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ROLE OF THE MONKEY VENTROLATERAL PREFRONTAL CORTEX IN THE INTEGRATION OF CONTEXTUAL INFORMATION FOR THE SELECTION AND UNDERSTANDING OF GOAL-DIRECTED ACTIONS

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1. INTRODUCTION

1.1. Selection, organization and understanding of actions in the prefrontal cortex

> *"To select relevant information for action, the prefrontal cortex must have access to the sensory and spatial aspects of the environment, mnemonic information acquired through experience, and linkage with motor control structures. In addition, decisions and actions taken in behavioural settings are inextricably embedded within the context of the internal, or emotional, environment of the individual, commonly expressed*"
> (Barbas, 2000).

To deal with daily activities, to plan and to execute actions which allow us to achieve different behavioural goals, we have to evaluate many variables. Action selection and organization are very complex processes that need to exploit the integration of contextual information and the retrieval of previously memorized information (Tanji and Hoshi, 2001). What is the neural basis of this context-dependent flexibility?

Recent studies conducted on monkeys (Fogassi et al., 2005; Bonini et al., 2010) demonstrated that areas PFG and F5 provide a different contribution to action goal coding: very likely, area PFG plays a more important role in organizing motor acts into actions, while F5 appears to be more involved in coding the goal of the single motor acts in a more abstract fashion (Rizzolatti et al., 1988; Umiltà et al., 2008). In particular, Fogassi and co-workers (2005) showed that inferior parietal neurons can code differently the same motor act (i.e. grasping), depending on the global action (grasp-to-eat or grasp-to-place) in which the act is embedded. Similar findings have been reported by neurophysiological investigations carried out on the ventral premotor (vPM) cortex (Bonini et al., 2010). Interestingly, the same neuronal properties emerged even when the monkey was required to observe the same motor acts performed by an experimenter, suggesting that these neurons not only code the observed motor act but also allow the observer to understand the agent's intentions. More recent experiments have shown that, particularly in the inferior parietal lobule (IPL), grasping neurons discharge can reflect the final goal of the action at higher level of abstraction (Bonini et al., 2011), suggesting the possible presence of a neural mechanism capable of integrating available contextual information used by the monkey to decide which action to perform.

Thus, motor acts embedded into an action are linked one to the other in order to achieve the final action goal. These links could depend on an underlying organization in intentional neuronal chains in which a neuron coding a given motor act is facilitated by that coding the previously executed one (Fogassi et al., 2005; Rizzolatti et al., 2006; Chersi et al., 2011). Anatomical connections between the cortical areas and functional results investigated in the literature suggest that cortico-cortical projections of different cortical areas can play a crucial role in the selection of inferior parietal and ventral premotor neuronal pools for the organization of intentional actions. The ventrolateral prefrontal cortex (VLPFC) appears one of the most plausible candidates to fulfill this function. Indeed, both parietal and premotor areas are anatomically connected with sectors of prefrontal cortex (PFC) and it is well known that this area is involved in associative learning (White and Wise, 1999). Furthermore, several studies evidenced that the VLPFC plays a role in arranging motor chunks - such as pushing, pulling or turning - into over-trained motor sequences based on learned sensory instructions (Hoshi et al., 1998; 2000; White and Wise, 1999; Wise and Murray, 2000; Miller and Cohen, 2001; Shima et al., 2007; Tanji and Hoshi, 2008).

On the basis of the of the anatomical connections of parietal and premotor areas (Borra et al., 2008, 2011; Gerbella et al., 2013b) and even on the data about functional properties reported in the literature that will be described in detail in the following sections, it seems very appropriate to suppose that one of the most plausible candidate involved in the selection of neuronal pools for the selection and organization of intentional actions is the prefrontal cortex.

1.3. Anatomical connections of the ventrolateral prefrontal cortex

The PFC constitutes the rostral field of the frontal lobe and it is mainly divided into three surfaces: orbital (or ventral), directly lying over the orbit; medial, in the medial surface; lateral, which occupies the dorsal and ventral convexity of the PFC. The prefrontal cortex is caudally delimited by a clearly identifiable anatomical structure, namely the arcuate sulcus (AS); medially, it is bordered by the cingulate sulcus and faces the medial surface of the contralateral prefrontal area; and horizontally, it is crossed by the principal sulcus (PS). Its anatomical organization places the PFC in a unique position and highlights its critical role in processing and integrating multimodal information. Besides this general partition, PFC is fractioned into distinct areas: the macaque prefrontal cortex has been subject of numerous anatomical studies (Walker, 1940; Barbas and Pandya, 1989; Preuss and Goldman Rakic, 1991; Petrides and Pandya, 1999, 2002) in which different architectonic subdivisions have been proposed (Figure 1). Furthermore, the lateral prefrontal cortex (LPFC), through its connections to premotor areas, the basal ganglia and the cerebellum is supposed to control broad aspects of motor behaviour. Specifically, by means of its connectivity, the LPFC modulates the flow of information in other areas of the central nervous system, depending on the behavioural requirements, providing a resource for adaptive control of information flow through cortical and subcortical structures (Miller, 2000).

The prefrontal area of major interest for this study largely corresponds to the ventral part of area 46 (area 46v) as defined by Walker (1940). On a functional point of view, the area 46v hosts neurons involved in action selection, learning processes and exploitation of behavioural rules for the execution of goal directed actions (Miller and Cohen 2001; Tanji and Hoshi 2008). In the monkey brain, area 46v occupies almost the entire rostro-caudal extent of the ventral bank of the PS and the immediately adjacent convexity cortex. The well-known rich connections of this sector with the IPL and the parietal operculum, on one side, and with the vPM and prearcuate oculomotor areas, on the other, allow us to consider this area the neural substrate for functional properties described above (Tanji and Hoshi, 2008).

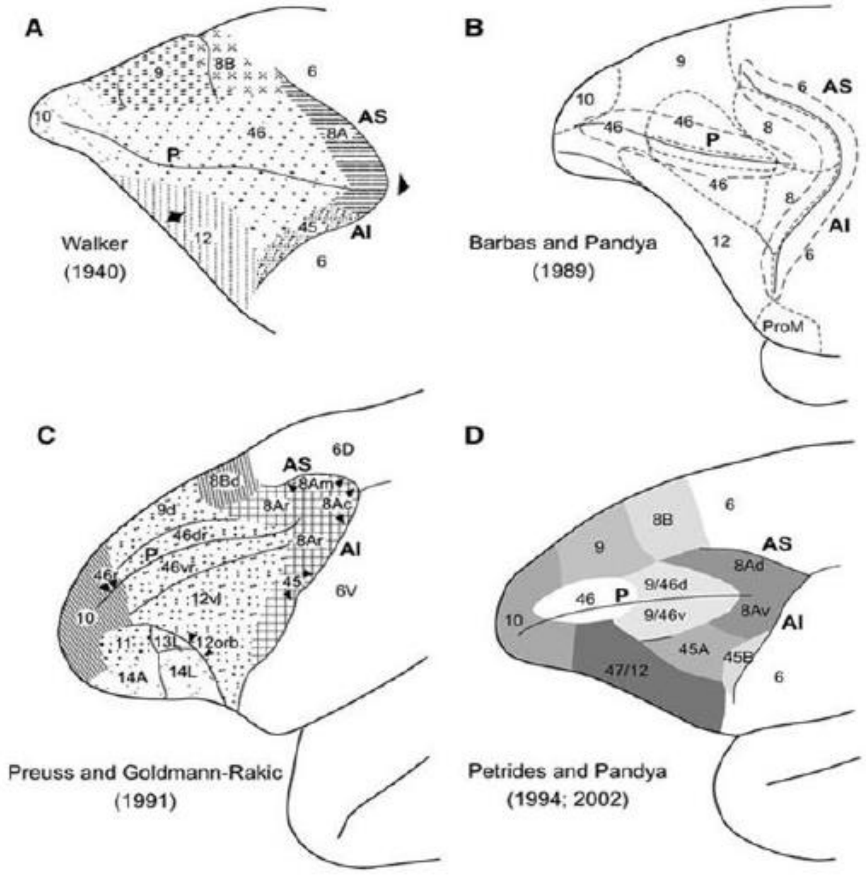

Figure 1. Four different architectonic maps of the macaque prefrontal cortex, defined by: A) Walker (1940); B) Barbas and Pandya (1989); C) Preuss and Goldman-Rakic (1991); D) Petrides and Pandya (1994, 2002).

Recent direct (Gerbella et al., 2007; 2013b) and indirect data (Rozzi et al., 2006; Borra et al., 2008; Gerbella et al., 2011) showed that area 46v is connectionally heterogeneous and that its half caudal part (46vc) mostly displayed intra-prefrontal connectivity with ventrolateral areas and robust connectivity with frontal and parietal sensorimotor areas. Based on a topographic organization of these connections, three distinct fields were identified in area 46vc.

The caudal field (caudal 46vc) is preferentially connected to oculomotor prearcuate (8/FEF, 45B, and 8r) and parietal areas (LIP), and rostral (IPa, Tea/m) and caudal (FST, MT) part of superior temporal sulcus (STS).

The other two fields, located more rostrally in the bank of the PS (rostral 46vc/bank) and in the convexity of the VLPFC (rostral 46vc/convexity), show pronounced labeling in the rostralmost sector of area 46v (area 46vr) and the caudal sector of dorsal area 46 (area 46dc); moreover, both sections are densely linked to the prefrontal area12r, to hand/mouth-related (F5a, 44) ventral premotor areas, area SII, insula and cingulate area 24. The rostral 46vc/convexity is even connected to the hand-related area AIP, and weakly to the premotor areas F5p and F5c. In addition, the rostral 46vc/bank is also connected to the prefrontal areas 45A and 45B, hand/arm-related parietal areas PFG and PG, to PGop, and to area 11 and the cingulate area 24.

Finally, injections of tracers in area 46vr revealed a dense intraprefrontal connectivity. Area 46vr is richly connected to the ventral areas 46vc and 12r, and to the dorsal areas 9/8b and 46d; in the orbitofrontal cortex, strong connections are found with area 11. Outside the PFC, it is connected with IPa and Tea/m of the STS, and with the area 24 in the cingulate cortex.

The present data, showing distinct connectivity patterns, suggest a differential involvement of different parts of area 46vc in high level integration for oculomotor behaviour and goal-directed arm, hand, and mouth actions. Taking into consideration the crucial role of the PFC in cognitive functions, in particular in action planning and behavioural organization, it comes as no surprise that this region is massively connected with a wide variety of cerebral structures, both cortical and subcortical, implicated in sensory and motor processing.

1.2 Functional properties of ventrolateral prefrontal neurons

Until a few decades ago, frontal lobes were considered “silent areas” of the brain, since lesions in these regions do not produce immediately noticeable changes of overt behaviour, in contrast to what happens when other cerebral areas are injured. Patients with damage confined to the prefrontal cortex typically present intact perceptual ability and general motor behaviour, and may perform standard results on intelligence tests, yet they are unable to function effectively in daily life. Overall, several investigations on lesions and electrophysiological studies have shown that the LPFC is involved in distinct aspects of behaviour as sensory, motor, visceral/emotional and executive processes (Jacobsen, 1935; Fuster, 2015). Toward the end of the 20th century, the primary research interests concerning LPFC examined the attentional functions (Desimone and Duncan, 1995) and the processing of information stored in short-term memory (Baddeley, 1986; Goldman-Rakic, 1987), providing additional evidence to the ability of this area in keeping information temporarily in mind to perform delayed tasks (Jacobsen, 1936).

The LPFC includes at least three different functional domains: one located in correspondence of the anterior bank of the arcuate sulcus (AS) and involved in oculomotor functions in the frontal eye field (Bruce and Goldberg, 1984), a second one close and within the PS, involved in visuo-spatial information processing (Levy and Goldman-Rakic 1999), and a third one located in the region of the inferior prefrontal convexity and involved in high-order processing of non-spatial information (Levy and Goldman-Rakic, 1999; Passingham et al., 2000; Romanski et al., 2004).

Raos and co-workers (1997) showed how “what”- and “where”-tuned neurons are widely represented in the dorsolateral prefrontal cortex (DLPFC), and that a significant number of registered neurons code both information, suggesting the capability of the integration of object and spatial information at the single neuron level.

Based on a series of lesion studies in monkeys (Petrides, 1991) and of brain imaging studies in humans (Petrides et al., 1993), Petrides (1995) proposed that the DLPFC is involved in the monitoring and manipulation of retrieved information for planning and execution of the behaviour. The ventral sector of the LPFC, in contrast, was proposed to be involved in active encoding and retrieval of specific information held in visual, auditory and somatosensory association areas, allowing selection, comparison and decision processes based on such information (Tanji and Hoshi, 2008). In the 1960s, a great number of investigations provided electrophysiological evidence for a role in the integration of sensorial inputs (see Fuster, 2015): neurons of monkey PFC react to visual, auditory, somatic, olfactory and gustatory stimuli according to anatomical connections.

Tanila and co-workers (1993) systematically mapped the representations of visual, auditory, somatosensory, somatomotor and oculomotor functions on the DLPFC. The neurons were functionally classified according to their responsiveness to visual, auditory and somatosensory stimulation and to correlation of their activity with spontaneous eyes or limbs movements. Visually responsive neurons were broadly distributed throughout the DLPFC, well in agreement with the extensive connections between this cortical area and the visual ones. Of all visually responsive neurons, less than half responded preferably to moving stimuli and were located more dorsally and caudally than the rest of the visually responsive neurons. Somatosensory and motor neurons were located more ventrally, but no clear somatotopy could be described. Oculomotor neurons were found caudally, in both the superior and inferior banks of the PS and in a narrow band on the dorsal convexity, corresponding with the projections fields of posterior parietal areas.

Besides this evidence of a somehow “basic” encoding of sensory and motor properties, many studies support an integrative role of stimuli processing in the PFC rather than a simple reflection of sensory information per se. In particular, the VLPFC, given its connections with the temporal lobe, can use information about the visual and auditory context to generate more complex behaviours, as rule encoding, categorization or action selection.

Hoshi and co-workers (1998) carried out an experiment to study movement-related neuronal activity in the dorsal and ventral convexity of PS, from the perspective of a general role for the PFC in controlling motor behaviour to achieve a specific goal according to the requirements of a given task. Monkeys were trained to perform two delayed motor tasks depending on a choice: many of the recorded neurons were selectively active depending on the target, while activity of many other recorded neurons depended on the type of task required (matching the target shape or its location). These data suggest that activity of PF neurons reflects task rule and not movement per se.

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

In another experiment, Saga and co-workers (2011) investigated the role of the LPFC in transforming external signals of multiple sensory modalities (visual, auditory or tactile) into information suitable for monitoring successive events across behavioural phases until an intended action is prompted and started. Thus, animals had to receive a series of signals, each one in a in a single sensory modality, and to respond by releasing a button when the fourth signal appeared. They found that the initial, short latency responses of recorded neurons reflected mainly the sensory modality rather than the phase or progress of the task. However, a task phase-selective activity was updated with the appearance of each cue until the planned action was initiated and reflected not merely a particular phase of the task but also multiple successive phases. Furthermore, they found combined representations of task phase and sensory modality in the activity of individual neurons, suggesting how information representing multiple phases of behavioural events develops in the LPFC to provide a basis for the temporal regulation of behaviour.

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

An interesting recent and more controlled study (Simone et al., 2015) investigated the role of VLPFC in grasping actions in four different contextual conditions: with and without visual control of hand-object interaction, memory-guided and, finally, by means of a naturalistic paradigm, namely in absence of abstract learned rules. The findings indicated the presence of a population of VLPF neurons that are active during the execution of reaching-grasping goal-directed actions in a wide range of contexts. Arguably, this neuronal population plays a role in controlling goal-directed grasping actions in several contexts on the basis of contextual information, providing a representation of the motor goal of the intended action.

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

1.4. Aims of the studies

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

The ventrolateral prefrontal cortex may play this role. However, almost all experiments previously described rely on cognitive capacities that do not appear to have an immediate correspondence among the relatively simple primates' behaviour in the wild. In contrast, a few studies investigated the role of the prefrontal cortex adopting a more naturalistic approach (Tanila et al., 1993; Simone et al., 2015), but it seems appropriate to examine in a closer way the issue of what could be the contribution of this cortical sector to the organization of natural actions.

To this purpose, we investigated the activity of VLPF single neurons by employing a paradigm similar to the one used in studies on parietal (Fogassi et al., 2005) and premotor neurons (Bonini et al., 2010, 2011), but strictly behaviourally controlled. In this study, we were able to both evaluate and clarify the processing and integration of distinct contextual (visual and auditory) information in order to select the simple and complex forthcoming action to perform and to examine the possible presence of goal-related activity in this portion of cortex. Moreover, we performed an observation task to clarify the possible contribution of VLPF neurons to the understanding of others' goal-directed actions.

2. STUDY ONE

2.1. INTRODUCTION

Contextual information available in the environment, and the internal plans and goals of an agent constitute the two poles of a continuum along which the brain orchestrates action selection in natural contexts (Koechlin and Summerfield, 2007). Most of the existing literature on high order action selection focused on the PFC (Tanji and Hoshi, 2008), and the bulk of the evidence have been collected with arm-reaching or oculomotor tasks (Rainer et al., 1998; Freedman et al., 2001; Wallis et al., 2001; Shima et al., 2007). These studies provided crucial contributions to clarify both the mechanisms underlying PFC executive functions and the possible evolutionary trends leading to the origin of uniquely human cognitive capacities (Wise, 2008; Genovesio et al., 2014). However, it remains unclear whether and to which extent the PFC is also involved in manipulative hand behaviours, such as reaching-and-grasping actions, in which motor and sensory brain regions located caudally to PFC have been shown to play a major role (see Cisek and Kalaska, 2010; Rizzolatti et al., 2014).

Recent neurophysiological studies in monkeys (Fogassi et al., 2005; Bonini et al., 2010, 2011, 2012) demonstrated that IPL and vPM areas underlie the organization of forelimb action sequences as those commonly performed by monkeys in their natural environment (e.g. grasping an object to eat it or to place it).

In particular, Fogassi and co-workers (2005) tested PFG grasping neurons in two main conditions, namely, grasp-to-eat and grasp-to-place. In the grasp-to-eat condition, the monkey reached and grasped a piece of food located in front of it and brought it to the mouth, while in grasp-to-place condition it reached and grasped an object and placed it into a container in order to receive a food reward. Note that, although in both conditions the motor act the monkey had to perform was the same (i.e. grasping), part of grasping neurons discharged differently depending on the type of action. Control experiments demonstrated that neuronal selectivity could neither be accounted for by different kinematics associated to the two actions, nor by the type of target grasped, and regardless the type of the reward obtained by the monkey, enabling to conclude that the crucial factor for determining the observed selectivity was the final goal of the action. Similar experiments have been also carried out in the vPM area F5 (Bonini et al., 2010, 2011), showing that, although the parietal cortex plays a leading role in coding motor acts depending on the action goal, also F5 grasping neurons can reflect the goal of the action in which the coded act is embedded. These studies showed that IPL and vPM neurons, besides primarily encoding specific motor acts (e.g. grasping), discharge differently during grasping execution depending on the monkey's final behavioural goal (i.e. eating or placing the grasped object), thus showing the capacity of predictive goal coding.

This capacity crucially relies on the availability of context-depending stimuli (Bonini et al., 2011), whose processing is considered a main function of the VLPFC (see Fuster, 2015), which in turn can influence parietal and premotor neurons activity through its well documented reciprocal connections with these regions (Petrides and Pandya, 1984; Barbas and Mesulam, 1985; Borra et al., 2011; Gerbella et al., 2013a). However, the possible contribution of prefrontal neurons to the processing of contextual information relevant for the organization of simple manual actions on solid objects has never been investigated.

To address this issue, we recorded single VLPF neurons activity by employing a modified, and more strictly controlled, go/no-go version of the grasp-to-eat/grasp-to-place paradigm used in previous studies on parietal and premotor neurons. With this task, we have been able to segment and independently evaluate the processing and integration of the visual and auditory contextual cues allowing the monkey to make a decision on whether or not to act and what action to perform.

2.2. MATERIALS AND METHODS

The experiments were carried out on two 4 years old female *Macaca mulatta* (M1, 3 Kg; M2, 4 Kg). Before starting the recording sessions, each monkey was habituated to sit in a primate chair, to interact with the experimenters and to become familiarized with the experimental setup. Then, they were trained to perform the task described below using the hand (right) contralateral to the hemisphere to be recorded (left; see Figure 2A). At the end of training period, a surgery was performed in order to implant a head fixation system (Crist Instrument, Hagerstown, MD) and, furthermore, a plastic recording chamber (18x18mm, AlphaOmega Engineering, Nazareth, Israel) based on the stereotaxic coordinates of the cortical regions to be recorded, derived from previously obtained functional magnetic resonance images of each monkeys' brain.

All surgeries were performed under general anaesthesia (ketamine hydrocloride, 5 mg/Kg intramuscular [i.m.] and medetomidine hydrocloride, 0.1 mg/Kg i.m.). Dexamethasone, prophylactic broad-spectrum antibiotics as well as appropriate pain medications were administered intra- and postoperatively (Borra et al., 2011; Bonini et al., 2010). All the experimental protocols complied with the European law on the humane care and use of laboratory animals (directives 86/609/EEC, 2003/65/CE, and 2010/63/EU), they were authorized by the Italian Ministry of Health (D.M. 294/2012-C, 11/12/2012), and approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12, 17/07/2012).

2.2.1. Apparatus and behavioural paradigm

The monkeys were trained to perform a sensory-cued go/no-go action sequence task, requiring them to grasp a target in order to eat it or to place it into a container. A success rate of at least 80% correctly performed trials per session was adopted as a criterion for training completion. The task included different experimental conditions, similar to those previously employed for experiments carried out on other cortical areas (Fogassi et al., 2005; Bonini et al., 2010). Nevertheless, the apparatus (Figure 2B) and the behavioural paradigm (Figure 2C) here employed enabled to more carefully manipulate and control the contextual elements crucial for action selection and decision.

The monkey was seated on a primate chair in front of a black plexiglas box, divided horizontally into two sectors by a half-mirror. The lower sector hosted two plastic containers: an empty plastic jar (inner diameter 4 cm) and the other used to present the monkey with one of two possible targets, namely, a piece of food (ochre spherical pellet of 0.6 cm of diameter, weight 19 mg), or an object (a white plastic sphere, of the same size, shape and weight of the food). On the basis of the colour, the monkey could easily discriminate the type of target. The target was located into a groove at the center of the container: the bottom of the groove was closed with a computer-controlled trap-door with a cavity in the middle that enabled the precise positioning of the target, so that its center of mass was at exactly 11 cm from the lower surface of the half mirror. The target was positioned at the center of the groove in complete darkness, and in presence of a constant white noise, in order to prevent the monkey from obtaining any visual or auditory cues during set preparation. The container holding the target was positioned along the monkey body midline, at 16 cm from its hand starting position. The monkey's hand starting position was constituted by a metal cylinder (diameter 3 cm, height 2.5 cm), fixed to the plane close to the monkey's body. The empty plastic jar, used as a container for placing the object, was located at the halfway point between the hand starting position and the target. The jar was endowed with a funnel-shaped pierced bottom: in this way, when the object was placed into the jar, it immediately felt down in a box unreachable and not visible by the monkey.

The upper sector of the task box hosted a small black tube fixed to the roof, containing a white light-emitting diode (LED) located 11 cm above the surface of the half mirror. When the LED was turned on (in complete darkness), the half-mirror reflected the spot of light, so that it appeared to the monkey as located in the lower sector, in the exact position of the center of mass of the not-yet-visible target (fixation point).

A stripe of white LEDs located on the lower sector of the box (and not directly visible to the monkey) allowed us to illuminate it during specific phases of the task. Note that, because of the half-mirror, the fixation point remained visible even when the lower sector of the box was illuminated.

Simple Visuo-Motor Task (sVMT)

The task was run in two modes, depending on the order of presentation of the auditory and visual cues (Figure 2C): in the Visual Decision mode (VDm), the cue sound was presented first and then the target became visible, while in the Auditory Decision mode (ADm) the target was presented first and the cue sound subsequently. In both modes, the task included two cue sounds (go/no-go) and two targets (food or object), thus resulting in a total of 8 different conditions, randomly interleaved, each of which recorded in 12 independent trials (96 trials in total).

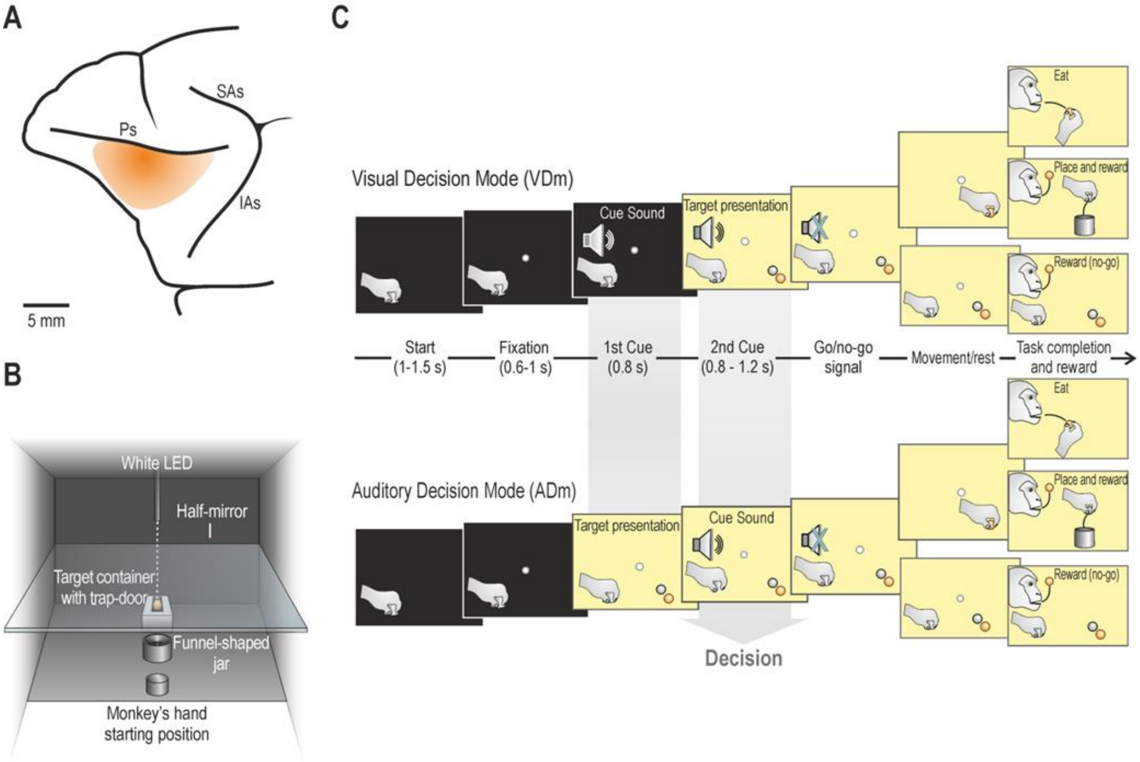

Figure 2. Recorded region, apparatus, and temporal sequence of task events. ***A***. Schematic view of the VLPF region investigated in the present study. Ps, principal sulcus; SAs, superior arcuate sulcus; IAs, inferior arcuate sulcus. ***B***. Box and apparatus settled for carrying out the action sequence task, seen from the monkey point of view. ***C***. The task was constituted by a fixed sequence of events that could be run in two modes, depending on the order of presentation of the two cues (sound and target) whose integration allowed the monkey to decide what to do next, namely, 1) grasp-to-eat, 2) grasp-to-place, 3) refrain from grasping an object, or 4) refrain from grasping a food pellet. The monkey could select what to do next (decision) either following the visual presentation of the target (in the VDm), or following the presentation of the cue sound (in the ADm).

Each trial started in a completely dark environment, when the monkey held its hand on the starting position for a variable interval of time, ranging from 1 to 1.5 s (inter-trial period). The temporal sequence of task events (see Figure 2C) was as follows.

Go condition in VDm. Following presentation of the fixation point, the monkey was required to start fixating it (tolerance window 3.5°) within 1.5 s. After a variable time lag from fixation onset (0.6-1 s), the first cue - a high tone constituted by a 1200 Hz sine wave, associated with go trials - was presented. After 0.8 s the second cue was provided: the lower sector of the box was illuminated, and one of the two possible targets (food or object) became visible (target presentation). Then, after a variable time lag (0.8-1.2 s), the cue sound ceased (go signal), and the monkey was required to reach and grasp the target within 1.2 s from the end of the sound: in case of the food pellet (Food trials), the monkey brought it to the mouth and ate it (grasp-to-eat), while in case of the plastic sphere (Object trials), the monkey had to place it into the jar (grasp-to-place). An almost natural bring-to-the mouth movement was possible in spite of the presence of the half-mirror (see Figure 1B) because its border did not interfere with the insertion of the food into the mouth. Correctly performed Food trials were self-rewarded by the brought-to-the-mouth food pellet, while correctly performed Object trials were automatically rewarded with a food pellet (identical to the one used during Food trials) delivered into the monkey’s mouth by a customized, computer-controlled pellet dispenser (Sandown Scientific, Hampton, UK), activated by the contact of the monkey’s hand with the metallic border of the jar. The pellet reward contacted the monkey’s mouth 500ms after its delivery.

No-go condition in VDm. The temporal sequence of events in this condition was the same as in the *go* condition. Following presentation of the fixation point, the monkey was required to start fixating it within 1.5 s. After a variable time lag from fixation onset (0.6-1 s), the first cue - a low tone constituted by a 300 Hz sine wave, associated with no-go trials - was presented. After 0.8 s the second cue was provided: the lower sector of the box was illuminated, and one of the two possible targets (food or object) became visible (target presentation). Then, after a variable time lag (0.8-1.2 s), the cue sound ceased (no-go signal), and the monkey had to remain still with the hand on the starting position, without performing any movement, maintaining fixation for 1.2 s, during both Food and Object trials. After correct task accomplishment, the monkey was automatically rewarded with a food pellet as described above for Object trials.

In the ADm, the temporal sequence of events in the two conditions was the same as in the VDm, but the order of presentation of the two cue stimuli was inverted: in this way, target presentation occurred before any go/no-go instruction was provided, while the go/no-go cue subsequently presented enabled the monkey to decide whether to act or not. Importantly, in both the ADm and VDm, only the second cue allowed the monkey to make the final decision on what to do next, regardless of its sensory modality, by integrating the information conveyed by it with that previously provided.

All the trials of the experimental session were run based on a list organized in a random order. All the trials in which the monkey broke fixation made an incorrect movement or did not respect the time intervals of the task stages were aborted. In all these cases, the trap-door below the target was automatically opened so that the target felt down and became unreachable by the monkey. The incorrect trials were not rewarded and re-presented until the list was completed and 96 correct trials acquired.

2.2.2. Recording techniques

Recording of neuronal data

Neuronal recordings were performed by means of 8 and 16 channels multielectrode linear arrays: U-probes (Plexon, Dallas, TX) and silicon probes developed in the EU project NeuroProbes (Ruther et al., 2010; Herwik et al., 2011), distributed by ATLAS NeuroEngineering (Belgium), respectively. Both types of probes were inserted through the intact dura by means of a manually driven stereotaxic micromanipulator mounted on the recording chamber (AlphaOmega, Nazareth, Israel). All penetrations were performed perpendicularly to the cortical surface, with a penetration angle of approximately 40° relative to the sagittal plane.

The recordings were performed by means of a 8 channels AlphaLab system (AlphaOmega, Nazareth, Israel), and of a 16 channels Omniplex system (Plexon, Dallas, Texas). The wide band (300-7000 Hz) neuronal signal was amplified and sampled in parallel with the main behavioural events and digital signals defining the task stages. The collected raw data were then sorted off-line by means of commercial spike sorting software (Plexon).

Recording of the behavioural events and definition of epochs of interest

Distinct contact-sensitive devices (Crist Instruments, Hagerstown, MD) were used to detect when the monkey touched the metal surface of the starting position, the metallic floor of the groove hosting the target (food or object) during grasping, and the metallic border of the plastic jar during placing of the object. Each of these devices provided a TTL signal, which was used by LabView-based software to monitor the monkey/experimenter performance.

Eye position was monitored by an eye tracking system composed by a 50Hz infrared CCD video camera (Ganz, F11CH4) and two spots of infrared light projected on the monkey eye. The analog signal related to horizontal and vertical eye position was fed to a computer equipped with dedicated software (Pupil), enabling calibration and basic processing of eye position signals. The eye position signal, together with the TTL events generated during task execution, were sent to the LabView software to monitor task unfolding and to control the presentation of auditory and visual cues of the behavioural paradigm. Based on TTL and eye position signals, the software enabled us to automatically interrupt the trial if the monkey broke fixation, made an incorrect movement or did not respect the temporal constrains of the behavioural paradigm. In all these cases, no reward was delivered: all the cues were switched off and, at the same time, the trap-door bearing the target opened so that the monkey could not grab it. Note that the monkey always received the same food pellet as a reward after correct accomplishment of each type of trial.

Based on the digital signals related to the main behavioural events, we defined different epochs of interest for statistical analysis of neuronal responses: (1) sensory cue (target presentation/cue sound) epoch, including the 400 ms after the onset of each cue; (2) movement epoch, ranging from 100 ms before the detachment of monkey's hand from the starting position to 100 ms after the contact with the target; (3) resting epoch, ranging from 0 ms to 400 ms after the end of the low sound (no-go signal). In order to assess whether neurons significantly responded to sensory cues, we compared the activity during the sensory cue epoch with that of the 500 ms preceding the onset of the cue (baseline epoch).

2.2.3. Data analyses and classification of recorded neurons

Single units were isolated using standard principal component and template matching techniques, provided by dedicated offline sorting software (Plexon).

After identification of single units that remained stable over the entire duration of the experiment, neurons were defined as "task-related" if they significantly varied their discharge during at least one of the epoch of interest, investigated by means of the following repeated-measures ANOVAs (significance criterion of $p<0.01$).

Sensory response to the first cue: the presence of responses related to the presentation of sounds (low and high tone) and target objects (food and sphere) as first cue, were assessed with a 2x2 repeated measures ANOVA (factors: Sound/Target, Epoch) followed by Bonferroni post-hoc tests in case of significant interaction effects. Neuronal activity during the cue presentation epoch was compared with that of the baseline epoch.

Sensory response to the second cue: since each stimulus (sound or target) presented as second cue occurred within the context established by the previously presented one, we employed 2x2x2 repeated measure ANOVA (factors: Sound, Target, Epoch), followed by a Bonferroni post-hoc tests in case of significant interaction effects, to explore not only possible activity changes induced by the cue, but also possible differences in stimulus processing caused by the context in which it occurred. The same analysis was applied to the neuron response tested in the ADm and VDm, separately. In order to verify a possible activity change specifically induced by the second cue, neuronal activity during cue presentation was compared with that of the 500 ms period before stimulus onset. Since when a stimulus was presented as second cue it could be influenced by the previously presented one, while this did not occur when it was presented as first, we compared the neurons response evoked by stimulus presentation in the cued and un-cued contexts by employing paired samples t-test ($p<0.05$,Bonferroni corrected).

Response during the movement/resting epoch: the possible modulation of single neuron activity during the movement/resting epoch (in the go and no-go condition, respectively) has been assessed by means of 2x2x2 repeated-measures ANOVA (factors: Target, Condition, Epoch). In addition to the movement/resting epoch defined above, here we considered as baseline the 500 ms epoch preceding the presentation of the first sensory cue. We considered as motor-related all the neurons showing significant interaction effects ($p<0.01$, followed by Bonferroni post-hoc tests) of the factors Condition and Epoch, with possible additional interaction with the factor Target.

Population analyses: based on the results provided by the analyses described above, task-related neurons were classified as sensory-driven (activated only by some sensory cue), motor-related (activated only during the movement/resting epoch), or sensory-and-motor (activated significantly during both sensory cue and movement/resting epochs). Population analyses were performed on specific sets of neurons, classified based on the results of the above described analyses, and taking into account single neuron responses calculated as averaged activity (spk/s) in 20 ms bins across trials of the same condition. The same epochs employed for single unit data were used for population analyses as well, except for motor-related responses (analysed on a trial-by-trial basis in single neurons), which have been analysed considering a 400 ms epoch ranging from 300 ms before hand-target contact to 100 ms after this event.

In order to identify the start/end of population selectivity for specific variables (i.e. target or condition), paired samples t-test were used to establish the first/last of a series of at least 5 consecutive 80 ms bins (slid forward in steps of 20 ms) in which the activity significantly differed (uncorrected $p<0.05$) between the two compared conditions.

2.3. RESULTS

We recorded 403 task-related neurons from the VLPFC of the left hemisphere of two monkeys (251 from M1 and 152 from M2), during execution of sVMT. According to the criteria described above (see Materials and Methods), almost half of them ($N=186$, 46.2%) have been classified as “sensory-driven” (127 in M1, 59 in M2), 84 (20.8%) “motor-related” (47 in M1 and 37 in M2), and 133 (33.0%) “sensory-and-motor” (77 in M1, 56 in M2) (see Figure 3A and Table 1). Figure 3B reproduces the anatomical location of the two investigated regions: it is clearly shown that, in both monkeys, sensory-driven, motor-related, and sensory-and-motor neurons were not anatomically segregated in the explored VLPF region.

	Response to sensory cues			No sensory response	Total
	Auditory	Visual	Auditory and visual		
Sensory-driven neurons	6	145	35	0	186
Motor-related neurons	0	0	0	84	84
Sensory-and-motor neurons	6	85	42	0	133
Total	12	230	77	84	403

Table 1. Number of sensory-driven, motor-related and sensory-and-motor neurons responding to visual and auditory cues presented in both task modes (VDm and ADm).

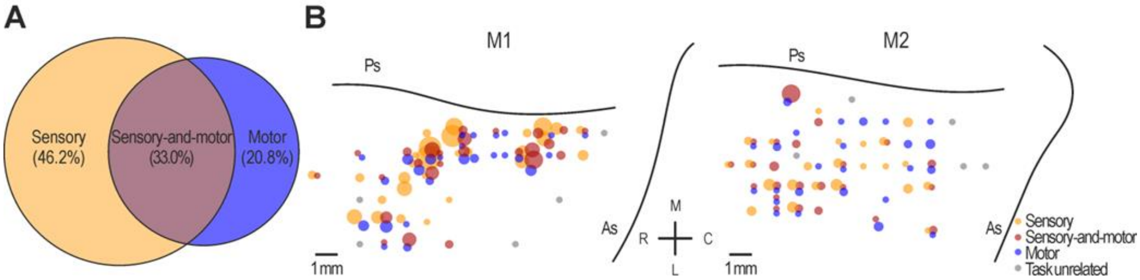

Figure 3. Types and anatomical distribution of task-related and task-unrelated neurons. ***A***. The Venn charts represent the proportion of task-related neurons classified as sensory, motor, or sensory-and-motor. ***B***. Reconstruction of the anatomical distribution of sensory, motor, and sensory-and-motor neurons in the explored VLPF sector of both monkeys (M1 and M2): the size of each circle is proportional to the number of single neurons (from N=1 to N=18) isolated in each penetration, and characterized by the property indicated by the colour code. Grey circles indicated penetrations in which neurons did not show task-related responses. Ps, principal sulcus; As, arcuate sulcus; M, medial; L, lateral; R, rostral; C, caudal.

In the following sections the properties of these three main neuronal categories will be described in detail.

2.3.1. Sensory-driven neurons

The majority of sensory-driven neurons (151/186, 81.2%) responded to the presentation of cue stimuli in only one of the two tested sensory modalities, namely, auditory or visual: in this section, we will focus on the description of this set of neurons. The remaining sensory-driven neurons (35/186, 18.8%) responded to both the cue sound and the visually presented target, and their properties will be described in a dedicated section on integrative multisensory responses.

Neurons responding only to auditory cues were rarely found (N=6). Two of them showed task-specific activation, responding to the sound only when it was presented as first (in the VDm), while the remaining 4 responded to cue sounds during both task modes in a similar way, as one would expect in the case of a purely auditory response (Figure 4A, Neuron 1).

In contrast to auditory-responsive neurons, those discharging only during visual presentation of the target were widely represented (N=145): 30 of them (20.7%) discharged in a similar way regardless of the context in which target presentation occurred, while the great majority (115/145, 79.3%), although selectively activated during visual presentation, discharged differently depending on the previously presented auditory instruction (see Table 2). Indeed, none of these neurons discharged stronger in the ADm, in which the target was presented as first cue, devoid of association with any previous instruction stimulus, while 95 neurons discharged stronger (N=86) or even exclusively (N=9) in the VDm, when target presentation followed the go/no-go cue. The remaining 20 neurons activated similarly in the two task modes, but in the VDm their visual response was always different depending on the previously presented cue sound (go/no-go).

	Go > No-go			No-go > Go			Go = No-go			Total
	Food	Object	Ns	Food	Object	Ns	Food	Object	Ns	
VDm>ADm	3	14	19	3	3	13	2	6	32	95
VDm=ADm	1	4	13	0	1	1	0	0	30	50
Subtotal	4	18	32	3	4	14	2	6	62	
Total		54			21			70		145

Table 2. Task mode (VDm/ADm), condition (go/no-go), and target (food/object) selectivity of visually-responsive neurons.

Examples of contextual selectivity of visually responsive neurons are shown in Figure 4A. Neuron 2 discharged stronger in the VDm than in the ADm, but its discharge did not encode any of the specific aspects of the task (go/no-go condition or type of target). Thirty-two neurons showed this behaviour (see Table 2). Neuron 3 responded stronger in the VDm than in the ADm, particularly during go-trials, but also showed a clear selectivity for the food item. Three neurons showed a similar behaviour (see Table 2). Finally, Neuron 4 responded selectively to the visual presentation of the object during go-trials in the VDm. This pattern of discharge is representative of 14 of the recorded neurons (see Table 2). Note that neurons activated differently during go and no-go trials also show target selectivity more often (29/75) than the other visually-responsive neurons (8/70, Fisher's exact probability test *p*=0.0001).

In order to better understand the possible interaction between target selectivity and the different task contexts, we focused on those neurons showing both target selectivity and a preference for one of the two (go/no-go) conditions (N=29). Then, we performed a population analysis in which we compared visual responses to the preferred (red line) and not preferred (black line) target (food N=7, object N=22) in three different contexts, namely: 1) instructed by the preferred cue sound, 2) instructed by the not preferred cue sound, or 3) not instructed by any previously presented sound in the ADm (Figure 3B). A 3x2x2 repeated measures ANOVA (factors: Context, Object and Epoch) revealed significant main effects of the factors Context [F(2,56)=18.02, *p*<0.001], Object [F(1,28)=57.17, *p*<0.001], and Epoch [F(1,28)=49.69, *p*<0.001], as well as a significant interaction of all factors [F(2,56)=36.16, *p*<0.001]. Bonferroni post-hoc tests indicated that the population activity during target presentation in the preferred (go/no-go) condition was stronger relative to that during all the other epochs (*p*<0.001). In addition, we found a clear-cut target preference in the preferred context (*p*<0.001), a barely significant one in the un-cued context (p<0.05), and no preference in the not preferred context (*p*=0.30). These findings indicate that the discharge of VLPF visually-responsive neurons is not simply related to the sight of a given object, but it is strongly modulated by previously available contextual information allowing the designation of visually presented objects as potential targets of forthcoming behaviours.

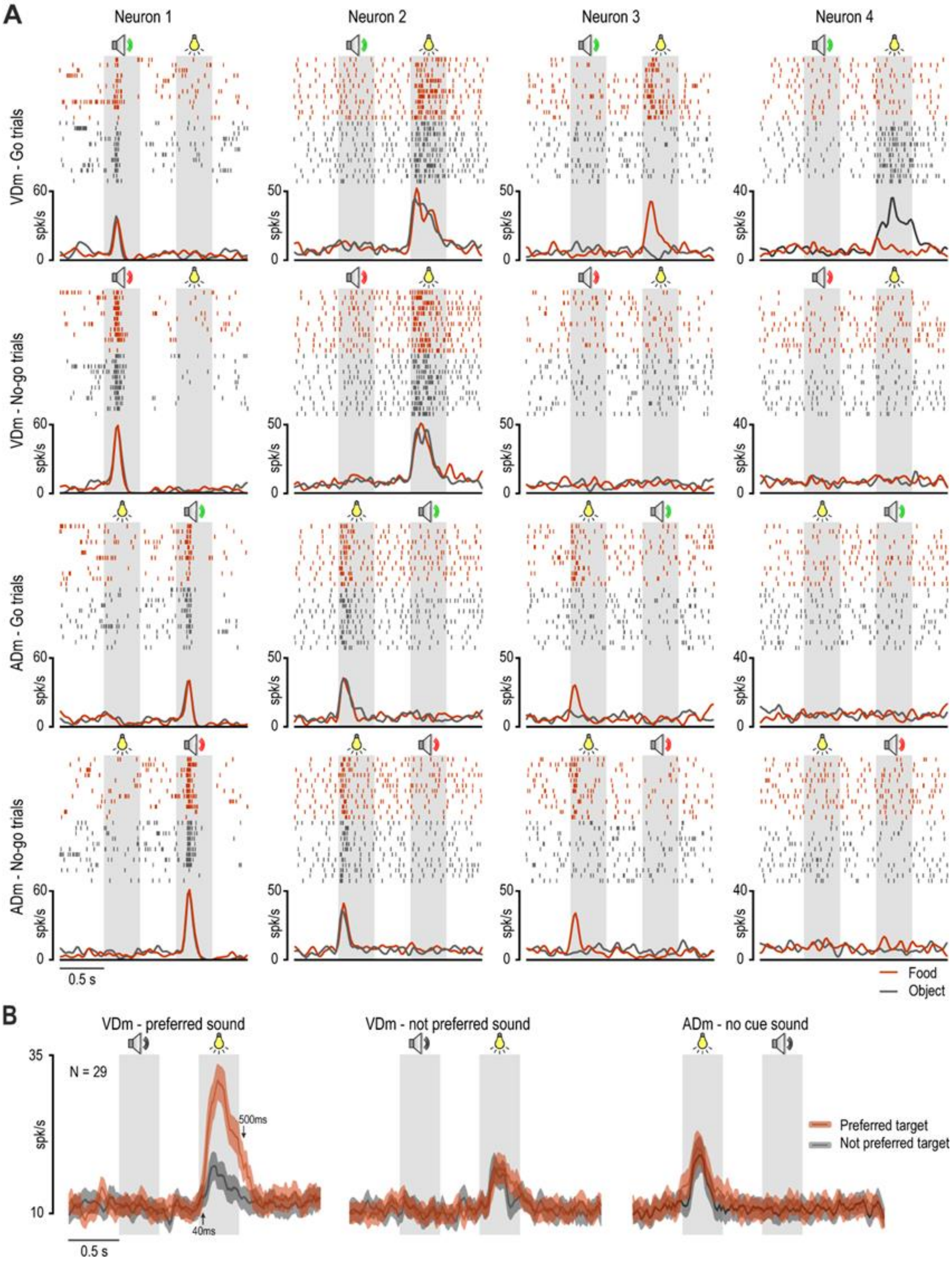

Figure 4. Examples of sensory-driven neurons and population responses. ***A***. Examples of four different sensory-driven neurons. Rasters and histograms are aligned on target presentation and cue sound onset, which were separated by a fixed interval of 800 ms. Rasters and histograms of single neuron response with different targets are shown in different colours. The grey shaded areas indicate the 400 ms time windows used for statistical analysis of neuronal sensory responses during (symbols) 1) target presentation (light bulb), 2) high cue tone (green speaker), and 3) low cue tone (red speaker). ***B***. Population activity of 29 visually-responsive neurons showing selectivity for both condition (go/no-go)

and target (food/object). The discharge of the same neuronal population is shown during target presentation cued by the preferred and not preferred sound in the VDm, as well as before the presentation of the cue sound, in the ADm. The grey speaker symbol identifies the epoch in which the cue sound was presented.

2.3.2. Motor-related neurons

Neurons classified as motor-related (N=84) could activate either during the movement epoch of the go condition (72/84), or during the corresponding epoch of the no-go condition (12/84), in which the monkey was required to refrain from moving (see Table 3). All these neurons did not respond during any of the previous cue epochs in both task modes.

	Target selectivity			
	Food > Object	Object > Food	Food = Object	Total
Go > No-go	14	23	35	72
No-go > Go	2	4	6	12
Total	16	27	41	84

Table 3. Selectivity for target and/or go/no-go condition of motor-related neurons.

Examples of motor-related neurons are shown in Figure 5A. Neuron 1 discharged during reaching-grasping actions regardless of the type of target (food or object), while it did not activate during no-go trials. Of the 72 motor-related neurons discharging specifically during go-trials, 35 displayed this type of activation pattern, while the remaining 37 neurons showed target selectivity (see Table 3). Neuron 2 provides an example of target-selective response: it discharged stronger during grasping of the food relative to the object, and it did not activate during no-go trials. Neuron 3 exhibited the opposite behaviour, becoming active selectively during grasp-to-place actions.

It might be interesting to note that target selectivity was overall much more represented in motor-related (43/84, 51.2%) than sensory-driven (37/115, 32.2%)

neurons (Fisher’s exact probability test, *p*=0.0053), suggesting that it more strictly depends on the behavioural relevance of the target than on its perceptual features.

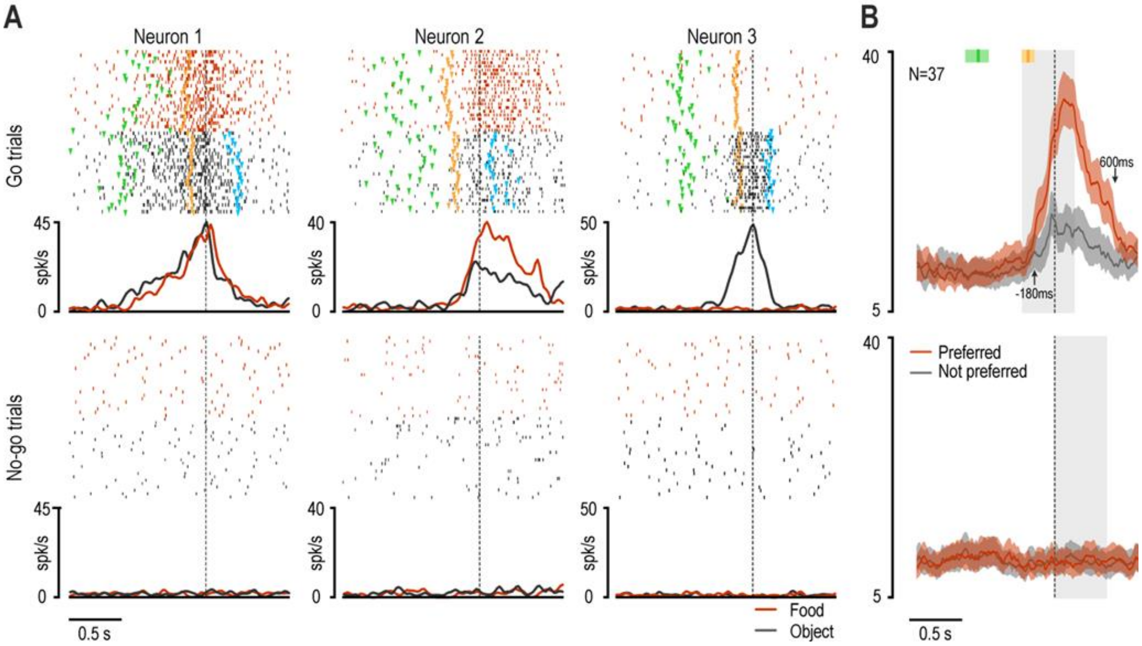

Figure 5. Examples of motor-related neurons and population responses. ***A***. Examples of three motor-related neurons. Raster and histograms of go trials are aligned on the hand-target contact (grasping), while those of no-go trials are aligned on the no-go signal. Markers colour code: green, go-signal; orange, detachment of monkey’s hand from the starting position (movement onset); light blue, contact of the monkey’s hand with the border of the jar (placing). ***B***. Population activity of all motor-related neurons with target selectivity. The red and grey lines indicate the average discharge intensity of neurons during grasping of the preferred and not preferred target, respectively, aligned as the single neurons example in A. The coloured shaded regions around each line indicate 1 SEM. The grey shaded regions indicate the windows used for statistical analysis of the population response. The median times of go-signal onset and movement onset are indicated with the green and orange markers, respectively, above each population plot. Shaded areas around each marker represent the 25th and 75th percentile times of other events of the same type. The black arrows indicate the time of onset (upward arrow) and end (downward arrow) of significant separation between the two compared conditions.

At the population level, the response of motor-related neurons endowed with target selectivity (Fig. 5B) clearly showed a peak at 100 ms after hand-target contact, thus being tuned on target acquisition. Furthermore, although most of the difference between the preferred and not preferred response concerned the post-contact epoch, in line with previous studies with similar paradigms in different cortical areas (Bonini et al., 2010, 2012), target selectivity emerged since 180 ms prior to hand-target contact, allowing us to exclude that it depended on different sensory feed-back from the grasped objects (Tanila et al., 1992).

2.3.3. Sensory-and-motor neurons

Sensory-and-motor neurons responded during the motor epoch of the task as well as during the presentation of cue stimuli in only one (91/133, 68.4%) or both (42/133, 31.6%) the two tested sensory modalities (auditory and visual). In this section we will focus on the description of the former set of neurons, while the properties of those responding to both the cue sound and the visually presented target will be described (together with those of sensory-driven neurons with the same feature) in the subsequent section on integrative multisensory responses.

A few unimodal sensory-and-motor neurons (N=6) were activated by the auditory cue, while the majority (N=85) responded to target presentation (see Table 4).

	Go > No-go			No-go > Go			Go = No-go			Total
	Food	Object	Ns	Food	Object	Ns	Food	Object	Ns	
VDm>ADm	11	16	15	0	0	1	0	0	15	58
VDm=ADm	5	8	9	0	0	4	0	0	1	27
Subtotal	16	24	24	0	0	5	0	0	16	
Total		64			5			16		85

Table 4. Task mode (VDm/ADm), condition (go/no-go), and target (food/object) selectivity of the visual response of sensory-and-motor neurons.

Examples of this latter type of sensory-and-motor neurons are shown in Figure 6. Neuron 1 responded to target presentation, particularly when it occurred after the go cue in the VDm, as well as during grasping execution: a preferential discharge during Food trials was evident during both target presentation (p=0.04, Bonferroni corrected) and grasping execution (p<0.001). Neuron 2 exemplifies the opposite target preference: it responded selectively to the presentation of the object following the go cue in the

VDm, and it also fired with remarkable preference during the execution of grasp-to-place, since the earliest phase of movement onset. Both these example neurons showed transient activation during specific phases of the task. In contrast, Neuron 3 responded to the visual presentation of the target during both the VDm and the ADm: it did not exhibit selectivity neither for the type of target nor for the go/no-go condition, but it showed sustained activation from target presentation till the end of the trial, with further significant increase of its firing rate following the go relative to the no-go signals.

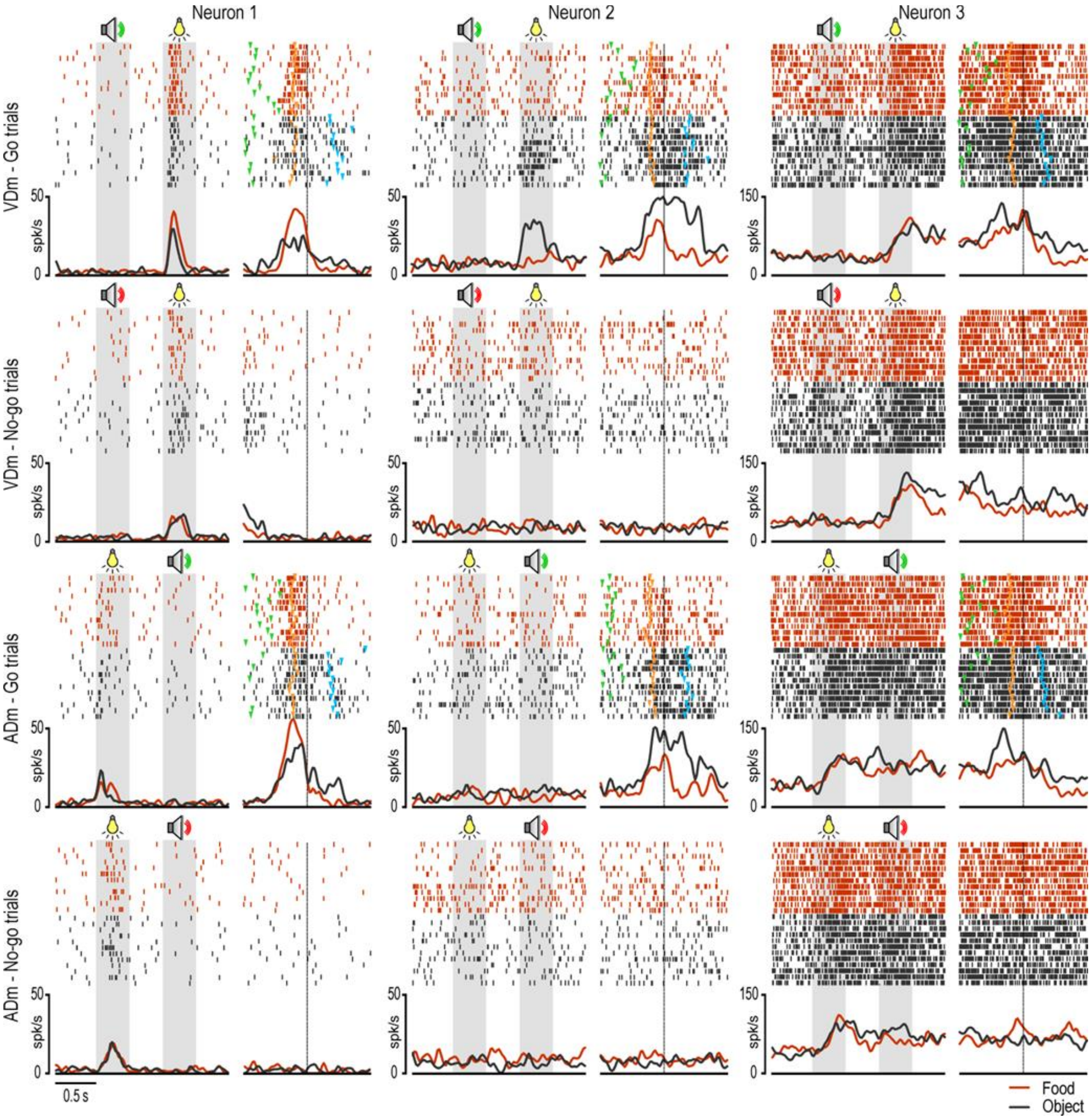

Figure 6. Examples of sensory-and-motor neurons. For each neuron, the left part of the panels illustrates the response during the presentation of the sensory cues, while the right part (after the gap) illustrates the motor-related activity aligned on hand-target contact. Conventions as in Figures 4A and 5A.

Interestingly, the properties of the visual response of sensory-and-motor neurons in terms of target selectivity and preference for the go condition were more similar to those of the motor response of motor-related neurons than to those of the visual response of sensory-driven neurons (Fig. 7), suggesting that this set of neurons encode the incoming visual information in relation to the upcoming motor action. This raises the issue of what might be the specific relationship between the visual and motor selectivity of sensory-and-motor neurons. To answer this question, we explored the target selectivity of the motor response of sensory-and-motor neurons preliminarily classified based on the selectivity of their visual response.

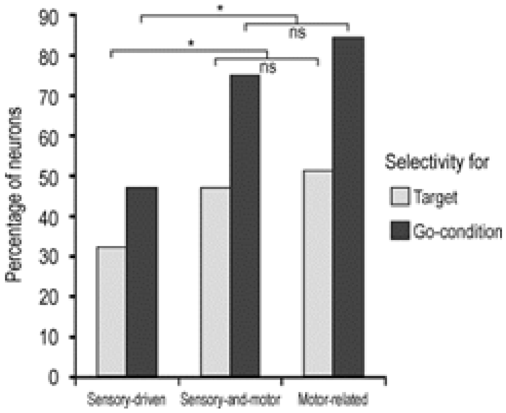

Figure 7. Percentage of target selectivity and selectivity for the go-condition in sensory-driven and sensory-and-motor neurons' visual response, and in motor-related neurons' motor response. * Fisher exact probability test with *p*<0.05.

Figure 8A shows that most of the sensory-and-motor neurons with a given target selectivity of their visual response tended to show the same selectivity (or no significant selectivity) in their motor response: virtually none of them exhibited incongruent visuo-motor preference for the target. Figure 8B shows the time course and intensity of the population activity of the same neuronal subpopulations shown in Figure 8A. It is clear that, even at the population level, neurons showing visual preference for food or object (*p*<0.001) during go trials in the VDm exhibited an overall significant grasp-to-eat (*p*<0.001) or grasp-to-place (*p*<0.001) preference in their motor response as well. It is interesting to note, however, that while the motor selectivity was present regardless of the order of presentation of the contextual cues, the visual selectivity emerged only when the target was presented as second cue, thus enabling the monkey to decide which action to perform based on visual information.

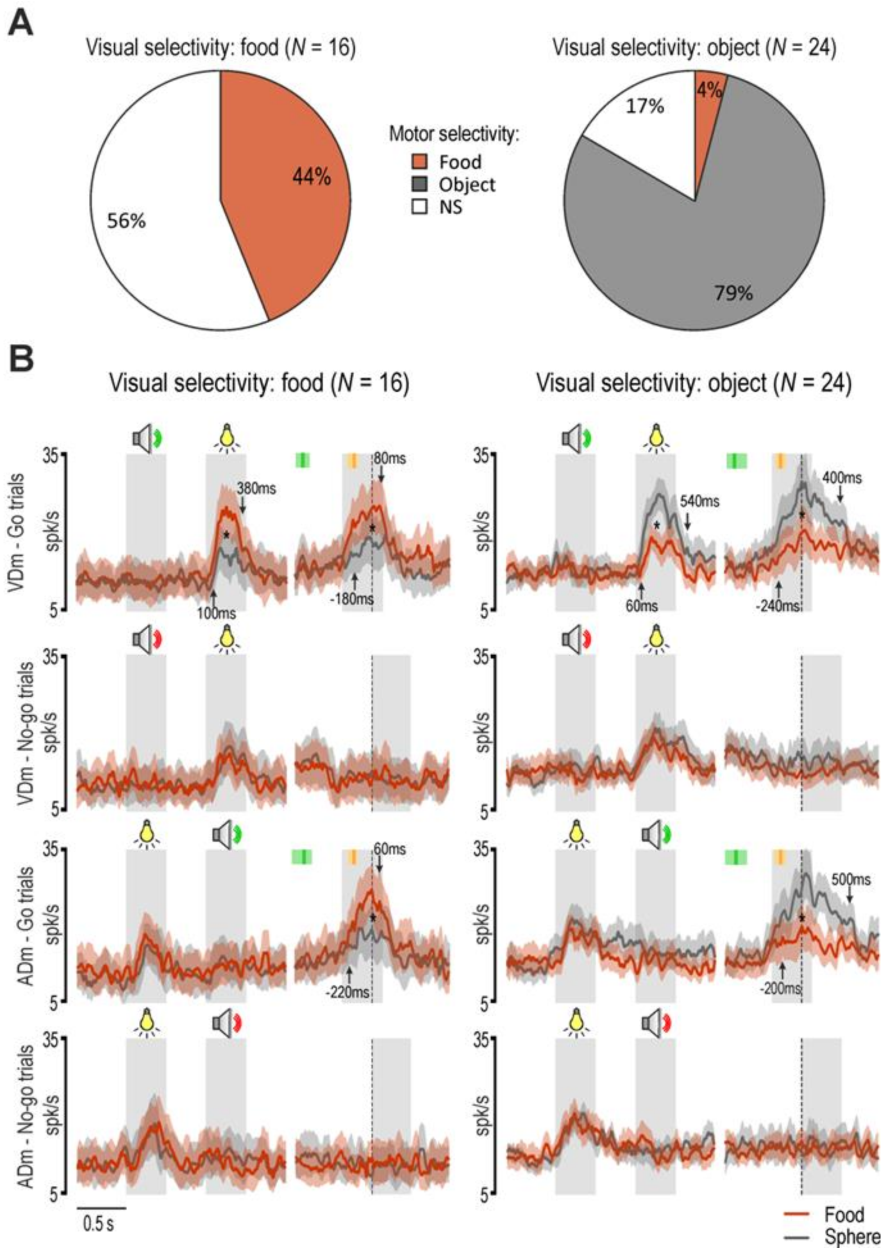

Figure 8. Visuo-motor congruence of target selectivity in sensory-and-motor neurons. ***A***. Motor selectivity of sensory-and-motor neurons showing visual selectivity for food or object. ***B***. Population response of the two subpopulations of sensory-and-motor neurons shown in A. In each population, the left part of the panels illustrates the response during the presentation of the sensory cues, while the right part (after the gap) illustrates the motor-related activity aligned on hand-target contact. * $p<0.001$ for all comparisons with Bonferroni post-hoc tests. Other conventions as in Figures 4 and 5.

2.3.4. Integrative multisensory responses

As described in previous sections, some sensory-driven (N=35) and sensory-and-motor (N=42) neurons showed more complex multisensory properties: they discharged both to the presentation of the auditory cues and to the visual presentation of the target, especially when these stimuli allowed the monkey to decide what to do next. Indeed, it is interesting to note that all these neurons showed at least a preference for the go or the no-go condition, with possible additional target selectivity (see Table 5).

	Target selectivity			
	Food > Object	Object > Food	Food = Object	Total
Go > No-go	8	23	36	67
No-go > Go	1	2	7	10
Go = No-go	0	0	0	0
Total	9	25	43	77

Table 5. Selectivity for go/no-go condition and target of multisensory neurons response to the second cue stimulus in both decision modes.

Examples of these two types of behaviour are shown in Figure 9. Neuron 1 discharged to the presentation of the second sensory cue, regardless of whether it was visual or auditory, but only during the go trials: indeed, no significant response was observed to the second cue during no-go trials, and to the presentation of the go cue as first. In addition, the lack of selectivity for the type of target indicates that this neuron discharges when the cue allows the monkey to decide to perform an action, whatever action will be performed. Neuron 2 behaved in a similar way, but also showed target selectivity with a clear preference for object relative to Food trials. These findings suggest that multisensory neurons specifically contribute to decide whether or not to act and, in some cases (e.g. Neuron 2), even what action to perform.

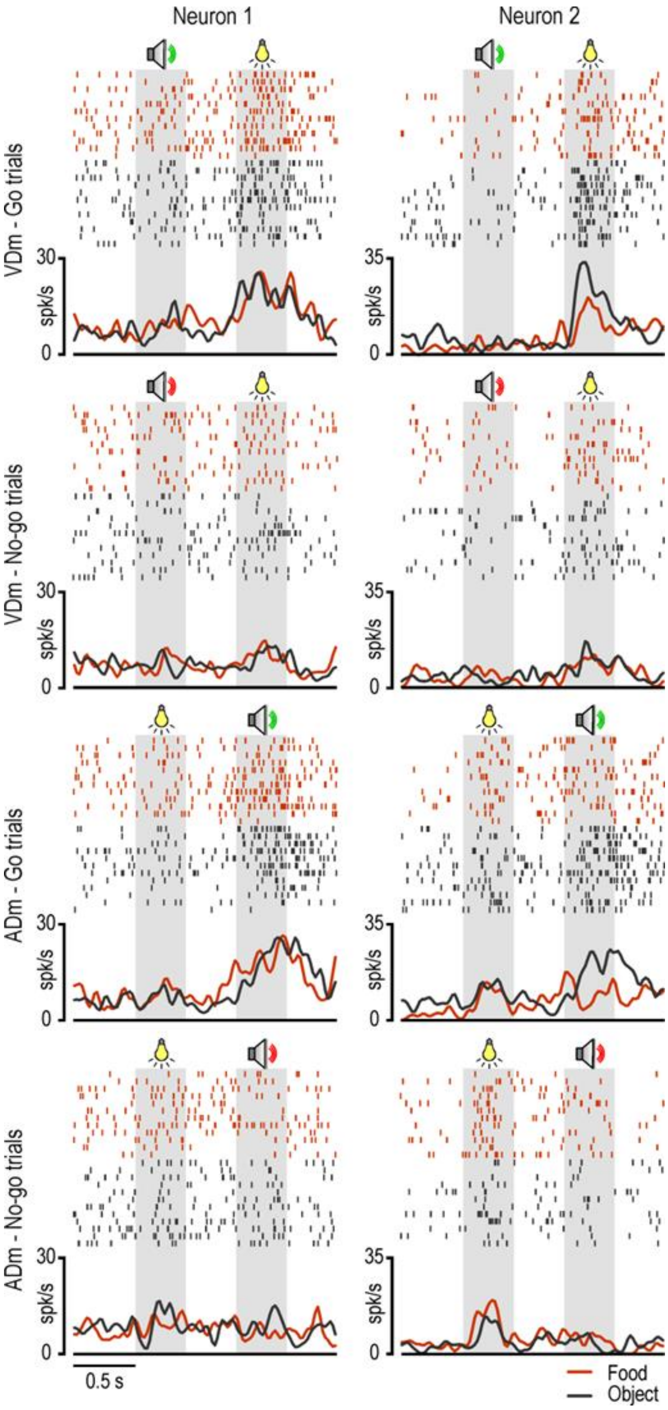

Figure 9. Examples of multisensory neurons. Conventions as in Figures 4A and 5A.

It is interesting to note that, during the VDm, whether or not to act could be already established based on the first auditory cue, though no final decision about the specific action to perform can be made yet. To better investigate whether and to which extent the auditory and visual cues are processed in an integrated manner by multisensory VLPF neurons, we separately studied the population activity of neurons with go selectivity only (see Figure10, N=36, out of which 12 were sensory-driven while 24 sensory-and-motor) as well as of those showing, in addition, a selective response for a given action (see Figure 11, N=31, out of which 17 were sensory-driven while 14 sensory-and-motor). The results clearly confirm that both populations encode a motor decision when the second cue is presented, regardless of its sensory modality. However, they also clearly show that while the visual cue (i.e. target presentation) can evoke a significant response ($p<0.001$, Bonferroni post-hoc test) even when presented before the auditory cue, although devoid of any selectivity, the auditory cue did not produce any modulation of populations activity when presented as first. It is interesting to note that although a significant modulation during the execution epochs was present in only some of the multisensory neurons included in the analyses shown in Figure 9 (24/36) and Figure 10 (14/31), their contribution to the population activity was clearly strong enough to generate, in both populations, a significant motor response with the same selectivity exhibited by the neurons during the sensory cue periods. These findings strongly support the idea that multisensory neurons discharge is associated with the emergence of a specific motor decision.

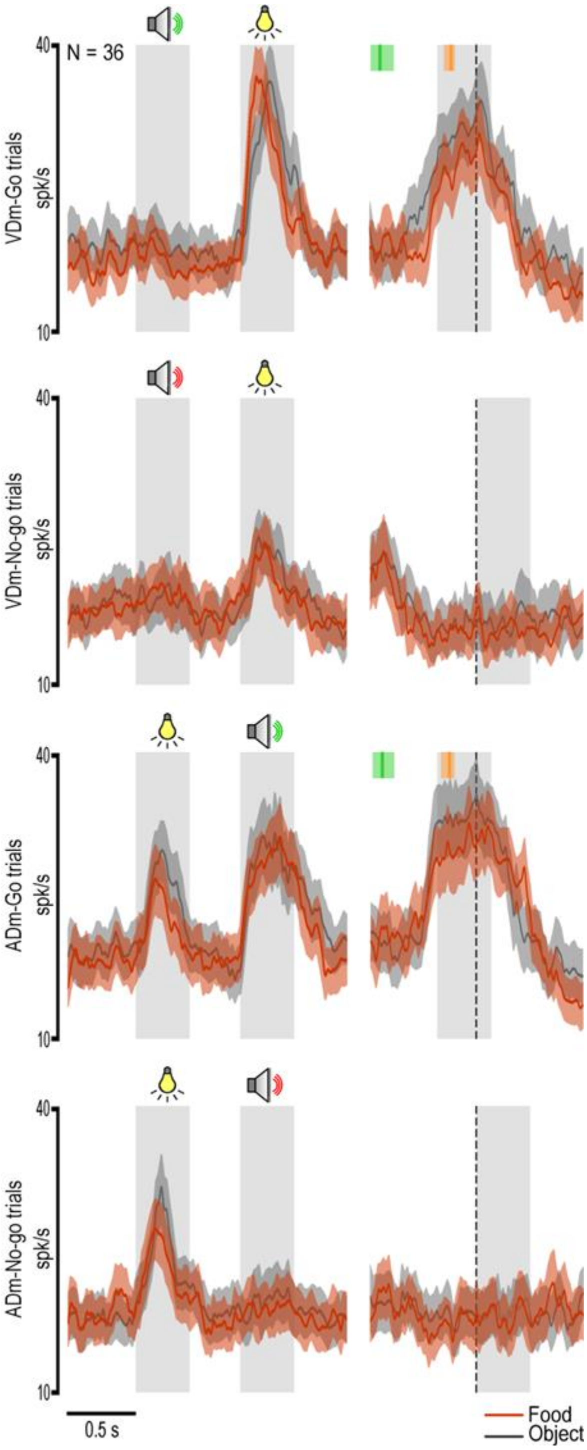

Figure 10. Population response of multisensory neurons showing preferential activation during the cue period of the go condition (see Table 5). Note that population activity was also significant during the execution epoch [in VDm, interaction Condition x Epoch F(1,35)=46.02, *p*<0.001; in ADm, interaction Condition x Epoch F(1,35)=56.01, *p*<0.001]. Bonferroni post-hoc tests also indicated the presence of significantly stronger discharge during object than food grasping (*p*<0.001). Other conventions as in Figures 4 and 8.

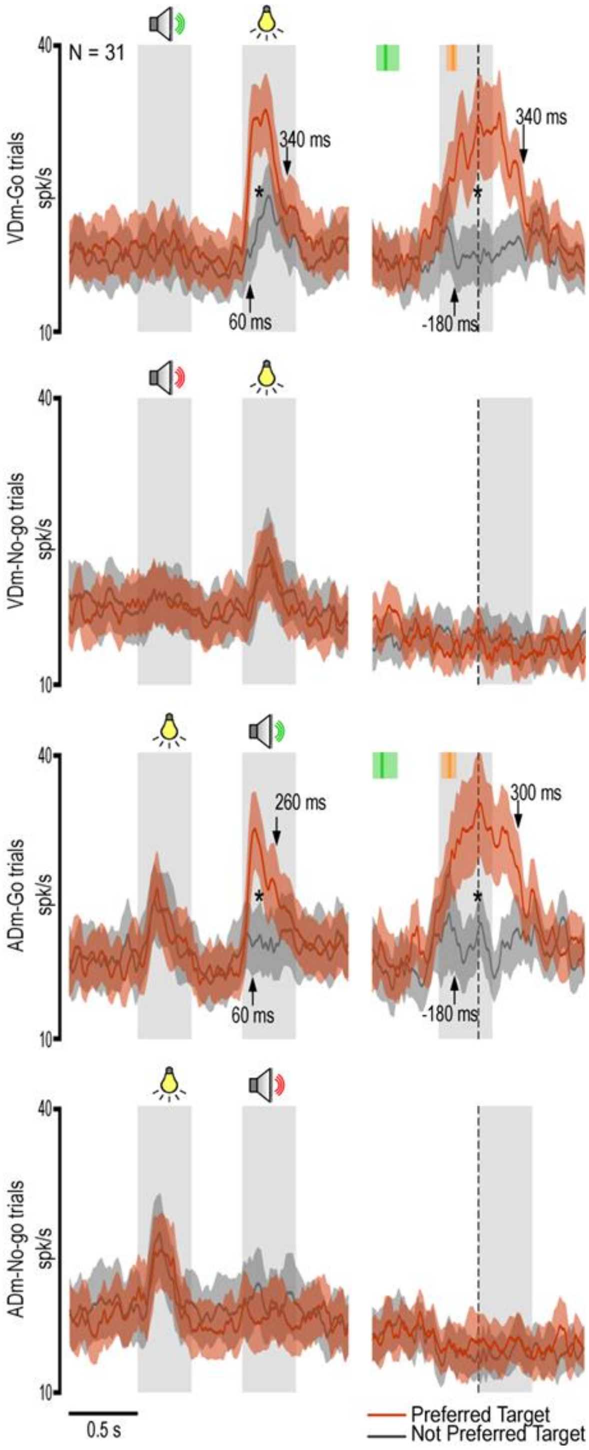

Figure 11. Population response of multisensory neurons showing selectivity for a specific motor decision (see Table 5). Note that population activity was also significant during the execution epoch, and showed the same selectivity as during the presentation of the second sensory cue [in VDm, interaction Target x Condition x Epoch F(1,30) = 7.73, *p*<0.01; in ADm, interaction Target x Condition x Epoch F(1,30) = 4.99, *p*<0.05]. * *p*<0.001 for all comparisons with Bonferroni post-hoc tests. Other conventions as in Figures 4 and 8.

2.4. DISCUSSION

In this study we investigated the contribution of VLPF neurons to the processing of contextual information allowing the monkey to select one among four behavioural alternatives: grasp/not to grasp a food morsel to eat it, and grasp/not to grasp an object to place it into a container. Crucially, the monkey's decision required to integrate two sequentially presented cues: a visually presented target, indicating *what* action to perform, and an auditory cue, indicating *whether* or not to act. Depending on the order of presentation of the two cues, the monkey made its final decision based on visual (Visual Decision mode, VDm) or auditory (Auditory Decision mode, ADm) information. Thus, if neuronal activity reflects the monkey's decision to perform a specific action, it should do so regardless of the decision mode.

We found four main types of neurons, with no clear segregation within the recorded region: unimodal sensory-driven (37%), motor-related (21%), unimodal sensory-and-motor (23%), and multisensory (19%) neurons. No-go neurons were poorly represented in all categories, likely because the trap-door below the target systematically prevented the monkey from grasping it during no-go trials, thus reducing both the automatic tendency of the animal to reach for potentially graspable objects and the need for active neural inhibition of this behaviour. Interestingly the discharge of multisensory neurons reflected a behavioural decision independently from the sensory modality of the stimulus allowing the monkey to make it: some encoded a decision to act/refraining from acting (56%), while others (44%) specified one among the four behavioural alternatives (grasp/not to grasp a food morsel to eat it, and grasp/not to grasp an object to place it into a container).

Contextual modulations of the processing of observed objects

Most unimodal sensory-driven neurons responded to the visually presented target (96%), while only a few (4%) encoded the auditory cue, in line with previous studies (Tanila et al., 1992; Saga et al., 2011). The wealth of visually-responsive neurons in VLPFC is not surprising. However, they were typically studied by presenting monkeys with abstract visual cues of different size, shapes, colours or spatial positions on a screen (see Fuster, 2008). Here, we showed that VLPF neurons respond to the presentation of real solid objects, often showing differential activation depending on the type of object and/or the behavioural context.

The great majority of visually triggered neurons discharged stronger and displayed target preference when the monkey was allowed to make a decision, by integrating the visual information with a previously presented cue (VDm). In contrast, when target presentation occurred before a go/no-go instruction (ADm), the response was generally weaker and devoid of any target selectivity, suggesting that VLPF neurons mainly encode real objects depending on what the monkey is going to do with them (see also Watanabe 1986; Sakagami and Niki, 1994). These findings extend previous evidence of categorical representation of visual stimuli in VLPFC (Freedman et al., 2001; Kusunoki et al., 2010) to solid objects belonging to behaviourally-relevant, natural categories (i.e. edible vs inedible).

It is interesting to note that the majority of target-selective visually-triggered neurons exhibited preference for the object (76%) relative to the food (24%): since monkeys needed an explicit training to learn what to do with the object (placing), while it did not need it for food trials, the prevalence of object-selective neurons likely reflects the role of VLPFC in the acquisition of visuo-motor associations underlying action selection (Wise and Murray, 2000).

Visuomotor selectivity for target objects in VLPF neurons

Motor-related discharge of VLPF neurons has been described in previous studies (Kubota and Funahashi, 1982; Quintana et al., 1988; Tanila et al., 1992), and it was proposed that these responses “reflect behavioural factors such as the *goal* or concept of the motor activity” (Hoshi et al., 1998), operationally identifying “goals” with different locations or shapes (e.g. circle or triangle) of a target. Here we showed that a similar coding principle also applies to motor responses occurring during forelimb object-directed actions. Indeed, about half of motor-related neurons showed target selectivity since 180ms before hand-target contact, suggesting that they might take part in the encoding of the final action goal previously described in IPL and vPM neurons (Fogassi et al., 2005; Bonini et al., 2010).

Interestingly, we also found a substantial number of VLPF neurons showing both a motor-related discharge and a visual presentation response (sensory-and-motor neurons), with remarkable visuo-motor congruence for the preferred target. Furthermore, similarly to what observed for sensory-driven neurons, we found that the visual target selectivity clearly appears when the target is presented after the auditory cue in the go condition (see Figure 8), thus suggesting it plays a role in the selection of the upcoming motor action. This proposal fits well with the rich anatomical connections linking VLPFC with IPL and vPM regions involved in the organization of goal-directed forelimb actions (Borra et al., 2011).

By comparing target selectivity during action execution observed in the present study with that described in previous reports on parietal and premotor neurons, it emerges that the selectivity for object/placing actions relative to food/eating actions forms a rostro-caudal gradient, with more object selectivity in PFC (63%, present data) relative to vPM (40%) and IPL (21%) regions (Bonini et al., 2010). This trend might reflect an overall major role of frontal regions in the encoding of learned sensory-motor associations between objects and actions relative to parietal cortex, which appears more devoted to the organization of hand-to-mouth actions automatically afforded by the intrinsic meaning of objects (Yokochi et al., 2003; Fogassi et al., 2005; Rozzi et al., 2008).

A further interesting finding is that the great majority of single cells, as well as populations activity, clearly show transient activation in response to contextual cues and executed actions rather than sustained activity during the delay period. This could certainly occur because, in our task, both the auditory and visual information remained available for the entire duration of the delay period, thus rendering unnecessary to rely on memory. Nevertheless, remarkably similar behavioural paradigms applied to parietal (Sakata et al., 1995) and premotor (Murata et al., 1997; Raos et al., 2006; Bonini et al., 2014a) grasping neurons evidenced sustained neuronal activity and grip/object selectivity during the delay period, although no memory load was required. Very likely, other brain regions such as dPM cortex or basal ganglia (Hoshi, 2013), which are anatomically connected with VLPFC (Borra et al., 2011; Gerbella et al., 2013a, b), underlie the generation of sustained context-dependent activation during the selection of goal-directed actions.

Multimodal integration of contextual information underlying motor decision

One of our most interesting findings is that almost 20% of the recorded neurons integrated the visual and auditory cues, providing a signal reflecting the decision of the monkey to act or refrain from acting, or even the decision to perform/withhold a *specific* action, such as grasp-to-eat or grasp-to-place. Crucially, their response and selectivity appeared only when sufficient sensory evidence was accumulated to enable the monkey to make a final decision, regardless of whether the last piece of evidence was conveyed by auditory (go/no-go) or visual (food/object) information. Interestingly, when the cue sound was presented first, it did not evoke virtually any significant response (see Figure 9 and 10). Nevertheless, it evoked a vigorous response when it allowed the monkey to make a decision based on the previously presented target. Where does this multisensory integration come from?

One possibility is that other brain regions, anatomically linked with the VLPFC, perform this integration, select a specific action, and send their input to VLPFC. For example, neural correlates of motor decision based on sequentially presented instructions have been demonstrated in dPM (Hoshi and Tanji, 2000; Cisek and Kalaska 2005). However, single neuron evidence also supports the possibility that audio-visual integration occurs in PFC (Benevento et al., 1977; Fuster et al., 2000). Although further studies are needed to address this issue, the present and previous findings indicate a key role of VLPFC in the multimodal integration of contextual information underlying the selection of goal-directed forelimb actions.

A final important question concerns what exactly multisensory neurons discharge represents. A plausible interpretation is that they encode conceptual-like representation of the monkey’s final behavioural goal. Indeed, previous studies showed that VLPF neurons can code motor goals at different level of abstraction (Saito et al., 2005; Mushiake et al., 2006), and even conceptual representations of sequential actions (Shima et al., 2007): the present findings suggest that a similar interpretation also applies to the activation of VLPF multimodal neurons prior to goal-directed manual actions.

2.5. CONCLUSIONS

The PFC underwent a considerable expansion during phylogeny (Fuster, 2008), which appears to parallel the increasing complexity, goal-directedness, and flexibility of animals’ behaviour (Sigala et al. 2008; Stokes et al. 2013). In this study, we explored the neural underpinnings of the organization and execution of goal-directed actions resembling some of the most widespread foraging behaviours of primates. Our findings expand the knowledge on prefrontal functions by showing that the majority of prefrontal neurons encode target objects and manual actions in a context-dependent manner, and a set of them even exhibits multimodal representation of the intended goal of a forthcoming action. On these bases, we propose that the PFC may host an abstract “vocabulary” of the intended goals pursued by primates in their environment.

3. STUDY TWO

3.1. INTRODUCTION

Human and nonhuman primates are capable to perform intentional actions to different degrees of complexity (Koechlin and Jubault, 2006; Botvinick, 2007; Fuster, 2008). Neurophysiological studies have shown that the discharge of neurons in the ventral premotor (Rizzolatti et al., 1988; Kakei et al., 2003; Umiltà et al., 2008) and inferior parietal (Gardner et al., 2007; Rozzi et al., 2008) cortices encodes the goal of single motor acts, the basic building blocks for organizing actions. Furthermore, grasping neurons in the inferior parietal area and in the ventral premotor area, can discharge differently according to the final goal (i.e., eating or placing) of the action in which the coded act is embedded (Fogassi et al., 2005; Bonini et al., 2010).

The organization of simple actions could relies on sensory information associated with the target (Toth and Assad, 2002) in a given environmental context (Salinas, 2004; Baumann et al., 2009) for selecting the final goal. However, complex action sequences performed in our daily life could not rely only on such a sensory-cued selection of the action goal because information about the final goal may not be available before action onset. Thus, the agent has to form an internal representation of the goal and keep it in mind to shape action unfolding. Contextual information available in the environment and internal plans and goals of an agent constitute the two poles of a continuum along which the brain orchestrates action selection in a variety of contexts (Koechlin and Summerfield, 2007). Previous studies showed that PFC is also involved in manipulative hand behaviors, such as reaching-and-grasping actions, in which motor and sensory brain regions located caudally to PFC have been shown to play a major role (Bruni et al., 2015; Simone et al., 2015; Cisek and Kalaska 2010; Rizzolatti et al., 2014). However, the possible contribution of prefrontal neurons to the processing of contextual information relevant for the organization of complex actions on solid objects has never been investigated.

To address this issue, we recorded single VLPF neurons activity while monkeys performed complex manipulative action sequences aimed at distinct final goals, by employing a modified, and more strictly controlled, go/no-go version of the grasp-to-eat/grasp-to-place paradigm used in previous studies on parietal and premotor neurons.

Each action sequence included two subsequent grasping acts differing from each other in terms of availability or not of information relative to the final goal.

3.2. MATERIALS AND METHODS

The experiments were carried out on two female monkeys (M1 and M2). All the experimental protocols complied with the European law on the humane care and use of laboratory animals (directives 86/609/EEC, 2003/65/CE, and 2010/63/EU), they were authorized by the Italian Ministry of Health (D.M. 294/2012-C, 11/12/2012), and approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012). For more details about familiarization and training of monkeys and surgical procedures, see materials and methods section of Study One.

3.2.1. Apparatus and behavioural paradigm

The experimental setup and basic procedure were the same as those used for the sVMT, but the container in which the target was located was covered with a lid by the experimenter, before the beginning of each trial. The lid had a small handle of spherical shape and 0.6 cm of diameter located in the center of a groove (3.5 × 1 × 1 cm): this feature forced the monkey to grasp the handle with a precision grip identical to that used for the grasping of the target.

The monkeys were trained to perform a sensory-cued go/no-go action sequence tasks of different degree of complexity, which we will refer to as Simple Visuo-Motor Task (sVMT) and Complex Visuo-Motor Task (cVMT). The sVMT is the same as that described in previous study: for details see *Simple Visuo-Motor Task (sVMT)* section of the Study One.

Complex Visuo-Motor Task (cVMT)

The cVMT was run in a unique mode, in which the cue sound was presented first, and then the lid became visible. The task included two cue sounds (go/no-go) and two targets (food or object), thus resulting in a total of 4 different conditions, randomly interleaved, each of which recorded in 12 independent trials (48 trials in total).

As described for the sVMT, each trial started when the monkey held its hand on the starting position for a variable period of time, ranging from 1 to 1.5 s (inter-trial period). The temporal sequence of task events (see Figure 12) was as follows.

Go condition. Following presentation of the fixation point, the monkey was required to start fixating it within 1.5 s, and after a variable time lag (0.6-1 s) from fixation onset, the first cue (a high tone) was presented. After 0.8 s, the second cue was provided: the lower sector of the box was illuminated and, differently to the sVMT, the lid became visible (visual presentation). Thus, when the cue sound ceased (go signal), the monkey was required to reach and grasp the handle of the lid within 1.2 s and to remove it. Then, it grasped the food or the object located inside and in case of the food pellet (Food trials), the monkey brought it to the mouth and ate it (grasp-to-eat), while in case of the plastic sphere (Object trials), the monkey had to place it into the jar (grasp-to-place). It is important to note that from target grasping to the end of the action sequence, the cVMT is identical to the corresponding phase of the sVMT. The reward contingency associated with correct task accomplishment was the same as in the simple action.

No-go condition. The temporal sequence of events in this condition was identical to that described for the sVMT, except for the target of the fixation, namely food or object in sVMT and the handle of the lid in the cVMT.

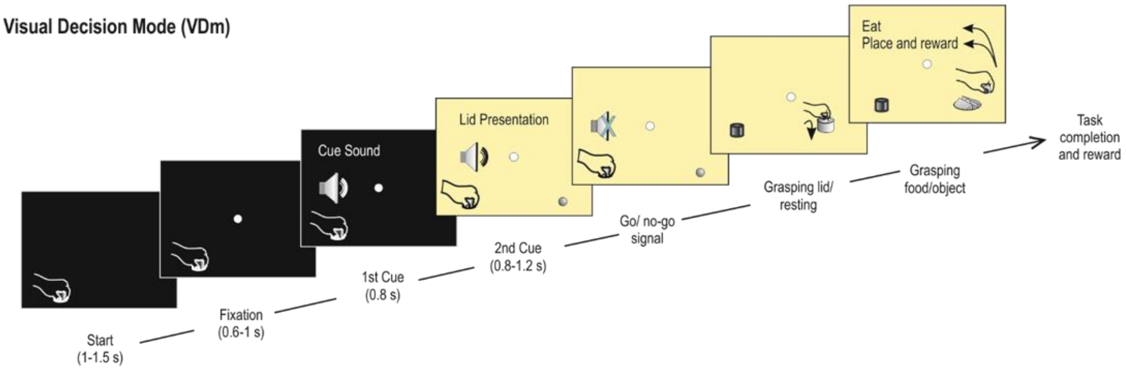

Figure 12. The cVMT was run in in a unique mode (VDm) in which the cue sound was presented first, and then the lid became visible. The integration of the two cues allowed the monkey to decide whether grasp or refrain from grasping a target. In the go condition, monkey could select the action to be performed only after removing the lid; in the no-go condition, monkey merely fixated the lid. In a version of the cVMT run in block, the monkey could decide what to do based on memory driven information.

Furthermore, the monkeys performed a version of the cVMT by running Food-trials and Object-trials in blocks. This allowed the monkey to establish the action to be performed after a few trials based on memory-driven information.

3.2.2. Recording techniques

Recording of neuronal data, behavioural events and definition of epochs of interest

Recording of neuronal activity and of behavioural events were performed as described in the previous study and I will briefly summarize.

Neuronal recordings were performed by means of 16 channels silicon probes developed in the EU project NeuroProbes (Ruther et al., 2010; Herwik et al., 2011) and distributed by ATLAS NeuroEngineering (Belgium). The recordings were carried out by means of an 8 channels AlphaLab system (AlphaOmega, Nazareth, Israel). The wide band (300-7000 Hz) neuronal signal was amplified and sampled in parallel with the main behavioural events and digital signals defining the task stages.

Concerning the behavioural events, here we detected a further one corresponding to the grasping of the lid, while the remaining are identical to that above described.

Based on the digital signals related to the main behavioural events, we defined different epochs of interest for statistical analysis of neuronal responses: (1) sensory cue

(cue sound/lid presentation) epoch, including the 500 ms after the onset of each cue; (2) movement epoch 1, ranging from 100 ms before the detachment of monkey's hand from the starting position to 100 ms after the contact with the handle of the lid; this epoch was also used for the analysis of target-grasping activity in the sVMT; (3) movement epoch 2, ranging from 100 ms before the monkey's hand contact with the target to 200 ms after (second grasping act in the cVMT).

3.2.3. Data analyses and classification of recorded neurons

Single units were isolated using standard principal component and template matching techniques, provided by dedicated offline sorting software (Plexon).

After identification of single units that remained stable over the entire duration of the experiment, neurons were defined as "task-related" if they significantly varied their discharge during at least one of the epoch of interest, investigated by means of the following repeated-measures ANOVAs (significance criterion of $p<0.01$).

Sensory response to the first cue: the presence of responses related to the presentation of sounds (low and high tone) as first cue were assessed with a 2x2 repeated measures ANOVA (factors: Sound, Epoch) followed by Bonferroni post-hoc tests in case of significant interaction effects. Neuronal activity during the cue presentation epoch was compared with that of the baseline epoch.

Sensory response to the second cue: the presence of responses related to the lid presentation as second cue were assessed by means two different 2x2 repeated measures ANOVA (factors: Sound, Epoch), separately for Food and Object trials, followed by Bonferroni post-hoc tests in case of significant interaction effects. In order to verify a possible activity change specifically induced by the second cue, neuronal activity during the second cue presentation (presentation of the lid) was compared with that of the 500 ms period before stimulus onset. Since the lid prevented the monkey to see the target, we compared the neurons response evoked by lid presentation in the Food and Object trials by employing paired samples t-test ($p<0.05$,Bonferroni corrected).

Response during the movement epochs: the possible modulation of single neurons activity during movement epoch 1 and movement epoch 2 in the go condition, has been assessed by means of 2x3 repeated measures ANOVA (factors: Target, Epoch). Besides the movement epochs defined above, here we considered as a baseline the 500 ms epoch preceding the presentation of the first sensory cue. We considered as motor related all the neurons showing significant main effect of the factor Epoch, with possible additional interaction with the factor Target ($p<0.01$, followed by Bonferroni post-hoc tests).

3.3. RESULTS

We recorded 61 VLPF neurons of the left hemisphere of both monkeys during the execution of all experimental tasks (sVMT, cVMTrandomized and cVMTblock). Depending on the stage of the tasks in which neurons changed their intensity of activity, only during the visual presentation (target or lid) epoch, only during movement epoch or both epochs, we classified all recorded neurons as “sensory-driven” (N=5, 8%), “motor-related” (N=26, 43%), and “sensory-and-motor” (N=30, 49%), respectively (see Table 6). All the recorded neurons were also tested during the cVMT: this task was carried out by randomizing Food and Object trials, so that the monkey was not aware of what was the target of the ongoing trial until it removed the lid.

	Response to sensory cues			No sensory response	Total
	Auditory	Visual	Auditory and visual		
Sensory-driven neurons	0	5	0	0	5
Motor-related neurons	0	0	0	26	26
Sensory-and-motor neurons	0	30	0	0	30
Total	0	35	0	26	61

Table 6. Number of sensory-driven, motor-related and sensory-and-motor neurons tested in all experimental tasks responding to auditory and visual cues.

Since sensory-driven neurons are virtually absent and the aim of this study was motor intention and action organization, we did not include them in further statistical analysis and we focused on the motor response of motor-related and sensory-and-motor neurons. Properties of these neuronal categories will be described in the following section.

3.3.1. Motor responses of motor-related and sensory-and-motor neurons

Neurons classified as motor-related (N=26) activated during the movement epoch of the go-condition of the sVMT, in which the monkey was required to perform one of the two actions. All these neurons did not respond during any of the previous cue epochs. On the contrary, sensory-and-motor neurons (N=30) responded during the motor epoch as well as during the presentation of the sensory cue of the sVMT and cVMT. Note that sensory-and-motor neurons showing a target preference in the visual responses during the sVMT, lost their visual target selectivity during the cVMTrandomized, but it was restored when the monkey grasped the target. Thus, we analyzed motor responses of both neuronal categories evoked during the cVMTrandomized, pooling together motor-related and sensory-and-motor neurons.

By comparing neuronal activity of these neurons recorded during the cVMTrandomized while the monkey removed a lid to grasp-to-eat or grasp-to-place, we found that half of them (29 out 56, 52%) activated also during the grasping of the handle of the lid (see Table 7). Note that these neurons did not show any preference for a specific target when the monkey grasp the handle of the lid and, among of them, not any discharged exclusively or stronger during the first compared with the second grasping motor act. Concerning the second grasping motor act, 45 out 56 showed a target selectivity for the object (N=32, 57%) or the food (N=13, 23%), while the remaining (N=11, 20%) activated with the same intensity of discharge during grasping of both targets (see Table 7).

	Target selectivity during the II grasping			
	Food > Object	Object > Food	Food = Object	Total
Grasp 1=2	3	6	2	11
Grasp 2>1	2	12	4	18
Only 2	8	14	5	27
Total	13	32	11	56

Table 7. Target selectivity shown in the motor response by motor-related and sensory-and-motor neurons tested in all experimental tasks, during grasping the target in cVMT carried out by randomizing Food and Object trials.

In order to verify whether the target preference could also depend on an internally generated representation of the final goal based on previously acquired information about the target, we tested these neurons during cVMTblock: in this version of the complex task, Food trials and Object trials were run in blocks, so that the monkey performed the same sequence of motor acts, but being aware of what was the target of the ongoing trials even before grasping the lid. Interestingly, we found that the motor target selectivity showed by these neurons during the second grasping was strictly congruent with that showed by the same neurons during the grasping motor act tested in sVMT. It is noteworthy that many neurons discharged also during the grasping of the lid (see Table 8).

	Target selectivity during the second grasping			
	Food > Object	Object > Food	Food = Object	Total
Grasp 1=2	8	14	3	25
Grasp 2>1	3	14	2	19
Only 2	1	4	7	12
Total	12	32	12	56

Table 8. Target selectivity shown in the motor response by motor-related and sensory-and-motor neurons tested in all experimental tasks, during grasping the target in cVMTblock.

Furthermore, it is striking that neuronal preference is maintained despite the lack of direct visual information cueing the action goal. Indeed, we found that this neuron discharged selectively in correspondence of both grasping execution, lid and target (see Table 9).

	Target selectivity during the first grasping			
	Food > Object	Object > Food	Food = Object	Total
Grasp 1=2	8	12	5	25
Grasp 2>1	2	8	9	19
Total	10	20	14	44

Table 9. Target selectivity shown in the motor response by motor-related and sensory-and-motor neurons tested in all experimental tasks, during grasping the lid in cVMTblock.

An example of these neurons is shown in Figure 13. In particular, it represents a motor-related neurons tested in all tasks. This neuron discharged preferentially during the grasping of the object, when it was tested during both the sVMT and the cVMTrandomized. Furthermore, when the complex task was run fully randomized, this neuron did not activate during grasping of the handle of the groove; in contrast, when the complex task was run in blocks, this neuron showed a preference for the object also during the grasping of the lid. Indeed, in each trial of this version of the complex task, the monkey could predict the target of the action based on its memory of the previously executed trials. This behaviour was displayed by 30 recorded neurons and in particular the majority was selective for the object (N=20), while the remaining for the food (N=10).

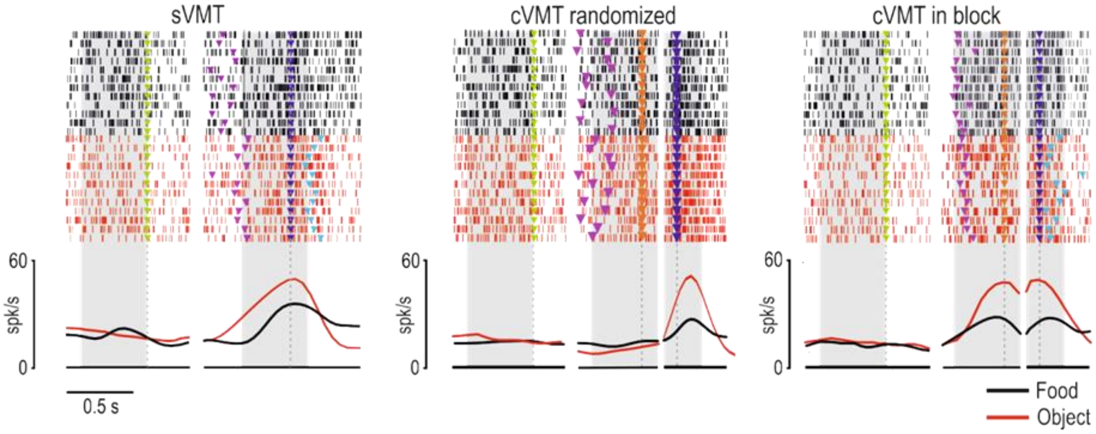

Figure 13. Example of a neuron activated during the execution of the sVMT (left panel) and the cVMT run in both randomized (central panel) and block (right panel) version. Conventions: light green, cue sound onset; purple, detachment of the monkey's hand starting position (movement onset); orange, grasping the handle of the lid; dark blue, grasping of the target. Other conventions as in Figure 5.

Taken together, these findings suggest that visual information concerning the target to be grasped, which during the cVMT was available only after the removing of the lid, could be necessary to trigger the observed neuronal preference during the final stages of the task. However, also memory-driven information relative to a graspable target could be able to produce a neuronal preference during final stages of the task and this preference is shown before the container had been uncovered.

3.4. DISCUSSION

In this study, we investigated the contribution of ventrolateral prefrontal neurons to the selection and organization of complex actions. In order to address this issue, we recorded single neurons from the VLPFC of two monkeys during the execution of a simple visuo-motor task (sVMT). The same neurons were also tested during a complex visuo-motor task (cVMT), which was similar to the sVMT, but included a further grasping motor act. Finally, the cVMT was run in two versions, randomized and in block. All experimental tasks were designed in order to study manipulative grasping actions (i.e. grasping a piece of food and bringing it to the mouth and grasping an object and placing it into a container) of different degree of complexity.

The results of this electrophysiological study confirmed that there are different sets of neurons responding in different phases of the tasks: a low number of neurons activated only during the visual presentation of the target or the lid, some neurons responded only during the motor epochs, and others were activated by both visual presentation and action execution.

In order to investigate the motor intention and action organization, in this study we focused on motor-related and sensory-and-motor neurons. A general feature, which applies to both neuronal categories responses, is a greater selectivity for the object compared with that for the food. This finding is in line with the results of the Study One.

Motor responses of motor-related and sensory-and-motor neurons

Most of the recorded neurons of this dataset (45 out 56) displayed a target selectivity: in particular, the majority of these neurons showed a preference for the object (N=32) rather than the food (N=13), during the motor response.

It is clear that these neurons showed a similar behavior during the second grasping of both versions of the complex task (randomized and in block). Furthermore, target selectivity showed during the second grasping motor act in the cVMTrandomized and cVMTblock is the same as that displayed during the grasping of the sVMT. In contrast, it is noteworthy that these neurons activated differently during grasping of the lid in the cVMTrandomized and cVMTblock. In particular, when the complex task was run randomized, half of the motor-related and sensory-and-motor neurons discharged during grasping of the lid. It is clear that all these neurons tested in the cVMTrandomized could be activated already during the first grasping motor act, but the selectivity for one of the two graspable targets emerges only during the execution of the second grasping. This is easy to explain because before the removing of the lid, monkeys do not know what is the target located in the container and cannot know the final goal of the action. In contrast, when the cVMT was run in block, almost all these neurons not only discharged during the first grasping motor act, but also displayed the same target selectivity shown in correspondence of the hand contact with the target. This finding could be explained because in this experimental setting, monkeys after a small number of trials run in block, can predict what will be the target placed under the lid. Thus, the information stored in memory could be enough to evoke or modulate the neuronal response also during the grasping of the lid. Furthermore, in a set of neurons (N=12) the mnemonic information did not evoke a significant activation during the execution of the first grasping. Among these neurons, 42% showed a target selectivity during the second grasping when monkeys could access to the sensory information about the target.

Finally, all neurons are exclusively or more strongly activated during grasping of the lid. This could be explained in terms of coding the final goal of the action: the discharge during the grasping of the lid could be a representation of the graspable target.

These results are in line with findings reported in a previous study carried out in the parietal and premotor cortices, in order to investigate the contribution of these neurons to the coding of complex actions (Bonini et al., 2011). This study suggests that ventral premotor and inferior parietal neurons can code the goal of intentional actions at different levels of motor abstraction. In particular, two different categories of action goal-related neurons, as previously characterized using simple actions (Fogassi et al., 2005; Bonini et al., 2010), were identified: late preference (LP) neurons, that showed their preference only during target grasping, and early preference (EP) neurons, discharging differently in the two conditions during both grasping of the lid and grasping of the target. LP neurons reflect a specific action goal only in the late phase of action unfolding, whereas EP neurons encode the action goal already during the early phase of the action sequence. Thus, the authors of this study concluded that, on one side, LP neurons display a prospective encoding of the action goal, very likely facilitating subsequent acts. On the other side, EP neurons reflect the action goal at higher level of abstraction, keeping active the representation of the individual's motor intention during action unfolding. In this study, we found a set of neurons similar to EP neurons only when the complex task was run in block because only in this behavioral paradigm monkeys could select the action to perform before removing of the lid. Furthermore, this study show that memory information could modify the neuronal pattern shown in correspondence of the same grasping of the lid embedded in two different contexts, cVMTrandomized and cVMTblock, respectively.

3.5. CONCLUSIONS

The present study suggests that also ventrolateral prefrontal neurons have a role in the organization of complex actions and in the coding of a final goal depending on the available or memorized information relative to a graspable target.

This hypothesis is coherent with many studies that have assigned a major role to the prefrontal cortex in sequential aspects of motor cognition, such as the acquisition of motor sequences (Koechlin et al., 2002; Averbeck et al., 2006) and the achievement of behavioral goals (Charron and Koechlin, 2010). Furthermore, prefrontal neurons have been described, encoding action categories (Shima et al., 2007; Tanji et al., 2007) and behavioral goals (Saito et al., 2005) at different level of abstraction.

Altogether, the present findings and previous data could support the idea that the prefrontal cortex works together with parieto-premotor circuits to organize motor acts into action sequences and to keep active internal representations of the individual's motor intention.

4. STUDY THREE

4.1 INTRODUCTION

Predicting others' intended behaviours constitutes one of the most crucial and challenging tasks that social animals, like primates, have to deal with. How does the primates' brain solve this problem? Two main theoretical frameworks have been formulated to address this issue (van Overwalle and Baetens, 2009; Rizzolatti and Sinigaglia, 2010; Bonini et al., 2013): the "simulation" and the "mentalizing" theories.

According to "simulation theories", we understand the intentions of others as we activate, depending on contextual information (Fogassi et al., 2005; Iacoboni et al., 2005), the same neuronal repertoires we would recruit ourselves to act in that context, thus mirroring other's forthcoming motor acts onto our own motor system. In contrast, according to "mentalizing theories", understanding others' intentions depends on contextually-driven inferential processes, which rely on brain areas that do not belong to the motor system (Frith and Frith, 2003; Brass et al., 2007). Although both these theories attribute a crucial role to the context in which an action is about to occur for inferring others' intention, the brain mechanisms underlying this function remain still largely unknown.

A series of recent studies reported that monkey vPM (Rizzolatti et al., 1996; Gallese et al., 1996; Bonini et al., 2010) and IPL (Fogassi et al., 2005) host a set of neurons (mirror neurons, MNs) whose peculiar property is to discharge during both the execution and the observation of goal-directed motor acts. Moreover, besides the goal of single motor acts, MNs discharge can also reflect action goals at a higher level. In fact, a set of studies tested parietal (Fogassi et al., 2005) and premotor (Bonini et al., 2010) MNs activity while the monkey performed a go/no-go task and observed the experimenter performing the same task. Most of the recorded MNs were differentially activated during the observation of the same motor act (grasping), depending on whether the observed grasping was followed by bringing the target to the mouth or by placing it into a container. Thus, MNs activity is not simply driven by the visual representation of an observed motor act: elements present within the contextual setting in which action is performed and observed, crucially contribute to the possibility by the observer to decode what other individuals are doing or intentioned to do. Concerning to studies carried out on human subjects, a recent fMRI study suggests that premotor MNs, previously thought to be involved only in action recognition, also contribute to the understanding of others' motor intention, when enough contextual information are available (Iacoboni et al., 2005).

Here we addressed the issue of whether neurons in the monkey VLPFC, a cortical region that does not belong to the motor system but is tightly connected with it (Borra et al., 2011; Gerbella et al., 2013b), can activate both when the monkey faces a specific action context, and when it observes another agent facing the same context. To this purpose, we trained two monkeys to perform a contextually-cued action sequence task (see Study One, Figure 2C) and to remain still and to observe an experimenter performing the same task in their extrapersonal space. In this situation, the same contextual auditory and visual cues are clearly behaviourally irrelevant for the monkey, but they might enable it to predict what the observed agent will do next.

4.2. MATERIALS AND METHODS

The experiments were carried out on two female monkeys (M1 and M2). All the experimental protocols complied with the European law on the humane care and use of laboratory animals (directives 86/609/EEC, 2003/65/CE, and 2010/63/EU), they were authorized by the Italian Ministry of Health (D.M. 294/2012-C, 11/12/2012), and approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012). For more details about familiarization and training of monkeys and surgical procedures, see materials and methods section of Study One.

4.2.1. Apparatus and behavioural paradigm

The experimental setup and basic procedure were the same as those used for the sVMT.

The monkeys were trained to seat in front the plexiglas box used to perform the sVMT. On the back wall of the box, in the upper right quadrant of the monkey visual field and at 45 cm from the monkey face, a black plastic plane for the execution of the observation task (OT) was located. The plane was separated from the inside of the box by a vertical half-mirror (l 25,5 x h 25 cm), which enabled the monkey to see what happened outside it only when the external ambient light was turned on. Three elements were aligned on the sagittal plane of the experimenter and on the perpendicular plane respecting to the monkey. On the left side of the plane a plastic cylinder with a metal surface on its top was located: the target (a peanut for Food trials, a grey metal object of 1 x 1 x 1 cm for Object trials) was placed on the cylinder during the inter-trial period, in complete darkness and in presence of a constant white noise; in front of it an empty plastic jar, used to place the object after grasping, and a small metal plate used as experimenter's hand starting position were located. Between the two containers a small white LED was located, to be used as the fixation point for the monkey.

Observation Task (OT)

The OT was run in a separate block of 96 randomized trials (48 go trials and 48 no-go trials) but, in this case, the experimenter performed the task with exactly the same conditions as those used in the sVMT by the monkeys (Figure 2C).

Through the half mirror, the monkey could see the experimenter's hand, the containers and the actions executed by the experimenter: grasping an object or a piece of food, to place it in the container or to eat it, respectively.

Each trial started in complete darkness, when both the monkey and the experimenter had their own hand on the respective starting positions. After the switching on of the fixation point, the monkey was required to engage fixation within 1.5 s. The temporal structure and events following the beginning of each trials were exactly the same as in the sVMT but, in this case, the sounds constituted behaviourally relevant cues for the experimenter, since the monkey was required to remain still with its hand on the starting position for the entire duration of all trials and to maintain fixation, until the reward (a food pellet identical to those used in the sVMT and in the cVMT) was automatically delivered at the end of each trial performed by the experimenter. In all cases in which the monkey broke fixation or detached its hand from the starting position, the trial was aborted and re-presented at the end of the list, and the reward was not delivered.

4.2.2. Recording techniques

Recording of neuronal data, behavioural events and definition of epochs of interest

Recording of neuronal data were performed as described in the Study One.

Concerning the behavioural events, here we detected the detachment of the experimenter's hand from the starting point, the contact of the monkey/experimenter's hand with the metallic floor of the container hosting the target (food or object), the contact of the experimenter's hand with the metallic border of the jar in which the object had to be placed. Simultaneously, monkey's eye movement were monitored by dedicated software.

Based on the digital signals related to the main behavioural events, we defined different epochs of interest for statistical analysis of neuronal responses, which correspond to that identified and described in Study One (for details, see the section *Recording behavioural events and definition of epochs of interest* in Study One).

4.2.3. Data analyses and classification of recorded neurons

In order to analyze neuronal activation during the observation of the task performed by the experimenter, we investigated the neuronal response to the first cue, the second cue and that evoked during the observation of the action execution/resting epoch. We applied the same statistical analysis carried out in Study One (for details see *Data analyses and classification of recorded neurons* section of the Study One).

4.3. RESULTS

We recorded neuronal activity from the ventrolateral sector of the prefrontal cortex of the left hemisphere of both monkeys during the execution of the sVMT and the OT. Statistical analyses revealed that 250 neurons responded during some phases of the OT. In particular, 112 neurons activated specifically in relation to the sensory cues presentation; 80 neurons were differentially activated during the observation of the experimenter's action compared with the corresponding target fixation period; and 58 neurons discharged stronger during both the observation of action compared with the fixation condition and the presentation of cue stimuli preceding experimenter's action (see Table 11). In the following sections, the properties of these main neuronal categories will be described in detail.

	Response to sensory cues			No sensory response	Total
	Auditory	Visual	Auditory and visual		
Sensory-driven neurons	11	86	15	0	112
Motor-related neurons	0	0	0	80	80
Sensory-and-motor neurons	6	42	10	0	58
Total	17	128	25	80	250

Table 11. Number of sensory-driven, motor-related and sensory-and-motor neurons responding to visual and auditory cues presented in both task modes (VDm and ADm) during the execution of the OT.

4.3.1. Sensory-driven neurons

Among all recorded neurons, 112 (41%) activated significantly during the presentation of cue stimuli, but not during the subsequent movement epoch. A great part of these neurons (86%) responded to the presentation of cue stimuli provided by only one of the two modalities, auditory or visual (mainly visual presentation). The remaining sensory-driven neurons (N=15, 14%) responded to both the cue sound and the visually presented target, and their properties will be described in a dedicated section.

Only a small number of neurons (N=11) responded to the auditory cue, while the majority of sensory-driven neurons increased their activity exclusively during the visual presentation of the target (N=86). The majority of them (65 out 86, 76%) activated with the same intensity during presentation of both targets, and only few (21 out 86, 24%,) showed a different discharge according to the type of target presented, the food (N=11, 52%), or the object (N=10, 48%).

4.3.2. Motor response of neurons activated during action observation

We classified as action observation-related neurons, those neurons discharging during the observation of experimenter's action (N=80, 32%), and not during any previous cue epochs. Almost half of this set of neurons (N=31) presented a selectivity for the target: in particular, 16 neurons (51%) discharged preferentially for the food, whereas 15 (49%) for the object.

In Figure 14 is represented an example of neuron active during both execution and observation of the same task. This neuron increases its firing rate exclusively during the movement epoch of the go condition in the sVMT and during the same epoch of the observation of the experimenter's action. The neuron discharges intensely during action observation in the OT: the increase of activity is detectable immediately after the detachment of the experimenter's hand from his starting position. Notably, a selectivity for the type of grasped object is not present. During the execution of the sVMT, the same cell also fires when the monkey actively performs a reaching-grasping action, with a similar discharge profile and a complete lack of selectivity for the type of grasped object.

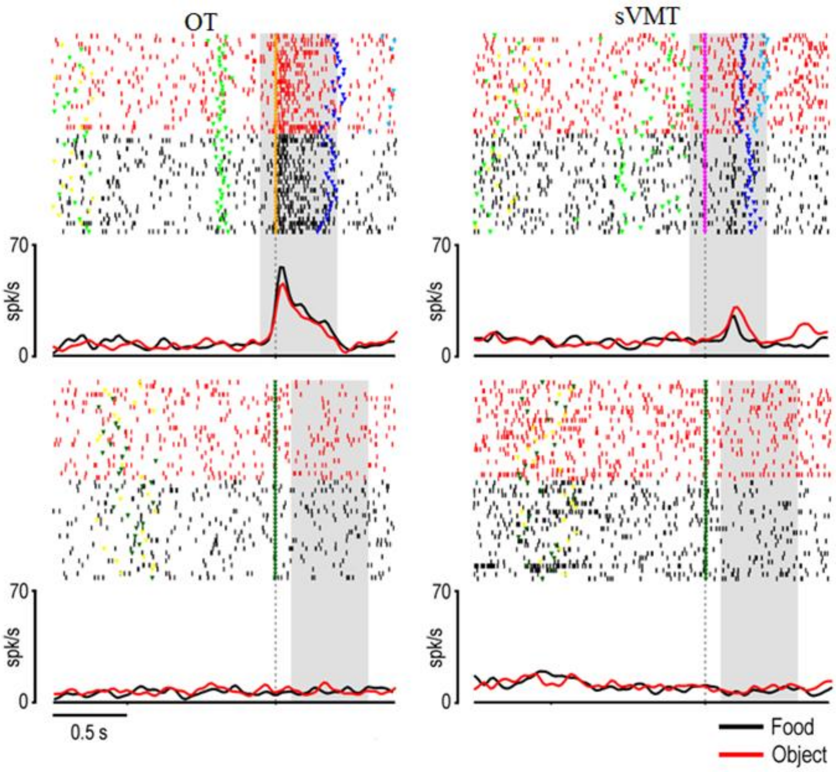

Figure 14. Example of a neuron activated during the movement epoch, during both the execution of the sVMT and the observation of the same task done by the experimenter (OT). Conventions: orange, detachment of experimenter's hand from starting position (movement onset); purple, detachment of monkey's hand from starting position (movement onset); dark green, no-go signal. Other conventions as in Figure 5.

A set of neurons (58/250, 23%) showed a significant activation during the motor epoch of the task performed by the experimenter as well as the presentation of cue stimuli in one (N=48) or both (N=10) of the two tested sensory modalities. The properties of those neurons responding both to the cue sound and to the visual presentation will be described in a following dedicated section.

Concerning the sensory response of neurons of this category, only a few (N=6, 12%) activated in correspondence of the auditory cue onset, while the great majority (N=42, 88%) of them were activated by the target presentation, with a greater preference for the object (N=11, 26%) than for the food (N=5, 12%). Interestingly, the majority of visually-selective neurons showed a preference for the go condition (80% of food-selective and 64% of object-selective neurons).

To better understand the relationship between the visual selectivity and the target preference, we explored the target selectivity emerged during the action observation in those neurons preliminarily classified based on the selectivity of their visual response. About the half of the sensory-and-action observation-related neurons neurons with a given target selectivity in their visual response tended to show the same selectivity in their motor-related response.

4.3.3. Integrative multisensory responses

Some sensory-driven (N=15) and sensory-and-action observation-related neurons (N=10) responded to sensory stimuli presented by more than a modality, exhibiting multisensory properties: they discharged both to the presentation of the auditory stimulus and to the visual presentation of the target, especially when these stimuli allowed the monkey to decide what to do next.

Among all multisensory neurons activated during the execution of the sVMT, few responded also during the observation of the action performed by the experimenter (Figure 15).

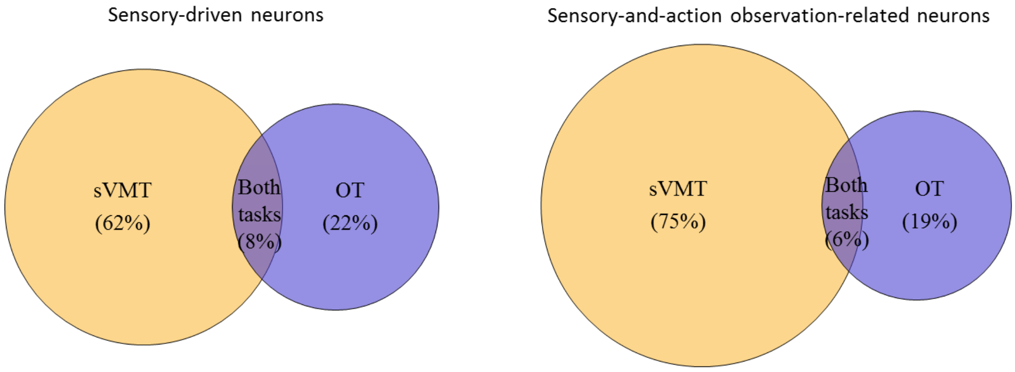

Figure 15. The Venn charts represent the proportion of sensory-driven and sensory-and-action observation-related multisensory neurons discharging during both the tasks, the sVMT and the OT.

The target selectivity is barely represented: 3 (12%) neurons are selective for the food, while 5 (20%) discharge stronger when the target is the object. In addition, 44% of these neurons show a preference for the go condition, whereas the majority of them discharge with the same intensity regardless the condition.

4.4. DISCUSSION

In this study, we investigated the contribution of VLPF neurons in the processing of contextual information allowing the monkey to predict others' actions. To address this issue, two monkeys were trained both to perform a simple visuo-motor task and to observe an experimenter performing the same motor task. Crucially, both the experimental tasks required the integration of two sequentially presented cues: depending on the order of presentation, the monkey could decide what to do (sVMT) and infer what the other agent has the intention to do (OT), based on visual (VDm) or auditory (ADm) information present within the contextual setting in which action is performed.

Some of the recorded neurons that activated during some phases of the sVMT also responded during the OT. In particular, we found three main categories of neurons: sensory-driven, action observation-related and sensory-and-action observation-related neurons. Furthermore, we identified a subset of neurons, which displayed multisensory properties. Here we were interested to study the neuronal response during action observation. Thus, we focused on the response during action observation of "action observation-related" and "sensory-and-action observation-related" neurons.

Neurons activation during action observation

A great part of the neurons active during the OT (138/250) specifically modulated at least during the action observation period. In particular, the majority of these neurons (N=80) significantly activated only during the observation of the experimenter's action; while the remaining (N=58) responded not only during the action observation phase, but also during cue presentation. Among all neurons responding during OT, one third (N=47) displayed a target selectivity, with a not clear preference for one of the two targets, at difference with the percentages found in Study One and Two.

Interestingly, these neurons varied their activity in close correspondence with the experimenter's action, with a temporal firing pattern similar to that of ventral premotor and inferior parietal MNs (Gallese et al., 1996; Fogassi et al., 2005). The crucial feature of MNs of parietal and premotor areas is that they encode both the observed and the executed action that suggested the interpretation of the possible role of this class of cells, namely that the activation of one’s own motor repertoire during the observation of others’ action enables the observer to understand the observed action well beyond the mere perceptual level, that is, ‘from the inside’ (Sinigaglia and Rizzolatti, 2011). Clearly this congruence is present also in VLPF neurons responding during action observation, although this is limited to the action tested in the present study. An interesting aspect to be compared between the neurons discharge during execution and that during observation is the temporal pattern of the discharge. In fact, a qualitative analysis reveals that the visual response to action observation was more time-locked to the reaching/shaping phase of the experimenter’s action than to the actual grasping phase (see Figure 14). Thus, although in principle these neurons show the main feature typical of mirror neurons, the difference in the timing of the visual and motor discharge raises the question of whether they can be considered ‘prefrontal analogous’ of the premotor and parietal MNs. However, we have to consider that these neurons have not been studied also in the naturalistic setting that was typically used for the description of mirror responses, therefore it is not possible to make a direct comparison. Furthermore, in the task used in the present study, monkeys received instructional cues that enabled them to predict whether and what action the experimenter would have subsequently performed: this expectancy could influence the behavior of these neurons that begin to change their activity in correspondence with the experimenter movement onset. This finding is in line with a recent study carried out in the premotor cortex (Maranesi et al., 2014). In this work, the authors demonstrated that the average onset timing of the mirror neurons population activity relative to the go signal was -340 ms, that is hundreds of milliseconds before the timing of mirror neurons visual response reported in previous studies (+60 ms relative to the onset of the agent's reaching movement; Maranesi et al., 2013), in which monkeys were not provided with any auditory or visual cue enabling them to predict the onset of the observed actions.

Finally, a surprising result is that the response during action observation reveals a low target preference: this finding is partially in contrast with the results reported in the Study One and previous studies carried out in parietal and premotor cortices (Fogassi, et al., 2005; Bonini et al., 2010), which showed a greater selectivity for the two types of actions. This lack of selectivity could be attributed to the high perceptual similarity between the two targets, which were identical in size and shape. However, this explanation seems unlikely, since the monkey had clearly no difficulties in discriminating their identity, likely based on their different color, and easily and rapidly could recognize the correct action (eating or placing) associated with each of them.

4.5. CONCLUSIONS

In this study, we recorded neurons activated during both execution and observation of actions and this result suggests that ventrolateral prefrontal cortex may host neurons with properties similar to those of mirror neurons. However, in spite of this similarity with premotor and parietal mirror neurons, prefrontal neurons showed some differences with them: anticipation of time onset of the discharge during action observation with respect to action execution and a low selectivity for the grasped target.

Taken together, these observations suggest that the role in the mirror neuron system of the neurons of the VLPFC recorded in the present work could be related to the understanding of a general intention of an agent (i.e. to grasp) regardless of the specific action final goal. Further study are needed to better investigate responses to action observation using several types of actions.

5. GENERAL CONCLUSIONS

Goal-directed grasping actions and object manipulation are skills that depend on sensory control in which predictive feedforward mechanism integrate somatosensory and visual signals with sensory-motor memory system (Johansson and Cole, 1992).

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

The aims of these studies were to explore the neural underpinnings of the organization and execution of simple and complex manipulative goal-directed actions, and to investigate the understanding of intention during observation of actions performed by others.

A particular finding of these studies is that a pool of VLPF neurons seems to integrate the contextual information to select one of two possible actions to perform. Interestingly, a set of neurons activates both during visual and motor response, showing in some cases a preference for the same target, as the visual response could be considered as a transformation of contextual information turned into specific motor intentions, subserving the selection and organization of the parieto-premotor neuronal pools underlying the execution of manipulative goal directed actions. During the active action unfolding, the selectivity for placing actions is more represented relative to eating action, and forms a rostro-caudal gradient with higher object selectivity in PFC relative to vPM and IPL regions (Bonini et al., 2010), reflecting a major role of frontal regions in encoding learned sensory-motor association. Nevertheless, the selectivity for the type of action seems to be less represented in neurons active during the observation of the action performed by another agent.

By comparing these findings with the results reported in previous studies, we note the presence of a higher number of grasping neurons selective for grasp-to-eat than grasp-to-place in the parietal cortex (Fogassi et al., 2005), a more balanced frequency of these neuronal types in the premotor cortex (Bonini et al., 2011, 2012), and a considerable prevalence of the representation of the object-related action in the prefrontal cortex. It appears that moving rostrally in the parieto-frontal motor system the relevance of learned aspects increases, while it decreases that of behavioural affordances in the action selection and control. Further studies, however, should be carried out by applying the same motor task used in this experiment to the premotor and parietal areas which have been subject to previous investigations, in order to directly verify whether a gradient in the selectivity for actions triggered by natural stimuli vs learned rules does exist in the parieto-frontal system.

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