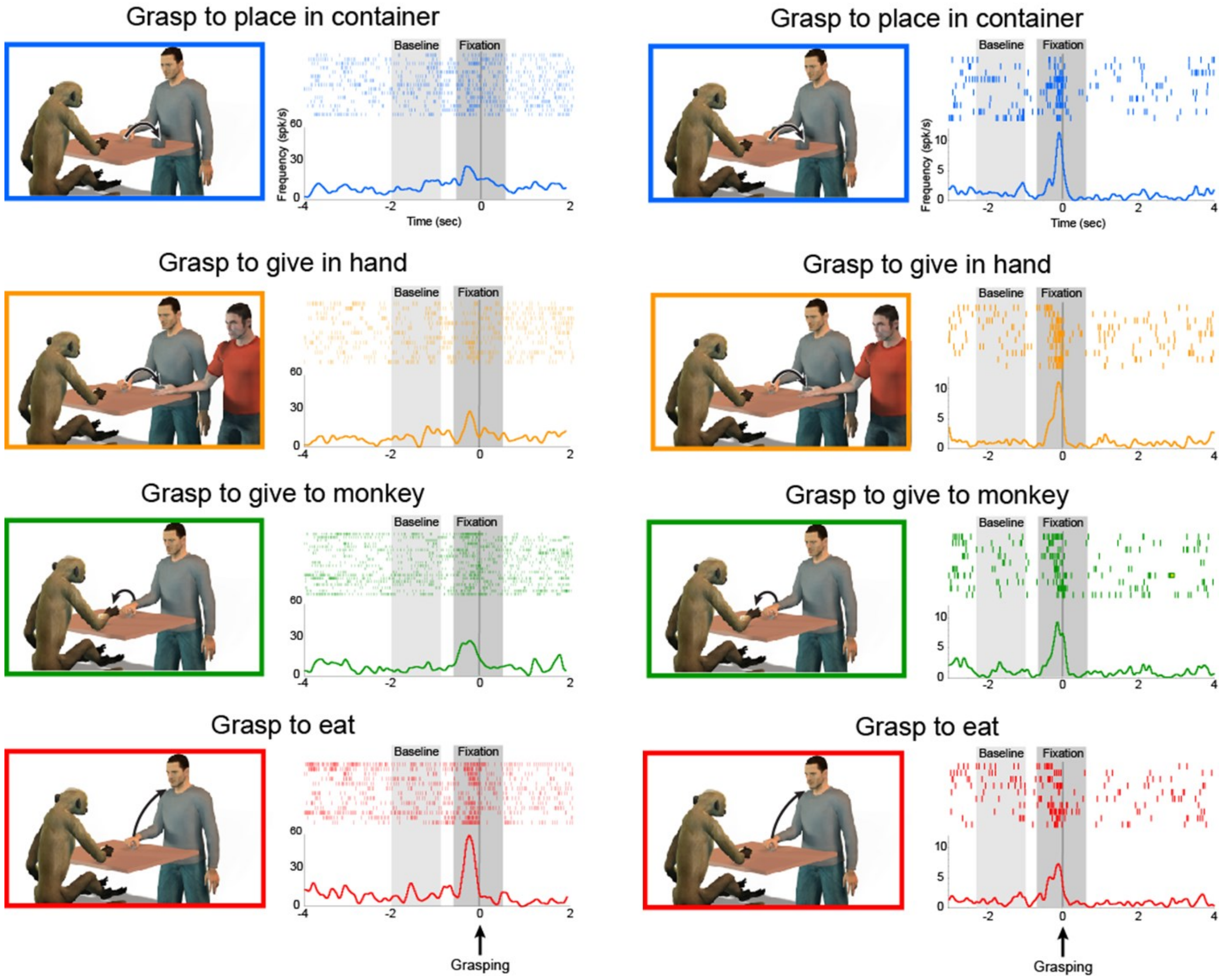

Figure 17 Example of visual responses of MNs modulated by one condition (on the left) and by more condition (on the right). *x* axis: Time, *y* axis: Frequency. The grey vertical line represents the grasping event and in grey shades the two temporal epochs, *Baseline* and *Grasping,* are indicated.

3.4.2 Electromyography control

In order to test any monkey hand muscle movements during the action observation task, or if any preparatory movements occurred during the *Monkey* condition, we performed EMG recordings in different sessions with respect to those performed for the acquisition of the neuronal activity. *Figure 18* shows the muscular activity recorded during the action observation task for all conditions. The graphic represents the muscular activity (μV) in relation to the time (seconds, s). As it can be noted, the muscular activity of the monkey is totally absent during the observation of the action performed by the agent (t=0). Moreover, our results show the absence of any preparatory movement of the monkey during the *Monkey* condition in which the animal had to prepare a movement to interact with the agent.

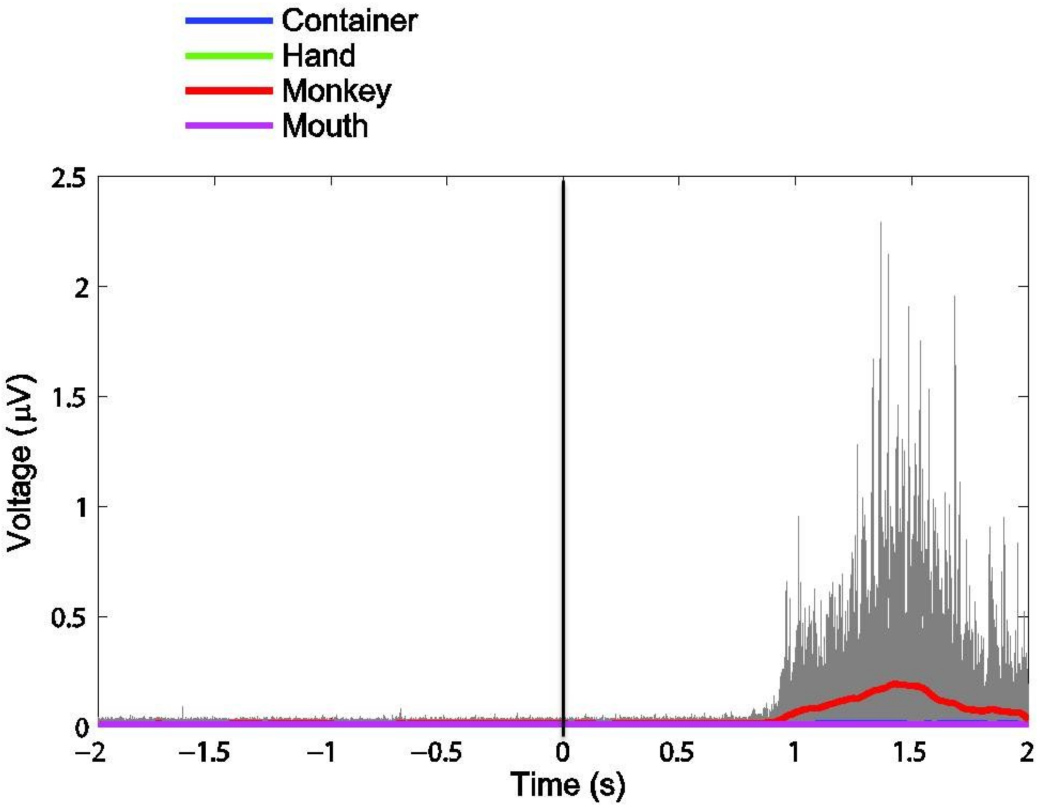

Figure 18 Muscular activity (rectified signal) during the action observation task in the four conditions. *x* axis: Time, *y* axis: Voltage. The black line indicates the agent's grasping action for all conditions.

3.4.3 Population analysis

Once we recorded all the MNs, regardless from their preference, we verified any significant differences at the population level. We considered all the 84 MNs, independently of their modulation, and we compared their firing rates (spk/s) for each condition during all the trials of all penetrations we performed. *Figure 19* shows the four population curves: for the statistical analysis, we considered, for each population, the *Grasping* epoch from 3700 ms to 4300 ms. The one-way repeated-measure *ANOVA* results show significant differences between conditions among the MNs ($F_{(3.332)}$=5.87; p<0.01); *post hoc* analyses show that MNs, independently of their preference pattern, had a significant higher firing rate in the *Mouth* condition with respect to the others conditions in the fixation epoch (p<0.01). No significant differences were found between the others conditions.

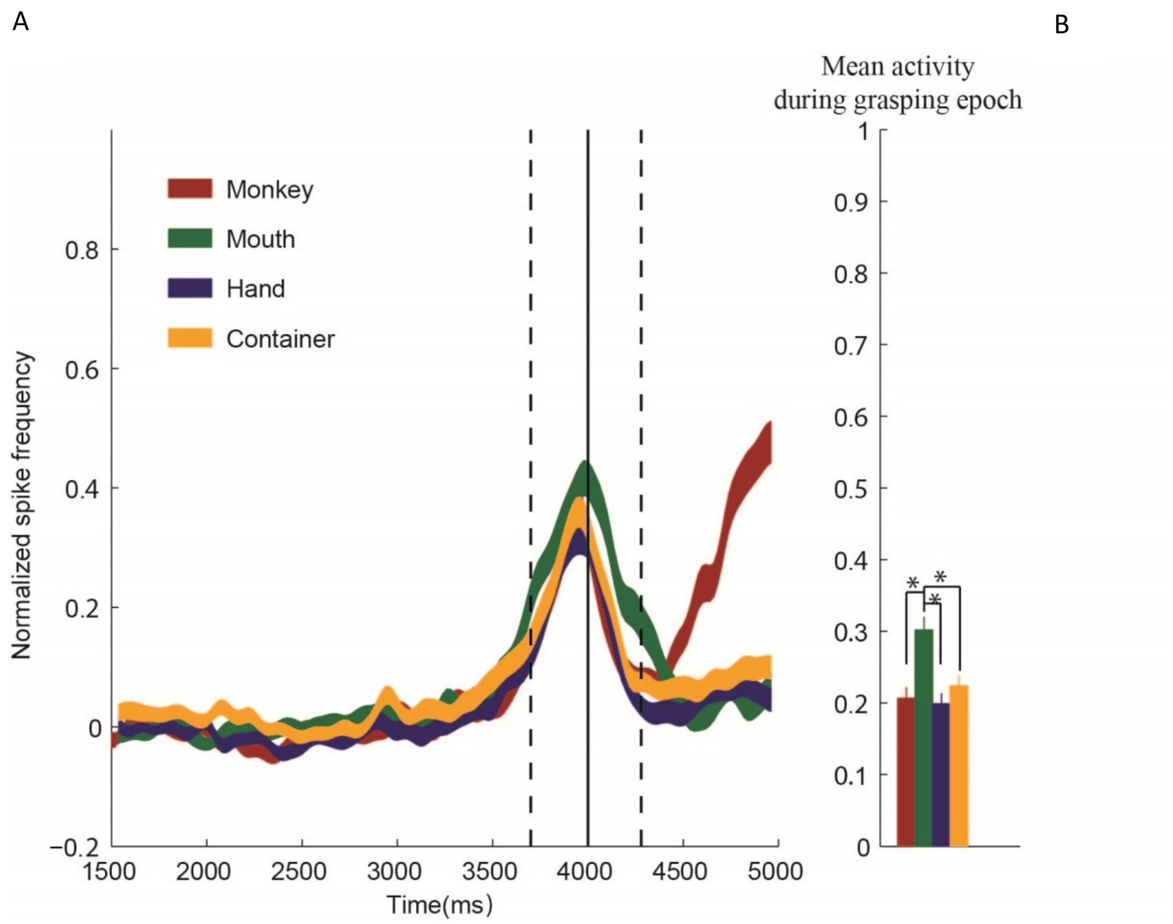

Figure 19 **A**: Mirror neurons population (n=84) in the four condition of the Visual task. *x* axis: Time; *y* axis: Normalized spike frequency. The black line indicates the agent's hand touch of the target object (t=4000ms). The dashed lines indicate the *Grasping* epoch considered for the statistical analysis. **B**: mean activity during the grasping fixation epoch. The error bars indicate ±2 standard error. Repeated measure *ANOVA* *p<0.01.

We then carried out a population analysis on MNs which preferred one condition during the visual task. That is, for each condition, we considered the activity of the 14 *Container* MNs (Figure 20), the activity of the 12 *Hand* MNs (Figure 21) and the activity of the 16 *Mouth* MNs (Figure 22). For each of the three populations (*Container*, *Hand* , *Mouth*), we compared the firing rate in the *Grasping* epoch for all the conditions, the preferred one and the others (one way Repeated measure *ANOVA*). Our statistical results show that the preferred condition had the higher firing rate respect the others, for each single population (*Container* population: $F_{(3.13)}$=22.41, p<0.01, *post hoc* analyses: *Container* vs *Hand* p<0.01, *Container* vs *Mouth* p<0.01; *Container* vs *Monkey* p<0.01. *Hand* population: $F_{(3.11)}$=14.24, p<0.01, *post hoc* analyses: *Hand* vs *Container* p<0.01, *Hand* vs *Mouth* p<0.01; *Hand* vs *Monkey* p<0.01. *Mouth* population: $F_{(3.15)}$=25.06, p<0.01, *post hoc* analyses: *Mouth* vs *Container* p<0.01, *Mouth* vs *Hand* p<0.01; *Mouth* vs *Monkey* p<0.01). We didn't perform a population analysis on *Monkey* condition since only one neuron had a preference for that condition.

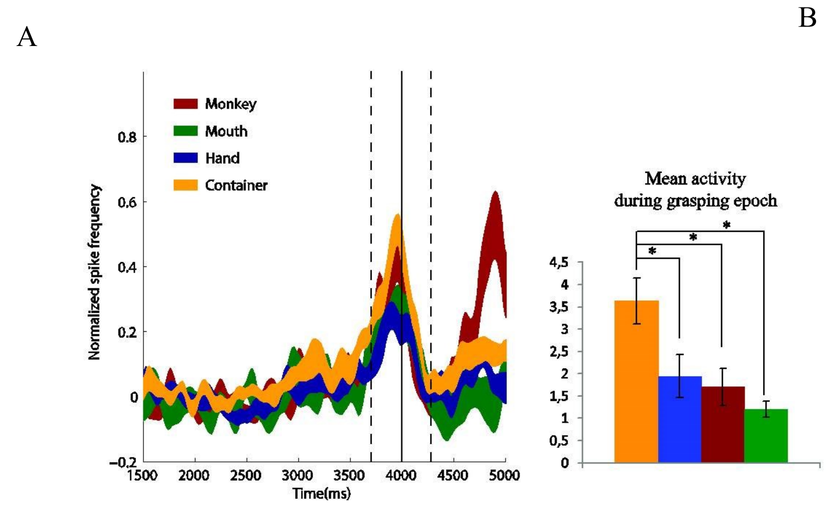

Figure 20 **A**: 14 MNs *Container* population in all the visual conditions. Same conventions as in Figure 12. **B**: Mean activity during the *Grasping* epoch. Same conventions as in Figure 19. One way Repeated measure *ANOVA*. *p<0.01.

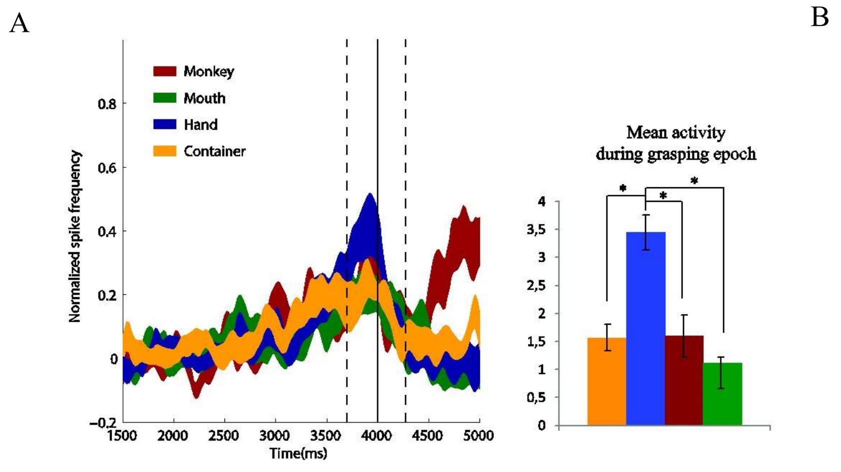

Figure 21 **A**: 12 MNs *Hand* population in all the visual conditions. Same conventions as in Figure 12 **B**: Mean activity during the *Grasping* epoch. Same conventions as in Figure 19. One way Repeated measure *ANOVA*. *p<0.01.

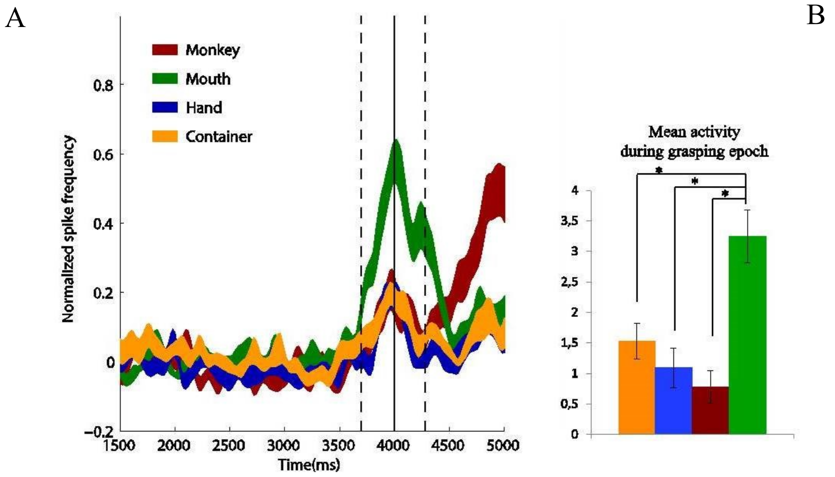

Figure 22 **A**: 16 MNs *Mouth* population in all the visual conditions Same conventions as in Figure 19. **B**: Mean activity during the *Grasping* epoch. Same conventions as in Figure 19. One way Repeated measure *ANOVA.* *p<0.01.

3.4.4 Correlation Analysis

Our statistical results show a very low number of MNs modulated by the *Monkey* condition, namely the only one in which the monkey had to observe the action and plan the motor act aimed at interacting with the observed agent. As mentioned in previous sections (introduction and aims) previous studies in humans have shown that the preparation of a complementary action in interacting tasks might interfere with the process of motor facilitation occurring while the observer is observing an action performed by another individual (Sartori et al. 2011, 2012, 2013a, 2013b). The lack of a population of neurons preferring the *Monkey* condition, seem to suggest that the monkey's preparation of an action that must be coordinated with the agent could have interfered with the visual response of MNs in the preceding observing phase. We hypothesize that preparing an action and its simultaneous suppression during the observation phase could produce an inhibitory effect on the activity of MNs in F5.

We, therefore, performed a correlation analysis for each MNs, independently of their modulation, and for each trial of this condition, between the normalized spike frequency (spk/s) in the *Grasping* epoch and the time difference between the agent's hand grasping of the target object and the monkey's hand release from the handle (monkey movement latency, second, s), ready to interact with the agent.

Our results show the presence of a non-linear inverse correlation between these two parameters (Spearman correlation, *r*= -0.11; p=0.03) (Figure 23). This relationship shows that the more the monkey has to suppress its action before the onset of the movement aimed at grasping the object from the agent, the weaker is the neuronal firing of the MNs. In *Figure 24* we plotted with a same color the trials pertaining to the same neurons. Such layout shows that the inverse relationship between neuronal firing and monkey movement latency does not depend on the specific behaviors of few neurons that had either very strong or very weak activity, but rather corresponds to a more general pattern of inhibition that is affected by the length of time in which the monkey was suppressing the movement.

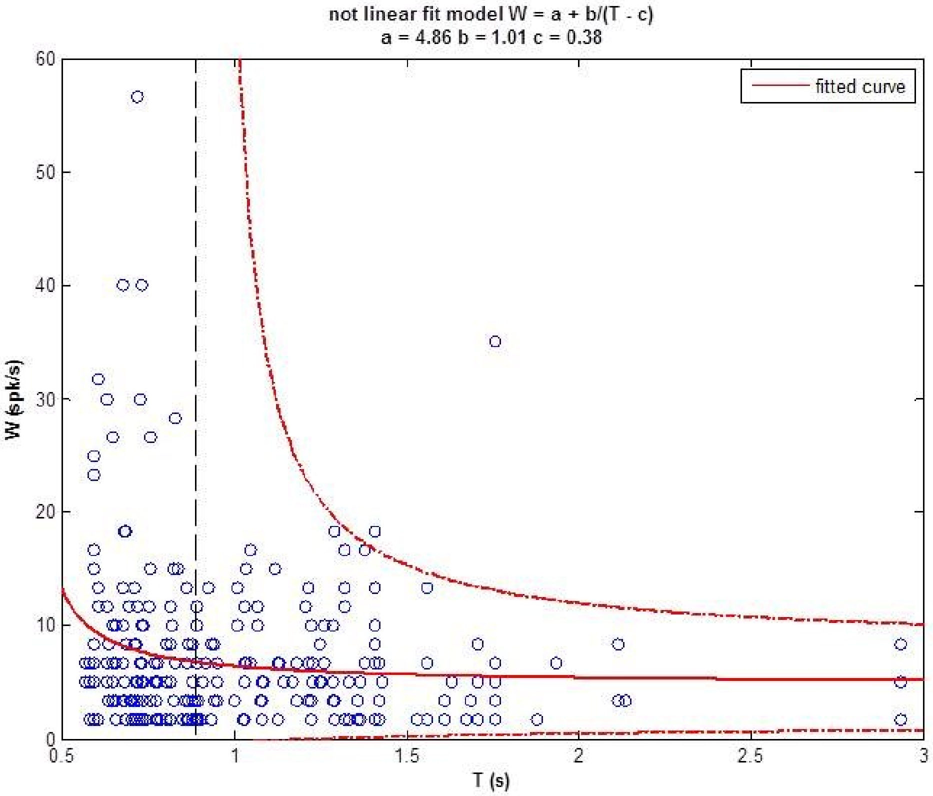

Figure 23 Graphic of inverse, not linear correlation. *x* axis: T (movement latency); *y* axis: W (spike frequency). Each dot indicates a trial. In red, the not linear fitted curve is indicatated; the red dashed lines represents the confidence level of 95%. The black dashed line indicates the median of the time. Spearman correlation: *r*= -0.11, p=0.03.

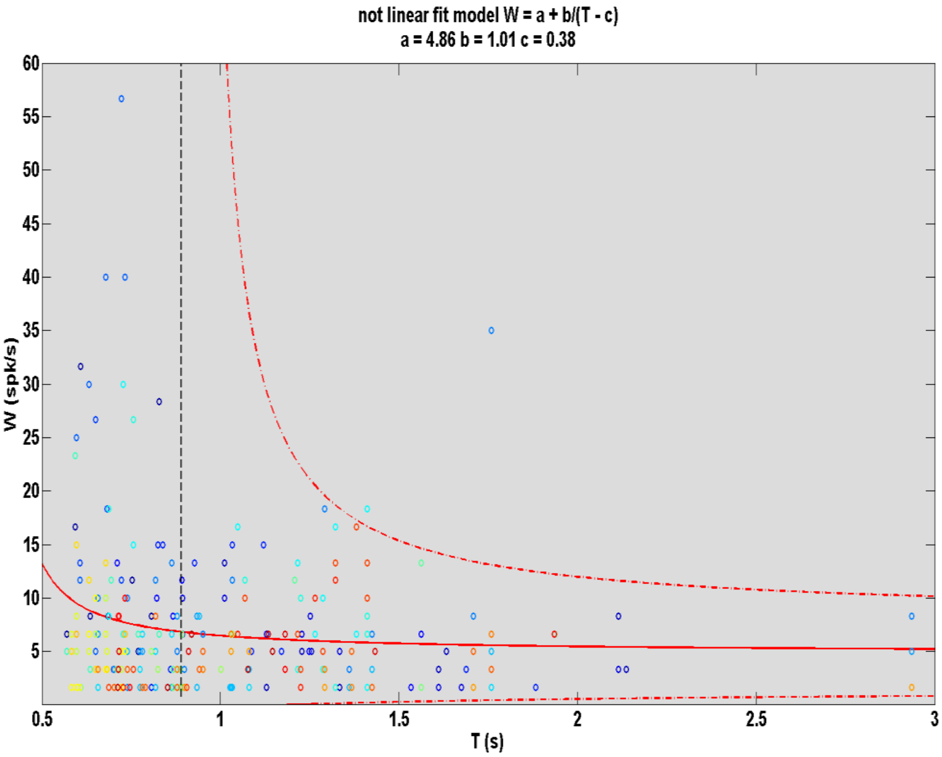

Figure 24 Graphic of inverse, not linear correlation. *x* axis: T (movement latency); *y* axis: W (spike frequency). Each dot indicates a trial, trials with the same color refer to the same neuron. Same convention as in Figure 23.

Since we found that there is a relationship between the neuronal discharge and the latency of the monkey movement, it was important to verify if this was not due to the confounding factor of a delayed reward associated with trials with longer movement latencies. In order to verify this possibility, we calculated the correlation between spike frequency (spk/s) and time difference between agent grasping and reward delivery (second, s).

Our results (Figure 25) show that there is no significant correlation between the firing rate on the MNs and the delayed at which the reward is delivered (Pearson correlation: *r*= -0.14, p=0.071,N.S.). They are, also, confirmed by the model of our visual task designed by the Software Labview (National Instruments), in which the reward delivering occurs at a fix time after the agent’s hand touch of the target object for all conditions and, specifically for the *Monkey* condition, independently if the monkey interacted early or late with the agent.

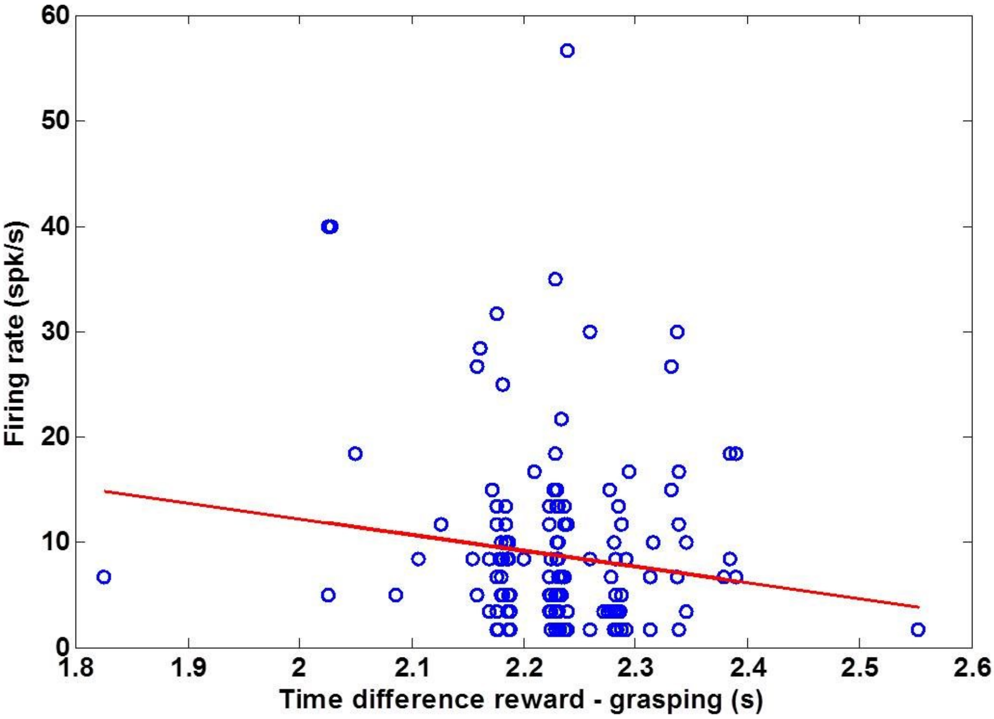

Figure 25 Graphic of correlation between spiking activity during grasping observation and time difference between reward delivery and the observed grasping. *x* axis: Time difference reward-grasping, *y* axis: Firing rate. In red, the fitted linear curve is indicated. Pearson correlation: *r* = -0.14, p=0.07, N.S.

3.4.5 Local Field Potential analysis

To better investigate the link between MNs activity and interaction movement latency, we analyzed the LFP signals recorded in the channels where MNs were found. If the interaction movement latency is link with a mechanism that reduces spiking frequency, it might be possible to observe such effects on the LFPs recorded during grasping observation. We first consider all the trials performed during the *Monkey* condition: our results are shown in the *Figure 26*. The spectrogram shows, in correspondence of the grasping event (t=0), an increase of the signal power in the high-gamma band (60-120 Hz) with respect to the baseline (one way t-test, *t*=14.2, p<0.01) and, on the other hand, a decrease of the signal power in the beta band (15-30 Hz) with respect to the baseline (one way t-test, *t*=-4.44, p<0.01). Note that, changes in high-gamma and beta frequency bands occur also at 500-600 ms after the grasping event, corresponding to the monkey movement onset aimed at grasping the object offered by the experimenter and the subsequent act of placing it into the container (see details in the method section).

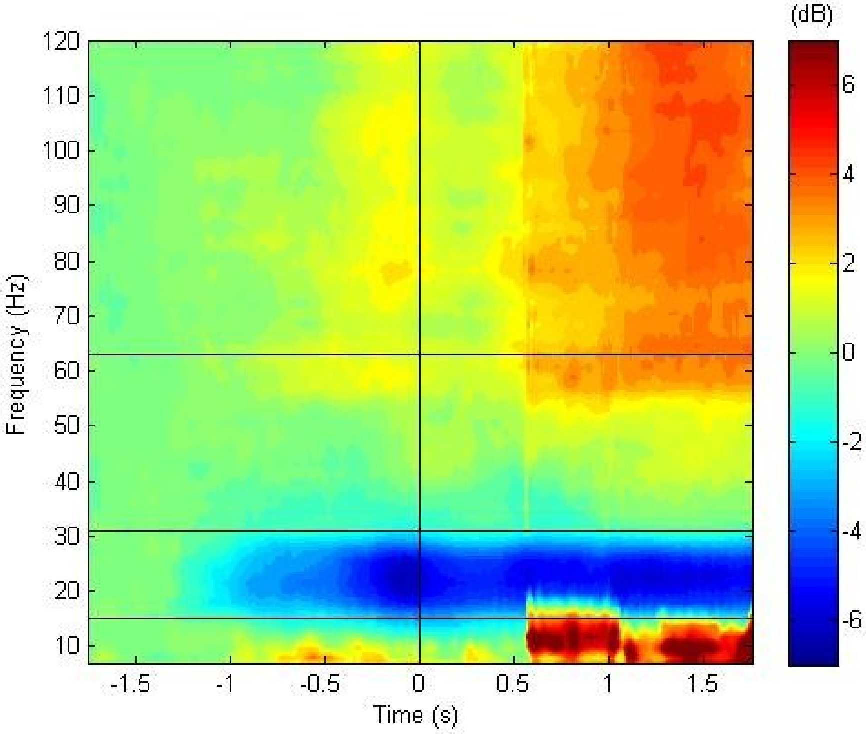

Figure 26 LFP spectrogram of all Monkey condition trials. *x* axis indicates the trial time (T), *y* axis indicates the signal Frequencies (LFP Freq). The black vertical line indicates agent's hand touch of the target object. The black horizontal lines indicate the frequency bands: alpha (7-15 Hz), beta (15-31 Hz), gamma (31-63 Hz), high-gamma (63-127 Hz). All trials are aligned with the grasping event (when the agent has contacted the object).

Since the LFPs frequency bands are associated with different neuronal phenomena, it is interesting to see if the LFP patterns associated with Short and Long movement latencies are different. The latency is calculated as the time difference occurring between the observed grasping phase (aligned at the moment when the experimenter has contacted the object) and the onset of the monkey movement (calculated from the release of the monkey's hand from the handle) aimed at grasping the object offered by the experimenter. We divided the Short and Long movement latencies based on the median latency (890 ms) from our sample. *Figure 27* and *Figure 28* show the two spectrograms we obtained. Both spectrograms, show an increase of the signal power in the high gamma band (60-120 Hz) and a decrease of the power in the beta band (15-30 Hz) both during the *Grasping* epoch and with respect to the baseline epoch (one way t-test. Short movement latency: high gamma t=8.78 p<0.01, beta t=-2.63 p<0.01. Long movement latency: high gamma t=12.1 p<0.01, beta t=-3.76 p<0.01), although the relative intensity is different between the short and long movement latencies (see analysis below). Note that, both spectrograms show, as the previous one, a high gamma synchronization and a beta desynchronization during the movement phase, in correspondence to the monkey movement onset. But, in this case, the high gamma activity occurs earlier in the Short latencies (about 500 ms and more after the grasping) and later in the Long latencies (about 900 ms and more after the grasping), reflecting different monkey movement onset due to selection criteria based on the latency median.

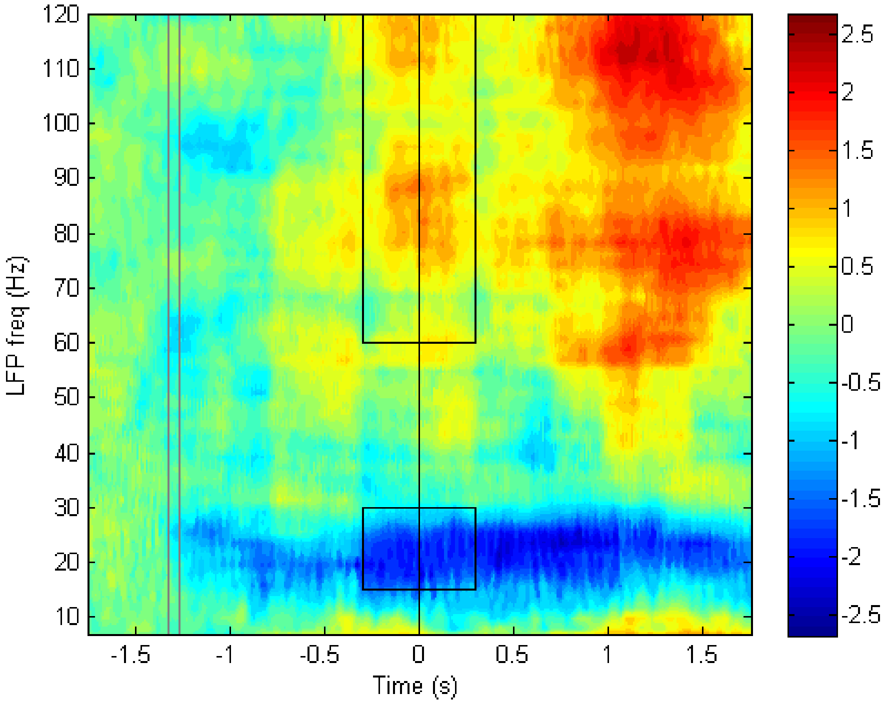

Figure 27 **Short movement Latencies** LFP Spectrogram: all trials monkey with movement time difference < 0.89s. *x* axis: Time (trial time, s); *y* axis: LFP freq (signal frequencies, Hz). The black line in correspondence of $t=0$ indicates the agent's hand touch of the target object and the grey lines indicate the appearance of fixation light. The black rectangles indicate the frequency ranges and the time epoch considered for the statistical analysis.

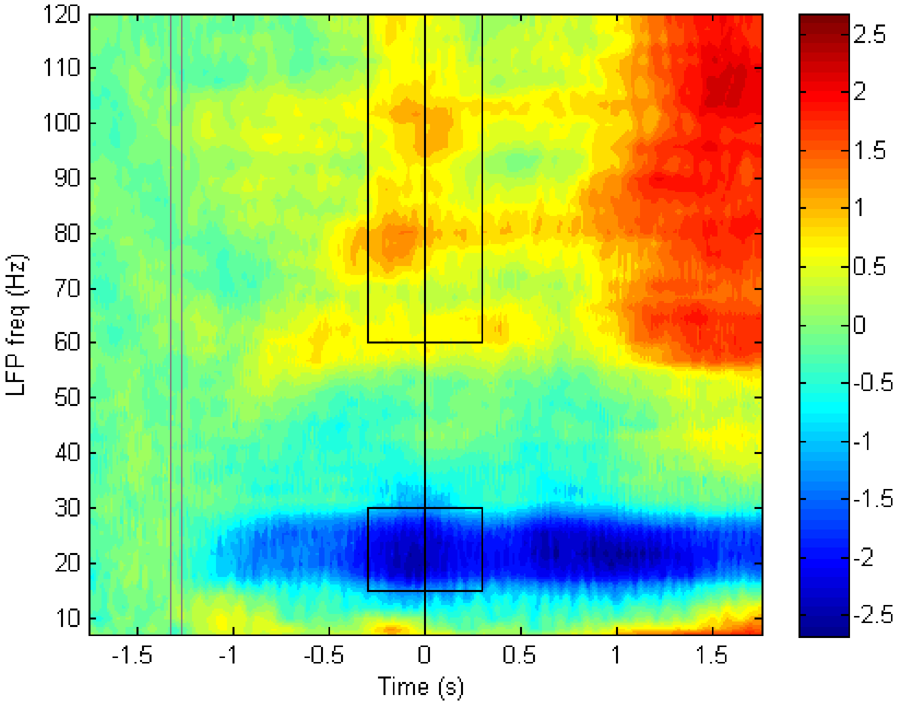

Figure 28 **Long movement Latencies** LFP Spectrogram: all trials monkey with movement time difference > 0.89s. *x* axis: Time (trial time, s); *y* axis: LFP freq (signal frequencies, Hz). The black line in correspondence of *t=0* indicates the agent's hand touch of the target object and the grey lines indicate the appearance of fixation light. The black rectangles indicate the frequency ranges and the time epoch considered for the statistical analysis.

Interestingly, we found significant differences between the Short and Long movement latencies in both beta and high gamma frequency bands. Specifically, Long movement latencies are associated with lower high-gamma synchronization than Short movement latencies (t test for independent variables, $t = 13,71$; $p < 0.01$), probably reflecting a reduced MN spiking activity taking place when the monkey has to refrain the planned movement onset for a longer period. Similarly, Long movement latencies are associated with stronger beta desynchronization than Short movement latencies (t test for independent variables ,$t = 12.25$; $p < 0.01$), which will be discussed in the following sections. The results are shown in *Figure 29*: the ERSs and the ERDs calculated respectively in the high gamma and beta band for the two LFP are statistically different ($p < 0.01$).

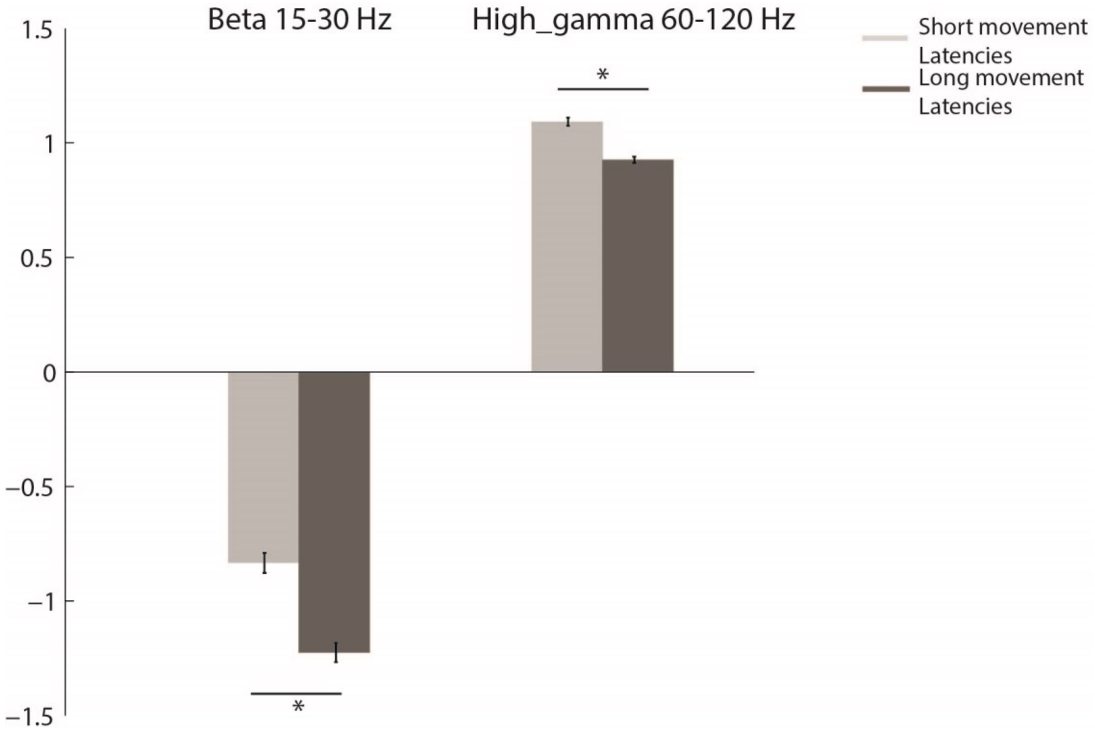

Figure 29 On the left: ERDs calculated in the beta band (15-30 Hz) in action observation epoch (600 ms). On the right: ERSs calculated in the high gamma band (60-120 Hz) in action observation epoch (600 ms). In the light grey and in the dark grey, respectively Short movement latencies LFP and Long movement Latencies LFP are indicated. t-test for independent variables, $^*p < 0.01$.

3.5. DISCUSSION

3.5.1 The role of social context on the action intention's understanding

The results of our study confirm and extend the findings obtained by previous investigations (Fogassi et al. 2005; Bonini et al. 2010; Bonini et al. 2011). In fact, previous studies assessed the activity of MNs and motor neurons during the execution and observation of grasping motor acts aimed at different goals (eating or placing). The idea behind these experiments was that during the execution of a sequence of motor acts, such as grasping a piece of food, bringing it to the mouth and eating it, the motor system can code goals at different levels. Therefore, the coding of a grasping motor act is not independent from its final goal. By testing neurons in the posterior parietal cortex and then in the ventral premotor cortex (F5) it has been demonstrated that, both during the observation and the execution task, some neurons had a differential discharge during the first phase of the motor sequence (i.e. grasping the object). Similarly to these studies (Fogassi et al. 2005; Bonini et al. 2010), our results show that the differential activity of MNs occurs during the first motor act of the motor sequence, the grasping act, which is identical in all conditions although it is aimed at a different goal. Moreover, we found that the majority of tested neurons showed a preference for at least one of the four conditions.

These neuronal activity modulations may be explained in part by a possible motor modulation (especially in the *Monkey* condition), due to the activation of the monkey's arm during the action observation. However, the electromyographic control study we carried out, demonstrate the absence of the muscle activation of the monkey's arm, during the observation of actions performed by the agent. In this way, we could confirm that, during action observation in all different experimental conditions, the differential neuronal activity recorded in F5 is not due to the muscle activation but rather to the activation of the mirror neurons, which are differently modulated by the contextual stimuli.

These data are also consistent with the previous research suggesting that the modulation of MNs in different experimental conditions may reflect the capacity of these neurons to predict the final goal of an action, which corresponds to the motor intention of the observed agent. Contextual elements could be particularly important because they could provide significant visual inputs to eliminate ambiguous situations in which it is difficult to discriminate, from the observer's point of view, the observed agent's intention. Previous findings showed in fact, that the presence of a container close to the target object could inform the observer that the grasping action, performed by the observed agent, was aimed at placing the object into the container. According to the chain model proposed by Fogassi et al. (2005) and Chersi et al. (2011), visual inputs related to the context could contribute to the modulation of the activity of specific chains of neurons and to the activity propagation along these chains. In other words, external cues could facilitate the activation of the most appropriate neuronal chain for a specific scope and inhibit other chains specific for different goals, thus preventing the simultaneous activation of multiple and different motor chains, incompatible from each other’s. During the observation of the grasping action, specific populations of neurons encoding for the grasping motor act are activated, and they, in turn, could pre-activate those neuronal chains that specify the scope of the action (for example, grasping to eat), allowing the observer to predict the final goal, or the motor intention, of the action performed by the observed agent (Chersi et al. 2011). Our findings are therefore in line with this early proposal, and show that in F5 there is convergence of different visual inputs, not only linked to the decoding of actions but also related to context, which may provide key information to support the prediction of the goal of the ongoing action.

Furthermore, our results show that the differential activity of mirror neurons could also reflect their ability to code the value and the importance that the observer attributes to the target object of the grasping, confirming the previous research by Caggiano and colleagues (2012). In fact, at the population level, it is clear that the firing activity of MNs in the *Mouth* condition is stronger than the other conditions. Despite the monkey received the same reward (juice) in all conditions, such stronger response might reflect either potential attentional factors elicited by a more salient object (i.e. food) compared to the objects used in the other three conditions (i.e. a metallic cube) or the subjective value of the food. Since the monkey had to fixate the target in all conditions, we exclude the former hypothesis, even though further analyses should be performed in order to better understand possible modulatory effects of neuronal discharge due to attention. Conversely, the selection of an appropriate response to the actions of others may require the assessment of the value that the object, on which the observed action is performed, has for the observer (Caggiano et al., 2012). As this value also depends on the specific needs, preferences, and desires of the observing monkey, it is commonly referred to as "subjective" value. Thus, the stronger discharge of the *Mouth* population than the other neuronal populations, seems to reflect a modulation of the "subjective" value, in terms of reward, that the observer attributed to the piece of food relative to the object used in the other conditions. Taken together, these results suggest that the discharge of F5 mirror neurons is influenced by the information on the value that the target object of the motor act has for the observer and this information may help the observer to interpret the action meaning and to read the motor intention of an observed agent.

A novel finding emerging from the current study is that the visual discharge of a number of MNs could be modulated also by the social context, i.e. the presence of a second agent. So that, these first results seem to support the idea that mirror neurons may play an important role not only in coding the others' actions, but also in the encoding of others' intentions in relation to social goals. In particular, some of these mirror neurons code actions depending on whether the observed actions are performed in order to interact with the monkey or with another individual and, then, aimed at social interaction. This ability of mirror neurons to code others' social intentions seems to be fundamental, because survival in a social group is inherently related to the ability to understand and predict others' behavior and this happens, often, on the basis of cues provided by other individuals and by the type of interaction occurring between group members (Pelpherey et al., 2004). Tracking and understanding social relations are crucial in highly social species, such as humans and macaques. Successful engagement in these relationship requires a sophisticated understanding of one's social position, and of the type of relations occurring between group members (Iacoboni et al., 2004). In addition, the capacity to read social intentions is key to anticipate others' behaviors in both competitive or cooperative tasks. For example, in order to investigate neural and cognitive mechanisms for understanding social relations, *Iacoboni* and colleagues (2004) performed an fMRI study while subjects were observing video clips depicting every-day social scenes. Their results suggest that human facility in understanding observed social relationships is provided by the combined activity of an action recognition system in the inferior frontal and superior temporal cortices, the combined activity of the anterior STS system that interprets the social significance of actions and the combined activity of the dorsomedial prefrontal cortex and medial parietal cortex system, which have been proposed to be involved in the analyses of social relationships and in the assessment of their implications. The dorsomedial prefrontal cortex and the medial parietal cortex system, for thinking about social relationships, is apparently part of the brain's default state circuitry; it may continuously, often without effort or intention, assess and analyse past, present, or possible future social relationships. Given the complexity and pervasive importance of social relationships, this ongoing social processing may be crucial to sustaining adaptive social relations (Iacoboni et al., 2004). Recent studies suggest that several brain regions such as the anterior cingulate cortex (Iacoboni et al., 2004; Rudebeck et al 2006), the insula (Caruana et al., 2011), the lateral intraparietal area (Fujii et al., 2007) and the amygdala (Gothard et al., 2007; Kuraoka & Nakamura 2006) are involved in the processing of social information and/or the production of emotional/social behaviors. Among these brain regions processing social information, the lateral prefrontal cortex (LPFC) is likely to be involved in the categorization of social behaviors and this discrimination may be adaptive for monkeys when they are required to simultaneously classify social information into more than one dimension (Tsunada et al., 2012). It has been demonstrated that monkeys simultaneously classify others according to both the individual rank and the kinship in the field (Bergaman et al., 2003). Also the orbitofrontal cortex (OFC) seems to play a key role in the coding of others' behaviour and choices. Studies performed in the OFC indicate that neurons in this cortex encode the identity and the subjective value of different goods and that during economic choices, subjective values computed in the OFC are causally related to the decision (Padoa-Schioppa & Cai 2011; Padoa-Schioppa 2007). Taken together, these findings demonstrate that, probably, visual information regarding the context, the social status of individuals, the type of social interaction, the subjective values of the action target and the final intention of action performed by the observed agents is operated by a plethora of brain regions which contribute, together with the premotor cortex, to the encoding of the action's intention when this is finalized to social goals. While the motor aspects of the action, in terms of type of action and the goal, are specified in the parietal-premotor circuits, other aspects related to the salience of the action and the subjective value of an action are coded by prefrontal and cingulate cortical regions, which are known to be connected to the premotor cortex (Matelli et al., 1986; Luppino et al., 1993; Rizzolatti and Luppino, 2001, Hoshi and Tanji, 2008).

3.5.2 Passive action observation vs active action observation

It is interesting to note that, the number of neurons which prefers the *Monkey* condition is virtually absent. This is an unexpected result because it could be predicted that in the *Monkey* condition, the planning of the subsequent grasping action performed by the monkey (in this condition the monkey knows it has to receive the object offered by the agent and interact with him) could pre-activate MNs firing during the action observation. Or that, the motor planning of a grasping action consequent to the observation of a similar action performed by the agent, could increase the MNs visual discharge during the first phase of the observation, as hypothesized by other authors (Sartori et al., 2011; 2012; 2013a, 2013b).

Contrary to what was expected, the planning of a motor act during the observation of the action performed by the agent likely exert an inhibitory effect on the visual discharge of some MNs. This finding could be interpreted by three different hypotheses:

1. *Attentional hypothesis*: the monkey, since it has to prepare an action in coordination with the agent who gives the object to the animal, has not only to prepare the action, but also to pay attention to how the agent grasps the object and gives it to the animal, because this aspect determines critical adjustments in the motor coordination of the action. This "displacement" of neural resources could have a secondary effect on the visual mirror neurons discharge during the observation, resulting in a decrease of the mirror neurons activity.
2. *Reward delay hypothesis*: the variability in the timing of onset movement following grasping by the agent may determine delays in juice delivery to the monkey. Thus, higher delays may determine significant decrease in the firing activity of MNs during grasping observation.
3. *Motor inhibition on the visual discharge of MNs hypothesis*: the activation of local inhibitory circuits of neurons involved in the motor preparation of the subsequent action, may have a consequent inhibitory

effect on the visual input that reaches the mirror neurons, and thus, reducing their activation during the action observation.

The first hypothesis seems not be supported by our results. In fact, a second analysis, which was performed on a single monkey and in which we correlated, trial by trial and only for the *Monkey* condition, the times of the monkey's hand release from its handle following the agent grasping action (monkey movement latency) to the neuronal discharge, revealed that the visual mirror neuron activity during the action observation is more intense when the monkey quickly released the handle, ready to receive the object, and less intense when the monkey had to actively inhibit the reaching-grasping behavior for a longer period of time, holding with its hand the handle. It's important to note, that this relationship is not due to a possible confounding factor of a delayed reward, associated with trials with longer movement latencies: in fact, the visual task was designed and controlled by the Software Labview (National Instruments) to deliver the reward always at a fix time after the agent's hand touch of the target object in all conditions and, specifically, for the *Monkey* condition, independently if the monkey interacts earlier or later with the agent. The analysis of time difference between reward and grasping observation (see Figure 25) further support the idea that time of reward delivery (Hypothesis 2) cannot explain such inverse relationship.

Moreover, according to the first hypothesis, from an attentional perspective, the monkey, in the active phase of inhibition, should pay attention to the action performed by the agent, to be able to be coordinated with him, independently from whether the subsequent interaction occurs earlier or later in the course of the trial. Further, the inhibitory effect, according to the *attentional hypothesis*, should be present, both for low and high movement latencies, for the entire duration of active inhibition, that is immediately after the agent's grasping, when the monkey remained with its hand on the handle, ready for the subsequent interaction. But this is not supported by the inverse relationship we found between the spiking activity and the monkey movement latencies.

On the contrary, our results seem to be compatible with the third hypothesis, that is the active inhibition of a motor plan could have a suppression effect on the mirror neurons visual discharge during the action observation, especially when the monkey waits longer to interact with the agent. In particular, in our study, the monkey has to observe the action and prepare a complementary action in order to be coordinated with the agent and it has to maintain suppressed the action until the agent starts his movement toward the monkey. The suppressive effect on the MNs visual discharge could be interpreted as the result of an interference mechanism, which occurs when an individual observes and simultaneously prepares an action. In fact, in line with our hypothesis, some kinematics studies, performed in human, have shown that the observation of movements performed by another individual could produce motor interference if the observer has to perform actions that are different from those observed (Brass et al. 2001; Castiello et al. 2002; Sturmer et al. 2000a; 2000b). More specifically, they showed, through TMS and EMG, that under such circumstances there is an effect of interference of the complementary action preparation on the cortical motor excitability of the observer, during the observation phase (Sartori et al. 2011, 2012, 2013a, 2013b). In our study, such motor interference between the observation and the motor planning of an incongruent action could emerge during the action observation, in the *Monkey* condition. Probably this interference phenomenon could reflect the activation of local inhibitory circuits in which, the activation of a number of neurons, involved in a specific motor program, may interfere with the MNs activity inducing, at the same time, an inhibitory effect, to avoid a possible conflict of simultaneous motor commands. Interestingly, this interference and suppressive effects on the visual MNs discharge is not present or attenuated when the monkey interacts with the agent, immediately after the action observation. Since the mirror neurons are visual-motor neurons, their contribute to the action is also present during the execution of the grasping action performed by the monkey. However, it is not clear how their contribute, from a motor point of view, could interfere with the visual encoding of the action when the visual input is activated concurrently to a motor command.

In order to investigate more in depth the relationship between the visual discharge of MNs during the preparatory phase of a grasping movement to be performed in coordination with the agent, we analysed the Local Field Potential (LFP) during the observed grasping phase. LFPs mainly reflect the local synaptic activity, with contributions from spike-after potentials and intrinsic transmembrane current changes (Mitzdorf 1985; Logothetis et al. 2007). Several studies have used LFPs to investigate cortical network mechanisms involved in sensory processing, motor planning (Sherberger et al.,2005; Roux et al., 2006), and higher cognitive processes, including attention, memory and perception (Persan et al., 2002; Liebe et al., 2011). Generally, the two frequency bands more investigated are the high gamma (60-120 Hz) and beta (15-30 Hz) bands. While the LFP high gamma activity could be considered as a measure of neuronal activity of the population near the electrode (Ray et al., 2008), the LFP beta activity could reflect several neuronal phenomena, such as efficient motor maintenance, sensorimotor binding and motor planning (Sanes and Donoghue 1993; Kilavik et al., 2012). Moreover, recent studies have investigated the modulation of specific LFP frequency bands during action observation and execution (Waldert et al., 2015; Caggiano et al., 2015). Our results, in line with previous studies, show that the frequency bands which are more sensible during the action observation are the high gamma (60-120 Hz) band and beta (15-30 Hz) band.

In addition, to better investigate the possible inhibitory effect on the mirror neurons visual discharge during the *active* action observation, we have split the analysis of LFPs on the basis of two categories of trials: short and long movement latencies, that is, when the monkey releases its hand early or late in order to grasp the object offered by the agent. We found that LPFs were differently modulated based on the movement latencies: shorter latencies were associated with a more intense high gamma synchronization and a less intense beta desynchronization, while longer latencies showed an opposite LFP pattern in both high gamma and beta frequencies. It is worth noting that the monkey moved its hand only when the agent starts the arm extension movement toward the monkey in order to offer the object to her. The agent, after the grasping action, could decide arbitrarily when to extend his arm and give the object toward the monkey, so that the monkey movement latencies are different and dependent from the agent's motor behaviour trial by trial. Therefore, in this condition, it should be taken into account that the monkey has to prepare the action and to maintain suppressed the action until the agent starts his arm extension movement toward the monkey, otherwise the trial was aborted. Another important aspect of the task, that requires further analyses, relates to the fact that in the task, some aspects of the agent's movement kinematics clearly suggested to the monkey whether the agent was going to give the object to the monkey soon after it was grasped or whether the agent intended to hold the object for a longer period of time before giving it to the monkey. It is likely that the velocity to perform the movement or other kinematic parameters could provide such information to the monkey. We are currently analysing the agent's kinematic in order to better understanding how the monkey was able to predict, approximately, during the grasping phase these subtle aspects of the timing of the sequence.

It is well known that the LFP high gamma activity could represent the average firing of neurons near the microelectrode from which the LFP activity is recorded. The LFP high gamma activity could therefore be considered as a measure of spiking activity of the neuronal population near the electrode, in line with what other studies have reported (Ray et al., 2008). Our results show a significant high gamma power decrease during the action observation, in all the trials in which the monkey interacts later with the agent, relative to when the interaction occurs immediately after the action observation. Since the correlation analysis demonstrated that long movement latencies before the interaction between the agent and the monkey correlate with a lower visual discharge in the action observation , compared to when the interaction occurs immediately after the grasping, this inhibitory effect on the MNs activity could reflect the lower high gamma power observed in the LFP for the Long latencies, confirming the previous studies that show a direct correlation between high gamma and spiking activity (Ray et al., 2008).

Conversely, the LFP beta band activity could reflect several neuronal phenomena, ranging from a ''resting'' state of the motor cortex (Jasper and Penfield 1949; Neuper and Pfurtscheller 1996), efficient motor maintenance (Baker et al. 1999), or more generally the maintenance of the ''status quo'' (Engel and Fries 2010), attentional processing, sensorimotor binding, and motor planning (Sanes and Donoghue 1993; Kilavik et al., 2012; Waldert et al., 2015). Furthermore, the beta band also desynchronizes during the execution and observation of an action (Waldert et al., 2015). In particular, this latter study was carried out through neurophysiological recordings in the ventral premotor cortex and primary motor cortex, during a task with execution trials in which the monkey performed a reach to grasp movement toward an object (ring or sphere) and observation trials in which the monkey observed the same action performed by the agent. Results revealed that the beta power deviated from baseline level (in terms of power) as soon as the information about the type of grasp and who was going to perform the movement became available to the monkey. Most importantly, they found a lower level of LFP activity (both for high gamma band and beta band) for action observation respect to action execution and this could mean that some subnetworks, although recruited during action execution, are less active or inactive during observation. Probably, non-identical but partially overlapping networks are involved during execution and observation: probably, the neuronal resources used to actively control a particular movement are different to those responding to the same movement during observation (Waldert et al., 2015). This could be in line with our study: while the individual has to observe the action and simultaneously prepare a complementary action to that observed (*Monkey* condition), two "partially non overlapping networks" could be activated: one characterized by the activation of mirror neurons, in order to code the action's final goal, the other one characterized by the activation of local motor neurons involved in the planning of the subsequent action. And, such networks overlapping could probably be at the basis of the interference mechanism, occurring when the individual simultaneously observes an action and prepares a complementary action (Sartori et al 2011), which probably affects the MNs activity during the action observation.

In particular, our results show a beta desynchronization more intense when the monkey interacts with the agent later during the trial, with respect to when the interaction occurs immediately after the end of the grasping observation. Both for short and long movement latencies, the monkey has to observe the action and at the same time it has to plan a motor act in order to interact with the agent. But, when the interaction occurs later, the action should be planned and maintained suppressed for a longer time. There are several aspects we should consider in order to understand this phenomenon: 1) the observation of a grasping action (a precision grip) activates some mirror neurons in F5 that are involved in the decoding of that specific action; 2) the monkey prepares simultaneously a grasping action that is coordinated with that of the agent. However, there is a degree of uncertainty that depends on the fact that the space location where the object will be given is only roughly defined and this should imply an online control of the hand that must make adjustments in the trajectory, hand shape and orientation depending on the agent's action. It is possible that the recruitment of neurons involved in a specific grasping could somehow locally interfere with the neuronal activity generated by MNs during grasping observation; 3) the monkey has to suppress the action until the agent has not started his arm extension to give the object to the monkey. Thus, the stronger beta desynchronization found in the long movement latencies could reflect several neuronal phenomena: increased neuronal activity during the planning phase, inhibitory inputs reaching the PMv that block the generation of the movement, or conflict motor signals related to motor preparation and visual activation, which are simultaneously activated and contribute to the desynchronization of the LFP signal. Probably, the stronger beta desynchronization in the long movement latencies could be correlated with the interference phenomenon which occurs during the planning of an action complementary to that observed, described by the other kinematic studies, that have been mentioned above (Chinellato et al., 2015; Sartori et al., 2011, 2012, 2013a, 2013b). In this context, in the study performed by Chinellato and colleagues, participants were asked to perform a reach-to-grasp movement adopting a precision grip (PG) while observing a fixation cross, observing an actor performing a PG with interactive purposes, and observing an actor performing a PG without interactive purposes. Fine-grained kinematic analysis for interactive condition revealed a specific delay in the grasping and reaching components and an increased trajectory deviation despite the observed and executed movement's congruency. These data suggest that interactive contexts can determine a prompt modulation of stimulus-response compatibility effects. In addition, the results show that, when a subject is required to make a qualitatively different (incongruent) but complementary gesture with respect to that observed, the motor program (or representation) associated with the incongruent movement interferes with both the outgoing motor output and the observed movement: this interference arises as a general delay in the grasping and reaching component and as an increase of variance in movement trajectory (Chinellato et al., 2015). Thus, planning a complementary action, related to the one observed, could have the power to elicit interference effects during the action observation. According to Schubo (2001) and Dolk (2014) the representations that underlie perceptual and motor activities, such as producing a movement while concurrently encoding an independent stimulus motion, must be “kept separate” so that the two activities can be carried out without interfering. This is in line with our results, in fact in the *Monkey* condition, observing an interactive gesture automatically generates an internal representation of the required movement: this internal representation could be in conflict and cause interference with the internal representation of the observed action. The results of this conflict could induce the inhibitory effect on the MNs activity during action observation.

It is, also, interesting to note that this inhibitory effect on the visual discharge of the mirror neurons during the action observation is not present in the other conditions (*Container*, *Mouth*, *Hand*) in which the monkey had to simply observe the action holding its hand on the handle, without plan any interactive movement (*passive* action observation). In fact, the only condition in which the monkey was allowed to release its hand from the handle was the *Monkey* condition, in which, after the observation of the grasping performed by the agent, the animal took the object offered by the agent and interact with him (*active* action observation). Moreover, even if the *Hand* condition is aimed at social goal (interaction between the two agents), we didn't find the same inhibitory effect on the mirror neurons activity as in the *Monkey* condition. Thus, it seems that, only during the *active* action observation, which requires a motor action plan in order to interact with the observed agent, this inhibitory effect emerges on the activity of mirror neurons.

3.5.3 Conclusions

Overall, this study provides evidence that social context, in particular, social interactions between two individuals or with the observer, can modulate the activity of mirror neurons in F5c. This allows the observer to understand other's intention in relation to social goals. This ability seems to be fundamental in a complex social context, where others' behavior and intentions provide a rich supply of information, such as outcomes (rewards or punishments directly experienced and observed) and actions (directly executed and observed): humans and other animals have the capability of learning from these information, such as learning from others and learning about others (Hayden et al., 2009; Frith C. and Frith U., 2010; Yoshida et al. 2011).

This study also provides evidence that online interference occurs when an observed movement requires to plan an incongruent movement with respect to that observed: during the observation, the internal representation of the observed action could interfere with the internal representation of the congruent action which is going to be executed (Sartori et al., 2011; Chinellato et al., 2015). This interference phenomenon could affect the visual discharge of MNs during the action observation, resulting in a inhibitory effect on their activity.

In conclusion, our study doesn’t support the hypothesis according to which the activity of mirror neurons could reflect a motor preparation or anticipation of the action that an individual will perform, but rather confirm and extend the idea that MNs could facilitate the understanding of others’ intentions by discriminating the contextual, biological and social factors available in the environment that are crucial to decode the final goal of an observed action.

4. REFERENCES

- Alexander G.E. and Crutcher M.D. (1990). Neural representations of the target (goal) of visually guided arm movements in three motor areas of the monkey. J Neurophysiology 64(1):164-78.

- Anderson J.R., Montant M. e Schmitt D. (1996). Rhesus monkeys fail to use gaze direction as an experimenter-given cue in an object choice tasks. Behavioral processes 37(47-55).

- Azzi J.C.B., Sirigu A., Duhamel J. R. (2011). Modulation of value representation by social context in the primate orbitofrontal cortex. PNAS.

- Baker S.N., Kilner J.M., Pinches E.M., Lemon R.N. (1999). The role of synchrony and oscillations in the motor output. Exp. Brain Res. 128(1-2):109-17.

- Baker S.N., Olivier E., Lemon R.N. (1997). Coherent oscillations in monkey motor cortex and hand muscle EMG show task-dependent modulation. J Physiol. 501:225-41.

- Ballesta S. and Duhamel J.R. (2015). Rudimentary empathy in macaques' social decision-making. PNAS 112(50):15516-21.

- Barbas H., Pandya D.N., (1987). Architecture and frontal cortical connections of the premotor cortex (area 6) in the rhesus monkey. J Comp Neurol. 256(2):211-28.

- Bault N., Coricelli G., Rustichini A. (2008). Interdependent utilities: How social ranking affects choice behavior. PLoS ONE 3:e3477.

- Belmalih A., Borra E., Contini M., Gerbella M., Rozzi S.,Luppino G. (2009). Multimodal architectonic subvision of the rostral part (area F5) of the macaque ventral premotor cortex. The Journal of Comparative Neurology 512: 183-217.

- Bergman T.J., Beehner J.C., Cheney D.L., Seyfarth R.M. (2003). Hierarchical classification by rank and kinship in baboons. Science 302:1234-1236.

- Bonini L., Rozzi S., Serventi F.U., Simone L., Ferrari P.F., Fogassi L. (2010). Ventral premotor and inferior parietal cortices make distinct contribution to action organization and intention understanding. Cerebral Cortex 20:1372-1385.

- Bonini L., Serventi FU, Simone L., Rozzi S., Ferrari P.F., Fogassi L. (2011). Grasping neurons of monkey parietal and premotor cortices encode action goals at distinct levels of abstraction during complex action sequences. J. Neurosci. 31(15): 5876-86.

- Bonini L. , Maranesi M., Livi A., Fogassi L, Rizzolatti G. (2014). Ventral premotor neurons encoding representations of action during self and others' inaction. Curr Biol.

- Bonini L, Maranesi M, Livi A, Fogassi L, Rizzolatti G. (2014). Space-dependent representation of objects and other's action in monkey ventral premotor grasping neurons. J Neurosci.

- Borra E., Belmalih A., Calzavara R., Gerbella M., Murata A., Rozzi S., Luppino G. (2008). Cortical connections of the macaque anterior intraparietal (AIP) area. Cereb Cortex 18: 1094-1111.

- Brass M., Bekkering H., Prinz W. (2001). Movement observation effects movement execution in a simple response task. Acta Psychol. 106:3-22.

- Brodmann K., (1909). Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues, Johann Ambrosius Barth Verlag, Leipzig.

- Byrne R.W. and Whiten A. (1988). Machiavellian intelligence: social expertise and the evolution of intellect in monkeys, apes, and humans. New York: Oxford Univeristy Press.

- Caggiano V., Fogassi L., Rizzolatti G., Thier P., Casile A. (2009). Mirror neurons differentially encode the peripersonal and extrapersonal space of monkeys. Science. 2009 324(5925):403-6.

- Caggiano V., Fogassi L., Rizzolatti G., Casile A., Giese M.A., Their P. (2012). Mirror neurons encode the subjective value of an observed action. PNAS 109(29):11848-53.

- Caggiano V., Giese M., Thier P., Casile A. (2015) Encoding of point of view during action observation in the local field potentials of macaque area F5. Eur. J. Neurosci. 41(4):466-76.

- Caruana F., Jezzini A., Sbriscia-Fioretti B., Rizzolatti G., Gallese V. (2011). Emotional and social behaviors elicited by electrical stimulation of the insula in the macaque monkey. Curr. Biol 21: 195-199.

- Castiello U., Lusher D., Mari M., Edwards M.G., Humphreys G.W. (2002). “Observing an human or a robotic hand grasping an object: differential motor priming effects”. In Attention and Performance, Vol 19, eds Prinz and B. Hommel (Cambridge: MIT Press).

- Chang S., Garièpy J., Platt M. (2013). Neuronal reference frames for social decisions in primate frontal cortex. Nature neuroscience.

- Cheney D.L. and Seyfarth R.M. (1990). How monkeys see the world: inside the mind of another species. Chicago: University of Chicago Press.

- Cheney D.L. and Seyfarth R.M. (2007). Baboon Metaphysics: the evolution of social minds. Chiacago: University of Chicago Press.

- Chersi F., Ferrari P.F., Fogassi L. (2011). Neuronal chains for actions in the parietal lobe: a computational model. PLoS One. 6(11):e27652.

- Chinellato E., Castiello U., Sartori L. (2015). Motor interference in interactive context. Front. Psychol. 6:791.

- Cisek P., Kalaska J.F. (2005). Neural correlates of reaching decision in dorsal premotor cortex: specification of multiple direction choices and final selection of action. Neuron 45: 801-814.

- Coudè G., Ferrari P.f., Rozzi S., Borelli, Bovini L., Rizzolatti G. and Fogassi L. (2005). Motor control, sensorimotor integration, and cognitive functions of the cortical ventral motor areas of macaque monkey: a mapping study. Soc Neurosc Abs 194.2.

- Coudé G., Festante F., Cilia A., Loiacono V., Bimbi M., Fogassi L., Ferrari P.F. (2016). Mirror Neurons of ventral premotor cortex are modulated by social cues provided by others' gaze. J. Neuroscience. 36(11):3145-3156.

- Crawford J.D., Medendorp W.P., Marotta J.J. (2004). Spatial transformations for eye–hand coordination. J Neurophysiol 92:10–19.

- Deaner R.O., Khera A.V., Platt M.L. (2005). Monkeys pay per view: Adaptive valuation of social images by rhesus macaques. Curr Biol 15:543–548.

- Di Pellegrino G., Fadiga L., Fogassi, L., Gallese V., Rizzolatti G. (1992). Understanding motor events: a neurophysiological study. Exp Brain Res.;91(1):176-80.

- Dolk T., Hommel B., Colzato L.S., Schutz-Bosbach S., Prinz W., Liepelt R. (2014).The joint Simon effect: a review and theoretical integration. Front. Psychol. 5:974.

- Donoghue J.P., Sanes J.N., Hatsopoulos N.G., Gaal G. (1998). Neural discharge and local field potential oscillations in primate motor cortex during voluntary movements. J. Neurophysiol. 79(1):159-73.

- DunbarR.I.M. (1983). Structure of gelada baboon reproductive. Units 3. The male's relationship with his females. Anim Behav 31:565-575.

- Edwards M.G., Humpreys G.W., Castiello U. (2002). Motor facilitation following action observation: a behavioural study in prehensile action. Brain Cogn 53:495-502.

- Emery N.J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. Neurosci Biobehav Rev 24(6): 581-604.

- Engels A.K. and Fries P. (2010). Beta-band oscillations—signalling the status quo? Curr Opin Neurobiol. 20(2):156-65.

- Fadiga L., Fogassi L., Gallese V., Rizzolatti G. (2000). Visuomotor neurons: ambiguity of the discharge or 'motor' perception. Int J Psychophysiol.;35(2-3):165-77.

- Fehr E. and Fischbaker U. (2003). The nature of human altruism. Nature 425(6960):785-91.

- Ferrari P.F., Kohler, E., Fogassi, L., Gallese, V. (2000). The ability to follow eye gaze and its emergence during development in macaque monkeys. Proc Natl Acad Sci U S A.; 5;97(25):13997-4002.

- Ferrari P.F., Gallese V., Rizzolatti G. e Fogassi L. (2003). Mirror neurons responding to the observation of ingestive and communicative mouth actions in the monkey ventral premotor cortex. Eur J Neurosci 17(8): 1703-14.

- Ferrari P. F., Rozzi S. e Fogassi L. (2005). Mirror neurons responding to observation of actions made with tools in monkey ventral premotor cortex. J Cogn Neurosci 17(2): 212-26.

- Ferrari P.F., Coudé G., Gallese V., Fogassi L. (2008) . Having access to others' mind through gaze: the role of ontogenetic and learning processes in gaze following behavior of macaques. Social Neuroscience, 3, 239-249.

- Ferrari P. F. and Rozzi S. (2012). Mirror neurons, action and relation. The acting brain as the building block of the social mind. RSF VOL. CXXXVI- N°1.

- Fogassi L., Ferrari P.F., Gesierich B., Rozzi S., Chersi F., Rizzolatti G. (2005). Parietal lobe: from action organization to intention understanding. Science; 29;308(5722):662-7.

- Fogassi L., Gallese V., Buccino G., Craighero L., Fadiga L. Rizzolatti G. (2001). Cortical mechanism for the visual guidance of hand grasping movements in the monkey: A reversible inactivation study. Brain;124(Pt 3):571-86.

- Fogassi L., Gallese V., di Pellegrino G., Fadiga L., Gentilucci M., Luppino, G., Matelli, M., Pedotti A., Rizzolatti G. (1992). Space coding by premotor cortex. Exp Brain Res.;89(3):686-90.

- Fogassi L., Gallese V., Fadiga L., Luppino G., Matelli M., Rizzolatti G. (1996). Coding of peripersonal space in inferior premotor cortex (area F4). J Neurophysiol.;76(1):141-57.

- Fogassi L., Luppino G. (2005). Motor functions of the parietal lobe. Curr Opin Neurobiol. 2005 Dec;15(6):626-31. Epub.

- Fontana A.P., Kilner J.M., Rodrigues E.C., Joffily M., Nighoghossian N., Vargas C.D., Sirigu A. (2012). Role of the parietal cortex in prediction incoming action. Neuorimage 59(1):556-64.

- Frith U., Frith C. (2010). Learning from others: introduction to the special review series on social neuroscience. Neuron 65:739-743.

- Fuji N., Hihara S., Iriki A. (2007). Dynamic social adaptation of motion-related neurons in primate parietal cortex. PloS one 2:e397.

- Gallese V., Fadiga L., Fogassi L., Rizzolatti G. (1996). Action recognition in the premotor cortex. Brain;119 (Pt 2):593-609.

- Gallese V., Murata A., Kaseda M., Niki N., Sakata H. (1994). Deficit of hand reshaping after muscimol injection in monkey parietal cortex. Neuroreport; 21;5(12):1525-9.

- Gallese V., Fadiga L., Fogassi L. and Rizzolatti G. (2002). Action representation and the inferior parietal lobule. Common Mechanism in Perception and Action: Attention and Performance. W. Prinz e B. Hommel, Oxford University Press. 19: 334-355.

- Gallese V., Keysers C. and Rizzolatti G. (2004). A unifying view of the basis of social cognition. Trends in Cognitive Sciences; 8(9): 396-403.

- Gentilucci M., Fogassi L., Luppino G., Matelli M., Camarda R., Rizzolatti G. (1988). Functional organization of inferior area 6 in the macaque monkey. I. Somatotopy and the control of proximal movements. Exp Brain Res; 71(3):475-90.

- Gentilucci M., Fogassi L., Luppino G., Matelli M., Camarda R., Rizzolatti G. (1989). Somatotopic representation in inferior area 6 of the macaque monkey. Brain Behav Evol; 33(2-3):118-21.

- Gothard K.M., Battaglia F.P., Erickson C.A., Spitler K.M., Amaral D.G. (2007). Neural responses to facial expression and face identy in the monkey amygdala. J. Neurophysiol. 97:1671-83.

- Graziano M.S.A., Aflalo T.N.S., Cooke D.F. (2005). Arm movements evoked by electrical stimulation in the motor cortex of monkeys. J Neurophysiol 94:4209–4223 Available at: http://www.ncbi.nlm.nih.gov/pubmed/16120657 [Accessed August 27, 2016].

- Graziano M.S.A., Taylor C.S.R., Moore T., Cooke D.F. (2002). The cortical control of movement revisited. Neuron 36:349–362.

- Hampton A.N., Bossaerts P., O'Doherty J.P. (2008). Neural correlates of mentalizing-related computations during strategic interactions in humans. PNAS 105(18):6741-6.

- Hasson U., Ghazanfar A.A., Galantucci B., Garrod S., Keysers C. (2012). Brain-to_brain coupling: a mechanism for creating and sharing a social word. Trends Cogn Sci. 16(2): 114-121.

- Hayden B.Y., Pearson J.M., Platt M.L. (2009). Fictive reward signals in the anterior cingulate cortex. Science, 324:948–950.

- He S.Q., Dum R.P., Strick P.L. (1993). Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. J Neurosci.;13(3):952-80.

- Helms S.I., Flanders M., Soechting J.F. (1991). Information system of visual and kinesthetic. J Neurosci 11:770–778.

- Henriques D.Y., Klier E.M., Smith M.A., Lowy D., Crawford J.D. (1998). Gaze-centered remapping of remembered visual space in an open-loop pointing task. J Neurosci 18:1583–1594.

- Henriques D.Y., Medendorp W.P., Gielen C.C., Smith A.M., Crawford J.D. (2003). Geometric computations underlying eye–hand coordination: orientations of the two eyes and the head. Exp Brain Res 152:70–78, doi:10.1007/s00221-003-1523-4.

- Hoshi E. and Tanji J. (2004). Area-selective neuronal activity in the dorsolateral prefrontal cortex for information retrieval and action planning. J Neurophysiol 91(6): 2707-22.

- Hoshi E. and Tanji J. (2008). Role of the Lateral Prefrontal Cortex in Executive Behavioral Control. Physiol. Rev. 88: 37-57.

- Hyvarinen J. (1981). Regional distribution of functions in parietal association area 7 of the monkey. Brain Res 206(2): 287-303.

- Iacoboni M., Lieberman M.D., Knowlton B.J., Molnar-Szakacs I., Moritz M., Throop J., Fiske A.P. (2004). Watching social interactions produces dorsomedial prefrontal and medial parietal BOLD fMRI signal increase compared to resting baseline. NeuroImage 1167-1173.

- Isbaine F., Demolliens M., Belmalih A., Brovelli A., Boussaoud D. (2015). Learning by observation in the macaque monkey under high experimental constraints. Behav Brain Res 289:141-8.

- Jasper H. and Penfield W. (1949). Electrocorticograms in man: effect of voluntary movement upon the electrical activity of the precentral gyrus. Arch. F. Psychiatr. U. Z. Neur. 183:163.

- Jeannerod M., Arbib M.A., Rizzolatti G., Sakata H. (1995). Grasping objets: The cortical mechanisms of visuomotor transformation. Trends Neurosci 18:314–320.

- Jellema T., Perrett DI. (2003). Perceptual history influences neural responses to face and body postures. J Cogn Neurosci. 15(7):961-71.

- Johansson R.S., Westling G., Bäckström A., Flanagan J.R. (2001). Eye–hand coordination in object manipulation. J Neurosci 21:6917–6932.

- Keysers C., Kohler E., Umiltà M. A., Nanetti L., Fogassi L. e Gallese, V. (2003). Audiovisual mirror neurons and action recognition. Exp Brain Res 153(4): 628-36.

- Kilavik B.E., Ponce-Alvarez A., Trachel R., Confais J., Takerkart S., Riehle A. (2012). Context-related frequency modulations of macaque motor cortical LFP beta oscillations. Cerebral Cortex 22(9): 2148-59.

- Kilner J.M., Vargas C., Duval S., Blakemore S.J., Sirigu A. (2004). Motor activation prior to observation of a predicted movement. Nat Neuroscience 7(12):1299-301.

- Klein, J.T., Shepherd, S. V, Platt, M.L. (2009). Social attention and the brain. Curr. Biol. 19, R958-62. doi:10.1016/j.cub.2009.08.010

- Knoblich G. and Jordan J.S. (2003). Action coordination in groups and individuals: learning anticipatory control. J Exp Psychol Learn Mem Cogn 29:1006–1016.

- Knoblich G. (2011). Psychological research on joint action: theory and data the psychology of learning and motivation. Ross, B editor. Academic Press.

- Kohler E., Keysers C., Umiltà M.A., Fogassi L., Gallese V., Rizzolatti G. (2002). Hearing sounds, understanding actions: action representation in mirror neurons. Science; 2;297(5582):846-8.

- Kraskov A., Dancause N., Quallo M., Shepherd S., And Lemon R. (2009). Cortical neurons in macaque ventral premotor cortex with mirror properties: a potential mechanism for action suppression? J. Neurosci 12.010.

- Kraskov A., Philipp R., Waldert S., Vigheswaren G., Quallo M.M., Lemon R.N. (2014). Corticospinal mirror neurons. Philos Trans R Soc Lond B Biol Sci 369(1644):20130174.

- Kritikos A., Bennett K.M., Dunai J., Castiello U. (2000). Interference from distractors in reach-to-grasp movements. Q. J. Exp. Psychol A. 53(1):131-51.

- Kuraoka K. and Nakamura K. (2007). Impacts of facial identity and type of emotion on responses of amygdala neurons. Neuroreport 17:9-12.

- Lemon R.N., Mantel G.W. Muir R.B. (1986). Corticospinal facilitation of hand muscles during voluntary movement in the conscious monkey. J. Physiology 381: 497-527.

- Liebe S., Logothetis N.K., Rainer G. (2011). Dissociable effects of natural image structure and color on LFP and spiking activity in the lateral prefrontal cortex and extrastriate visual area V4. J. Neurosci. 31(28):10215-27.

- Logothetis N.K., Kayser C., Oelterman A. (2007). In vivo measurements of cortical impedance spectrum in monkeys: implications for signal propagations. Neuron 55:809-823.

- Luppino G., Matelli M., Camarda R.M., Gallese V., Rizzolatti G. (1991). Multiple representations of body movements in mesial area 6 and the adjacent cingulate cortex: an intracortical microstimulation study in the macaque monkey. J Comp Neurol.; 22;311(4):463-82.

- Luppino G., Matelli M., Camarda R., Rizzolatti G. (1993). Corticocortical connections of area F3 (SMA-proper) and area F6 (pre-SMA) in the macaque monkey. J Comp Neurol.; 1;338(1):114-40.

- Luppino G. e Rizzolatti G. (2000). The Organization of the Frontal Motor Cortex. News Physiol Sci 15: 219-224.

- Maranesi M., Rodà F., Bonini L., Rozzi S., Ferrari P.F.,Fogassi L., Coudè G. (2012. Anatomo-functional organization of the ventral primary motor and premotor cortex in the macaque monkey. European Journal of Neuroscience, Vol. 36, pp. 3376-3378.

- Matelli M., Camarda R., Glickstein M., Rizzolatti G. (1986). Afferent and efferent projections of the inferior area 6 in the macaque monkey. J Comp Neurol.; 15;251(3):281-98.

- Matelli M. and Luppino G. (2000) Parietofrontal circuits: parallel channels for sensory-motor integrations. Adv Neurol.;84:51-61. Review.

- Matelli M., Luppino G. (2001). Parietofrontal circuits for action and space perception in the macaque monkey. Neuroimage; 14(1 Pt 2):S27-32.

- Matelli M., Luppino G., Rizzolatti G. (1985). Patterns of cytochrome oxidase activity in the frontal agranular cortex of the macaque monkey. Behav Brain Res.;18(2):125-36.

- Matelli M., Luppino G., Rizzolatti G. (1991). Architecture of superior and mesial area 6 and the adjacent cingulate cortex in the macaque monkey. J Comp Neurol.; 22;311(4):445-62.

- Matelli M. and Luppino G. (1997). Functional anatomy of human motor cortical areas. In: F. Boller and J. Grafman (Eds.), Handbook of Neuropsychology, Vol. 11. Elsevier, Amsterdam pp. 9–26.

- Meunier M, Monfardini E., Boussaud D. (2007). Learning by observation in rhesus monkeys. Neurobiol Learn Mem. 88(2):243-8.

- Mitzdorf U. (1985). Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol Rev 65(1):37-100.

- Mountcastle V.B., Lynch J.C., Georgopoulos A., Sakata H. e Acuna C. Posterior parietal association cortex of the monkey: command functions for operations within extrapersonal space. J Neurophysiol 38(4): 871-908.

- Murata A., Fadiga L., Fogassi L., Gallese V., Raos V., Rizzolatti G. (1997). Object representation in the ventral premotor cortex (area F5) of the monkey. J. Neurophysiol. 78: 2226-2230.

- Nelissen K., Luppino G., Vanduffel W., Rizzolatti G., Orban GA. (2005). Observing Others: Multiple Action Representation in the Frontal Lobe. Science; 310:332 – 336.

- Nelissen K., Borra E., Gerbella M., Rozzi S., Luppino G., Vanduffel W., Rizzolatti G. and Orban G.A. (2011). Action Observation circuits in the macaque monkey cortex. The Journal of Neuroscience 31 (10): 3743-3756.

- Neuper C. and Pfurtscheller G. (1996). Post-movement synchronization of beta rhythms in the EEG over the cortical foot area in man.

- Nummenmaa, L., Calder, A.J., (2009). Neural mechanisms of social attention. Trends Cogn. Sci. 13, 135–143. doi:10.1016/j.tics.2008.12.006

- Padoa-Schioppa C. (2007). Orbitofrontal cortex and the computation of economic value. Ann N Y Acad Sci 1121:232-53.

- Padoa-Schioppa C. and Cai X. (2011). The orbitofrontal cortex and the computation of subjective value: consolidated concepts and new perspectives. Ann N Y Acad Sci. Review.

- Pelphrey K. A., Singerman J. D., Allison, T. e McCarthy, G. (2003). Brain activation evoked by perception of gaze shifts: the influence of context. Neuropsychologia 41(2): 156-70.

- Pelphrey K.A., Morris J.P., McCarthy G. (2004). Grasping the intentions of others: the perceived intentionality of an action influences activity in the superior temporal sulcus during social perception. J Cogn Neurosci.;16(10):1706-16.

- Penfield W., Welch K. (1951). The supplementary motor area of the cerebral cortex; a clinical and experimental study. AMA Arch Neurol Psychiatry; 66(3):289-317.

- Perrett D.I., Harries M.H., Bevan R., Thomas S., Benson P.J., Mistlin, A.J., Chitty A.J., Hietanen, J.K., Ortega, J.E. (1989). Frameworks of analysis for the neural representation of animate objects and actions. J Exp Biol.;146:87-113. Review.

- Pesaran B., Pezaris J.S., Sahani M., Mitra P.P., Andersen R.A. (2002). Temporal structure in neuronal activity during working memory in macaque parietal cortex. Nat. Neurosci. 5:805-811.

- Petrides M. e Pandya D.N. (1994). Comparative architectonic analysis of the human and the macaque frontal cortex. In: Handbook of neuropsychology, Vol. IX. F. Boller e J. GRafman. New York, Elsevier.

- Petrides M. e Pandya D.N. (1984). Projections to the frontal cortex from the posterior parietal region in the rhesus monkey. The Journal of Comparative Neurology; 228 (1):105–116.

- Porter R. e Lemon R. (1993). Corticospinal function and voluntary movment. Oxford, Clarendon Press.

- Raos V., Franchi G., Gallese V., Fogassi L. (2003). Somatotopic organization of the lateral part of area F2 (dorsal premotor cortex) of the macaque monkey. J Neurophysiol.; 89(3):1503-18.

- Raos, V., (2005). Functional Properties of Grasping-Related Neurons in the Ventral Premotor Area F5 of the Macaque Monkey. J. Neurophysiol. 95, 709–729. doi:10.1152/jn.00463.2005

- Ray S., Crone N.E., Niebur E., Franaszczuk P.J., Hsiao S.S. (2008). Neural correlates of high-gamma oscillations (60-120 Hz) in macaque local field potential and their potential implications in electrocorticography. J. Neurosci. 28(45):11526-11536.

- Rilling J., Gutman D., Zeh T., Pagnoni G., Berns G., Kilts C. (2002). A neural basis for social cooperation. Neuron 35(2):395-405.

- Rizzolatti G., Cattaneo L., Fabbri-Destro M., Rozzi S. (2014). Cortical mechanisms underlying the organization of goal-directed actions and mirror neuron-based action understanding. Physiol Rev 94:655–706.

- Rizzolatti G, Fogassi L, Gallese V (2009). The mirror neuron system: a motor-based mechanism for action and intention understanding. In: The cognitive neuroscience IV (Gazzaniga M, ed), pp 625–640. Cambridge, MA: MIT Press.

- Rizzolatti G. and Craighero L. (2004). The mirror-neuron system. Annu Rev Neurosci. 2004;27:169-92.

- Rizzolatti G., Gentilucci M., Fogassi L., Luppino, G., Matelli M., Ponzoni-Maggi S. (1987). Neurons related to goal-directed motor acts in inferior area 6 of the macaque monkey. Exp Brain Res.;67(1):220-4.

- Rizzolatti G., Camarda, R., Fogassi L., Gentilucci, M., Luppino, G. e Matelli M. (1988). Functional organization of inferior area 6 in the macaque monkey. II. Area F5 and the control of distal movements. Exp Brain Res 71(3): 491-507.

- Rizzolatti G., Fadiga L., Gallese, V., Fogassi L. (1996). Premotor cortex and the recognition of motor actions. Brain Res Cogn Brain Res.;3(2):131-41.

- Rizzolatti G., Fogassi L., Gallese, V. (1997). Parietal cortex: from sight to action. Curr Opin Neurobiol.;7(4):562-7. Review.

- Rizzolatti G. and Luppino G. (2001). The cortical motor system. Neuron; 27;31(6):889-901. Review.

- Rizzolatti G., Fogassi L., and Gallese V. (2002). Motor and cognitive functions of the ventral premotor cortex. Curr Opin Neurobiol. 12(2):149-54.

- Rizzolatti G., Luppino G., Matelli M. (1998). The organization of the cortical motor system: new concepts. Electroencephalogr Clin Neurophysiol.;106(4):283-96. Review.

- Rizzolatti G. and Sinigaglia C. (2010). The functional role of the parieto-frontal mirror circuit: interpretations and misinterpretetions. Nat Rev Neurosc 11:264-274.

- Roux S., Mackay W.A., Riehle A. (2006). The pre-movement component of motor cortical local field potentials reflects the level of expectancy. Behav Brain Re. 169(2):335-51.

- Roy, A.C., Paulignan, Y., Farnè, A., Jouffrais, C., Boussaoud, D., 2000. Hand kinematics during reaching and grasping in the macaque monkey. Behav. Brain Res. 117, 75–82. doi:10.1016/S0166-4328(00)00284-9.

- Rozzi S., Calzavara R., Belmalih A., Borra E., Gregoriou G., Matelli M. e Luppino G. (2006). Cortical connections of the inferior parietal cortical convexity of the macaque monkey. Cereb Cortex 16(10): 1389-417.

- Rozzi S., Ferrari P.F., Bonini L., Rizzolatti G., Fogassi L. (2008). Functional organization of inferior parietal lobule convexity in the macaque monkey: electrophysiological characterization of motor, sensory, and mirror responses and their correlation with cytoarchitectonic areas. Eur. J Neurosci. 28(8):1569-88.

- Rudebeck P.H., Buckley M.J., Walton M.E., Rushworth M.F. (2006). A role of the macaque anterior cingulate gyrus in social valuation. Science 13:1310-2.

- Sanes J.N. and Donoghue J.P. (1993) Oscillations in local field potential of the primate motor cortex during voluntary movement. PNAS 90(10):4470-4474.

- Sartori L., Cavallo A., Bucchioni G., Castiello U. (2011). Corticospinal exicitability is specifically modulated by the social dimension of observed actions. Exp. Brain. Res. 211:557-568.

- Sartori L., Cavallo A., Bucchioni G., Castiello U. (2012). From simulation to reciprocity: the case of complementary actions. Soc. Neurrosci. 7:146-158.

- Sartori L. Betti S., Castiello U. (2013a). When mirroring is not enough: that is, when only a complementary action will do (the trick). Neuroreport 24:601-604.

- Sartori L., Betti S., Castiello U. (2013b). Corticospinal excitability modulation during action observation. J. Vis. Exp. 82:e51001.

- Scherberger H., Jarvis M.R., Andersen R.A. (2005). Cortical local field potential movement intention in the posterior parietal cortex. Neuron 46(2):347-54.

- Schubo A., Aschersleben G., Prinz W. (2001). Interactions between perception and action in a reaction task with overlapping S-R assignments. Psychol. Res. 65:145-157.

- Sebanz N., Bekkering H., Knoblich G. (2006). Joint action: Bodies and minds moving together. Trends Cogn Sci 10:70–76.

- Sescousse G., Redouté J., Dreher J. C. (2010). The architecture of reward value coding in the human orbitofrontal cortex. J Neurosci 30: 13095-13104.

- Shamay-Tsoory S.G., Tomer R., Goldsher D., Berger B.D., Aharon-Peretz J. (2004). Impairment in cognitive and affective empathy in patients with brain lesions: Anatomicaland cognitive correlates. J Clin Exp Neuropsychol 26:1113-1127.

- Shepherd V., Kleinb T., Deanerc R. e Plat L. (2009). Mirroring of attention by neurons in macaque parietal cortex. PNAS. 106(23):9489-9494.

- Stűrmer B., Siggelkow S., Dengler R., Leuthold H. (2000a). Response priming in the Simon paradigm. A transcranial magnetic stimulation study. Exp Brain Res 135:353-9.

- Stűrmer B., Aschersleben G., Prinza W. (2000b). Correspondence effects with manual gestures and postures: a study of imitation. J Exp Psychol Hum Percept Perform 26(6):1746-59.

- Subial F., Clanton J., Holloway R.L., Terrace H.S. (2004). Cognitive imitation in rhesus macaques. Science 305(5682):407-10.

- Tanji J. (2001). Sequential organization of multiple movements: involvement of cortical motor areas. Annu Rev Neurosci. 24:631-51. Review.

- Tanji J. and Hoshi E. (2008). Role of the lateral prefrontal cortex in executive behavioural control. Physiol Rev 88(1):37-57.

- Terao Y., Andersson N.E., Flanagan J.R., Johansson R. (2002). Engagement of gaze in capturing targets for future sequential manual actions. J Neurophysiol 88:1716–1725.

- Tsujimoto S., Genovesio A., Wise S.P. (2009). Monkey orbitofrontal cortex encodes response choices near feedback time. J. Neuroscience 29(8):2569-74.

- Tsunada J., Sawaguchi T. (2012). Neuronal categorization and discrimination of social behaviors in primate prefrontal cortex. PloS one 12:e52610.

- Umiltà M.A., Kohler E., Gallese V., Fogassi L., Fadiga L., Keysers C., Rizzolatti G. (2001). I know what you are doing. a neurophysiological study. Neuron; 19;31(1):155-65.

- Umilta, M.A., Brochier, T., Spinks, R.L., Lemon, R.N. (2007). Simultaneous recording of macaque premotor and primary motor cortex neuronal populations reveals different functional contributions to visuomotor grasp. J. Neurophysiol. 98, 488–501. doi:10.1152/jn.01094.2006.

- Umiltà M.A., Escola L., Intskirveli I., Grammont F., Rochat M., Caruana F., Jezzini A., Gallese V., Rizzolatti G. (2008). When pliers become fingers in the monkey motor system. Proc Natl Acad Sci U S A 105:2209–2213.

- Vigneswaran G., Philipp R., Lemon R.N., Kraskov A. (2013). M1 corticospinal mirror neurons and their role in movement suppression during action observation. Curr. Biol. 23(3):235-43.

- Waldert S., Vigneswaren G., Philipp R., Lemon R.N., Kraskov A. (2015). Modulation of the intracortical LFP during action execution and observation. J. Neurosci. 35(22):8451-8461.

- Woolsey C.N., Settlage, P.H., Meyer D.R., Sencer W., Pinto Hamuy T., Travis A.M. (1952). Patterns of localization in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. Res Publ Assoc Res Nerv Ment Dis. 1952;30:238-64.

- Yoshida K., Saito N., Iriki A., Isoda M. 2011. Representation of Others’ Action by Neurons in Monkey Medial Frontal Cortex. Current Biolgy 21, 249-253.