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RUOLO DELLA CORTECCIA PREFRONTALE VENTROLATERALE E PREMOTORIA VENTRALE DELLA SCIMMIA NELLA GUIDA DEL COMPORTAMENTO BASATA SULLE INFORMAZIONI SOCIALI

ROLE OF MONKEY VENTROLATERAL PREFRONTAL AND VENTRAL PREMOTOR CORTICES IN GUIDING BEHAVIOR BASED ON SOCIAL INFORMATION

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1. INTRODUCTION

The ability to use contextual cues for the processing of coherent behavioral responses is fundamental for human and non-human primates. Among those cues, information about the social environment are crucial for action planning. This kind of information mostly derives from the understanding of others' actions, together with the knowledge about the agents' identity. In the last years, a large body of evidence described a neural mechanism involved in coding other's actions, the "Mirror Mechanism", that is theorized to play a crucial role in action understanding (Gallese et al. 1996; Rizzolatti et al. 1996; Ferrari et al. 2003, Rizzolatti & Sinigaglia, 2010; Rizzolatti & Fogassi, 2014).

Neurons with mirror-like properties have been found in a large network including premotor, parietal, and prefrontal cortex (Bonini, 2016; Rizzolatti & Fogassi, 2014), whose lateral sector, the Lateral Prefrontal Cortex (LPFC), is known to be crucial for processing and exploitation of contextual cues for the selection, planning, and guidance of behavioral responses (Tanji & Hoshi, 2008; Yamagata et al. 2012; Yoshida et al. 2011, 2012; Simone et al., 2015, 2017; Rozzi & Fogassi, 2017, Bruni et al., 2015).

Specifically, the ventrolateral part of LPFC is involved in first-order executive processes such as active retrieval and selection of information, whereas the dorsolateral part is more involved in higher-order executive components of behavioral planning, including monitoring, manipulation, and integration of multiple pieces of information (see Tanji & Hoshi, 2008; Fuster, 2001).

Until recently, the prefrontal cortex was deemed to be a part of the so called "associative" cortices (Fuster, 2001). The role traditionally assigned to these cortices is related to the integration of the converging multimodal sensory stimuli, already processed at lower levels, in order to form higher order sensory representations that ultimately enable the perception of space, the categorization of stimuli and the processing of behavioral strategies (Simpson & Fitch, 1988, Corbetta & Shulman, 2002, Gilbert & Sigman, 2007).

Studies conducted on both human and non-human primates undermined a simplistic view of hierarchical organization of mental functions, leading to the proposal of a more likely functional model in which different cortical areas (including LPFC) provide specific contributions to different functions, and specific group of areas are organized and integrated in a series

of neural networks underlying different cognitive, emotional and behavioral functions.

Each mental function, for this model, is thus based on a series of processes that are distributed on a network that involves different cortical areas, each cooperating and sending information and inputs to the other, rather than a hierarchical unidirectional flow of information in which higher order areas control lower level areas, which simply execute the commands.

Note, however, that this does not mean that each area can play each type of role: within a specific network, each area has a specific role, and contributes to the general network function, and often an area participates to multiple functions being involved in different networks. Here we present a study based on this general theoretical framework, aimed at assessing the specific role of different areas of VLPFC in action planning and executing based on biological and non-biological cues.

1.1 The Mirror mechanism

Behavior guidance based on the processing of contextual cues relies (like already said above), in a fundamental way, on information extracted from the social environment, in particular from the observation and

understanding of others' actions. According to a classical conception of the evolution of social cognition, the reading of others' behavior is mediated by processes of inference and mentalization that presuppose the existence of dedicated modules of the mind. These processes would part of a mind-reading mechanism called "Theory of Mind" (Premack & Woodruff, 1978; Baron-Cohen et al., 2013; Meunier, 2017). According to this formulation, individuals would be able to read the behavior of others through the ability to attribute mental states such as emotions, desires, intentions etc. However, this view poses several problems because, from a neurobiological point of view, it does not propose any model on the possible neural mechanism operating in mentalization processes. In fact, although several neuroimaging studies indicate the activation of specific areas of the brain in human subjects subjected to inference tasks, they do not give us any information about how mentalization processes operate at the neuronal level.

According to another hypothesis, it is possible to recognize an action observed (thus, to exploit this information to guide behavior) because its observation activates, in our brain, the motor representation of that particular action (see Gallese, 2006). A crucial role in action understanding, thus, would be played by the "Mirror mechanism", defined as the neural

mechanism that unifies perception and action, transforming sensory representations of the behavior of others into motor representations of the same behavior in the brain of the observer (Rizzolatti & Sinigaglia, 2010. This mechanism has been firstly discovered in the ventral premotor cortex of the macaque monkey (area F5) (Rizzolatti et al. 1996; Gallese et al. 1996; Ferrari et al. 2003, Rizzolatti & Fogassi, 2014), particularly in its cortical convexity, and later in the convexity of the inferior parietal lobule (PFG) (Fogassi et al., 2005; Rozzi et al., 2008, Bonini et al., 2010), where populations of mirror neurons, endowed with the property of discharging both when executing an act or observing the same act, have been described.

The discovery of mirror neurons occurred during a series of studies aimed at investigating, by recording single neurons, the functional properties of the macaque's ventral motor cortex (area F5), an area involved in the execution of finalized motor acts performed with the hand and mouth.

During these experiments, it was observed that a subpopulation of motor neurons, which were activated during the grasp performed by the monkey, were also activated during the observation of the same action performed by an experimenter, as if the monkey itself had grasped it.

After excluding that these responses could depend on imperceptible movements performed by the monkey, or on food expectation, it was

assumed that their coding concerned the motor representation of the motor act, regardless of who was performing it.

Mirror neurons, therefore, are a class of neurons that is activated both when the monkey performs a finalized motor act, and when it observes another individual or its conspecific performing the same motor act (Gallese et al. 1996; Rizzolatti et al. 1996; Ferrari et al. 2003, Rizzolatti & Fogassi, 2014). Neurons with this property have been observed in several cortical areas that are anatomically connected, forming a functionally integrated circuit endowed with the "Mirror mechanism". (Rizzolatti & Sinigaglia, 2010; Bonini, 2016).

1.2 Properties of F5 mirror neurons

1.2.1 Coding of the actions' goal, action understanding and the concept of motor representation in the cerebral cortex

One of the most important challenges that researchers have faced, following the discovery of the mirror mechanism, has been to shed light on its possible functional role played. After rejecting the possibility that they are only a preparatory class of neurons, it has been proposed that it underlies

the recognition and understanding of the meaning of observed motor acts (Gallese et al., 1996; Rizzolatti et al., 1996, Rizzolatti, & Fogassi, 2014).

Planning and executing an action involve having an intention, selecting an appropriate sequence of motor acts, each of which has its own immediate goal, and executing the sequence of movements that constitute each motor act. This view on action structuring implies that different elements (movement, motor act, action) are organized in different hierarchical levels. To achieve the whole action's goal, the individual elements must be linked to each other in a precise temporal structure to generate the "kinetic melody" (Luria, 1973) that characterizes normal behavior.

While the primary motor area F1 is fundamental for the execution of the action, the ventral premotor area F5 plays an important role in coding the goal of motor acts and motor intention. Single neuron recordings show that neurons in the monkeys' ventral premotor and posterior parietal cortices (area F5 and PFG) are activated when the monkey performs purposeful actions, such as grasping an object. Specifically, they are activated following the performance and/or observation of motor acts such as grasping, holding, manipulating or ripping.

The first studies conducted on F5 mirror neurons (Rizzolatti et al., 1996; Gallese et al., 1996) found that they are activated only when the observed action involves an interaction between effector (hand or mouth) and object. These neurons, however, do not present any discharge during the observation of a mimed action (absence of actual interaction with the object) or following the "mere" presentation of an object (before any interaction has occurred). Furthermore, the discharge is weak when grasping occurs through motor sequences outside the monkey's motor repertoire (e.g. using tools such as pliers).

F5 mirror neurons, based on the comparison between visual and motor responses, have been mainly distinguished into strictly congruent and broadly sense (Rizzolatti et al., 1996). In the former we see a high correspondence between the act observed and performed, for example when the animal observes a specific rotation of the experimenter's hand manipulating a piece of food and when it grasps food by performing the same type of rotation. In the latter, however, we can see a congruent but not identical encoding in visual and motor terms, presenting different levels of generalization (Rizzolatti and Sinigaglia, 2006), for example a neuron that is activated when the monkey observes the experimenter grasping an object with a precision grip or with a force grip, while at the motor level it

responds only when the animal grasps the object with a precision grip. Thus, the neuron encodes the general purpose of the action, but the motor response is more specific than the visual response (Rizzolatti et al., 1996).

These neurons do not simply code movements involving specific muscle bundles: movement is represented in a more generalized way, and this information is used for other processes that do not necessarily have to be purely motor in nature (Bonini et al. 2010).

An example of how F5 neurons code motor acts in terms of goal, rather than specific movements, was provided by a study in which the same goal (taking possession of food) was achieved with completely opposite movements (Umiltà et al., 2008). Monkeys took food using normal pliers (similar to ice pliers) that require closing the hand to take possession of the object, and inverted pliers (similar to escargot pliers) that instead require opening the hand to achieve the same purpose. A population of F5 neurons coded the achievement of the goal (taking possession of the target food) regardless of the specific movement required to achieve it (finger flexion or extension).

To understand whether mirror neurons code the purpose of a motor act, it is necessary to test their activation during the observation of an action and, subsequently, whether they also respond to sensory inputs not

dependent on the observation of the same motor act. This aspect is crucial for establishing the "generalization" ability of mirror neurons.

Thus, if these neurons were involved in the process of understanding the goal of a motor act in a general sense, their activity should reflect the meaning of the observed motor action, not simply its visual characteristics. In this regard, two experiments were conducted: the first investigated whether F5 mirror neurons were able to recognize the motor act by its sound, while the second investigated whether these neurons where active even if the action execution was not fully visible and, thus, the understanding of the actions' goal was based on memory clues.

Kohler and coworkers (2002) studied mirror neurons while the monkey was observing a motor act characterized by a typical sound, such as tearing a sheet of paper or breaking a peanut, and while the same sound was presented without viewing the corresponding motor act. The results show that about 15% of mirror neurons respond to the presentation of motor acts accompanied by the corresponding sounds and also respond to the presentation of the sound alone.

The second experiment, conducted by Umiltà and collaborators (2001), started from the assumption that, if mirror neurons respond to the purpose of the motor act, then they should be activated even when the monkey does

not see the action, but has sufficient clues to create a mental representation of what the experimenter is performing. The authors demonstrated how cells that discharge during the observation of a grasp, continue to discharge even when the final part of the action (the moment of interaction between hand and object) is obscured.

It seems, therefore, that the meaning of the action is "extracted" regardless of the complete vision of the action, thanks to a prior knowledge of the context, and in particular the memory of the presence of the object. The motor act and its purpose are coded by mirror neurons even if, during execution, the vision of the hand-object interaction is missing: the discharge of these neurons, therefore, reflects the activation of an internal representation of a "potential motor act" that allows to integrate the missing part of the action itself by recognizing its overall meaning. Consequently, the response of mirror neurons during the observation of others' motor acts has been interpreted as a fundamental element in the process of recognition of a specific motor act, in the sequence of movements observed, and differentiation of the type of action observed, recognizing it as part of their motor vocabulary.

On the basis of this evidence, it has been proposed that the F5 area contains a "vocabulary" of motor acts (Rizzolatti et al., 1988) made up of

"words" each of which is represented by a population of neurons. Some encode the general purpose of the motor act, others the specific mode of execution, and still others specify the temporal aspects of the act to be performed (Jeannerod et al., 1995).

1.2.2 Coding of space and point of view

Object and others' actions may have different relevance for the observer and therefore lead to different behavioral responses, depending on the regions of space in which they occur. In a recent study, Caggiano and collaborators (2009) wanted to investigate whether the activity of mirror neurons is modulated by the position in space of the action being observed, showing that about half of them are activated differently when the observed action is performed in the peripersonal or extrapersonal space of the monkey.

Moreover, part of these mirror neurons, seems to encode space according to a metric representation, while others in operational terms, namely encoding space in terms of the possibility of acting on it. The space in which the motor act takes place therefore plays a fundamental role in the choice of behavioral reaction to the stimulus with which the subject interacts.

To test this hypothesis, the authors analyzed the effects of the relative distance between the observer and the person performing the action. Although, in fact, it is completely irrelevant to the understanding of the action itself, precise knowledge of distance is crucial for choosing the most appropriate reaction and assessing the possibility of interaction.

Some neurons, which responded exclusively during observation of the act in extrapersonal space, were also studied by performing the same act in the monkey's reaching space, with the interposition of a transparent screen that allowed the monkey to observe the action, but made it impossible for it to physically act in that sector of space, thus transforming a metrically close space into a pragmatically distant one.

In this setting, some of the “extrapersonal” selective neurons would discharge during observation, as if the action were taking place in the extrapersonal space. We can therefore conclude that it is the possibility of action, and not simply geometric distance, that determines how we encode actions performed by others and their spatial location. These considerations thus suggest that mirror neurons respond differentially to motor acts performed in different regions of space. The distance between observer and actor actually plays no role in understanding the meaning of an observed motor act; however, it is important for assessing subsequent behaviors.

Many experiments on mirror neurons have been performed using what is called "naturalistic testing". It consists of recreating, during the experiment, a realistic interactive condition, with the experimenter placed in front of the monkey and manipulating food or performing a variety of other actions. However, the accuracy of this type of study is limited by the variability of human movements, making it difficult to have standardized trials. To address these issues, Caggiano and collaborators (2011) investigated, in the first part of their study, if there was a difference in mirror neurons' discharge between the observation of an action in a naturalistic setting (executed by an experimenter placed in front of the monkey) and the observation of filmed actions, depicted on the screen. The authors found a subpopulation of neurons that were equally responded to filmed actions and to action executed by the experimenter placed in front of the observer. This allowed them to demonstrate the validity of the "artificial" approach, given that filmed actions, presented on the screen, can elicit responses in mirror neurons similar to those observed during a "naturalistic" setting.

This study also aimed to understand whether mirror neurons also contribute in coding the perspective from which one observes the motor acts of others. To do so, the visual responses of mirror neurons in the

monkey's F5 area were recorded using the presentation actions filmed from different visual perspectives: Monkeys' point of view (subjective point of view: 0°), Lateral perspective (90°) and Frontal point of view (180°). From the study, it was found that more than half of the neurons activated during stimulus presentation showed a significant discharge preference for at least one perspective, and only a minority showed viewpoint-independent responses.

The presence in the F5 area of mirror neurons that are activated independently of the perspective of observation of an action performed by others could be important in encoding the observed action, potentially in terms of the motor goal and independently of the characteristics of its visual details. More difficult, however, is the interpretation of the responses of neurons dependent on the perspective of observation (Caggiano et al., 2011).

1.3 Properties of PFG mirror neurons

Mirror neurons having functional properties similar to those recorded in F5 have also been identified in the rostral portion of the inferior parietal cortex (PFG area). In the early 1980s, Hyvarinen and collaborators (1981, 1982) observed, in this area, the presence of neurons responding both to

sensory stimuli and during the execution of movements, Successively, it was demonstrated that motor neurons in the inferior parietal cortex code for finalized motor acts and that visual and tactile properties are, almost always, also present at the level of single neurons (Rozzi et al., 2008).

In particular, visual neurons in the PFG area respond to the observation of stimuli in the peripersonal field and to the observation of biological movements, including finalized actions (Gallese et al., 2002; Fogassi et al., 2005; Rozzi et al., 2008). Furthermore, an influential experiment on parietal mirror neurons showed that, during the execution of a grasping act, the activity of a subpopulation of them is modulated depending on whether the monkey eats the target food of the movement or puts it in a container. The same type of modulation also occurs when the monkey observes the experimenter performing either action (Fogassi et al., 2005).

The differential discharge of mirror neurons could reveal a mechanism by which the monkey can predict the final purpose of the observed action (placing or eating). It has been proposed, therefore, that such activation requires that mirror neurons also receive contextual information independent of the observed movements.

These parietal regions, as the premotor cortex (and in particular with the F5 area), have important connections also with specific prefrontal and

temporal areas located at the level of the superior temporal sulcus (Pandya & Seltzer, 1982; Rozzi et al., 2006). This network could constitute the nervous circuitry by which the visual description of the observed action is associated with the motor program that the observer uses to actively perform the same action.

1.4 Visual properties of STS neurons

The presence of neurons in the premotor cortex that are activated both during the observation and during the execution of the same motor act raises the question of how visual information related to observed movement can be combined with their motor representation. The origin of visual information could be found in a high-order multisensory area located at the level of the Superior Temporal sulcus (STS), which is connected to F5 by means of PFG (see below), where neurons selectively activated during the observation of biological movements have been observed (Perrett et al. 1989; Allison et al. 2000; Puce & Perrett 2003).

It has also been shown that some STS neurons not only respond to the observation of different purpose-directed hand actions, such as grasping, tearing, and manipulating objects (Perrett, 1989,1990 ; Jellema et al., 2000), but also appear to be modulated by the shape of the object involved in the

action observed. These neurons also show no response when the experimenter's hand is replaced by a tool, such as pliers.

STS neurons have been studied in relation to their visual responses, however the presence of any motor responses has never been researched and demonstrated (Keysers & Perrett, 2004). For these reasons, therefore, it is still unclear what role these cells play in understanding observed actions, if we accept the hypothesis that understanding of actions is mediated by the mirror neuron system.

1.5 Neural circuits involved in action observation

Since the 1990s, numerous works have been conducted with the aim of studying the neuronal mechanisms responsible for action observation in non-human primates and the neural circuits involved. After the identification of mirror neurons in the premotor and parietal cortex, functional magnetic resonance imaging (fMRI) studies (Nelissen et al., 2005, 2011) showed that several regions are activated during the observation of grasping actions. Specifically, the monkey was shown several videos depicting finalized actions, conducted by different agents or non-biological effectors, together with their static counterparts: objects and controls obtained from "scrambling" procedures of the above.

The results show that action observation activates the ventral premotor cortex, the inferior parietal cortex, a large region of the temporal lobe located at the level of the STS, and a large region of the ventrolateral prefrontal cortex (which will be discussed in more detail below). In particular, in the periarcuate region the observation of videos showing decontextualized hands grasping objects (after subtraction of the relative static contrast) activated several cortical areas, such as the premotor areas F5a and F5c and the prefrontal area 45B; the observation of videos showing a full-length subject grasping objects (after subtraction of the relative static contrast), instead, activated only the area F5c (Nelissen et al., 2005). In a subsequent study, conducted by the same team (Nelissen et al., 2011), the activation of the monkey's superior temporal sulcus and posterior parietal lobe regions during observation of the same videos was examined.

The results show that extensive regions of the STS, such as MT/V5 (middle temporal cortex), FST (fundus of the superior temporal area), LST (Lower Superior Temporal Region**),** LB2 (lower bank of superior temporal sulcus), STPm (superior temporal polysensory area), and some parietal areas such as PFG and AIP (anterior intraparietal area), were activated during the observation of both stimuli described above. In order to understand which of the different activated areas of STS was sending

information to the two parietal areas involved in encoding the grasping act, retrograde neural tracers were injected, which allowed tracing the cortico-cortical connections of the injected areas of interest (Rozzi et al., 2006; Borra et al; 2008; Gerbella et al, 2010; Nelissen et al., 2011). These works indicate that areas with a similar activation profile are also anatomically connected to each other, and these connections indicate that the observed action information, encoded by STS, is sent to the ventral premotor cortex (F5) via two distinct circuits.

The first one connects the upper bank of STS with the PFG area, which in turn is connected to the premotor area F5c; the second circuit instead connects the ventral part of the lower bank of STS with the premotor areas F5a and F5p through AIP.

Although both temporo-parieto-frontal functional circuits transmit visual information regarding the coding of the grasping act, the STPm-PFG-F5c pathway appears to be more sensitive to the presence of the agent in the video, and thus could play a role in contextualizing the action and extracting its underlying intention; instead, the LB2-AIP-F5p/F5a circuit seems to be more focused on the target of the action, and therefore fundamental in understanding the goal of the motor act, not only in terms

of how to grasp an object, based on its intrinsic properties, but also in terms of different motor actions performed with the hand.

An additional possible circuit responsible for action observation is represented by LB1-LIP-45B (Nelissen et al., 2011), suggesting that information about observed actions can be used for oculomotor control and also involves the ventrolateral prefrontal cortex (Flanagan & Johansson, 2003; Gerbella et al., 2010). Neuroimaging studies in macaques show that a large prefrontal region including areas 45A, 45B, 46v, and 12 is activated during action observation. The area corresponding to area 45A is activated during observation of others' motor acts (Nelissen et al., 2005).

Projections reaching area 45A from the multisensory area STP, which is also activated during the viewing of different biological movements (Oram & Perrett 1994; Barraclough et al., 2005; Jellema & Perrett 2006), suggest that these two areas are part of a network involved in the processing of visual aspects present in the communicative behaviors of the species. Area 45B, on the other hand, is activated following the presentation of images of objects and the observation biological actions performed on them (Nelissen et al., 2005) and the presentation of images of faces (Tsao et al., 2008): this is in agreement with the connections that this area presents with

the TEa (anterior part of the inferior temporal cortex), site of visual processing of three-dimensional objects (Janssen et al., 2000a).

1.5.1 Recent findings about LPFC involvement in action observation

Recent functional (Nelissen 2005; Simone et al., 2015, 2017) and anatomical (Borra et al., 2015; Gerbella et al., 2010, 2013; Saleem et al., 2014) evidence showed that the lateral sector of the prefrontal cortex can be considered as a crucial node of the "Mirror System", with a possible specific role in the organization of socially driven behavior, based on the exploitation of contextual cues such as others' actions (Bonini, 2016, Rozzi & Fogassi, 2017). The details about the anatomical and functional properties of the prefrontal node of the mirror system, specifically the ventral sector of LPFC, will be described in the following sections of this introduction.

1.6 Prefrontal Cortex

1.6.1 Phylogeny and ontogeny of the prefrontal cortex

The development of the nervous system starts in a relatively late phase of the embryogenesis, preceded by the generation of three principal cell layers, namely the endoderm, mesoderm and ectoderm, from which derive the principal structures of the peripheral and central nervous system. The ectoderm gives rise to the neural tube starting from which, in turn, originate three cerebral vesicles: forebrain, midbrain and hindbrain.

The development of the vesicles, thereafter, gives rise to the principal regions of the central nervous system of the adult brain, among which the cerebral cortex is the last to develop fully. The cortex, in adult subjects, is divided in four lobes: occipital, temporal, parietal and frontal, each of which contains several functionally distinct regions that subserve different roles in the processing of informations. The frontal lobe is the most anterior portion of the brain, and in humans it's traditionally divided in two portions:

- agranular motor frontal cortex, which is in turn subdivided in primary motor cortex (BA 4) and secondary motor areas (BA 6, including pre-motor cortex and both supplementary and pre-supplementary motor areas - SMA and Pre-SMA)

- granular prefrontal cortex, which constitutes an extended network linking motor regions with regions that process perceptive stimuli and emotions (Goldman-Rakic, 1995; Passingham, 1995).

The phylogenetic development of the prefrontal cortex, and its increase in relative size in human primates' brain compared to non-human primates' brain, can be inferred by studying the latter. The "regio frontalis", which correspond to the PFC (Brodmann, 1909, 1912), occupies 29 percent of the cortex in humans, 17 percent of the cortex in chimpanzees and 11.5 percent of the cortex in macaques. Another comparative study (Semendeferi et al., 2001), focused on the anterior or fronto-polar prefrontal cortex (BA 10), an area involved in complex cognitive functions.

The frontal pole can be found in various primates like bonobos, orangutans and gibbons, presenting similar cytoarchitectonic characteristics but varying in its organization between species (specifically there area variations regarding the width of its cortical layers and the space available for connections). The frontal pole of an hominoid like the gorilla appears highly specialized, while area 10 in the gibbon occupies only the orbital sector of this region. In humans, area 10 is larger ,relative to the rest of the brain, than it is in the other apes and presents more space for connections with higher order association areas.

The relative growth in dimension (and connectivity with other regions) of the PFC in humans, compared to the other apes, suggests that this region could represent the neural substrate underlying the various complex cognitive processes that constitute a "patrimony" of our species and that resulted from the phylogenetic differentiation with the other apes (J. Fuster, 2015b).

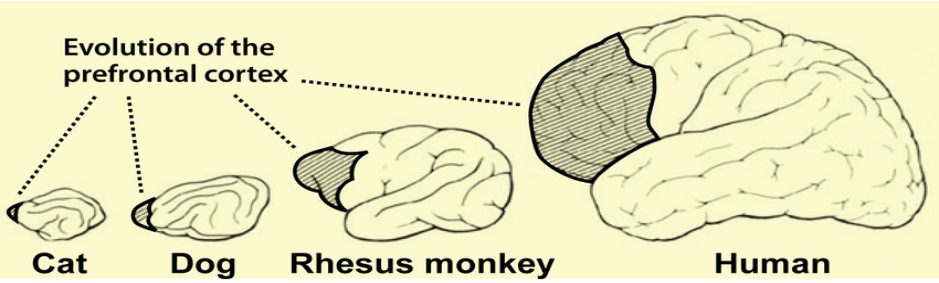

Figure 1: Cortical area occupied by the PFC in various species. (Wunsch, 2017)

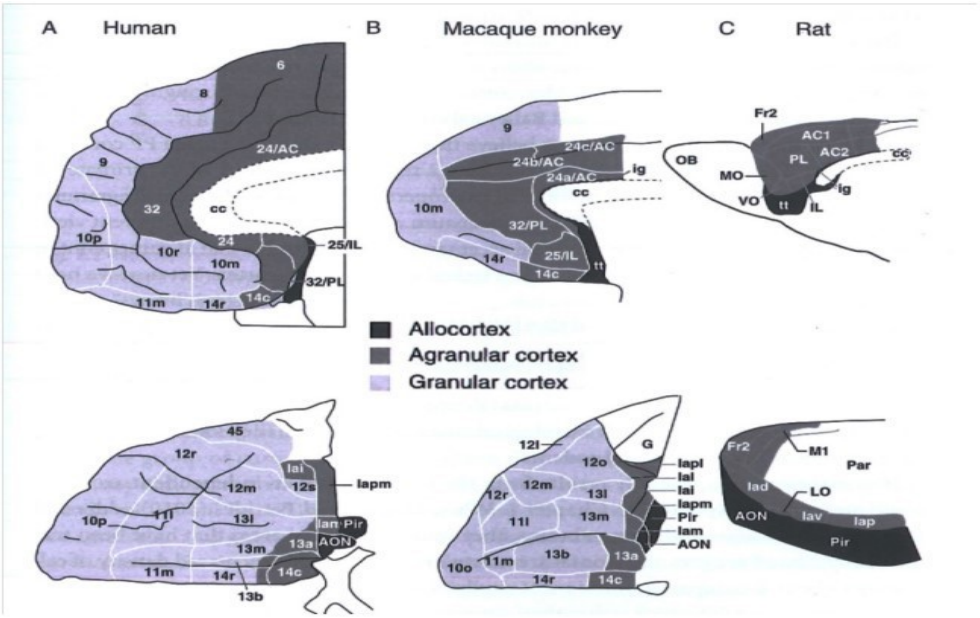

Figure 2: Differences in the dimension of the medial (in the upper part) and orbital (lower part) PFC in human, macaque and rat brain.

Other than representing the last part of the cortex to develop, both phylogenetically and ontogenetically, the PFC can be defined as the most complex region of the brain. Its complexity is represented by the numerous gyri and sulci that are present in this region, which become more evident and irregular when proceeding through the evolutionary scale of the mammals. The complexity of this region progresses until reaching a grade of differentiation and development such as the one that characterizes the hominoid PFC and especially the human primates, in which this region occupies (as said at the beginning) almost a third of the entire cortex.

Evaluating the ratio between the volume of the frontal lobe and the volume of the entire cortex in various species such as gorillas, bonobos, chimpanzees, orangutans and macaques, it has been demonstrated that the frontal lobe always occupies from 20 to 30 percent of the entire cortex (Table 1). It is obvious, given the data described above, that these regions subserve a fundamental role not only in humans but in other apes too, being in the latter cases strongly developed and occupying a significant part of the entire cortex.

	(Brodmann, 1909)*	(Blinkov & Il'ja, 1968)*	(Semendeferi, et al., 2002)**
Human	36.3	32.8	37.7 (± 0.9)
Chimpanzee	30.5	22.1	35.4 (± 1.9)
Bonobo	NA	NA	34.7 (± 0.6)
Gorilla	NA	ND	35 and 36.9
Orangutan	NA	21.3	37.6 (± 1.1)
Gibbon	21.4	21.2	29.4 (± 9.8)
Macaque	NA	NA	30.6 (± 1.5)
Cebus	22.5	NA	29.6 and 31.5

*Table 1: Relative size of the frontal cortex, expressed in percentage of the size of the entire cortex (Semendeferi et al., 2002). *Surface of frontal cortex in percentage of surface of cortex of cerebral hemispheres. **Volume of frontal cortex in percentage of volume of cortex of cerebral hemispheres. NA, not available*

Neuroimaging studies indicated that, in humans, the prefrontal cortex does not develop fully until adolescence (Chugani et al., 1987; Paus et al., 1999; Sowell et al., 1999). These results are in line with experimental evidence indicating that the higher order cognitive functions, which are subserved by the PFC, such as abstract reasoning, are indeed the last to emerge in the developmental processes (J. M. Fuster, 2001).

Neurophysiological studies in monkeys and neuroimaging studies in humans have provided a wide view upon the basic activity of PFC, the functions subserved by the various areas found in this region and its numerous connections with other areas throughout the brain. The PFC is composed of various interconnected areas, which, in turn, are connected and communicate virtually with all cortical sensory and motor system and even with subcortical areas (E. K. Miller & Cohen, 2001).

Thanks to the strong interconnection with other networks and systems throughout the brain, the PFC is involved in numerous high order cognitive function such as planning and execution of goal-directed behaviors, organization and regulation of emotional behavior, integration of information acquired from the environment, processing in the short-term memory. The heterogeneity of this region entails a variety of processes and

outputs, which constitute the so-called "executive functions", that cannot be traced back to specific areas and that allow the individual to adapt its behavior to different social contexts and in a changing environment, full of information to be acquired and used to plan actions directed to specific goals.

1.6.2 Anatomo-functional organization of the macaque prefrontal cortex

The primates' PFC is divided in three regions: lateral, medial and ventral or orbitofrontal. The lateral prefrontal cortex (LPFC) of human primates is characterized by the presence of two sulci originating from the precentral sulcus: the superior frontal sulcus, which separates the superior frontal gyrus from the middle frontal gyrus, and the inferior frontal sulcus, which separates the middle from the inferior frontal gyrus.

One of the most useful experimental models available to study the anatomo-functional organization of the primate brain is the macaque, given the similarity of its brain with the human counterpart. The macaque PFC is indeed characterized by the three great subdivisions described in the human brain (Passingham, 1995; J. Fuster, 1997), but, other than this homology,

there are also significant differences: the lateral portion of the prefrontal cortex of macaques is subdivided in two subregions (dorsal and ventral) by the principal sulcus.

Starting from the first quarter of the 20th century, several authors focused on studying the architectonic structure of the cerebral cortex (including the PFC) of humans and macaques. The different parcellations of the PFC available at the moment are not entirely congruent.

The discrepancy between the architectonic "descriptions" of human and macaques PFC is due to the fact that, in experimental investigations of the macaque monkey performed during the last 50 years, the architectonic nomenclature and criteria used to describe the areas found in the prefrontal cortex has been largely based on the map by Walker (Walker, 1940), which was not based on a comparative investigation of the cytoarchitecture of the human and macaque monkey prefrontal cortex. As a result of this, the criteria frequently used for demarcating areas in humans and macaques are not always consistent. The discrepancies existing between different parcellations of the macaque PFC is instead determined mainly by the progressive improvement of histological techniques and the development of new techniques through time.

Petrides and Pandya (Petrides & Pandya, 1994,1999,2002a) proposed a parcellation of the PFC, specifically comparing the macaque and human prefrontal cortices (Figure 1.3), as to resolve the discrepancies existing between the descriptions of this region in the two primate species, due to the problems described above.

If we consider the architectonic structure of the PFC in the human and macaque brain, as defined by Petrides and Pandya, it is clear that the two are very similar since the only differences consist in the total area occupied by the whole prefrontal cortex (higher in the human brain) and the presence of few areas that have been localized in the PFC of only one of the two species: area 44, found in the human LPFC but not in the macaque LPFC, area 25, found only in the macaques orbital PFC, and area 45A, found only in both lateral and orbital human prefrontal cortices (while it is localized only in the LPFC of the macaques brain).

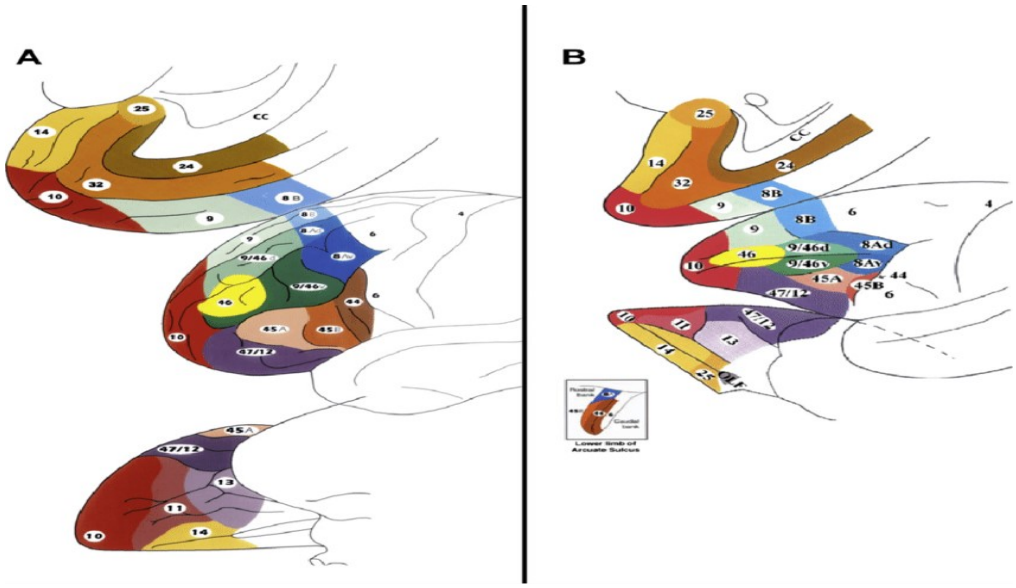

Figure 3: Cytoarchitectonic maps of the PFC in human (a) and macaque (b) brain as parcellated by Petrides and Pandya in 1994 (Petrides & Pandya,1994)

The prefrontal cortex is extensively connected with various areas, including the parieto-premotor circuits (and therefore, with the mirror neurons contained in these areas). The LPFC represents the region of the prefrontal cortex most connected to motor areas, basal ganglia and cerebellum, and it is through these extensive interconnections that it exerts its control on motor behaviors (Tanji & Hoshi, 2008)

This region is anatomically connected, both directly and indirectly, also to other cortical areas such as the associative temporo-parietal cortices, the

limbic cortex and several subcortical structures. The LPFC can be divided in a dorsal and ventral portion (DLPFC and VLPFC) which are involved in different networks (Figure 1.4). The mediodorsal network (that includes DLPF) receives inputs from multimodal areas situated in the temporal cortex or auditory areas of the superior temporal gyrus and it is involved in the processing of spatial informations. The orbitoventral network (which includes VLPF) mostly receives sensory inputs from visual, auditory, somato-sensory, gustatory and olfactory areas, and it is involved in the processing of non-spatial information.

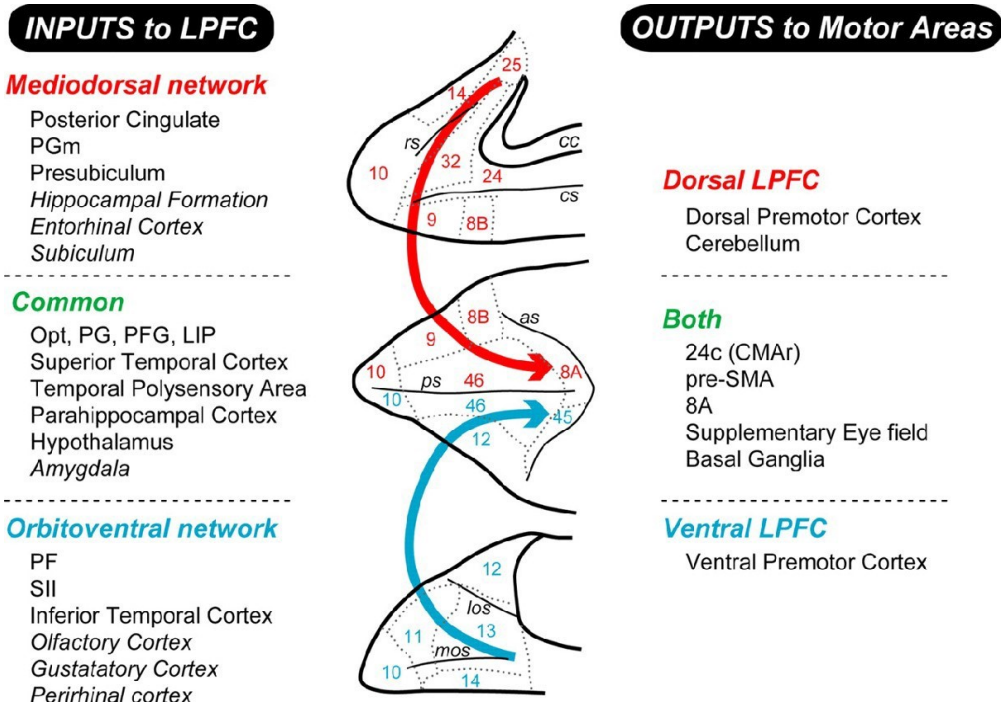

Figure 4: Schematic illustration of the cytoarchitecture of the prefrontal cortex and input-output organization of the lateral prefrontal cortex (LPFC). (Tanji & Hoshi, 2008). Rs, rostral sulcus; cs, cingulate sulcus; cc, corpus callosum; as, arcuate sulcus; ps, principal sulcus; mos, medial orbital sulcus; los, lateral orbital sulcus. PF, PFG, PG, PGm, and Opt are subareas in the parietal cortex (Pandya & Seltzer, 1982). SII, secondary somatosensory area; LIP, lateral intraparietal area; CMAr, rostral cingulated motor area; pre-SMA, pre-supplementary motor area.

In the middle column of figure 1.4, the middle panel shows the cytoarchitectonic boundaries of the LPFC (Walker, 1940). The top and

bottom panels show the cytoarchitectonic boundaries of the medial and orbital prefrontal cortices, respectively (Carmichael & Price, 1994). The medial cortex (top panel) is shown upside down. In the orbital cortex (bottom panel), the midline is along the bottom. Rostral is to the left in all views.

The red and blue arrows indicate the mediodorsal and orbitoventral trend, respectively, of cytoarchitectonic differentiation, according to Barbas and Pandya (Barbas & Pandya, 1989). The red-colored areas in the medial prefrontal cortex and the dorsal LPFC belong to the mediodorsal network and the blue-colored areas in the orbital prefrontal cortex and the ventral LPFC belong to the orbitoventral network. In the left column, trends of inputs to these networks are summarized. Inputs are classified into three categories: areas preferentially projecting to the mediodorsal network (top), areas preferentially projecting to the orbitoventral network (bottom), and areas commonly projecting to both networks (middle).

Areas chiefly projecting to the orbital or medial prefrontal cortex, but less to the LPFC, are italicized. In the right column, trends of outputs from the lateral prefrontal cortex to major motor areas are summarized: areas to which the dorsal LPFC preferentially projects (top), areas to which the

ventral LPFC preferentially projects (bottom), and areas to which both parts of the LPFC project (middle).

The LPFC is directly or indirectly connected with widespread structures in the brain through the mediodorsal and orbitoventral networks. Additionally, the two networks are also extensively interconnected, and this organization allows the lateral prefrontal cortex to integrate multiple sets of information on a large scale, playing a fundamental role in collecting, integrating, sorting and modulating the diverse sets of "data" processed in other parts of the brain (Tanji & Hoshi, 2008; Preuss & Goldman-Rakic, 1989; Pandya & Yeterian, 1991; Petrides & Pandya, 2002).

The LPFC is interconnected with premotor areas, the basal ganglia, and the cerebellum. Through these connections, the LPFC can control broad aspects of finalized motor behavior. Moreover, the LPFC modulates the flow of information in other areas of the central nervous system, in conforming to behavioral requirements, serving as a center for the control and sorting of information flowing through cortical and subcortical structures (Tanji & Hoshi, 2008).

1.6.3 Functions of the prefrontal cortex

Thanks to its extended interconnection with other cortical and subcortical areas, the prefrontal cortex has access to a diverse set of data regarding both the internal state of the subject and the external world, being therefore fundamentally involved in a broad spectrum of emotional and cognitive processes such as planning and temporal organization of actions (process in which the integration of multisensory informations plays a fundamental role), selection of appropriate behavioral responses in relation to the social context (included in the broader category of the executive functions), inhibitory control, emotion regulation and expression, attention, working memory (Barbas et al., 2003; Tanji & Hoshi, 2008; Gray et al., 2002). This broad spectrum of functions will be further exposed in the next paragraphs.

Sensory functions

Several studies demonstrated the presence of neurons in the monkey PFC that code visual (L. M. Romanski, 2007), acoustic (Sugihara et al., 2006; Genevieve & Petrides, 2007; Belmalih et al., 2009) and somatic (L. M. Romanski, 2007) stimuli. One of the regions most involved with the

processing of sensory stimuli is the ventrolateral prefrontal cortex, in that neural cells showing a strong response to visual stimuli have been found specifically in the pre-arcuate regions (including area 8/FEF) and in the lateral surface of the prefrontal convexity (Sugihara et al., 2006). Some neurons show a response to complex stimuli such as faces (O'Scalaidhe et al., 1997) or to the presentation of food in both visual and gustatory modalities (Thorpe et al., 1983a). In particular, the ventrolateral prefrontal cortex seems to be designated to the coding of others identity. Neurophysiological studies have in fact evidenced the presence of neurons in this region coding faces that are either static (O'Scalaidhe et al., 1997; O'Scalaidhe et al., 1999) or associated to congruent vocalizations (Sugihara et al., 2006; L. Romanski & Diehl, 2011). The latter response described is related to multisensory neurons specifically coding complex audiovisual communication stimuli, which activity is thus related to the presentation of a conspecific vocalization matched to the corresponding facial gesture (L. M. Romanski, 2007; L. Romanski & Diehl, 2011; Sugihara et al., 2006).

In addition, other authors have found PFC neurons both during the presentation of visual and auditory stimuli and during the execution of motor tasks (Nelson & Bignall, 1973; Schechter & Murphy, 1975; Benevento et al., 1977; Ito, 1982). The evidence here described clearly the fundamental

involvement of the prefrontal cortex, specifically LPFC (which receives multiples inputs from sensory areas and multimodal association areas), in the integration and processing of multisensory inputs aimed to efficiently plan and execute behavioral responses appropriate to the context in which one operates and to the information available in it. This aspect will be further discussed later in this introduction

Executive functions

The term "executive functions" refers to the processes that allow to set behavioral goals, plan, execute and monitor the output of a sequence of responses aimed to reach those goals and, if necessary, to modify ones behavioral responses in order to adapt it to a new situation and new conditions. This broad category included numerous coordinated "sub-processes" aimed principally at selecting actions appropriate to the context (and aimed to reach a specific goal). Neuropsychological data (among other evidences derived electrophysiological studies) allowed to localize executive functions in the prefrontal cortex, in that a lesion in this region determines the so called "disesecutive syndromes" which are characterized by significant deficits in elaborating a behavioral strategy in new or unusual situations.

Working memory

The term "working memory" refers to a system that allows us to acquire, memorize and manipulate information that are fundamental for the complex cognitive processes necessary to the control of behavior (Collette & Van der Linden, 2002). Although there is still no clear evidence about the specific localization of the central components of the working memory, it is possible to assume that a great part of the neural substrate that subserves this system is localized in the lateral prefrontal cortex. This assumption is based on electrophysiological studies performed on monkeys which provided evidence regarding the presence of neurons in the LPFC that are activated during the latency period that follows the presentation of visual (J. M. Fuster & Alexander, 1971; Funahashi et al., 1989; Di Pellegrino & Wise, 1993b; E. K. Miller et al., 1996) or acoustic (Bodner et al., 1996) cues indicating the goal to achieve or the action to perform.

On this matter, it seems that the dorsal LPFC is less likely to be involved on information storing and retention, being more likely more involved on the processing of information aimed at the correct execution of the task. Thus, it seems that the ability to store the information for a brief period of time is not essential for the functional role of the LPFC. The contribute of

this region to the working memory system is most likely related to the processes involved in the control of behavior that operate at an abstract level with respect to the elaboration of single sensory inputs. Attentional modulation and control, interpretation and use of the instruction stored in the memory, selection of appropriate response, generation (through a series of trials) of a response model to guide behavior and interferences modulation are the working memory aspects in which LPFC seems fundamentally involved (Tanji & Hoshi, 2008).

Attention for action

The involvement of LPFC in the processes related to attentional modulation and control has long been known (Di Pellegrino & Wise, 1993a; Boussaoud & Wise, 1993; Wise et al., 1997). The prefrontal cortex plays an essential role in orienting attention in order to efficiently code relevant information for the current behavior, thus filtering irrelevant signals (Desimone, 1996; E. K. Miller et al., 1996; Lumer et al., 1998; E. K. Miller & Cohen, 2001). These results are congruent with a top-down "model" in which the attentive processes, controlled by the frontal cortex, modulate the activity of posterior areas in order to promote the flow of information relevant for the goal directed action to perform (Pessoa & Desimone, 2003; B.

T. Miller & D'Esposito, 2005), blocking those that are irrelevant. The role of LPFC in maintaining the attention "fixed" on an object, rather than maintaining the object in memory, has been reported in fMRI studies in human subjects (Rowe & Passingham, 2001; Lau et al., 2004); However, it is difficult to pinpoint the different components of this cognitive process in specific anatomical areas (Nagahama et al., 2001).

Preparatory set and regulation of cross-temporal contingencies

The idea that the general function of the LPFC is the temporal organization of behavior is a traditional concept (Jacobsen, 1935; Pribram & Tubbs, 1967; Milner & Petrides, 1984) that has been re-emphasized by authors such as Fuster (J. M. Fuster, 1997). The preparatory set is a prospective cognitive function that specifies the occurrence of a forthcoming action with a proper timing and order; it regulates the temporal relations between the occurrence of different events and action, that is, it regulates the cross temporal contingencies (J. M. Fuster & Alexander, 1971; Fukushima, 2003).

Considering the results of a study carried out by Fukushima, the representations of temporal contingencies may be updated in agreement with the instructions used to reach a specific goal (Fukushima, 2003). Genovesio and collaborators (2006) observed that two distinct group of PFC neurons coded preceding or future goals. It has been demonstrated that the interaction between PFC and the inferior temporal cortex plays a crucial role in associating a visual stimulus with an action during a visuomotor task (Bussey et al., 2002) and in strategy implementation in which the subject could maximize the gain in terms of reward by following a planned way to make a choice of objects (Gaffan et al., 2002). These results indicate that PFC has a central role in defining the temporal relations that occur between actions or, in general, between relevant events, in accordance with the current behavioral context (Tanji & Hoshi, 2008).

Behavioral vs motor planning

The fact that a substantial part of prefrontal cortex neurons are involved in preparing a movement has received significant support from studies that examined the neural activity of LPFC (Boch & Goldberg, 1989; Requin et al., 1990; Sakagami & Niki, 1994; Iba & Sawaguchi, 2003). Until recently, the results of several studies led to the consolidation of the idea that neurons in

the LPFC play a considerable role in preparing or planning an intended movement.

White e Wise (White & Wise, 1999) demonstrated that the movement related activity of LPFC neurons, as well as the activity related to the presentation of instructional cue signals or to the delay periods, were significantly modulated by the rule guiding the experimental paradigm. Thus, it is t possible that behavioral factors not directly relevant for the specification of motor variables constituting the planned movement (such as the motor parameters) are the principal elements processed by the PFC, which may not be involved in the specification of "characteristics" of the movements.

In a series of studies aimed to solve this issue, monkeys were trained to move a cursor on a video monitor by operating two manipolandums with either hand (Mushiake et al., 2006). The instruction given indicated the final position to be occupied by the cursor; the results of these studies indicate that for a great part of the neurons recorded in PFC, the activity during the period of movement preparation reflected the movement of the cursor (or its localization) on the screen, but not the movement to execute (for example which hand was used or the direction of the movement).

The neurons recorded primarily represented the movement of an object that would result from the finalized movement of the limb, rather than the limb movement per se. These results suggest the possibility that the planning of motor behavior in the PFC is generally executed in terms of an end result, which occurs as a consequence of an action, rather than in terms of motor parameters and selection of movements.

Reward expectancy and reward-based control of behavior

Other than the orbito-frontal cortex, which represents the primary and most studied reward coding region (Rolls, 2000; Thorpe et al., 1983b), it has been observed that there are neurons in LPFC whose activity is modulated by the reinforcers (Niki & Watanabe, 1979; Rosenkilde et al., 1981; Ono et al., 1984). Watanabe (1996) found that the activity of LPFC neurons during a delay period reflected not only reward expectancy but also the reward type. Both the orbito-ventral and dorsolateral portion of the PFC present neurons encoding reward quantity, while neurons modulated by reward expectancy and reward quality seem to be localized only in the dorsolateral portion (Tremblay & Schultz, 1999; Hollerman et al., 2000). Furthermore, it has been described that neuronal activity in the LPFC could also reflect the discrepancy between the expectancy of a specific reward and

the reward actually obtained (Leon & Shadlen, 1999). These results appear to be congruent with the hypothesis that the orbito-frontal cortex codes primarily the reward per se, while LPFC uses reward related informations to control behavior Kobayashi and collaborators (Kobayashi et al., 2002) conducted further studies in which they verified that the information processing in the LPFC differs depending on whether the expected reward following the execution of a behavior is positive or negative.

Temporal sequencing of multiple actions

Since many intentional behaviors are composed of sequences of actions, the brain needs to put together all the sequence related information before planning to execute it. Neural activity reflecting specific sequence related information has been observed in many areas of the frontal cortex (Mushiake et al., 1990; Nakamura et al., 1998; Procyk et al., 2000; Shima & Tanji, 2000; Lu & Ashe, 2005). This kind of activity has been considered essential to the temporal sequencing of actions (Tanji, 2001). By studying the involvement of LPFC in these processes, it was observed that neurons of LPFC represent the temporal order of the objects that the monkey planned to capture, without considering their spatial position.

These results are congruent with those reported clinical studies reporting disturbances in the temporal ordering of events (Milner, 1971; Petrides & Milner, 1982; Shima & Tanji, 2000) and with neuroimaging results (Paus et al., 1993; Cabeza et al., 1997) relative to the activity of LPFC during behavioral task requiring a temporal structuring of visual information. Based on the dynamic properties of neurons whose activity is related to the temporal structuring of action sequences, Averbeck and collaborators (2006) proposed that the PFC effectively implied in the representation of the subjective knowledge of the correct sequence of actions

Response inhibition

Dias and coll. observed that lesions of LPFC in monkeys caused the loss on inhibitory control in attentional selection, while lesions of the orbitofrontal cortex caused the loss in inhibitory control of affective processing (Dias, et al., 1996). In human subjects, LPFC lesions are associated with deficit in performing the Wisconsin Card Sort Test (WCST). A following fMRI study highlighted that the area, localized in VLPFC, active during the process of set shifting (thus during the WCST) coincided with the area active during a No-Go response (Konishi et al., 1999), suggesting that VLPFC could be involved in the inhibition of different targets (the go

response in the latter tasks and the cognitive set during WCST). These findings indicate the presence of multiple inhibitory mechanisms in the LPFC.

1.7 Ventrolateral prefrontal cortex

Recently, Luppino and collaborators (Borra et al., 2015; Gerbella et al., 2010; Gerbella et al., 2013; Saleem et al., 2014) observed that ventrolateral prefrontal areas 12r and 46v are characterized by connectional heterogeneity which allows to distinguish between rostral and ventral parts of both areas (and an additional intermediate region for 12r).

The caudal part of area 12r displays strong connections with the caudal part of the VLPF, including oculomotor areas 8/FEF and 45B, weak orbitofrontal connections and extra-prefrontal connections limited to the inferotemporal cortex. The rostral part of area 12r is mostly connected with rostral prefrontal and orbitofrontal areas and relatively weaker connections with the fundus and the upper bank of the superior temporal sulcus. The intermediate part of area 12r is strongly connected with the caudal half of area 46v, orbitofrontal areas, ventral premotor area F5, anterior intraparietal (AIP) area (both involved in visuomotor transformations for grasping) and with subcortical areas involved in reaching and grasping movements. Given

its connections, the intermediate part of area 12r has been indicated as a possible prefrontal node for the grasping network (Borra et al., 2011, 2014, 2017).

With regards to 46v, its rostral part displays an extensive and almost exclusive intraprefrontal connectivity, with extraprefrontal connections limited to area 24 and inferotemporal areas. In contrast, the caudal part of 46v mostly displays intraprefrontal connectivity with ventrolateral areas 8r, 44 and 45b and robust connectivity with frontal (F5a, FEF and SEF) and parietal (LIP, AIP, PG, PFG, PF, SII) sensorimotor areas. These patterns of connections suggest that between, the two "subregions" of 46v, the caudal part is the most involved in motor control, specifically as a part of circuits controlling oculomotor behavior, arm, hand, or mouth actions, other than action recognition (Gerbella et al., 2013).

VLPFC is particularly characterized by projections to premotor areas, principally F5a and F6, involved in the cited grasping network. Areas F5a and F6, which receive inputs from the VLPFC, are in turn connected to regions of the limbic system: F5a displays connections with the frontal operculum and the central part of the insula, while F6 is connected with the anterior cingulate gyrus (Gerbella et al., 2011; Luppino et al., 1993).

Yamagata and collaborators (2012) proposed that distinct processes of information representation and elaboration underlying goal directed behavior occur in the dorsal and ventral portion of the lateral prefrontal cortex: The DLPFC appeared to encode (together with the dorsal premotor cortex) the behavioral goal through the delay period, even after action specification and during execution, while the VLPFC encoded object features of the instruction cues for behavioral goal retrieval and, subsequently, spatial locations of the choice cues for specifying the actions. The two regions thus operated at different stages of the process of perception-action transformation underlying goal directed behavior, having each one a specific role.

1.7.1 VLPFC and action execution

The activity of VLPFC neurons has been analyzed during a Go-No Go task in which the monkeys were required to observe or execute grasping actions in different conditions (Simone et al., 2015). At the beginning of each trial (excluding the blocked motor and naturalistic conditions) a cue was turned on, indicating the monkey which condition to perform: a red LED instructed the monkey to fixate the object during the whole task, a green LED instructed the monkey to reach and grasp the presented object.

Subsequently, while the LED was still on, the object was presented to the monkey. The LED was then turned off, indicating the monkey to either execute a grasp or maintain fixation.

The grasping actions were executed in different conditions: the object to be grasped could be either illuminated (motor condition), thus allowing the execution of a visually guided action, or not (dark motor condition), in which case the action was executed without visual control. In the blocked motor condition, the object was not presented during each trial, but only at the beginning of each block of trials.

In this case, the monkey after the green LED was turned off, had to grasp the object under mnemonic guidance. Two naturalistic condition, grasping in light and in dark, were added to better evaluate the properties of the neurons studied in the motor conditions. In the grasping in light condition, the experimenter presented a piece of food to the monkey, who freely reached for and grasped it. In the grasping in dark condition, the monkey was prevented from seeing the scene, and the food was introduced near the monkey in a fixed position, so that it could know the position of the food to be grasped.

The authors found that a sector of the VLPF cortex hosts neurons that are active during the execution of goal-directed reaching-grasping actions. These movement-related neurons were typically activated both with and

without visual control of hand-object interaction, when the object had to be grasped under mnemonic guidance, and in a naturalistic context in the absence of learned rules. Some of them were active during object presentation, generally discharging more strongly when the object had to be grasped rather than simply observed. Finally, although some movement-related neurons showed a preference for a grip type, none of them showed selectivity during object presentation.

This study demonstrated that movement-related neurons are activated during grasping in different behavioral situations (grasping under visual control, grasping in darkness, memory-guided grasping, and simple grasping of food), indicating that VLPF neuronal activity is not necessarily dependent on the learned relationship between instruction and motor output. Many of these neurons displayed a response during task epochs preceding movement execution, in line with several studies showing that the VLPF cortex employs information about the visual context to generate goals by forming associations between cues and goals (White & Wise, 1999; Asaad et al., 2000; E. K. Miller, 2000; Wallis et al., 2001).

A high percentage of movement related neurons responded during set (250 ms before the offset of the cue) and/or go (from the offset to the release of the hand) epochs, in agreement with studies describing the role of the

VLPF cortex in movement planning (Quintana & Fuster, 1992; Funahashi et al., 1993; Averbeck et al., 2002; Shima et al., 2007; Yamagata et al., 2012). Among those, many show prolonged differential activity starting from object presentation. This discharge is not affected by the different contextual conditions (Motor condition in light and darkness; Blocked Motor condition), as shown by the population analyses, and could thus represent a type of preparation related to object "graspability" or the maintenance of action goal representation. This supports the idea that the VLPF cortex could play a role in action planning and execution, extending this role to the case of natural actions. It has been proposed that neurons found in this study play a role in a wider network subserving grasping action, given the connections of the VLPFC sector analyzed in this study with parietal and premotor areas involved in higher hand motor control (Petrides & Pandya, 1999; Borra et al., 2011; Yeterian et al., 2012; Gerbella et al., 2013).

From a functional point of view, the neurons described in this study show similarities but also differences with parieto-premotor neurons. In the sector of the VLPF cortex from which movement-related neurons were recorded, there are many fewer grip-selective neurons than in premotor area F5 and the anterior intraparietal (AIP) area. Furthermore, the response to object

presentation of prefrontal grip-selective neurons is not object-specific, and thus lacks the basic prerequisite for establishing congruence between visual and motor preference that is typical of canonical neurons of F5 and object-type neurons of AIP (Murata et al., 1997; Murata et al., 2000; Raos et al., 2006).

In addition, most VLPFC movement-related neurons are not affected by the absence of visual control during action execution. This evidence supports the idea that VLPF movement-related neurons, unlike parietal and premotor grasping neurons, are not involved in coding visuomotor transformations or in the visual control of hand-object interactions, but, rather, appear to encode the action goal and, partly, the way to achieve it (Simone et al., 2015).

1.7.2 VLPFC and action observation

Several studies have described the presence of neurons in the VLPF cortex of macaques responding to the observation of actions performed by others (Nelissen et al., 2005; Falcone et al., 2016; Sliwa & Freiwald, 2017; Simone et al, 2017), suggesting that this region could be part of a broader network activated during action observation, being specifically involved in "social information processing", that is the processing of cues provided by

others behavior during interaction, fundamental for selecting and executing an appropriate response.

Simone and collaborators (2017) investigated VLPFC neurons response to biological movements and object motion using a paradigm in which the monkeys were required to observe six different types of videos depicting different scenes with varying agents and perspectives: A monkey grasping a piece of food seen from a first (Monkey Grasping I, MGI) or third (Monkey Grasping III, MGIII) person perspective, a human actor (seen from a lateral view) grasping (Human Grasping, HG), or mimicking to grasp (Human Mimicking, HM) or extending his forelimb in front of himself (Biological movement, BM), and the motion of an object (Object Motion, OM). The experiment also included a modified version of the task in which the first or the second phase of the videos showing goal directed or mimicked actions could be partially obscured, allowing to compare the neuron response recorded during the observation of the masked action was with that obtained during the observation of the non-masked stimuli.

The results of this study showed the presence of VLPF there are neurons responding to observation of biological movements performed with the forelimb, a majority of which showed a stimulus-specific activity, responding best or exclusively to one of the presented stimuli (HS). Most

of these selective neurons presented their strongest discharge during observation of goal-directed actions: the most effective were those performed by a conspecific, while a lower number of neurons responded to human goal-directed actions. Most tested neurons (2/3) did not change their activity when the action was obscured, suggesting that visual information was not the crucial aspect of their coding.

As suggested by Umiltà and collaborators (2001), the permanence of the response during the obscured phase could be interpreted as the generation of an internal motor representation that includes the action outcome. This response thus suggests that neurons in this area code high order representation of the observed action rather than a simple visual description of it. Furthermore, several VLPF cortex neurons tested in this study discharge also before movement onset. This behavior could indicate that these neurons are able to "predict" the type of action the agent is going to perform and its outcome. In this way, based on the context, the monkey could try to interpret other's actions even before their beginning and use it for planning its behavior.

In conclusion, the VLPF cortex seems to play a fundamental role in the planning, organization and selection of behavioral output based on cues provided by the social environment, that are processed at an higher order

level compared to the parieto-premotor areas. Furthermore, besides having a role in the processing of abstract information finalized to accomplish a specific task, this region seems to use contextual cues to plan and guide behavior responses also in natural situations.

In fact, some authors have found neurons encoding both self and another agents past and future goal during human- monkey interactions (Falcone et al., 2016) or specifically responding to the observation of an interaction between conspecifics (Sliwa & Freiwald, 2017), indicating that the VLPF cortex of macaques is involved in monitoring others choices, evaluating their past goals and predicting their future goals in relation to self past and future goals, in order to generate an appropriate response to others actions or intentions during the interaction in the natural environment.

1.8 Prefrontal cortex lesions

1.8.1 Prefrontal cortex lesions in non-human primates

One of the typical behavioral manifestations associated with prefrontal cortex lesions in monkeys is hyperactivity, mostly characterized by an aimless locomotion and a generalized activation of the muscles (French, 1959; Jacobsen, 1931; Mettler, 1944). These behaviors can be frequently observed after orbitofrontal lesions rather than lesions located more dorsally, which usually determine deficits in ocular motility consisting specifically in an ipsilateral deviation of the eyes and thus a contralateral neglect (Kennard & Ectors, 1938; Kennard, 1939; Ruch & Shenkin, 1943).

Orbitofrontal lesions often result in distraction easiness and incapacity of inhibiting behavioral reactions. Various studies have in fact shown that, following a lesion in this region, monkeys repeatedly failed in task that required them to stay still after the appearance of a target stimulus, rather executing a response after each stimulus (Grueninger & Pribram, 1969; Oscar, 1975).

Lesions of the prefrontal cortex also induce deficit of social behaviors in monkeys, as demonstrated by Myers and collaborators (1973), which

observed that PFC lesions resulted in an alteration of social expressions and gestures, a reduction of vocalizations and a general loss of affiliative behaviors such as grooming and maternal care, which were significantly less frequent in monkeys with PFC lesion.

Prefrontal lesions results in deficits of the working memory, such as an inability to correctly perform delayed match-to-sample tasks (Harlow & Spaet, 1943), in which monkeys have to execute a response after a certain delay period following the instructive cue. The principal sulcus and the dorsolateral prefrontal cortex are particularly important in the execution of tasks requiring the processing of spatial information. Lesions of these areas indeed determine significant deficits in the execution of visuospatial tasks (Mishkin et al., 1969; Goldman & Rosvold, 1970; Mishkin & Manning, 1978). Monkeys with prefrontal lesions display deficits in learning procedures, employing more time than necessary to learn how to correctly execute a task. These consequences show that the PFC is involved in understanding the organization of a task and in defining and setting motor procedures according to given rules (J. Fuster, 2015a). The control of inhibitory responses is compromised after lesions of this region, which indeed determine persevering behaviors, contextual inappropriate behaviors and inability to learn from errors (Mishkin, 1964; Kane & Engle, 2002). Finally, several

studies have described how lesions localized below the principal sulcus cause deficits in visual (Stamm, 1973), auditory (Lawicka et al., 1975), tactile (Semmes et al., 1969) and olfactive (Tanabe et al., 1975) discriminatory tasks.

1.8.2 Prefrontal cortex lesions in human primates

One of the most studied cases in neuropsychology is that of Phineas Gage, who suffered from a bilateral lesion of frontal areas determined by a work incident, after which the subject did not show any motor or linguistic deficits but rather complex behavioral alterations, such as unstable and violent traits. Other specific traits of the disorders arising from frontal, prefrontal and orbitofrontal lesions have been thoroughly described by Grossi and Trojano (Grossi & Trojano, 2002, 2013), with a particular focus on clinical and behavioral manifestations.

1.8.3 Behavioral and emotional disorders

Disinhibition syndrome

Patients often appear to be "inadequate" with respect to their context, acting impulsively or alluding to sexual themes. They do not manage to

accurately maintain their attention, being easily distracted and irritable, and show an uncontrolled verbal production, with an almost compulsive use of word plays (H. Damasio et al., 1994).

Apathetic syndrome

Patients suffering from this syndrome present apathy, lack of motivation, abulia and hypokinesia, all symptoms that determine a loss of initiative in various contexts. They often show indifference and poor non-verbal communication (Marin, 1991).

Acquired sociopathy

A very evident problem in patients with frontal syndromes is the lack of respect for social and ethical norms, showing inappropriate and unusual behavior compared to the contexts in which they are inserted. They show insensitivity to others and uncontrolled aggressive acts (Damasio A.R. et al., 1990; Damasio H. et al., 1994).

Compulsive behaviors

Patients with frontal damage develop childish and capricious behavior, as well as compulsive behaviors such as gambling, hypersexuality and object accumulation disorder without a particular value (Irle et al., 1998; Swoboda & Jenike, 1995).

1.8.4 Cognitive disorders

Attention deficits

The easy distraction is manifested by an attentive capture by relevant stimuli, both external and internal, which does not allow the patient to follow, for example, the interview with the examiner. They often show difficulty in identifying relevant stimuli (selective attention deficit), or in keeping the attention for the time necessary to accomplish a task (sustained attention deficit). Sometimes, deficits emerge when it is required to perform multiple tasks simultaneously, making clear the inability of the patient to divide the attentive resources on the different tasks (divided attention deficit) (Wilkins et al., 1987).

Memory impairment

Patients often tend to produce totally invented or improbable stories, referring to themselves or to particular events, thus manifesting a confabulatory behavior. They frequently use this strategy because they fail to chronologically sort events and differentiate them with appropriate strategies (Grossi & Trojano, 2002).

Perseveration and rule violations

Perseveration in psychology is the repetition of a previously given response that is no longer adequate to the current environmental demands. Patients appear repetitive and rigid because they adopts habitual behaviors and strategies in all contexts, violating the rules imposed by the specific environment (Luria, 1965; Vilkki, 1989).

Planning deficits

The inability to execute effective strategies manifests itself during tasks involving the planning of actions in a concatenated and coherent manner in order to achieve an objective. In these cases, patients often fail because they

are precipitous or superficial, omitting passages that are fundamental to achieve the final goal (Owen et al., 1990).

Deficit of abstract logical abilities

Patients find it particularly difficult to diverge and abstract from concrete information, showing deficits in abstract reasoning thus in the construction of hypotheses and in the definition of the consequences of a particular event. A very clear example is observed when they are asked to explain the meaning of a proverb or draw a conclusion from a story told (Grossi & Trojano, 2002).

1.8.6 Motor disorders

Utilization behavior and compulsive imitation

A poor control and a lack of inhibition of behaviors activated by environmental stimuli are at the base of the disorder in question. Patients uncontrollably use the objects they find in the surrounding environment. Moreover, this lack of inhibition is also manifested through the compulsive imitation of gestures (echoprassy) and word (echolalia) of other individuals. These manifestations are due to the lack of inhibitory control on the chains of motor acts, which are executed spontaneously and uncontrolled by the

patient who is guided, rather than by appropriate motor schemes encoded internally, from external stimuli such as the behavior of others (Grossi & Trojano, 2002).

Grasping reflex

Patients compulsively grasp in response to stimuli given on the palm of the hand, a reflex naturally present only in infants that normally disappears in adulthood. If an object is presented, patients will tend to reach and grasp it, regardless of environmental demands, simply "triggered" by the presentation of the stimulus (Lhermitte, 1983).

1.9 Hypothesis and aim of the study

The evidence described above suggests that VLPFC could play a fundamental role in guiding behavior based on the contextual cues available. In social animals, such as primates, informations gathered from the social environment are of fundamental importance.

Recent functional and anatomical studies showed that the VLPFC, that is known to be involved in action planning, also processes information

related to others' actions (Nelissen et al., 2005; Simone et al., 2017; Falcone et al., 2016; Sliwa & Freiwald, 2017, Rozzi & Fogassi, 2017).

Accordingly, it has been proposed that one possible source of this information is the "mirror neuron system" (Rozzi & Fogassi, 2017). This is also supported by anatomical evidence, showing the presence of strong interconnection between the prefrontal areas 46v and 12 r and parietal and premotor nodes of the mirror system (Borra et al., 2011, 2017; Borra et al., 2014, Gerbella et al., 2013).

Up to now, however, it is not clear the specific role of prefrontal and premotor neurons in exploiting information about the social context (such as other's goals and intentions) to select and guide appropriate behaviors. It is also not clear whether the information about social and non-social context is processed at the level of the same neurons and areas, or whether there is functional segregation.

In light of the above considerations, the general aim of this study is to assess the role of neurons of area 46v, 12r and F5 in guiding actions based on social and non-social information. In particular, we aim at verifying if, and in which areas there are neurons a) capable to specifically code visual biological or non-biological stimuli; b) coding visual stimuli based on the behavioral goal associated to them also independent of their visual features;

c) differentially coding action planning and execution based on social or non-social information.

2. Materials and methods

One female rhesus monkey (Macaca mulatta), weighing about 7 kg, aged 11 years was used in the present experiment. The animal handling, and the surgical and experimental procedures, complied with European guidelines (2010/63/EU) and Italian laws in force on the care and use of laboratory animals, and were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012, and Prot. 91/OPBA/2015) and authorized by the Italian Health Ministry (D.M. 294/2012-C, 11/12/2012 and 48/2016-PR, 20/01/2016).

2.1 Training and surgical procedures

Before recordings, the monkey was habituated to sit comfortably in a primate chair, to interact with the experimenters, and to become familiar with the experimental setup. At the end of habituation sessions, a head fixation system composed of a titanium cylinder perpendicular to its base, anchored to the cranial vault by means of self-tapping titanium screws, was implanted under general anesthesia (see below for procedure). Subsequently, the monkey was trained to perform the tasks described below,

using the right hand. Before electrodes implantation, the monkey underwent a MRI scan (Figure 4.1) with a General Electric 7-T tomograph, under general anesthesia (described above). The MRI scan allowed to identify the anatomical reference points needed for planning the surgery necessary to implant the recording electrodes.

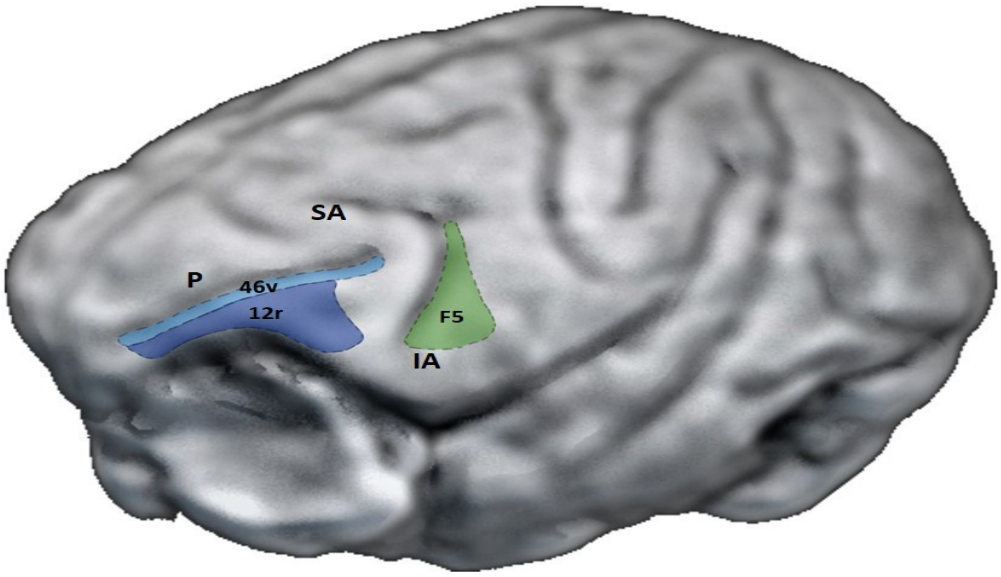

Figure 5: 3D rendering of the left hemisphere of the macaque obtained through the use of a 7-tesla tomograph with an 8 channel receiver coil. The highlighted areas indicate the cortical areas of interest for the placement of arrays: 12r, 46v and F5. P =Principal Sulcus, SA = Superior Arcuate Sulcus, IA = Inferiore Arcuate Sulcus.

The radiological study of the monkey's brain anatomy allowed us to identify the optimal target for electrode implantation and extract their

coordinates in terms of stereotaxic coordinate. Based on these coordinates, we carried out a surgery to implant the electrodes. First, we opened a bone breach above the foreseen coordinates, then we opened a dural flap, above the ventrolateral prefrontal cortex (areas 46v and 12r) and the ventral premotor cortex (area F5). Finally, we implanted the recording electrodes arrays. The microelectrodes were connected, by means of a cable composed of wires isolated in a silicone envelope, to a metal connector cemented on the skull by means of an antibiotic acrylic resin (Antibiotic Simplex).

All surgical procedures were carried out under general anesthesia. Specifically, the anesthesia was preceded by the administration of Atropine, 0.03 mg/kg i.m., followed after about 10 minutes by sedation with i.m. injection of Ketamine hydrocloride, 5-10 mg/kg and Medetomidine hydrocloride, 40-60 μg/kg. An intravenous catheter was then placed for administration of isotonic solutions (NaCl 0.9% saline or Ringer's lactate 3-5 ml/kg/h). Induction of general anesthesia was performed with sodium thiopental 1.25% at a dose of 1-3 mg/kg i.v. General anesthesia was maintained with isoflurane vaporized in oxygen after orotracheal intubation with Magill's tube. General anesthesia was followed by postsurgical antibiotic and pain medications (Fogassi et al., 1996; Rozzi et al., 2006).

2.2 Experimental Apparatus

During training and recording, a headband was fixated to the primate chair, in which the monkey was previously seated, and then attached to the head-fixation system. In front of the monkey a shelf (80x60cm) was placed, at the level of the abdomen. Above this shelf, at about 6 cm from the monkey's chest, there was an aluminum cylinder, which constituted the starting position.

At 26 cm from the chest of the monkey there was a transparent plexiglass box hosting on top an aluminum sphere (diameter 1 cm), centered with respect to the box. Behind the box, at 34 cm from the chest of the monkey, a 19-inch screen with a resolution of 1440 x 900 pixels was placed. The aluminum cylinder and sphere were connected to two different contact detection circuits.

The plexiglass box contained a LED which could produce a green or red light, serving as an instructive cue relative to the type of task to perform, and a photodiode, i.e., a device that converts light into an electrical signal, that allowed to accurately detect the onset and offset of the stimuli presented on the monitor. Above the monitor there was an infrared camera (resolution 120Hz), connected to a dedicated computer, part of the ISCAN ETL-200

system (I-scan inc., Cambridge, MA, USA; 120hz). This system allows to constantly monitor the eye's position. On the right side of the monitor a laser device was located, that could project a light beam into the center of the screen, used as fixation point.

Before beginning each training and recording session, a cannula was placed near the mouth of the animal to administer fruit juices or water, to reinforce correct trials.

Eye position calibration

To precisely assess the eye position, it is necessary to make a preliminary calibration of the instrument. The calibration procedure, repeated at the beginning of each training and recording session, consisted in presenting a set of five light points, at the center and in the four corners of a virtual 10 x 10 degrees square window.

The position of the pupil was detected and recorded following the fixation of each presented individual point. At least 16 acquisitions per point were performed, after which the software automatically calculated the average and standard deviation of the recorded signal, in volts. Based on known parameters (i.e., the distance between the eyes and the monitor and the

distance among copies of the presented points) the signal acquired by the oculometer can be transformed into eye position, expressed in degrees with respect to the center of the screen/fixation point, by a program created *ad hoc* in the Lab View environment. Subsequently, an 8 x 8 degrees window, inside which the monkey had to keep fixation during the task, was defined around the central fixation point.

2.3 Stimuli

The database of stimuli used during the experiment consisted of a set of "biological" videos and another set of "non-biological" video clips. All of the videos had a size of 12x12 degrees and a duration of 880 milliseconds. They were projected at the center of the screen, in a 10 x 10 degrees window, with "the area of interest" of the video (in which the object was grasped/touched or the square/triangle moved) falling the 8 x 8 degrees fixation window previously defined.

The biological stimuli depicted a human subject that either reached for and touched an object or reached for, grasped and lifted the same object placed in front of him. The action was presented in an allocentric (third person) perspective, i.e., as observed by an executant located in front of us looking toward us, depicted on a black background; the face of the agent

was obscured, since it is known that there are prefrontal neurons sensitive to face.

The non-biological stimuli consisted in two yellow geometric shapes, a triangle and a square (1 x 1 degrees of size), moving vertically on the screen from a central starting position to a final position corresponding to that occupied by the object in the biological condition. Each non-biological stimulus included, in the bottom left part, a white circle (non-visible to the monkey) placed perfectly in line with the photodiode, that allowe- to accurately track the timing of presentation of the stimuli, specifically detecting the onset and offset of the stimuli.

Figure 6: Stimuli proposed within the experimental paradigm. Black arrows indicate the direction of movement followed by the figures, which, starting from the center of the window, reached the same position occupied by the object grasped/touched in the biological stimuli.

2.4 Experimental paradigm

The experimental paradigm is characterized by two conditions: "Imitation" and "Observation" (Figure 7 and 8). Note that the term "Imitation" was only used to highlight the fact that the monkey was asked to perform an action similar to that observed (or an action associated to a specific non biological stimuli), after learning to do so through conditioning. It is not a real process of imitation, which is indeed only found in humans.

These conditions are instructed by a green or red instructive light (produced by the LED contained in the plexiglass box) respectively. Each trial began when the monkey placed his hand on the starting position, after which the fixation point was projected in the center of the monitor and the monkey had to fixate it. After a randomized time of 500 to 750 ms, if the monkey kept fixating and did not move the hand from starting position, one of the two instructing cues was presented. If the monkey continued to maintain its gaze within the limits of the fixation window, after a further time interval randomized between 500 and 750 ms, one of the four stimuli was presented. Following a randomized time between 500 and 900 ms after

stimulus presentation, the cue and fixation point were switched off (Go signal).

In the "Imitation" condition, the monkey had to release the hand from the starting point and touch or grasp and pull the object, starting in less than 1 second. Specifically, the monkey had to grasp the object if it had observed either a video depicting a human subject grasping an object or a vertically moving triangle, whilst it had to reach for it if it had seen either the video depicting a human subject reaching for it or a vertically moving square. In the "Observation" condition, the monkey had to simply release the hand from the starting point and remain still, regardless of the presented stimulus.

The order of stimuli presentation was randomized. The trial was considered null if the fixation was not maintained for at least 90 percent of the time during each phase of the task, if the monkey released the hand from the starting position too early (before the go signal) or too late (more than 1s after the go signal) or if the monkey's response was not correct in relation to the instructive cue or the presented stimulus. Under all conditions, every correct test was rewarded with the release of a few drops of fruit juice. If the monkey performed an incorrect trial, reinforcement was not delivered. Incorrect trials were repeated, in a random order, after all the set of stimuli

were presented, to ensure a minimum of 11 correct trials for each stimulus/condition.

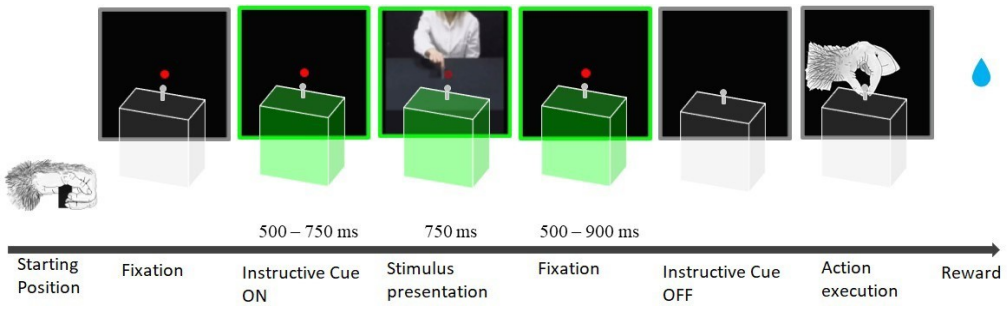

Figure 7: Temporal sequence of events in the "Imitation" condition.

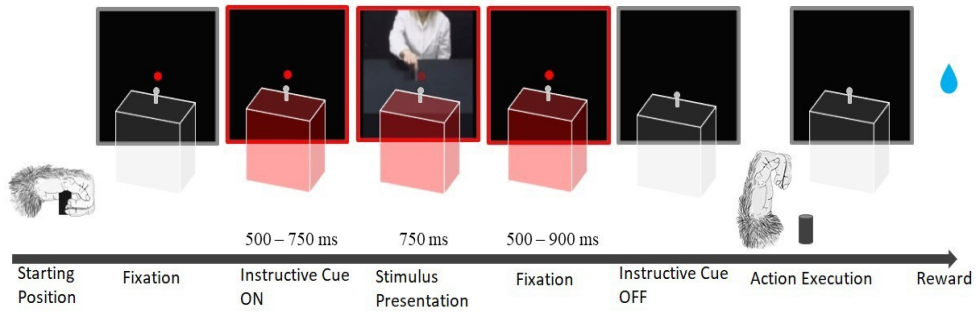

Figure 8: Temporal sequence of events in the "Observation" condition

2.5 Recording techniques and signal acquisition

Figure 9 shows an example of the used type of arrays, a Floating Microelectrode Array (FMA MicroProbes for Life Science, Gaithersburg, MD, USA). The arrays consist of a ceramic platform of 4 x 1.8 mm, 125 microns thick, containing 36 rigid platinum microelectrodes with a diameter of 25 microns, arranged in triangles 400 μm apart, and with a length comprised between the 0.5 and 5 mm. The different length of the electrodes was chosen in order to cover the different depths of the recorded regions, which include convexities and sulci banks. Out of the 36 microelectrodes of each micro-array, 32 were actual recording microelectrodes with an impedance of about 0.5Ω, 2 correspond to reference channels and 2 to ground channels. While the length of the recording electrodes was such to allow to record in a cortical convexity (ranging from 0.5 to 1.7 mm) or in the bank of sulci (ranging from 0.5 to 5 mm) ground and reference electrodes were longer in order not to record cortical activity: 3 mm for convexity arrays; 5 and 6 mm for mixed convexity sulci arrays. Figure 10 illustrates the anatomical location where the three arrays were positioned.

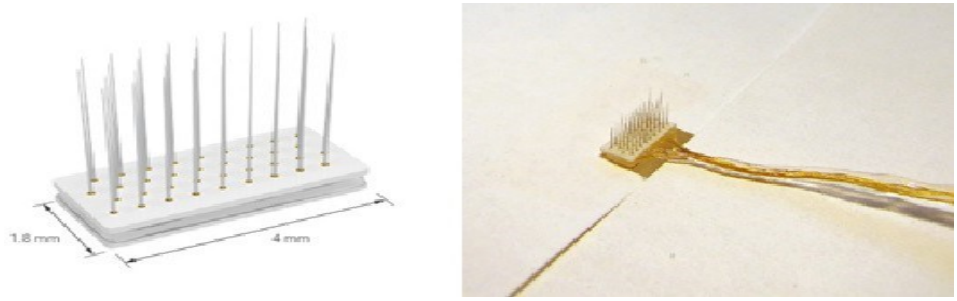

Figure 9: A. Example of Floating Micro Electrode Array composed of 36 electrodes with a diameter of 25 microns each. B. Microarray and connection made of wires insulated by means of a silicone sheath

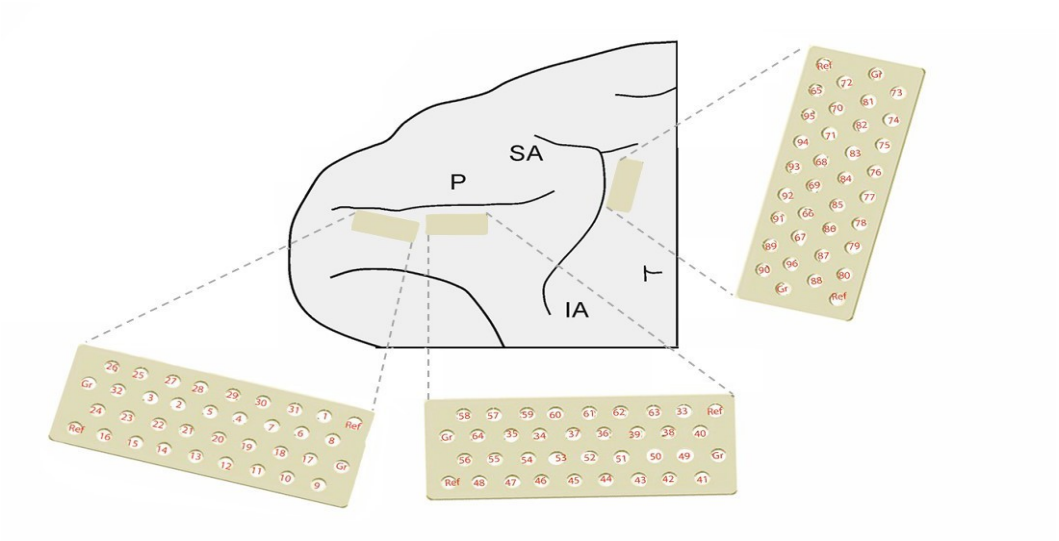

Figure 10: Representation of the lateral cortex of the macaque with the indication of the position of the multielectrode arrays. The numbers indicate the position of each recording, reference or ground channel. The rectangles indicate the positioning of the electrodes in areas 12r, 46v and F5. P = Principal Sulcus, SA = Superior Arcuate Sulcus, IA = Inferior Arcuate Sulcus.

Neuronal activity was recorded and monitored through the integrated hardware-software "Open Ephys" acquisition system (Siegle et al., 2017). The software consists of a downloadable specialized electrophysiological recording software, the Open Ephys GUI (graphical user interface) which is fully integrated with the acquisition board and implements a plugin-based architecture useful for acquisition, processing and visualization of the activity detected by extra-cellular electrodes.

The experimental paradigm was controlled by a software programmed in LABVIEW (National Instruments, Austin, Texas, USA), installed in a second computer (with respect to the one connected to the acquisition board), which guided the different behavioral events: *appearance* and *disappearance of the fixation point; appearance of the instructive light (cue onset)* and *go-signal (cue offset), stimulus presentation onset and offset* and finally *reward delivery,* simultaneously sending the digital signals associated to all these events to the acquisition system. The signal acquired by the circuits connected to the starting position and the sphere were also converted in digital signals (*release from the starting point and hand-object contact*) and recorded, in order to digitize the moment of contact/detachment with/from the starting position and the beginning/end of reaching and

grasping. Finally, the analog signal recorded by the oculometer were also acquired and later sent to the "recording" computer.

2.6 Data Analysis

Offline processing of the neural signal and extraction of single neurons

The neural activity acquired has been aligned to the different digital and analog signals using specific, on programs such as Neuro Explorer and MATLAB. In particular, the digital signals used for alignment corresponded to the onset and offset of fixation point, instructive led and stimulus presentation, the detachment from the starting position and, only for the imitation condition, the contact between hand and object. Neuronal activity, according to the various alignments, was displayed in the form of rasters and histograms.

Neuronal activity was firstly analyzed through "Mountainsort", an automatic offline sorting software (Chung et al., 2017), an open-source Mountainlab plugin package for processing and visualization of the activity, subsequently identifying and isolating the waveforms of single neurons.

Statistical analysis

In order to assess whether neurons significantly responded during task unfolding, we performed a one-way ANOVA (Factor: Epoch, $p<0.01$), followed by Newman-Keuls post hoc tests. To this aim we identified six epochs based on the acquired digital signals, as follows;

Fixation, 500 ms following the appearance of the fixation point;

Cue, 500 ms following the appearance of the instructive light (cue onset);

Stimulus, 500 ms following stimulus presentation onset;

Delay, 500 ms following stimulus presentation offset;

Go, 250 ms following the disappearance of the instructive light (cue offset);

Action, 500 ms following release from the starting point.

Neurons were considered task related when the one-way ANOVA (Factor: Epoch, $p<0.01$, see Methods) revealed a significant effect and, the following post-hoc test, revealed a significant difference in activity between at least one of the main task epochs (*Fixation, Cue, Stimulus, Delay, Go, Action*) and the *Baseline* epoch.

Best response computation

The neurons best responses were calculated by considering the mean firing rate in the baseline and in the different epochs, as follows: if a given epoch's mean firing rate was lower than the baseline, we subtracted the mean firing rate of that epoch from the baseline mean firing rate (Baseline-Epoch); if a given epoch's mean firing rate was higher than the baseline mean firing rate, we subtracted the baseline mean firing rate from the mean firing rate of that epoch (Epoch-Baseline). We then defined the epoch of best response to be the epoch in which this difference was highest.

Principal Component Analysis

Trial-averaged firing rates of each unit were calculated by binning the spiking activity in 20 ms time windows and smoothing the resulting firing rates with a 60-100 ms Gaussian kernel. PCAs were performed on these averaged firing rates using three different alignments designated as: "*Cue onset"* consisting in a time period ranging from 500 ms before to 2 second after the appearance of the instructive light; "*Stimulus presentation"* consisting in a time period ranging from 500 ms before to 1 second after stimulus presentation onset; "*Release from starting point"*, consisting in a

time period ranging from 500 ms before to 1 second after release of the hand from the starting point. Each alignment includes all conditions, thus allowing to compare the behavior of neural populations during different conditions across the recorded areas (12r, 46v and F5).

In PCA analysis, for each unit, the smoothed firing rates were first divided by the maximum firing rate across all conditions and tasks, and then the overall average firing rate was subtracted bin-by-bin to obtain a normalized firing rate. After this pre-processing, the normalized firing rates were considered as an N-dimensional neural population state space. Furthermore, since the amplitude of a generic population vector segment length in an N-dimensional cube grows as $\sqrt{N}$, (see Anderssen et al., 1976), each firing rate was normalized dividing it for $\sqrt{N}$ to compare reliably PCA projections of different subpopulations even if they were made up of a different number of units. Then, for each task and condition, the corresponding full-dimensional neural trajectory was projected onto the plane of the first two PCs.

3. Results

Neuronal activity was recorded from areas 12r, 46v and F5 (figure 4.4). Both multi and single unit activity were extracted through an offline analysis of the recorded signal (see Methods). Here, we will consider only single unit activity defined as “single unit”.

Offline processing and sorting of the signal allowed to isolate 423 single units divided as follows: 191 (45,15% of the total single units extracted) were recorded from the 12r array, 189 (44,68% of the total) from the 46v array and 43 (10,16% of the total) from the F5 array. Out of the 96 channels used to record the neural activity during a total of 30 sessions, 42 were classified as active (recording at least one single unit during the sessions considered), subdivided as follows: 23 in area 12r, 12 in area 46v and 7 in area F5.

According to the criterion used to identify task-related neurons (see Methods), 253 single units (59,81% of the total single units extracted) were identified as task related, divided as follows: 123 single units recorded from 12r array, 103 from 46v array and 27 from F5 array.

3.1 Neural response in the different task epochs

To define the epoch selectivity of task-related neurons coding, we calculated, for each neuron, the number of epochs in which the activity was significantly different from the baseline. Figure 11 shows the percentage of neurons responding to one or more epochs. It can be noted that the number of neurons responding in all 6 epochs (n=87, 34,39% of the total) is the largest, immediately followed by neurons responding to 5 epochs (n=84, 33,20%). No task-related neurons responded in one epoch only.

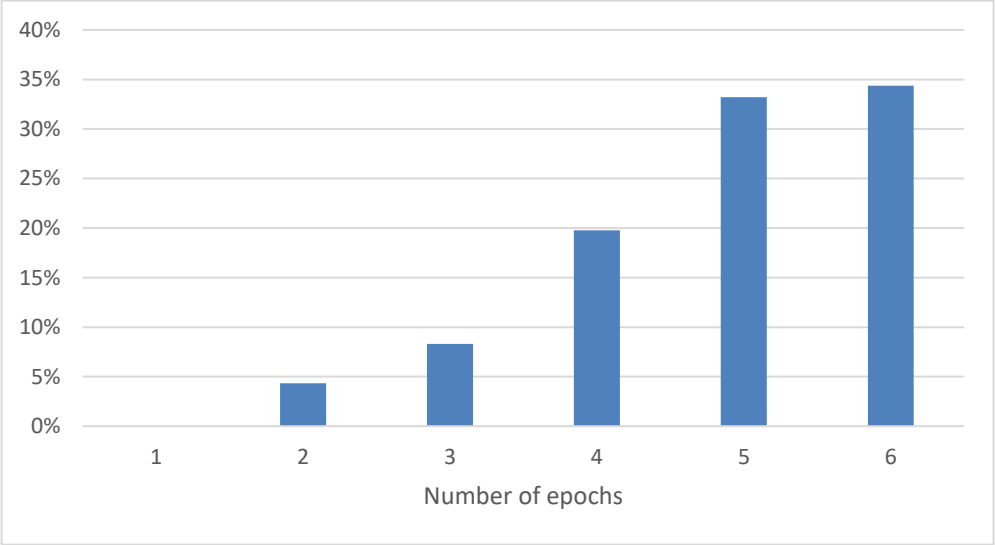

Figure 11 Percentage of task-related neurons responding in one or more epoch.

The post-hoc analysis also allowed us to evaluate the number of neurons responding in each epoch, thus identifying which epochs were the most frequently coded. 182 neurons (72%) showed a response during the *Fixation* epoch, 223 neurons (88% of the neurons responsive to the task) were modulated during the *Cue* epoch, 206 neurons (81%) responded during the *Presentation* epoch, 213 (84%) in the *Delay* epoch, 211 (83%) in the *Go* epoch and 191 (75%) during the *Action* epoch. Note that the sum of the percentages is higher than 100 because each neuron can be active during different epochs, as described above.

The post-hoc analysis described above identifies as responsive either neurons that increase their discharge frequency compared to the baseline activity (excited), or those that reduce it (inhibited). The distribution of excited or inhibited neurons for each epoch is shown in Figure 12.

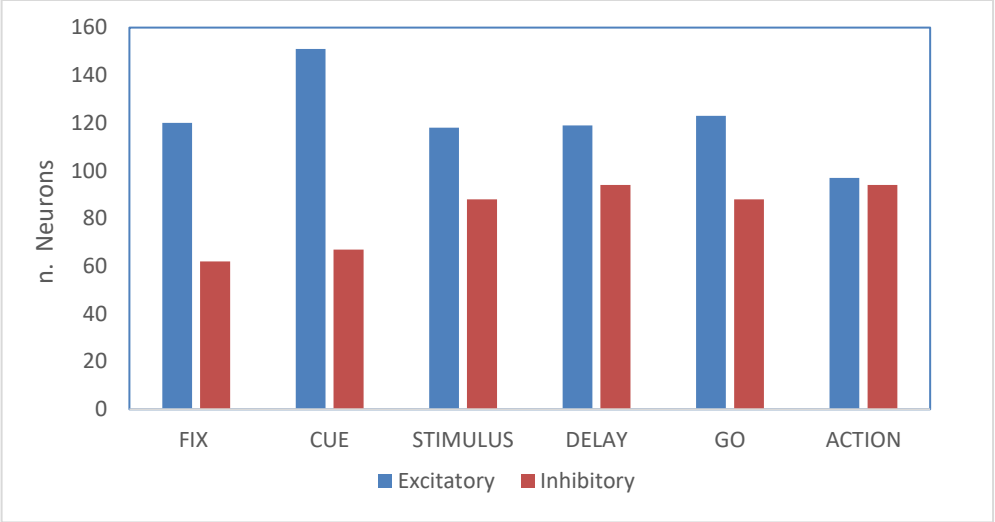

Figure 12. Number of excitatory (blue) and inhibitory (red) responses in different epochs.

3.2 Classification of neuronal responses according to the criterion of "best response"

Finally, we classified the neuronal responses on the basis of the epoch in which each neuron showed the best response, considered as the response that maximally differed from baseline activity (see Methods). Thus, every responsive neuron has its “best” response associated to only one epoch.

The epoch most frequently associated with a best response (considering both excitatory and inhibitory responses) is that of *Cue* (N=57, 22,35%), the epoch less frequently associated with a best response is that of *Delay* (N=25, 9,09%). Concerning the other epochs,11.45% of neurons (N=29)

have their best response during the *Fixation* epoch, 19.76% (N=50) in the *Stimulus presentation* epoch, 18,97% (N=48) in the *Go* epoch and 18,18% (N=46) in the *Action* epoch (Figure 13).

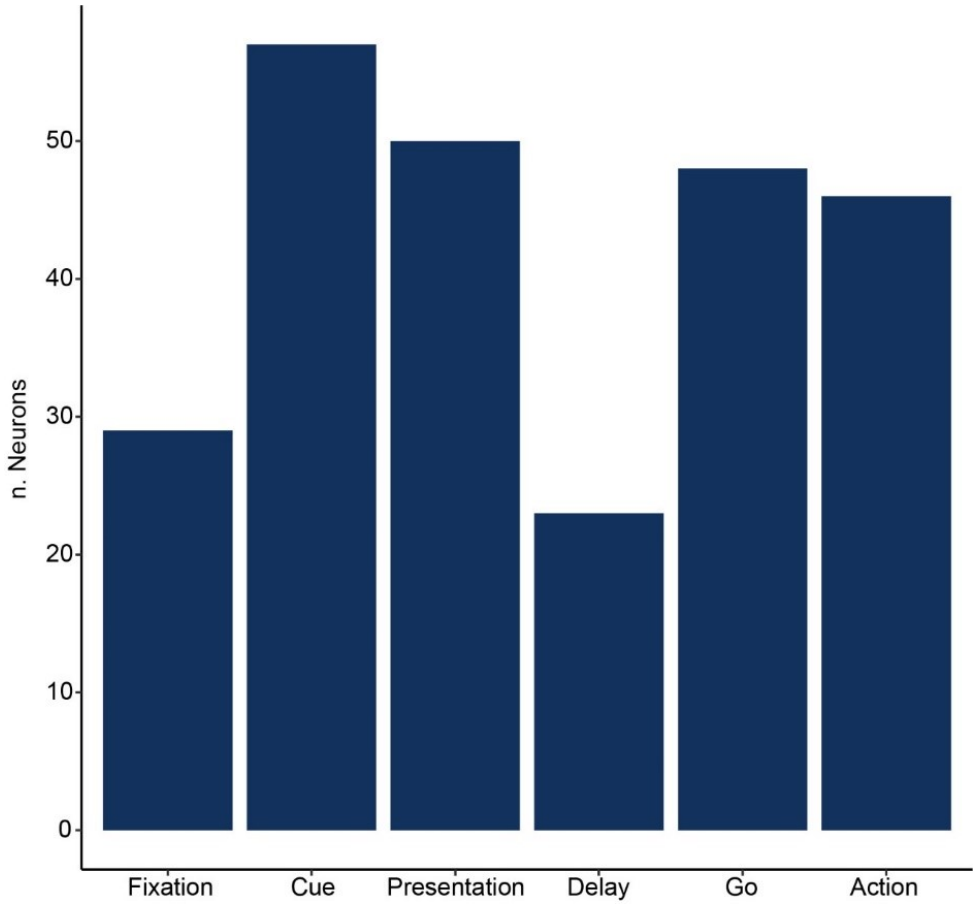

Figure 13. Number of best responses per epoch.

To better assess these responses, excitation and inhibition best responses have been considered separately for each epoch, as shown in figure 14. It

is possible to note that, except for the Go epoch, most of them are represented by excitatory responses.

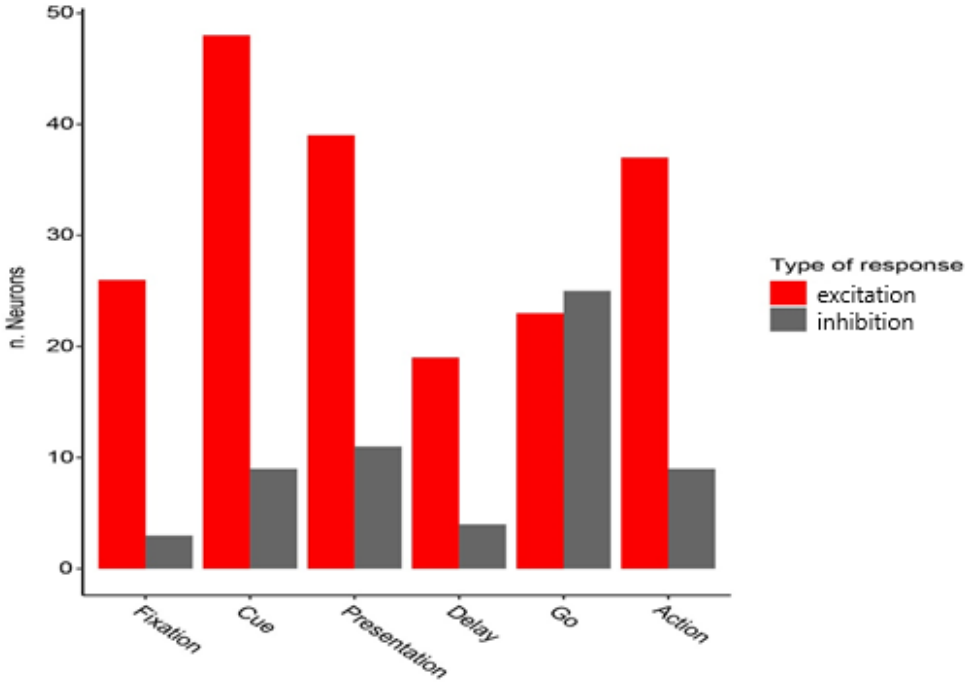

Figure 14 Number of excitatory and inhibitory neurons subdivided on the basis of their best responses for each epoch.

Figure 15 shows the activity of an area 46v excitatory neuron responding in different task epochs and showing its best response in the Action epoch. The neuron starts discharging above baseline about 200 ms after the presentation of the instructive light, then the discharge strongly

increases after the Go signal and peaks during movement execution. The discharge drops at baseline level after reward delivery. Note that in the Imitation condition, the discharge reaches its peak after hand object contact.

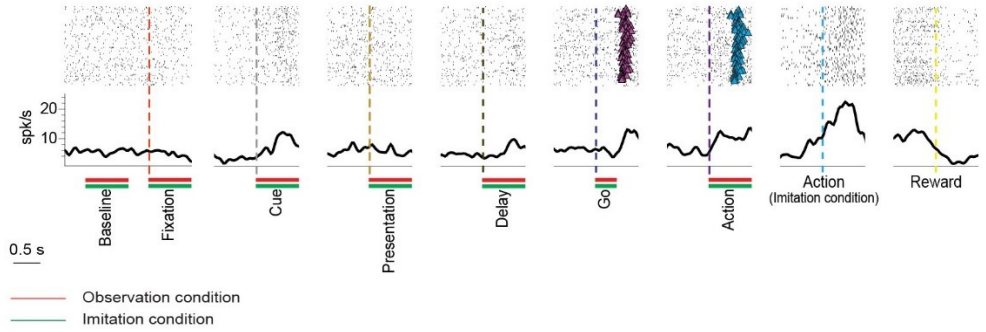

Figure 15. Raster and histogram showing the activity of an area 46v neuron recorded in 88 correct trials of the behavioral task. Rasters and histograms are aligned on different behavioral events, shown by vertical dashed lines. Orange : onset of fixation point; gray : onset of instructive light, yellow : stimulus presentation onset; dark green : stimulus presentation offset; blue : go-signal; violet: release of starting point; light blue: hand-object contact, yellow : reward delivery. Violet and light blue triangles indicate release of starting point and hand-object contact, respectively. The green and red lines below the histograms represent the time epoch used for statistical analysis. Abscissae: time (s). Ordinate: firing rate (spikes/s).

3.3 Frequency of best responses in the different areas

To verify whether there were differences between the three registered areas, the categorization described above was carried out separately for areas 12, 46v and F5. Since the number of task-related neurons recorded from the three micro-arrays was different, for each area (12r, 46v and F5) the percentage of neurons that have their best response in relation to the different epochs, compared to the total number of task related neurons recorded in that area, has been considered (Figure 16).

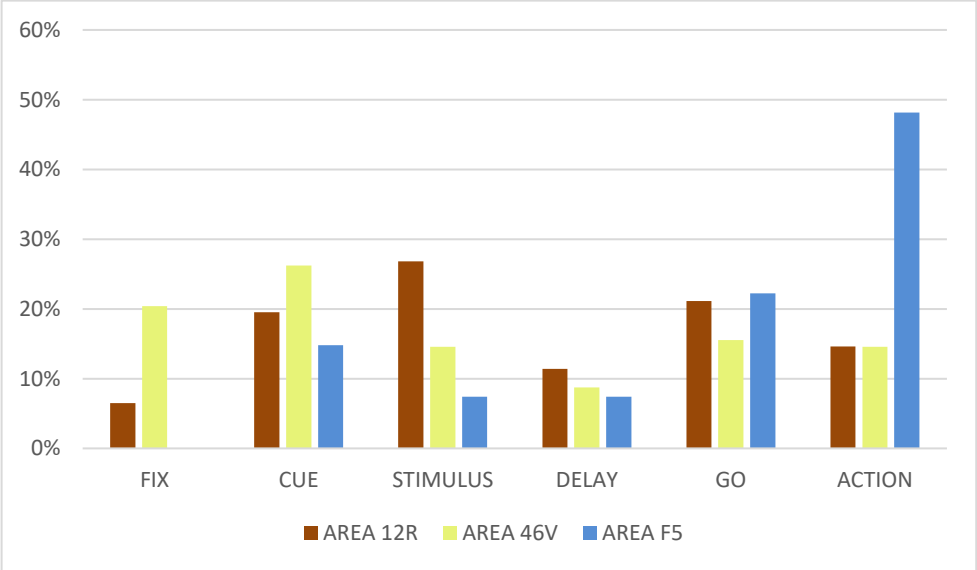

Figure 16. Percentage of "best" responses for each epoch in areas 12r, 46v and F5.

It is evident that the distribution of the best responses in the various epochs is remarkably different between the three areas. Specifically, in area 12r the distribution of the best responses in the different epochs is the following: *Cue* (n = 24, 19%), *Stimulus* (n = 33, 27%), *Delay* (n = 14, 11%), *Go* (n = 26, 21%) and *Action* (n = 18, 16%). In the *Fixation* epoch (n = 8, 6%), the percentage of best responses is lower than the others.

Figure 17 shows the discharge of an area 12r neuron showing its best excitatory activity in the Presentation epoch. The neuron start firing after stimulus presentation and the activity peaks about 200 ms after stimulus presentation. The peak is followed by a brisk reduction in discharge returning to baseline level within 500 ms.

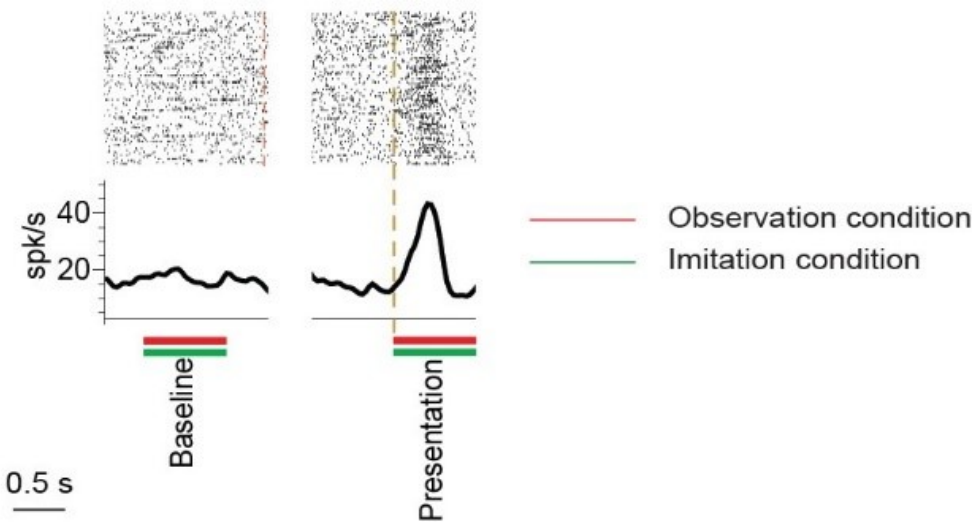

Figure 17. Raster and histogram showing the activity of an area 12r neuron, showing its best excitatory response in the Presentation epoch. The activity during baseline and fixation epochs are aligned on the appearance of the fixation point. The activity during presentation epoch is aligned on stimulus presentation. Conventions as in Figure 15.

The "best" responses detected by the array placed in 46v are, in percentage, more numerous in *Fixation* (n=21, 20%) and *Cue* (n=27, 26%) epochs, while the epochs of *Stimulus presentation*, (n=15, 15%), *Delay* (n=9, 9%), *Go* (n=16, 16%) and *Action* (n=15, 14%) are less represented.

Figure 18 shows the discharge of an area 46 neuron showing its best activity in the Cue epoch. The neuron slightly increases its firing rate about 200 ms before Cue onset, but the neuron discharge strongly increases just after Cue appearance, peaking about 100 ms after the event. The peak is

followed by a rapid reduction in activity, and returns at baseline level before the end of the Cue epoch.

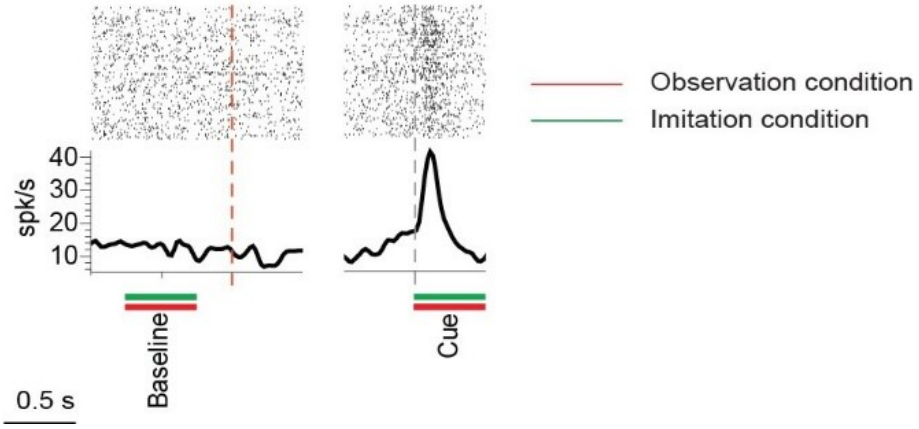

Figure 18. Raster and histogram showing the activity of an area 46 neuron, showing its best excitatory response in the Cue epoch. The activity during baseline and fixation epochs are aligned on the appearance of the fixation point. The activity during cue epoch is aligned on stimulus presentation. Conventions as in Figure 15.

In F5, finally, one can note a higher representation of best answers in the *Action* epoch (n = 13, 48%) while the other epochs are associated with a significantly lower percentage of "best" responses, in particular: *Go* (n=6, 23%), *Cue* (4, 15%), *Delay* and *Stimulus* (n= 2 in both, 7%). Finally, no "best" response was found during the *Fixation* epoch in area F5.

3.4 Principal Component analysis

Principal component analysis has been carried out in order to evaluate the neural population dynamics of the recorded areas, during different phases and conditions of the experimental task (see Data analysis).

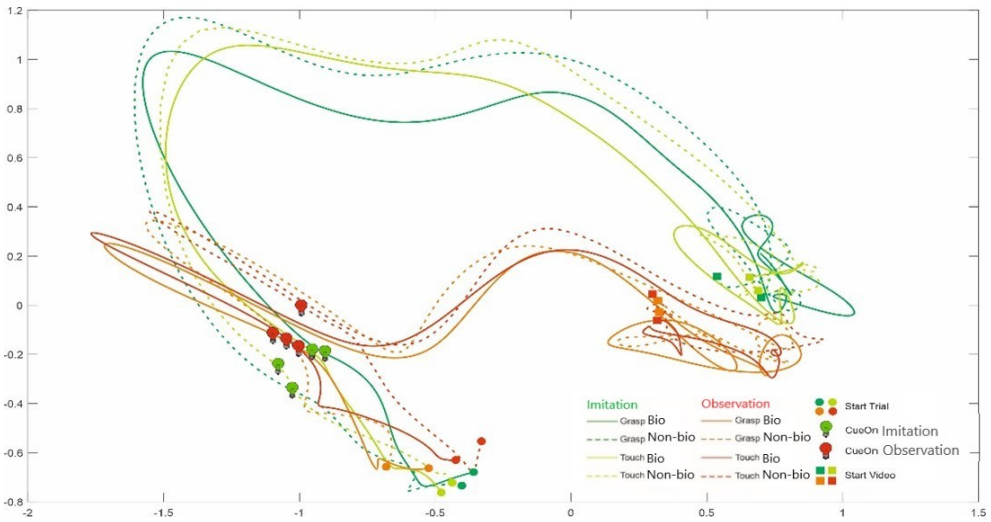

Figure 19. "Cue onset" alignmen, area 12r. All conditions are simultaneously shown in this graphic.

Figure 19 shows the area 12r neural signal behavior during the initial phases of the trial, when the green or red cue is presented to the monkey. It is possible to notice a clear distinction between trajectories representing the "Imitation" and "Observation" conditions, starting after the cue is presented. This separation is maintained until stimulus presentation.

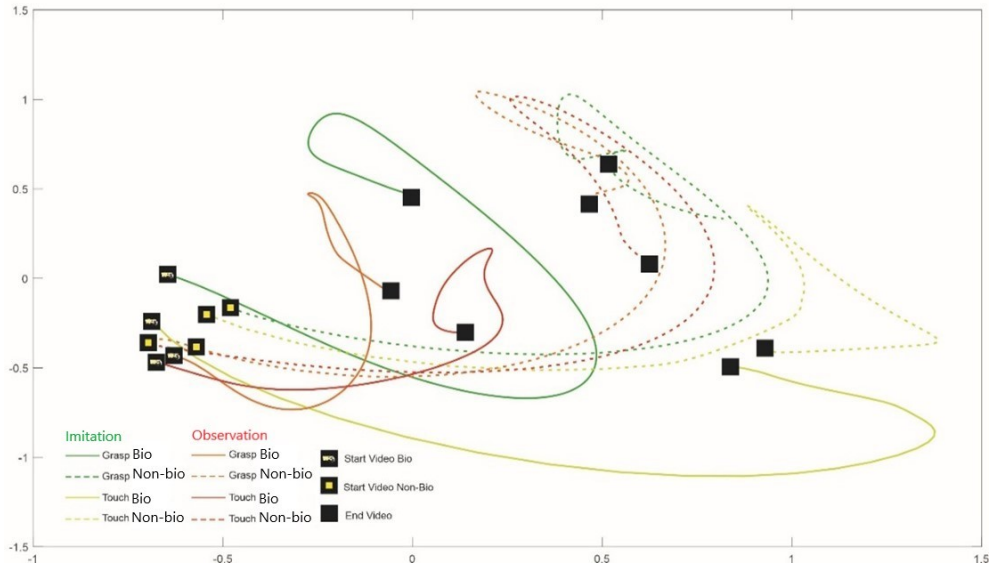

Figure 20. "Stimulus presentation" alignment, area 12r. All conditions are simultaneously shown in this graphic.

Looking at the neural signal behavior of the same area, during stimulus presentation (figure 20), it is possible to see that the differentiation between trajectories becomes more specific and complex. At the starting point (here shown at the level of stimulus presentation onset) all the trajectories is relatively similar. After stimulus presentation, the trajectories representing the "Observation" conditions show a differentiation only between biological and non-biological videos, while the trajectories representing the "Imitation" condition show a differentiation between both the two type of

stimuli (biological vs. non biological) and the two behavioral goals (grasp vs. touch).

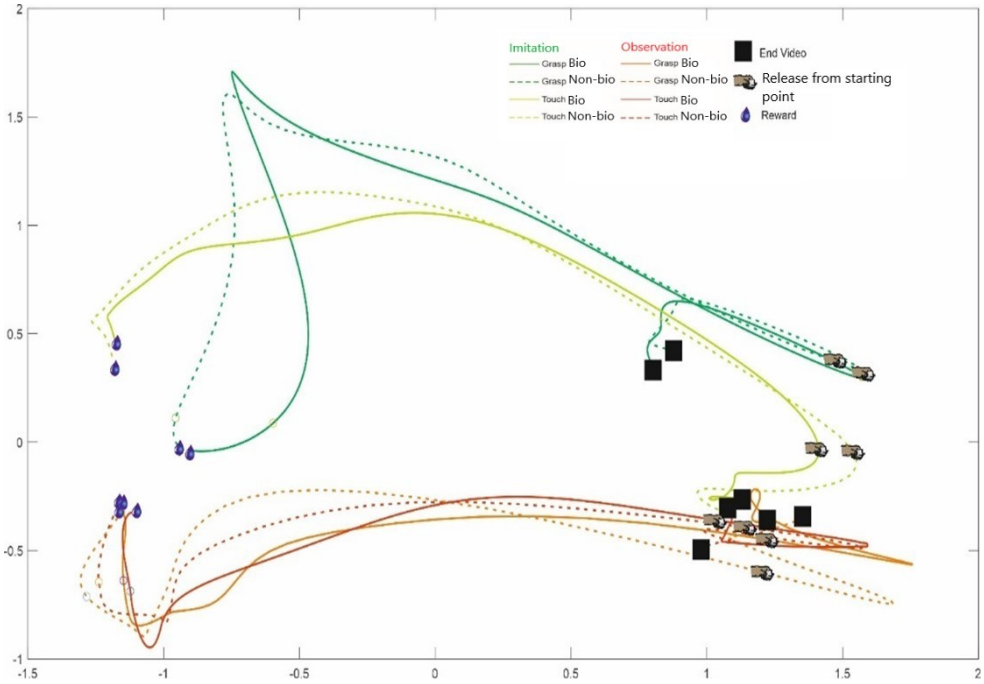

Figure 21. "Release from starting point" alignment, area 12r. All conditions are simultaneously shown in this graphic.

When we consider the behavior of area 12r neural signal during the final phases of the task, it is possible to see that there is a different starting point for the "grasp" and "touch" trajectories in the Imitation condition. These trajectories are thus already separated, before the end of the video (stimulus presentation offset), as showed above, and maintain this separation until the end of the task (reward delivery). There is instead no separation between

the type of stimuli presented in the “Imitation” condition. The observation condition shows no separation between grasp and touch: all the trajectories follow the same direction through this phase of the task.

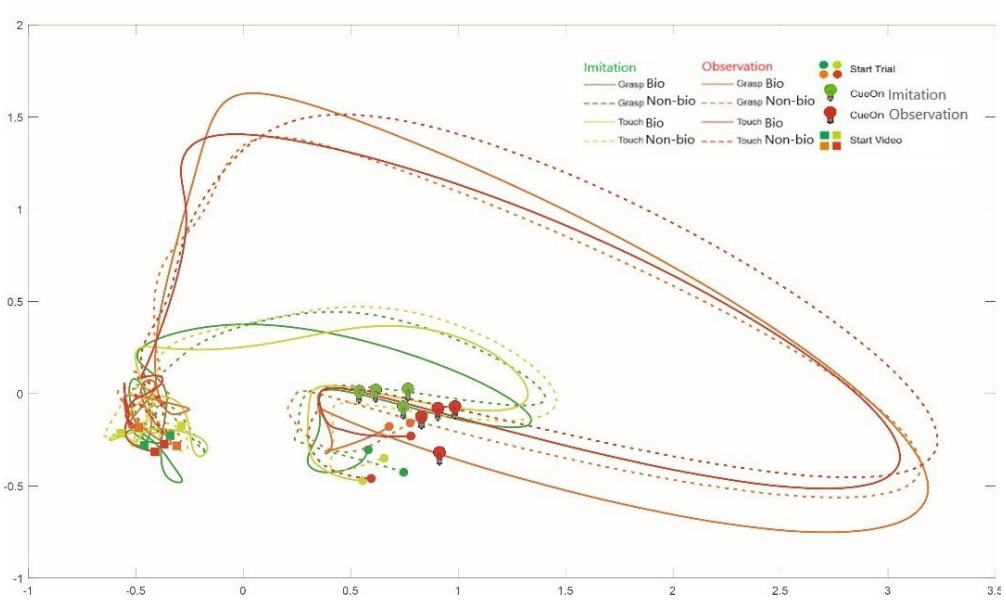

Figure 22. “Cue onset” alignment, area 46v. All conditions are simultaneously shown in this graphic.

Figure 22 shows area 46v neural signal behavior during the initial phase of the task (*Cue onset*). There is, as previously shown for area 12r, a very clear separation between “Imitation” and “Observation” trajectories. In this case, it is possible to notice that, unlike the case of area 12r, the trajectories tend to converge to the same point right before stimulus presentation onset. Moreover, while for area 12r the trajectories representing the “Imitation”

condition were significantly larger than those representing the "Observation" condition,

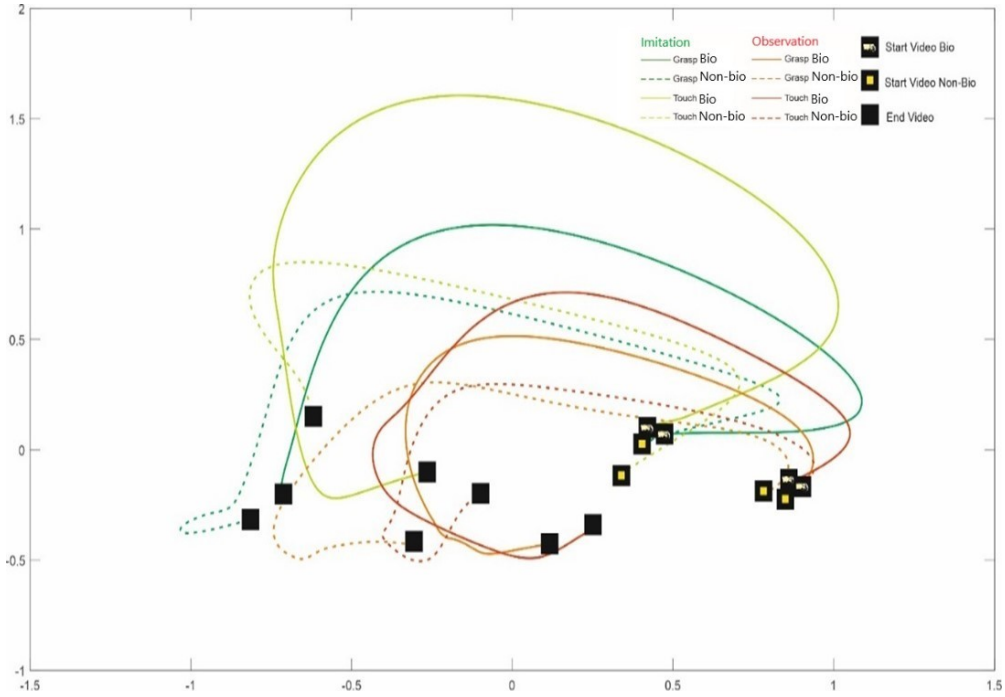

Figure 23. "Stimulus presentation" alignment, area 46v. All conditions are simultaneously shown in this graphic.

Looking at area 46v neural signal behavior After stimulus presentation (figure 23), it's possible to notice firstly that "Imitation" and "Observation" condition trajectories start in different points, being already separated from before stimulus presentation onset, maintaining this separation until the end of the video. Furthermore, there is a separation between the trajectories representing the two types of stimuli presented (biological vs. non

biological) in both the “Imitation” and “Observation” conditions. Finally, the “Imitation” condition trajectories also show a differentiation between the two behavioral goals (grasp vs. touch).

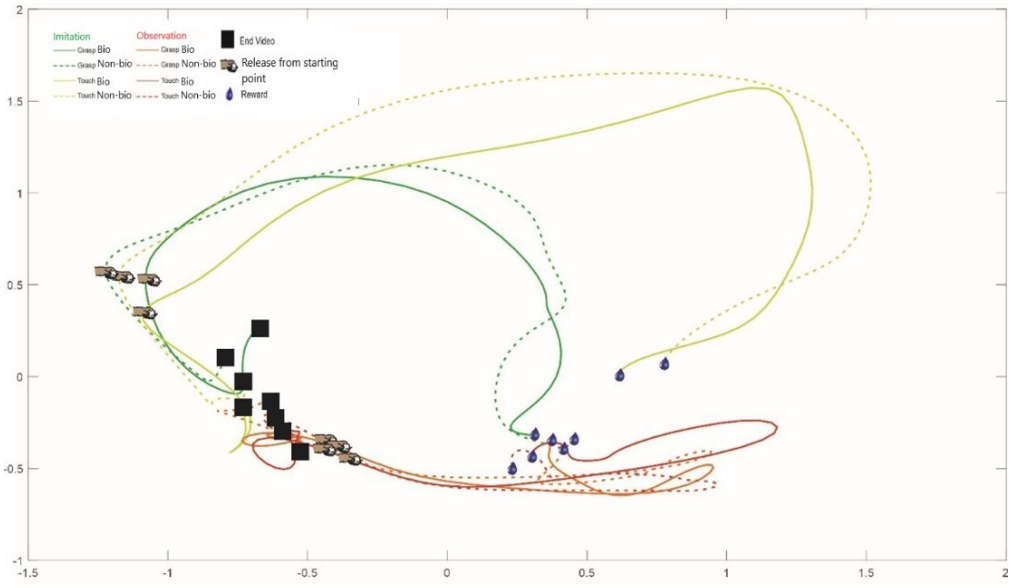

Figure 24. “Release from starting point” alignment, area 46v. All conditions are simultaneously shown in this graphic.

In figure 24, representing the area 46v neuronal signal behavior during the final phases of the task, it is possible to see that the trajectories associated with the “Imitation” and “Observation” conditions, starting from the end of the stimulus presentation, follow opposite direction up to the final reward, where they tend to converge to the same position.

Furthermore, it is possible to notice that after the release of the hand from the starting position, there is a general differentiation between grasp and touch (independently whether cued by biological or non-biological videos) in the "Imitation" condition, whereas for the "Observation" condition there is no such differentiation.

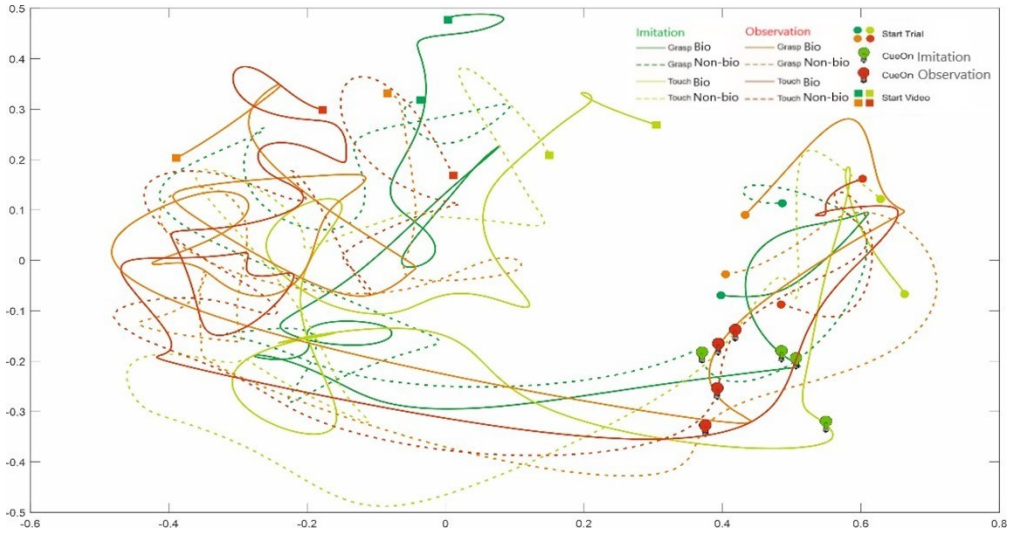

Figure 25. "Cue onset" alignment, area F5. All conditions are simultaneously shown in this graphic.

Looking at the neural signal behavior of area F5 during the initial phases of the task, it's possible to notice that they are not as manifest as for the other areas, possibly because of the small number of neurons considered for this area. Specifically, there is no evident differentiation between the trajectories representing the "Imitation" and "Observation" conditions.

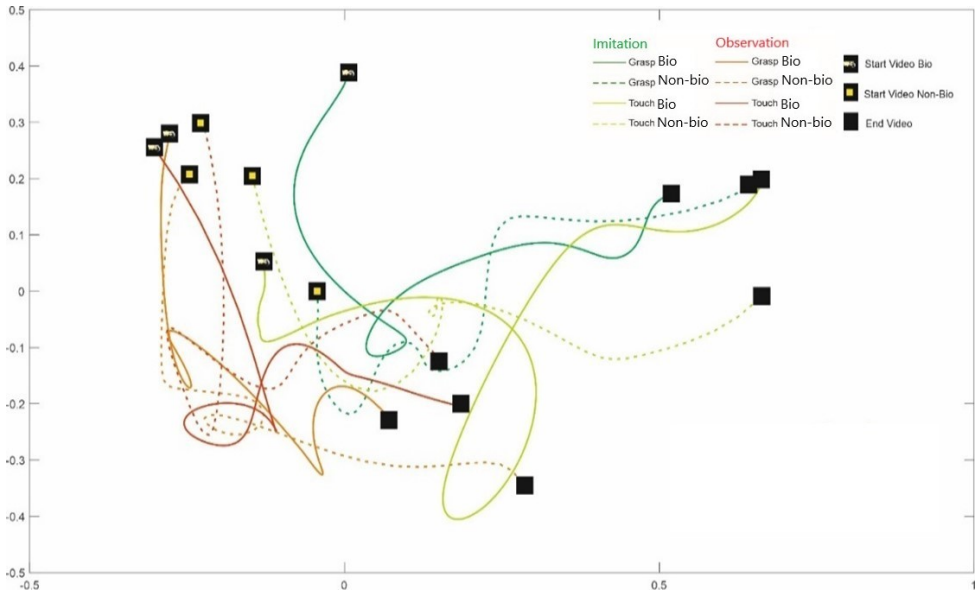

Figure 26. "Stimulus presentation" alignment, area F5. All conditions are simultaneously shown in this graphic.

Looking at F5 neural signal dynamics during stimulus presentation, it is possible to notice that the "Observation" condition trajectories seemingly show no difference between neither the two behavior goals nor the two types of stimuli presented. As for the "Imitation" condition, there also seems to be no difference between the type of stimuli presented (biological vs. non biological) or the goal associated to them (grasp vs. touch). The only notable difference is relative to a more general separation between the trajectories representing the "Imitation" and "Observation" conditions

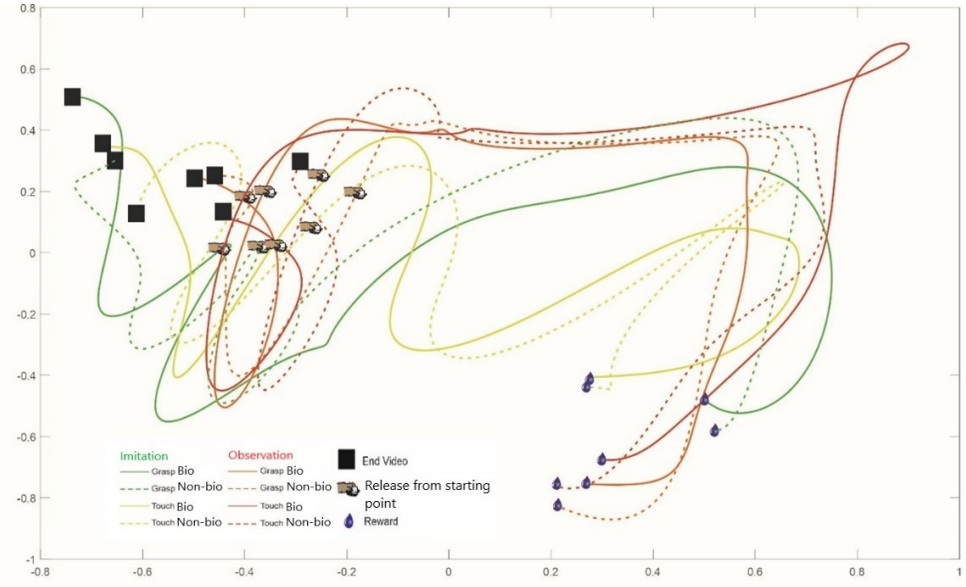

Figure 27. "Release from starting position" alignment, area F5. All conditions are simultaneously shown in this graphic.

Finally, in F5 it is possible to notice, despite being less distinct, the same pattern described for the other two areas during he last phases of the task, in that the trajectories associated to the "Observation" condition appear to follow the same direction from the end of the video to the final reward, whereas for the "Imitation" condition there is, after the release of the hand from the starting position, a distinction between grasp and touch trajectories.

Further comparison between conditions have been made to emphasize specific patterns and differences between areas. To this aim we performed PCA analysis by grouping and merging task conditions in three different modalities (Imitation vs. Observation, Biological vs Non-Biological stimuli, Grasp vs. Touch). Thus, for example, in Imitation vs. Observation we merged the two types of stimuli and the two behavioral goals.

Looking at the comparison between "Imitation" and "Observation", Figure 29 shows that the separation between the two conditions is clear and noticeable in the three analyzed epochs, in all areas, with the exception of the *Cue* epoch in F5. Thus, a strict separation between the two different general condition is confirmed by these comparisons, as seen by the preceding graphics.

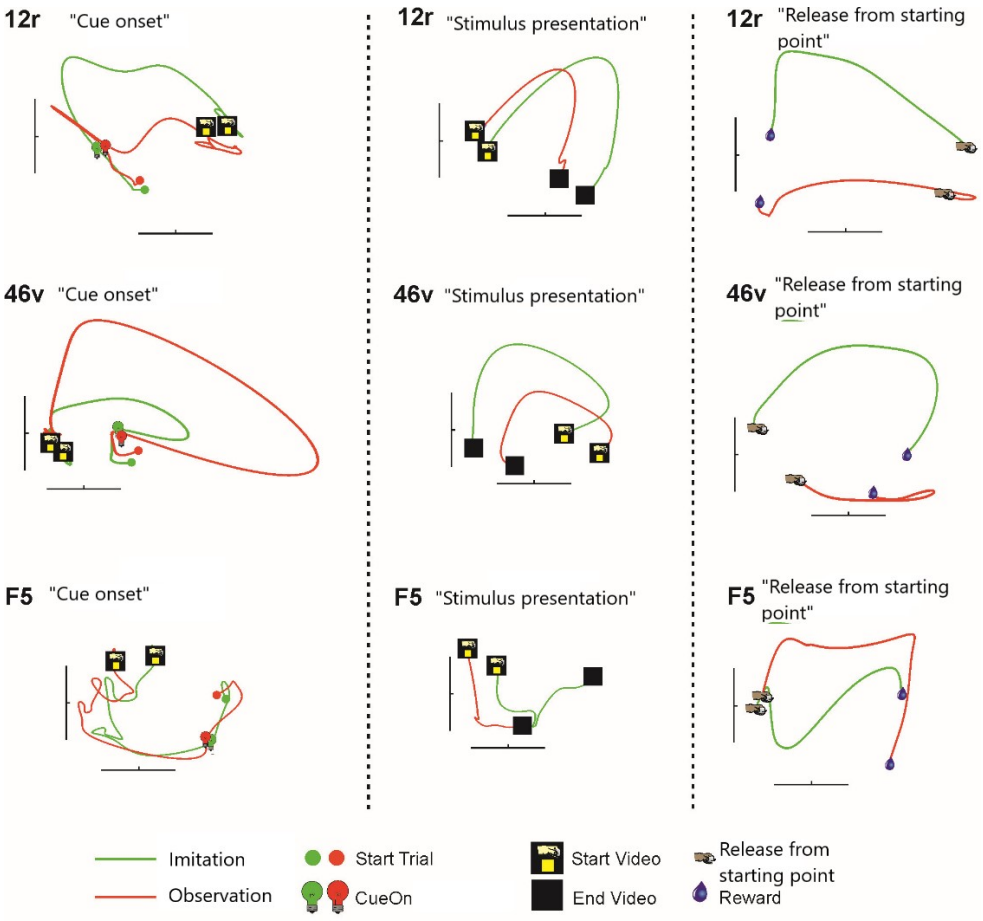

Figure 28. Imitation vs. Observation comparison, all areas.

The PCA shows a different behavior of the overall recorded signal but fails in precisely assessing its neural basis. Thus, it is important to assess and verify if the "general" neural signal dynamics described using principal component analysis are confirmed by specific analysis at the level of single neurons and population.

Even if the present study is based on preliminary analyses, and more specific statistical analyses at the single neuron or population level have not been performed yet, we observed several neurons differentially coding the two used conditions (Imitation vs. Observation), an example of which is shown in Figure 29, which shows the discharge of an area 46v neuron displaying its best, or optimal, excitatory activity in the *Cue* epoch and differentially coding the "Imitation" and "Observation" conditions during the same epoch. In particular, the neuron starts firing just after cue onset, both in the "Imitation" and "Observation" conditions, but, in this latter, the peak is more than two times higher. The discharge of neurons with the same neural specificity could produce the difference in signal behavior shown by the PCA analysis.

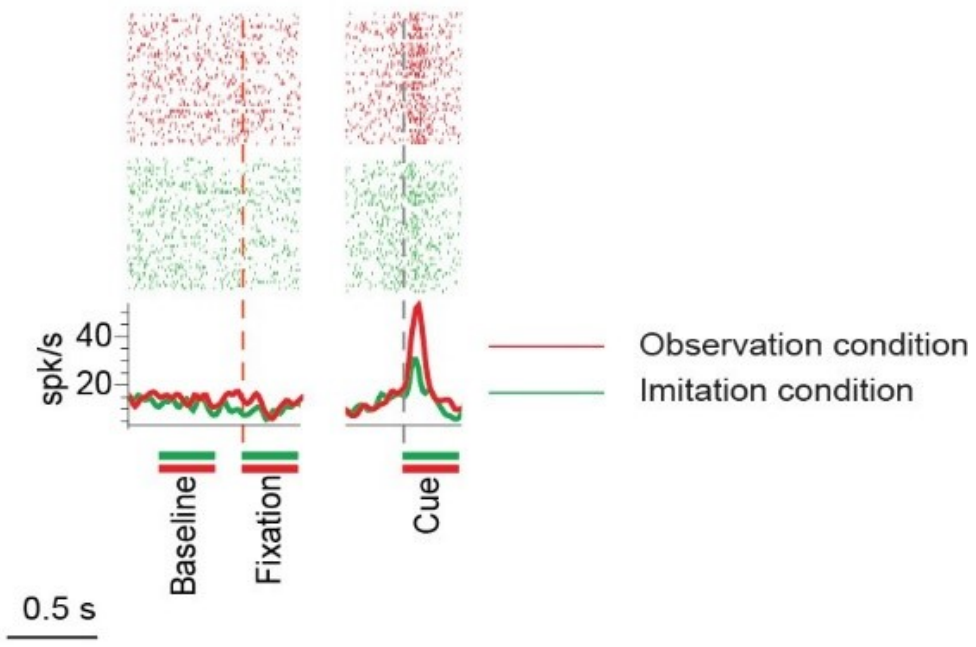

Figure 29. Raster and histogram showing the activity of an area 46v neuron, differentially coding Imitation and observation conditions in the Cue epoch. The activity on baseline and fixation epochs are aligned on the onset of the fixation point. The activity on cue epoch is aligned on Cue onset. Conventions as in Figure 15.

Looking at the comparison between "Grasp" and "Touch" (figure 30), it is possible to observe that, considering the "Cue onset" alignment, there are no differences in the areas between the trajectories. Instead, looking at the "Stimulus presentation" alignment, it is possible to see that the neural trajectories representing "Grasp" and "Touch" start to separate from each other in areas 12r and 46v, while F5 shows no particular separation. Finally, by

examining the last alignment ("release from starting position") it is possible to notice that the neural trajectories for 12r show no differentiation between grasp and touch (thus, the preceding separation has ended), while both 46v and F5 show a difference between the two behavioral goals. This particular behavior of the neural signals recorded in the three areas will be further discussed below.

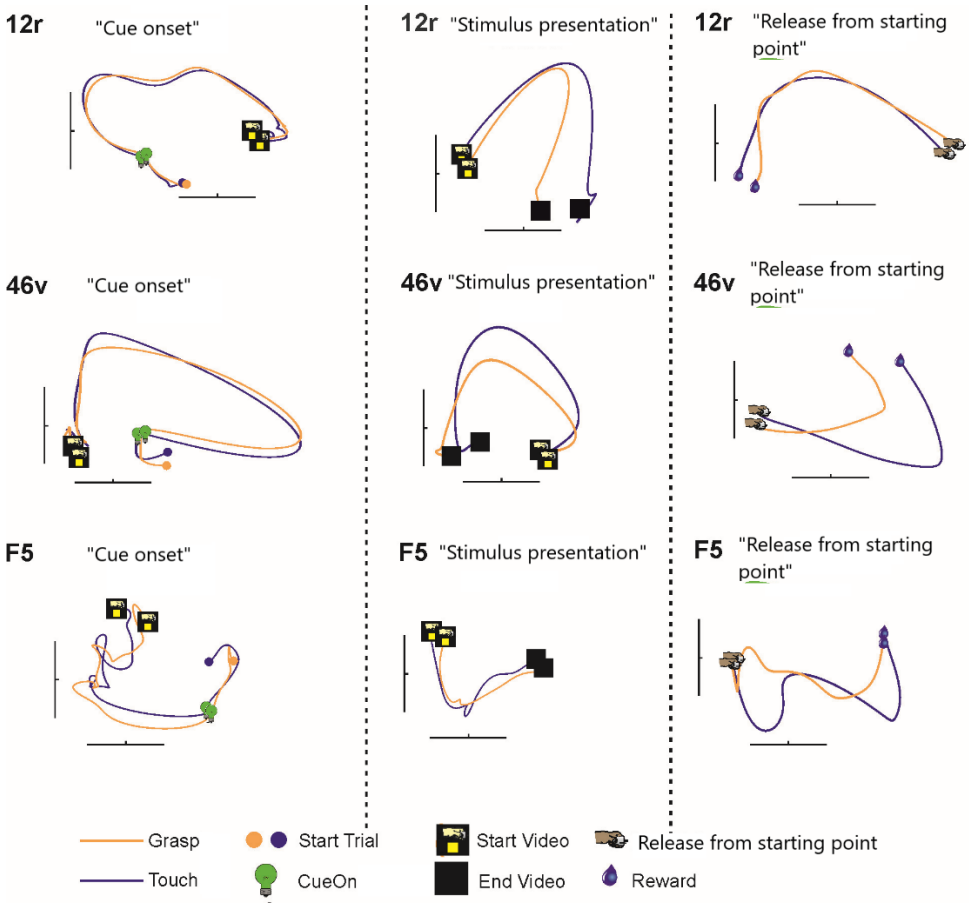

Figure 30. Grasp vs Touch comparison in the "Imitation" condition, all areas.

Figure 31 shows the discharge of an area 12r neuron which presents its best excitatory activity in the Action epoch and differentially codes the two behavioral goals (Grasp vs. Touch). In the Imitation condition, the neuron starts firing before the beginning of actual movement (release of the hand from the starting position) and the peak of discharge occurs around hand-object contact. It is evident that the discharge occurring during actions aimed at grasping and pulling the object is much stronger than that recorded during touching actions. Though specific statistical analyses are still needed, it appears that the discharge is already different just before the beginning of movement.

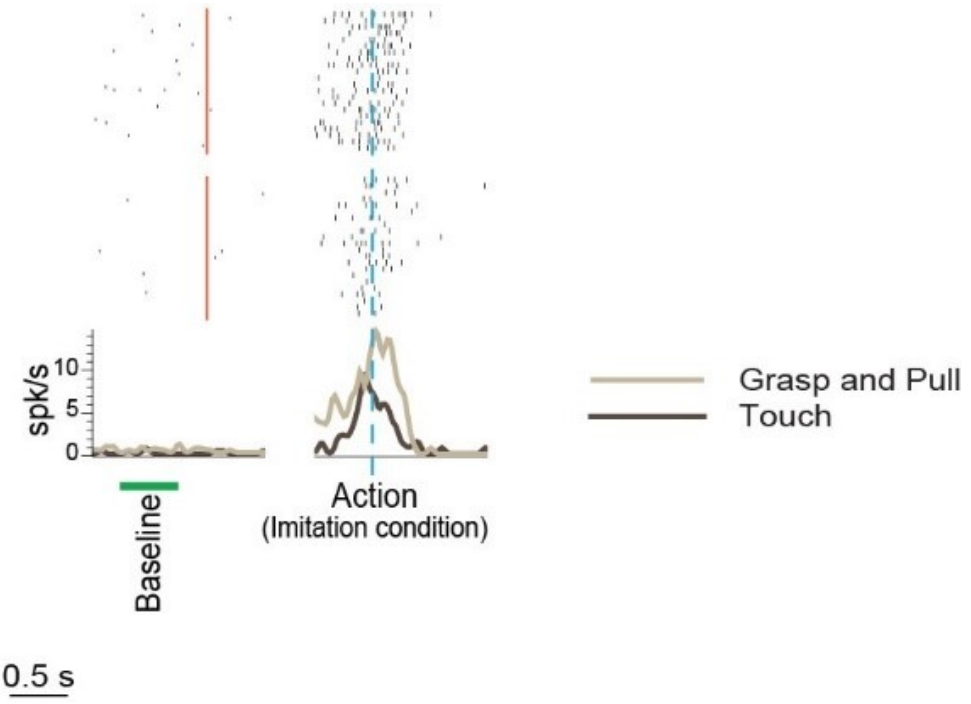

Figure 31. Raster and histogram showing the activity of an area 46v neuron, differentially coding Imitation and observation conditions in the Cue epoch. The activities on baseline and fixation epochs are aligned on the onset of the fixation point. The activity on Cue epoch is aligned on Cue onset. Conventions as in Figure 15.

Finally, the comparison between Biological vs. Non-biological stimuli, showed that differences in neural signal behavior emerge particularly at the level of the prefrontal areas (figure 32). Specifically, while the "Cue onset" alignment shows no differences between the trajectories, in the following alignment ("Stimulus presentation") areas 12r and 46v show a clear separation between the neural trajectories relative to the type of stimuli

presented (Biological vs. Non-biological stimuli). This separation is not maintained in the last phase of the task, as its possible to see that, considering the "Release from starting point" alignment, all the areas show no differentiation between the trajectories representing the type of stimuli presented. F5 area shows no differentiation between Biological and Non-Biological stimuli in neither of the considered alignments.

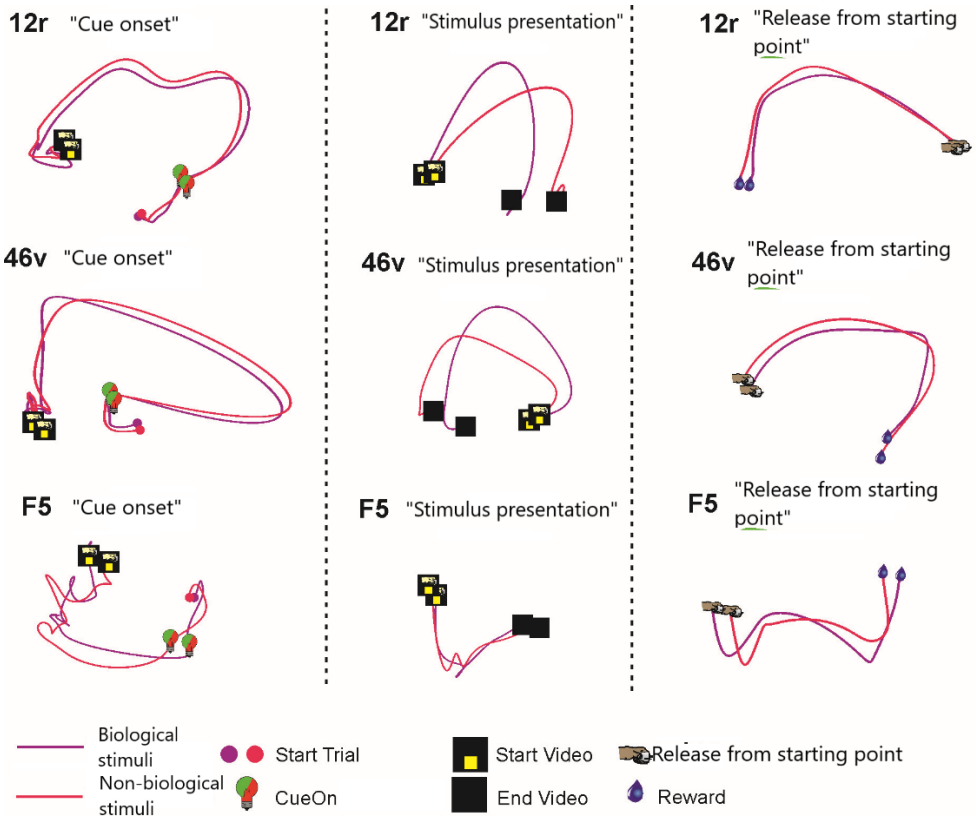

Figure 32. Biological vs Non-biological stimuli comparison, all areas.

Figure 33 shows the discharge of an area 12r neuron showing its best excitatory activity in the Presentation epoch and differentially coding the Type of visual stimulus (Biological vs. Non-biological). It is evident that in both the Imitation and Observation conditions, the neuron has a strong discharge during the presentation of videos representing the biological stimuli but do not fire at levels above baseline during the observation of the moving shapes.

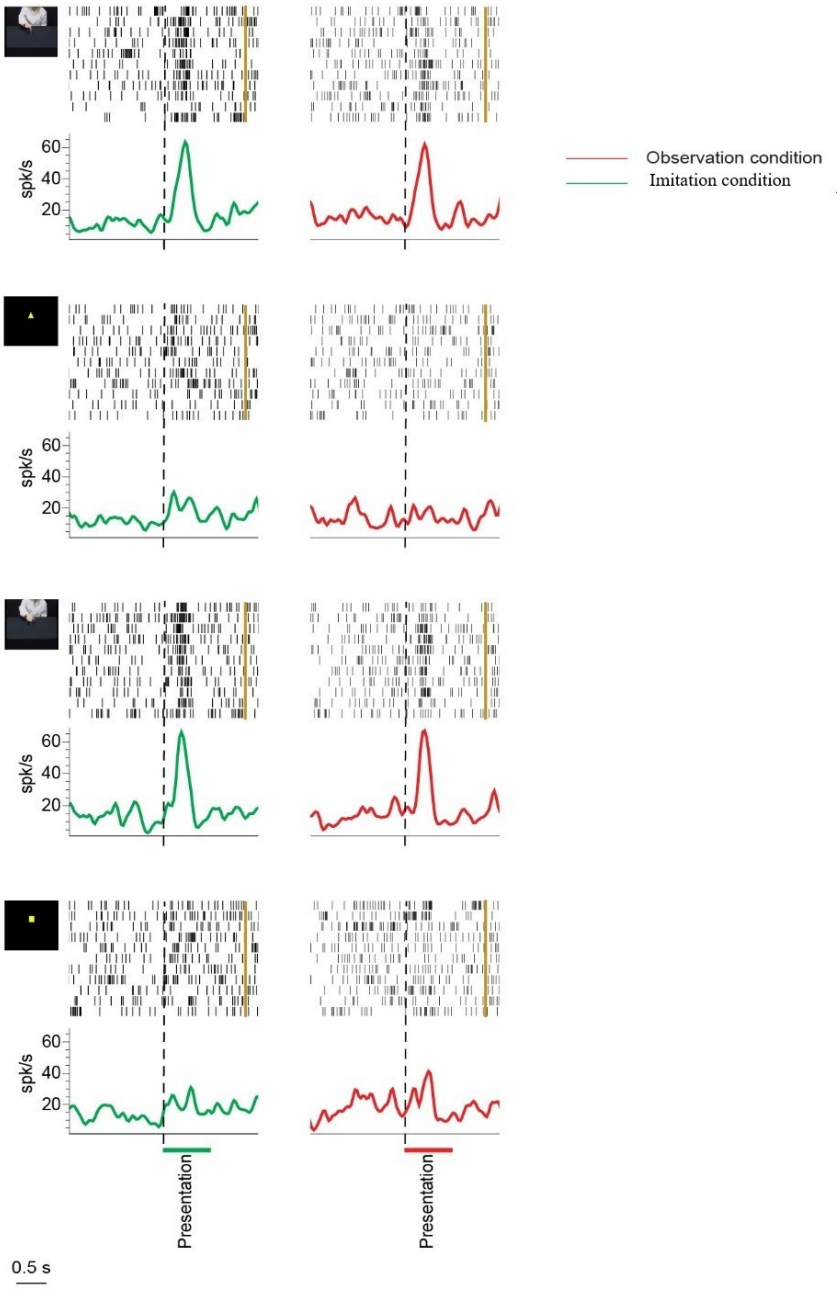

Figure 33. Raster and histogram showing the activity of an area 12r neuron, specifically responding to the observation of biological stimuli, in both used conditions.

Rasters and Histograms are aligned on the stimulus presentation. Solid dark yellow line indicates Cue offset. Conventions as in Figure 15.

4. Discussion

This work is intended as an initial investigation aimed at identifying neurons recorded simultaneously from different areas, active during the same behavioral task (task-related neurons). The study is therefore preliminary to a subsequent phase in which we will assess the differences in the discharge of task related neurons due to different conditions and stimuli employed in the task.

4.1 Technical considerations

The three microarrays used in the study contained an adequate number of recording electrodes from which it was possible to obtain numerous single neurons. However, the microarray placed in the ventral premotor cortex revealed a smaller number of recording channels, compared to the other two prefrontal arrays, probably for reasons related to the surgical phase of implantation. The recording system used is more complex than those employed up to now, and has achieved its intended purpose, allowing

to chronically record activity from different areas simultaneously for more than a year.

In particular, the number of task-related neurons to be further analyzed is very high. It remains to be clarified, in a statistically sound way, how stable is the recorded activity, by comparing different sessions (even very distant in time) and, in particular, by evaluating whether the activity recorded from the same channels in several days is related to the same neurons, or changes occurred revealing loss of some units and/or the addition of new ones. Moreover, the success of the approach allows us to plan new studies of learning phenomena at the level of single units or populations

4.2 Neural response distribution between the epochs of the task

Our results show that the majority of task-related neurons are active during a large number of epochs (87% of them show a significant response during 4 or more epochs, see results). This could indicate that, typically, neurons did not show a particular selectivity for specific phases of the task.

Note, however, that this could depend on the very basic statistical approach employed, aimed at identifying neurons for further, more specific, analyses.

Noteworthy, our data show a fairly balanced distribution of neural responses in the different task epochs taken into consideration. In fact, about 70-80% of neurons activates to some degree in each of the considered epochs.

The picture changes if one takes into consideration each neuron best response, that results in a more unbalanced distribution. In particular, the Fixation and Delay epochs are coded by a much lower number of neurons with respect to the other epochs. Among the other epochs, Cue is the most frequently coded, followed by the Presentation epoch and by the last epochs (Go and Action), in which movement is actually prepared and executed (see below for a possible interpretation of this data)

4.3 Type of neural response during the different task epochs

Our data also reveal that task-related neurons respond increasing or decreasing their firing rate, and that the majority of responses in each epoch are excitatory, with a predominance of excitatory neural responses in the in

initial phases of the task. Nonetheless, the presence of many inhibitory responses, indicates that, at the level of a single neuron, there can be an excitation during a phase of the task and an inhibition in another phase. Focusing on the best responses, it is evident that in the crucial epochs of the task (Cue, Presentation and Action) the ratio between the number of neurons with excitation and inhibition is clearly in favor of excitatory responses.

4.4 Comparison between the distribution of optimal response of task related neurons in the three areas recorded

The comparison between the best responses in the three areas recorded during the different phases of the task, showed that in F5 there is a high concentration of optimal responses (48%) during the final phases of the task (i.e. during action execution). The strong tuning of premotor neurons to action preparation and execution is in line with previous literature (CIT). On the other hand, the prefrontal areas show a relatively more balanced distribution of optimal responses in the various phases. Our data reveal that area 12r neurons most frequently encode (as best response) the epochs of

Presentation, Cue and Go, while area 46v neurons mayly encode the initial phases of the task, i.e. Fixation and Cue epochs.

Furthermore, the distribution of best responses in the various epochs observed in the prefrontal areas is in agreement with previous observations on the same prefrontal region, showing that the responses in the initial (equivalent to our *Cue* and *Presentation* epochs) and final phases (*Action*) of a task are more represented than the *Delay* epoch (Yamagata et al., 2012; Simone et al., 2015, 2017). This seems partially in contrast with the literature, which has greatly emphasized the role of prefrontal cortex in coding delay periods (J. M. Fuster & Alexander, 1971; Funahashi et al., 1989; Di Pellegrino & Wise, 1993b; E. K. Miller et al., 1996; Bodner et al., 1996). However, this discrepancy may be due to the type of task employed by different authors. Our behavioral paradigm was planned to keep clearly separated stimulus presentation, response preparation, and action execution, whereas in many of the previously used tasks the delay period mainly required an active maintenance of mnemonic track, and was very relevant to the final decision, partly including response preparation. Our data, showing a lower number of prefrontal neurons active in the delay period, do not imply that the prefrontal cortex does not play a crucial role in mnemonic processes, but rather stands for the fact that neural response

during delay periods is present mainly when these delays are relevant for guiding behavior. Note also that the *Go* epoch of our tasks, during which many neurons have their best responses, may contain part of the information present in the delay periods of previous studies. Subsequent analyses will be needed to better verify the information encoded in the go epoch by making comparisons across conditions or action purposes.

The different representation of epochs associated to an optimal response, when comparing areas 46v and 12r on the one hand and area F5 on the other, confirms the previous observations present in literature, in particular those resulting from the application of naturalistic motor tasks requiring actions similar to those used here (Simone et al., 2015, 2017; Rozzi & Fogassi, 2017, Bruni et al., 2015) on the different role of these areas. In other words, our data confirm the role of F5 as a motor area, while prefrontal areas show responses related to a large number of components and phases characterizing the task, suggesting a major role in the coding and use of contextual information, and in the maintenance of the action goal during its execution.

4.5 Neural signal dynamics observed during the task

The results emerging from the Principal component analysis (PCA) of the signal recorded in the different areas is in line with the evidence just described. The PCA, which represents a preliminary descriptive analysis to the statistical assessment of individual neuron response, allowed us to identify differences in the "behavior" of neurons (neural dynamics) in relation to the different conditions characterizing the task and to the types of stimuli presented. Although the biological meaning of this analysis has still to be precisely defined, there are some dynamics that are evident by observing the graphs related to the PCA (see figures 29, 30 and 31): both prefrontal areas seem to differently code the general instruction of the task (Action vs Observation) during all phases of the task, whereas in F5 this difference is evident only in the *Presentation* and *Action* epochs.

In addition, concerning the "Imitation" condition, the information related to the behavioral goal (touch vs. grasp) influences the neuron behavior during different epochs, depending on the areas considered: area 46v seems to differently encode the behavioral goal during both *Presentation* and *Action*, area 12r shows this differentiation only during the *Presentation* epoch, while area F5 only during the *Action* epoch. Finally,

encoding of stimulus type (Biological vs. Non-biological) is observed only in the *Presentation* epoch in the two prefrontal areas, but not in F5.

4.6 Conclusion

From the preliminary results of this study, we can conclude that contextual information is processed at the VLPFC level. In particular, our data show that prefrontal neurons can respond differently to the presented stimuli on the basis of their visual characteristics (Biological vs Non-biological stimuli) and exploit them to guide behavior. In addition, we show that prefrontal areas 46v and 12r and premotor area F5 likely have a different role in this process. Indeed, PCA analysis reveals that the behavioral goal biases neural signal in the early phases of the task in area 12r, during both early and late phases in area 46 and in the late phases in area F5. This prompts us to hypothesize that the described processing and integration of contextual cues for behavior guidance, occurs in the 12r-46v-F5 direction.

Future analysis and experiments are necessary to assess whether single neurons and neural populations differentially code the two used conditions (Imitation and Observation), the behavioral goals (Grasp and Touch) and

the two types of stimuli presented (Biological and Non-biological) in different epochs.

Finally, for a better characterization of prefrontal and premotor areas in social cognition, and taking advantage of the possibility to chronically record neural activity, it will be important to assess possible changes in neuronal discharge during the process of learning and generalization of the task.

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