SAPIENZA
UNIVERSITÀ DI ROMA

PhD program in Behavioral Neuroscience

Curriculum: Behavioral Neurophysiology

NEURAL CORRELATES OF THE DISTINCTION BETWEEN SELF AND OTHERS IN THE MACAQUE'S FRONTAL CORTEX

Lorenzo Ferrucci

Supervisor: Prof. Aldo Genovesio

CONTENTS

AIM OF THE WORK

Social interaction is a fundamental prerogative of primate's life. Different abilities are part of the repertoire that is necessary to fulfill a complex social behavior. Many of these abilities are shared between human and monkeys: non-human primates are capable of cooperating (Haroush & Williams 2015), monitor each other's actions (Falcone et al. 2012a), learn from observation (Subiaul et al. 2004, Falcone et al. 2012b, Chang et al. 2011, Monfardini et al. 2014). One of the bases of social behavior is certainly the ability to understand other's actions. In this respect, one of the major discovery in neurophysiology in the last decades is that of 'mirror neurons' in monkey's parietofrontal circuits (di Pellegrino et al. 1992, Rizzolatti et al. 1996). These neurons are activated both when an action is performed and when the same action it is just observed. It has been suggested that they could play a critical role in providing the bases for understanding the action of others through the same neural mechanisms which activate during the execution of a specific motor act. Despite that, the activity of mirror neurons is not able to provide a neural signal able to distinguish between self and others. Recently, some studies attempted to investigate the neural correlates of self-others differentiation, looking for evidence of a non-overlapping neural representation of self and others action. In this thesis, I will discuss the results of three distinct neurophysiology experiments that investigated the role played by different areas of the macaque frontal cortex in providing such distinction. Through a task design that required the interaction between humans and monkeys, the aim of these experiments was to explore the distinct neural correlates which allow the prediction or the anticipation of someone else actions.

In Section 1, a general description of the experimental design adopted by the three experiments discussed here is provided. The non-match-to-goal task required the interaction between the monkey and the experimenter, which alternated their role as actor and observer along the different trials.

In Section 2, the methods and the main results of two previous neurophysiology experiments (Falcone et al. 2016, Falcone et al. 2017) are discussed. These experiments investigated the property of single neurons recorded in the lateral prefrontal cortex and in the medial frontal cortex.

In Section 3 are discussed the methods and the main results of our study (Cirillo et al. 2018), which adopted the same task design as the two previous experiments, recording the activity of neurons in the dorsal premotor cortex.

Section 4 presents an overview of the overall results, discussing the role played by different categories of cells identified within the frontal cortex in the three experiments that investigated the neural correlates of representing others' future and past behavior in a separate way from one's own.

SECTION 1

THE NONMATCH-TO-GOAL TASK IN SOCIAL INTERACTION

1.1 THE MATCH-TO-GOAL RULE

Analogical reasoning is considered a key feature of the human's cognition. This concerns the ability to identify similarities and correspondences between exemplars, getting to know the relationship that occurs between them (Gentner 1983). Reasoning by analogies is considered a core high level function of the human mind that enables to learn information about the relationship between objects and to transfer this knowledge whenever a similar and new situation is presented (Holyoak et al. 1984). Studies with human children highlighted the important role played by different executive functions in the development of such ability (Krawczyk 2012, Simms et al. 2018, Whitaker et al. 2018).

In comparative research, many studies tried to address the question whether the ability of abstract thinking and making logical inference is an exclusively human competence or whether it is shared with animals. Since the use of language is a key feature in human for the ability to acquire abstract concepts, the first studies that tried to address this question proposed that nonverbal being could not develop such a competence (Premack, 1978). Several studies over the years suggested that this is not the case: analogical reason has been found in great apes (Gillian et al. 1981, Thompson et al.1997), monkeys (Katz et al. 2002, Fagot et al. 2011, Truppa et al. 2011), crows (Smirnova et al. 2015) and parrots (Obozova et al. 2015). To investigate these processes in a laboratory setup, one of the most common experimental paradigm used is the match-to-goal task (MTG). In this type of task usually two different stimuli are presented. The sample stimulus has to be compared with two or more comparison stimuli in order to give a judgment about the difference between them. Consider for example a geometrical figure used as sample stimulus, a red square. The comparison stimuli in the test phase could be a green square and a green triangle: in this case, the correct response may be the stimulus with the same geometrical shape, the green square. However, another case is possible, in which two different comparison stimuli can be presented, a red circle and a green triangle: the underlying rule in this case may be to select the same color, which would make the red circle the correct response. Indeed, in the MTG the correct response is untied to the specific features of a stimulus, but rather it relies on the rule of 'same/different', that could be varied experimentally. In order to recognize whether two stimuli match with each other, it is necessary to have the notion of 'sameness', which is an abstract concept. Different versions of the MTG task can test more difficult abstraction rules, like the relational match (RMTG). In the case of the MTG, the 'sameness' relies on a perceptual judgment about the physical features of the stimuli; in the RMTG, it is required to understand if objects share the same relation rather than the same perceptual attributes. Consider for example a sample stimulus composed by 2 letters (AA) and two comparison stimuli also composed by two letter, the stimulus (BB) and the stimulus (CD). Identifying the first comparison stimulus (BB)

as the correct response requires the ability to understand relationships; in this case the fact that the correct comparison stimuli is the one composed by a couple of identical objects, as the sample stimulus, even if the objects are perceptually and physically different.

1.2 NONMATCH-TO-GOAL AND SOCIAL INTERACTION

We analyzed three different datasets recorded in three different frontal areas of the rhesus monkey's brain. The aim of the experiments was to study the neural activity of single neurons during a social interaction task. For this purpose, a variant of MTG task was used in all the three experiments, the nonmatch-to-goal-task (NMTG). This task provides the same underlying rule of the MTG task except that, in the case of the NMTG, the correct response is given by the selection of the stimulus that doesn't match, instead of the one which is the same. Here I provide a brief general description of the task design used in those experiments; a more specific description along with the details of the specific versions and their variants used in each experiment will be discussed in the further sections.

In the NMTG task, in each trial a couple of stimuli is presented on the touchscreen. The task's rule required the monkeys to discard the stimulus selected in the previous trial and to select the other one. In each trial, one of the two stimuli presented on the screen was thus the correct stimulus in the previous trial, and the other stimulus was either a new one or the one previously not selected. The peculiarity of the NMTG task is that choosing the different stimulus instead of the same, as in the MTG task, makes it possible to have a sequence of trials linked with each other, in which the correct response changes from trial to trial. This feature becomes crucial because it introduces the necessity to keep in mind the selection carried out in the previous trial in order to be successful in the current trial. Therefore it is possible to measure the ability of the monkeys to follow the nonmatch rule, which is expressed in terms of the proportion between correct and error trials after a correct trial.

In this framework the experiments discussed in this work introduce a variant in the NMTG task to study social interaction. In the social version, monkeys have to interact with a human partner to perform the task, alternating the role of actor and observer during the experiment. At the end of a trial performed by the monkey, the human agent could intervene in the task, performing himself the next trial with monkey observing his choices and actions. In the trial performed by the human agent, the correct choice still depends on which stimulus was chosen in the previous trial by the monkey: the human agent follows the same nonmatch rule as the monkey, taking in account the previous monkey's choice and selecting the different target. At the end of the trial performed by the human agent, the monkey can perform the next trial. The underlying idea behind the social variant is that the monkey

has to keep in mind the stimulus previously chosen by the human partner in order to discard it and to choose the new one of the couple, as well as he performs the task alone.

The NMTG task described above represents the basic task designed behind all the three different experiments that will be discussed in this work. However, two different variants were adopted, that differed in the 'domain' in which they operate: an 'object' version and a 'spatial' version. In the object nonmatch-to-goal version (ONMTG), four objects of different shapes presented in a couple are used as stimuli and the rule consisted in discarding the previously chosen object. In the spatial nonmatch-to-goal version (SNMTG), a unique stimulus is presented in two out of four possible different positions and the rule consisted in discarding the previously chosen position. The same NMTG rule was applied in both cases, a rule that required to identify the different stimulus and to discard it, but within a different context. For instance, the information about the position of the stimulus was uninformative in the ONMTG version, while it was fundamental in the SNMTG. Nevertheless, Falcone et al. (2013) found that monkeys were capable of transferring the abstract rule underlying the NMTG between the two different versions, despite they appear to belong to two different cognitive domains. For the purpose of this work, the ability of trained monkeys to switch easily between the two task versions can offer a good chance to compare the results obtained with the two of them.

1.3 ADVANTAGES AND LIMITATIONS OF THE TASK DESIGN

This task design offers various advantages in the attempt to study a complex and naturalistic behavior like social interaction in the laboratory environment. First, it introduces the requirement of monitoring other's actions. Previous studies that investigated others' actions observation often adopted experimental paradigms in which no active monitoring was required to the monkeys. This is the case, for example, of the classical studies about mirror neurons (di Pellegrino et al. 1992, Rizzolatti et al. 1996, Casile et al. 2013), in which monkeys observed passively the execution of different movements performed by the experimenters. In the NMTG task instead, for its interactive aspect, it becomes critical for the monkey to monitor the action of the human partner during his turn, focusing on the choice that the partner makes, and to retain the information until it is time to make a choice in the next trial. Introducing an active monitoring of the partner's actions permits to have a behavioral measure of the ability of the monkeys in the task, in terms of correct trials performed after a trial performed by the human partner. Second, a social interaction with a real physical agent was introduced. Some previous studies investigated action observation when a cursor moved on a screen

(Cisek & Kalaska 2004, Tkach et al. 2007). It is not clear how the monkeys can interpret the automatic movement performed by a computer of a cursor on a screen, without an observable agent that physically performs the action, and in any case it is far from the typical interaction that a monkey experiences daily in its social life. Furthermore, the animacy of a rival agent seems to play a role in the modulation of the activity of neurons recorded during a competitive social task (Hosokawa & Watanabe, 2012). Finally, the interaction with a human partner, instead of another monkey, offers the chance to have the performance of the agent under experimental control. In the NMTG task used in the three experiments that we will further discuss, the human partner always performed the correct choice, without making mistakes. The absence of errors allowed the creation of a stable predictive context, in which the monkeys always know what the human partner will do next without ambiguities, offering the base for a possible prediction of his actions. For this particular reason, the main analysis on neural activity has been carried out in a delay period of the task, occurring between the presentation of the stimuli and the go-signal that allows the movement toward one of them. The introduction of this task period is important to study the neural activity without the possible confound of the perception of the movement either of the monkey or of the human agent. The purpose of this task is to investigate not only the neural mechanisms that lead to the differentiation between self and others' actions during the choice of a behavioral goal, but also the anticipation, or prediction, of these actions. Knowing the underlying rule of the task and creating a strong stable predictive contest can facilitate the prediction of other's choices in this period of the task, where the information that defines the correct behavioral goal is already present but the movement necessary for its selection is not allowed yet.

However, a major limitation arises with the use of this task design. This is related to the impossibility to study error related activity, at least during the trial performed by the human agent. Whilst a stable predictive context enhances the ability of the monkeys to predict what the human agent will do, because his actions do not lend themselves to any ambiguities, the absence of errors as controls leads to an uncertainty in the clear interpretation of the results as an actual predictive activity. This possibility will be further addressed in details in the conclusive section.

SECTION 2

LATERAL PREFRONTAL CORTEX AND MEDIAL FRONTAL CORTEX

2.1 lPFC AND MFC IN SOCIAL INTERACTION

lPFC

The prefrontal cortex (PFC) in the macaque brain can be divided into three main regions: the medial prefrontal cortex (mPFC), the ventral prefrontal cortex (vPFC) and the lateral prefrontal cortex (lPFC). The latter can be subdivided into two further regions: the dorsolateral prefrontal cortex (dlPFC), which includes the Broadmann's areas 9 and 8b, and the ventrolateral prefrontal cortex (vlPFC), which includes The Broadmann's areas 12/47, 45 and 46 (Walker 1940). Of the many cognitive functions that have been associated with the activity of the lPFC, many neurophysiological studies focused on its role in the representation of behavioral goals. The activity of the neurons in this area represented the position of the behavioral goal during the delay period of different tasks (Rainer et al. 1998, Saito 2005). The sustained activity during the delay suggests that lPFC is involved in retaining the necessary information to achieve behavioral goals mediating working memory mechanisms (Goldman-Rakic 1988, Tanji & Hoshi 2008). Yamagata et al. (2012) investigated different aspects of the generation of a goal-directed behavior: analyzing the activity of lPFC during and after the presentation of an instruction cue (IC) the authors found that neurons in the vlPFC encoded the visual features of the IC, while neurons in the dlPFC did not. On the contrary, during the presentation of the IC and during the following delay period, neurons in the dlPFC directly encoded the behavioral goal represented by the IC (in this case, right or left position), to a greater extent than neurons in vlPFC. Such difference seems to be in line with the idea proposed by Goldman-Rakic (1988) that different information domain are processed by the two different areas, with the vlPFC involved in features processes, and the dlPFC involved in spatial processes. In addition, the involvement of lPFC has been investigated not only in the coding of future goals, but also for coding past goals. Genovesio et al. (2006) found that neurons in the lPFC had activity that encoded future and previous goal in a task in which it was necessary, similarly to the NMTG, to remember the previous chosen goal in order to succeed in the successive trial. Interestingly, only a few neurons encoded both, leading to two separate subpopulations in lPFC that clearly distinguish between future and previous goals.

All these evidence of the ability of the lPFC to represent so many specific aspects in the process of selection of a behavioral goal lead to the question whether similar processes can take place in this area during social interaction. The ability to distinguish between their own goals and those of others is a key feature in primate social life. So far, evidence of the involvement of the lPFC in social cognition mechanisms comes from functional neuroimaging studies with humans, where these areas have been observed to encode other's actions prediction error (Burke et al 2010, Suzuki et al. 2012).

MFC

The medial frontal cortex (MFC) as a whole has been suggested to play an important role in different cognitive functions, but a special attention has been dedicated to study social cognition (Ridderinkhof et al. 2004, Amodio & Frith 2006). Numerous studies outlined the importance of several areas of the MFC in the processes of action (Carter et al. 1998, Botvinick et al. 2001) and errors monitoring (Ito et al. 2003), with a specific involvement of the anterior cingulate cortex, in the ventral part of the medial wall. The dorsal part of the medial wall includes numerous areas. The posterior part belongs to the Broadmann areas 4 and 6 and includes the primary motor cortex M1, the supplementary motor area (SMA) and the pre-supplementary motor area (pre-SMA). The anterior part lies in the prefrontal cortex, and the Broadmann's areas 8 and 9 occupy the more posterior region (posterior medial prefrontal cortex, pmPFC). Recording sites in the experiment of Falcone et al. (2017) were located in these areas.

In the macaque brain, the supplementary (SMA) and pre-supplementary motor areas (pre-SMA) are located on the dorsomedial frontal cortex in Broadmann area 6, rostrally to the primary motor cortex. The caudal part of area 6 corresponds to the SMA and the anterior part to the pre-SMA (respectively area 6aα and area 6aβ, Matelli et al. 1991, Pickard & Strick 1996). These regions are involved in different fundamental aspects of motor behavior (Nachev et al. 2008). Neurons in these areas discharge before movements of specific parts of the body (Brinkman & Porter 1979, Tanji & Kurata 1982), responds to movements cued by specific sensory cues (Tanji & Kurata 1985) and are active during learning of movement's sequences (Nakamura et al. 1998). Only recently, the role of these areas has been investigated in social interaction paradigms, to better understand their involvement in the representation of self and others' behavior. Yoshida et al. (2011) recorded the activity of single neurons in two rhesus macaques using a role-reversal task. In this task, monkeys were required to press one of two buttons, green or yellow, in order to receive a reward. Pressing one button led to reward delivery while pressing the other did not. The correct response was associated with the pression of a specific button for blocks of 5-17 trials, then switched unpredictably. Monkeys alternated the role of actor and observer every 2 trials. When the correct button was pressed, reward was delivered to both the actor and the observer. When the partner chose the wrong button because of the block change, the observer monkey had to use this information to switch color and press the other button when it was his turn to be the actor. Recordings were obtained in two areas of the medial cortex: a dorsomedial region, including the SMA and pre-SMA areas, and a more ventral region, including the cingulate sulcus regions. The authors found a significant proportion of cells that selectively encoded the agent who performed the trial during the action, 'partner type' neurons and

'self type' neurons. The proportion of partner and self type neurons was greater in the dorsomedial area compared to the cingulate sulcus region. In the same dataset, authors found cells that selectively encoded partner's errors (Yoshida et al. 2012), proving that the supplemental motor complex is highly involved at different levels in the processes of self-others's actions differentiation. The advantages of this kind of experimental paradigm are multiple. In the first place, similar to what happens with the NMTG task, monkeys have to actively monitor the actions of the partner to make a choice when they become the actors. This feature distinguishes it from previous paradigms in which the monitoring was essentially passive, where it was not necessary to extract information during the observing phase to succeed during the execution phase. In the second place, the task includes a real interaction with another living agent instead of an inanimate one such a cursor. However, this particular experiment comes with the limitation of not having a delay period, since the neural activity was investigated during the movement period of the task, as previously done in studies about mirror neurons activity (di Pellegrino et al. 1992, Rizzolatti et al. 1996). The introduction of a delay period allowed a better understanding of those mechanisms which permit the self-others differentiation in terms of prediction of others' actions, without the possible interference due to the observation of the movements.

To what extent the pmPFC is involved in social interactions mechanisms appears to be nowadays less clear. The few neurophysiological studies with primates that targeted these areas showed neurons which were modulated by the selection of specific tactics necessary to select an action (Matsuzaka et al. 2012, Matsuzaka et al. 2016), involving this area in the processes of supervisory control during the execution of a specific motor behavior.

2.2 MATERIALS AND METHODS

Here are reported the experimental procedures and the details of the tasks used by Falcone et al. (2016, 2017) in their studies on social interaction in the lateral prefrontal cortex and medial frontal cortex. In the lPFC (Falcone 2016), two male rhesus monkeys (*macaca mulatta*) performed an ONMTG task version: Monkey 1, average weight 9.5 Kg, and Monkey 2, average weight 7.5 Kg. In the MFC (Falcone 2017), two male rhesus monkeys (*macaca mulatta*) performed instead a SNMTG task version: Monkey 1, 10 years old, average weight 9 kg, and Monkey 2, 7 years old, average weight 8 kg.

Sequence of task events

lPFC

Each monkey sat in a primate chair facing a monitor touch screen (Microtouch monitor, 19 inches, 800x600 pixel resolution) used to perform the ONMTG task (Fig 2.1). The task started with the presentation of a white circle (central stimulus, CS, 7° visual angle) in the center of the screen: the monkeys were required to touch it and to hold the touch to start the trial. If the CS was not touched within 2 s, the trial was aborted. After the touch of the CS, a rectangular horizontal grey bar (18° x 10°) immediately appeared on the screen, above the CS (14° above the position of the CS). The monkeys were required to hold the touch on the CS for a randomly chosen period of 1 or 1.5 s (*holding CS period*) after which the object-goals (OG) appeared on the screen. The OGs were 4 different figures (13°x13°, Fig 2.1, on the top) which were presented in couples, one on the right and one on the left of the CS (24° from the CS position). After the appearance of the OGs, a *delay period* started, randomly lasting 0, 1 or 1.5 s. During the *delay period* the CS, the horizontal bar and the OGs were on the screen, and the monkeys were still required to hold the touch on the CS. At the end of the delay period, the horizontal bar disappeared: this represented the go-signal that inform the monkeys to move toward one of the two OGs, making a choice. After the monkeys reached one of the two objects, they had to maintain the touch for a randomly chosen period of 0.8 or 1.2 s (*holding goal period*). After that, a visual feedback, that was informative about the correctness of the choice just made, appeared around the chosen object. A white square (28°) or a red triangle (30° x 25°) informed the animals that the choice was correct; a white circle (28°) or a blue triangle (30° x 25°) informed the animals that the choice was incorrect (Fig 2.1, on the top). Positive and negative feedbacks were presented in couple based on color (white square and circle) or shape (red and blue triangle) for blocks of 21 correct trials, then the couple was switched. Since the appearance of the feedback around the chosen object, monkeys had to continue to maintain the touch on the OG for a randomly chosen period of

0.8 or 1.2 s (*feedback period*). At the end of the feedback period, reward was delivered after correct choice, then the screen turned black. After a randomly *intertrial period* of 1 or 1.5 s, the next trial started. In the successive trial, the chosen object was presented again, together with one of the two objects of the four not presented before or with the same object discarded in the previous trial. After incorrect choices, no reward was delivered and after the intertrial period the next trial followed, which was always a correction trial. In correction trials, the same couple of objects was re-presented, so as the monkeys could switch their choice and select the correct object. Perseverative errors in correction trials led to the repetition of the correction trial. In the ONMTG the rule was to discard the object that was chosen in the previous trial and select the other one. In the first trial of each session, which was not preceded by any trial, each of the two object was accepted as correct choice.

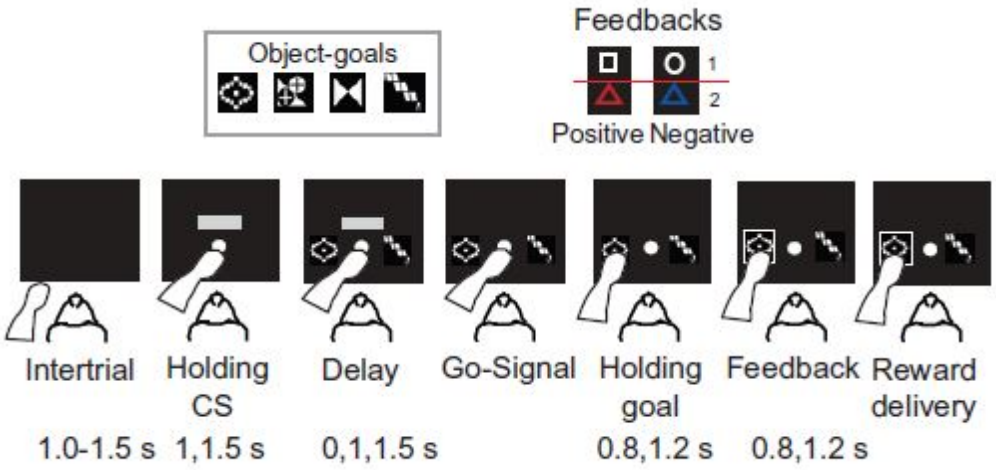


Fig. 2.1 Sequence of the task events in the ONMTG task used by Falcone et al. (2016). Each black square represents the monitor screen in a period of a trial. On the top the four different objects and the feedbacks used in the task are represented.

MFC

In this study Falcone et al. used a very similar experimental paradigm to that used in the lPFC, with the difference that monkeys carried out a SNMTG task (Fig 2.2). Using the same experimental setup previously described, a trial started with the presentation of a CS represented by a white circle (7°) in the center of the touch screen monitor. After the touch, the go-signal horizontal grey bar (18° x 10) appeared above the CS, initiating the *holding CS period* in which the monkeys had to hold the touch on the CS for a randomly chosen period of 1 or 1.5 s. At the end of this period, two peripheral targets

(PT) represented by identical filled grey rectangle (7.1° x 7.7°) appeared on the touch screen in two different positions out of four. The four possible positions were a combination of two spatial coordinates, right or left and bottom or center: bottom right (17.5 ° and 23.5° below and right from the center), bottom left (17.5 ° and 23.5° below and left from the center), center right (23.5° left from the center) and center left (23.5° left from the center). The *delay period* duration of 0 or 1s started after the presentation of the PT, during which the animals were not allowed yet to move toward one target but instead they had to maintain the touch on the CS. As in the other experiment, at the end of the delay period the disappearance of the horizontal bar served as a go signal for the monkeys. Within 3s animals had to select one of the two PT and to continue touching it for a *holding target period*, lasting 0.8 or 1.2 s. Finally, the same couples of feedback used in the other experiment (Fig. 2.2, on the top) were used to inform the monkeys whether the choice was correct or not: the shape feedbacks were a white square (14.8°) for the correct response and a white circle (16.7°) for the incorrect response; the color feedback were a red triangle for the correct response and a blue triangle (19.3° x 15.9°) for the incorrect response. The feedback surrounded the chosen PT appearing at the end of the *holding target period.* In the *feedback period,* monkeys continued touching the target for 0.8 or 1.2 s. At the end, reward was delivered in correct trials and after 1 or 1.5 s of *intertrial period* the successive trial could start. In the successive trial, the chosen position was presented again, together with the two position not presented or the positions not chosen in the previous trial. In the SNMTG the correct response was not related to the shape of the object, as in the ONMTG previously described, but rather it was related to its position. Indeed, monkeys had to choose the target that was not presented in the same position as the chosen target of the previous trial. In incorrect trial, reward was not delivered and after the *intertrial period* a correction trial was presented. In correction trials, targets were presented in the same positions of the previous trial, to allow the monkeys to correct their choice. Errors in a correction trial led to another correction trial.

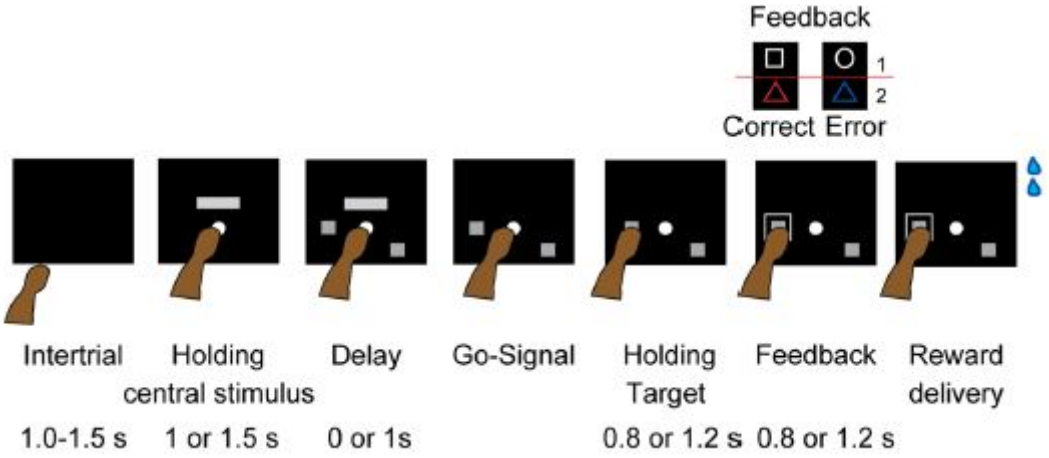


Fig. 2.2 Sequence of the task events in the SNMTG task used by Falcone et al. (2017). Each black square represents the monitor screen in a period of a trial. On the top the feedbacks used in the task are represented.

Monkey Human interaction

In both experiments, in a subset of trials the monkeys interacted with a human partner to perform the task, alternating their role as observer and agent. The human partner was sitting next to the animals on their right side. After a complete and correct trial performed by the monkey, the human could decide to intervene in the successive trial, moving his hand toward the center of the screen during the *intertrial period.* Monkeys were trained to let the human perform the trial when it was his turn without intervening and just observing his actions. The sequence of the events of a trial was identical whether the monkey or the human performed it. The human partner could perform randomly between 1 and 4 consecutive trials, and he performed always the correct choice. At the end of every trial, the human agent could decide to leave his hand at the center of the screen during the *intertrial period*, to make it clear that he was going to perform another trial. Otherwise, he could decide to remove the hand from the center of the screen, so that the monkeys could know that they should perform the next trial. In this way, it was possible to distinguish three type of trial, based on the agent who performed it. The *human trials* were all the trials in which the human was acting as agent and the monkey as observer. Instead, the trials in which the monkey was acting as agent and the human as observer were classified as *monkey trials*. Furthermore, the *monkey trials* were divided into two different subgroups. The *monkey trials not interactive* were all the trials performed by a monkey when the previous trial was also performed by the monkey; the *monkey trial interactive* were all the trials performed by a monkey when the previous trial was instead performed by the human agent (Fig 2.3).

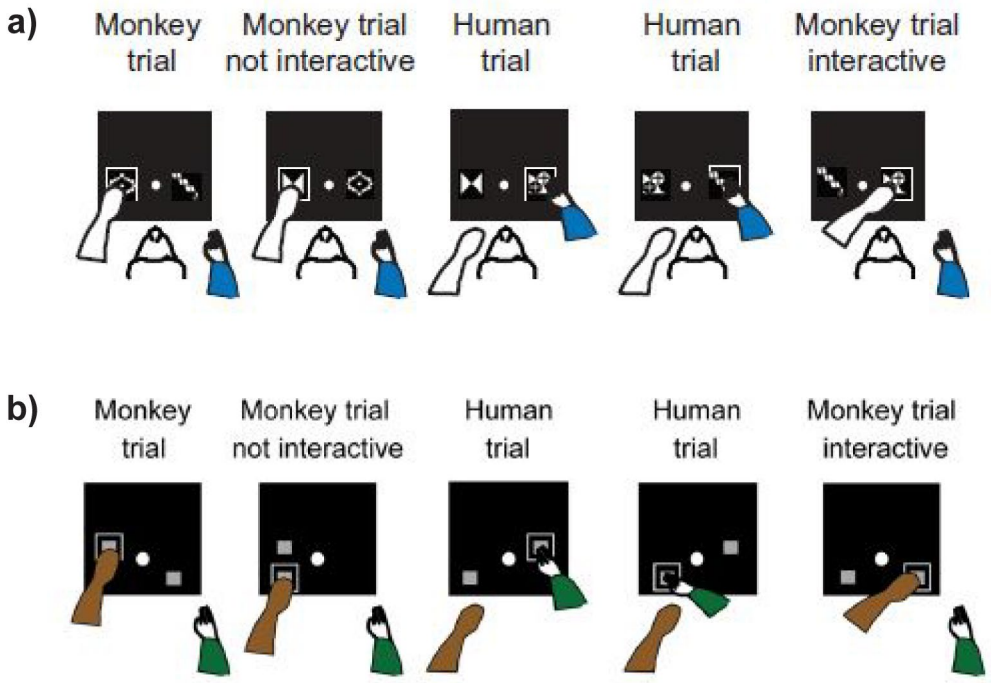


Fig. 2.3 Example of a sequence of trials in the ONMTG (a) used by Falcone et al. (2016) and in SNMTG (b) used by Falcone et al. (2017). The monkey and the human alternate as actor and observer. Each black square represents the monitor screen in the feedback period in an example set of five consecutive trials. Above each black square is described the type of trial.

Surgical Procedures and data collection

Animals were surgically implanted with a head holding device during the training phase. In the lPFC experiment, before the recording sessions started, two recording cylinder (18mm diameter) were implanted in both monkeys, over the right frontal lobe for Monkey 1 and over the left frontal lobe for Monkey 2. Recordings were obtained extracellularly with a 5-channel multielectrode system (Thomas Recording); the electrodes were inserted transdurally with a microdrive. Penetrations reached the lateral prefrontal cortex in both monkeys in different hemisphere (Fig 2.4a). In the MFC experiment, recordings were obtained with the same methods from three different areas: the posterior medial prefrontal cortex (pmPFC), the pre-supplementary motor area (pre-SMA) and the supplementary motor area (SMA). Penetrations sites for both monkeys in the medial prefrontal cortex are shown in Fig. 2.4b.

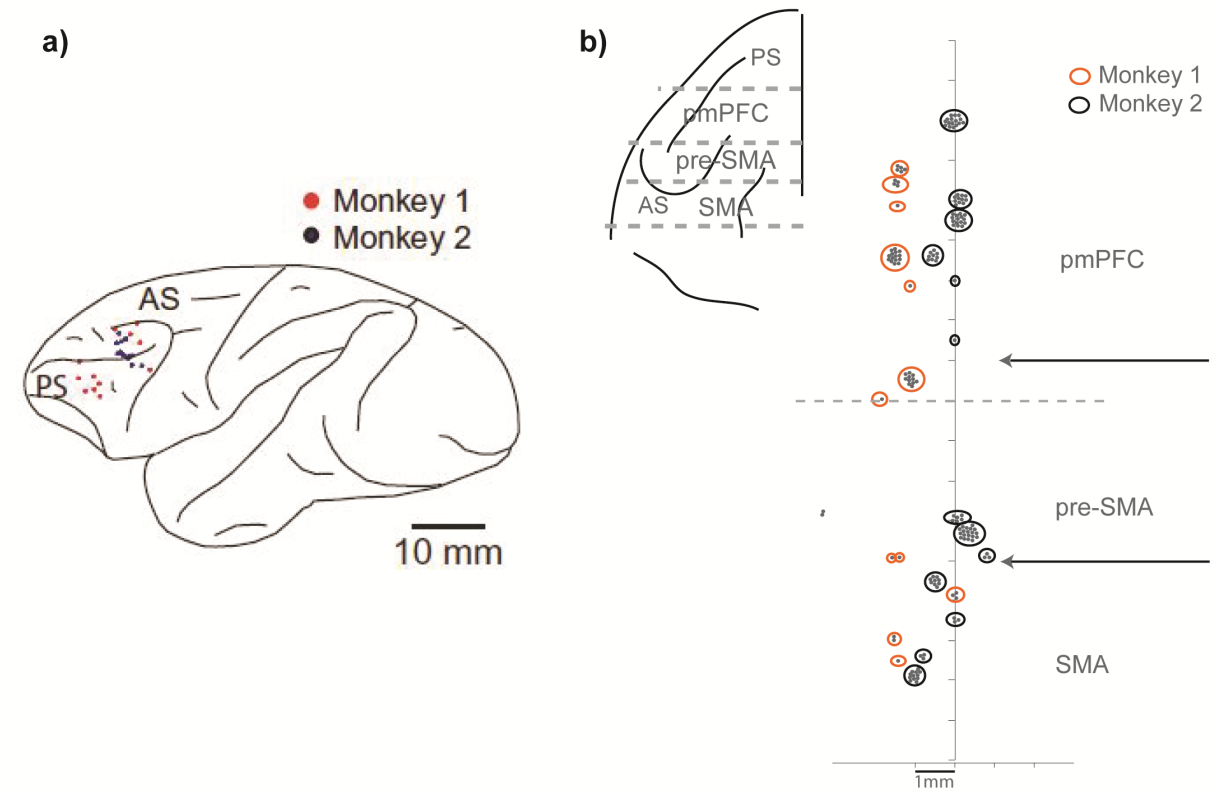


Fig. 2.4 (a) Penetration sites for both monkeys in the experiment of Falcone et al. (2016), lateral view. Penetrations in the right hemisphere of Monkey 1 are plotted on the left hemisphere for a better comparison with Monkey 2. (b) Penetration sites for both monkeys in the experiment of Falcone et al. (2017), top left – top view.

2.3 RESULTS

Behavior

In both experiments, to assess the ability of the monkeys to follow the task rule and monitor the actions of the human agent, the percentage of correctly performed trials was calculated in the two conditions, i.e. not interactive and interactive. In the ONMTG task in lPFC, Monkey 1 performed 93% and 91% of correct trials; Monkey 2 performed 95% and 91% of correct trials, in not interactive trials and in interactive trials, respectively. In the SNMTG task in MFC, Monkey 1 performed 97% and 91% of correct trials; Monkey 2 performed 98% and 95% of correct trials, in not interactive trials and in interactive trials, respectively. These results showed in both experiments that the monkeys were able to monitor the choice made by the human partner and to remember the goal or the spatial information contained in this choice in order to give the correct response in the trial after, with a high degree of accuracy.

Neural data

The aim of the two experiments here described was to investigate the properties of single neurons in the frontal areas of the macaque brain during a social interaction task. For this purpose, neural activity was recorded extracellularly through a multielectrode system, and single unit activity was identified checking the recorded waveforms through an offline sorting system (OpenSorter, TDT). Here we discuss the main findings of the two experiments separately.

lPFC

In the experiment with the ONMTG task, Falcone et al. (2016) recorded the activity of 184 single neurons from both monkeys (81 from Monkey 1 and 103 from Monkey 2) in the lPFC.

Neural activity was analyzed during 5 different periods of the task, in all correct trials preceded by a correct trial: the *holding CS period*, from 80 to 1000 ms; the *delay period*, from 80 to 1000 ms; the *reaction and movement period*, extended from the go signal to the touch of the OG; the *holding goal period*, from the touch of the OG to 800 ms later; the *feedback period,* from 80 to 800 ms after the appearance of the feedback around the chosen goal. Here we will focus in particular on the analysis performed in the first two periods listed above, the *holding CS period* and the *delay period*. Because the appearance of the OG on the screen is straddling these two periods, this offers the chance to study the modulation of neurons in two different processes. In the *delay period,* monkeys were asked to remain with their hand on the CS until the go-signal, while the two object goals, one on the right and one on the left, had already appeared on the screen. In this way, the information about the spatial

position of the correct choice is already available for the monkeys, in monkey trials as well as in human trials. This delay between the appearance of the OG and the go signal allowed the authors to investigate a possible encoding of the future correct response in terms of modulation of the neural activity between trials with the correct target on the left and trials with the correct target on the right, excluding the possible interference due to the movement of the hand toward the object. This is possible because in this period monkeys possess the necessary information to select the correct object during monkey trials and to predict the future choice of the human partner before he performs it during human trials. In the *holding CS period* instead, monkeys are still waiting for the presentation of the object-goals on the screen, hence they do not have a clue yet about their position in the current trial. The absence of the information about the spatial position of the correct object in this period of the task makes it impossible to study the future goal position selectivity. Instead during this period, the authors investigated whether the neurons of the lPFC could somehow represent information related to the previous trial. In the NMTG paradigm indeed, it is necessary to retain in memory what happened in the previous trial in order to succeed in the current one.

In the *delay period,* a two-way Anova was performed on the firing rate using as factor the current agent performing the task and the current position of the correct goal. Out of a total of 184 single neurons, 41 (22%) selectively encoded the agent that was performing the task, the monkey or the human agent, independently of the correct goal position; 27 (15%) selectively encoded the correct goal position, right or left, no matter which agent performed the trial; 15 (8%) showed a significant interaction between the factors agent and correct goal position. Furthermore, the authors investigated whether some neurons could encode the correct goal position with an agent specificity. Through a post-hoc analysis single neurons were therefore classified into three distinct group: the monkey-only cells, namely those neurons that encoded the correct goal position only during monkey trials; the human-only cells, which encoded the correct goal position only in human trials; the both-agents cells, which were the neurons that encoded the the correct goal position during both monkey trials and human trials. 40 cells were grouped into these three groups. The 68% of these cells coded the spatial position only when the monkeys were performing the trial, the group of monkey-only cells. The 23% of cells were classified as human-only cells. Finally, only the 10% of cells showed a goal position effect both in monkey and in human trials.

In the *holding CS period,* a two-way Anova was performed on the firing rate distribution using as factors the previous agent and the previous goal position, to test whether neurons encoded these information related to the trial before at the beginning of the trial. Although to a lesser extent compared to the delay period, a percentage of cells encoded the agent and the target position of the

previous trial: 9% for the previous agent and 14% for the previous correct goal position. As for the delay period, the authors divided into three classes those cells that showed a goal position selectivity for at least one agent. Out of 32 cells in total, 47% were classified as monkey only, i.e., the cells that encoded the previous correct goal position only when the trial before was performed by the monkey; 41 % human-only, which encoded the previous correct goal position only when the trial before was performed by the human agents; both-agents cells, which encoded the previous correct goal position in both cases.

MFC

In the experiment with the SNMTG task, Falcone et al. (2017) recorded a total amount of 273 cells from both monkeys (87 from Monkey 1 and 186 from Monkey 2), divided between three different areas: 128 in the pmPFC, 81 in the pre-SMA and 64 in the SMA (Fig. 2.4).

Likewise the analysis performed in the other experiment, the authors focused on the *delay period* to investigate whether some neurons could encode the correct target position or the agent who performed the task. A two-way Anova using the current agent (human or monkey) and the current correct target position (bottom right, bottom left, center right and center left) as factors showed that in all the three areas more than half of the recorded cells were modulated by the agent performing the task (51% in pmPFC, 56% in pre-SMA and 53% in SMA, Fig. 2.5).

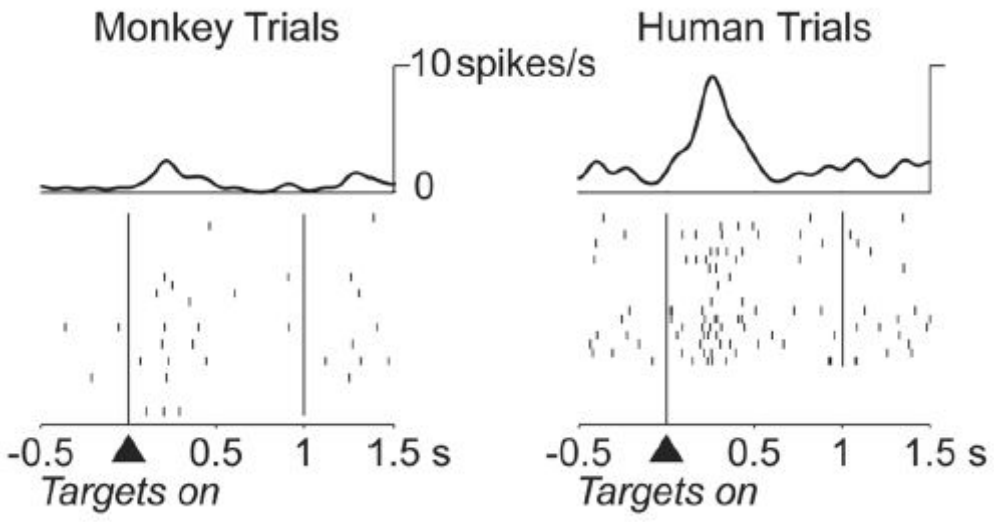


Fig. 2.5 Example of a neuron encoding the agent who performed the task in the delay period in the experiment of Falcone et al. (2017). In the raster plot each dot represents a spike and each line represents a trial. Neural activity is aligned to the beginning of the delay period when the targets appeared.

Conversely a minority of cells were modulated by the correct target position (25% in pmPFC, 19% in pre-SMA and 14 % in SMA) and by the interaction between the two factors agent and target position (23% in pmPFC, 17% in pre-SMA and SMA). Post hoc analysis revealed the presence of those cells that encoded the correct target position with an agent specificity or for both agents, dividing the population into three groups: monkey-only, human-only or both-agents cells. The proportion of monkey-only and human-only was similar across the three different areas. On the total amount of cells that showed a modulation for the correct target position for at least one agent, 40% were classified as monkey only in the pmPFC, 41% in the pre-SMA and 61% in SMA, while 40% were classified as human-only in the pmPFC, 41% in the pre-SMA and 33% in the SMA. The proportion of both-agents cells was lower compared to the other two groups: 24% of cells in the pmPFC, 18% in the pre-SMA and 6% in the SMA.

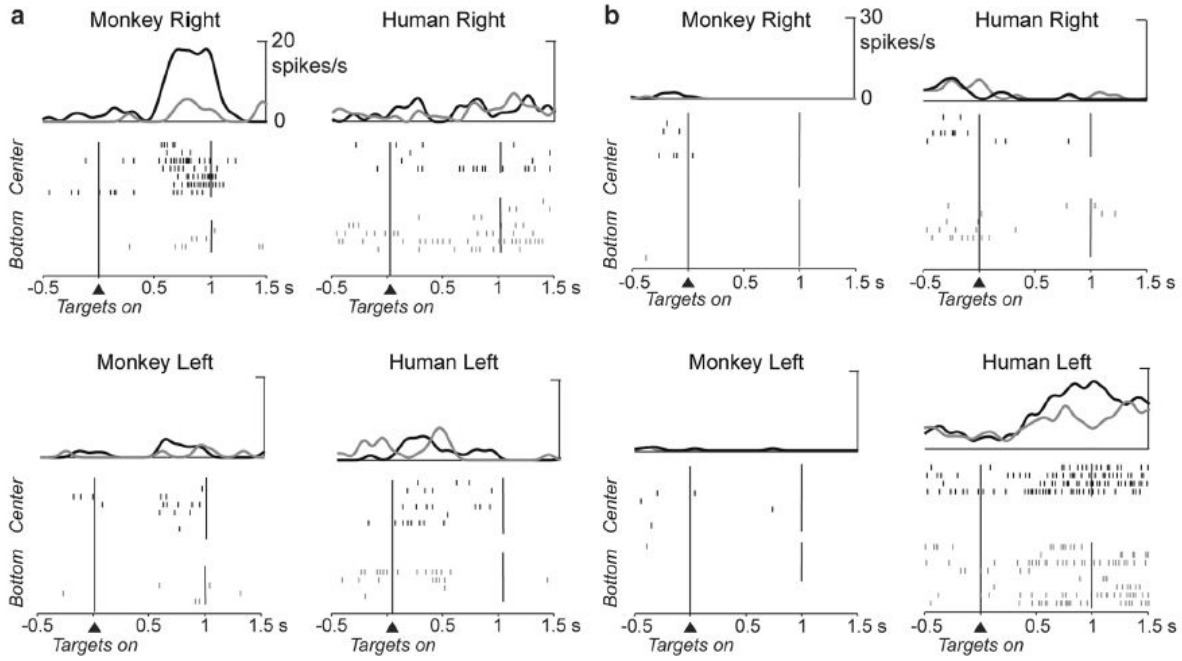


Fig. 2.6 (a) Example of a neuron that shows a higher activity for the center right position in monkey trials in the delay period. The same neuron do not shows selectivity for any target position in human trials (monkey-only cell). (b) Example of a neuron that shows a higher activity for the center left position in human trials in the delay period. The same neuron do not shows selectivity for any target position in monkey trials (human-only cell).

Moreover, the authors investigated the monitoring of the previous trial at the beginning of the current trial as was done in the lPFC experiment. A two-way Anova using the previous agent (human or monkey) and the previous correct target position (bottom right, bottom left, center right and center left) as factors was performed on the firing rate during the *holding CS period* from 80 to 1000 ms. On

a smaller scale compared to the *delay period,* they found a larger proportion of cells that encoded the previous agent: 13% in the pmPFC, 12% in the pre-SMA and 9 % in the SMA. The number of neurons encoding the previous correct target location was smaller but comparable between the three areas: 8% in the pmPFC, 7% in the pre-SMA and 8% in the SMA. The authors found with post-hoc analysis the proportions of cells which showed a modulation for a specific previous correct target position for at least one agent: monkey-only cells (56% in pmPFC, 43% in pre-SMA, 27% in SMA), human-only cells (40% in pmPFC, 39% in SMA, 41% in pre-SMA) and both-agents cells (4% in pmPFC, 18% in pre-SMA, 32% in SMA).

SECTION 3

THE DORSAL PREMOTOR CORTEX

3.1 PMd IN SOCIAL INTERACTION

The premotor cortex (PM) in the macaque brain is part of a wide frontal region, which corresponds to the Broadmann Area 6 (Broadmann, 1908), lying in a caudal position relative to the arcuate sulcus and in a rostral position relative to the principal sulcus. The PM cortex is one of the different motor areas that are placed on the medial and lateral surface of the area 6, which compose the cortical motor system, projecting directly not only to the primary motor cortex (M1), but also to the spinal cord (Dum 2002). PM is placed on the lateral surface of area 6 and it is usually divided into two main parts, based on anatomical and functional differences: a dorsal region (PMd) and a ventral region (PMv) (Barbas & Pandya 1987, Kurata 1994). A further distinction can be made based on the rostro-caudal axis, distinguishing between an anterior and posterior part of PMd (PMdr, or F7, and PMdc, or F2, respectively) and an anterior and posterior part of PMv (PMvr, or F5 , and PMvc, or F4, respectively) (Rizzolatti & Luppino 2001). The first studies on premotor cortex outlined the importance of both the dorsal and the ventral regions in the control of motor functions (Fulton 1935, Weinrich & Wise 1982). PM receives inputs from parietal and frontal lobes and transmits them to M1, and it represents different aspects of motor behavior: neurons show movement related activity (Wise 1985), signal related activity after the presentation of a visual instruction stimulus and set related activity during a delay period (Kurata & Wise 1988, Boussaoud & Wise 1993). As the studies on PM continue, it becomes clear that this area, and more generally all the motor areas, are not simply involved in the generation and execution of motor acts, but rather they participate also in the elaboration of higher cognitive functions, such as the production of goal-direct behavior or action understanding (Rizzolatti et al. 2014).

The seminal idea that premotor cortex could play a role in ‘social’ mechanisms like action understanding comes from one of the major breakthroughs in the last decades in neuroscience, that is the discovery of ‘mirror neurons’. These particular neurons, observed for the first time in the F5 area of the macaque ventral premotor cortex, show an increased activity in their firing rate in response to both executed and observed actions (di Pellegrino et al. 1992, Rizzolatti et al. 1996). The properties of this class of cells were deeply investigated through many studies over the years. Gallese et al. (1996) showed that the great majority of mirror neurons showed their mirror activity only when there was a correspondence between the type of observed and executed action, such as grasping, manipulating or placing a specific object. A strong evidence in favor of the idea that these neurons are involved in actions’ understanding and they are specific for goal-directed behavior comes from the fact that a strong modulation was found even when the final part of the action was covered from view (Umiltà et al. 2001). All these findings led to the idea that the activation of the mirror system in

the brain could represents a bridge between the perception of an action and its understanding. The 'direct match hypothesis' proposed by Rizzolatti et al. (2001) claimed that the visual analysis of the components of an action has to be mapped onto the motor representation of the very same action in our brain in order to understand it. Nevertheless, social interaction is a complex behavior which requires not only the ability to understand others' actions and intentions, but also the capacity to distinguish between self and others. A neural network that is responsible for the execution and at the same time the observation and the understating of others' action it may fail in providing such distinction. It is therefore why we wondered whether beside the mirror network could exist a neural substrate that is able to recognize self and other's behavior without overlapping and whether the activity of single neurons in the dorsal premotor cortex could reveal it.

To our knowledge, the first study to report the activity of single neurons in the dorsal premotor cortex during a performance and observation task was the one of Cisek and Kalaska (2004). They used a center-out reaching task to investigate how the PMd could represent different alternative reaching direction. During the task, two spatial cues of different colors were displaced in two out of eight different possible positions, located in a circle around the center of the screen. After the disappearance of the spatial cues, a color cue of the same color as one of the two previous spatial cues was presented at the center of the screen. The color cue served as an instruction stimulus for the monkey: at the end of a delay period, after a go signal, they had to move a cursor on the screen with a manipulandum toward the location in which the spatial cue of the same color was presented (execution condition). As a control task, monkeys had to observe the same task sequence and events without intervening, while the computer was moving automatically the manipulandum (observation condition). The authors reported neurons with 'mirror like' properties: the majority (84%) of those cells that were directionally tuned in the delay period for a specific direction during the execution condition, showed the same selective tuning also in the observation condition. The activity of those cells is defined 'mirror like' because, although not during the movement period but during the delay period, in which there is no movement in either condition on the part of the monkey, it is possible to observe the representation of the intended motion during both the execution and the observation condition. In a successive study (Tkach et al. 2007) single neurons activity from M1 and PMd was collected during a similar task with an execution and an observation condition. The monkeys had to use a manipulandum to move a cursor on the screen toward a target that was moving between different locations (execution condition). Successively, they had to observe passively the automatic movements of the cursor on the screen (observation condition). This task differed from the previous one for the lack of a delay period, since the neural activity was recorded during the actual movement

and during the observed movement. Also in this case, authors discovered that the neural responses were similar in the two conditions, with neurons that showed mirror properties.

These studies taken together seems to suggest that in PMd the action execution and the action observation activate similar networks that overlap with each other. However, these results and their interpretation might be the product of some specific task features and analysis criteria. First, in these experiments the authors were looking for those cells that showed a modulation in both the execution and the observation conditions. This led to the selection of those neurons activated during the movement or the pre-movement period, which were tested after to check whether they showed the same activation in the observation or not. This bias in the selection criterion made impossible to find neurons that were selectively activated during the observation condition only. In our experiment we aim to discover whether in the PMd a separated representation of others action and behavior exists, which is independent of one's own. Second, monkeys had to observe the movement of a cursor on a screen, the same cursor that was moved during the execution condition. This could have enhanced the activation of the same neurons activated during the execution, due to an underlying 'simulation' mechanism from the monkeys. Indeed, Cisek and Kalaska interpreted this activity as an evidence of a 'mental rehearsal' process. Furthermore, observing a non-real agent such a cursor instead of a physical real agent, may lead to a different response from the neurons, that has been observed to modulate their activity based on the animacy of the partner (Hosokawa & Hatanabe 2012). Introducing a real agent, which is visible and sitting close to the monkeys, might produce a more natural social interaction and might enhance the differentiation between self and other's actions. Furthermore, the interaction with the human partner comes with another advantage, which is the necessity to actively interact with him. In the previous tasks, the observation was passive, i.e. it was not necessary to monitor the action of the cursor. The NMTG task introduces a behavioral measure which gave us the chance to know how well the monkeys are able to monitor the action of the human agent, represented by the proportion of correct trials performed by the monkey after an observed trial performed by the human.

All these differences in the experimental settings are studied to better investigate social interaction mechanisms in a task with a real physical agent, which requires an active monitoring of other's actions. Our aim is to investigate whether a different neural substrate capable of recognizing self and others behavior in a separate way is present in the dorsal premotor cortex, besides the well-known mirror network which is fundamental for the understanding of other's actions.

3.2 MATERIALS AND METHODS

Here we report the experimental procedures and the details of the task used in our experiment to investigate social interaction in the dorsal premotor cortex. Two rhesus monkeys participated in this study performing two versions of a NMTG task: a spatial version (SNMTG) and an object version (ONMTG): Monkey 1 (8 years old, 8 Kg average weight) and Monkey 2 (12 years old, 9.5 kg average weight).

Sequence of task events

Monkeys faced a monitor touch screen (Microtouch 19 inches, 800x600 pixel resolution) with the head fixed, sitting on a primate chair. The animals performed two versions of a NMTG task (Fig 3.1). The two tasks used different peripheral stimuli as targets (Fig. 3.1 on top), but the underlying rule to solve the task was identical in both cases. The other stimuli and the duration of the task periods were the same in both versions. Both animals performed the SNMTG, while the second one performed also the ONMTG version. To allow a better comparison we decided to analyze the data of the SNMTG in Monkey 1 and of the ONMTG task in Monkey 2, since the second monkey did not reach the criterion (>70% of correct *monkey trials interactive*) in the interaction with the human agent in the spatial version.

A trial started with the presentation of a central stimulus (CS) represented by a red circle in the center of the screen (7° visual angle). Monkeys were required to touch it within 2s, otherwise the trial was aborted and a new trial started. After the touch of the CT, monkeys had to maintain the touch for a *holding CS period* randomly lasting for 0.5 or 0.8 s. After this period, the peripheral targets (PT) appeared on the screen. In the SNMTG version, the PTs were two identical grey rectangles (7.1 x 7.7°) that were presented in two out of four possible locations on the screen: bottom right (17.5 ° below and 23. 5° right from the center), bottom left (17.5 ° below and 23. 5° left from the center), center right (23. 5° right from the center) or center left (23. 5° left from the center). In the ONMTG version, 4 different objects were presented in couples as PT, one on the right (23.5 ° right from the center) and one on the left (23.5 ° left from the center) of the CS. The objects were four different geometrical shapes: a blue rectangle, a pink square, a green triangle and a yellow cross. A *delay period* started after the presentation of the PTs, lasting for a randomly chosen period of 0.8 or 1.2 s. During the *delay period* monkeys were required to hold the touch on the CS. The disappearance of the CS served as a go signal for the monkeys, allowing them to move the hand from the CS toward one of the two PTs. The touch on the PT had to be maintained for 0.4 or 0.6 s, the *holding target period*. At the end of the holding target period, reward was delivered for correct trial and it was

missing for incorrect trials. After an *intertrial period* of 1 or 1.5 s in which the screen turned black, the next trial started. In case of a correct choice in the previous trial, the same PT chosen before was presented again coupled with one of the other PTs not presented in the previous trial or with the same PT that was not chosen before. In case of incorrect choice, a correction trial was presented, with the same PTs in the same positions to allow the monkey to correct their choice. Incorrect choices in a correction trial led to another correction trial. As outlined above, the two tasks shared the same ground rule: in each trial, the monkeys had to discard the PT chosen in the trial before and select the new one. In the case of the SNMTG, they had to discard the previous chosen position; in the ONMTG they had to discard the previously chosen object. In both cases, it was necessary to keep track of the choice in the previous trial to succeed in the current trial.

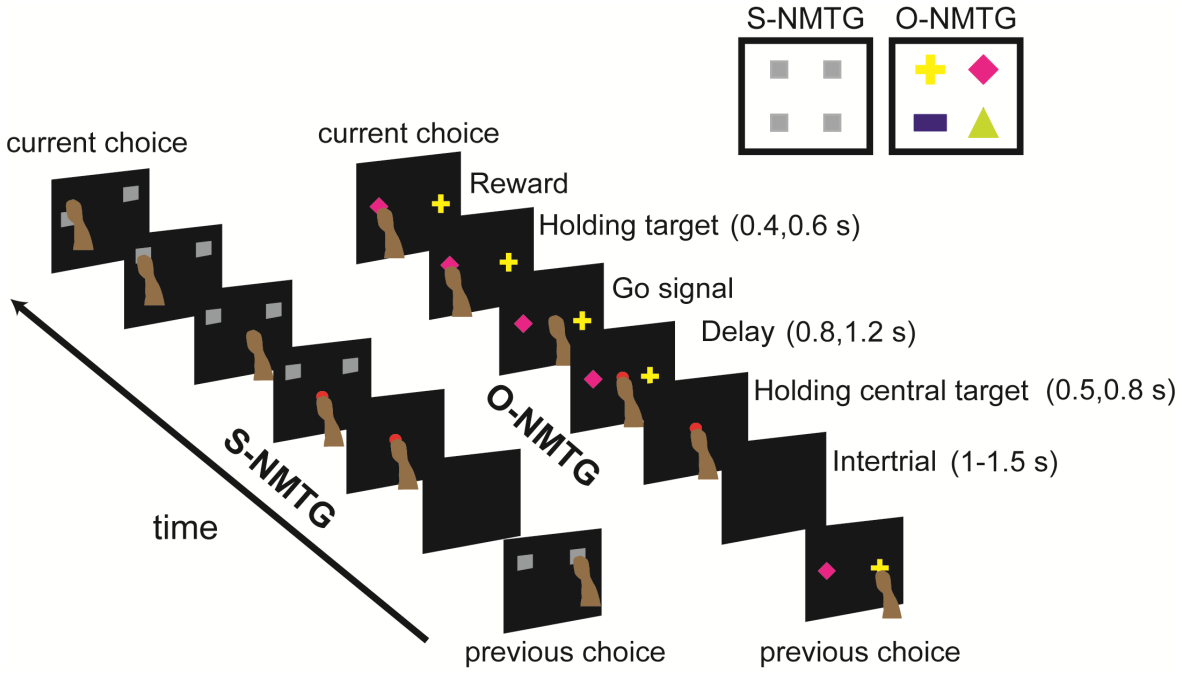


Fig. 3.1 Sequence of the task events in the SNMTG and ONMTG task used in this study. On the top the peripheral target used in the two versions are represented.

Monkey Human interaction

Both monkeys interacted with a human partner in a subset of trials. In this paradigm the animals were trained to switch their role during the task with the human, acting in some trial as the agent and in some trials as the observer. The human partner was sitting to the right of the monkey, facing the touchscreen monitor. After a correct trial performed by the monkey, he could decide to intervene in the task by placing his hand at the center of the screen during the *intertrial period.* At this point, monkeys recognized the 'shift' and observed the human performing the trial in his place. The human

performed only correct trials and at the end of every trial monkeys received the reward as in the trials performed by themselves. The human could perform only 1 trial or a sequence of trials, up to 4 consecutive trials, before removing his hand from the screen during the *intertrial period* at the end of his last trial. At this point the monkey could start a new trial. Behavioral performances were analyzed during two type of trials: the *monkey trials not interactive*, the trials performed by the monkey after a trial performed by the monkey itself, and the *monkey trials interactive,* the trials performed by the monkey after a human trial. Neural activity was instead analysed during two type of trials: correct *monkey trials,* the trials performed by the monkey, and correct *human trials,* the trials performed by the human, in both cases preceded by a correct trial.

Surgical Procedures and data collection

Both animals were implanted during the training period with a head holder device. At the end of the training period, a chronic recording system (Utah array, Blackrock Microsystems) was surgically implanted over the premotor dorsal cortex in the left hemisphere in both monkeys (Fig 3.2). With the chronic implant it was possible to record the neural activity extracellularly through 96 different channels in each session. We obtained 3 recording sessions from Monkey 1 and 4 recording sessions from Monkey 2.

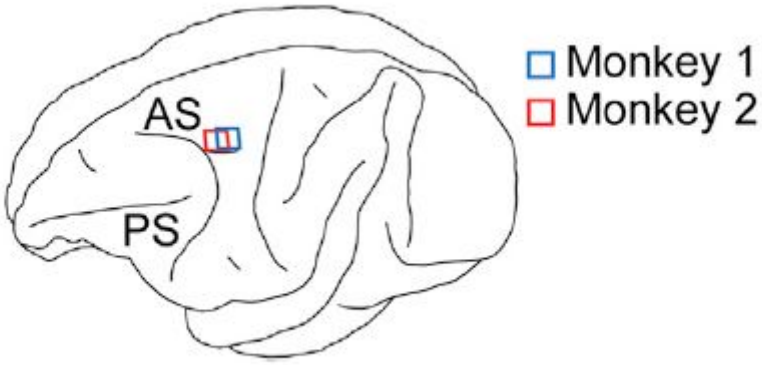


Fig. 3.2 Location of the chronic implants in both monkeys relative to the Arcuate Sulcus (AS) and the Principal Sulcus (PS).

3.3 RESULTS

Behavior

As previously described, behavioral performance was analysed in monkey trials, *not interactive* and *interactive*. In the first case, the percentage of correctly performed trials was informative about the ability of the monkey to follow the basic task rule, i.e. keeping in mind its last choice and discarding it. In *not interactive trials*, Monkey 1 performed 90.9 % ± 1.2%; Monkey 2 performed 90.1 ± 1.0% (±SEM). In the second case, the performance in the *interactive trials* tested the ability of the monkeys to monitor the human choice. The performance for Monkey 1 was 86.0% ± 1.2%, while the performance for Monkey 2 was 75.3% ± 2.4% (±SEM). Reaction times, defined as the time from the go signal to the detach of the CS, were not significantly different for Monkey 1 in the not interactive and interactive condition (t-test, $p=0.07$, $t_{[2]}=3.58$), whereas it was significantly different for Monkey 2 (t test, $p=0.02$, $t_{[3]}=4.87$), being lower in not interactive trials.

Neural data

Single unit activity

Our recording dataset was composed of 400 single neurons, obtained through 4 sessions from Monkey 1 (258 neurons) and 3 sessions from Monkey 2 (152 neurons). Single unit activity was isolated through an offline sorting software (OpenSorter, TDT). Since recordings were obtained with chronic implants, there was the possibility to record with a specific electrode the same cells among different sessions. Therefore, from the recording dataset, we excluded those cells that turned out to be the same across sessions. The single unity stability method (Fraser and Schwarz 2012) was utilized after the sorting that led to the selection of the 400 single units. To determine a score of similarity, this method considers, for each recorded neuron, four different parameters: the shape of the waveforms, the mean firing rate, the autocorrelation function and the cross correlograms with the others neurons from the dataset. These different scores were combined with a quadratic classifier in order to find a decision boundary. If the combined similarity exceeds the decision boundary, two neurons were classified as the same. This analysis was performed for neurons recorded in consecutive sessions; when a neurons was classified as the same among two consecutive sessions, we considered it only in the session with the highest number of trials, discarding the other. Thus, our final dataset was composed of 328 single neurons, 210 recorded from Monkey 1 and 118 recorded from Monkey 2.

We decided to focus our analysis to the same periods which were matter of interest in the previous works of Falcone et al. (2016, 2017), the *delay period* and the *holding CS period.*

In the *delay period* we selected trials with both delay durations (0.8 s or 1.2) and we analyzed the firing rate in the interval between 0.4 and 0.8 s after the appearance of the PTs. A two-way Anova was performed on the discharge rate using as factors the agent performing the task (monkey or human) and the spatial position of the correct target (right or left). For the SNMTG task, since there were two possible positions for right and left targets (bottom or center), we decided to combine these positions and assign them either to the right or left position, analysing the activity only in those trials in which the two targets were presented in non-ipsilateral locations. We found that 48% of cells selectively encoded the agent who performed the task while 26% selectively encoded the spatial position of the correct target.

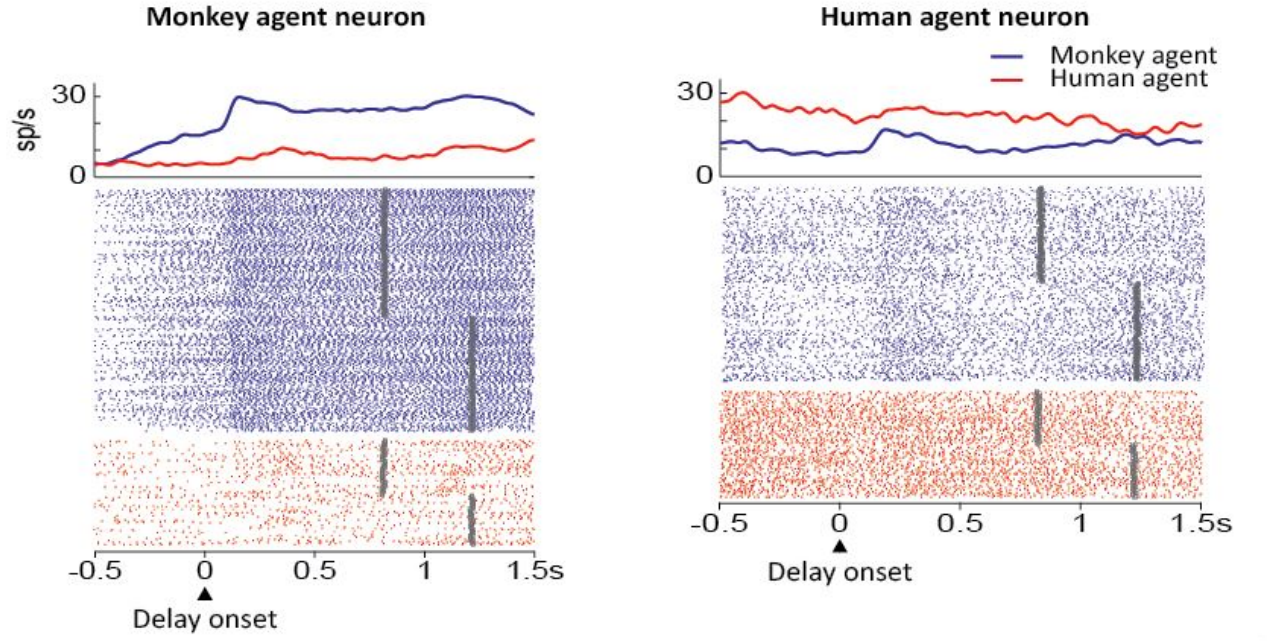


Fig. 3.3 Example of two agent selective neurons. Neural activity is aligned to the appearance of the peripheral targets and the analysis of selectivity was performed in the window 0.4 – 0.8 s of the delay period. Nevertheless, it is possible to observe that the agent selectivity is also extended in other periods of the trial, before and after the period of interest.

We investigated whether some cells could encode the target position exclusively for a specific agent, monkey or human, or for both. Post hoc analysis (Fisher's least significant difference [LSD] test, $p<0.05$) revealed those cells that we classified accordingly to the subgroups described by Falcone et al. in the previous experiments. The monkey-only cells were those cells that showed the encoding of the correct target position in the delay period only when the monkey was performing the trial, but not when the monkey was the observer. Human-only cells viceversa, were the group of cells that encoded the target position when monkeys were observing the human agent performing the trial, but not when

they were performing it by themselves. Finally, both-agents cells encoded the target position independently from the agent who performed the trial. During the delay period 80 cells out of 328 coded the target position for at least one agent: the majority (64%) was classified as monkey-only cells, while the proportion of human-only and both-agents cells were similar (17% and 19% respectively).

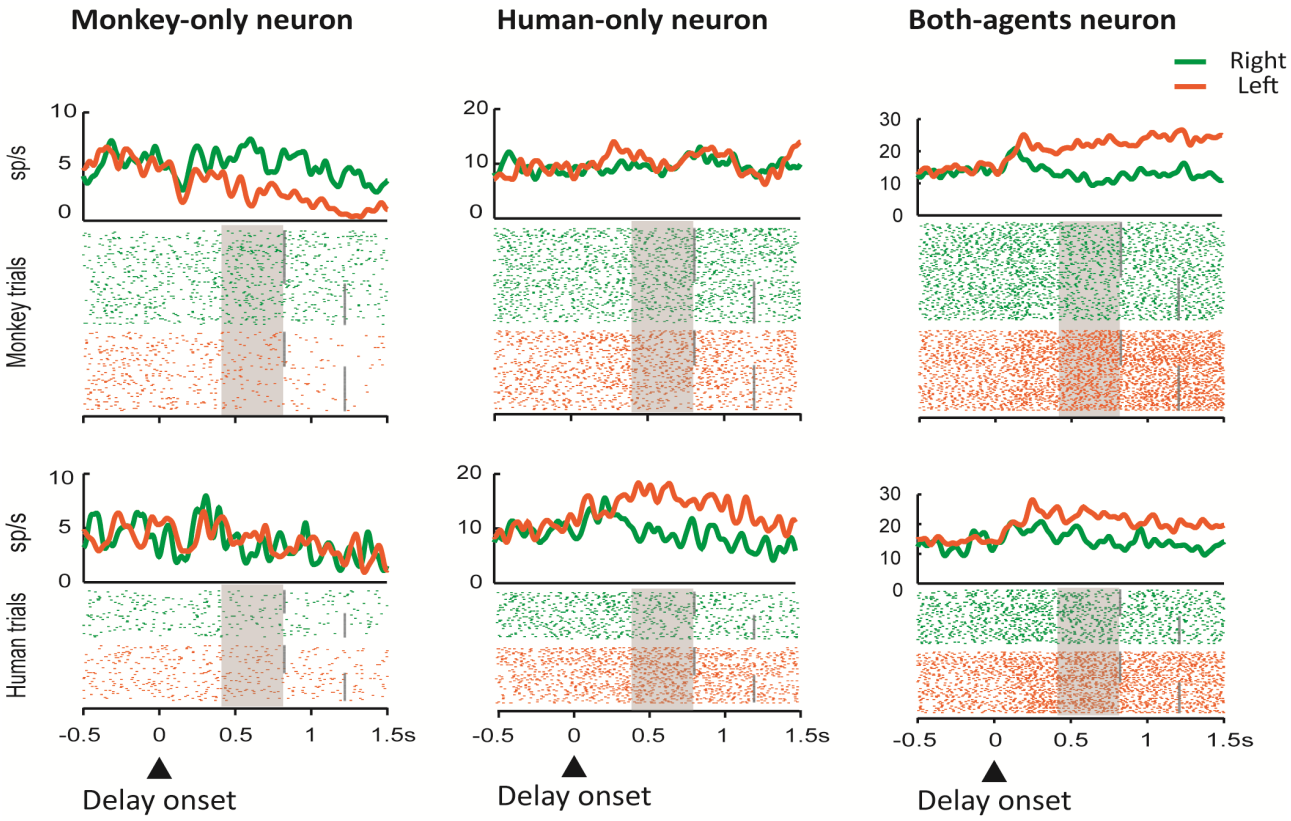


Fig. 3.4 Raster plot of three example neurons, one per group. Neural activity is aligned to the beginning of the delay period. Grey shaded areas indicate the analyzed period. Grey vertical bars in the raster plots represent the end of the delay period.

In the *holding CS period* we examined whether a monitoring activity of the previous trials was occurring. We performed a two-way Anova on the firing rate in this period using as factors the agent who performed the previous trial (monkey or human) and the previous correct target position (right or left). We selected the current trials performed either by the monkey or by the human. This period was chosen because is at the very beginning of the trial and the current targets have not appeared on the screen yet, excluding in this way any possible influence on the neural activity. As a result of the analysis, we found that the majority of cells (18%) was selectively encoding the previous agent who performed the trial, while a smaller but comparable number (15%) encoded the previous correct target position. Going along with the criterion that led to the subdivision into the three subgroups of cells

previously described in the *delay period*, we performed the post hoc analysis (Fisher's least significant difference [LSD] test, $p<0.05$) in the *holding CS period* too, in order to find those cells that showed a clear modulation for the previous correct spatial position for at least one previous agent, or for both. We discovered that in this group of cells the great majority was classified as monkey-only cells (76%), while the two others subgroup were significantly lower in comparison (12% each of human-only cells and both-agents cells).

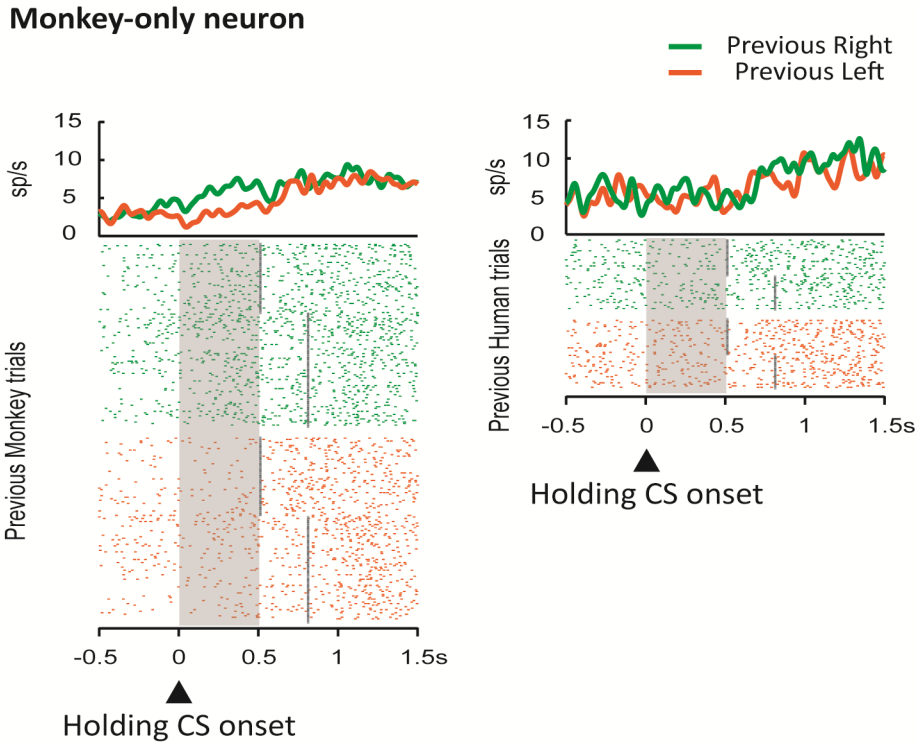


Fig. 3.5 Raster plot of a monkey only cells that encode the previous target position only when the monkey performed the previous trial and not the human. Neural activity is aligned to the touch of the CS. Grey shaded areas represents the analyzed period. Grey vertical bars represent the end of the holding CS period and the appearance of the peripheral targets.

Population activity

We investigated the strength of the spatial tuning for these classes of cells looking at their population activity during these two periods, the *delay* and the *holding CS.*

The population histogram reported in Figure 3.6a represents the activity of the categories of cells that showed a spatial selectivity for a specific agent during the *delay period* (0.4 – 0.8 s): the monkey-only cells (N=51) and the human-only cells (N=14). For each cell the analysis was performed by selecting the trials on the basis of the preferred and the anti-preferred locations, in monkey trials for the monkey-only cells and in human trials for the human-only cells. The rank which determined the

preferred location was assigned to each cell individually comparing the mean firing rate during the *delay period* (0.4 – 0.8 s after the appearance of the PT) in right and left trials and selecting the highest of the two. The two categories of cells developed a spatial selectivity that began after the appearance of the peripheral targets, persisted during the delay and then disappeared at the end of the trial. As a further control, we plotted the population histograms of the firing rates of monkey-only cells and human-only cells in human and monkey trials respectively, assigning the same preferred and anti-preferred locations derived from their original trials (monkey trials for monkey-only and human-trials for human-only). This control allowed us to assess whether the spatial tuning of these two categories of cells was fully specific for the significant agent. Indeed, in principle the monkey-only cells could share the same spatial tuning in monkey trials and human trials with a smaller and non significant effect. Similarly, the human-only cells might how at least the tendency to share the same spatial selectivity in monkey trials, although not being significant. If that was the case, we should expect to see, in the delay period of the population histogram, a higher activity for the preferred location defined in the monkey-only cells also for the same location in the human trials. Similarly we would expect a higher activity for the preferred location defined in the human-only cells also for the same location in the monkey trials. On the opposite, we found that, within the two groups, there was no tendency to share the same spatial tuning between the trials performed by the two different agents (Fig. 3.6b).

Figure 3.7a shows the population histograms for the both-agents cells (N=15). The same method was applied and this group of cells is plotted in both monkey trials and human trials, with the rank assigned individually to each cell based on the firing rate in the corresponding trials. In the control, we plotted again the two groups but switching the ranks, to assess whether the both-agents cells showed the same spatial selectivity in monkey and human trials. Contrary to the results of the monkey-only and human-only groups, both-agents cells did not show an agent specific spatial tuning (Fig. 3.7b). Out of 15 cells, 14 showed the same spatial tuning between the trials performed by the two different agents, proved by the fact that the preferred location is still showing a higher firing rate than the anti-preferred when the ranks are switched.

We reported the population histogram of the monkey-only group (Fig. 3.8), the only group of cells with a signifant effect (N=33), in the *holding CS period*. As described for the *delay period*, the preferred and the anti-preferred ranks were assigned to each cell individually by computing its mean firing rate during the *holding CS period* (0 – 0.5 s after the touch of the CS) and assigning for each cell a trial to the preferred or the antipreferred category based on which previous position was associtaed to the highest activity.

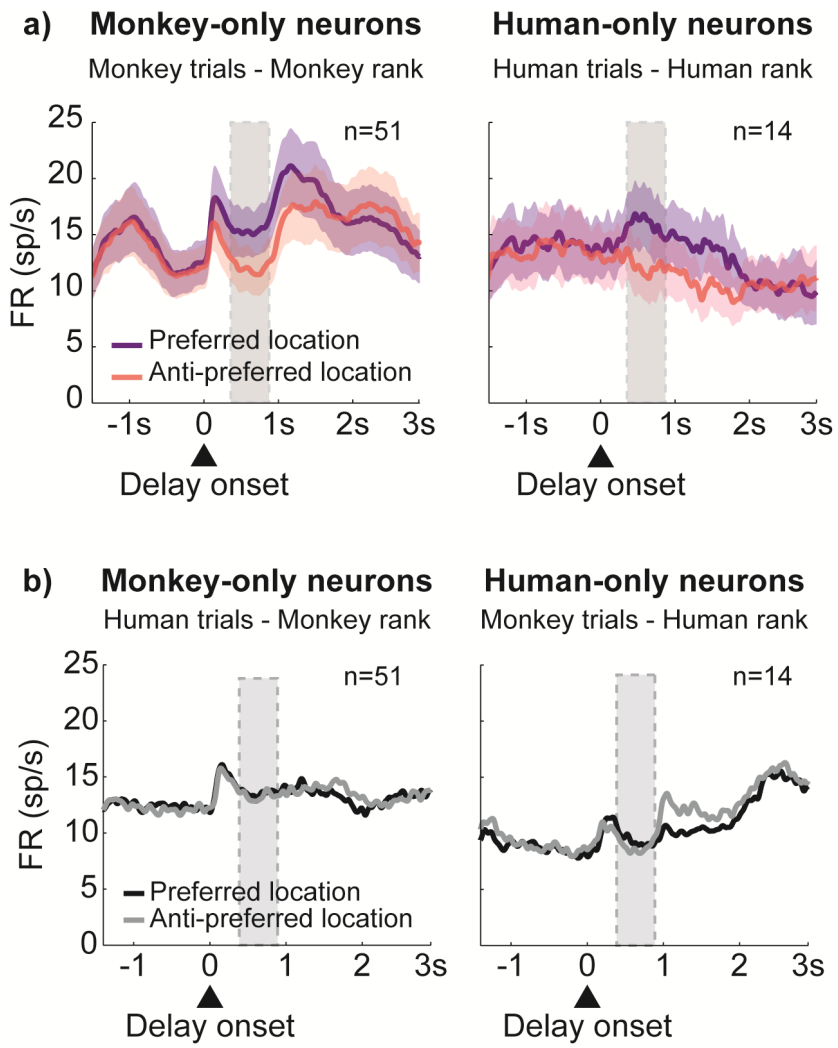


Fig. 3.6 a) Population histograms of mean firing rate for the groups of monkey-only and human-only cells in the delay period. Neural activity is aligned to the delay onset. Grey shaded areas indicates the period of analysis (0.4 – 0.8 s in the delay period). Error bars are ± SEM. b) Population histograms of mean firing rate for the groups of monkey-only and human-only cells in human trials and monkey trials respectively, assigning the same preferred and anti-preferred location derived from their original trials. Grey shaded areas indicates the period of analysis (0.4 – 0.8 s in the delay period).

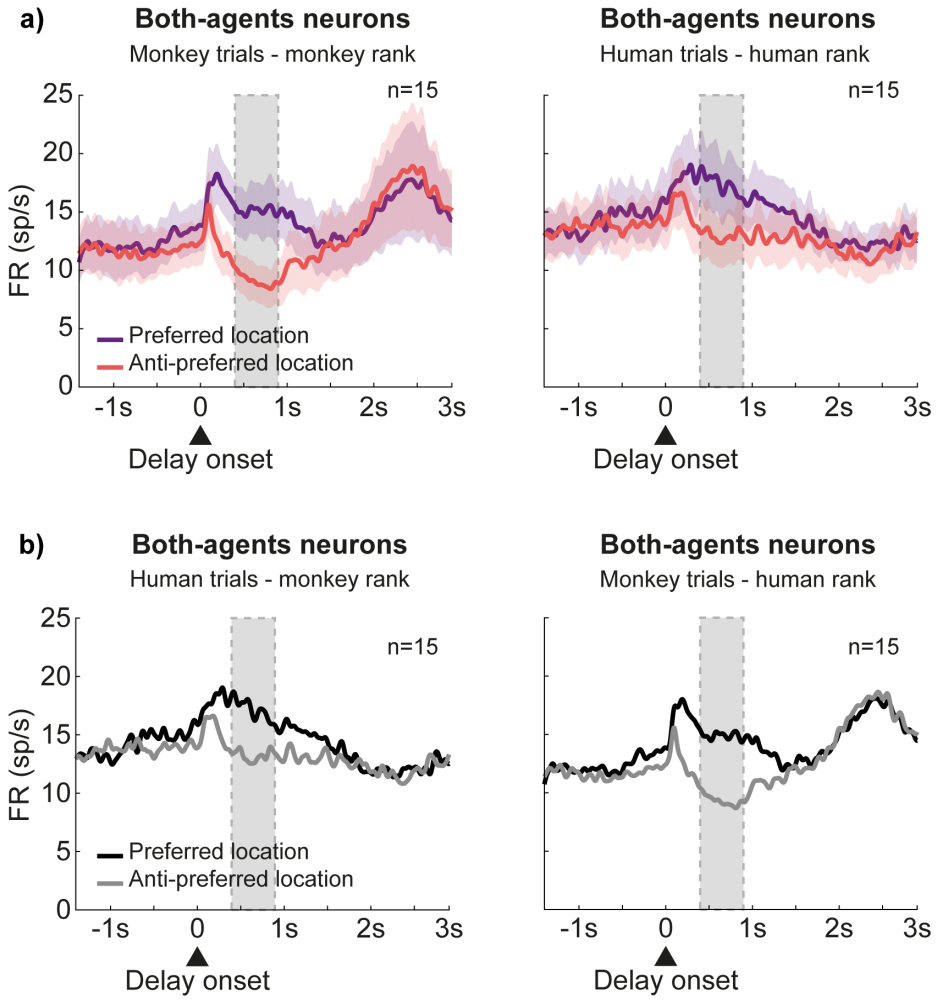


Fig. 3.7 Population histograms of mean firing rate for the group of both-agents cells in the delay period. Neural activity is aligned to delay onset. Grey shaded areas indicates the period of analysis (0.4 – 0.8 s in the delay period). Error bars are ± SEM. b) Population histograms of mean firing rate for the group of the both-agents cells, switching the ranks. Grey shaded areas indicates the period of analysis (0.4 – 0.8 s in the delay period).

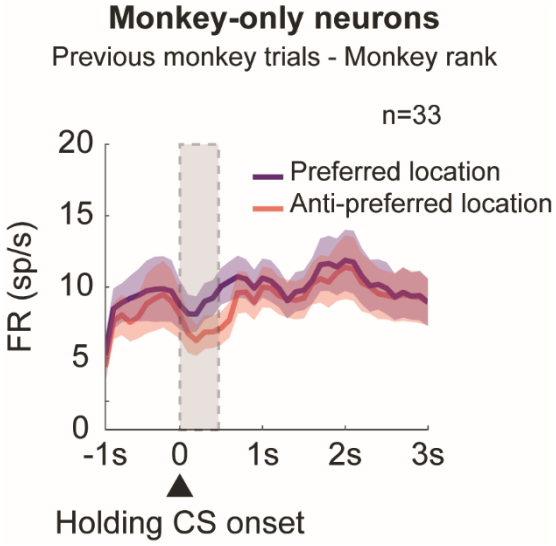


Fig. 3.8 Population histogram of mean firing rate for the groups of monkey-only in the holding CS period. Neural activity is aligned to the touch of the CS. Grey shaded areas indicates the period of analysis (0 – 0.5 s in the holding CS period). Error bars are ± SEM.

Neuron dropping analysis

To further investigate the representation of the spatial position of the correct targets for the three categories of cells we perfomed a classification procedure using a neuron dropping analysis (Foffani & Moxon, 2004). The aim of this analysis is to represent how well it is possible to decode, from the activity of a given subpopulation of cells, a specific spatial position, depending on the size of the sample. We started selecting randomly *n* neurons from the neural populations of the three groups of cells (monkey-only, human-only and both agents). For a given subpopulation a test trial was randomly selected from each neuron. The remaining trials were sorted by condition (right or left) and neuron and then the mean firing rate was computed in the delay period. The difference between the firing rate of the test trial and the mean firing rate of the remaining trials in each condition obtained from the same neuron of the test trial was computed. The test trial was classified as belonging to the condition in which the difference between the firing rates was the lowest. If the actual and the classified condition of the test trial matched, the classification was correct. This procedure was repeated 1000 times in order to assess how often this computation led to a correct estimation of the target position. The procedure was done separately in monkey and human trials for the three group of cells.

The results of the neuron dropping analysis is shown in Fig 3.9. The estimation of the target position in monkey trials was higher for monkey-only cells compared to human only cells. On the contrary, human-only cells show a better estimation of the target position in human trials compared to monkey-

only cells. The neuron dropping curves for both agents cells provide a correct classification of the target position in both kind of trials. These results shows that the two agent selective groups of cells, monkey and human only, provided an estimation higher than chance in the delay period in monkey and human trials respectively, an estimation that increased as the number of neurons in the population increased. Moreover, the curves show that there is no tendency to share the same spatial tuning between monkey-only and human-only cells, while the both agents cells provide a similar estimation in both monkey and human trials.

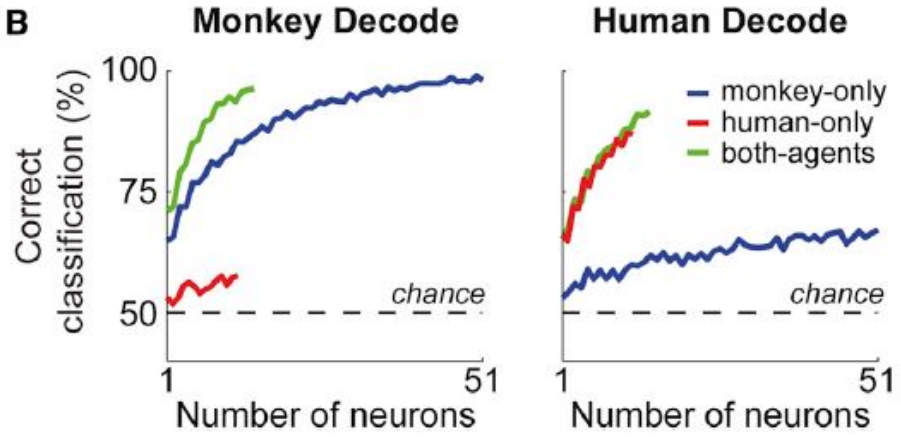


Fig. 3.9 Neuron dropping curves for monkey-only, human-only and both agents cells in monkey (left) and human (right) trials (0.4 – 0.8 s in the delay period). Dashed lines indicates chance level.

SECTION 4

DISCUSSION

4.1 DELAY PERIOD: PLANNING SELF OR REPRESENTING OTHERS FUTURE CHOICES

In the previous chapter, we presented the procedures and the main results of our experiment aimed at investigating the neural substrates of social interaction in the dorsal premotor cortex (Cirillo et al. 2018). The same experimental paradigm was adopted in two previous studies also discussed in this work, which investigate two distinct brain areas in the frontal lobe of the macaque brain: the lateral prefrontal cortex (Falcone et al. 2016) and the medial frontal cortex (Falcone et al. 2017). In all the three experiments, a human-monkey interaction paradigm was adopted in the context of a NMTG task, in which the monkeys were required to monitor the human's actions in order to choose the correct target in the successive trial. In this section, we will provide a comparison of the main results of the three studies, in order to gain a clearer view about how these different areas of the frontal cortex are engaged during a social interaction task. The comparison is strengthened by the chance we have to confront the properties showed by neurons that were recorded through the same experimental paradigm and measured with the same method of analysis in different areas of the macaque brain.

All the versions of the NMTG task used in the three previously described experiments included a pre movement period, i.e. the delay period. This period was defined as the time in the trial occurring between the appearance of the peripheral targets and the go signal. The go signal instructed the monkeys to start the movement toward one of the peripheral target and select it. The choice of the delay period as a principal period for the analysis is thus driven by two main reasons. First, it is possible in this period to study neural activity without the possible modulation due to any movement. The monkeys are required to keep their hand on the CS throughout the duration of the delay period, and any movement led to an abort and at the start of a new trial. The same happens during the human trials: the hand of the human agent is on the CS until the go signal, and the monkeys are not observing any movement. Second, during the delay period the relevant information about the correct target is available to the monkeys to plan their future movement toward the correct target in monkey trials or to predict or anticipate the choice that the human agent will do in human trials.

For these reasons, in all the three experiment the main analysis on the firing rate of the neurons focused on the delay period. The two-way analysis of variance tested the effects of the factors agent (monkey or human) and position (right or left) on neuronal activity in this period. Figure 4.1 reports the percentage on the total of recorded cells for each area that showed a selectivity for these factors.

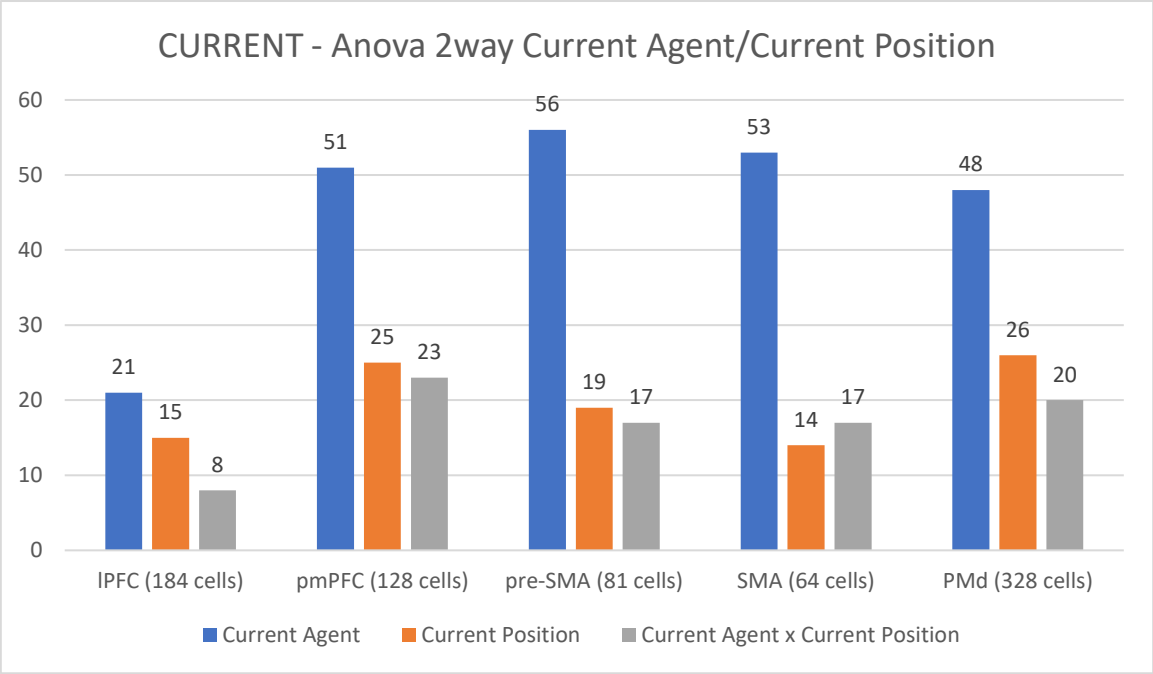
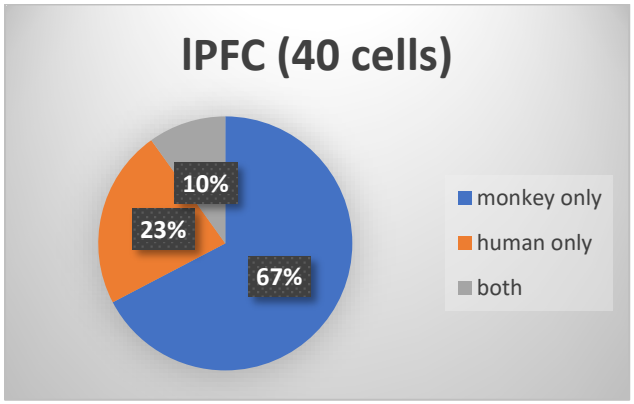


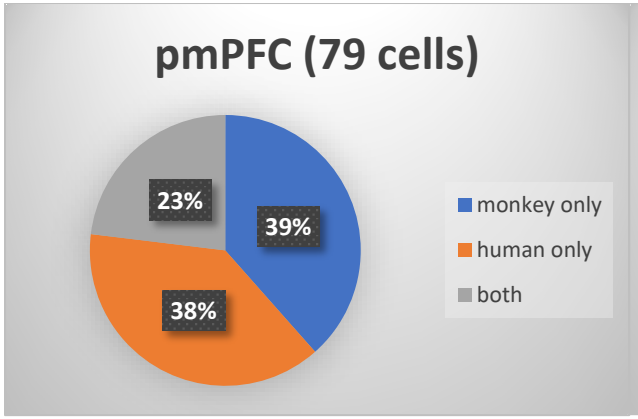
Fig. 4.1 Results of the Anova two-way during the delay period in each of the recorded area.

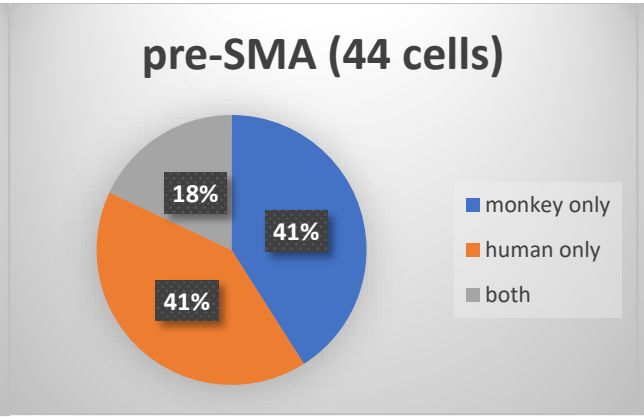
Figure 4.1 shows that in each area the majority of cells is modulated by the agent who performed the task during the delay period. The areas more involved in this distinction between self and other are the areas of the medial prefrontal cortex (pmPFC, pre-SMA, SMA), that showed a significant effect for the agent factor in more than the half of the cells recorded. The dorsal premotor cortex showed a similar percentage of cells that are able to distinguish between the two agents as well. Interestingly, in PMd the majority of the agents cells showed a preference for the human agents intended as an higher activity during the observation of the human trials (67% of agent selective cells), while the opposite trend was observed in the areas of the medial prefrontal cortex, where the majority of cells showed a higher firing rate for monkey trials (55% in pmPFC, 60% in pre-SMA, 59% in SMA). The function of these specific neurons is difficult to assess. The example cells of Fig. 3.3 represents two type of cells that during the delay period showed a difference in their firing rate depending on the actor that was performing the trial. Nevertheless, we can see that this modulation is present also beyond the delay period, in the periods before and after it. It is possible that the agent-cells take part in the coordination of actions between two interacting agents, helping to take turns in a task such as the interactive NMTG. From this perspective, the activity of the human-agent neurons could underly the inhibition of the action that is required to let intervene the other agent in the task.

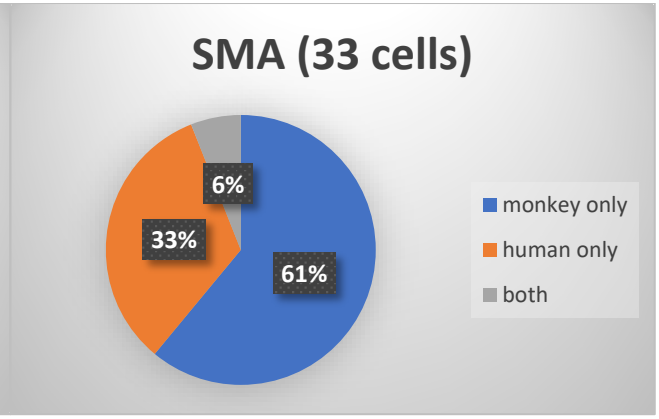
The main purpose of these experiments is to investigate whether it could exist a separate neural representation of self and others' actions. In the delay period the action is not performed yet, thus it

can be prepared in the monkey trials and predicted or anticipated in human trials, relying on the combination of knowledge of the task rules and of the human agent behavior, who performs always the correct choice. For this purpose, these experiments investigated whether a representation of the correct behavioral goal could be specific to one of the agents who performed the trial, either the monkey or the human, instead of being just a shared representation for both. This led to the identification of three categories of cells: the monkey-only cells encoded the position of the correct behavioral goal exclusively when the monkey was performing the trial; the human-only cells encoded the position of the correct behavioral goal exclusively when the human was performing the trial; and finally the both-agents cells encoded the position of the correct behavioral in both monkey and human trials. Here I provide a comparison of the proportion of these three category of neurons in the different areas investigated by these experiments (Fig. 4.2). The percentages are obtained in each area from the total amount of cells that show a spatial selectivity for at least one agent in the delay period.









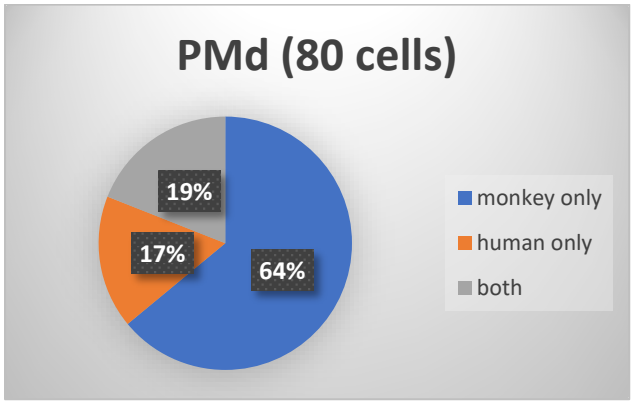


Fig. 4.2 **Percentages of the three group of cells (monkey-only, human-only and both-agents) in the delay period for each of the recorded areas.**

Monkey-only

The first category of cells is the monkey-only cells. These specific neurons in the delay period show a spatial selectivity for one of the position of the behavioral goal only when the monkey is performing the trial, but not when the human agent is performing it (Fig. 2.6a, Fig. 3.4). For example, in the figure 3.4, in the ONMTG task recorded in the PMd, the cell was showing a higher firing rate when the correct behavioral goal was on the right than on the left position, but only in monkey trials. In human trials, no difference could be found between the two spatial positions. The pie chart of Fig. 4.2 shows that more than half of the spatial selective cells in the lPFC and in the PMd are classified as monkey-only cells. In these two areas, it is possible that neurons that show the same features are actually coding different cognitive and behavioral functions. For example, it is possible that in the lPFC these cells are representing more abstractly the behavioral goal, processing the visual information about the correct target to reflect the behavioral goal in terms of spatial position, but specifically only for the monkey. In the PMd, given the connection of this area with other areas related to movement functions, the activity of monkey-only cells can reflect the preparation of a movement toward a specific location.

Human-only

The human-only cells act in the opposite way compared to the monkey-only cells, showing a spatial selectivity in the delay period only when the human agent is performing the action. In Fig. 3.4 the raster plot in the center shows an example of a human-only cells recorded in the PMd, that shows a higher firing rate for the left than for the right behavioral goal; this difference is present only in the trials performed by the human agent, and not in monkey trials. Fig 4.2 shows that the greatest percentages of this type of neurons can be found in the medial frontal cortex, especially in its anterior parts, the pmPFC and the pre-SMA areas, where they are found in a similar proportion to the monkey-only cells (38% and 41% of human-only cells respectively). A lower percentage can be found in the lPFC and in the PMd, where the percentage of monkey-only cells is largely predominant. These results confirm the prominent role played by the MFC in the processes of self others differentiation. The results of Yoshida et al. (2012) indicates that this differentiation can be found in the activity of single neurons in the MFC at least in the domain of motor action. The authors discovered that during the observation of motor acts a specific population of neurons was selectively activated (partner type neurons, 46% of the agent related cells). These neurons were not active when the action was executed instead of observed. In the experiment of Falcone et al. (2017), these results are extended beyond the motor domain, because the activity of human-only cells is selective for the partner's action before the action actually occurs. Putting these results together, it is clear that the medial frontal cortex plays a

major role at different stages of the processes of self-others differentiation, not only during the observation of the movement of another agent, but also in representing the imminent choice or behavior goal of another agent.

In summary, the activity of these cells can be described as predictive of others' choices. These neurons indeed 'predict' the upcoming action of the human agent, anticipating his choice based on the clear knowledge of the monkeys of the fact that the human always performs the correct choice. However, the lack of errors from the human agent makes difficult to interpret thoroughly this predictive activity. In simple words, it is not clear whether the activity of these neurons is reflecting what the human agent 'will do' or what the human agent 'should do'. To investigate further these possibilities, it would help to study error-related activity. One would expect that, in a stable predictive context in which the monkey is always expecting the choice of the incorrect behavioral goal from the human agent, these neurons would show the same spatial selectivity for the position of the incorrect target. Anyway, in this case of course there would be no chance to confront it with correct trials. Intermixing correct and error trial from the human agent would lead to the loss of the stability in the predictive context from the monkeys, that would not be able anymore to have a clear expectation of what the human agent will do, losing the potential ability to 'predict' his behavior.

Both-agents

The monkey-only and the human-only cells are the groups of neurons that can represent the neural substrates of the self-other differentiation. Indeed the last group, the both-agents cells, showed a modulation for a specific position of the behavioral target in monkey trials as well in human trials. Fig. 3.4 shows an example of this type of neurons in the delay period, with a preference for the left position regardless of the agent who is performing the trial. We can define this kind of activity as 'mirror like', because the properties of these neurons resemble the mirror neurons that were found for the first time in the ventral premotor cortex (di Pellegrino et al. 1992), showing the same activation during action execution and action observation. Although they are similar, we define it as 'mirror like' and not simply 'mirror' because this kind of modulation in these tasks is not observed during the movement, as in the original experiment, but before, in a delay period that preceded the actual movement. In Fig. 4.2 is possible to see that in each one of the recorded area the amount of both-agents cells is lower compared to the other two groups, with the exception of the PMd, which has similar percentages of human-only and both-agents cells. Nevertheless, this result in the PMd it is of great interest. Different studies in the past contributed to establish the common view that mirror like activity is a general feature of the dorsal premotor cortex (Hatsopoulos & Suminski 2011, Mendoza & Merchant 2014), leading to the idea that the PMd is part of an action-observation network

(Andrieux & Proteau, 2016) in which the same neural mechanisms can be activated when an action is performed or observed. This overlap between the encoding of self and other's actions has been observed in PMd by Cisek & Kalaska (2004): they found that an overwhelming proportion of neurons (84%) that were directionally tuned before movement when the monkey was performing a motor task, did so also when the monkeys observed a cursor on the screen. Although the evidence of these findings is strong, and it is well established that this area shows clear mirror properties, our results anyway suggest that also a different interpretation of the role played by the PMd in the processes of self-others differentiation is possible. The experimental paradigm of Cisek & Kalaska and the NMTG used in these experiments differed in the type of observed external agent (an inanimate cursor vs a real physical agent) and in the requirement for monitoring (passive observation vs active monitoring). These differences with the previous task of Cisek & Kalaska may have elicited a departure from 'real' social interaction, promoting more the activation of an underlying simulation mechanism rather than the activation of some neural processes related to self-others differentiation. Our task design promotes a real face-to-face interaction, in which the two agents actively interact monitoring each other's choices. In this more natural context, it seems that in the PMd only a minority of cells exhibits mirror like properties, while it is present a neural substrate that is able to distinguish between self and others.

Finally, a further consideration has to be done when considering the role played by the both-agents cells. We considered before that the activity of the monkey-only and of the human-only cells represent the neural signal that allows the distinction between self and others, because their preference for one of the spatial positions of the correct behavioral target is unique for a specific agent. Instead, the both-agents cells show a spatial preference during both monkey and human trials. However, it is possible that the preferred spatial position it is not the same between the two agents. For example, it is possible that during the monkey trials a particular neuron shows a preference for the correct behavioral goal in the left position, while it can show during human trial a preference for the correct behavioral goal in the right position. This cell is classified as both-agents, because in its firing rate there is a difference between the spatial position in both monkey and human trial, though it is not the same. A different instance is when the preference for a specific spatial position is shared between monkey and human trials. In the latter case, the activity of such a 'congruent' both-agents cell is not carrying any information useful for the distinction between self and others, while in the other case, the activity of the 'incongruent' both-agents cell is providing a signal of distinction. In the dorsal premotor cortex, we classified the vast majority of both-agents cells as congruent; with the exception of one neuron, the others showed a mirror like activity in a narrow sense, with no distinction between agents. In the experiment of Falcone et al. (2017) in the medial frontal cortex, a spatial modulation index was calculated to assess whether the both-agents cells could be classified as congruent or incongruent. A

position rank was calculated in monkey trial and human trials separately on the firing rate in the four different possible position of the targets, to assess the preferred and the anti-preferred position. They found that, similarly to the results in the PMd, a prevalence of congruent activity was present in the both-agents cells recorded in the pre-SMA and SMA areas. Instead, in the pmPFC, more than the half (63%) of the recorded both-agents cells showed an incongruent activity between monkey and human trials. This result suggests that neurons in prefrontal areas such as the pmPFC show a higher level of flexibility compared to neurons in areas that are located more posteriorly, such as the pre-SMA, SMA and PMd. Their ability to switch their spatial preference depending on the actor that is performing the trial could make them suitable to participate in more complex neural computations, compared to the other groups of cells that instead show a more strict coding scheme.

4.2 REPRESENTING PREVIOUS CHOICES

One of the most important task requirements in the interactive version of the NMTG adopted by these experiments was the requirement to remember the correct behavioral target chosen in the previously performed trial in order to discard it in the successive and choose the new one. To perform correctly in a given trial therefore the monkeys needed to remember not only their own choices, but also to monitor and remember the human agent's choices. To this end, in all the three experiments the analysis was extended in another period, the *holding CS period*, to study the neural activity related to the monitoring of the previous trial. The 2-way analysis of variance performed in the different experiments tested the effect of the factors previous agent (monkey or human) and previous position (right or left) on the firing rate. Figure 4.3 reports the percentage on the total of the recorded cells in each area that showed a selectivity for these factors.

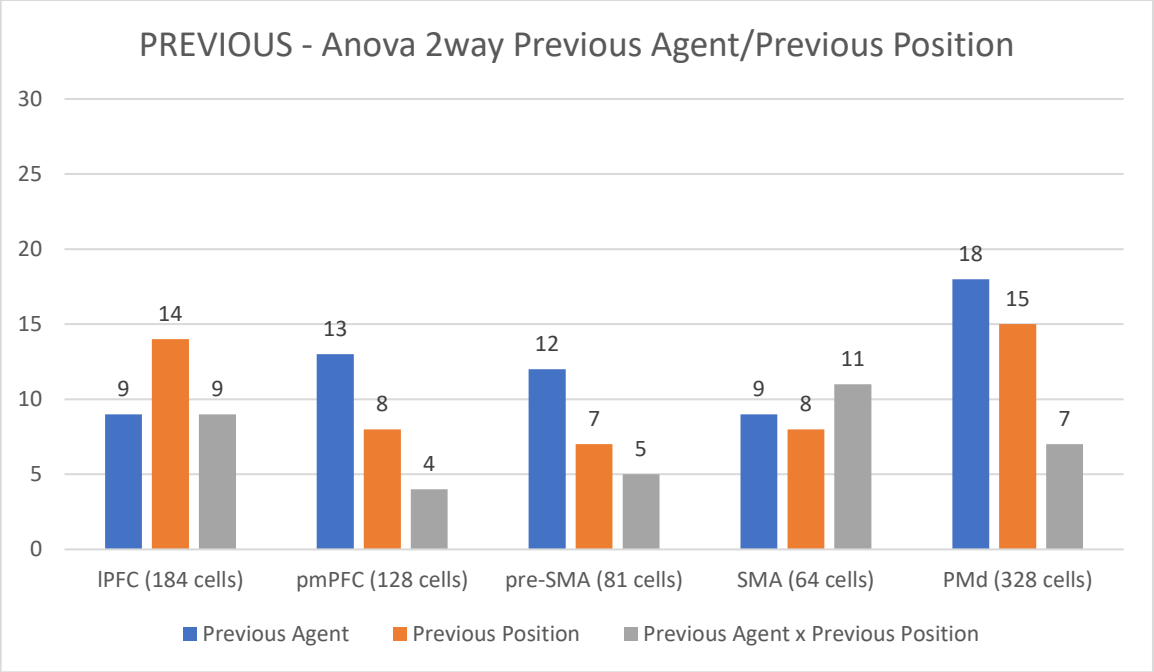


Fig. 4.3 Results of the Anova 2way during the holding CS period in each of the recorded area.

The figure shows a smaller number of selective neurons compared to those obtained by the 2-way Anova in the current trial (Fig. 4.1), with a general decrease of the amount of cells that responded to the agent that performed the previous trial compared to the delay period results.

As for the analysis in the delay period, three groups of cells were identified, as shown in Fig. 4.4.

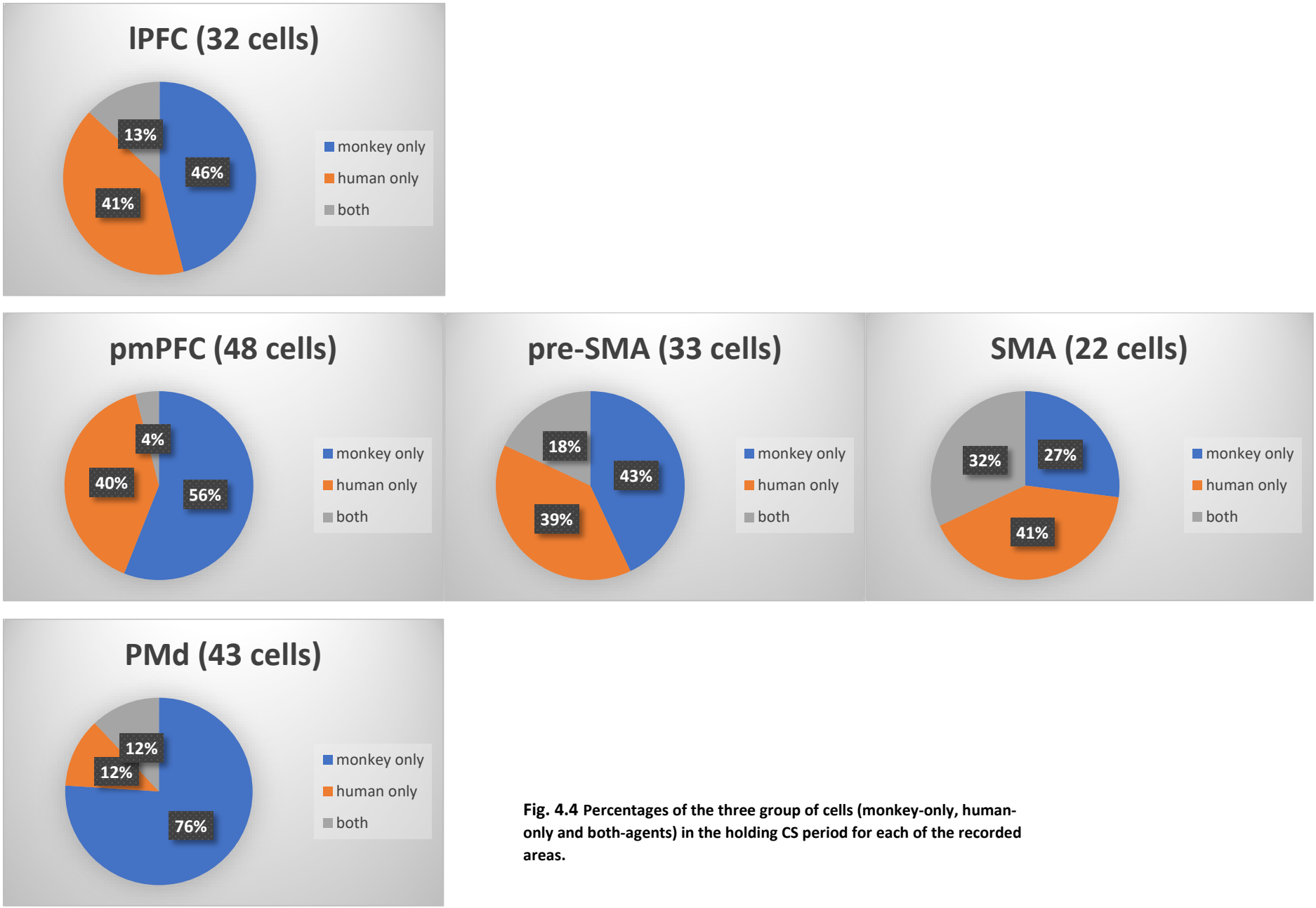


Fig. 4.4 **Percentages of the three group of cells (monkey-only, human-only and both-agents) in the holding CS period for each of the recorded areas.**

The monkey-only cells and human-cells represent the previously chosen position only when the previous trials was performed by the monkeys or the human agent respectively; the both-agents cells represent the previously chosen position irrespective of the previous agent. While these three groups of cells in the delay period can provide a representation of the future behavior of a specific agent, their activity at the beginning of the trial can represent in a similar manner the past choice of a specific agent. Being able to distinguish who performed an action and to remember the outcome of this action is a fundamental skill in social interaction, especially when coordinating with other individuals.

Previous studies investigated the role of the lPFC in monitoring previous trials. Genovesio et al. (2006) found that prefrontal neurons that encoded the previous behavioral goal position, in tasks in which, similarly to the NMTG, monkeys had to choose the goal in the current trial based on the choice made in the previous trial. Interestingly, they found no overlapping between the population of neurons that encoded the behavioral goal in the current trial and those that encoded the behavioral goal of the previous trial, suggesting that these functions are processed by two separate neural networks. Moreover, it has been reported that the lPFC encodes not only information from the previous trial relevant for guiding behavior, but also information regardless of their task relevance (Tsujimoto et al. 2012, Genovesio et al. 2014), such as previous spatial position or visual features of the objects. These findings indicates that lateral prefrontal cortex is deeply involved in the representation of multiple aspects related to the previous trials at different levels. In line with these results, the proportion of monkey, human and both-agents cells identified in the *holding CS period* was similar to that in the *delay period* (32 vs 40), but while in the delay the majority of neurons encoded the future position selectively for the monkey, at the beginning of the trial we observed an increase of the percentage of cells that encoded the previous goal position for the human agent only. In the dorsal premotor cortex instead, we observed a prevalence of monkey-only cells, with the almost total absence of cells coding for the human agent or for both-agents. This suggests that this area is not specifically involved in coding the other's action beyond the limits of the current trial, in contrast to the high percentage of previous human-only cells found in the pre-SMA and SMA areas. Although these areas are also part of the motor complex as the PMd, it seems that the encoding of other's action remains a fundamental prerogative of the medial cortex, where the proportions of human-only cells are comparable with those found in the delay period.

4.3 FURTHER INVESTIGATIONS

To achieve a more complete understanding of the neural mechanisms underlying the differentiation between self and others, further studies will have to focus on three main aspects. First, comparing these results with the past literature on social cognition, we observed that studies that fail to report a clear neural substrate able to distinguish between self and others, also adopted experimental paradigms in which the observed action was carried out by an inanimate agent such a cursor on the screen. It is fundamental to have the chance to compare within the same experimental paradigm the effect of interacting with both an animate and an inanimate interactive agent. In this regard, the NMTG paradigm lends itself well for this purpose. Besides the interaction with the human agent, the same task paradigm can be applied with a cursor moving on the screen. The cursor can act in the same way the human agent does, intervening in the task and moving from the center to the correct behavioral target. Monkeys would still have to monitor the action of the cursor in order to succeed in the trial after, as they do with the human agent. Second, it will be necessary to study error-related activity. Training the monkeys in a stable predictive context in which the human agent always choose the incorrect target would allow to control whether a predictive activity develops also under this condition in the delay period, helping to dispel the doubts about the activity of the human-only cells. Finally, it will be necessary to test the interaction with multiple agents. In the experiments described, the human-agent with whom each monkey interacted during the task was always the same, usually the experimenter who trained them and with whom they had a high level of familiarity. Introducing multiple agents will clarify whether the 'other' related activity is a general response to the interaction with a real physical agent or is specific to a particular agent.

4.3 CONCLUSIONS

In this work, we investigated through three complementary neurophysiological experiments, the neural correlates of self-others distinction. To understand clearly the underlying mechanisms of social interaction, neurophysiological experiments with macaque model are a powerful tool. Firstly, they allow to study the activity of specific brain areas by investigating the pattern of activation of single neurons, the very basic unit of the nervous system, with a high degree of spatial and temporal resolution. Moreover, macaque monkeys represent an excellent experimental model because of their proximity to humans and their ability to fullfill complex cognitive and social behaviors. Our experiments investigated the single-unit activity of different areas of the frontal cortex while the monkeys were performing a social interaction task during which they were required to monitor others' behavior. Through the use of the same experimental paradigm we compared the activity of the PMd neurons with the one collected in two previous studies in the lPFC and MFC, extending to the premotor cortex a role in social interaction. The connectivity of the PMd with the prefrontal areas investigated in the previous studies suggests that this area can be included in a more general and already described 'social brain', which includes brain areas involved in social aspects of cognition. The neurons of these areas contribute differently to create a a distinct representation of self and others. This specificity we higlighted here is fundamental to fullfill complex social behaviors as understanding other's action, intentions or goal.

BIBLIOGRAPHY

Amodio DM & Frith CD. Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci* **7,** 268–277 (2006).

Andrieux M & Proteau L. Observational learning: tell beginners what they are about to watch and they will learn better. *Front Psychol* **7,** 1–9 (2016).

Barbas H & Pandya DN. Architecture and frontal cortical connections of the premotor cortex (area 6) in the rhesus monkey. *J Comp Neurol* **256,** 211–228 (1987).

Botvinick MM, Braver TS, Barch DM, Carter CS & Cohen JD. Conflict monitoring and cognitive control. *Psychol Rev* **108,** 624–652 (2001).

Boussaoud D & Wise SP. Primate frontal cortex: effects of stimulus and movement. *Exp Brain Res* **95,** 28–40 (1993).

Brinkman C & Porter R. Supplementary motor area in the monkey: activity of neurons during performance of a learned motor task. *J Neurophysiol* **42,** 681–709 (1979).

Broadmann K. Beitraege zur histologischen lokalisation der grosshirnrinde. III. Mitteilung. Die cortexgliederung des menschen. *Journal fur Psychologie und Neruologie,* **10**, 231-246 (1908)

Burke CJ, Tobler PN, Baddeley M & Schultz W. Neural mechanisms of observational learning. *Proc Natl Acad Sci* **107,** 14431–14436 (2010).

Carter A, Braver TS, Barch DM, Botvinick MM, Noll D & Cohen JD. Anterior cingulate cortex, error detection, and the online monitoring of performance. *Science* **280,** 747–749 (1998).

Casile, A. Mirror neurons (and beyond) in the macaque brain: An overview of 20 years of research. *Neurosci Lett* **540,** 3–14 (2013).

Chang SWC, Winecoff AA & Platt ML. Vicarious reinforcement in rhesus macaques (macaca mulatta). *Front Neurosci* **5,** 1–10 (2011).

Cirillo R, Ferrucci L, Marcos E, Ferraina S & Genovesio A. Coding of self and other's future choices in dorsal premotor cortex during social interaction. *Cell Rep* **24,** 1679–1686 (2018).

Cisek P & Kalaska JF. Neural correlates of mental rehearsal in dorsal premotor cortex. *Nature* **431,** 993–996 (2004).

di Pellegrino G, Fadiga L, Fogassi L, Gallese V & Rizzolatti G. Understanding motor events: a neurophysiological study. *Exp Brain Res* **91** 176–180 (1992).

Dum RP, & Strick PL. Motor areas of the frontal lobe of the primate. *Phys & Behav* **77,** 677-682 (2002).

Fagot J & Thompson RKR. Generalized relational matching by Guinea baboons (papio papio) in two-by-two-item analogy problems. *Psychol Sci* **22,** 1304–1309 (2011).

Falcone R, Brunamonti E, Ferraina S & Genovesio A. Monkeys monitor human goals in a nonmatch-to-goal interactive task. *PLoS One* **7(2),** e32209 (2012a).

Falcone R, Brunamonti E & Genovesio A. Vicarious learning from human models in monkeys. *PLoS One* **7(7),** e40283 (2012b).

Falcone R, Bevacqua S, Cerasti E, Brunamonti E, Cervelloni M, Genovesio A.. Transfer of the nonmatch-to-goal rule in monkeys across cognitive domains. *PLoS One* **8,** e84100 (2013).

Falcone R, Brunamonti E, Ferraina S & Genovesio A. Neural encoding of self and another agent's goal in the primate prefrontal cortex: Human-monkey interactions. *Cereb Cortex* **26,** 4613–4622 (2016).

Falcone R, Cirillo R, Ferraina S & Genovesio A. Neural activity in macaque medial frontal cortex represents others' choices. *Sci Rep.* **7,** 12663 (2017).

Foffani G & Moxon KA. PSTH-based classification of sensory stimuli using ensembles of single neurons. *J. Neurosci. Methods* **135,** 107-120 (2004).

Fulton JF. A note on the definition of the motor and premotor areas. *Brain* **58,** 311-316 (1935)

Gallese V, Fadiga L, Fogassi L & Rizzolatti G. Action recognition in the premotor cortex. *Brain* **119,** 593–609 (1996).

Genovesio A, Brasted PJ & Wise SP. Representation of future and previous spatial goals by separate neural populations in prefrontal cortex. *J Neurosci* **26,** 7305–7316 (2006).

Genovesio A, Tsujimoto S, Navarra G, Falcone R & Wise SP. Autonomous encoding of irrelevant goals and outcomes by prefrontal cortex neurons. *J Neurosci* **34,** 1970–1978 (2014).

Gentner, D. Structure-mapping: a theoretical framework for analogy. *Cog Sci* **7,** 155-170 (1983).

Gillan DJ, Premack D & Woodruff G. Reasoning in the chimpanzee: i. analogical reasoning. *J Exp Psychol* **7,** 1 (1981).

Goldman-Rakic PS. Topography of cognition: parallel distributed networks in primate association cortex. *Annu Rev Neurosci* **11**, 137-156 (1988)

Haroush K & Williams ZM. Neuronal prediction of opponent's behavior during cooperative social interchange in primates. *Cell* **160,** 1233–1245 (2015).

Hatsopoulos NG & Suminski AJ. Sensing with the motor cortex. *Neuron* **72,** 477–487 (2011).

Holyoak KJ, Junn EN & Billman DO. Development of analogical problem-solving skill. *Child Development* **55**, 2042–2055 (1984).

Hosokawa T & Watanabe M. Prefrontal neurons represent winning and losing during competitive video shooting games between monkeys. *J Neurosci* **32,** 7662–7671 (2012).

Ito S, Stuphorn V, Brown JW & Schall JD. Performance monitoring by anterior cingulate cortex during saccade countermanding. *Science* **302,** 120–122 (2003).

Katz JS, Wright AA. & Bachevalier J. Mechanisms of same/different abstract-concept learning by rhesus monkeys (Macaca mulatta). *J Exp Psychol Anim Behav Process* **28,** 358–368 (2002).

Krawczyk DC. The cognition and neuroscience of relational reasoning. *Brain Res* **1428,** 13–23 (2012)

Kurata K & Wise SP. Premotor cortex of rhesus monkeys: set-related activity during two conditional motor tasks. *Exp Brain Res* **69,** 327–343 (1988).

Kurata K. Information processing for motor control in primate premotor cortex. *Behav Brain Res* **61,** 135–142 (1994).

Matelli M, Luppino G & Rizzolatti G. Architecture of superior and mesial area 6 and the adjacent cingulate cortex in the macaque monkey. *J Comp Neurol* **311,** 445–462 (1991).

Matsuzaka Y, Akiyama T, Tanji J & Mushiake H. Neuronal activity in the primate dorsomedial prefrontal cortex contributes to strategic selection of response tactics. *Proc Nat Acad Sci* **109,** 4633–4638 (2012).

Matsuzaka Y, Tanji J & Mushiake H. Representation of behavioral tactics and tactics-action transformation in the primate medial prefrontal cortex. *J Neurosci* **36,** 5974–5987 (2016).

Mendoza G & Merchant H. Motor system evolution and the emergence of high cognitive functions. *Prog Neurobiol* **122,** 73–93 (2014).

Monfardini E, Hadj-Bouziane F & Meunier M. Model-observer similarity, error modeling and social learning in rhesus macaques. *PLoS One* **9(2),** e89825 (2014).

Nachev P, Kennard C & Husain M. Functional role of the supplementary and pre-supplementary motor areas. *Nat Rev Neurosci* **9,** 856–869 (2008).

Nakamura K, Sakai K & Hikosaka O. Neuronal activity in medial frontal cortex during learning of sequential procedures. *J Neurophysio* **80,** 2671–87 (1998).

Obozova T, Smirnova A, Zorina Z & Wasserman E. Analogical reasoning in amazons. *Anim Cogn* **18,** 1363–1371 (2015).

Picard N & Strick PL. Motor areas of the medial wall : a review of their location and functional activation. *Cereb Cortex* **6**, 342–353 (1996).

Premack D. On the abstractness of human concepts: why it would be difficult to talk to a pigeon. In: Hulse, S.H., Fowler, H., Honig, W.K. (Eds.), *Cognitive Processes in Animal Behavior*. Erlbaum, Hillsdale, NJ, pp. 423–451.

Rainer G, Asaad WF & Miller EK. Selective representation of relevant information by neurons in the primate prefrontal cortex. *Nature* **393,** 577–579 (1998).

Ridderinkhof KR, Ullsperger M, Crone EA. & Nieuwenhuis S. the role of the medial frontal cortex in cognitive control. *Science* **306,** 443–447 (2004).

Rizzolatti G, Fadiga L, Gallese V & Fogassi L. Premotor cortex and the recognition of motor actions. *Cogn Brain Res* **3,** 131–141 (1996).

Rizzolatti G, Fogassi L. & Gallese V. Neurophysiological mechanisms underlying the understanding and imitation of action. *Nat Rev* **2,** 661– 670 (2001).

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

Rizzolatti G & Luppino G. The cortical motor system. *Neuron* **31,** 889–901 (2001).

Saito N, Mushiake H, Sakamoto K, Itoyama Y & Tanji J. Representation of immediate and final behavioral goals in the monkey prefrontal cortex during an instructed delay period. *Cereb Cortex* **15,** 1535–1546 (2005).

Simms NK, Frausel RR & Richland LE. Working memory predicts children's analogical reasoning. *J Exp Child Psychol* **166,** 160-177 (2018)

Smirnova A, Zorina Z, Obozova T & Wasserman E. Crows spontaneously exhibit analogical reasoning. *Curr Biol* **25,** 256–260 (2015).

Subiaul F & Clanton JF. Cognitive imitation in rhesus macaques. *Science* **305,** 70–74 (2004).

Suzuki S, Harasawa N, Ueno K, Gardner JL, Ichinohe N, Haruno M, Cheng K & Nakahara H. Learning to simulate others' decisions. *Neuron* **74,** 1125–1137 (2012).

Tanji J & Kurata K. Comparison of movement-related activity in two cortical motor areas of primates. *J Neurophysiol* **48,** 633–653 (1982).

Tanji J & Kurata K. Contrasting neuronal activity in supplementary and precentral motor cortex of monkeys. I. Responses to instructions determining motor responses to forthcoming signals of different modalities. *J. Neurophysiol* **53,** 142–52 (1985).

Tanji J & Hoshi E. Role of the lateral prefrontal cortex in executive behavioral control. *Physiol Rev* **88,** 37–57 (2008).

Thompson RKR, Oden DL & Boysen ST. Language-naive chimpanzees (pan troglodytes) judge relations between relations in a conceptual matching-to-sample task. *J Exp Psychol Anim Behav Process* **23,** 31–43 (1997).

Tkach D, Reimer J & Hatsopoulos NG. Congruent activity during action and action observation in motor cortex. *J Neurosci* **27,** 13241–13250 (2007).

Truppa V, Mortari EP, Garofoli D, Privitera S & Visalberghi E. Same/different concept learning by capuchin monkeys in matching-to-sample tasks. *PLoS One* **6(8),** e23809 (2011).

Tsujimoto S, Genovesio A & Wise SP. Neuronal activity during a cued strategy task: comparison of dorsolateral, orbital, and polar prefrontal cortex. *J Neurosci* **32,** 11017–11031 (2012).

Umiltà MA, Kohler E, Gallese V, Fogassi L, Fadiga L, Keysers C & Rizzolatti G. I know what you are doing: a neurophysiological study. *Neuron* **31,** 155–165 (2001).

Walker AE. A cytoarchitectural study of the prefrontal area of the macaque monkey. *J Comp Neurol* **73,** 59–86 (1940).

Weinrich M & Wise SP. The premotor cortex of the monkey. *J Neurosci* **2,** 1329–1345 (1982).

Whitaker KJ, Vendetti MS, Wendelken C, Bunge SA. Neuroscientific insights into the development of analogical reasoning. *Dev Sci* **21**, e12531 (2018)

Wise SP. The primate premotor cortex: past, present and preparatory *Ann Rev Neurosci* **8**,1–19 (1985).

Yamagata T, Nakayama Y, Tanji J & Hoshi E. Distinct information representation and processing for goal-directed behavior in the dorsolateral and ventrolateral prefrontal cortex and the dorsal premotor cortex. *J Neurosci* **32,** 12934–12949 (2012).

Yoshida K, Saito N, Iriki A & Isoda M. Representation of others' action by neurons in monkey medial frontal cortex. *Curr Biol* **21,** 249–253 (2011).

Yoshida K, Saito N, Iriki A & Isoda M. Social error monitoring in macaque frontal cortex. *Nat Neurosci* **15,** 1307–1312 (2012).