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THE ROLE OF THE MONKEY VENTROLATERAL PREFRONTAL CORTEX IN THE ORGANIZATION OF INTENTIONAL ACTIONS

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1. INTRODUCTION

1.1. Hierarchical organization of the motor system: movements, motor acts, actions and intentions.

Before the 19th century, voluntary actions were considered as a variety of reflexes with some degree of autonomy with respect to external stimuli (Jeannerod, 2006). The idea that the cerebral cortex might be involved in controlling movements began to emerge during the second half of the 19th century thanks to the work of physiologists and neurologists who made direct observations on animals and human patients.

The contribution of clinical studies largely depended on the pioneering works of John Hughlings Jackson. He described patients with unilateral epileptic seizures that started from one body part and then spread with the characteristic "march" to adjacent body parts. These observations provided strong support to his idea that specific lesions of the cerebral cortex could cause the seizures, and that the clinical features of these phenomena supported a role for the cerebral cortex in the control of body movements. This latter function was typically assigned to the spinal cord, and the cerebral cortex was previously considered devoid of motor functions and specifically involved in cognitive and perceptual processes.

The first direct demonstration of the causal role of the cerebral cortex in the control of movement derived from the work of Gustav Theodor Fritsch and Julius Eduard Hitzig. In 1870, these physiologists discovered that an electrical stimulation delivered on the cortical surface of different regions of the precentral gyrus in the dog caused movements of specific body parts, contralateral to the stimulated site. A few years later, David Ferrier (1876) confirmed this result in the monkey. These experiments not only demonstrated that the cerebral cortex is actually involved in the control of movement, but also evidenced for the first time the existence of a somatotopic map of the controlled body parts on the cortical surface.

Since the very first experiments of Fritsch and Hitzig, it appeared there was a clearly different distribution of motor functions in the frontal lobe: "*a part of the convexity of the hemisphere of the brain (of the dog) is motor [. . .] another part is not*

motor. The motor part, in general, is more in front, the non-motor part more behind. By electrical stimulation of the motor part, one obtains combined muscular contractions of the opposite side of the body" (Fritsch and Hitzig, 1870). As more recent proposals claim, it can be envisioned a rostro-caudal, hierarchical organization even within the frontal lobe (Badre et al., 2007) and, more specifically, of the motor system.

In the field of neurophysiology, a huge amount of data from human and animal studies point now to the idea that different areas of the motor system encode movement at different levels of complexity. At the lowest levels, the type of coding provided by the primary motor cortex can be studied not only through extracellular recording of neuronal activity, but also by mean of direct electrical stimulation of the neural tissue. The results of several years of experiments clearly indicate that this region encode simple *movements, which are* the result of the activation of a limited muscular district that produces the displacement in space of one or more joints, as for example the flexion of the index finger, regardless of the purpose for which the finger has been flexed. In fact, the immediate motor goal is irrelevant at the level of simple movements. In contrast, the concept of goal is crucial for the higher levels of the hierarchy. *Motor acts,* for example, can be formed by one or many simple movements, which are smoothly organized in a synergistic way in order to achieve a specific, immediate motor goal, such as reaching, grasping or bringing something to the mouth. Therefore, while a movement such as the flexion of the index finger could be used to perform several distinct motor acts, such as scratching, grasping or digging out, each of these acts appears to be specific in terms of the motor goal it enables to achieve. However, in everyday life it is usually necessary to select and organize different motor acts into longer and more complex sequences in order to achieve a final goal: "*action may be defined as a sequence of movements which, when executed, allows one to reach his goal. Although the action is unitary in terms of its final aim, from the motor point of view it appears to be formed by various segments, each having its own limited aim. These action segments are the motor acts. Reaching, grasping with the hand, holding, bringing to the mouth, grasping with the mouth are the various motor acts which together form the action the final goal of which is to ingest food*" (Rizzolatti et al., 1988). Thus, compared with motor acts, actions constitute a further step of motor organization in which goals identify the individual's behavioural purposes.

1.1.1. Primary motor cortex and the encoding of simple movements

The primary motor cortex is located in the caudalmost part of the frontal lobe, largely lying in the anterior bank of the central sulcus. Since the half of the '80s a series of anatomical studies (Matelli et al., 1985; Barbas and Pandya, 1987; Matelli et al., 1991; Petrides and Pandya, 1994) had revealed a lack of homogeneity in the agranular frontal cortex of the monkey, leading to the characterization of 7 distinct cytoarchitectonic areas (Matelli et al., 1985). The primary motor cortex (Brodmann area 4) corresponds to area F1, while areas from F2 to F7 correspond the ventral (F4 and F5), dorsal (F2 and F7) and mesial (F3 and F6) portions of Brodmann premotor area 6, respectively.

Several architectonic maps of the agranular cortex of the macaque monkey have been published (Brodmann, 1909; Vogt and Vogt, 1919; Von Bonin and Bailey, 1947; Matelli et al., 1985; Barbas and Pandya, 1987; Matelli et al., 1991), and most investigators agree that the primary motor cortex, unlike premotor cortices, is basically homogeneous. Some distinctive cytoarchitectonic features (Von Bonin and Bailey, 1948; Geyer et al., 1998, 2000; Rivara et al., 2003; Sherwood et al., 2004; Belmalih et al., 2007) permit to recognize it in all primates: it is agranular, poorly laminated and characterized by low cell density with a prevalence of pyramidal type cells of big size, among which giant Betz cells in layer V have been described.

Area F1 is the only motor area showing cortico-spinal projections ending in lamina IX of the spinal cord, where motor neurons are located. This anatomical feature enables F1 to exert a direct control on discrete body movements (Rizzolatti and Luppino, 2001). The critical role of the primary motor cortex in the control of voluntary movements of the body is well established since several decades (Woolsey et al., 1952; Philips and Porter, 1977; Evarts and Fromm, 1980; Porter, 1985; Humphrey, 1986; Lemon, 1988). Its functional properties have been extensively studied during hand and arm movement tasks, and several experiments demonstrated that F1 neurons are tuned to specific kinematic parameters of movement, in particular the force (Evarts et al., 1983; Taira et al., 1996; Sergio and Kalaska, 1998) and, at the population level, even hand position (Georgopoulos et al., 1984; Kettner et al., 1988), speed (Ashe and

Georgopoulos, 1994; Moran and Schwartz, 1999a) and direction (Georgopoulos et al., 1982; Ashe and Georgopoulos, 1994; Fu et al., 1995).

Another crucial aspect of F1 motor properties is the possibility to control individual dexterous fingers movement (Porter and Lemon, 1993), due to its direct access to spinal motor neurons: in fact, lesions of the primary motor cortex cause severe paresis of the controlateral hand (Kuypers et al., 1978; Matsumura et al., 1991; Liu and Rouiller, 1999).

Converging experimental evidences (Hoshi et al., 1998; 2000; Umiltà et al., 2008) indicate that this area, compared with other premotor or prefrontal regions, provide a crucial and specific contribution to the encoding of simple movements and motor parameters regardless of the motor goals, rules or, more generally, behavioural context in which movements are performed.

1.1.2. Premotor and parietal neurons encode goal directed motor acts

The monkey ventral premotor cortex hosts neurons coding specific goal-directed motor acts (Rizzolatti et al., 1987; 1988; Gentilucci et al., 1988). A set of these neurons, found in area F4, discharge during proximal arm-related motor acts ("reaching" and "bringing to the mouth" neurons), while others, recorded in area F5, encode hand and mouth motor acts ("grasping", "holding" and "tearing" neurons). The most peculiar aspect of these latter cells is that they specifically encode *the goal* of the motor act, and not the specific movements involved. In fact, some of them fire both when the monkey grasp an object with the controlateral hand, the ipsilateral hand or even with the mouth (Rizzolatti et al., 1987, 1988). More recent and controlled studies confirmed this concept, by training monkeys to grasp food morsels with two different types of pliers: normal pliers, that can be used by a sequence of extension-flexion of the fingers, and reverse pliers, that can be used by an opposite sequence of flexion-extension of the fingers (Umiltà et al., 2008). Results showed that most neurons in the ventral premotor area F5 encode the immediate motor goal (taking possession of the food) with the same firing pattern, regardless of whether the food was grasped with the hand, the normal pliers or the reverse pliers. In particular these neurons discharged, for example, when

the monkey flexed the fingers to grasp the object with the normal pliers, or when it extended the fingers to grasp it with the reverse pliers

Since the first studies on ventral premotor cortex neurons it was reported that part of grasping neurons could also be selective for the type of grip employed by the monkey to grasp the object (precision grip, finger or whole hand prehension). Thus, it was suggested that area F5 contains a "vocabulary" of motor acts. This vocabulary is formed by different neurons, each coding a specific motor goal and, possibly, also the way in which this goal can be achieved (Rizzolatti et al., 1988).

Neurons encoding motor acts have also been recorded in the monkey inferior parietal lobule (IPL) (Fogassi et al., 2005; Rozzi et al., 2008; Bonini et al., 2010). Similarly to premotor neurons, they are active during a specific motor act and not when a similar movement is performed with another purpose. A considerable number of grasping neurons in area PFG (Gregoriou et al., 2006), as in area F5, also encode how a grasping act has to be performed, showing selectivity for a specific type of grip (Rozzi et al., 2008; Bonini et al., 2012).

The functional similarity in the encoding of goal directed motor acts and the way in which these acts have to be performed between PFG and F5 neurons is strongly supported by the strict and reciprocal connections between these areas (Rozzi et al., 2006; Bonini et al., 2010), suggesting that IPL convexity should be considered as part of the motor system.

1.1.3. From motor acts to intentional actions

The concepts of "intention" and "intentionality" have long been the object of philosophical debate and some fundamental neuroscientific and theoretical issues still remain unresolved, mainly because of the lack of clear and unitary operational definition of what is meant with terms such as "action", "intention" and "motor intention". The philosopher John Searle (1983) distinguished a "prior intention", that is, a motor plan to be carried out in an undetermined future and built on the basis of a current goal that the individual wants to achieve, from an "intention in action", which accompanies the actual action execution during its unfolding. Several neuroscientific

approaches have dealt with the concept of intention and motor intentionality, implicitly or explicitly assuming as central one of these basic aspects of the concept. In particular, motor intention could either designate 1) the cause of a willed action, thus being something preceding the movement onset, or 2) 'why' an action is performed, thus guiding and shaping the whole action unfolding.

Concerning the first aspect of motor intention, neuroscientific studies focused on the preparatory activity of the brain prior to actually start the execution of internally generated actions (Libet, 1985). Richard Andersen with his co-workers has been among the first authors to directly investigate, at the single neuron level, the substrates of preparatory activity specifically linked to intentional plans for movement. These authors defined intention as the formulation of an anticipated motor program specifying what movement to perform (Andersen and Buneo, 2002). Their studies investigated neuronal activity in the lateral intraparietal area (LIP) of the monkey IPL, showing that most of the recorded neurons encode specifically *what* (either an arm reach or a saccade) the animal intends to do next. Other authors demonstrated, in different brain regions, neuronal activity more generally tuned to *when* an upcoming movement was about to be performed. In particular, Hoshi and Tanji (2005) showed that neuronal activity in the anterior cingulate cortex reflect the emergence of a general intention to move, prior to movement onset, but devoid of any clear motor specificity.

As far as the second aspect of intentional actions is concerned, that is, why an action is being performed, only recent studies (Fogassi et al., 2005; Bonini et al., 2010, 2011) provided evidence that parietal and premotor cortices can encode differently the same motor act (i.e. grasping) depending on the goal of the action in which the act is embedded, that is, the overall motor intention of the acting individual. In particular, in the study by Fogassi and co-workers (2005) PFG grasping neurons were tested in two main conditions: grasp-to-eat and grasp-to-place. In the grasp-to-eat condition, the monkey reached and grasped a piece of food located in front of it and brought it to the mouth, while in grasp-to-place condition it reached and grasped an object and then placed it into a container in order to receive a food reward. Note that, although in both conditions the motor act the monkey had to perform was the same (i.e. grasping), many grasping neurons discharged differently during grasp-to-eat and grasp-to-place actions. Control experiments demonstrated that neuronal selectivity could neither be accounted

for by different kinematics associated to grasp-to-eat or grasp-to-place actions, nor by the type of target grasped (i.e. a piece of food or an object). The same neuronal selectivity was also observed when the reward obtained by the monkey for the different actions was varied, enabling to conclude that the crucial factor for determining the observed selectivity was the final goal of the action. Similar experiments have been also carried out in the ventral premotor area F5 (Bonini et al., 2010, 2011), showing that, although the parietal cortex plays a leading role in coding motor acts depending on the action goal, also F5 grasping neurons can discharge differently during grasp-to-eat and grasp-to-place actions.

What is the link between motor acts and the overall action goal? And why are motor acts recruited specifically depending on the action goal, rather than forming a multipurpose motor repertoire enabling to save neuronal and metabolic resources? Although more economic, a multipurpose system might be more difficult to organize, considering the need of providing the high motor fluidity and smoothness that characterize natural actions. Luria (1973) defines the temporal organization of acts into actions as a "kinetic melody", which requires a close link between the different motor acts forming an action. The system identified in the parietal lobe might thus fulfil exactly this function: motor acts embedded into an action are linked one to the other in order to achieve the final action goal. These links could depend on an underlying organization in intentional neuronal chains in which a neurons coding a given motor act is facilitated by that coding the previously executed one (Fogassi et al., 2005; Rizzolatti et al., 2006; Chersi et al., 2011). A similar model has been recently directly demonstrated at the synaptic level as the neural substrate for the generation of complex sequential behaviour, such as bird singing (Long et al., 2010).

1.2. Perceptuo-cognitive functions of areas of the cortical motor system

For a long time, it has been thought that sensory information flows from the posterior part of the brain, subserving perceptual functions, to the frontal areas, where it is exploited by the prefrontal association cortex for cognitive processing and evaluations. Subsequently, specific motor plans would be activated in the posterior

frontal motor area, leading to the execution of overt actions. Currently, a huge set of evidences stand in contrast with this classical, serial model.

In fact, on one hand, neurons were discovered in the premotor cortex responding not only during motor actions, but also to visual and tactile stimulation (Rizzolatti et al., 1988; Rizzolatti, 1981; Matelli, 1985; Gentilucci et al., 1988). On the other hand, neurons responding to arm and hand voluntary movements were recorded in the parietal areas 5 and 7 of awake behaving monkeys (Mountcastle et al. 1975; Hyvärinen, 1981). Subsequent studies revealed that sensory and motor properties are integrated, at the single neuron level, in a specific way, depending on the anatomical connections of distinct cortical sector. As shown by several neurophysiological and neuroanatomical studies (see Rizzolatti and Luppino 2001), a mosaic of distinct frontal and parietal areas, reciprocally connected, forms a complex set of circuits in which 1) neurons with motor properties are present both in the frontal and the parietal nodes; 2) both parietal and premotor neurons encoding motor acts also receive sensory information, suggesting their involvement in perceptuo-cognitive function well beyond those traditionally assigned to them in the perspective of the perceptuo-motor dichotomy; 3) the parieto-frontal circuits work in parallel, performing different types of sensori-motor transformation for actions, turning sensory information into the format of one's own motor knowledge, thus representing the basic elements of an extended and more complex cortical motor system than previously thought (Rizzolatti and Luppino, 2001; Cisek and Kalaska, 2010).

1.2.1. Visuo-motor neurons

Besides their motor response, a considerable part of F5 neurons also activate during the visual presentation of different types of stimuli, such as three-dimensional objects (canonical neurons, see Murata et al., 1997) and actions performed by other agents (mirror neurons, see Gallese et al., 1996; Rizzolatti et al., 1996).

A class of visuomotor neurons, typically hosted in the sector or area F5 lying in the posterior bank of the inferior arcuate sulcus (area F5p, see Belmalih et al. 2009), respond when the monkey grasps an object with a particular type of grip as well as when it observes an object with size and shape affording the type of grip encoded by the neuron (Murata et al. 1997; Raos et al. 2006).

Raos and co-workers (2006) investigated the motor and visual properties of F5 grasping neurons using a controlled paradigm in which monkeys had to grasp or observe different three dimensional objects. Each object had to be grasped with a specific type of grip. The results showed that neurons can display different preference for grasping an object or a set of objects with specific grip types. Importantly, about half of these neurons also show a visual response to the simple object presentation, even when it is not required a subsequent grasping act. Importantly, the preferred object or set of objects as defined on the basis of their neuronal motor response were the same also on the basis of the visual response. The congruence between the motor and visual discharge is expressed by the fact that the neural representation of a certain type of grip is activated also by the mere sight of an object (or set of objects) that could be grasped by employing that grip. James Gibson called "affordances" the motor possibilities to interact with objects, and the neurophysiological experiments so far reviewed demonstrate that object affordances are encoded in the motor system as potential motor acts to be performed upon an object, on the basis of its visual features.

Recent studies also showed that visuo-motor neurons can, more in general, operate a transformation of visual stimuli into motor programs that the monkey has learned to employ as appropriate responses to those stimuli, even if each stimulus, *per se*, does not afford any specific motor representation. Scherberger and co-workers (Baumann et al. 2009) trained monkeys to grasp the same handle with either a precision grip or a whole hand prehension, depending on the colour of a visually presented cue (a spot of red or green light). They showed that both premotor (Fluet et al. 2010) and, to some extent, even parietal area AIP (Baumann et al. 2009) neurons, can respond during the cue period even if no graspable object is visible, likely because of a learned association between the visual cue and an appropriate grip type.

Area F5 is tightly anatomically connected with area AIP (Luppino et al., 1999; Borra et al., 2008), in which neurons with similar properties were originally described (Sakata et al., 1995; Murata et al., 1996; Murata et al., 2000). These findings prompted a model in which area AIP would receive visual information directly from the temporal lobe. Then, these information are would be fed to area F5, where canonical neurons appear to constitute a crucial node through which the visual description of an object is turned into a motor representation suitable for interacting with it (Jeannerod et al., 1995; Sakata et al., 1997), revealing that visual perception of real object of the outside world is a widespread process, which involves different areas of the cortical motor system.

Mirror neurons and the representation of others' motor acts and actions

Mirror neurons (MNs) are a particular class of visuomotor neurons discovered in the sector of ventral premotor area F5 lying on the postarcuate convexity cortex (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996). Subsequently, neurons with similar properties have been found also in the parietal area PFG (Fogassi et al., 2005; Bonini et al., 2010).

These neurons, similarly to other neurons of these areas lacking mirror properties, discharge when the monkey actively executes motor acts having a specific goal (e.g. grasping). However, they also fire during the observation of the same motor act performed by another monkey (Rizzolatti et al., 1996) or by an experimenter (di Pellegrino et al., 1992; Gallese et al., 1996), but not during the simple visual presentation of the target object or during the observation of motor acts mimicked in the absence of a target. While some MNs also showed a strict congruence between the type of executed and observed motor acts, the majority of them showed a broader congruence, leading to hypothesize that the basic features of an observed act that is matched with its internal motor representation is its motor goal. This suggested that a basic function of these neurons could be that of enabling an automatic and direct form of understanding of the goal of other's acts.

Several studies have been then carried out to directly investigate the hypothesis that MNs encode the goal of motor acts. Umiltà and co-workers (2001) showed that a

subset of MNs become active also when the final part of an observed goal-directed motor act (i.e. the hand-object interaction), which is crucial for triggering the neuron response, occurred behind an opaque screen that prevented the monkey from seeing it. However, the same neurons did not respond to the observation of the same grasping act when it was simply mimicked behind the screen in the absence of any object. Interestingly, from the visual point of view goal-directed and mimicked acts behind the screen were identical, thus MNs can infer what the agent is doing (the goal) even in the absence of a full visual description of the motor act (Umiltà et al., 2001). Other studies (Kohler et al., 2002; Keysers et al., 2003) showed that some MNs of the ventral premotor area F5 can respond to the sound of a motor act, such as breaking a peanut, even in the absence of any visual information, but simply listening to the characteristic sound produced by the action done by another agent. The existence of these audio-visual MNs demonstrates that the individual's motor repertoire can be activated by a multiplicity of sensory inputs, provided that they can evoke the motor representation of the motor act that generated them. Moreover, it has been demonstrated that MNs can code the goal of observed motor acts that do not belong to the monkey's motor repertoire, such as grasping acts performed by a tool (a pliers or a stick) that the monkey has not been trained to use (Ferrari et al., 2005).

More recent studies also showed that, besides the goal of single motor acts, MNs discharge can also reflect action goals at a higher level. Fogassi and co-workers tested MNs activity by mean of a visual task in which the experimenter performed, in front of the monkey, the same grasp-to-eat and grasp-to-place actions that the monkey did during the motor task previously described (see ch. 1.1.3). Most of the recorded MNs, both in the parietal (Fogassi et al., 2005) and premotor cortex (Bonini et al., 2010), were differentially activated depending on whether the observed grasping was followed by bringing to the mouth or by placing the target into a container. Importantly, they showed a high congruence in their visuo-motor selectivity for one of the two actions. For example, MNs neurons that discharged selectively for grasping when the monkey's intention was that of eating (or placing) the grasped food, showed the same selectivity also when the monkey observed the experimenter grasping a piece of food with the intent to eat it (or placing it, respectively). Control experiments ruled out that the type of target per se or other spurious factors, such as movement kinematics, could explain the

observed differences, thus suggesting that MNs can also underlie an automatic form of other's intention understanding.

One of the main problems to tackle when dealing with the mechanism enabling the differential activation of these neuronal pools is how they can be specifically recruited during the observation of an identical grasping act aimed at different final goals. In the experiments so far reviewed there are at least two factors, not explicitly studied by the authors, that may be crucial for neuron selection: the first is the context in which the action is performed (e.g. the presence or absence of the container in which the target has to be placed, or the repeated sequence of identical trials performed in a block), the second is the target of the action (i.e. the use of a metallic solid as target automatically suggests a grasping-to-place action). Probably, these two factors interact with each other or with other elements, but no study has been carried out to specifically address this issue. A very plausible cortical area that could play a role in this selection process based on contextual information is the prefrontal cortex.

1.3 The ventrolateral prefrontal cortex: processing of contextual information for action selection and organization.

"Grasping an apple which is on the table": this is one of the many common actions we may execute during our everyday life.

I could grasp an apple for eating or putting it inside a basket: these are two potential goals of my action. But, what can drive me toward an action goal rather than toward another one? How can I decide if grasping to eat or to put the apple inside a basket? If I am very hungry I can choose to eat the apple after seeing it; instead, if I have a stomach-ache, I can decide to put the apple in the basket. I can also choose not to eat the apple because it is dented. In the first two situations an internal state is driving my behaviour, while in the last it is the value of an external factor. Also, the presence or not of a basket is a factor that could elicit an action rather than another. However, I can decide not to grasp that apple because I don't like this fruit or because I am in the kitchen of a person with which I have not much confidence and, even if I'm hungry, I don't grasp the apple for not being impolite.

Action selection and organization are very complex processes that need to exploit contextual information and the retrieval of previously memorized information, as well as the integration of these different types of data.

According to what has it been written so far, areas F5 and PFG provide a different contribution to action goal coding. Very likely, area PFG plays a more important role in organizing motor acts into actions, while F5 appears to be more involved in coding the goal of the single motor acts in a more abstract fashion (Rizzolatti et al., 1988; Umiltà et al., 2008). Both these areas are anatomically connected with sectors of prefrontal cortex (PFC).

Thus, on the basis of the anatomical connections of the hand fields of areas F5 and PFG with prefrontal cortex and on the basis of the data reported in the literature on the functional properties of this area, it seemed very appropriate to record from ventrolateral prefrontal cortex (VLPF) of the macaque monkey, using an experimental paradigm similar to the above described one (Fogassi et al., 2005; Bonini et al., 2010).

1.3.1. Anatomical connections of the ventrolateral prefrontal cortex

The most anterior region of cerebral cortex of the mammalian brain is commonly called the prefrontal cortex. The prefrontal cortex has increased its size along phylogenetic development, as shown by the studies on existent animals' brains, as well as from paleoneurological data (Papez, 1929). In particular, its expansion is most evident in the primate order, where the cortical sector named by Brodmann (1909; 1912) "region frontalis" (which approximately corresponds to what is nowadays called prefrontal cortex) constitutes 29% of the total extension of the cortex in the human brain, 17% in the chimpanzee, 11.5% in the macaque, 7% in the dog and 3,5% in the cat (Brodmann, 1912).

Traditionally, the prefrontal cortex of primates has been classified into at least three major subdivisions: lateral, medial, and ventral (orbitofrontal). In the monkey, the prefrontal cortex is bordered by the inferior arcuate sulcus, caudally and ventrally, by the cingulate sulcus, medially, and it is crossed for the whole lateral surface by the

principal sulcus, which remains the best anatomical landmark in macaque monkey (Fuster, 2008).

 The macaque prefrontal cortex has been subject to many anatomical studies that proposed anatomical subdivisions not completely corresponding one with each other (i.e. Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991; Gerbella et al., 2007; Petrides and Pandya, 1999). Moreover, the areas of major interest for this study largely correspond to areas 45, 12 and ventral part of area 46 as defined in a basic anatomic study by Walker (1940).

Cortical connections

The prefrontal cortex is among the cortical regions with the highest number of connections with other cortical area and subcortical centers, implicated in sensory and motor processing. From a general point of view, the dorsal and ventral sectors of the LPFC can be considered as part of two distinct networks: the dorsal sector is part of a mediodorsal network originating from the periallocortex in the medial PFC, and the lateral sector is part of an orbitoventral network originating from the periallocortex in the orbital PFC (Tanji and Hoshi, 2008).

The dorsolateral prefrontal cortex receives from multimodal temporal areas a large set of inputs which, in part, have already been processed and integrated, while the ventromedial portion receives lower levels sensory inputs (including visual, auditory, somatosensory, gustatory, and olfactory inputs) for a first integration. However, the two portions are widely interconnected and work in parallel integrating different sets of information in a larger pattern and more abstract way.

Generally, the prefrontal cortex does not receive directly from primary sensory cortices, but only from secondary cortices, as described by Jones and Powell (1970): each primary sensory cortex (somatosensory, visual and auditory) projects to the correspondent associative cortex (parietal, occipital, temporal), which in turn project to a very specific prefrontal area (Barbas and Mesulam, 1985; Ungerleider et al., 1989). As in the case of other sensory inputs, olfactory and gustatory inputs also reach the prefrontal cortex. Nevertheless, the cortical connections to the prefrontal do not

converge all on identical locations but show a degree of differential distribution: the lateral prefrontal cortex is the target of visual, auditory and somatic afferents whereas the orbitomedial prefrontal cortex is mainly the target of olfactory and gustatory afferents (Prince, 1993).

The most important cortical outputs of the lateral prefrontal cortex are directed to the motor areas. Connections with premotor cortex differ between the dorsal and ventral parts of the lateral prefrontal cortex. The dorsal part is linked with the dorsal premotor cortex, medial to the spur of the arcuate sulcus, whereas the ventral part is linked with the ventral premotor cortex, lateral to the spur (Barbas and Pandya, 1987; Charmichael and Price, 1995; Luppino et al; 2003). Instead, both dorsal and ventral parts of the lateral prefrontal cortex are linked with the rostral cingulate motor area (area 24c), presupplementary motor area and supplementary eye field (Charmichael and Price, 1995; Luppino et al., 1993). In light of these connections, the PFC is regarded as crucial in cognitive control of motor behavior (Mishkin and Manning, 1978; Lu et al., 1994, Takada et al., 2004; Hoshi, 2006).

Subcortical efferents and afferents

As a general rule, the prefrontal cortex sends fibers to almost all of the structures from which it receives (Clark, 1932; Goldman, 1979; Siwek and Pandya, 1991). Concerning subcortical efferents, either direct or mediated by motor and premotor areas, the prefrontal cortex sends to the basal ganglia and to the cerebellum for motor control. In particular, the conspicuous efferents that depart from the prefrontal cortex to the basal ganglia, especially to the caudate nucleus and the putamen, seem to be organized in a topographic manner (Kemp and Powell, 1970; Schultz, 2006). The dorsal sector sends preferentially to the dorsal part of the caudate nucleus, while the ventral sector to its ventral one. The central part instead receives from both of the caudate subdivisions (Yeterian and Pandya, 1991). In general, there seem to be different sub-circuits through which the lateral prefrontal cortex sends to the different nuclei of the basal ganglia, and each of them seems to play a different role in motor control (Tanji and Hoshi, 2008). Concerning subcortical efferents the lateral prefrontal cortex receives, directly or

relayed by thalamic nuclei, input from the basal ganglia, cerebellum and numerous limbic structures, including the hippocampus and the amygdala (Goldman-Rakic e Porrino, 1985; Barbas et al., 1991; Ray and Price,1993)

This organization places the prefrontal cortex in a unique position and highlights its critical role in processing and integrating multimodal information. Furthemore, the lateral prefrontal cortex through its connections with premotor areas, the basal ganglia, and the cerebellum can control broad aspects of motor behavior. Specifically, the lateral prefrontal cortex, by means of its connectivity, modulates the flow of information in other areas of the central nervous system, depending on the behavioral requirements, providing a resource for adaptive control of information flow through cortical and subcortical structures (Miller, 2000).

1.3.2. Anatomical organization of the ventrolateral prefrontal areas

The ventrolateral prefrontal areas of major interest for this study largely corresponds to area 45 and to the ventral part of area 46 (area 46v) as defined by Walker (1940) and to the area 12 sector rostral to area 45 (area 12r) as defined by Carmichael and Price (1994) and Gerbella et al., (2007).

Area 45 was defined by Petrides and Pandya (1994, 2002) as the caudal ventrolateral prefrontal sector formed by two slightly different architectonic subdivisions: a caudal one, 45B, lying ventrally in the prearcuate bank and a rostral one, 45A, extending on the rostrally adjacent inferior frontal convexity (Gerbella et al., 2010). Petrides and Pandya (2002, 2009) showed that area 45, as a whole, is connected to several cortical areas, including auditory-related and multisensory areas of the superior temporal gyrus and caudal areas of the inferior parietal lobule. However, recent evidence showed that 45A and 45B are two distinct cortical entities each with its own connectivity patterns clearly, suggesting a different involvement of these areas in non-spatial information processing: area 45A corresponds to the prefrontal sector for which a role in communication behavior was proposed; whereas area 45B is a distinct prearcuate area, possibly affiliated with the oculomotor frontal system (Gerbella et al., 2007, 2010).

Area 12r lies in the ventrolateral prefrontal cortex, ventrally to area 46 and rostrally to area 45A (Carmichael and Price, 1994; Gerbella et al., 2007). This area is the target of projections from higher order visual areas of the inferotemporal cortex (Webster et al., 1994) and hosts visual neurons tuned specifically to the identity or features of objects (Wilson et al., 1993). Furthermore, lesion studies have highlighted on an involvement of this area in behavioral tasks in which information on object identity plays a key role (Passingham, 1975; Mishkin and Manning, 1978; Wang et al., 2000). Together, these data have suggested a role for this prefrontal sector in higher-order aspects of nonspatial information processing (Levy and Goldman-Rakic, 2000; Passingham et al., 2000; Tanji and Hoshi, 2008). Moreover, recent direct (Borra et., 2012) and indirect data (Borra et al., 2008; Gerbella et al., 2011), showing that the intermediate part of this area is connected to the anterior intraparietal area (AIP) and the anterior subdivision of the ventral premotor area F5 (F5a), have suggested a role, not previously hypothesized, for the intermediate 12r sector in the neural mechanisms for selecting and controlling hand actions.

Area 46v occupies almost the entire rostrocaudal extent of the ventral bank of the principal sulcus and the immediately adjacent convexity cortex. Area 46v hosts neurons involved in action selection, learning processes and the exploitation of behavioral rules for the execution of goal directed actions (Miller and Cohen 2001; Tanji and Hoshi 2008). The well-known rich connections of area 46v with the inferior parietal lobule (IPL) and the parietal operculum, on one side, and with the ventral premotor (PMv) and prearcuate oculomotor areas, on the other, allow to consider this area the neural substrate for functional properties described above (Tanji and Hoshi 2008). Recent direct (Gerbella et al., 2012) and indirect data (Rozzi et al., 2006; Borra et al., 2008; Gerbella et al., 2011) showed that area 46v is connectionally heterogeneous and specifically that its half caudal part (46vc) mostly displayed intraprefrontal connectivity with ventrolateral areas and robust connectivity with frontal and parietal sensorimotor areas. Based on a topographic organization of these connections, 3 fields were identified in area 46vc. A caudal field (caudal 46vc) was preferentially connected to oculomotor prearcuate (8/FEF, 45B, and 8r) and inferior parietal areas. The other 2 fields, located more rostrally, in the bank of the principal sulcus (rostral 46vc/bank) and on the ventrolateral convexity cortex (rostral 46vc/convexity), respectively, were

connected with hand/mouth-related (F5a, 44) ventral premotor areas, area SII, and the insula. However, rostral 46vc/convexity was also connected to the hand-related area AIP, whereas rostral 46vc/bank to hand/arm-related areas PFG and PG, to PGop, and to areas 11 and 24. The present data suggest a differential involvement of different parts of area 46vc in higher level integration for oculomotor behavior and goal-directed arm, hand, and mouth actions.

1.3.3. Functional properties of ventrolateral prefrontal neurons

The LPFC includes at least three different functional domains: one located in correspondence of the anterior bank of the arcuate sulcus and involved in oculomotor functions (Bruce and Goldberg 1985), a second one close and within the PS, involved in visuo-spatial information processing (Levy and Goldman-Rakic 1999), and a third one located in the region of the inferior prefrontal convexity and involved in high-order processing of non-spatial information (Levy and Goldman-Rakic, 1999; Passingham et al., 2000; Romanski, 2004).

Several lesion and electrophysiological studies have shown that the LPFC is involved in many aspects of behavior, as diverse as sensory, motor, visceral/emotional and executive processes (Jacobsen, 1935; Fuster, 2008). Toward the end of the 20th century, the primary research interests concerning LPFC were the processing of information stored in short-term memory (Buddeley, 1986; Goldman-Rakic, 1987) and attentional function (Desimone and Duncan, 1995). A major debate regarding this cortical area has been whether it should be considered functionally unitary or heterogeneous. This dispute has been characterized by two alternative points of view: the domain-specific (Goldman-Rakic, 1988) versus the process-specific (Petrides, 1995) hypothesis. The first model is partially based on the dichotomy originally proposed for the processing of visual information between a ventral streams, carrying object-centered visual information conveyed to temporal lobe structures, and a dorsal stream, conveying spatial information to the posterior parietal cortex (Ungerleider and Haxby, 1994). Goldman-Rakic (1988), based on connectional data, proposed that spatial processing takes place in DLPF, while feature processing is performed by VLPF. This hypothesis

was initially supported by reports of functional studies in the monkeys by Wilson and co-workers (1993). In fact, they showed that distinct primate prefrontal cortex neurons can provide specific encoding of either stimulus identity or stimulus location, and the different neuronal population are anatomically segregated one from the other, suggesting that PFC contains separate processing mechanisms for remembering "what" and "where" an object is.

However, the results of subsequent functional studies stand in contrast with this view. Rao et al. (1997) examined neurons of the LPFC in monkeys during a task that required processing of variables concerning both "what" and "where". Object- (what) or location- (where) tuned neurons were widely distributed in both the ventral and dorsal part of the LPFC. Furthermore, over half of the prefrontal cortex neurons with delay activity showed both what and where tuning, suggesting the need of a single neuron integration of object and spatial information to guide behavior. On the other hand, based on a series of lesion studies in monkeys (Petrides, 1991) and of brain imaging studies in humans (Petrides et al., 1993), Petrides (1995) proposed that the dorsal part of LPFC is involved in the monitoring and manipulation of retrieved information for planning and execution of behavior. The ventral part of the LPFC, in contrast, was proposed to be involved in active encoding and retrieval of specific information held in visual, auditory, and somatosensory association areas, allowing selection, comparison, and decision processes based on such information (Tanji and Hoshi, 2008).

There is sufficient evidence to reveal functional heterogeneity within the LPFC. As long as behavioral tasks do not require much information processing, neuronal activity may primarily represent the information received from sensory signals (Tanji and Hoshi, 2008). In the 1960s, a great number of investigations provided electrophysiological evidence for a role in the integration of sensorial input (Fuster, 2008). Neurons of monkey prefrontal cortex react to visual, auditory, somatic, olfactory and gustatory stimuli according to anatomical connections.

Tanila and co-workers (1993) carried out an experiment to verify whether the distribution of functional properties in the Walker's area 9 and 46 (DLPF) corresponded to the anatomical parcellation proposed by Barbas and Pandya (1989), that made a distinction between basoventral and mediodorsal trends in the prefrontal cortex on the basis of slight differences in laminar organization and intrinsic connections. In

particular, basoventral prefrontal regions receive visual projections mainly from inferotemporal cortex implicated in pattern recognition, whereas the mediodorsal regions receive most of their visual input from parietal areas associated with visuo-spatial functions. The authors systematically mapped the representations of visual, auditory, somatosensory, somatomotor and oculomotor functions on the dorsolateral cortex. The neurons were functionally classified according to their responsiveness to visual, auditory and somatosensory stimulation, and to correlation of their activity with spontaneous eyes or limbs movements. Furthermore, the authors in the same study compared the distribution of different functions showed by recorded neurons with modality-specific anatomical connections to various sectors of this area, showing a good correspondence between the functional and connectional maps. From their results, it emerged that half of the recorded neurons responded to visual stimulation, either alone or in combination with other stimulus modalities. Visually responsive neurons were broadly distributed throughout the DLPF, well in agreement with the extensive connections between this cortical area and the visual ones. Of all visually responsive neurons, less than half responded preferably to moving stimuli and were located more dorsally and caudally than the rest of the visually responsive neurons. Somatosensory and motor neurons were located more ventrally, but no clear somatotopy could be described. Oculomotor neurons were found caudally, in both the superior and inferior banks of principal sulcus and in a narrow band on the dorsal convexity, corresponding with the projections fields of posterior parietal areas (Tanila et al., 1993).

Besides these evidences of a somehow "basic" encoding of sensory and motor properties, many studies support an integrative role of stimuli processing in the prefrontal cortex rather than a simple reflection of sensory information per se. In particular, the VLPF, given its connections with the temporal lobe, can use information about the visual and auditory context to generate more complex behaviors, as rule encoding, categorization or action selection.

Hoshi et al. (1998) carried out an experiment to study movement-related neuronal activity in the DLPF (more specifically, dorsal and ventral convexity of principal sulcus) from the perspective of a general role for the prefrontal cortex in controlling motor behavior to achieve a specific goal according to the requirements of a given task. Monkeys were trained to perform two delayed motor task depending on a

choice: if the choice cue was a combination of a triangle and a circle, monkey had to reach and press an object with the same shape as the sample cue (shape matching task); if the choice cue was a combination of three triangles or three circles (after a triangle or circle sample cue, respectively), monkey had to reach and press an object with the same location as the sample cue (location matching task). Many of the recorded neurons were selectively active whether the reaching target was a circle or a triangle; while activity of many other recorded neurons depended on type of task required (matching the shape or the location). These data suggest that activity of neurons prefrontal cortex reflects task rule, not movement per se.

It is generally assumed that the lateral part of PFC reacts to external signals that dictate forthcoming actions (Goldman-Rakic, 1987; Fuster, 1997; Tanji and Hoshi, 2001). Neurons in the prefrontal cortex show short-lived changes in activity in response to instructional cues, followed by long-lasting activity that persists throughout instructed delay period that precedes the start of a predetermined behavior. It has been established that activity during an instructed delay period reflects both the sensory information contained in the instruction cue (Fuster and Alexander 1971; Rao et al., 1997; Constantinidis et al., 2001) and the properties of behavioral responses that are planned in accordance with an instruction (Quintana and Fuster, 1999). However, it is not known to what extent such activity represents the motor attributes of prepared responses or cognitive processes that reflect planning during the instructed delay period or reward expectation or other cognitive processes. Saito and co-workers (2005) constructed a spatial maze task to induce monkeys to plan multiple behavioral goals to be attained in a temporal sequence. During a preparatory delay period, after monkey received an instruction signal and was waiting for a go signal, neurons showed two types of activity. The authors identified these two types of activity with neuronal correlates that represent immediate and final behavioral goals, suggesting that LPFC is implicated in governing goal-oriented sequential behavior rather than sensorimotor transformation (Saito, 2005).

In another experiment, Saga and co-workers (2011) investigated the role of the LPFC in transforming external signals of multiple sensory modalities into information suitable for monitoring successive events across behavioral phases until an intended action is prompted and started. The authors trained monkeys to receive a succession of

visual, auditory or tactile sensory signals, separated by variable intervals, and then to release a key as soon as the last (fourth) signal appeared. Thus, animals had to monitor and update information about the progress of the task upon receiving each signal preceding the key release in response to the fourth signal. They found that the initial, short latency responses of recorded neurons reflected mainly the sensory modality rather than the phase or progress of the task. However, a task phase-selective response developed within 500ms of signal reception and information about the task phase was maintained throughout the presentation of successive cues. The task phase-selective activity was updated with the appearance of each cue until the planned action was initiated. The phase-selective activity of individual neurons reflected not merely a particular phase of the task but also multiple successive phases. Furthermore, they found combined representations of task phase and sensory modality in the activity of individual neurons, suggesting how information representing multiple phases of behavioral events develops in the lateral prefrontal cortex to provide a basis for the temporal regulation of behavior.

Finally, the lateral prefrontal cortex is involved in goal-directed behavior. Yamagata et al. (2012) explored the role of DLPF and VLPF and compared it whit that of dorsal premotor cortex, which is involved in goal-directed behavior. They explored four aspects of information processing: encoding of visual signals, behavioral goal retrieval, action specification and maintenance of relevant information (Yamagata et al., 2012). The authors initially presented to monkeys a visual object (instruction cue) to indicate a behavioral goal (reaching to the right or left of a potential targets); after a subsequent delay, a choice cue appeared at one of six different locations on the screen. At this point, the animal could specify what to do (i.e., action) for the first time (Yamagata et al., 2012) and after the go signal monkey had to reach for the target with the right arm touching the correct square on the screen. They found that VLPF neurons amply encoded object features of the instruction cues for behavioral goal retrieval and, subsequently, spatial locations of the choice cues for specifying the actions. By contrast, DLPF neurons and dorsal premotor neurons rarely encoded the object features, but reflected the behavioral goals throughout the delay period. After the appearance of the choice cues, the dorsal premotor neurons held information for action throughout the preparation of reaching movements. Lateral prefrontal neurons represented information

for the behavioral goal continuously, even after the action specification as well as during its execution.

All these findings makes the VLPF an attractive area in which contextual information could be turned into specific motor intentions subserving the selection and organization of the parieto-premotor neuronal pools underling the execution of natural goal directed actions.

1.3.4. Aims of the study

A crucial aspect in the organization of intentional actions is the chaining of single motor acts into well organized motor sequences. A first pioneering experiment by Fogassi and co-workers (2005) have demonstrated that inferior parietal neurons can code grasping motor acts differently depending on the global action (grasp-to-eat or grasp-to-place) in which they are embedded, and similar findings have been reported by neurophysiological investigations carried out on the ventral premotor cortex (Bonini et al., 2010). More recent experiments have shown that, particularly in the inferior parietal lobule, grasping neurons discharge can reflect the final goal of the action at higher level of abstraction (Bonini et al., 2012), thus suggesting the possible presence of a neural mechanism capable of integrating contextual information used by the monkey to decide which action to perform. Functional results and anatomical connections between the cortical areas investigated in the studies above described suggest that cortico-cortical projections of different cortical areas can play a crucial role in the selection of inferior parietal and ventral premotor neuronal pools for the organization of intentional actions.

The ventrolateral prefrontal cortex appears one of the most plausible candidates to fulfill this function. In fact, several studies evidenced that the ventrolateral prefrontal cortex play a role in arranging motor chunks - such as pushing, pulling or turning – into over-trained motor sequences based on learned sensory instructions (Hoshi et al., 1998, 2000; White and Wise, 1999; Wise and Murray, 2000; Miller and Cohen, 2001; Shima et al., 2007; Tanji and Hohi, 2008). However, almost all tasks described in these studies rely on cognitive capacities that do not appear to have an immediate correspondence among the relatively simple primates' behavior in the wild. In contrast, a few studies

investigated the role of the prefrontal cortex adopting a more naturalistic approach (Tanila et al, 1993; Rozzi et al., 2011), but leaving unresolved the issue of what could be the contribution of this cortical sector to the organization of natural actions.

To this purpose, we carried out an electrophysiological study on this region using a controlled behavioral paradigm in order to clarify the possible contribution of ventrolateral prefrontal neurons to the integration of contextual information for the selection and generation of natural actions.

2. MATERIALS AND METHODS

The study was carried out on one macaque monkey (*Macaca mulatta*).

Before recordings, each monkey was habituated to comfortably sit in a primate chair, to interact with the experimenters and to become familiarized with the experimental setup. Then, a first surgery was performed in order to implant the head fixation system (Crist Instrument). The monkey was subsequently trained to perform the motor task described below using the hand contralateral to the hemisphere to be recorded (left). At the end of training, a second surgery was performed in order to implant a plastic square recording chamber (18x18mm, AlphaOmega Engineering, Nazareth, Israel), based on the stereotaxic coordinates of the cortical regions to be recorded derived from previously obtained functional magnetic resonance images of the monkey's brain.

All surgeries were performed under general anaesthesia (ketamine hydrocloride, 5 mg/Kg i.m. and medetomidine hydrocloride, 0.1 mg/Kg i.m.). Dexamethasone, prophylactic broad-spectrum antibiotics as well as appropriate pain medications were administered intra- and postoperatively (Borra et al. 2010; Bonini et al. 2010). All the experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma and complied with the European law on the humane care and use of laboratory animals.

2.1. Behavioral tasks and apparatus

The monkey was trained to perform a Visuo-Motor Task (VMT) involving simple action sequences (see Figure 1) similar to those previously employed for other experiments in different cortical areas (see Fogassi et al., 2005; Bonini et al., 2010). However, the experimental design and apparatus here employed enabled to more carefully manipulate and control the contextual elements crucial for action selection and decision. In addition, monkey was also trained to perform an Observation Task (OT) in which the experimenter performed the same VMT in front of the monkey.

The experiment was performed in a completely dark environment. The monkey sat in front of a black Plexiglas box, divided into two sectors by a half mirror. The lower
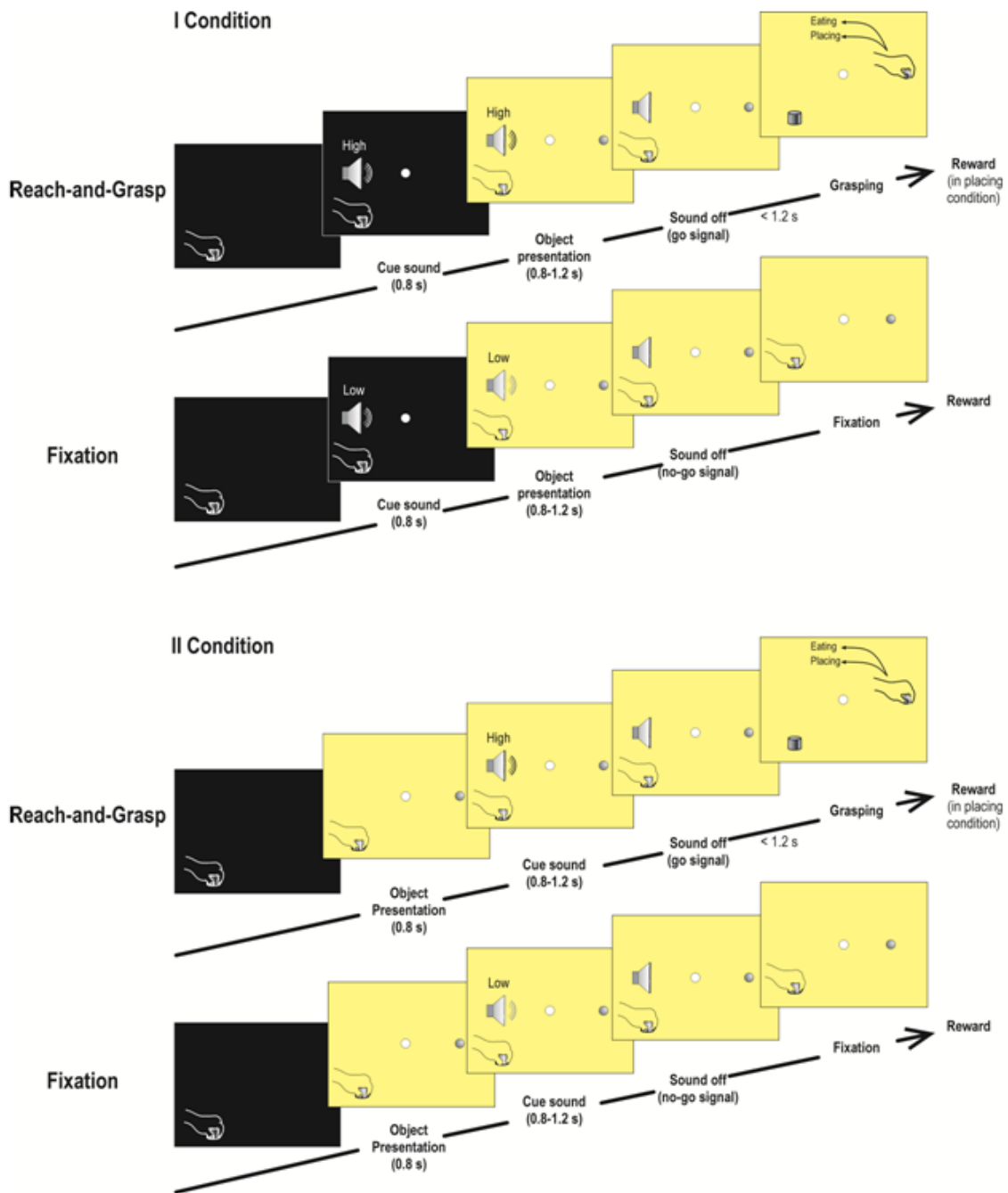
Figure1. Behavioral paradigm. During Visuo-Motor Task, the monkey, in the dark, fixed a bright spot. A sound instructs the monkey if she has to grasp (high sound) or simply fixate (low sound) a pellet or a plastic sphere. After 800 ms, one of the two objects is presented. At the end of the sound, the monkey must grasp to eat or grasp to place or simply fixate the pellets or plastic sphere, on the basis of the presented sound. Furthermore, behavioral paradigm was constituted by other two conditions with the order of presentation of the target and the sound was inverted, namely, with target presentation first and the cue sound second.

sector contained a plastic container endowed with a groove. The bottom of the groove was closed with a computer-controlled trap-door with a cavity in the middle, in which a

small sphere (target) of 6 mm of diameter could be placed, so that the center of mass of the sphere was at exactly 11 cm from the lower surface of the half mirror. On the upper sector of the box, a white LED was fixed at a distance of 15 cm from the upper surface of the half mirror, so that when the LED was turned on in complete darkness it was seen by the monkey as a spot of light located exactly in the position of the sphere, although this latter remained concealed. The container for the target was positioned along the monkey body midline, at 16 cm from its hand starting position. The hand starting position was located close to the monkey body and was constituted by a metal cylinder of 3 cm of diameter and 2.5 cm of height from the floor of the box. A further cylindrical container (inner diameter 4 cm) was located in between the hand starting position and the target: it had a funnel-shaped bottom, so that when small plastic spheres were placed into it they immediately felt down and could be gathered in a box unreachable by the monkey. On the back wall of the box, in the upper right quadrant of the monkey visual field and at 45 cm from the monkey face, a black Plexiglas plane for the OT was located. The plane was separated from the inside of the box by an half-mirror, which enabled the monkey to see what happened outside only when a light was turned on. On the right of the plane, a small metal plate was used as experimenter's starting position. In front of it, a plastic cylinder with a metal surface on its top was located. The target (either a grey metal object of 1 x 1 x 1 cm or a peanut) was placed on the cylinder during the inter-trial period, in complete darkness. In between the experimenter's hand starting position and the target a small white LED was located, to be used as a fixation point for the monkey during the OT trials.

Visuo-Motor Task (VMT)

Before each trial of the VMT, the experimenter positioned the target into the container. The target could be either a small ochre food pellet (sphere of 6 mm of diameter, weight 19 mg) or a white plastic sphere (of the same weight and diameter of the food pellet). The monkey could easily discriminate them. The target was positioned into the groove in complete darkness, and a constant white noise prevented the monkey from using any kind of acoustic cue on the type of target of the subsequent trial during set preparation.

The task included two basic conditions: reaching-and-grasping and fixation. Each reaching-and-grasping trial started in complete darkness when the monkey had its hand on the starting position. The white LED on the upper sector was switched on (Fig 1 – fixation point) and the monkey had to engage fixation (in a 3.5° x 3.5° space window) within 1.5 s after the presentation of the fixation point. After a variable time lag (from 600 ms to 1000 ms) from the onset of fixation, a cue sound (a sine-wave tone of 1200 Hz) was presented (Fig 1 – cue sound). The sound lasted from 800 to 1200 ms, and for its entire duration the monkey had to maintain fixation and to keep its hand still on the starting position. After 800 ms from cue sound onset, the light was switched on in the lower sector of the box (Fig 1 - target presentation), revealing which would have been the target of the ongoing trial. When the sound stopped (Fig 1 – Go signal), the monkey had to reach and grasp the target in light within 1.2s from the end of the sound. When the target was the food pellet, it had to bring it to the mouth and eat it, while when the target was the plastic sphere it had to place it into the container located in between the target and the hand starting position (Fig 1 – target grasping). After correctly performing reaching-and-grasping trials with food as target the monkey was self-rewarded by eating the food pellet (eating trials), while after correctly performing reaching-and-grasping trials with the plastic sphere as target (placing trials) the monkey was rewarded with an identical food pellet, automatically delivered into the monkey's mouth by a computer controlled pellet dispenser activated by the contact of monkey's hand with the border of the container. The pellet reward contacted the monkey's mouth 500ms after its delivery.

The temporal structure and events of fixation trials (Fig 1) were the same as those of reaching-and-grasping trials, but the cue sound was a low tone (sine wave tone of 300Hz). After target presentation, when the cue sound stopped, the monkey had to maintain fixation on the target for 1.2 s and to remain still with the hand on the starting position, without performing any movement. At the end of this period, all correct trials were automatically rewarded as reaching-and-grasping placing trials.

Each neuron was recorded for a total of 96 trials of this task, 48 reaching-and-grasping and 48 fixation trials, half of them (N=24) with the food pellet as target, the other half with the sphere. Furthermore, half of the trials for each condition and with each target type (N=12) were recorded by inverting the order of presentation of the

target and the sound, namely, with target presentation first and the cue sound after 800 ms. All the trials of VMT were run based on a trial list organized in a random order. After the presentation of all the trials of the list, all the wrong trials were re-presented until the list was completed and the desired number of trials acquired. All the trials in which the monkey broke fixation, made an incorrect movement or did not respect the time intervals of the task stages were aborted. In all these cases the trap-door below the target was automatically opened so that the target felt down and was unreachable by the monkey. All the wrong trials were not rewarded.

Observation Task (OT)

The OT was run in a separate block of 96 randomized trials (48 reaching-and-grasping and 48 fixation trials), with exactly the same conditions as those used in the VMT. In this task, each trial started, in complete darkness, when both the monkey and the experimenter had their hand on the respective starting position. As in the VMT, the fixation LED located in between the experimenter's hand starting position and the target switched on, and the monkey had 1.5s to engage fixation in a 3,5° x 3,5° space window centered on the spot. The temporal structure and events following the beginning of each trials were exactly the same as in the VMT but, in this case, the sounds constituted behaviorally relevant cues for the experimenter, since the monkey was required to remain still with its hand on the starting position for the entire duration of all trials and to maintain fixation until the reward (a food pellet identical to those used in the VMT) was automatically delivered at the end of the experimenter's reaching-and-grasping or fixation trials.

In all cases in which the monkey broke fixation or made an incorrect movement the trial was aborted and the reward was not delivered.

2.2. Recording techniques

Recording of the behavioral events

Contact detecting electric circuits (Crist Instruments) were employed to signal the main behavioral events, in order to monitor online the monkey performance and to subsequently align the neuronal activity for statistical analysis. The recorded events were: a) detachment of the monkey/experimenter's hand from the starting point; b) contact of the monkey/experimenter's hand with the target (food or object) of the action; c) contact of the hand with the rim of the container in which the object had to be placed.

A LabView based software enabled to control the behavioral paradigm and generate additional signals provided to the acquisition system in parallel with neuronal activity. In particular, dedicated digital lines were used to identify a) the switch on/off of the fixation LED, b) the switch on/off of the ambient light, the onset/offset of the c) high and d) low cue sound, e) the reward delivery and f) the moment when some error (resulting in trial abortion) occurred.

Eye position was monitored by mean of a 50Hz CCD video camera and two spots of infrared light projected on the monkey eye. The eye signal was processed through a dedicated software (Pupil) and fed to the LabView software to be monitored and acquired, in order to relate it with the other behavioral events of the paradigm.

Recording of neuronal data

Part of neuronal recording was carried out by mean of a 8 channels AlphaLab system (Alpha Omega, Nazareth, Israel), part with a 16 channels Omniplex system (Plexon, Dallas, Texas).

The whole study has been performed using linear multielectrode arrays inserted through the intact dura: 8 channels U-Probe (Plexon) and 16 channels Neuroprobe (Atlas NeuroEngineering), both with electrodes impedance ranging from 0.5 to 1.5MΩ measured at 1 kHz.

Probes were inserted by mean of an X-Y manipulator attached to the recording chamber. However, while U-Probes were inserted through guiding tube gently pressing the dura, Neuroprobes were inserted exploiting a vacuum system. This system, when turned on, allowed to keep the probe attached to a bar held by a mechanical micromanipulator until it penetrated inside the cortex and reached the requested depth. Then it was switched off in order to leave the inserted probe floating in the brain. This solution, by canceling the effect of brain pulsation, provided an optimal stability of the signal over several hours of recording. Furthermore, the possibility to avoid the use of a guiding tube, which exerts a certain pressure on the neural tissue (causing possible anoxic reduction of neural activity), enabled to obtain optimal physiological conditions for the recording and the highest quality of activity. Both probes had the further advantage of enabling to record multi- and single-unit activity during the phase of lowering into the cortex, enabling to easily and precisely locate all electrodes at the desired depth. The most proximal (more superficial) electrode (when recording with the Omniplex system) was usually left subdural, just outside the cortex, in order to use it as a reference for the remaining electrodes: this solution was extremely efficient in eliminating artifacts and reducing the noise.

The Omniplex system enabled to perform spike detection and sorting on-line, while this was not possible with the AlphaLab system. However, all the collected raw data (sampled at 22 kHz with Alphalab and 44 kHz with Omniplex) were high-pass filtered at 300Hz and then sorted off-line by mean of commercial spike sorting software (Plexon). Spike detection was performed by applying a negative threshold corresponding to 3 standard deviations from the mean peak height. Sorted spike shapes associated to distinct single units were prepared for statistical analyses using Neuroexplorer (Plexon).

2.3. Data analyses

Neuronal activity was acquired from 0.5 seconds before the switching on of the fixation point to 0.5 seconds after trial completion in both RG and F trials of the VMT and OT. In case of error during a trial, the recording was interrupted.

A first, preliminary analysis was performed in order to identify neurons significantly modulated during the task. To this purpose, we divided the entire acquisition period of correct trials into 200 ms bins (17 bins in total) centered around the main event of interest, as follows: 1st cue (4 bins; two before and two after cue onset), 2nd cue (4 bins; two before and two after cue onset), the onset of Go signal or No-Go signal (3 bins; one before, one during and one after signal onset), the onset of the movement (3 bins; one before, one during and one after movement onset), the onset of the reward (3 bins ranging from starting 200 ms after the onset of reward). Data were analyzed by mean of 1 way repeated measures ANOVA applied to each condition of the VMT and OT, using a significance criterion of $p < 0.001$. The null hypothesis was that a neuron does not vary its spontaneous activity during different task stages in any of the experimental conditions. Therefore, all neurons showing a significant main effect in this analysis in at least one condition of the VMT or the OT were considered as task-related.

Task related neurons were then included in 3 different analyses.

Reach-and-grasp responses

In order to verify the possible presence of movement-related responses (different activity during specific phases of RG trials compared with correspondent phases of F trials), we first defined two epochs of interest: 1) a 500 ms epoch before the go/no-go signal for both RG and F condition, and 2) a subsequent epoch of 500 ms after the no-go signal (in F condition) and a correspondent one (of variable duration) ranging from 100ms before movement onset to 100 ms after hand-target contact (in RG condition). Firing rate (spk/s) was normalized with respect to time, to avoid problems concerning differences in epoch duration. A 2x2x2 repeated measures ANOVA (factors: Object, Condition, Epoch) was applied. We considered as motor-related those neurons showing a significant main effect ($p < 0.01$) of the factor Condition and/or an interaction of the factor Condition with Epoch (followed by Bonferroni post-hoc tests). Possible selectivity for the factor Object was considered as motorically relavant only if it was associated (or in interaction) with, at least, the factor Condition.

The same analysis was applied to the OT, in order to establish the presence of possible responses to the observation of the task performed by the experimenter.

Responses related to reward delivery/mouth movements

In order to verify the possible presence of responses related to reward or mouth movements, we defined three epochs of interest in F trials of the VMT and the OT (analyzed separately): 1) a 500 ms epoch before reward delivery, 2) a 500 ms epoch from reward delivery to monkey reward acquisition, and 3) a 500 ms epoch corresponding to the phase of monkey reward acquisition. Neurons activity was analyzed by mean of a one-way repeated measures ANOVA ($p<0.01$).

Responses related to the presentation of sensory stimuli

In order to verify the possible presence of responses related to the presentation of sensory stimuli (cue sounds and target objects) we defined two basic epochs of interest: 1) a 350 ms epoch before stimulus onset and 2) a 350 ms epoch ranging from 50 ms to 450 ms after stimulus onset. We employed a 2x2x2 repeated measures ANOVA (factors: Object, Condition and Epoch) for each of the stimuli (ambient light onset and sound onset).

Population analysis

Population analyses have been carried out taking into account single neurons response expressed in terms of normalized activity, calculate as follows. The highest activity value among those of the compared conditions was taken to divide the value of each single bin (normalized activity): using this procedure each neuron is characterized by a mean baseline activity equal to 0 and a peak activity value of 1.

Population of reach-and-grasp responses was aligned on the onset of the movement considering an epoch of 500 milliseconds. Thus, the behavioural event of the monkey grasping was included. Population of responses related to the presentation of sensory stimuli was aligned on the onset of cue stimuli presentation, considering an epoch of 350 milliseconds.

In order to better identify a possible selectivity during the phase of the reach-and-grasp compared with the correspondent period of fixation, neuronal population responses were analyzed by ANOVA for repeated measures (factors: Condition, Target, Epoch), followed by Bonferroni post-hoc tests. All analysis were performed using a significance criterion of $p < 0.01$.

The same statistical analysis was applied to the neuronal populations of stimuli responses.

3. RESULTS

We recorded 474 neurons from the lateral aspect of the VLPF cortex (areas 46v and areas 12) of the left hemisphere of one monkey. Figure 2A shows the anatomical location of the two investigated regions.

Neuronal activity was recorded while monkeys performed the VMT and OT (see Figure 1A). Statistical analysis of the neuronal discharge revealed that, among all recorded neurons, 40 did not significantly change their activity during any phase of both the VMT and OT. Thus, the results will be focused on a total of 434 task-related neurons.

More specifically, concerning the VMT, 60 were differentially activated in the reach-and-grasp condition compared with the fixation condition, 169 responded only to the visual and/or auditory cue stimuli, while 113 discharged significantly at the presentation of sensory cue stimuli but were also differentially activated during reach-and-grasp compared with fixation conditions (see Figure 2B). Among these 342 neurons, 204 responded significantly *only* in the VMT, while 138 of them were also activated during one or more phases of the OT. In particular, 55 activated differently when monkey observed the experimenter's reaching-grasping actions compared with the fixation condition of the OT, 61 responded only to the visual and/or auditory cue stimuli of the OT, while 22 neurons discharged significantly at the presentation of sensory cue stimuli but were also differentially activated during reaching-grasping compared with fixation conditions of the OT (see Figure 2C).

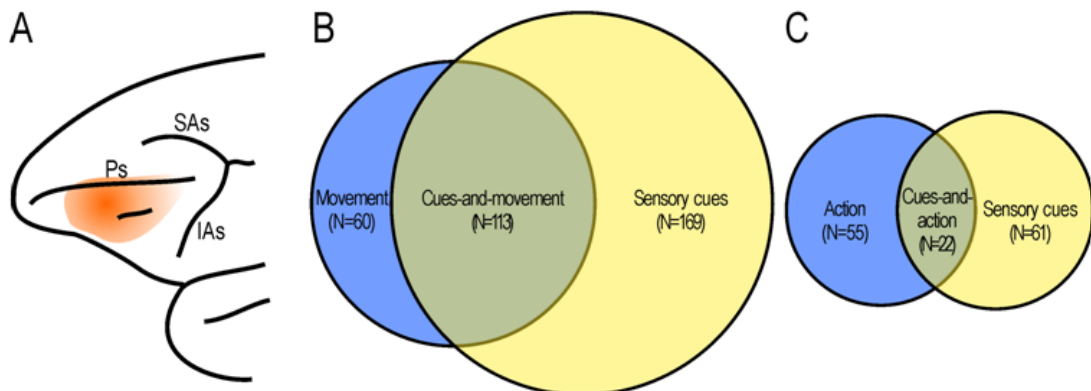**Figure2. A.** Schematic view of the lateral part of the left prefrontal cortex. The red shaded region indicates the recorded area. IAs, inferior arcuate sulcus; Ps, principal sulcus; SAs, superior arcuate sulcus. **B.** Distribution of neurons activated in the VMT based on their basic sensory and motor properties. **C.** Distribution of neurons activated in the VMT based on their properties as derived from the OT.

Finally, 17 neurons did not activate at all during the VMT but discharged significantly only during some epochs of the OT, while others (N=75) did not vary their activity during any phase of the VMT and the OT, but became active after reward delivery, regardless of task and experimental condition.

3.1. Movement-related neurons

Among task-related neurons, 60 were differentially activated in the reach-and-grasp condition compared with the fixation condition. In particular, most of them (58.3%) increased their activity after the go (reach-and-grasp condition) or no-go (fixation condition) signal (excited), while a few neurons (N=2) decreased their firing after the no-go signal (inhibited). Interestingly, 38.4% of these neurons did not significantly change their activity before and after the go/no-go signal (unchanged), thus showing a sustained activation in one of the two conditions compared with the other (see Table 1).

Table1. Number of movement-related neurons recorded with the VMT

	Excited	**Inhibited**	**Unchanged**	**Tot**
Reach and grasp	30 (50%)	0 (0%)	14 (23.4%)	44 (73.4%)
Fixation	5 (8.3 %)	2 (3.3%)	9 (15%)	16 (26.6%)
TOT	35 (58.3%)	2 (3.3%)	23 (38.4%)	60 (100%)

Half of movement-related neurons are activated after movement onset during the reach-and grasp condition compared with the fixation condition. An example is shown in figure 3A. This neuron clearly increased its firing after the go signal, but discharged stronger during reaching and grasping the food compared with that of the object. Figure 3B shows a neuron with the same pattern of firing but with the opposite selectivity, being more strongly activated during grasping of the sphere. Note that only 5

movement-related neurons among 60 were selectively activated when the target of the action was either the food pellet (N=2) or the plastic sphere (N=3). Figure 3C exemplifies the most frequently observed behavior of movement-related neurons. This neuron discharge during the reaching phase of the grasping act, regardless of the target was a food pellet or a not edible plastic sphere to be placed into the container. In contrast, it did not vary its activity neither after the no-go signal during the fixation condition or during previous cue stimuli presentation.
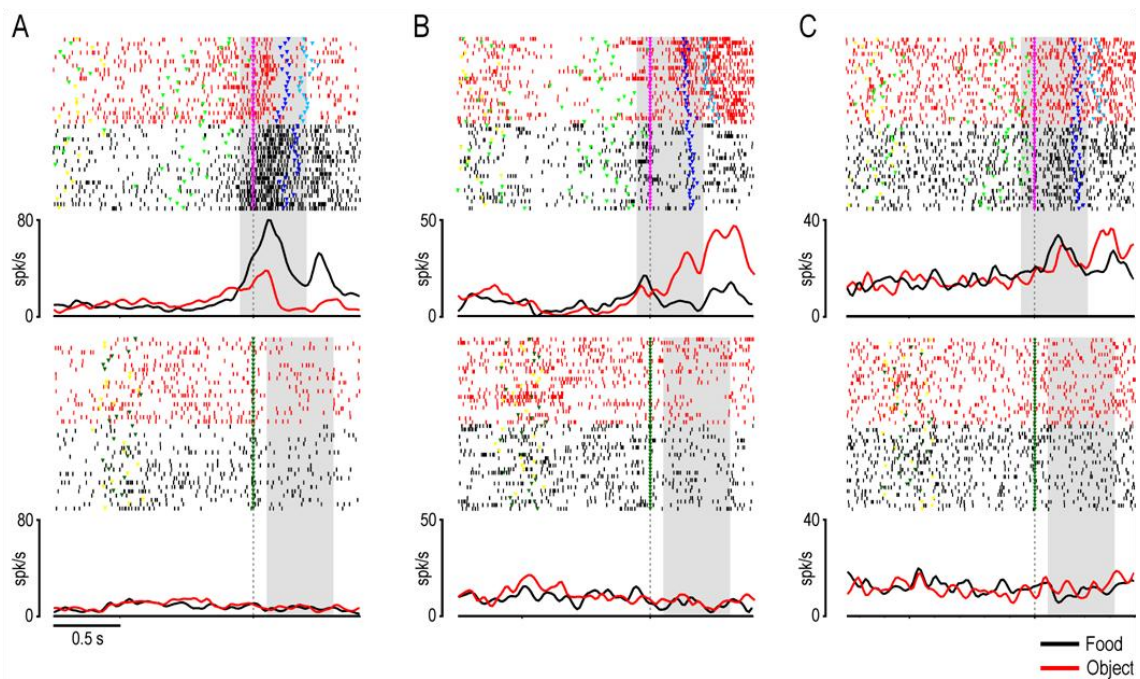

Figure3. Examples of movement-related neurons. The upper row shows the discharge of neurons aligned on the movement onset in the reach-and-grasp condition, while the lower row shows the activity of the same neurons aligned on the no-go signal in the fixation condition. The black line and the red line (as well as black rastergrams and red rastergrams) show the activity when the target was respectively food or a plastic sphere. **A.** Example of a neuron discharging stronger during grasping of the food pellet compared with that of the sphere. **B.** Example of a neuron discharging stronger during grasping of the sphere compared with that of the food pellet. **C.** Example of a neuron discharging similarly during both types of grasping actions. Colored markers indicate different behavioral events: yellow, light switch on (target presentation); light green, high tone on and high tone off (go signal); dark green, low tone on and low tone off (no-go signal); purple, movement onset; blue, hand contact with the object; light blue, hand contact with the rim of the container. The grey shaded region indicate the temporal window used for the statistical analysis of the neuron response.

To better understand the coding properties of these prefrontal neurons and their possible contribution to monkey's motor behavior, we studied the time course of

neuronal activity by pooling together all neurons which were more strongly activated either during reach-and-grasp (figure 4A) or fixation (figure 4B) condition. The populations activity was assessed by means of a 2x2x2 repeated measures ANOVA (factors: Condition, Target and Epoch) applied separately on reach-and-grasp and fixation selective neurons.
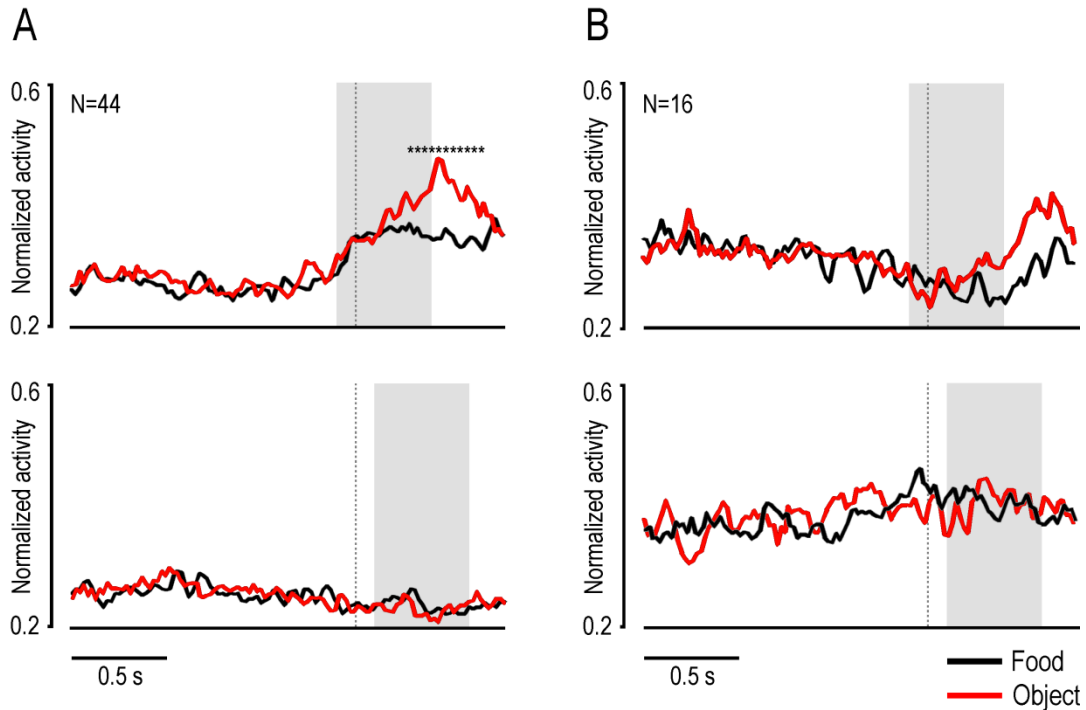

Figure4. Time course of the activity of neuronal populations aligned (gray dotted line) on the movement onset in the reach-and-grasp condition (first row) and on the no-go signal in the fixation condition (second row). Red lines indicate the time course of activity when the target was the sphere, while black lines represent the time course of activity when the target was food. **A.** Population of neurons activated strongly during reach-and-grasp compared with the fixation condition. **B.** Neuronal population activated stronger during fixation condition. The grey shaded regions indicate the temporal windows used for statistical analysis of the neurons response. Asterisks in figure A indicate the period in which paired samples t-tests evidenced a significant separation of the two curves ($p < 0.05$).

Concerning reach-and-grasp neurons (see Figure 4A) the analysis revealed significant main effects of the factors Condition [$F_{(1,43)}=59.25$, $p<0.001$], Epoch [$F_{(1,43)}=34.64$, $p<0.001$] and their interaction [$F_{(1,43)}=61.61$, $p<0.001$]. Bonferroni post-hoc tests revealed that the population activity is stronger during the movement epoch than in the pre-movement and fixation epochs. ANOVA also showed a barely significant effect of the interaction between Target and Condition [$F_{(1,43)}=4.19$,

p<0.05], and Bonferroni post-hoc test revealed that neuronal activity during both grasp-to-eat and grasp-to-place is significantly stronger than during fixation of the same targets in the no-go trials (p<0.001), while no significant difference emerges between the two types of grasping actions. This was likely due to the fact that most of this difference appears after grasping accomplishment, therefore outside the temporal window employed for the analysis. In order to verify this hypothesis, we applied paired samples t-test (p<0.05) to population activity in the grasp-to-eat and grasp-to-place conditions, starting from movement onset and sliding forward the analysis in steps of 20 ms bins. The results are shown by the asterisks in figure 4A: it is clear that a significant separation between the two curves occurred only 300 ms after the movement onset and it remains significant well beyond the end of the window employed for statistical analysis, thus fully justifying the lack of difference obtained with the ANOVA.

Concerning fixation neurons (see Figure 4B), ANOVA revealed a significant main effect of the factor Condition [F(1,43)= 24.48, p<0.001], indicating that the population activity is stronger during the fixation condition than during the reach-and-grasp condition.

3.2. Cue-related neurons

As described in the methods section, we analyzed sensory responses to the visual presentation of the target (light onset) or the cue sound (sound onset) when they were the second presented stimulus, so that the 1) pre-stimulus epoch was always instructed by the previous onset of the other stimulus (sound or light, respectively), 2) the monkey had its hand still on the starting position and 3) it was staring at the fixation point. Thus, neurons encoding a purely sensory representation of the target should discharge similarly regardless of the previously presented sound, while neurons encoding a purely sensory representation of the sound should discharge similarly regardless of the previously presented object.

We recorded a total of 169 cue-related neurons, which activated significantly during the VMT in relation to the presentation of cue stimuli but not during subsequent reaching-grasping or fixation phases. Among these neurons, most responded only to the

visual presentation of the target (N=133), some responded to the cue sound (N=13) while others encoded both these cue stimuli (N=23) (see Table 2).

Table2. Number and properties of cue-related neurons recorded in the VMT.

		Fixation			Reach-and-grasp			Unselective			Tot
		Food	**Sphere**	**Ns**	**Food**	**Sphere**	**Ns**	**Food**	**Sphere**	**Ns**	
Cue stimuli	**S**	0	0	3	0	0	1	1	0	8	13
	L	0	0	4	0	0	13	1	11	104	133
	L+S	0	0	2	0	1	3	0	1	16	23
Tot		0	0	9	0	1	17	2	12	128	169

(Header spanning row: **Task condition**)

While some cue-related neurons (10%) discharged stronger when the stimulus was presented in the context of a reaching-grasping trial and others (less than 5%) encoded stimuli specifically in the context of fixation trials, most of these neurons (87.6%) responded to the stimulus regardless of whether the trial in which it was presented required to reach and grasp the target or simply to fixate it (unselective – see Table 2). In addition, the selectivity of sensory responses for the type of stimulus appeared, in general, extremely low, since less than 10% of these neurons discharged differently depending on the use of food (N=2) or sphere (N=13) as target. Examples of this type of neurons are provided in Figure 5.

The neuron in Figure 5A is specifically activated during the visual presentation of the sphere compared with the food during both motor and fixation trials, with no difference between them. In contrast, the neuron shown in Figure 5B exemplifies the most frequently observed behavior, responding with similar intensity after the presentation of both targets, regardless of the context (reaching-grasping or fixation) in which the visual presentation occurred. Finally, the neuron shown in Figure 5C is specifically activated after the presentation of the cue sound, slightly stronger for the low tone which instructed target fixation, but regardless of the previously presented object (food or sphere).
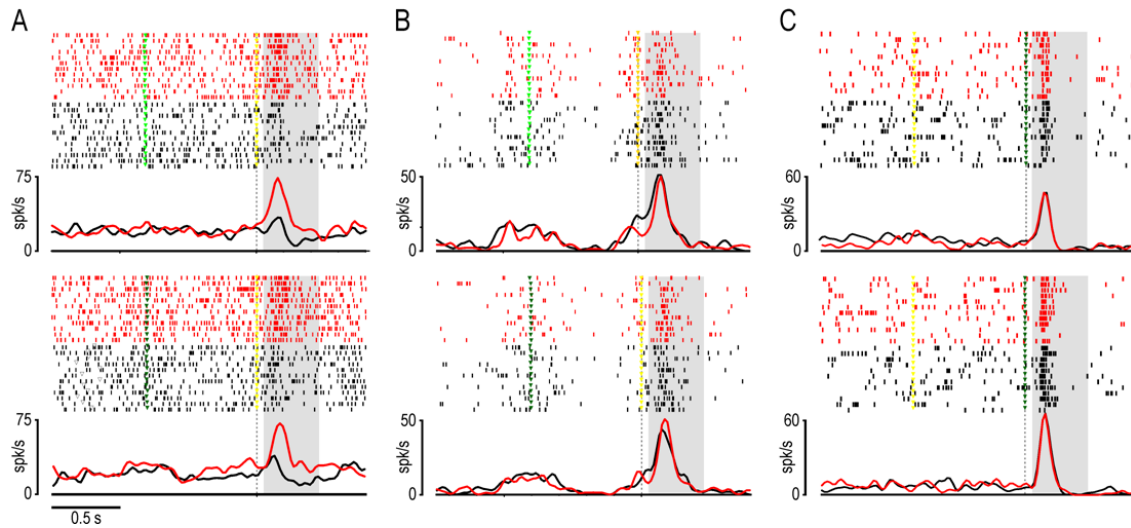
Figure5. Examples of cue-related neurons. **A**. Neuron responding to the visual presentation of the sphere but not of the food pellet. **B.** Neuron discharging similarly to the visual presentation of both target objects. **C.** Neuron responding to the presentation of the cue sound. Conventions as in Figure 3.
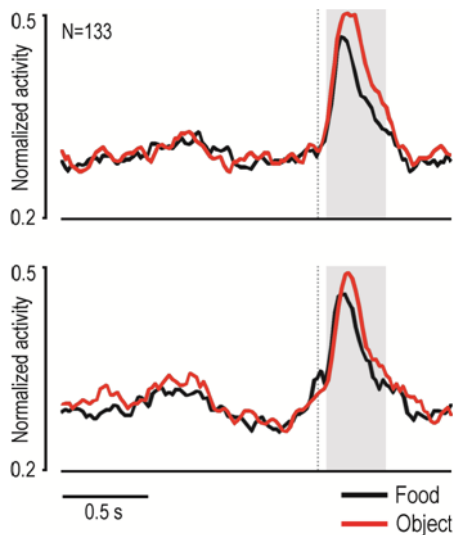

Figura 6. Time course of activity of cue-related neuronal population aligned on the light switch on (object presentation) in the reach and grasp condition (top) and on the no-go signal in the fixation condition (bottom). Conventions as in figure 4.

compared with fixation trials.

In order to analyze the time course and intensity of the activity of the most represented class of cue-related neurons, namely, those responding to the visual presentation of the target, we pooled together the normalized responses of these neurons, separately for each of the presented target and condition (reach-and-grasp and fixation). Results are shown in Figure 6. ANOVA revealed significant main effects for all factors as well as for the interaction between Object and

Epoch [F(1,132)=5.60, p< 0.05] and between all three factors [F(1,132)=4.92, p< 0.05]. Bonferroni post-hoc tests revealed that the population activity following light switch on is greater when the sphere rather than the food is presented, particularly in the context of reach-and-grasp trials

3.3. Movement-and-cue related neurons

Forty percent of the neurons responding after the presentation of the cue stimuli (light and/or sound) also showed a significant discharge modulation during reach-and-grasp conditions compared with fixation conditions. In particular, most of them (87%) varied their activity in relation to reach-and-grasp compared with fixation condition, while the remaining (13%) were modulated according to the fixation condition (see Table 3).

Table 3. Number of movement-and-cue related neurons recorded with the VMT

			Motor response						
			Reach-and-grasp			Fixation			Tot
			Excited	Inhibited	Unchanged	Excited	Inhibited	Unchanged	
Cue-stimuli response	Food	S	0	0	1	0	0	0	1
		L	0	0	0	0	0	0	0
		L&S	0	0	0	1	0	0	1
	Sphere	S	1	0	0	0	0	0	1
		L	2	0	2	0	0	0	4
		L&S	1	0	0	1	0	0	2
	Unselective	S	3	5	3	0	1	1	13
		L	33	1	22	1	1	6	64
		L&S	18	2	4	0	0	3	27
Tot			58	8	32	3	2	10	113

Among the 58 neurons discharging stronger during the movement epoch of the reach-and-grasp condition, most activated during object presentation (60%), some (6.9%) discharged after the cue sound, while the remaining (33.1%) encoded both auditory and visual stimuli. Neurons showing stronger discharge during the motor compared with the fixation condition, but with unchanged activity before and after the go signal, showed a similar distribution of sensory responses, mostly being activated by visual stimuli, alone (75%) or in combination with auditory responses (12.5%), while

auditory cues alone were rarely coded (12.5%). Examples of movement-and-cue related neurons are shown in Figure 7.
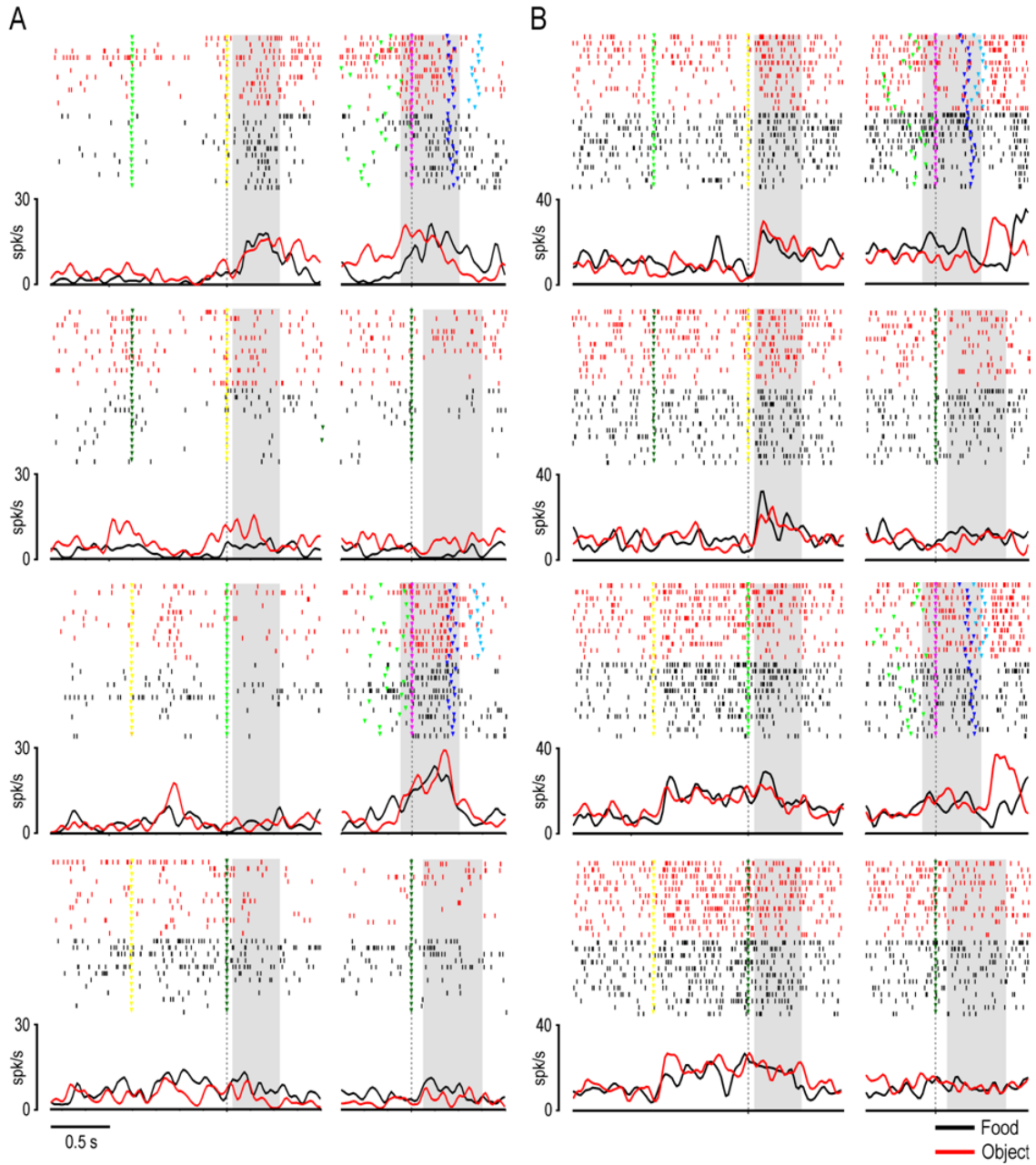
Figure7. Examples of movement-and-cue related neurons. **A.** Neuron responding to target presentation as well as during movement epoch of the reach-and-grasp condition, regardless of whether it was a food pellet or the sphere. **B.** Neuron responding to target presentation regardless of whether it was a food pellet or a sphere as well as during reach-and-grasp compared with fixation condition, but with unchanged activity before and after the go (and no-go) signal. Conventions as in Figure 3.

Figure8. Time course of activity of the neuronal population aligned on object presentation and movement onset (first row), object presentation and no-go signal (second row), high tone onset and movement onset (third row) and low tone and no-go signal (fourth row). Other conventions as in figure 4.

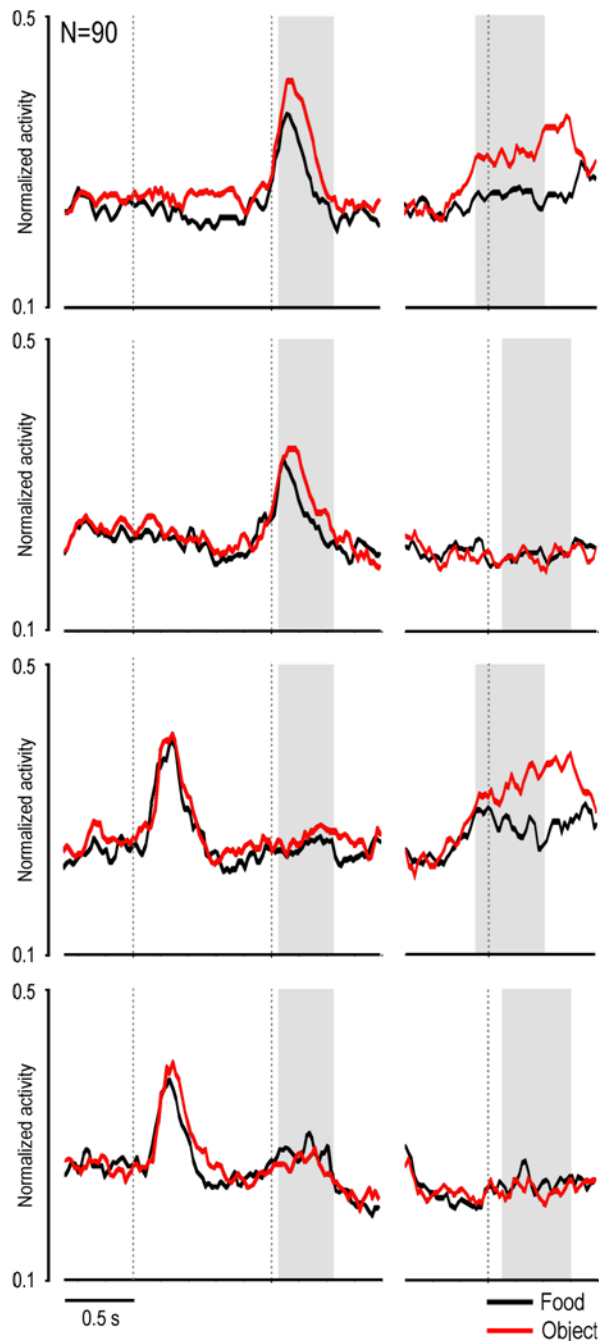The neuron shown in figure 7A displays a first increase of its discharge in correspondence to the switch on of the light, particularly when it is instructed by the previously presented high tone, and a further discharge beginning just before the monkey releases the starting position, both for grasping food and the sphere. This neuron does not respond to the cue sound, but nevertheless shows a clear motor-related discharge even when the sound is the second presented stimulus. The neuron shown in Figure 7B is tonically activated after the presentation of the target, regardless of whether it was preceded by a high or a low auditory stimulus, and even when the stimulus presentation occurs as a first cue. Noteworthy, it doesn't vary its activity in relation to the presentation of the cue sound, both when it is the first and the second presented stimulus. In addition, the sustained activity remains significantly higher in the reach-and-grasp condition compared with fixation condition, substantially unchanged before and after the go (and the no-go) signal.

In order to better study the time course and intensity of the activity of the most represented class of movement-and-cue related neurons, which is constituted by those responding stronger during reach-and-grasp than during fixation condition, we pooled

together the normalized responses of all these neurons regardless of their possible preference for either the sphere or the food, since this selectivity characterizes a large minority of the recorded cells. Results are shown in Figure 8.

Statistical analysis of the population response to target presentation (first two panels in figure 8) has been carried out by means of a 2x2x2 repeated measures ANOVA, revealing significant main effects of the factors Object [$F(1,89)=26,78$, $p<0.001$], Condition [$F(1,89)=9,27$, $p<0,01$] and Epoch [$F(1,89)=63,94$, $p<0,001$], indicating that the visual presentation response associated to the sphere is stronger than that evoked by the food pellet in both the reach-and-grasp and the fixation condition. In contrast, the same analysis carried out on the population response to the cue sound revealed a significant main effect of the factors Condition [$F(1,89)=26,16$, $p<0.001$], Epoch [$F(1,89)=22,04$, $p<0,001$] and their interaction [$F(1,89)=0,001$, $p<0,001$]. More specifically, Bonferroni post-hoc test showed that the activity significantly increases following cue sound presentation, particularly when the sound is the low tone associated to fixation trials.

Concerning the discharge of the same neuronal population during the epochs before and after the go and the no-go signal of the reach-and-grasp and of the fixation condition, ANOVA revealed significant main effects for all the factors, as well as for their interaction, both when the last cue stimulus was object presentation [$F(1,89)=16,24$, $p<0.001$] or the cue sound [$F(1,89)=26,38$, $p<0.001$]. Bonferroni post-hoc test indicated that, in both cases, the population activity increased during the movement epoch of the reach-and-grasp condition, particularly when the target was the sphere rather than the object ($p<0,01$ for all comparisons).

3.4. Neurons discharging during OT

Among all the neurons significantly activated during the VMT, 138 responded *also* during some phase of the OT. In particular, 55 neurons were differentially activated during the observation of the experimenter's action compared with the corresponding period of object fixation condition (see Table 4), 61 activated specifically in relation to the sensory cues provided during the OT, that instructed the experimenter on how to

perform the task (see Table 5), while 22 discharged differently during the observation of action compared with the fixation condition but also responded to the presentation of cue stimuli preceding experimenter's action.

Table4. Number of neurons responding differently during action observation compared with fixation during the OT.

	Excited	Inhibited	Unchanged	Tot
Reach and grasp	32 (58.2 %)	6 (10.9%)	8 (14.5%)	46 (83.6%)
Fixation	6 (10.9%)	0 (0%)	3 (5.5%)	9 (16.4%)
TOT	38 (69.1%)	6 (10.9%)	11 (20%)	55 (100%)

Table5. Number of neurons encoding cue-stimuli in the OT.

		Task condition									Tot
		Fixation			**Reaching-grasping**			**Unselective**			
		Food	Sphere	Ns	Food	Sphere	Ns	Food	Sphere	Ns	
Cue stimuli	**S**	0	0	1	0	0	2	0	0	4	7
	L	1	0	2	0	0	1	2	1	43	50
	L+S	0	0	0	0	0	0	0	0	4	4
Tot		1	0	3	0	0	3	2	1	51	61

The example of a neuron specifically activated during the observation of the experimenter's action and not during the corresponding phase of the fixation condition in the OT is shown in Figure 9. This neuron discharges intensely during action observation, increasing its firing immediately after the detachment of the experimenter's hand from the starting position, and reduces its activity when the object is about to be grasped. Notably, there is no selectivity of this response for the type of grasped object. Furthermore, the neuron is not activated during cue stimuli previously presented during the OT. Figure 9 also shows the response of this neuron during the VMT, evidencing that the same cell also fires when the monkey actively perform a reaching-grasping action, with a remarkably similar discharge profile and a complete lack of selectivity for

the type of grasped object and of sensory response to previously presented cue stimuli. The neuron discharge during action observation is also stronger compared with that recorded during action execution.
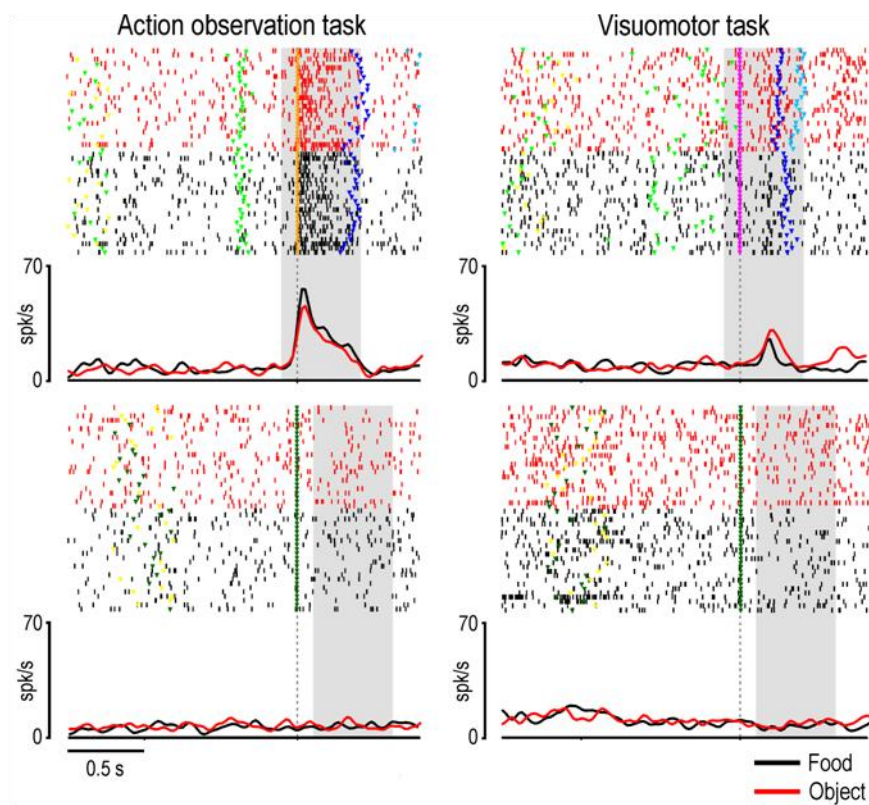

Figure9. Example of a neuron activated during reach-and-grasp in the VMT and during the observation of the same task done by the experimenter (OT). Conventions: orange, experimenter movement onset; other conventions as in Figure 3.

We investigated the time course and intensity of the activity of the neuronal population discharging differently during reach-and-grasp compared with fixation condition during the OT, by means of 2x2x2 repeated measures ANOVA (factors: Condition, Target and Epoch) applied to the visual presentation and the movement and no-movement phases of both the OT and the VMT.

Concerning the response to target presentation in the OT, ANOVA did not show any significant effect. In contrast, the same analysis applied to the neurons response during action observation in the same task evidenced a significant main effect of the factor Condition [$F(1,39)= 56.88$, $p<0.001$], Epoch [$F(1,39)= 9.79$, $p<0.01$] and of the interaction between Condition and Epoch [$F(1,39)= 18.20$, $p<0.001$]. Bonferroni post-

hoc tests revealed that population activity increases significantly after the onset of experimenter's action (p<0.01), and it is stronger during action observation epoch than during the corresponding phase of the fixation condition (p<0.01). In contrast, concerning the response to target presentation during VMT, ANOVA revealed a significant main effect of the factor Epoch [F(1.39)=34.39, p<0.001], indicating the presence of a clear sensory response which is specific for the VMT, although it does not differ between reach-and-grasp and fixation conditions. Instead, concerning the response during monkey's active movement, results of ANOVA showed the presence of a significant main effect of the factor Epoch [F(1,39)= 12.48, p<0.005] and of the interaction between Target and Condition [F(1,39)= 7.02, p<0.05] and Condition and Epoch [F(1,39)= 21.67, p<0.001]. Bonferroni post-hoc tests indicated that population activity increased significantly during the movement phase of the reach-and-grasp condition (p<0.01), and it was also significantly stronger compared with the activity of the same population during the corresponding phase of the fixation condition (p<0.01).
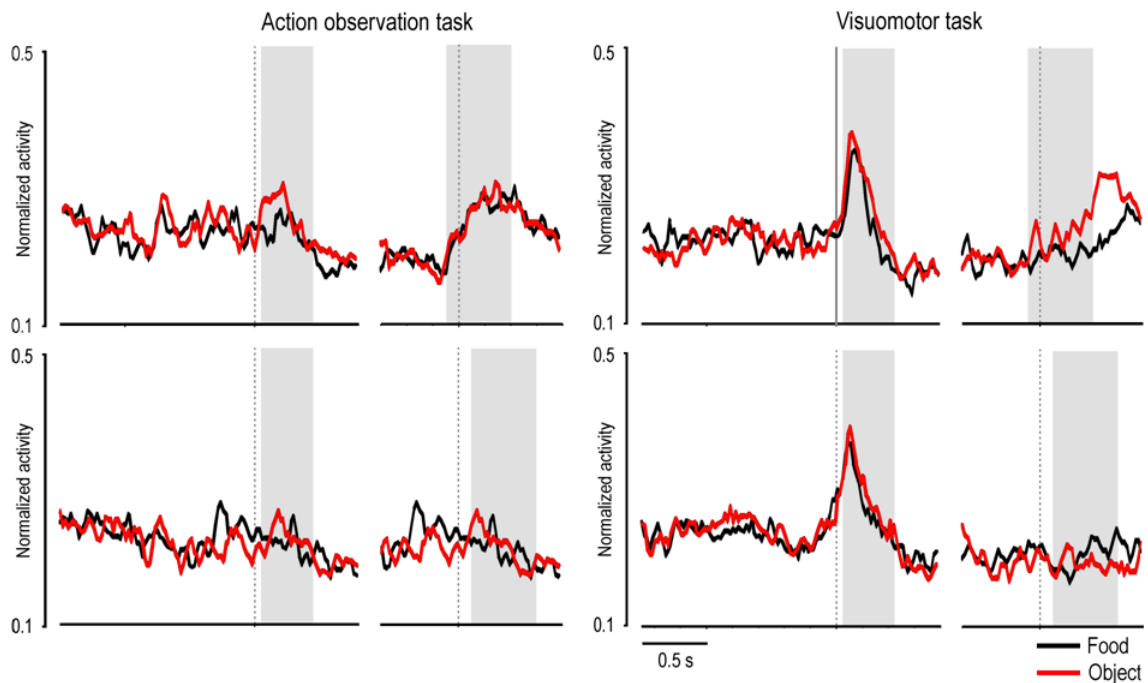

Figure10. Time course of activity of the neuronal population aligned on the light onset (on the left of each panel), movement onset in the reach and grasp condition (top right panel) and on the no-go signal in the fixation condition (bottom right panel). Conventions as in figure 4.

Based on the results of these analyses, it is clear that strong visual presentation responses observed during the VMT, regardless of the "motor" or "fixation" context in which they occur, are not present during the OT. Furthermore, during the OT clearly appears a significant population response during action observation which is paralleled by an increase of the population activity also during action execution in the VMT. It should be noted, however, that the temporal profile of the population activity in the VMT does not reflect neither the profile of activity observed in the same phase of the OT, nor that typically associated to the encoding of reaching and grasping acts in parietal and premotor areas. Thus, to better evaluate the possible congruence of the neuronal firing pattern between the OT and the VMT, we also focused on single neurons behavior.

More specifically, among the 40 neurons forming the above described neuronal population activated during the observation of reach-and-grasp compared with object fixation in the OT, N=11 varied their activity only in the movement phase of the VMT, 18 responded similarly but also encoded sensory stimuli, while the remaining 11 did not show any movement-related response in the VMT.

A considerable number of neurons responding during the OT did not vary their activity during action observation, but encoded stimulus presentation (N=61). Almost all of these neurons responded to the presentation of objects after the switch on of the light (82%), with no selectivity for the presented object, only a few responded to the auditory stimulus alone (11,5%), while in some cases the response was present for both the auditory and visual cues (6,5%). Interestingly, neurons responding to visual or auditory stimuli in the OT also encoded similarly the same stimuli when presented during the VMT. In particular, among 50 neurons responding to target presentation in the OT, most (80%) significantly responded to target presentation also during the VMT, while 20% of them were selective for the target presentation in the OT. In contrast, among 133 neurons showing target presentation responses during the VMT, most did not showed any visual presentation response in the OT (70%).

In order to investigate the time course and intensity of the activity of the neuronal population discharging differently to the visual presentation in both the OT and the VMT, we applied a 2x2x2 repeated measures ANOVA (factors: Condition, Target and Epoch). Results are shown in figure 11. Concerning the response to the

Visuomotor task   Observation task
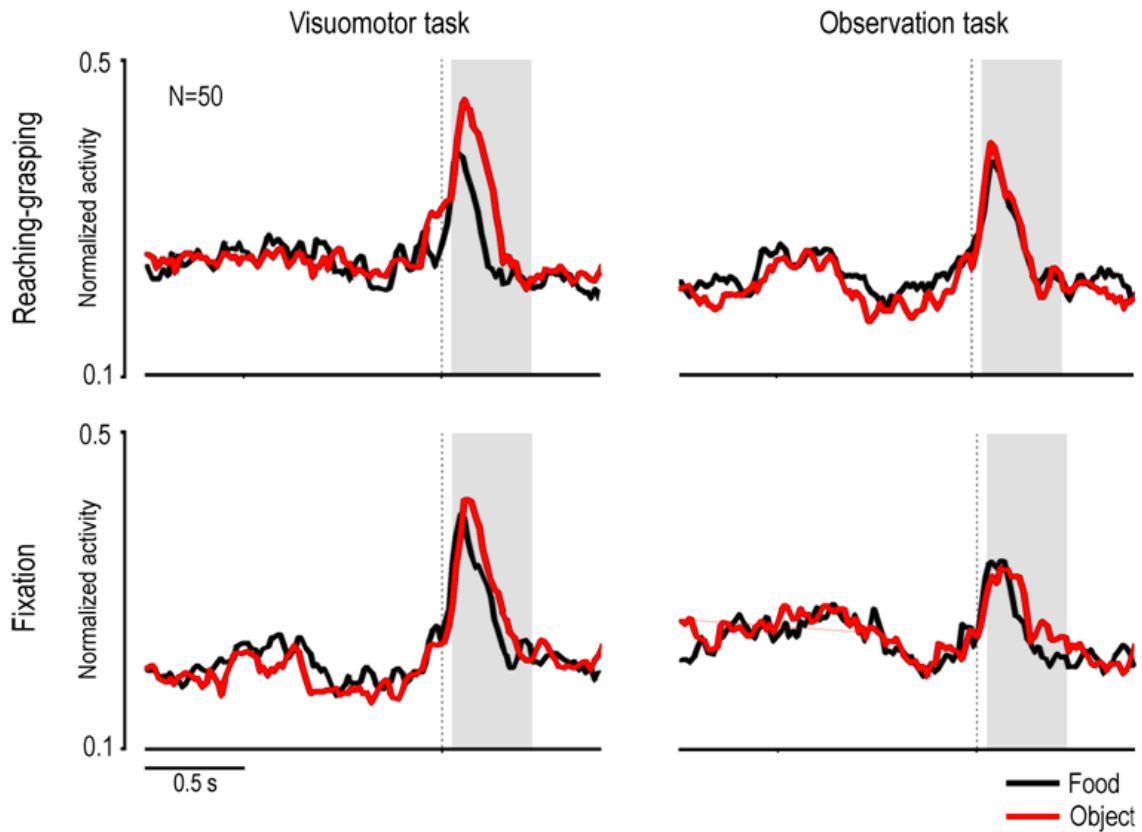
visual presentation in OT, this statistical analysis revealed significant main effect of the factor Epoch [F(1,49)=93.16, p<0.001] indicating the presence of a clear sensory response in both conditions of reaching-and-grasping and fixation regardless of the target presented. Concerning to the response to the visual presentation in VMT, the same statistical analysis revealed significant main effect of the factors Object [F(1,49)=7.92, p<0.005] and Epoch [F(1,49)=107.36, p<0.001] and the interaction between the factor Object and Condition [F(1,49)=7.23, p<0.01] and Object and Epoch [F(1,49)=19.55, p<0.001]. Bonferroni tests indicated that the population activity increase significantly when the sphere rather the pellet (p<0,001) was presented in the contest of reach-and-grasp trials. In order to verify if the intensity of the visual presentation was the same in both OT and VMT, we applied another 2x2x2x2 repeated measures ANOVA (factors: Task, Condition, Target and Epoch) and this analysis evidenced significant main effects of the factors Task [F(1,49)= 7.27, p<0.01], Condition [F(1,49)= 11.48, p<0.005], Epoch [F(1,49)= 112.35, p<0.001] and the interactions between Task and Epoch [F(1,49)= 12.77, p<0.001], Target and Epoch [F(1,49)= 18.54, p<0.001], Task Target and Epoch [F(1,49)= 4.36, p<0.05] and between

Target, Condition and Epoch [$F(1,49)= 4.74$, $p<0.05$]. Bonferroni showed that the population activity increased when a target was presented in both VMT and OT ($p<0.001$), although intensity of discharge was stronger when a target was presented during VMT performance ($p< 0.001$). In particular, visual presentation during OT evoked the same intensity of discharge regardless to the target presented (pellet or sphere) and the contest of trials (reach and grasp or fixation), while the visual presentation of a sphere in the contest of reach and grasp trials during VMT evoked a greater intensity of response than an identical sphere was presented in fixation trials or a pellet was presented in both reach and grasp trials during VMT or than a pellet or sphere was presented during OT regardless to action observation or fixation trials ($p<0,001$). These results indicated that there is a major activation when the target of reach and grasp or fixation is a sphere specially when the monkey is engaged in active performance.

4. DISCUSSION

In this study, single neurons were recorded from ventrolateral prefrontal cortex while the monkey was performing different grasping actions or fixating different potential target objects. The same neurons were recorded also when the monkey observed an experimenter performing the same task, which included both grasping observation and object fixation conditions. Both visuomotor and observation tasks were designed in order to study natural grasping actions (i.e. grasping a piece of food and bringing it to the mouth) and actions similar to those usually performed by monkeys in nature (i.e. grasping an object and placing it into a container, which remind the action of throwing away an object). None of these actions consisted in artificial movement sequences, often employed in studies on prefrontal cortex (Tanji et al., 2008). The only aspect that has been necessary to standardized in our task was the timing of presentation of the cue stimuli: this enabled us to assess whether and to which extent prefrontal neurons could process and integrate sensory information which are crucial for selecting and organizing natural actions.

The results of this electrophysiological study showed that there are different sets of neurons, some responding during reaching-grasping actions, others activated by the presentation of sensory stimuli (mainly visual ones), or modulated by both motor execution and stimulus presentation. A general feature which applies to both sensory- and motor-related responses is a rare and low selectivity for the type of target (food or sphere) at the single neuron level, although a higher selectivity emerges at the population level. Another relevant finding is that almost half of the recorded neurons that activated during some phase of the visuomotor task also responded during the action observation task, in addition to a small number of cells which were activated exclusively during the action observation task. An interesting aspect evidenced by comparing the sensory responses of single neurons between the visuomotor and the action observation task is that, even during fixation condition, a considerable set of visually-responsive neurons (70%) activated selectively for the presentation of food and/or object in the visuomotor but not in the action observation task, while a smaller number (30%) discharged in both conditions, although stronger in the visuomotor task

at the population level, suggesting a higher salience of objects when presented as potentially reachable targets.

The main conclusion suggested by these results is that the investigated sector of the ventrolateral prefrontal cortex uses sensory, mostly visual information about potential target object in order to plan and control simple natural actions, although this information does not appear to be linked to a goal-related action selection. In addition, the presence of visual responses during action observation suggests that also this cortical sector could play a role in the mirror circuit, although the available data do not allow to conclude for their possible involvement in intention understanding.

4.1. Movement-related prefrontal neurons

A set of the recorded neurons (about 14%) was differently activated in the reaching-grasping epochs compared with the corresponding epochs of the fixation condition, with no significant response to auditory or visual stimuli. Half of these neurons showed a discharge pattern similar to that of 'purely motor neurons' typical of motor and premotor cortices (Fogassi et al., 2005; Rozzi et al., 2008, Bonini et al., 2011, 2012), being activated *specifically* during monkey's active movement. Of the remaining neurons, a fraction discharged stronger during motor than fixation condition, however their activity was unchanged before and after the go-signal (23.4%). Neurons of the other fraction were more strongly activated during the fixation condition (26.6%).

The report of movement-related neurons in prefrontal cortex is not completely unexpected. In fact, a few studies are available in the literature in which the role of prefrontal cortex was assessed during the performance of natural actions. Tanila and co-workers (1992; 1993) recorded neurons from the monkey prefrontal cortex (areas 46 and 9) during the application of a wide set of stimuli as well as during motor actions actively performed by the monkey, showing that part of the recorded neurons appeared to be specifically related to active movement execution, and were located more ventrally than the remaining ones, concentrated along the middle third of the inferior bank of principal sulcus and the adjacent inferior prefrontal convexity. The above mentioned studies by Tanila and coworkers appear, however, poorly controlled, for

example they did not require the monkey to maintain fixation during movement execution, so that the motor-related discharge could depend on concurrent eye movements to the target during reaching, or on the coordination of eye and arm movement: these properties are known to be frequently encoded in different regions of the parietal cortex that project to the investigated prefrontal sectors. In the present study we can directly exclude these influences because the monkey was required to maintain fixation during all trials, thus convincingly showing that the motor-related response is actually specific for limb movement. Interestingly, a recent mapping study reported the presence of forelimb- and mouth-related motor responses during monkey execution of reaching-grasping task in a region similar to that investigated in the present work (Rozzi et al. 2011), with a percentage comparable with that found in our study.

Part of movement-related neurons was activated tonically before and after the go- with respect to the no-go signal. Since in the motor condition the task structure and reward contingency were identical to those of the fixation condition, the anticipated and sustained activation of these neurons before movement onset likely reflects a preparatory set. Similar firing patterns, although usually studied by means of delayed response paradigms, have been described in different premotor (Wise and Mauritz, 1985; Kurata and Wise, 1988) and parietal (Johnson et al., 1996; Wise et al., 2007) areas of the monkey. Many of these areas are known to have considerable projections to ventrolateral prefrontal cortex, which can justify the observed behaviour.

Population activity of reach-and-grasp neurons taken as a whole showed that their response is strictly related to the movement phase, since it rises immediately before the detachment of monkey's hand from the starting position and reaches its peak immediately after grasping accomplishment. Noteworthy, a clear discrepancy emerged when comparing the selectivity for grasp-to-eat or grasp-to-place actions at the single neuron and population level. In fact, when individually analyzed less than 8% of the all movement-related neurons showed some grasp-to-eat or grasp-to-place selectivity, in contrast with previous studies with a similar motor task performed in other cortical areas (Fogassi et al., 2005; Bonini et al., 2010, 2011, 2013), while at the population level it emerges a clear selectivity for grasping of the sphere. In fact, while the selectivity in many neurons did not reach statistical significance in the first level analysis, when the time course of the population activity was analyzed, it has been

possible to show that the discharge becomes significantly selective only in the final stages of the task, well after grasping accomplishment. This last phenomenon could be explained by different factors. First, it could be due to a sensory feed-back provided by the contact between the monkey's hand and the rim of the container, since it is known that neurons with somatosensory responses are present in this cortical region and co-localize with movement-related neurons (Tanila et al., 1993). Another possibility is that, because of the need to carefully consider neuronal activity in a time window which precisely includes the reaching and grasping acts without encompassing a later period, we did not include possible motor responses specifically due to placing the sphere into the container (see the neuron example in Figure 3B) or to the act of bringing the food into the mouth. A third, more speculative explanation is that, while grasping a piece of food only requires to wait until the go-signal is given and then the motor flow unfolds automatically as a daily 'routine activity' (Cooper and Shallice, 2000), reaching-and-grasping of a sphere more strongly rely on a deliberate selection of a learned, although very simple, motor sequence. This selection requirement may be sufficient to evoke a stronger activity at the population level during this behavior, and might suggest that the motor response of prefrontal neurons could reveal a distinction between learned and automatic actions.

Thus, movement-related responses by ventrolateral prefrontal neurons reveal a clear involvement of this sector in the control of actions, with a higher degree of 'neural effort' when a learned motor behavior is required. Furthermore, detailed analyses of the timing of the movement-related responses could reveal different neuronal categories and, probably, corresponding different functional roles during action unfolding.

4.2. Cue-related prefrontal neurons

About 40% of the recorded neurons varied their activity following the presentation of auditory and/or visual stimuli, but not during movement execution. In particular, almost all neurons classified as cue-related (79%) responded to the visual presentation of the target (food or object). This finding is in line with the robust bulk of evidence showing that ventrolateral prefrontal neurons encode visual stimuli (Tanila et

al., 1993; Romanski and Goldman-Rakic, 2002; Romanski, 2007; O'Scalaidhe et al., 1997; see also Fuster 2008). Typically, it is assumed that these cells respond better either to highly complex stimuli belonging to specific categories, such as faces (Pan and Sakagami, 2012), or to stimuli arbitrarily associated to specific motor response (Murray et al. 2000), but almost no study has convincingly investigated the prefrontal contribution to the encoding of stimuli linked with specific behavioral goals, such as physically and geometrically identical food and non-food items. These stimuli belong to different categories that, at least in the case of edible items, are linked to specific behavioral responses. The present data showed that, among visually responsive prefrontal neurons, only a few (9%) showed clear visual preference for the type of target, all but one being selective for the plastic sphere. This lack of selectivity could be attributed to the high perceptual similarity between the two targets, which were identical in size, shape and location during the task. However, this explanation seems unlikely, since the monkey had clearly no difficulties in discriminating their identity, likely based on their different color and surface texture, since it could easily and rapidly perform the correct action (eating or placing) associated with each of them. It appears, therefore, that a purely categorical coding of edible and non-edible target could not be performed by the visually-responsive prefrontal neurons included in our sample.

Nevertheless, we observed that, at the population level, the neuronal response was stronger at the presentation of the sphere, as compared with that of the food, both during reach-and-grasp and during object fixation trials. Noteworthy, this effect cannot depend on the small number of visually selective neurons included in the population, since it remained significant even when these neurons were removed (see Results). This clearly indicates that visually responsive prefrontal neurons have a subtle but systematic preference for the non-edible object, even when it is not the target of a subsequent action.

The same population analysis also revealed that a significant difference exists in the visual responses for the same target depending on the context in which the presentation occurred: in particular, population responses were stronger for visual presentation of the target during reach-and-grasp condition compared with fixation condition, even if only 11% of the recorded cells matched this behavior based on single neuron analysis. Even in this case, the population selectivity persists also when the

analysis is applied to the neurons that did not show selectivity with the first level analysis. Taken together, it appears that the population of recorded neurons can signal a specific target that, in the setting used in the present study, is related to a specific type of action. One could argue that the difference in the population response at the presentation of the sphere with respect to the food might be due to differences in terms of reward expectancy or action utility value between the two conditions (Glimcher, 2003; Schultz, 2004; Watanabe, 2007). However, these considerations would better apply to a preference for the food, rather than the sphere. Furthermore, these arguments hardly apply to the present experimental paradigm, in which the monkey was automatically rewarded with the same amount and type of food after correct accomplishment of all the motor and fixation trials. Thus, the observed preference for the sphere could be better explained by the fact that the monkey had to learn the association of the sphere with the placing motor response, while this was not the case for the food item in the grasp-to-eat action. It might be interesting to note that this selectivity fits well with that shown by the population of movement-related neurons, corroborating the idea that visually responsive prefrontal neurons might be more involved in the encoding of visual stimuli associated with learned actions.

A small number of cue-related neurons (8%) responded specifically to the cue sound, most of them with no difference between high and low tone. This finding is in line with previous studies (Tanila et al., 1992, 1993; Saga et al., 2011), showing that auditory-responsive neurons are quite rarely found, and usually distributed in a scattered manner. Altogether, these data suggest that neurons in this prefrontal sector mainly encode visual rather than auditory information on potential target of motor actions, largely independently from the action context (reach and grasp or fixation) in which the object is presented.

Another small percentage of cue-related neurons (13%) was represented by cells that responded to the presentation of both visual and acoustic cue stimuli, with a generally poor selectivity for target object and experimental condition (reach-and-grasp or fixation), as already shown for visually responsive neurons. Also for this category of neurons, the population analysis, showed the presence of a selectivity for the visual responses (related to the two types of actions), but not for the acoustic cues (related to the choice between go and no-go).

The different behaviour of the neuronal responses, at the population level, to the two types of sensory information may suggest that prefrontal cortex, in general, tends to exploit more visual information than other types of sensory inputs for several types of selection processes.

4.3. Sensory-and-motor related prefrontal neurons

A considerable fraction of the recorded neurons (26%), beyond responding after the presentation of cue stimuli, also discharged differently during reach-and-grasp compared with fixation condition.

Concerning the response to *cue stimuli* presentation, most of these neurons did not show any preference for the target object, the cue sound or the experimental condition, while at the population level it emerged a significant preference for the visual presentation of the sphere, particularly during reach-and-grasp trials. This scenario perfectly overlaps with that previously described concerning cue-related neurons.

As far as the *motor* responses of this neuronal class are concerned, although at the single neuron level a poor selectivity for the type of target object could be observed, population analysis revealed the presence of a marked selectivity for reaching and grasping of the sphere. Compared with movement-related neurons lacking sensory responses, in which we showed that motor selectivity for the sphere emerged in the later stage of grasping execution, here it is clear that selectivity starts earlier on in the movement epoch, rising even before the detachment of monkey's hand from the starting position in the case in which the visual presentation of the target was the second sensory cue before movement onset. Noteworthy, the visual and the motor discharges appear to be clearly segregated, insofar as there is no sustained activity linking the visual presentation of the target with the subsequent motor action directed toward it. This could be explained by the fact that the stimulus was always visible to the monkey for the entire duration of the waiting period, in which memory of the presented target was not required. Nevertheless, the observed pattern of response suggests that the presence of a sensory access to perceptual information on target object in addition to movement-related activity may reveal the emergence of a more clear-cut categorical representation

of learned (compared with more natural) actions in the prefrontal cortex. Since the timing of the movement-related response, and of its selectivity, is quite late when compared with the population responses of the parietal and premotor neurons studied with a very similar task, it is likely that the prefrontal population response indicates that a given action has been selected within the parieto-premotor circuit rather than contributing to the selection process.

4.4. Prefrontal neurons studied during the observation of others' action

Among all neurons activated during some phase of the visuomotor task, 40% responded also during some phase of the observation task. Interestingly, among neurons specifically modulated only during the action observation period, most (69%) varied their activity in close correspondence with the experimenter's action, with a temporal firing pattern similar to that of ventral premotor and inferior parietal mirror neurons (Gallese et al., 1996; Fogassi et al., 2005). Of the remaining neurons, a fraction discharged stronger during the observation of action than in the fixation condition, however their activity was unchanged before and after the go-signal (14.5%). Neurons of the other fraction were more strongly activated during the fixation condition (16.4%).

A remarkable feature of mirror neurons of parietal and premotor areas is that they encode both the observed and the executed action. This property has been considered crucial for the interpretation of the possible role of this class of cells, several studies indicating that the activation of one's own motor repertoire during the observation of others' action might enable the observer to understand the observed action well beyond the mere perceptual level, that is, 'from the inside' (Sinigaglia and Rizzolatti, 2011). Here we assessed the possible congruence between neurons discharge during execution and observation of actions, showing that among neurons discharging stronger during action observation than during object fixation in the observation task, 72.5% showed a similar pattern of activity also in the corresponding phases of reaching-grasping in the visuomotor task. At the population level, it emerged that neurons specifically activated when observing actions done by the experimenter increased their activity also when the monkey performed the task, although the visual response to

action observation was stronger and more time-locked to the experimenter's movement than the motor response. Thus, although in principle these neurons show the main feature typical of mirror neurons, the difference in the timing of the visual and motor discharge raises the question whether they can considered 'prefrontal analogous' of the premotor and parietal mirror neurons. It must also be considered, however, that these neurons have not been studied also in a naturalistic setting, that typically used for the description of mirror responses, so that it is not possible to make a direct comparison. The low selectivity found in this task for the two actions does not allow to conclude that the recorded neurons can play a crucial role in the circuit for understanding motor intention. This is in line with the results found during the execution of the motor task, in which the selectivity for the two types of actions was quite low.

The same population analysis also revealed an interesting dissociation concerning sensory responses to the presentation of cue (mainly visual) stimuli during the observation and the execution task. In fact, while this neuronal population was specifically activated during action observation and did not show any response to the presentation of the cue stimuli in the observation task, nevertheless it was strongly activated during visual presentation of the target object during the visuomotor task.

To better investigate possible differences between object presentation responses during the execution and the observation task, we analyzed neurons responding to the presentation of the cue visual stimuli during the observation task. Almost all neurons that responded to the target presentation during the observation task, discharged also during the target presentation in the visuomotor task. This suggests that these neurons, activated by stimuli presented in both the peripersonal and the extrapersonal space, could have a space-invariant coding of objects. In contrast, the majority (70%) of neurons responding to the visual presentation during the visuomotor task did not discharge during the observation task, suggesting that most visually-responsive prefrontal neurons code objects when they are potential target for the monkey's own action, regardless of whether monkey will actively grasp them or not. This proposal has been recently advanced also for ventral premotor neurons responding to the observation of objects and others' actions, showing a strong link of object presentation responses with the monkey's peripersonal space (Bonini et al., 2012). Further recent neurophysiological studies on several parietal areas (V6A, PF, PFG) showed encoding

of objects in the peripersonal space (Rozzi et al., 2008; Hadjidimitrakis et al., 2013, 2011). The well known anatomical link between many of these parietal areas and the ventrolateral prefrontal cortex (Borra et al., 2012; Gerbella et al., 2010) supports the idea that object processing by prefrontal neurons could play a role in the selection of potential targets for subsequent actions, provided that they are located in a reachable peripersonal space.

4.5. Cortical mechanism for the organization of intentional actions

The American psychologist James Gibson (1979) was the first to introduce the concept of 'affordance', which refers to the 'opportunities' provided by a certain object or environment to act on them. Several studies in the subsequent decades have convincingly demonstrated that this concept is not only behaviourally plausible, but it is confirmed at the neurophysiological level, in particular for visually guided grasping motor acts (Sakata et al., 1992, 1994; Murata et al., 1996, 1997, 2000).

For many years the attention of neurophysiologists focused on motor acts. However, motor acts are usually connected in sequences, in order to accomplish simple or complex actions, each endowed with a behavioural goal. The studies on motor sequences for many years concentrated on arbitrarily arranged motor chunks such as pushing, pulling (Hoshi et al., 2000; Wise and Murray, 2000; Miller and Cohen, 2001; Shima et al., 2007; Saga et al., 2011; Yamagata et al., 2012). Recently a series of work studied the role of neurons coding motor acts in natural action sequences and demonstrated that the discharge of grasping neurons in parietal and premotor cortex not only encodes the meaning of the motor act per se, but it is also modulated by the behavioural goal of the action in which grasping is embedded (Fogassi et al. 2005; Bonini et al. 2010, 2011, 2013). These data suggested that intentional actions are coded by neuronal chains in parietal and premotor cortex. These data, together with the results of a model of the functioning of these neuronal chains (Chersi et al. 2011), brought also to the proposal that the selection of a specific action chain in a given context could be achieved not directly within the parieto-premotor circuit, but through others cortical sectors connected with these areas, providing information about context and motivation.

Because of its strong anatomical connections with the parieto-premotor circuit, this sector was proposed to correspond to the ventrolateral prefrontal cortex.

The data presented here, however, do not confirm this hypothesis, suggesting that the role of ventrolateral prefrontal neurons could be not so crucial for the selection of the types of actions used in this study. This might be due to several factors. The first is related to whether an action is part of the usual monkey repertoire or is the result of a learning procedure. In fact, in our study, the stimuli associated to the more natural action (grasp food to eat) are not so specifically encoded by prefrontal neurons. In contrast, the plastic sphere, which is an object arbitrarily associated, through specific training, to the placing action, appears to elicit some selective responses in single neurons and, above all, significantly modulates the populations discharge. Second, while one could have expected an early involvement of the discharge of movement-related prefrontal neurons in the selectivity for the action type, this occurs instead mainly in the latest phases of its execution, thus suggesting that they cannot play a role in predictive selection of neuronal pools in the parietal and premotor cortex. Third, and more importantly, considering the whole population of neurons showing only motor or sensory-and-motor responses, there was no evidence of sustained and object-related activity between the object presentation epoch and the reaching-grasping epoch.

Taken together, these considerations lead to a different hypothesis, namely, that the automatic transformation of sensory information into correspondent motor plans concerns not only 'object affordances', but might be extended to 'behavioral affordances'. Behavioral affordances could be considered as the possible action alternatives prompted by the semantic information conveyed by the object, depending on the internal motivational state. For example, an apple could afford not only different ways of grasping it (with either a power grip or by precision grip on the petiole), but also different possible actions. Some of them could be directly linked to the behavioural meaning of the apple (i.e. grasp-to-eat), while others might more strongly depend by a larger context and further information that could be available (i.e. throwing it against something or someone, placing it in a basket or in the sink to wash it, etc.). Thus, it is possible that not only the object, but also some behavioural affordances are automatically encoded in the parieto-premotor system, enabling a fast and efficient recruitment of the neural machinery subserving the organization of the most common

and behaviourally relevant actions in the animals' repertoire. This would finely explain why previous studies reported an higher number of grasping neurons selective for grasp to eat than for grasp-to-place in the parietal cortex (Fogassi et al., 2005), while a more balanced frequency of these neuronal types was observed in the premotor cortex (Bonini et al., 2011, 2012) and, finally, a considerable prevalence of the representation of the non-food object and related action has been shown, in the present study, in the prefrontal cortex. It appears as if moving rostrally in the parieto-frontal motor system the relevance of learned aspects increases, while it decreases that of behavioural affordances in the action selection and control. Further studies, however, should be carried out by applying the same motor task used in this experiment to the premotor and parietal areas which have been subject to previous investigations, in order to verify the plausibility of the concept of object affordance, to formally study possible visuo-motor responses to the presentation of object with different behavioural meaning, and to empirically investigate whether a gradient in the selectivity for actions triggered by natural stimuli vs learned rules does exist in the parieto-frontal system.

5. CONCLUSIONS

The findings reported in this study do not appear to strongly support the hypothesis derived from the evidences reported by previous studies, because no clear selectivity emerges between the two studied actions, neither during active motor execution nor action observation. However, the selectivity for one of the two type of actions, grasping to place a sphere, emerges at a population level when they are actively executed. Furthermore, concerning the response to the visual presentation, emerges the same selectivity, always at population level, when a sphere was presented. In particular, the selectivity for the sphere was stronger when it represented a potential target of an action.

The movement-related response of ventrolateral prefrontal neurons seems too late to be considered as the representation of a motor intention coding. It probably could better represent a feed-back signal sent from parietal and premotor areas when an action goal is achieved. It is interesting to note that a kind of selectivity is revealed for a learned action, suggesting that the prefrontal cortex could play a role in the association of behavioural instructions to the visual stimuli, in agreement with previous studies using different behavioural paradigms. In contrast, the visual response could be considered as a transformation of contextual information could be turned into specific motor intentions subserving the selection and organization of the parieto-premotor neuronal pools underling the execution of natural goal directed actions.

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