UNIVERSITÀ DI PARMA

UNIVERSITA’ DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN

"Neuroscienze"

CICLO XXXV

Ventrolateral Prefrontal Neurons of the Monkey Encode Visual Instructions and Motor Behaviors in the Same Pragmatic Format

Coordinatore:

Chiar.mo Prof Luca Bonini

Tutore:

Chiar.mo Prof. Leonardo Fogassi

Chiar.mo Prof. Stefano Rozzi

Dottorando: Alfonso Gravante

Anni Accademici 2019/2020 – 2021/2022

1. INTRODUCTION

STUDY 1. VENTROLATERAL PREFRONTAL NEURONS OF THE MONKEY ENCODE INSTRUCTIONS IN THE 'PRAGMATIC' FORMAT OF THE ASSOCIATED BEHAVIORAL OUTCOMES

STUDY 2. VISUAL RESPONSE OF VENTROLATERAL PREFRONTAL NEURONS AND THEIR BEHAVIOR-RELATED MODULATION

Abstract

The lateral prefrontal cortex (LPF), because of its extended anatomical connections with other cortical and subcortical areas, has access to a wide set of information regarding both the internal state of the subject and the external world, that determine its involvement in a broad spectrum of sensorimotor and cognitive processes. The resulting multidimensional representations enable this cortical sector to produce flexible strategies to navigate into the complex, ever-changing social environment, exploiting contextual and motivational information for selecting and implementing appropriate behaviors, as well as for inhibiting unnecessary or inappropriate ones (Miller and Cohen 2001; Rozzi and Fogassi, 2017; Tanji and Hoshi, 2008).

Monkey electrophysiology and human fMRI studies suggested that information processing for action planning becomes more abstract when moving along a caudal-to-rostral gradient in the frontal cortex (Badre and D'Esposito, 2007, 2009; Koechlin *et al.*, 2003; Koechlin and Summerfield, 2007). Despite theoretical differences among the authors, there is general agreement that the mid-portion of LPF is involved not only in behavior selection but also in planning actions. These functions require a strict relation between the middle sector of LPF and the parieto-premotor circuits subserving sensorimotor transformations. Accordingly, anatomical studies in the monkey have indicated that this sector, in particular the part corresponding to areas 12r and 46v, is anatomically connected with the parieto-premotor circuits for grasping control (Barbas and Pandya, 1989; Borra *et al.*, 2011; Cavada and Goldman-Rakic, 1989; Cipolloni and Pandya, 1999; Gerbella *et al.*, 2013; Saleem *et al.*, 2014), suggesting that this prefrontal region could be an additional node of the *lateral grasping network*, involved in the context-based control of motor goals (see Rizzolatti *et al.*, 2014). In line with these anatomical data, previous work from our lab (Simone et al. 2015) demonstrated that the ventral part of the lateral prefrontal cortex (VLPF) contains movement-related neurons, active during grasping execution both when the behavior is instructed by abstract rules and in naturalistic situation (Simone *et al.*, 2015). Although these processes necessarily require the generation of goals based on the current context, it is largely unknown how VLPF neurons prospectively encode the instructing stimuli in relation to behavioral demands, and what is the specific format of the underlying neural representations.

To tackle this issue, in ***Study 1***, we analyzed the temporal dynamics of the responses VLPF neurons recorded during a Visuo-motor task instructed by visual cues, in which the monkey

had to observe real objects and, subsequently, to perform (Action condition) or refrain (Inaction condition) object-oriented grasping actions. Our data show that VLPF recorded sector contains neurons responding in different task phases, and that the neuronal population discharge is stronger in the Inaction condition when the instructing cue is presented, and in the Action condition in the subsequent phases, from object presentation to action execution. Decoding analyses performed on neuronal populations showed that the activity recorded during the initial phases of the task shares the same type of format with that recorded during the final phases, suggesting the *pragmatic* nature of this format and that instructions and goals are encoded by prefrontal neurons as predictions of the action outcome.

In ***Study 2***, we aimed at assessing whether prefrontal neurons visual responses exclusively depend on the visual properties of the observed stimuli or are modulated by their pragmatic features and by the environmental contingencies. To this purpose, we recorded neuronal activity of prefrontal neurons in a Visual task requiring the monkeys simply to observe images of objects on a monitor. The recording sessions were carried out in the same days as for *Study 1*, allowing us to compare the visual responses in the two tasks in the same neurons.

Our results indicate that part of VLPF neurons respond specifically to one stimulus or to a small set of stimuli, but there is no indication of a “passive” categorical coding. The comparison of neural responses recorded in the Visual and the Visuo-Motor tasks indicates that the visual responses to objects are often modulated by the task demands, with the strongest discharge when the object is target of an action.

Altogether, the data of the two studies indicate that VLPF neurons encode sensory stimuli (e.g., instructing cues and real objects) in relation to the current individual intention, and we propose that VLPF sensory-related responses are encoded at the neural level in terms of their behavioural outcome (pragmatic hypothesis).

Authors'note

This thesis is based upon one study published during my Phd program (***study 2***), and one study not submitted, yet, in which I am the co-first author (***study 1***).

1) *"Visual response of ventrolateral prefrontal neurons and their behavior-related modulation"*, published in *Scientific reports* in 2021.
   (https://doi.org/10.1038/s41598-021-89500-0)

Please note that, for the sake of readability, the supplementary figures cited in the test are shown in the appendix at the end of the thesis.

1. Introduction

1.1 Prefrontal cortex in mammals

Our mental attitude, our thoughts and, consequently, our behaviors, can be effectively modulated by our context (e.g. physical laws, space, objects, previous events, social relationships) and by information stored or created ex-novo in our brain (e.g. motivation, creativity, intention). A fundamental role in the elaboration of these cognitively complex processes, that form the basis of the evolutionary leap distinguishing man and the anthropomorphic apes from the remaining animal world, seems to be largely due to the phylogenetic development of the most anterior part of the neocortex: the prefrontal cortex (PFC). In the evolutionary history of mammals, the prefrontal cortex is a relatively recent structure: the prefrontal cortex covers only 3,5% of the cortical mantle in cats and just a little more, about 7%, in dogs. Only in primates prefrontal cortex significantly enlarges reaching 10% of the cerebral cortex in the case of gibbons and macaques and 16.9% in chimpanzees (Semendeferi *et al.*, 2002). It is in human being, however, where the development of this cortical region has occurred most considerably becoming about 30% of the total cortical mantle (Carlén, 2017). The development of the prefrontal cortex is late not only phylogenetically but also ontogenetically, in human being the maximum development of this cortical sector is reached only after adolescence (Chugani *et al.*, 1987; Paus, 1999; Sowell *et al.*, 1999). The protracted ontogenetic development of this cortical sector up to adulthood would also seem to be in line and correlated with the peculiar learning skills that characterize our early years of life (Kolk and Rakic, 2022). These results are consistent with the behavioral evidence that these areas are critical for those higher cognitive functions, such as propositional speech and reasoning, which are in fact the last to emerge from developmental processes.

The fundamental role that the prefrontal cortex plays in the implementation of cognitive complex behaviors, such as the elaboration of intentions, the formation of short- and long-term plans, the attribution of the correct affective value in different contextual situations, the ability to take decisions by exploiting environmental and mnemonic resources and the ability to abstraction, is evident in patients with focal lesions of prefrontal areas. These patients, while maintaining basic cognitive abilities, have evident deficits in executing and programming complex behaviours, such as organizing and planning long term behaviors. Until recently, the prefrontal cortex was considered the "associative" cortex of the frontal lobe (Fuster, 2001). Traditionally, the role assigned to these cortices is linked to the integration of multimodal sensory stimuli, already processed at lower levels, which converging into this region contribute to the formation of a higher order type of representation, that finally allows stimuli categorization and the development of behavioral strategies (Corbetta and Shulman, 2002; Gilbert and Sigman, 2007). Therefore, they are different from other cortical areas because they are not strictly sensory or motor areas. In this model, the prefrontal cortex assumes a "command" role within a structural organization in which mental functions are hierarchically distributed in the various cortical territories, from the sensory regions, throughout the prefrontal ones, and finally reaching ones the motor counterparts. Thus, sensory inputs that converge in the prefrontal areas would be used to generate commands sent to the motor areas, hierarchically lower, to plan actions directed to specific goals.

1.2 Anatomical organization of the prefrontal cortex

The prefrontal cortex of primates is not an anatomically and functionally homogeneous cerebral region but is cytoarchitecturally heterogeneous region, in which each subdivision is specialized in specific motor and cognitive tasks, the "higher functions" or "executive". From an architectural point of view, the PFC is a homotypic cortex with a highly developed internal granular layer (IV), which distinguishes it from the rest of the frontal cortex (Walker, 1940). Traditionally it is divided into three major regions: lateral, medial and orbital. Of the three, the lateral region develops last and most, and includes, both in humans and in non-human primates, areas 12/47, 45, 46 in the ventral part; and areas 8b and 9 in the dorsal portion (Petrides and Pandya, 1999, 2002). Subsequently, using a multiarchitectonic and connectional approaches, Luppino and coworkers (Gerbella *et al.*, 2007), further subdivided the caudal part of lateral prefrontal cortex of the macaque into eight areas: 1) three located caudally inside and near the arcuate sulcus, the areas 8FEF, 8r, 45B; 2) three located in the ventral convexity, the areas 45A, 12r and 12l; 3) two located in the dorsal convexity, the areas 8B and 9; 5) two located inside and around the ventral and the dorsal bank of the principal sulcus (PS), the areas 46v and 46d, respectively; and 6) one located in the frontal pole, the area 10.

The lateral prefrontal cortex is divided in a dorsal and a ventral sector by the Principal sulcus, and is directly or indirectly connected with widespread structures in the brain through the mediodorsal and orbito-ventral networks. The mediodorsal network (that includes the dorsolateral prefrontal cortex, DLPF) receives inputs from multimodal areas situated in the temporal cortex or auditory areas of the superior temporal gyrus and it is involved in the processing of spatial information. The orbito-ventral network (which includes the ventrolateral prefrontal cortex VLPF) mostly receives sensory inputs from visual, auditory, somato-sensory, gustatory and olfactory areas, and it is involved in the processing of non-spatial information. Additionally, the two networks are also extensively interconnected, and this organization allows the lateral prefrontal cortex to integrate multiple sets of information on a large scale, playing a fundamental role in collecting, integrating, sorting and modulating the diverse sets of "data" processed in other parts of the brain (Petrides and Pandya, 2002; Preuss and Goldman-Rakic, 1989; Tanji and Hoshi, 2008; Yeterian *et al.*, 2012).

On the basis of connectional studies (see Borra *et al.*, 2017; Gerbella *et al.*, 2017), the lateral LPFC can be subdivided at least into three vertical strips, caudo-rostrally located. The caudal-most strip is essentially connected with the parietal (mostly LIP) and frontal (FEF and SEF) areas and subcortical centers (i.e., the intermediate and superficial layers of the superior colliculus) involved in control of eye movements (Borra *et al.*, 2015; Gerbella *et al.*, 2010, 2013; (Saleem *et al.*, 2014). The rostral-most strip essentially shows intrinsic prefrontal connections, including the frontal pole and the orbital prefrontal areas (Borra *et al.*, 2011; Gerbella *et al.*, 2013; Saleem *et al.*, 2014). Finally, the intermediate strip is connected with parietal and premotor areas and subcortical structures involved in grasping and reaching movements (Borra *et al.*, 2011, 2014; Gerbella *et al.*, 2013, 2016; Saleem *et al.*, 2014).

Albeit the areas of the intermediate strips, areas 12r, 46v and part of area 46d, are connected to areas forming the parieto-frontal object-grasping circuit, each of them shows some specificity in their connections. Area 12r is connected with area F5, particularly with F5a, and AIP. In addition, it is also linked with the SII-complex and with the anterior part of the ventral bank of STS, possibly corresponding to area LB2, an area active during observation of hand grasping actions (Nelissen *et al.*, 2011). Additional connections were also observed in the middle part of the insula. The adjacent area 46v shows a pattern of connections, similar to that of area 12r, with areas F5a and AIP as well as with SII and the insula. In addition, it is strongly connected with area PFG, and to a lesser extent with area F6, cingulate motor area 24 and with the temporal lobe (Gerbella *et al.*, 2013). Area 46d shows relatively strong connections with dorsal and mesial premotor areas F7 and F6 and weaker ones with F2vr. Further 46d connections involve cingulate motor area 24 and parietal areas PG and V6A (Saleem *et al.*, 2014). Note that concerning area 12r and 46, the connections with the temporal lobe increase ventrally, suggesting a stronger role of ventral prefrontal areas in processing of object semantics and recognition.

1.3 Functional inhomogeneity of the Lateral Prefrontal cortex

Dorso-ventral organization

The physiological and behavioural studies conducted on macaques have evidenced that the prefrontal cortex plays a fundamental role in the modulation of the behavioural attitude according to the different contextual situations. Jacobsen's pioneering studies, based on lesions restricted to the lateral prefrontal region, first highlighted the involvement of this cortical sector in the development of behavioral strategies that require the use of short-term mnemonic representations, and, more recently Baddeley (1974) evidenced its role in "operative memory". The operating memory or "working memory" is a form of motor planning that involves the active maintenance of information important for the behavior or the ability to store information, concerning an object or a situation for a short period of time before the performance of a task for which such information is relevant (Fuster, 1997). Fuster and coworkers (Fuster and Alexander, 1971) were the first to observe, through electrophysiological recording studies of single units, the presence in the macaque prefrontal cortex of neurons whose activity is modulated in tasks requiring the use of operative memory such as delayed response tasks in which a movement of the arm or of the eyes towards a stimulus previously observed is required. Further and subsequent studies conducted mainly by Patricia Goldman-Rakic and coworkers (see Goldman-Rakic, 1987) led to the hypothesis that inside the prefrontal cortex there are various parallel systems of "working memory" able to use and process different sensory inputs in order to modulate specific behavioural responses in specific delayed response tasks. In particular, in analogy with the hypothesis of the existence of two distinct pathways within the visual stream, the way of "what" and "where", Goldman-Rakic hypothesized that also in the lateral prefrontal cortex there were two domains: one located in the dorsolateral prefrontal cortex and involved in processing spatial information (i.e. objects location) and one localized in the ventrolateral one involved in processing semantic information (objects features). Subsequently, other authors, based both on electrophysiological and lesion studies conducted on the monkey and on functional studies conducted on humans, proposed a dorso-ventral subdivision of the prefrontal cortex not based on the nature of the sensory elaboration but of operational type of process occurring in the two sectors. In particular, Passingham and coworkers (Passingham *et al.*, 2000) proposed that the dorsolateral portion of the prefrontal cortex is involved in "working memory" processes, while the ventrolateral portion is involved in exploiting working memories for implementing different behavioral strategies appropriate to the current environmental and contextual situations. The hypothesis proposed by Passingham is mainly based on the observation of the presence, in correspondence with the ventrolateral prefrontal cortex, of neurons active in behavioral tasks that require the use of specific operational rules, independent of the type of type of working memory (spatial or non-spatial) required. Petrides and collaborators (see Petrides, 2005) have further expanded the range of functional hypotheses on the role of the dorsal and ventral sectors of the prefrontal cortex. According to this author the dorsolateral prefrontal cortex is not involved in "working memory" processing, which would be coded in temporal and parietal areas, but would play a role in the control and monitoring of higher order aspects of complex cognitive behaviors. On the other side, the ventrolateral prefrontal cortex would be involved in first-order executive processes such as selection, comparison and judgment of the environmental and mnemonic stimuli necessary for the execution of intentional behaviours.

Rostro-caudal organization

In addition to the above described functional differences between dorsal and ventral prefrontal subdivisions, several studies evidenced also a rostro-caudal inhomogeneity in the functional organization of the prefrontal lobe (Koechlin *et al.*, 2003; Nee and D'Esposito, 2016; Riley *et al.*, 2016). In particular, these authors demonstrated the existence a rostro-caudal stimulus selectivity gradient through the entire extent of the lateral prefrontal cortex. In the study by Constantinidis and coworkers (Riley *et al.*, 2016), monkeys passively viewed visual stimuli belonging to three categories: a spatial set, a shape set, and a color set. None of these stimuli required any specific response by the monkeys, the only behavioral request being that of paying attention to the stimuli presented. The main conclusion of this study was that the posterior part of the prefrontal cortex is selective for stimuli, in contrast to the anterior region that appear to be engaged in more abstract operations. Several studies shows that neurons active in tasks requiring oculomotor responses are located caudally (e.g, Boch and Goldberg, 1989; Ichihara-Takeda and Funahashi, 2007), while neurons active during the execution of forelimb movement tend to be located more rostrally (e.g Funahashi *et al.*, 1993; Hoshi *et al.*, 1998), within the posterior two-thirds of the prefrontal cortex. These results are in agreement with anatomical evidence (see above).

1.4 Role of the Lateral prefrontal cortex in guidance and control of behaviour

Almost all the authors studying the PFC agrees on the fact that this cortical region plays an important role in guiding behaviors. In most of the functional studies carried out in monkeys to investigate the role of PFC in guiding behaviour, mainly focused on the delay period preceding the movement, or on conceptual aspects of the task, such as, for example, conditional sensorimotor behaviors that required selection of a movement arbitrarily associated with a sensory signal based on a learned rule. In a recent review, Genovesio and coworkers, proposed that PFC is crucial in monkeys for reducing "foraging errors by generating goals from current contexts and learning" to adapt to the current environment (Genovesio *et al.*, 2014). Accordingly, PFC should be involved also in guidance and control of natural actions. The role of this cortical region implementing *natural* object-oriented behaviors has been almost unexplored till recent years.

In a recent study, Simone and coworkers (2015) examined the activity of VLPF neurons with a Go-No Go task in which the monkeys were required to observe or execute grasping actions in different conditions, either requiring the use of learned rules or involving simple natural actions. The authors found that a sector of the VLPF, hosts neurons that are active during the execution of goal-directed reaching-grasping actions. These movement-related neurons were typically active during grasping execution both in the conditions instructed by abstract rules as well as in naturalistic actions. Some of them were also active during object presentation, generally discharging more strongly when the object had to be grasped rather than simply observed. Finally, although some movement-related neurons showed a preference for a grip type, none of them showed strong selectivity during object presentation. This study demonstrated that movement-related neurons are activated during grasping in different behavioral situations (grasping under visual control, grasping in darkness, memory-guided grasping, and simple grasping of food), indicating that prefrontal neuronal activity is not necessarily dependent on the learned relationship between instruction and motor output. Many of these neurons displayed a response during task epochs preceding movement execution, in line with several studies showing that the ventrolateral prefrontal cortex employs information about the visual context to generate goals by forming associations between cues and goals (Asaad *et al.*, 2000; Wallis *et al.*, 2001; White and Wise, 1999).

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

(Averbeck *et al.*, 2002; Funahashi *et al.*, 1993; Shima *et al.*, 2006; Yamagata *et al.*, 2012). Among those, many of them show prolonged differential activity starting from object presentation. This discharge is not affected by the different contextual conditions (Motor condition in light and darkness; Blocked Motor condition), as shown by the population analyses, and could thus represent a type of preparation related to object “graspability” or the maintenance of action goal representation.

1.5 Role of VLPF in the extended "lateral-grasping network"

Given the connections of the VLPF sector with parietal and premotor areas involved in hand motor control (see Borra *et al.*, 2017; Gerbella *et al.*, 2017), a working hypothesis is that prefrontal movement-related neurons play a role in a wider network subserving grasping action (see Borra *et al.*, 2011; Rizzolatti *et al.*, 20141). In fact, from a functional point of view, the neurons described by Simone et al., (2015) show similarities but also differences with parieto-premotor neurons of grasping network. In particular, the sector of the VLPF movement-related neurons showed lower grip-selectivity than that observed in premotor area F5 and in the AIP. Furthermore, the response to object presentation of VLPF grip-selective neurons is not object-specific, different from canonical neurons of F5 and object-type neurons of AIP (Murata *et al.*, 1997, 2000; Raos *et al.*, 2006). In addition, most VLPF movement-related neurons are not affected by the absence of visual control during action execution. Altogether, this evidence supports the idea that VLPF movement-related neurons, unlike parietal and premotor grasping neurons, are not involved in coding visuomotor transformations or in the visual control of hand-object interactions, but, rather, appear to encode the action goal and, partly, the way to achieve it.

Considering altogether the aforementioned studies, it is possible to conclude that VLPF cortex seems to play a role in the planning, organization and selection of behavioral output based on cues provided by the context, cues that are processed at a higher order level whether compared to the processes carried out in the parieto-premotor areas. Furthermore, besides having a role in the processing of abstract information finalized to accomplish a specific task, this region seems to use contextual cues to plan and guide behavior responses also in natural situations.

Aims

The brief description of the functional properties of PFC neurons presented above clearly indicates that this cortical region is involved in planning behavioral responses appropriate to the context. This process necessarily requires the analysis of the environment and the generation of goals based on the current context, but it is largely unknown how VLPF neurons encode the sensory stimuli in relation to behavioral demands, and which is the specific format of the underlying neural representations. To this purpose, we recorded neuronal activity of prefrontal neurons in two behavioral paradigms: 1) Visuo-motor task, in which the monkey had to observe real objects and, based on abstract cues, to perform (Action condition) or refrain (Inaction condition) object-oriented grasping actions; 2) Visual task, requiring the monkeys simply to observe a wide set of visual stimuli on a monitor.

Study 1. Ventrolateral Prefrontal Neurons of the monkey Encode Instructions in the 'Pragmatic' Format of the associated behavioral outcomes

2.1 Introduction

The term "executive functions" refers to the ability of selecting behaviours, anticipating their outcomes, and monitoring them to reach specific intended goals, and is crucial for flexibly adapting the behaviour to the continuously changing context (Funahashi and Andreau, 2013; Fuster, 2008; Genovesio *et al*., 2014; Miller and Cohen, 2001; Tanji and Hoshi, 2008). These functions rely on several more basic cognitive processes, deeply involving the prefrontal (PF) cortex, including working memory (Funahashi *et al.*, 1989; Fuster and Alexander, 1971; Goldman-Rakic, 1990; Miller *et al.*, 1996; see Constantinidis and Procyk, 2004; Miller *et al.* 2018), attention (Boussaoud and Wise, 1993; Desimone and Duncan, 1995; Everling *et al.*, 2002; Di Pellegrino and Wise, 1993; see; Katsuki and Constantinidis, 2014), response inhibition (Butter, 1969; Dias *et al.*, 1996; Nakahara *et al.*, 2002; Sakagami *et al.*, 2001; see Fuster, 2008; Levy and Wagner, 2011), rule coding (Hoshi *et al.*, 2000; Muhammad *et al.*, 2006; Wallis *et al.*, 2001; White and Wise, 1999; see Miller and Cohen, 2001), present and past objectives monitoring (Genovesio *et al*., 2006; Saito *et al*., 2005; see Fecteau and Munoz, 2003; Genovesio and Ferraina, 2014) and behavioural goal selection (Simone *et al.*, 2015; see Rozzi and Fogassi, 2017 and Tanji and Hoshi, 2008).

The role of PF in producing contextually appropriate behaviours and inhibiting the inappropriate ones is demonstrated by its lesions in humans and monkeys, producing syndromes characterized by a common feature: the executed behaviours are motorically correct, but inappropriate to the context (see Iaccarino *et al.*, 2014; Lhermitte, 1986).

Monkey electrophysiology and human fMRI studies evidenced a rostro-caudal gradient in the PF functional organization (Badre and D'Esposito, 2007; Koechlin *et al.*, 2003; Koechlin and Summerfield, 2007; Nee and D'Esposito, 2016; Riley *et al.*, 2016), suggesting the presence of a relation among neurons coding the different aspects and temporal phases of a behavioural task. Despite theoretical differences among the authors, there is general agreement that the mid-portion of PF is involved in behaviour selection and control. In line with this idea, anatomical studies in monkeys showed that this sector of the ventrolateral PF (VLPF) is anatomically connected with the parieto-premotor circuits (Barbas and Pandya, 1989; Borra *et al.*, 2011; Cavada and Goldman-Rakic, 1989; Cipolloni and Pandya, 1999; Gerbella *et al.*, 2013; Saleem *et al.*, 2014) involved in organization and control of grasping actions (see; Borra *et al.*, 2017; Rizzolatti *et al.*, 2014). The inclusion of PF in the grasping circuit suggests that this region plays a crucial role in the context-based control of motor goals (Miller, 2000; Rozzi and Fogassi, 2017; Tanji and Hoshi, 2008).

Most electrophysiological studies on monkey PF cortex adopted tasks involving the presentation of an instructing cue arbitrary associated to a specific behaviour, to be executed after a delay. There is clear evidence that PF neurons activity recorded during cue presentation, delay period and behaviour production is deeply influenced by the general rule governing the paradigm (Asaad *et al.*, 1998; Hoshi *et al.*, 2000; White and Wise, 1999). Generally, these studies employed as behavioural output the execution of eye movements (Funahashi *et al.*, 1989; 1982; Spaak *et al.*, 2017; see Funahashi, 2014) or simple forelimb movements (Hoshi *et al.*, 1998; Yamagata *et al.*, 2012), while the role of PF in the guidance of object-oriented natural actions has been rarely investigated (Bruni *et al.*, 2015; Simone *et al.*, 2015; Tanila *et al.*, 1992). Previous works from our lab demonstrated that VLPF contains grasping-related neurons (Simone *et al.*, 2015). These studies focused on specific neuronal populations responding to object observation and/or during grasping execution, while it was not investigated the role of prefrontal neurons in linking the context to these actions.

The first aim of the present study is to assess the role of VLPF in linking the abstract rules guiding a Visuo-motor task and the object features with the instructed behaviour. To this aim, we recorded VLPF neurons in a Go/NoGo task requiring executing or withholding object-oriented grasping actions.

The second aim of the study is to describe the format in which instructions and behaviours are encoded by VLPF neurons. To this aim, we described the temporal dynamics of PF neural activity during task unfolding and employed cross-temporal decoding analyses of specific VLPF populations.

2.2 Material and Methods

Subject

The experiment was carried out on two female Rhesus monkeys (*Macaca mulatta*, M1, M2) weighing about 4 kg. The animals have been previously employed in a series of experiments, whose results have already been published (Rozzi *et al.*, 2021; Simone *et al.*, 2015, 2017). All methods were carried out in accordance with the European (2010/63/EU) and the ARRIVE guidelines. The experimental protocols, the animal handling, and the surgical and experimental procedures complied with the European guidelines (2010/63/EU), and Italian laws in force on the care and use of laboratory animals, were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12, 17/07/2012; Prot. 91/OPBA/2015, 21/10/2015) and authorized by the Italian Health Ministry (D.M. 294/2012-C, 11/12/2012; 48/2016-PR, 20/01/2016).

Training and surgical procedures

The monkeys were first trained to seat on a primate chair and to familiarize with the experimental setup. At the end of the habituation sessions, a head fixation system (Crist Instruments Co. Inc.) was implanted. Then, they were trained to perform the task described below. After completion of the training, a recording chamber (32x18 mm, Alpha Omega, Nazareth, Israel) was implanted on VLPF, based on MRI scan. All surgeries were carried out under general anesthesia (ketamine hydrocloride, 5 mg/kg, i.m. and medetomidine hydrocloride, 0.1 mg/kg, i.m.), followed by postsurgical pain medication (Fogassi *et al.*, 1996; Rozzi *et al.*, 2006; Simone *et al.*, 2015).

Experimental apparatus

During training and recording sessions the monkeys seated on a monkey chair with the hand contralateral to the hemisphere to be recorded on a resting position, located 9 cm in front of the abdomen. A box containing three different objects was located in front of the monkey, 22 cm from the monkey’s chest. A small door (7x7 cm) facing the monkey at eye’s height allowed, when opened, to present the objects, one at the time. The objects were a small sphere (diameter 1 cm), a large cube (side 2 cm) and a horizontally oriented cylinder (length 4 cm, diameter 1.5 cm) and were chosen so as to elicit three different types of grip, i.e., precision grip, whole hand prehension and finger prehension, respectively. Two laser spots (instructing cues) of different colors (green and red) could be projected onto the box door or onto the object, depending on the task phase. Eye movements were recorded using an infrared pupil/corneal reflection tracking system (Iscan Inc., Cambridge, MA, USA) positioned above the box. Sampling rate was 120 Hz.

Visuo-Motor task

The Visuo-Motor task is the same as that described in Simone *et al.*, 2015. Briefly, the task consisted of two basic conditions: Action and Inaction (Fig. 1). Each trial started with the monkeys' hand on the starting position. Then, one of the two instructing cues (green=Action condition; red=Inaction condition) turned on and was projected onto the closed box door. In both conditions, the monkeys had to maintain fixation within a 6°x6° fixation window centered on the instructing cue for a randomized time interval (500-1100 ms). The, the box door opened allowing the monkeys to see one of the three objects.

In the Action condition, during object presentation, the monkeys had to maintain fixation with the green cue still on, projected onto the object. After a randomized time (700 to 1100 ms), the green cue turned off (Go signal), instructing the monkeys to reach for, grasp the object and pull it. In the Inaction condition, the monkeys were instructed by a red cue. The condition unfolding was the same as in the Action condition till the red cue turned off, requiring the monkeys to keep fixating the object for 600 ms, refraining from acting.

The order of presentation of both objects and conditions was randomized. If the monkeys correctly performed a trial, a drop of liquid reward was delivered at the end of it. A trial was discarded when the monkeys did not maintain fixation until the end of the trial, when they released the hand from the resting position during the Inaction condition or before the Go signal in the Action condition, or when they did not reach for and grasp the object with the correct prehension. Discarded trials were repeated at the end of the sequence to collect at least 30 correct trials for condition (10 trials x 3 objects).

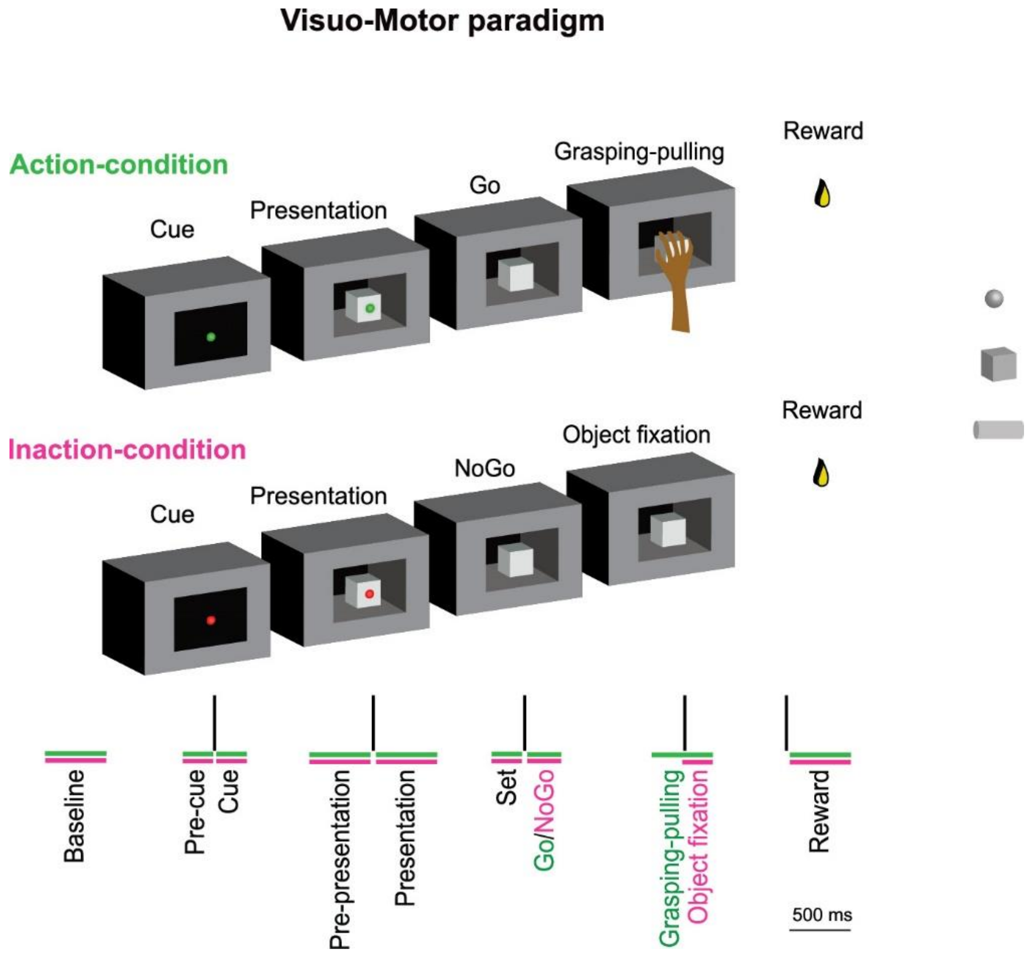


Figure 1 Temporal sequence of events in the two conditions of the behavioral paradigm. The objects used in the task are depicted on the right side. Red and green bars indicate the epochs used for statistical analysis carried out on single neurons and populations, aligned on the corresponding task event (vertical black line). For the detailed description of the paradigm, see Methods.

Recording techniques and task events acquisition

Neuronal recordings were performed by means of a multi-electrode recording system (AlphaLab Pro, Alpha Omega Engineering, Nazareth, Israel) employing glass-coated microelectrodes (impedance, 0.5-1 MOhm) inserted through the intact dura. The microelectrodes were mounted on an electrode holder (MT, Microdriving Terminal, Alpha Omega) allowing electrodes displacement, controlled by a dedicated software (EPS; Alpha Omega). The MT holder was directly mounted on the recording chamber. Neuronal activity was filtered, amplified, and monitored with a multichannel processor and sorted using a multi-spike detector (MCP Plus 8 and ASD, Alpha Omega Engineering). Spike sorting was performed using the Off-line Sorter (Plexon, Inc, Dallas TX, USA).

The experiment was controlled by a homemade Labview software. Digital output signals determined the onset and offset of laser spots, opening of the door and reward release, and contact-detecting electric circuits provided the digital signals related to monkey hand contact and release of the resting position and the beginning and end of object pulling. Analog signals provided information about eye position.

Neuronal analysis

The digital signals were also employed to align neuronal activity and to create the response histograms and the data files for statistical analysis.

The neural activity was recorded for at least 60 successful trials (thirty per condition, 10 for each object). For statistical analysis of the neural activity, nine epochs have been defined (see also Simone et al. 2015), based on the digital signals (Fig. 1) as follows:

1) Baseline: from 750 ms to 250 ms before the onset of the instructing cue; 2) Pre-cue: 250 ms preceding the onset of the instructing cue; 3) Cue: 250 ms following the onset of the instructing cue; 4) Pre-presentation: 500 ms preceding the opening of the box door; 5) Presentation: 500 ms following door opening (object presentation); 6) Set: 250 ms before the offset of the instructing cue; 7) Go/NoGo, from the offset of the instructing cue to the release of the hand starting position (Action condition) or 250 ms following the offset of the instructing cue (Inaction condition); 8) Grasping/Fixation: from 250 ms before to 250 ms after the Pulling onset (Action condition) or a time period ranging from 250 ms to 500 ms after the offset of the instructing cue (Inaction condition); 9) Reward: 500 ms following reward delivery.

Single-neuron responses were statistically evaluated by means of a 9X2 ANOVA (Factors: Epoch, Condition, $p<0.01$) followed by Newman-Keuls post hoc tests. Since trials were randomized, changes of the baseline activity across trials were not expected, and the neurons showing a significant different between baselines were discarded. Accordingly, neurons were included in our dataset and were defined as task related when the 9x2 ANOVA revealed at least one of the two following effects: 1) a significant main effect Epoch ($p<0.01$) with the relative post-hoc test showing a significant difference between the activity recorded in the Baseline epoch and in at least one of the other epochs (Condition-independent neurons); 2) an Interaction effect (Condition x Epoch, $p<0.01$), with the subsequent post-hoc test showing a significant difference between at least one epoch of one condition and both its baseline and the corresponding epoch of the other condition (Condition-dependent neurons). Considering that the epochs of Pre-cue and Reward fall in the inter-trial period, when eye movements are not controlled, we decided to consider, for our analysis, the remaining six epochs plus the Baseline.

In order to test Condition and Object selectivity in the neurons active during object presentation, all the neurons with significant responses in the Presentation epoch were further analyzed with a 3x2 ANOVA (factors: Object and Condition, $p<0.01$) followed by post hoc Newman-Keuls. Neurons were considered object selective when the analysis revealed a significant main effect Object ($p<0.01$) and the relative post-hoc test showed a significant difference between at least two objects.

Population analysis

To characterize the time course and the discharge rate of different neuronal populations with respect to the main task phases, the neuronal activity of each population was aligned with the main behavioral events. The population activity was computed as follows. The mean single neuron activity over trials, in term of firing rate, was calculated for each 20 ms bin in the two conditions. The average baseline activity was then subtracted from the mean single neuron activity over trials for each bin. Accordingly, in this analysis, 0 represents baseline activity. The net average discharge frequency of each neuron was used for subsequent statistical analysis. Each neuron contributed one entry to each data set. The statistical design adopted was the same as that employed for single neuron activity (see above). Statistical analysis was performed with a significance criterion of $p<0.01$.

Demixed Principal Components Analysis

In order to evaluate how the population of task-related neurons encodes the two conditions and the three objects during task unfolding, we adopted a data-simplification method: the demixed principal components analysis (dPCA), using freely available code provided by Kobak and coworkers (Kobak et al. 2016; url http://github.com/machenslab/dPCA).

Besides reducing the dimensionality of dataset, dPCA uses information related to specific task factors (condition and object) to calculate the percentage of variance explained by the identified factors of the task. In addition, this analysis allows to identify components non related to the chosen factors, reflecting the dynamic changes of the population activity in time which are similar for all factors. The toolbox uses a linear classifier (stratified Monte Carlo leave-group-out cross validation) to evaluate at which time points the given task elements belonging to a factor (i.e., Action vs. Inaction; Sphere vs. Cylinder vs. Cube) are significantly different from each other (see below).

First, since in our paradigm time intervals were randomized, we aligned the neural activity with different events and defined the following time periods: 1) Baseline : -750 to 250 before cue onset ; 2) Cue: 0 to 500 ms following cue onset; 3) Presentation: 0 to 500 ms following object presentation; 4) Decision: -200 to +200 ms around the Go/NoGO signal; 5) Behavioral response: -300 to +100 ms around the beginning of object holding in the Action condition and 200 to 600 ms after the NoGO signal in the Inaction condition. Subsequently we joined these time periods to create a matrix of the same time length for each trial.

Then, we classified the activity of task related neurons according to the six possible types of trial (i.e., the combination of 2 conditions and 3 objects), and calculated trial-by-trial the averaged 20-ms bins firing rate. The result was smoothed with a Gaussian-weighted moving average filter with a window of six 20 ms bins.

Starting from this dataset, we calculated the first 30 principal components. The number of repetitions used for optimal lambda calculation was 10, the number of iterations for cross-validation was 100, and the number of shuffles used to compute the Monte Carlo chance distribution was set to 100.

Finally, we plotted the time course of the two largest demixed principal components for which variance was mainly attributable to the Condition and the Object factors as well as to other possible factors unrelated to them. We considered a statistical separation of the curves when the actual classification accuracy exceeded all 100 shuffled decoding accuracies in at least 10 consecutive time bins.

Decoding Analysis

In order to estimate which type of information is coded by the different neuronal populations considered in this work and how this information is encoded in dynamic patterns of activity, we adopted a population decoding approach according to the methodology described by Meyers and coworkers (Meyers, 2013; url: http://www.readout.info/).

First, for each neuron, the activity was aligned with the main behavioral events as described in the PCA section and binned as follows: for each trial we calculated the average firing rate in bins of 60 ms, sampled at 20 ms intervals. Thus, each trial expressed in bins was defined as data point. We concatenated data points of each neuron to obtain a population of binned data characterized by a number of data points corresponding to the number of trials per decoding factor (30 data points x 2 conditions for condition decoding; 20 data points x 3 objects for object decoding). Labels were then assigned to each data point to identify the corresponding factors to be analyzed (condition or object) in an N-dimensional space (where N is the total number of neurons considered for each analysis). Next, we randomly grouped all the available data points for each neuron into *k* non-overlapping splits, where *k* is the number of data points per decoding factor (30 in the condition decoding, 20 in the object decoding). A split contained a number of data points corresponding to 2 (number of Conditions), in the Condition decoding or 3 (number of Objects), in the Object decoding, for each neuron used for the analysis. Note that each split contained a "pseudopopulation", namely, a population of neurons that were possibly recorded separately but treated as if they were recorded simultaneously. Then, we performed a cross-validation procedure consisting in training a *Poisson naïve Bayes classifier* on *k*-1 splits and testing it on the remaining one. This procedure was repeated *k* times, leaving out a different split each time. Finally, to increase the robustness of the results, the whole decoding procedure was repeated 50 times using different data points in the training and test splits, and the decoding accuracy from all these repetitions was averaged.

In order to evaluate the dynamics of the temporal evolution of information coding, we applied a temporal cross-decoding analysis (TCT), which consists in training the classifier at time t and testing it at all the other time bins.

The data alignment on task events and the binning procedure described above lead to merge in the same bin the activity at the border between two subsequent periods of the task (bins of 60 ms, sampled at 20 ms intervals). Accordingly, in our TCT, we removed the last two bins of each task period considered in the analysis.

Anatomical reconstruction of the neuronal properties.

The recording region was reconstructed based on the location of the penetrations in stereotaxic coordinates plotted onto the MRI scans of the brain of each investigated monkeys (see Simone *et al*., 2015). Penetration depth, as reported by the protocol, was matched with its location with respect to the sulci.

2.3 Results

Behavioral results

In order to calculate the task performance, we considered, for each monkey, more than 1500 trials from 21 recording sessions randomly chosen (M1: 1504; M2: 1531). The task performance was higher than 80% in both monkeys (M1: 84%; M2: 82%). The percentage of successful trials was higher for the Inaction (M1: 93,7%; M2 94,3%) than for the Action condition (M1: 77,1%; M2 75,4%).

Properties of task-related neurons

We recorded neural activity from the left VLPF of two monkeys during the execution of the Visuo-Motor task. The recorded sector covers a large cortical region including most of VLPF, excluding its rostralmost sector, and slightly extending in the dorsal prefrontal cortex (Fig. 2 A).

We recorded 1390 task-related neurons based on the criteria described in the Methods section. Of them, 672 had a significant response with respect to the baseline in only one epoch, while the remaining had a significant response in two or more epochs.

Figure 2 B shows the number of neural responses recorded in each of the considered epochs. The most represented epoch is that of Presentation followed by Grasping/Fixation, Go/NoGo and Cue. Out of all task-related neurons, 430 (31%) showed a significant differential discharge between Action and Inaction conditions in at least one epoch (Condition-dependent neurons; Interaction effect, followed by Newman Keuls post-hoc, $p<0.01$), while 960 (69%) did not show any significant difference between conditions (Condition-independent neurons). Figure 2 C depicts for each epoch the percentage of Condition-dependent neurons preferring the Action (green) or Inaction (red) condition. It is clear that, while in the Cue epoch there is a much higher percentage of neurons preferring the Inaction condition, this difference reverses from Presentation epoch onward.

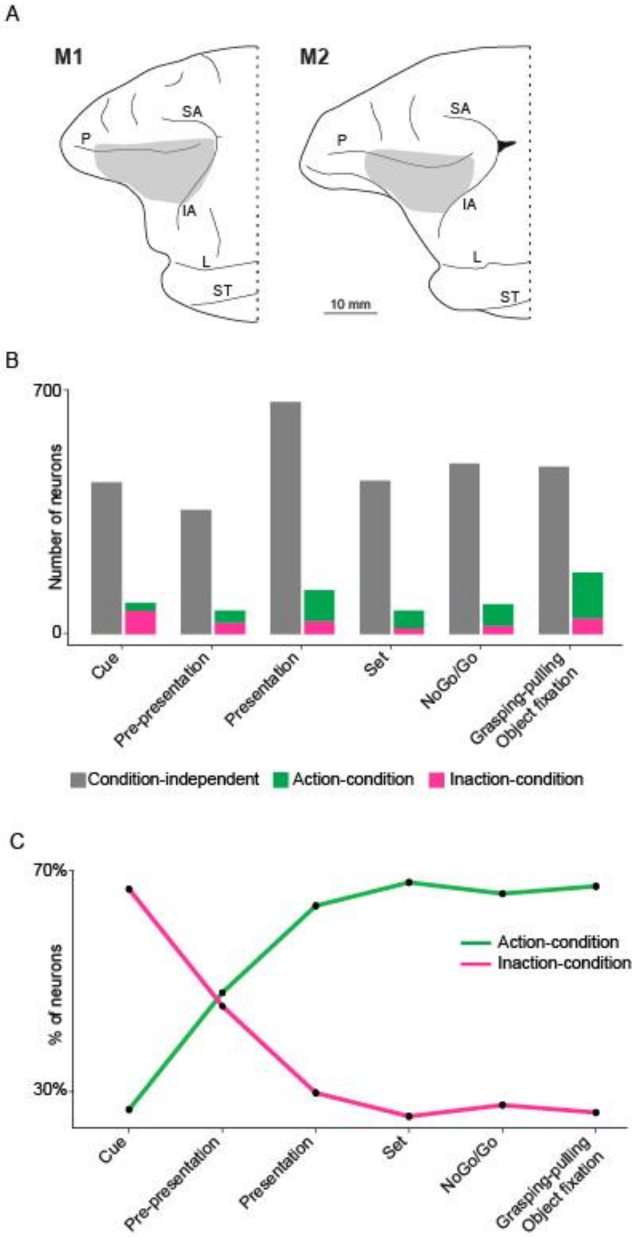


Figure 2 A Reconstructions of the left hemisphere of the two monkeys (M1 and M2), showing the recorded region (shaded area). IA: inferior arcuate sulcus; L: lateral fissure; P: principal sulcus; SA: superior arcuate sulcus; ST: superior temporal sulcus. B Distribution of neuronal responses in the different task epochs. For each epoch the grey bar indicates the number of Condition-independent neurons, the green bar that of Action-related neurons, and the magenta bar that of Inaction-related neurons. C Percentage of Condition-dependent neurons preferring the Action or Inaction condition in the different epochs. For each epoch, the ratio, expressed as percentage, is calculated as the number of neurons with preferential response in each condition divided by the total number of neurons showing an Interaction effect in that epoch.

Condition-dependency of task related neurons

We carried out a demixed principal component analysis (dPCA, see Methods) on task related neurons (n = 1240) to evaluate how this population encodes the two conditions and the three objects during task unfolding. Figure 3 A shows the distribution of variance among factors. Most of the variance of the activity (69%) is factor independent. For the rest, a larger part of the variance (18%) is captured by the Condition factor, a smaller one (9%) by the Object factor and the remaining (4%) depends on the interaction between the two factors.

Figure 3 B depicts the first two demixed principal components for which variance is mainly attributable to Factor-independent parameters (upper row), Condition factor (middle row), and Object factor (lower row). The trajectories of the first two principal components not dependent from the two considered factors show two peaks on cue and presentation onset. Note that component 2 remains modulated after object presentation in the late phase of the task, during decision and Behavioral response period (see Methods for the definition of the temporal period). In both the components related to the Condition factor the firing rate between the Action and Inaction conditions start differing just after cue onset. In Component 4 the difference between conditions gradually increases until the end of the task, reaching its maximum in the behavioral response period, in component 7 reverts a first time just before object presentation and a second time in the behavioral response phase. In both the components related to the Object factor, the firing rate difference among objects emerges after object presentation and remains significantly different till the end of the task.

Figure 3 C shows the net mean activity relative to the two conditions of the population of task-related neurons showing an increase in discharge in at least one of the considered statistical epochs ($n = 978$). In order to evaluate differences in the neuronal population discharge in different epochs and condition we carried out a 9x2ANOVA on the same factors used for single neuron analysis (see Methods). From the figure, it is evident that there are three main task phases that elicit a strong neural discharge. The first corresponds to cue onset in which the peak during the Inaction condition is higher than that of the Action condition (9x2 ANOVA, Interaction effect $p<0.01$); the second peak, the highest, occurs after object appearance, the activity being stronger in the Action condition. The third corresponds to the period going from the Go/NoGo signal to the beginning of object pulling, in which the activity is stronger in the Action condition. Note that the difference in activity between the two conditions abruptly ceases on object pulling.

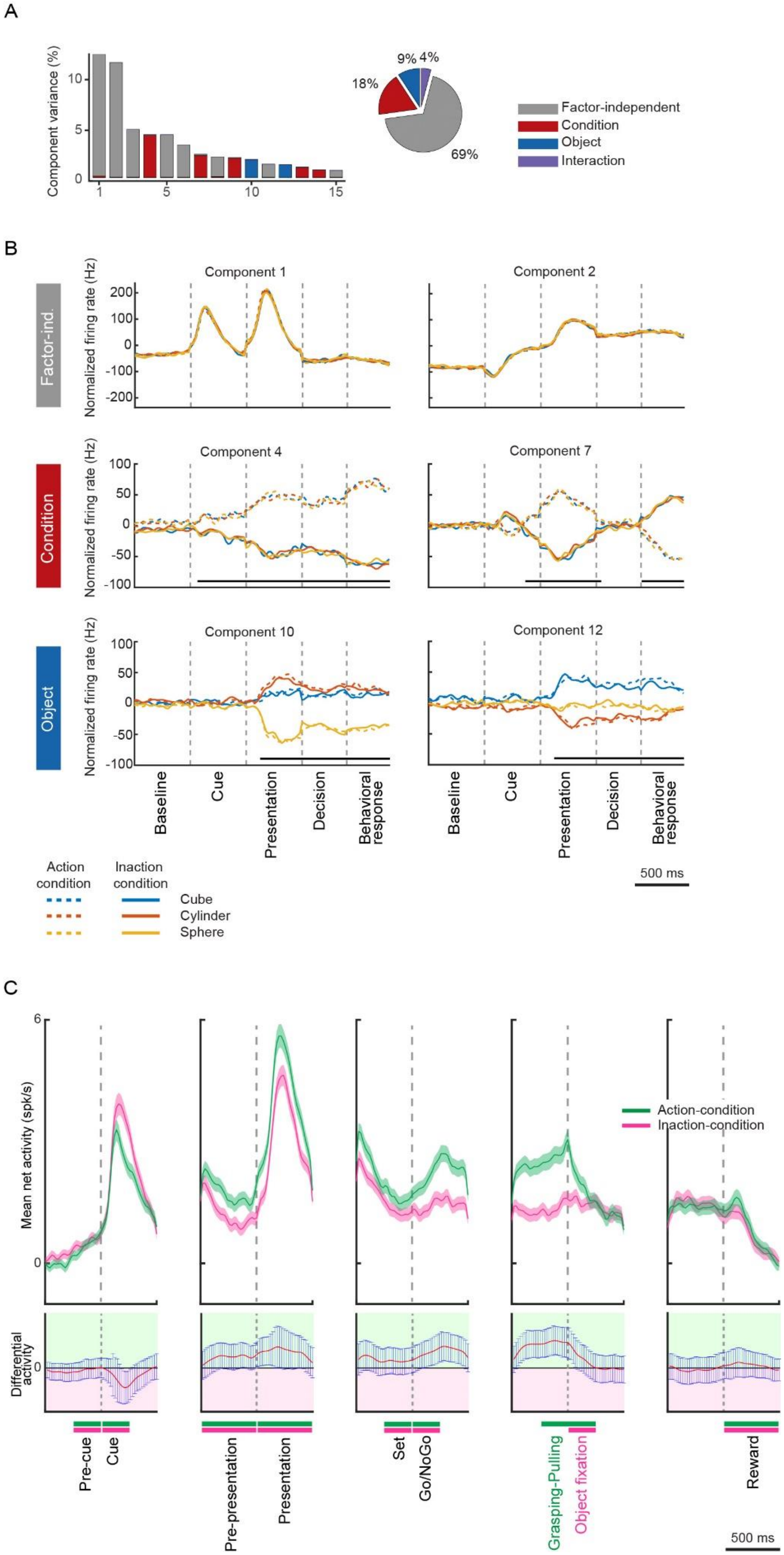

A
Component variance (%)
10
5
0
1
5
10
15
9%
4%
18%
69%
Factor-independent
Condition
Object
Interaction
B
Factor-ind.
Normalized firing rate (Hz)
Component 1
Component 2
200
100
0
-100
-200
Condition
Component 4
Component 7
100
50
0
-50
-100
Object
Component 10
Component 12
Baseline
Cue
Presentation
Decision
Behavioral response
Action condition
Inaction condition
Cube
Cylinder
Sphere
500 ms
C
6
0
Mean net activity (spk/s)
Action-condition
Inaction-condition
Differential activity
0
Pre-cue
Cue
Pre-presentation
Presentation
Set
Go/NoGo
Grasping-Pulling
Object fixation
Reward
500 ms

Figure 3 A, **B** Demixed principal component analysis. **A** Each bar of the histogram shows the percentage of explained variance of the individual demixed principal components. Pie chart shows how the total signal variance is split between the factors indicated on the right. **B** Each panel depicts the time course of the projections of the two largest demixed principal components that can be attributed to the Factor-independent (first row), Condition (second row) and Object (third row) factors. In each panel the dashed vertical lines indicate the beginning of the considered time periods (see Methods). Horizontal thick lines below the trajectories indicate the time intervals where task factors (Condition and Object) are reliably decoded (see Methods).

C Temporal profile of the discharge of the neuronal population. In the upper part of each panel, the magenta and green curves indicate the population mean net activity in the Inaction and Action condition, respectively, of task-related neurons showing a significant increase of discharge in at least one epoch. The shaded area around each curve represents standard errors. In the lower part of each panel, the magenta curve represents the differential activity (Action minus Inaction), and the blue bars represents three standards errors. The neuronal activity is aligned on the main task events (vertical dashed lines), that are used for the identification of statistical epochs (magenta and green bars). Abscissae: time. Ordinates: mean net activity.

Coding of the instructing Cue

Four hundred fifty-one neurons had a significant response in the Cue epoch (see Methods). Of them 359 (79.6%) were Condition-independent, while 92 (20.4%) were Condition-dependent, showing a preference for the Inaction (68) or the Action (24) condition. Figure 4 A shows the distribution of condition preference expressed in the different epochs by all neurons responding in the Cue epoch.

Figure 4 B-D shows examples of neurons responding to the appearance of the instructing cue. The neuron depicted in Figure 4 B responds equally well to the two cues, those in C and D have a clear preference for the green or red cue, respectively.

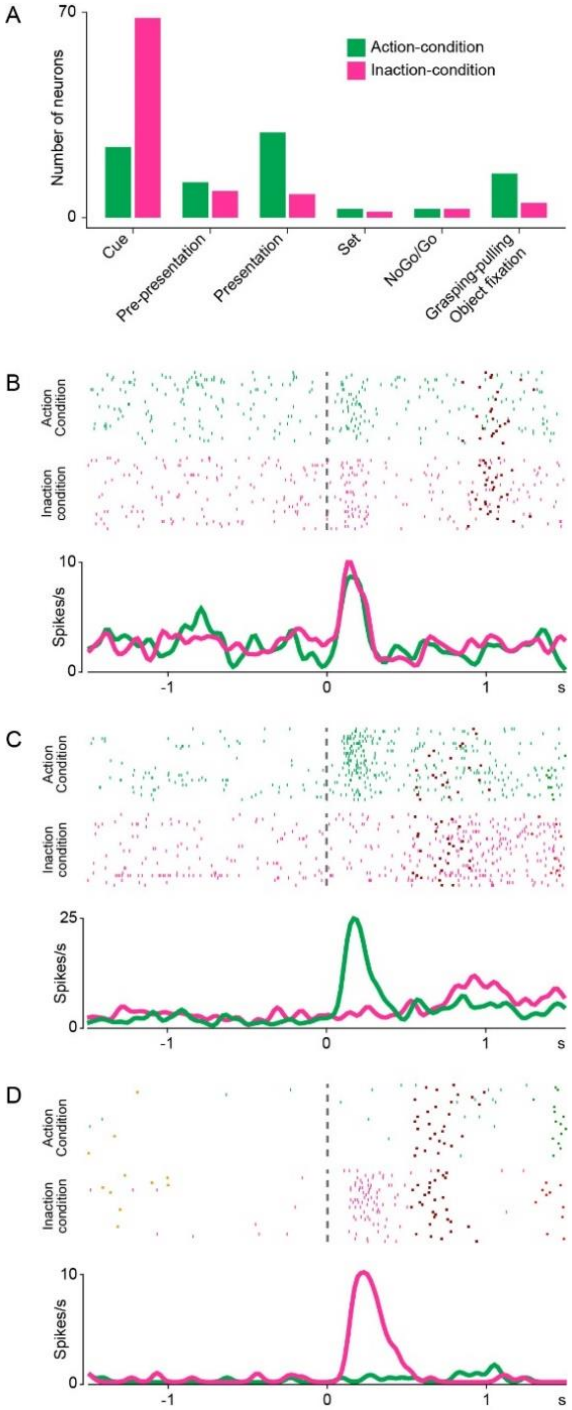


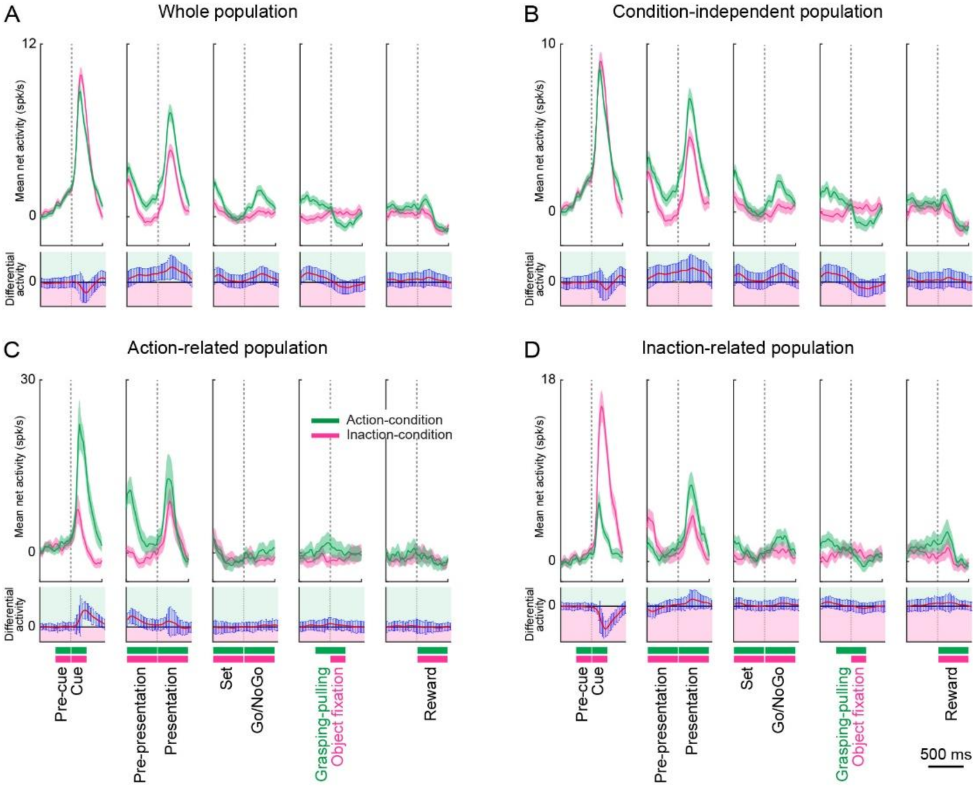
Figure 4 Neuronal responses in the Cue epoch. A Number of neurons showing Condition dependency in the Cue epoch, having a preference for the Action or Inaction condition in the different task epochs. B Example of neuron showing a similar discharge profile following cue onset in both conditions. C Example of neuron responding to cue appearance exclusively in the Action condition. D Example of neuron responding to cue appearance only in the Inaction condition. Rasters and histograms are aligned (vertical dashed line) with the onset of the instructing cues. Brown squares: object presentation; Green/Magenta circles: Go/NoGo signal. Abscissae: time (s); Ordinates: firing rate (spikes/s).

Figure 5 A-D shows the mean net activity of the populations of neurons responding with an increase in discharge to cue appearance. The four graphs correspond to the response of whole population of neurons (*n* = 402), the Condition-independent neurons (*n* = 310), the Condition-dependent neurons preferring the Action (*n* = 24) and Inaction (*n* = 68) condition, respectively.

The whole population of neurons (A) has a strong activity in the Cue and Presentation epochs in both conditions. The mean Inaction-related activity in the Cue epoch is significantly higher than the Action-related one, and this preference reverses in the Presentation epoch (9X2 ANOVA, Interaction effect, p<0.01, see Methods). It is also evident that the population shows, only in the Action condition, a prolonged response above baseline from the Go signal till the beginning of holding, after which the response has an abrupt decrease. A similar profile is also visible in the Condition-independent population (B). The two populations of Condition-dependent neurons show different profiles. In fact, while in Action-related neuronal population (C) there is a significant preference for the Action condition in the Cue Epoch, that tends to remain, though not statistically significant, in the Presentation Epoch, in the Inaction-related neurons (D) there is a preference for the Inaction condition in the Cue epoch that reverses in the Presentation epoch. The preference for the Action condition in the Presentation epoch in both Condition-dependent populations does not completely account for that observed in the whole population of cue-related neurons, since is also present in the population of Condition-independent neurons.

Figure 5 A'-D' shows the accuracy level for the decoding of the Condition factor when the classifier was trained and tested on different time periods (temporal-cross decoding plots, see Methods) for the four corresponding populations shown in Figure 5 A-D. In all populations, the highest decoding accuracies occur along the diagonal, with the lowest level of accuracy shown by the population of Condition-independent neurons (*n* = 358). In addition, along the diagonal there is a clear decrease in accuracy mainly around the Decision period in all populations, especially evident in that of Action-related neurons (*n* = 24). Finally, in this latter population of neurons, the decoding performance is also high when training on data from the Cue period and testing on data from the Behavioral response one and vice versa. This result might indicate that between the Cue and the Behavioral response periods there is a common pattern of activity encoding the Condition factor. Finally, in the population of Inaction-related neurons (*n* = 67), as well as in the whole population (*n* = 443), the decoding performance is quite high when training on data from the Decision period and testing on data from Cue period.

Cue-related populations



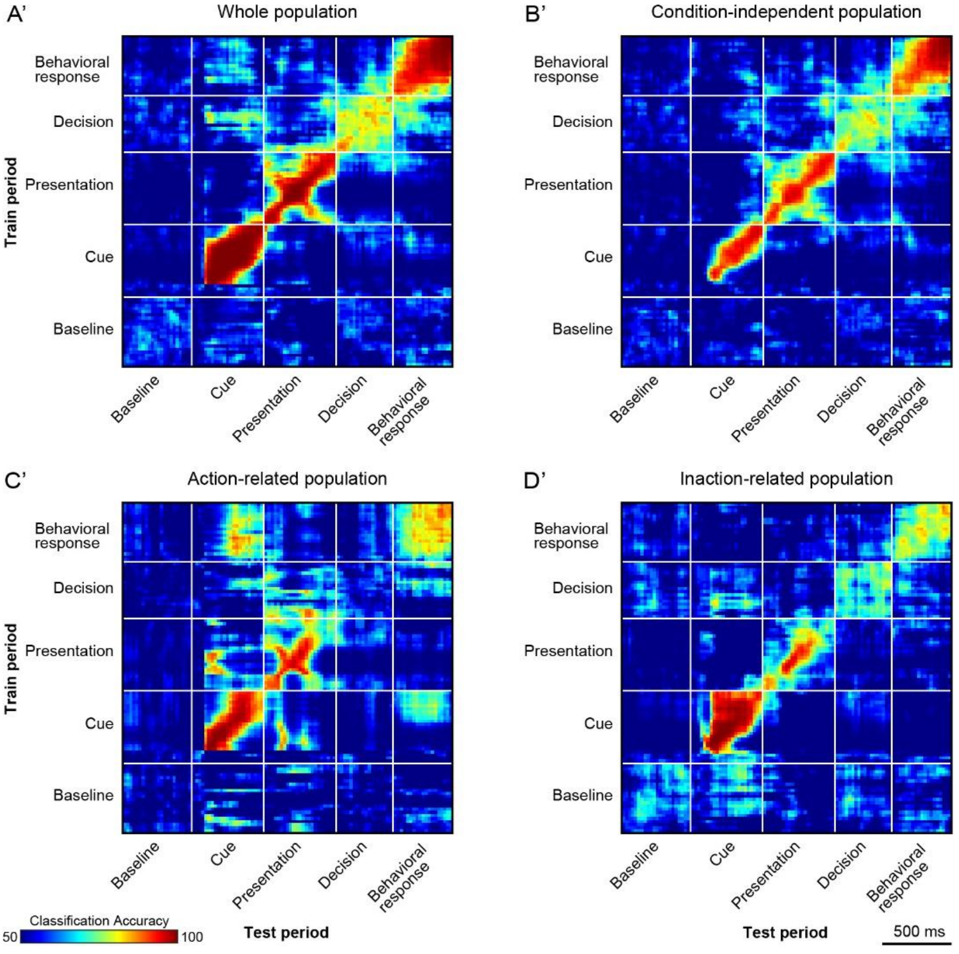


Figure 5

A, **B**, **C**, **D** Temporal profile of the mean net and differential activity of the populations of neurons responding with an increase in discharge to cue appearance. **A** Whole population; **B** Condition-independent neurons; **C** Action-related neurons; **D** Inaction-related neurons. Alignments and conventions as in Fig. 3 C.

A', **B'**, **C'**, **D'** Temporal-cross decoding analysis of the Condition factor (Action and Inaction) in the populations of neurons responding to cue appearance. **A'** Whole population; **B'** Condition-independent neurons; **C'** Action-related neurons; **D'** Inaction-related neurons. The decoding accuracy (color-coded) is computed in bins of 60 ms, sampled at 20 ms intervals. For each plot, the vertical and horizontal lines indicate the beginning of the considered time period (see Methods). Decoding periods of testing and training are indicated on the X and Y axes, respectively.

Neural response to Object presentation

Condition dependency. About half of task-related neurons showed a significant response in the Presentation epoch (678, 48.7%) Of them, 551 (81.2%) were Condition-independent, while 127 (18.7%) were Condition-dependent, 38 preferring the Inaction and 89 the Action condition. Figure 6 A shows the distribution of condition preference expressed in the different epochs by all neurons responding in the Presentation epoch.

Figure 6 B-D shows examples of neurons responding to object presentation, with no statistical difference between the two conditions (B), a clear preference for the Action (C) or Inaction condition (D), respectively.

Figure 7 A-D shows the net activity of the populations of neurons showing a significant increase in discharge in the Presentation with respect to the Baseline epoch. The depicted populations are presented in the same order as that of Figure 5.

In all populations the response to object presentation is the highest among all epochs. Note that in the whole population (n = 530) as well as in that of Condition-independent neurons (n = 407) the response to object presentation is significantly higher in the Action than in the Inaction condition (9X2 ANOVA, Interaction effect, $p<0.01$, see Methods). A second, although smaller, response present in all populations occurs in the Cue epoch. A third response present in all populations but that of Inaction-related neurons, occurs only in the Action condition and starts after the Go signal, abruptly ending after the beginning of object holding.

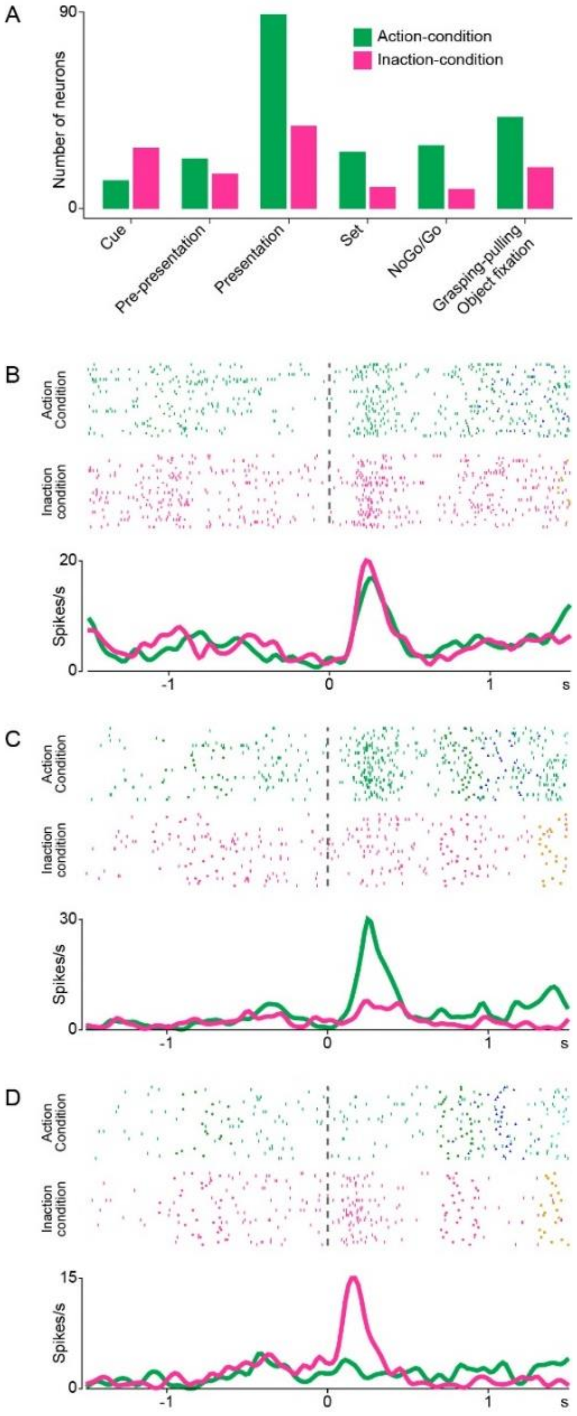


Figure 6 Neuronal responses in the Presentation epoch.

A Number of neurons showing Condition dependency in the Presentation epoch, having a preference for the Action or Inaction condition in the different task epochs. B Example of neuron showing a similar discharge in the two conditions. C Example of neuron responding to object presentation exclusively in the Action condition. D Example of neuron discharging for object presentation only in the Inaction condition. Rasters and histograms are aligned (vertical dashed line) with the onset of object presentation. Green circles: Action cue appearance/Go signal; Magenta circles: Inaction cue appearance/NoGo signal; Blue triangles: release of the hand starting position (Action condition); Cyan diamonds: beginning of object pulling; Ocher squares: reward delivery. Other conventions as in Fig. 4.

The two populations of Condition-dependent neurons (Action: n = 88; Inaction: n = 38) show markedly different profiles. In fact, while in Action-related neurons there is a significant preference for the Action condition in the Presentation, Go/NoGo and Grasping/Fixation epochs, in the Inaction-related neurons there is a preference for the Inaction condition only in the Presentation epoch. Figure 7 A'-D' shows the accuracy level for the decoding of the Condition factor when the classifier was trained and tested on different time periods (temporal-cross decoding plots, see Methods) for four different populations, presented in the same order as that of Figure 5. In all populations, the highest decoding accuracies occur along the diagonal, with the lowest level of accuracy shown by the population of Condition-independent neurons, and, along the diagonal, a clear decrease in accuracy mainly in the Decision period in the populations of Condition-independent (*n* = 551) and in that of Inaction-related neurons (*n* = 38). In this latter population the decrease in accuracy continues also in the Behavioral response period. In addition, in this population of neurons, the decoding performance is high also when training on data from the Cue period and testing on data from the Behavioral response one and vice versa. Concerning the population of Action-related neurons (*n* = 88), this is characterized by a high decoding accuracy when the classifier was trained on data from the initial and late phase of the Presentation period and tested on the Decision and Behavioral response period. The decoding performance is high also when training on data from the Decision period and testing on data from the Behavioral response one and vice versa. This suggests that in these two periods there is a common pattern of activity encoding the Condition factor. This static pattern also extends to specific phases of the Presentation and Cue periods, although the decoding performance is oscillating in terms of accuracy. The general decoding performance observed in the whole population (*n* = 673) is very similar to that of Action-related neurons.

Presentation-related populations

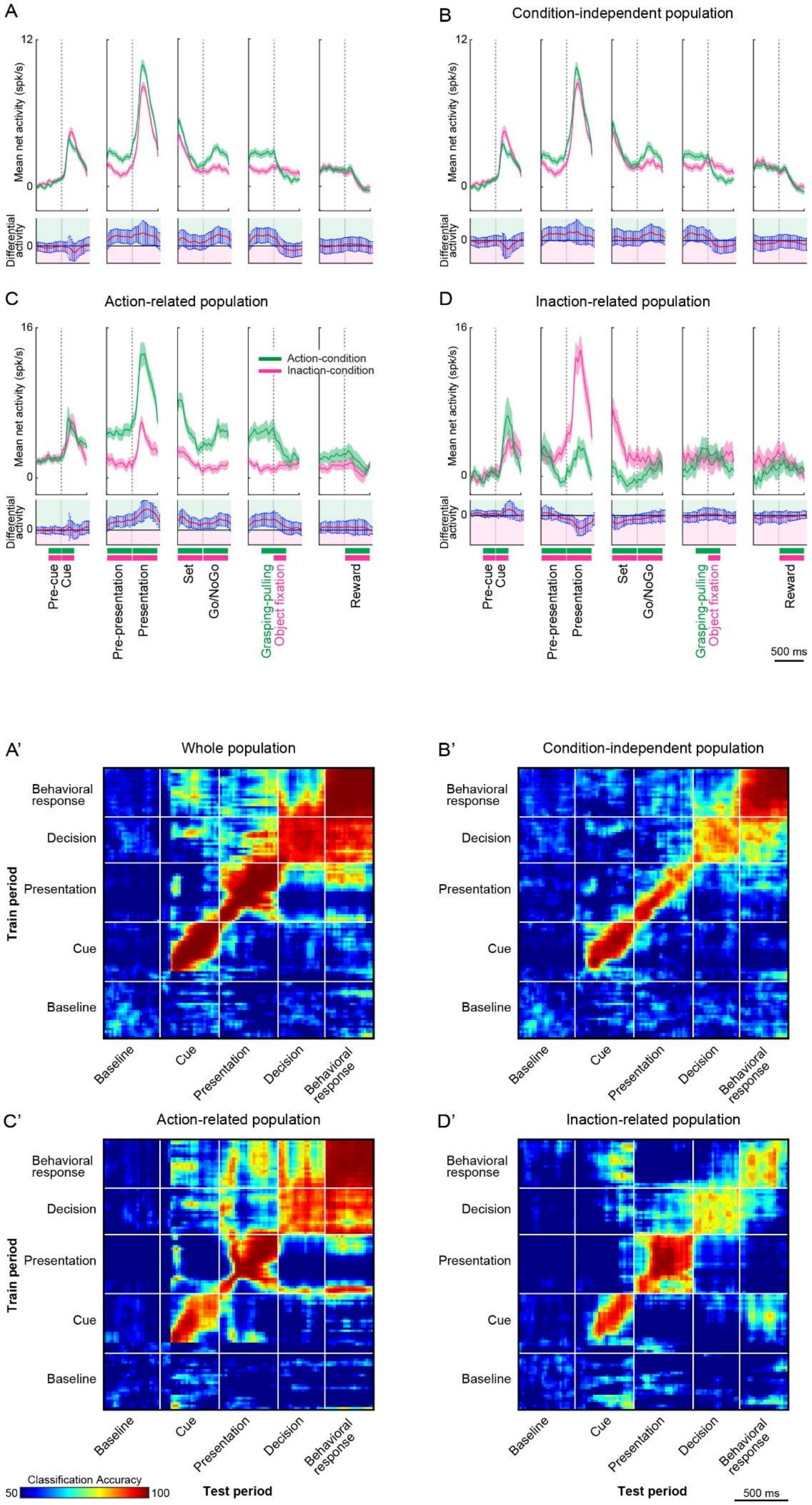


Figure 7

A, **B**, **C**, **D** Temporal profile of the mean net activity and differential activity of the populations of neurons responding with an increase in discharge to object presentation. Alignments and conventions as in Fig. 3 C.

A', **B'**, **C'**, **D'** Temporal-cross decoding analysis of the Condition factor (Action and Inaction) in the populations of neurons responding during object presentation. Alignments and conventions as in Fig. 5.

Object preference. In order to assess whether neurons responding to object presentation had some type of object and/or condition preference, we run a 3X2 ANOVA (factors: Object and Condition, p<0.01; see Methods). This analysis revealed that 26.8% of presentation neurons differentially respond in the two conditions, while a smaller percentage (11.2%) shows some type of object selectivity. This selectivity is almost equally distributed among the three objects (33, sphere; 23, cube; 21, cylinder). Figure 8 A shows an example of a neuron responding to the presentation of the three objects only in the Action condition. Figure 8 B depicts a neuron preferentially responding to the presentation of the cylinder in both conditions. Only 3% of presentation neurons show a significant Interaction (Condition x Object), indicating a high selectivity for a specific object in a given condition, as shown by the neuron in Fig. 8 C, responding strongest to the presentation of the cylinder in the Action condition.

Figure 8 D, E show the accuracy level for the decoding of the Object factor when the classifier is trained and tested on different time periods for the populations of neurons responding to object presentation with a differential response for conditions (n = 182) and objects (n = 76). In the population of neurons differentially responding in the two conditions, the decoding accuracy is high only along the diagonal in the second half of the presentation phase. In that of neurons differentially responding to objects, the highest decoding accuracy is present after object presentation, but it is also evident when training on data from the Presentation period and testing on data from the Behavioral response period and vice versa.

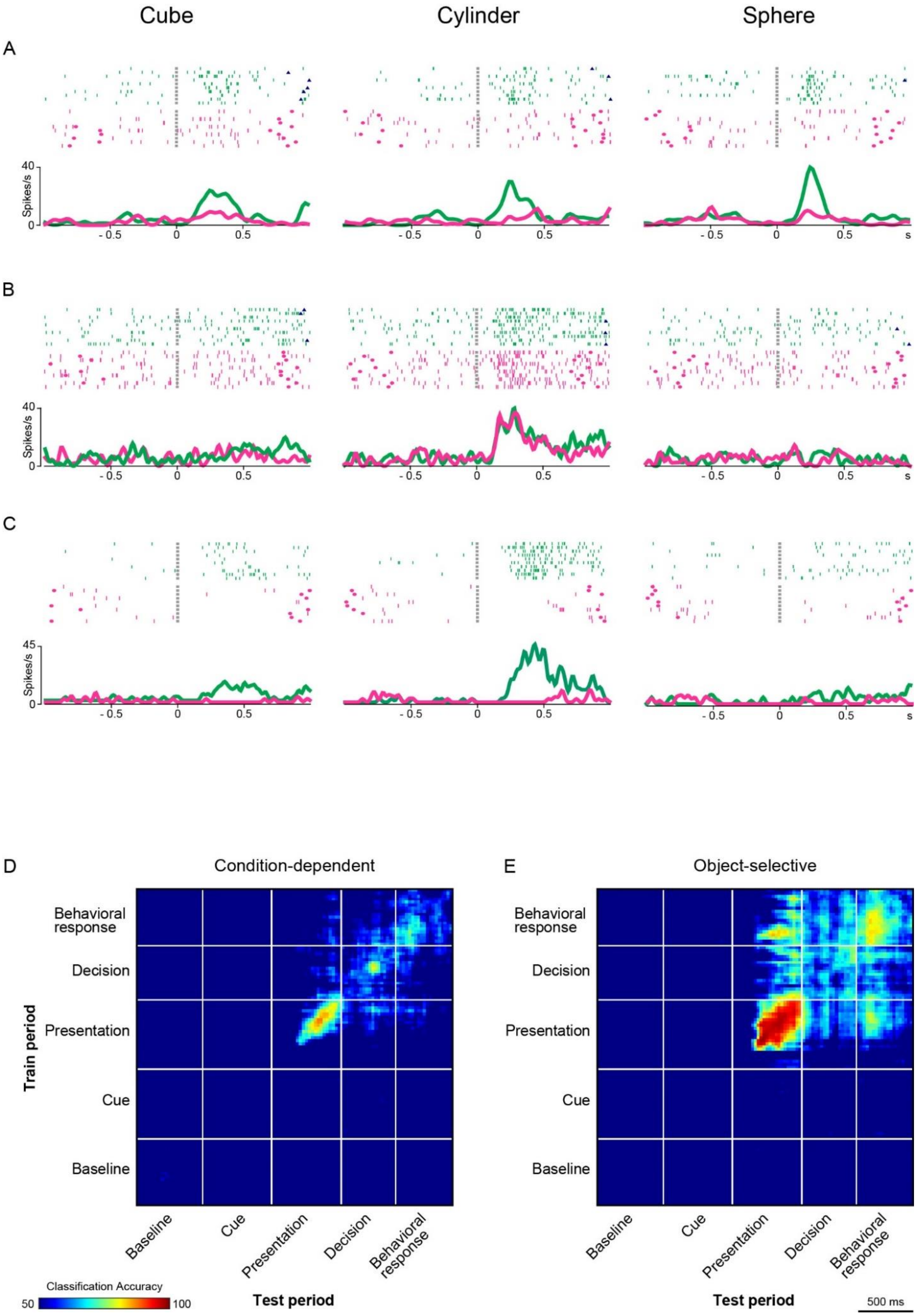

Cube
Cylinder
Sphere
A
B
C
40
45
Spikes/s
0
- 0.5
0
0.5
s
D
Condition-dependent
E
Object-selective
Train period
Behavioral response
Decision
Presentation
Cue
Baseline
Test period
Baseline
Cue
Presentation
Decision
Behavioral response
Classification Accuracy
50
100
500 ms

Figure 8

A Example of neuron responding during the observation of all presented objects only in the Action condition. **B** Example of neuron active during the observation of the Cylinder in both conditions. **C** Example of neuron activated exclusively during the observation of the cylinder, with a significantly higher discharge in the Action condition.

D, **E** Temporal-cross decoding analysis of the Object factor (Cube, Cylinder, Sphere) performed on the population of neurons responding during object presentation. **D** Subpopulation of Condition-dependent neurons; **E** Subpopulation of Object-selective neurons. Alignments and conventions as in Fig. 5.

Neural activity during the behavioral response phase

Since the pattern of activity of the population of task-related neurons is very similar in the Go/NoGo and Grasping/Fixation epochs (see Fig. 3 C), we decided to analyze together the neurons responding during these two epochs (behavioral response phase). Note also that the large majority (67.7%) of neurons activating in the Go/NoGo and/or Grasping/Fixation epochs, actually respond in both epochs.

About half of task-related neurons showed a significant response in the behavioral response phase (710, 51.4%). Of them, 494 (69.6%) were Condition-independent, while 216 (30.4%) were Condition-dependent, 52 preferring the Inaction and 164 the Action condition. Figure 9 A shows the distribution of condition preference expressed in the different epochs by all neurons responding in the behavioral response phase.

Figure 9 B-D shows examples of neurons responding in the behavioral response phase, with no statistical difference between the two conditions (Fig. 9 B), a clear preference for the Action (C) or Inaction (D) condition, respectively.

Figure 10 A-D shows the net mean activity of the populations of neurons showing a significant increase in discharge in the Go/NoGo and/or Grasping/Fixation epochs with respect to the Baseline epoch. The whole population (n = 424) shows a strong discharge in the Presentation epoch and in the behavioral response phase, but a significant difference between the two conditions emerges only in the Go/NoGo and/or Grasping/Fixation epochs. In the population of Condition-independent neurons (n = 222) the response to object presentation is the highest, while it decreases in the behavioral response phase, and no significant differences between conditions in any epoch is present (9x2 ANOVA, Interaction effect: n.s.). In the population of Action-related neurons (n = 160) the differential response starts growing during Presentation epoch, further increases in the subsequent epochs peaking on object pulling and decreases during object holding. In the population Inaction-related neurons (n = 44) the discharge increases during the behavioral response phase, although the statistical analysis does not reveal any significant difference between conditions (9x2 ANOVA, interaction effect: n.s.).

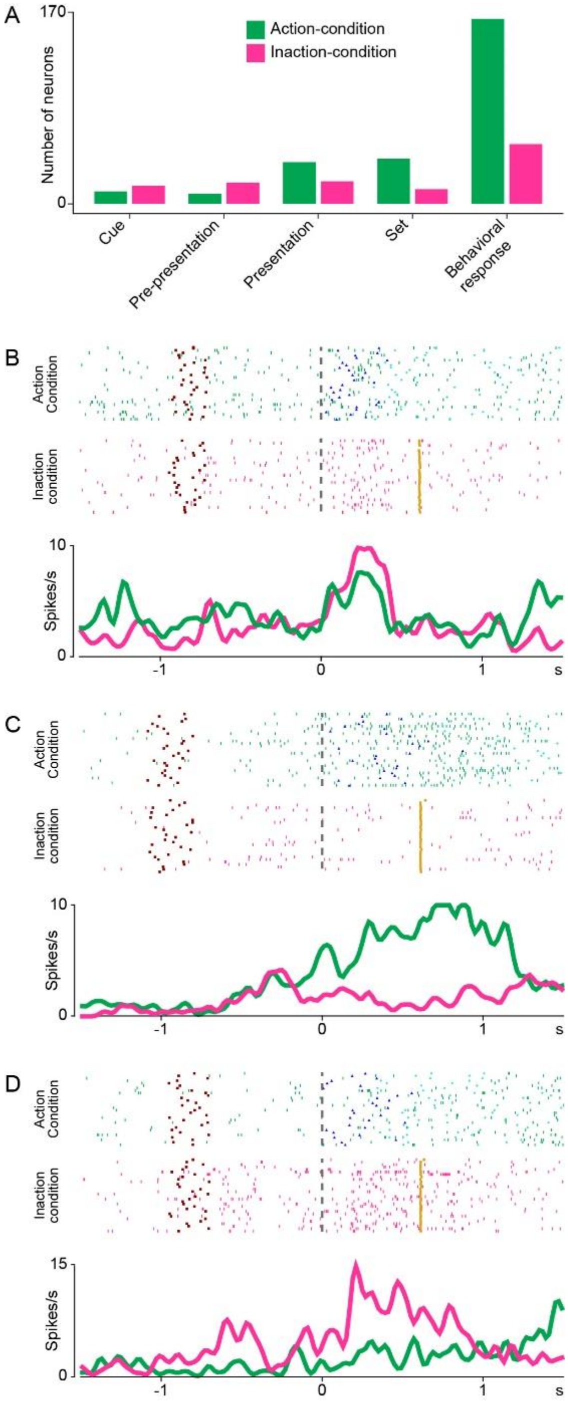
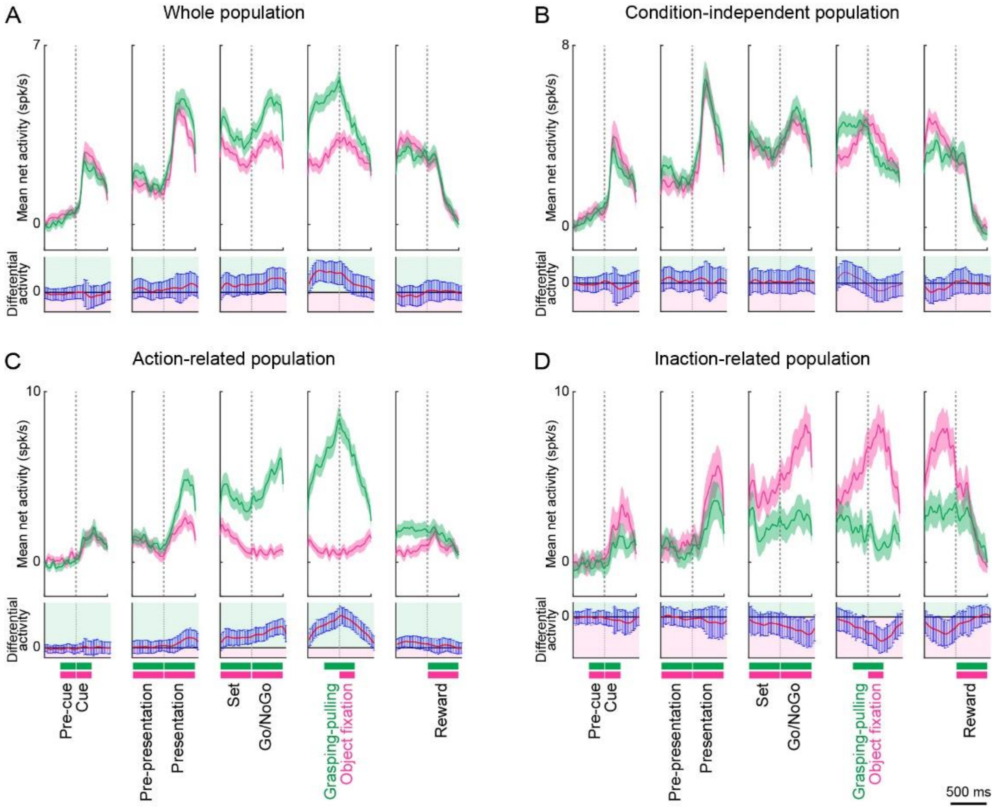


Figure 9 Neuronal responses in the Behavioural response phase.

A Number of neurons showing a Condition dependency in the Behavioral response phase, having a preference for the Action or Inaction condition in the different task epochs. **B** Example of neuron showing a similar discharge in the two conditions. **C** Example of neuron responding in the Behavioral response phase only in the Action condition. **D** Example of neuron discharging in the Behavioral response phase exclusively in the Inaction condition. Rasters and histograms are aligned (vertical dashed line) with the Go/NoGo signal (cue disappearance). Other conventions as in Fig. 4 and 6.

Behavioural reponse-related populations



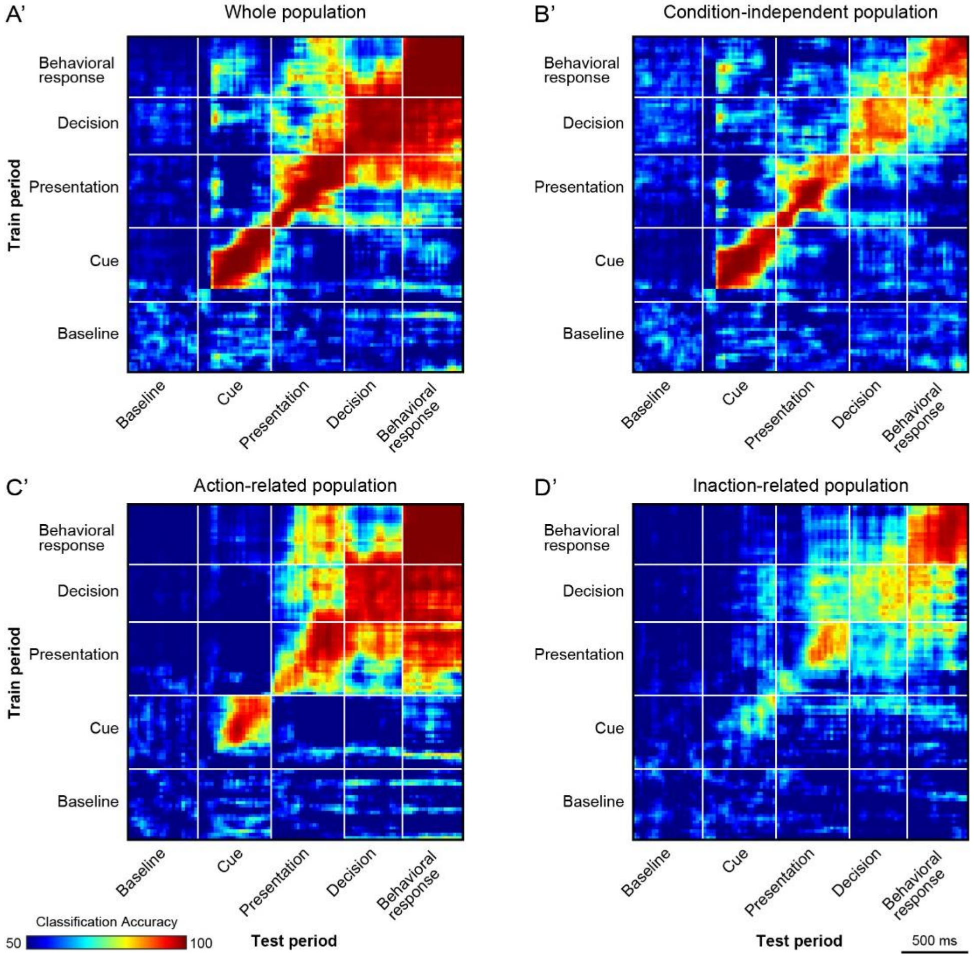


Figure 10

A, **B**, **C**, **D** temporal profile of the mean net activity and differential activity of the populations of neurons responding with an increase in discharge in the Behavioural response phase. Alignments and conventions as in Fig. 3C.

A', **B'**, **C'**, **D'** Temporal Cross-Time decoding analysis (TCT) of the condition factor (Action and Inaction) in the populations of neurons responding during the Behavioural response phase. Alignments and conventions as in Fig. 5.

2.4 Discussion

In this work, we recorded single neuron activity from ventral prefrontal cortex to investigate its role in coding execution or withholding of grasping actions instructed by abstract rules and based on object features. In particular, we focused on the temporal dynamics of functionally identified populations of neurons.

The results of our work show that: a) the main factor influencing neural discharge is the behavioral condition (Action/Inaction), while object coding is a less relevant factor; b) there is a clear preference of the whole neuronal population for the Inaction condition when the instructing cue is presented, while this preference inverts in favor of the Action condition, from object presentation onward; c) the study of the dynamic response of specific populations during the different phases of the task reveals that the same type of neural coding is shared between different epochs.

In order to study typical prefrontal functions such as action selection and inhibition, we chose a Go/NoGo paradigm. However, our Go/NoGo task implied necessarily a difference in complexity between conditions: while in the Inaction condition the choice of the behavioral response relied only on the instruction cue, in the Action condition, the type of object and the timing of action initiation were also relevant. Accordingly, the behavioral assessment clearly showed that the percentage of errors was higher in the Action condition. These considerations allow us to better interpret the results that show a difference in the dynamic of discharge between the two conditions (see below).

The cue instructing the task rule is prospectively encoded in the format of behaviour selection and execution

The majority of cue-responsive neurons are Condition-independent, while about 20% are Condition-dependent. The response of the first neuronal category could be triggered by attentional factors such as increase in luminosity or beginning of the task. Indeed, neural responses related to attentional factors or task phases have been described in prefrontal cortex (Everling *et al.*, 2002; Ninokura *et al.*, 2003; Di Pellegrino and Wise, 1993; Shima *et al.*, 2006). Note that the Condition-independent population develops, in the Presentation epoch, a preference for the Action condition, suggesting that part of its neurons achieve a specificity for this condition when additional object-related information is provided.

Most Condition-dependent neurons prefers the Inaction cue, very likely because at this time the associated behavior is already set. In fact, the subsequent object presentation and NoGo signal, although necessary for accomplishing the Inaction condition, are not relevant for the decision of which behavior to perform. On the other hand, the preference of the minority of Condition-dependent neurons for the Action cue could indicate that the monkey is going to actually execute an action and that subsequent events will be relevant for fulfilling the task. A similar neural pattern has already been described and interpreted in terms of rule coding (Asaad *et al.*, 2000; Hoshi *et al.*, 2000; Wallis *et al.*, 2001; White and Wise, 1999; see Miller and Cohen, 2001; Tanji and Hoshi, 2008).

The decoding analysis applied to Action and Inaction populations of cue-responsive neurons allowed us to determine the format of this coding. In fact, in the Action population the activity recorded in the Cue period allows an accurate condition decoding in the Behavioral response period, and vice versa, while in the Inaction population the decoding has a high performance when training the classifier on data on the Decision period and testing it on the Cue period. This indicates that the rule to act or not is coded in the same ‘pragmatic’ format of action execution and withholding.

Neural response to observed objects is coded in terms of behavioral outcome in both Action and inaction condition

During object presentation, the response in the Action condition is prevalent on that of the Inaction condition, both in terms of neurons numerosity and of average population discharge. The presentation of the object, in the Action condition, allows the monkey to progress from the general programming of behavioral goal (to act) to the specific motor program to be executed.

In the whole population of object presentation neurons, as well as in that of action-related neurons, the response is stronger in the Action condition not only in the Presentation epoch, but also from the Go signal to object pulling. The permanence of this preference until the end of the action is confirmed by the decoding analysis, showing a high performance when training the classifier on data from the Presentation period and testing on the Decision and Behavioral response periods and vice versa. Note also that the decoding accuracy, very high in the first and late phases of presentation, falls during its middle phase. This could be due to two possible, not mutually excluding factors: a) during this phase, some other feature is encoded (see below discussion on object preference); b) the high accuracy in decoding the first, very short phase of presentation is not actually related to presentation per se but is a sort of tail of the pre-presentation activity. We favor this interpretation for two main reasons: first, PF neurons activity recorded in delay periods preceding an event typically ceases or peaks and abruptly falls just after the event occurrence (Funahashi *et al.*, 1989; Saga *et al.*, 2011; Watanabe, 1996; see Funahashi, 2014); second, the duration of the first phase of presentation (about 60 ms) characterized by high accuracy decoding, occurs before the population activity reaches its peak (200 ms, for a similar timing see Freedman *et al.*, 2001; Rozzi *et al.*, 2021; Yamagata *et al.*, 2012). It is well known that VLPF neurons activate during the observation of visual stimuli (Ó Scalaidhe *et al.*, 1997; Romanski, 2007; Rozzi *et al.*, 2021; Wilson *et al.*, 1993). Indeed, our recorded region includes sectors connected with inferotemporal and/or parietal cortex (area 12, 45 and 46; Preuss and Goldman-Rakic, 1989; Petrides and Pandya, 2002; Saleem *et al.*, 2008; Borra *et al.*, 2011; Gerbella *et al.*, 2013). The prefrontal neurons described in this work, in general, do not show a marked object selectivity. This could be due to the fact that our task does not overtly require object discrimination. If the task had required object discrimination or categorization, the percentage of object-selective neurons could have been higher (Freedman *et al.*, 2001, 2002; Kusunoki *et al.*, 2010). Nonetheless, about 10% of neurons responding to object presentation have some type of object preference. In addition, the decoding of the Object factor in the population of neurons with object preference shows a high accuracy in the Presentation and Behavioral response periods, suggesting that this population contains neurons similar to visual and visuomotor neurons involved in the parieto-premotor grasping circuit (Murata *et al.*, 1997, 2000; Raos *et al.*, 2006; Rozzi *et al.*, 2021).

Accordingly, we propose that object coding, in the Action condition, is related to the motor implementation (‘pragmatic’ interpretation). This is demonstrated by the decoding of the Object factor in the population of neurons with a differential response for the type of object, that shows a high accuracy in the Object presentation and Behavioural response periods, suggesting that this population contains neurons similar to grasping neurons of inferior parietal and ventral premotor cortex. The ‘pragmatic’ interpretation is in line with the data and results of the *Study* 2 (see below) showing , that some movement related neurons also discharged during object presentation and that the visual response to objects could be modulated by their pragmatic relevance (Rozzi *et al.*, 2021; Simone *et al.*, 2015) . Thus, the object presentation phase in the Action condition has a double coding valence: general goal (acting) and specific goal (type of grip).

The ‘pragmatic’ interpretation also applies to the Inaction neurons whose visual response is maintained until the NoGo signal, when the monkeys must actually withhold the movement. These neurons discharge could be related to the process of inhibiting the unwanted action. This is also supported by clinical literature showing that PF damage in human patients leads to compulsory actions on objects (environmental dependency syndromes such as the Utilization Behaviour) and to behaviour disinhibition (see Iaccarino *et al.*, 2014; Lhermitte, 1986).

Encoding and monitoring behavioural goals

In the Behavioral response phase, the monkey, already instructed on the condition to perform and on the object to grasp (in the case of Action condition), at cue disappearance can complete the task. Among Condition-dependent neurons, the large majority showed a clear prevalence for the Action condition. This is also evident from the population of task-related neurons significantly active in this phase. These findings are in line with the widely accepted idea that prefrontal neurons prospectively encode the behavioral output (Rainer *et al.*, 1999; see Passingham and Sakai, 2004) . In addition, in the Action condition the population activity decreases abruptly during object holding, likely signaling goal achievement. As discussed above, a differential coding of the behavioral output is already evident from the presentation phase of the task, especially in the population of Action-related neurons. In fact, decoding analysis carried out on this population, as well as on that of all neurons responding in the Behavioral period, reveals high accuracy when training on the Presentation period and testing on the grasping/holding phase of the Behavioral response and vice versa, indicating that goal achievement is already predicted when enough information to fulfil the task requirements is provided.

Although less represented, there are neurons preferring the Inaction condition. The activity of this population of neurons, enhanced during the fixation period, falls to baseline when reward is delivered. This evidence is in agreement with the decoding analysis, showing that the highest accuracy is reached in the final phase of the behavioural period (that is just before reward delivery) and suggests that these neurons encode the Inaction condition in terms of goal of the task or reward achievement. Furthermore, the same analysis reveals that the reward/task goal is already predicted from the presentation period onward. In our task, it is not possible to disentangle whether this neuronal activity is more related to reward expectancy or to goal achievement. Based on the available literature, we favor the second interpretation. In fact, although in PF there are neurons coding the expectancy of reward already in the delay period preceding reward delivery (Watanabe, 1996), some of them encode both the reward amount and the monkeys' behavioural response, suggesting that PF may use reward-related information to monitor the goal achievement and thus control the behaviour (Wallis *et al.*, 2001; Watanabe, 1996).

Altogether, these observations indicate that the VLPF neurons responding in the behavioral phase are involved in coding the crucial aspects of the intended behavior and monitoring it until its goal is achieved (e.g. grasping/holding or keeping fixating without moving).

VLPF neurons encode general task goals in terms of their intended motor outcome

The observation of the pattern of activity of the whole population of task related neurons (Fig. 3 C) can provide a general picture of the role of the investigated sector of VLPF in encoding intentional actions execution and withholding. Both the temporal profile of activity and the results of the dPCA (Fig. 3) clearly show that the whole population codes differently the two conditions in the different phases of the task and that in the final phases the activity drops with different timing (taking possession and pulling the object in the Action condition and reward delivery in both conditions). Note that, in both conditions, reward delivery signals the correct execution of the trial, but only in the Action condition there is, before that, a further feedback signal about the accomplishment of the goal of the grasping action.

Noteworthy, the activity observed in the Action condition during the behavioral response resembles the post-saccadic activity described in PF by Funahashi and coworkers (Funahashi *et al.*, 1991; see Funahashi, 2014), since it begins with movement initiation, is context-dependent, and, in some cases, neurons also discharge in relation to an instructing cue. In agreement with the interpretation provided in these studies, we propose that VLPF neurons encode the goal of intended actions in terms of the prediction of the critical events signaling the behavioral outcome (i.e. taking possession and pulling the object or reward delivery). This internal representation of goals would be crucial for maintaining active the sensory-motor representation of an action during its selection, programming and execution. This process probably relies on a top-down modulation on the parieto-premotor grasping neurons anatomically connected with those of the investigated sector (Miller and Cohen, 2001). The feedback signals sent by the parietal and premotor areas would, in turn, confirm the outcome prediction (goal) and suppress VLPF activity related to goal representation, ending the action.

Study 2. Visual Response of Ventrolateral Prefrontal Neurons and their Behavior-related Modulation

3.1 Introduction

In the lateral prefrontal cortex (LPF) there are many neurons responding to visual stimuli. These visual responses have been generally interpreted in terms of categorical coding or as the result of an association with subsequent behavioral reactions (Freedman *et al.*, 2001; Miller and Cohen, 2001b; Saga *et al.*, 2011; Seger and Miller, 2010; Tanji and Hoshi, 2008; Yamagata *et al.*, 2009, 2012). In the latter case, visual responses are often followed by a memory-related sustained activity (Funahashi *et al.*, 1989; Funahashi and Andreau, 2013; Fuster, 2008; Levy and Goldman-Rakic, 2000; Passingham, 1993).

It is generally accepted that visual responses of prefrontal neurons can be related either to object features or to spatial characteristics. This distinction would correspond to a subdivision of LPF into two functional sectors: a dorsal part (dorsolateral prefrontal cortex, DLPF) mainly involved in the elaboration of spatial aspects of visual information, and a ventral part (ventrolateral prefrontal cortex, VLPF) more related to the analysis of objects features (Levy and Goldman-Rakic, 2000; Passingham *et al.*, 2000; Wilson, O'Scalaidhe, *et al.*, 1993). This subdivision is in line with neuroanatomical data, showing that the dorsal part is mainly connected to posterior parietal cortex, while the ventral part with inferotemporal areas (Borra *et al.*, 2011; Gerbella *et al.*, 2013; Petrides and Pandya, 2002; Saleem *et al.*, 2014; Yeterian *et al.*, 2012). However, this sharp dichotomy has been challenged by a series of electrophysiological studies using visual stimuli differing in spatial location, shape and color (Constantinidis and Qi, 2018; Riley *et al.*, 2016). These authors confirm that DLPF shows a higher selectivity for spatial location, while their studies do not clearly support a strong preference of VLPF for objects with respect to spatial features. An exception to this finding would be represented only by very specific visual stimuli, such as faces, that are usually reported to activate distinct patches of VLPF (Tsao *et al.*, 2008). In addition, space and object specificity appears more pronounced in the posterior sectors of both DLPF and VLPF, since going more rostrally the neural responses become more abstract and more tuned to the characteristics of the behavioral task (Constantinidis and Qi, 2018) (for a similar view in humans, see (Koechlin *et al.*, 2003; Koechlin and Summerfield, 2007). In line with this redefinition of the dichotomy, it stands the neuroanatomical evidence that VLPF, besides the inferotemporal afference, receives also a strong parietal input functionally related to eye, arm/hand and mouth fields (Borra *et al.*, 2008, 2011; Cavada and Goldman-Rakic, 1989; Gerbella *et al.*, 2013, 2014; Howells *et al.*, 2020; Petrides and Pandya, 1984; Rozzi *et al.*, 2006).

Previous studies on visual responses in LPF mainly focused on the manipulation of visual input for guiding behavior, with paradigms requiring monkeys to learn an arbitrary association between a specific visual stimulus and, for example, a saccade or a reaching movement (Averbeck *et al.*, 2006; Funahashi *et al.*, 1993; Hoshi *et al.*, 2000; Yamagata *et al.*, 2012), see (Funahashi, 2014; Fuster, 2008; Tanji and Hoshi, 2008). Another series of studies assessed the role of prefrontal neurons in the categorization of visual stimuli, for example requiring the monkey to actively discriminate between objects belonging to different categories (Freedman *et al.*, 2001; Miller *et al.*, 2003; Seger and Miller, 2010). On the contrary, few studies investigated the neuronal responses to passive presentation of visual stimuli (Constantinidis and Qi, 2018; Riley *et al.*, 2016; Tanila *et al.*, 1992), and many specifically focused on visual responses to faces, mainly in a restricted sector of caudal VLPF (Ó Scalaidhe *et al.*, 1997; O Scalaidhe, Wilson and Goldman-Rakic, 1999; Romanski and Diehl, 2011; Wilson, O'Scalaidhe, *et al.*, 1993).

Thus, the *first aim* of this study was to evaluate and map the responses of neurons of a large sector of VLPF to a wide set of visual stimuli, with the only requirement for the monkey to fixate the image, without using it for a specific behavior. To achieve this aim, we recorded the neuronal activity from VLPF of two monkeys while they observed static images presented on a monitor (Visual task). In order to assess whether prefrontal neurons categorize visual stimuli even in the absence of a specific instruction we chose stimuli belonging to four semantic macro-categories.

Previous studies demonstrated the presence of VLPF neurons responding when monkeys observed real objects that represented the targets of grasping actions and also during the actual grasping of these same object. This suggested a link between the visual and motor activation (Bruni *et al.*, 2015; Simone *et al.*, 2015). On the basis of these results, the *second aim* of this study is to verify whether prefrontal neurons 1) respond differently to pictures of graspable objects with respect to the same, real, objects; 2) have a different activation when objects are passively observed or become target of a grasping action. To this purpose, we analyzed the neuronal responses recorded during a visuo-motor task, in which the monkeys had to observe real objects and, instructed by a cue, to grasp them or refrain from acting. These responses have been then compared with those recorded, in the same neurons, during the visual task.

3.2 Material and Methods

Subjects

The experiment was carried out on two female Rhesus monkeys (*Macaca mulatta*, M1, M2) weighing about 4 kg. The animals have been previously employed in a series of experiments, whose results have been published (Simone *et al.*, 2015, 2017). All methods were carried out in accordance with the European (2010/63/EU) and the ARRIVE guidelines. The experimental protocols, the animal handling, and the surgical and experimental procedures, complied with the European guidelines (2010/63/EU), and Italian laws in force on the care and use of laboratory animals, and were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12, 17/07/2012; Prot. 91/OPBA/2015, 21/10/2015) and authorized by the Italian Health Ministry (D.M. 294/2012-C, 11/12/2012; 48/2016-PR, 20/01/2016).

Training and surgical procedures

The monkeys were first trained to seat on a primate chair and to familiarize with the experimental setup. At the end of the habituation sessions, a head fixation system (Crist Instruments Co. Inc.) was implanted. Then, they were trained to perform the visual tasks described below. After completion of the training, a recording chamber (32x18 mm, Alpha Omega, Nazareth, Israel) was implanted on VLPF, based on MRI scan. All surgeries were carried out under general anesthesia (ketamine hydrocloride, 5 mg/kg, i.m. and medetomidine hydrocloride, 0.1 mg/kg, i.m.), followed by postsurgical pain medication (Fogassi *et al.*, 1996; Rozzi *et al.*, 2006; Simone *et al.*, 2015, 2017).

Recording techniques and signal acquisition

Single unit recording was performed using a multi-electrode recording system (AlphaLab Pro, Alpha Omega Engineering, Nazareth, Israel). The microelectrodes, glass-coated (impedance, 0.5-1 MΩ), were mounted on an electrode holder (MT, Microdriving Terminal, Alpha Omega) that, by means of dedicated engines, controlled by a software (EPS; Alpha Omega), allowed electrodes vertical displacement. The MT holder was directly fixed to the recording chamber. Neuronal activity was filtered, amplified and monitored with a multichannel processor and sorted using a multi-spike detector (MCP Plus 8 and ASD, Alpha Omega Engineering). Spike sorting was performed using an Off-line Sorter (Plexon, Inc, Dallas TX, USA).

The experiment was controlled by a homemade Labview software. The digital signals provided time-related information of task phases (the onset and offset of fixation point, stimuli presentation and reward delivery) and behavioral events (monkey hand contact with the starting point, beginning and end of object pulling) and were then used for aligning the neural activity.

Analog signals provided information about eye position. Eye movements were recorded at 120 Hz using an infrared pupil/corneal reflection tracking system (Iscan Inc., Cambridge, MA, USA) positioned above the screen in front of the monkey.

Experimental apparatus

During training and recording sessions the monkeys seated on the monkey chair with the hand contralateral to the hemisphere to be recorded on a resting position. The monitor where visual stimuli were presented in the Visual task (see below) was positioned at 54 cm from monkey's eyes. Monitor resolution was of 1680x1050 pixel and its geometrical center was located at the level of monkey's eyes. A laser spot could be projected on the center of the screen as fixation point. A phototransistor was placed on the monitor in order to provide the onset and offset of the visual stimuli.

A box containing three objects was placed at 22 cm from the monkey's chest during the Visuo-Motor task (see below). The opening of a small door (7x7 cm) facing the monkey at eye's height allowed to present three objects, one at the time. Two laser spots (instructing cues) of different colors (green and red) were projected onto the box door or onto the object, signaling the task conditions and phases.

Behavioral paradigms and stimuli:

a) Visual task

To evaluate the response of VLPF neurons to observation of visual stimuli, we displayed images (6°x6°) depicting 12 stimuli (see below), while the monkeys had to keep their gaze within stimulus limits. Figure 1A shows the sequence of events occurring during each trial. The monkeys were required to keep their hand on the resting position; if this was accomplished, the trial started, and the fixation point was turned on. They were required to fixate it for a randomized time interval (500-900 ms), keeping the eye within a ±3° (X and Y) fixation window. If they kept fixation for this period of time, the fixation point turned off and one of the images was presented for 600 ms. The monkeys had to observe it throughout the presentation period, keeping fixation within the fixation window. Then, the image disappeared, the fixation point turned on again for a randomized period (500-900 ms) and the monkeys had to keep fixation on it. The trials were accepted as correct, and the monkeys were rewarded, if they kept their eyes within the fixation window for the duration of each phase of the task (first fixation, stimulus presentation and second fixation) and did not release the hand from the resting position. Discarded trials were repeated at the end of the sequence to collect at least 10 presentations for each stimulus. The order of stimuli presentation was randomized.

The 12 stimuli (Figure 1B) belonged to 4 different semantic categories:

Graspable solids (pictures of the objects employed in the motor task described in Simone et al. (2015)): cube, cylinder, sphere;

Fruits: apple, banana, peanut;

Faces: human face, monkey face, sketchy drawing of a face;

Laboratory furniture, geometric, but not graspable: shelf, monitor and clock.

The stimuli had a similar luminance and were presented on a homogeneous green background. Note that the fruits and solids could evoke similar affordances (apple and cube: power grip; banana and cylinder: finger prehension; sphere and peanut: precision grip).

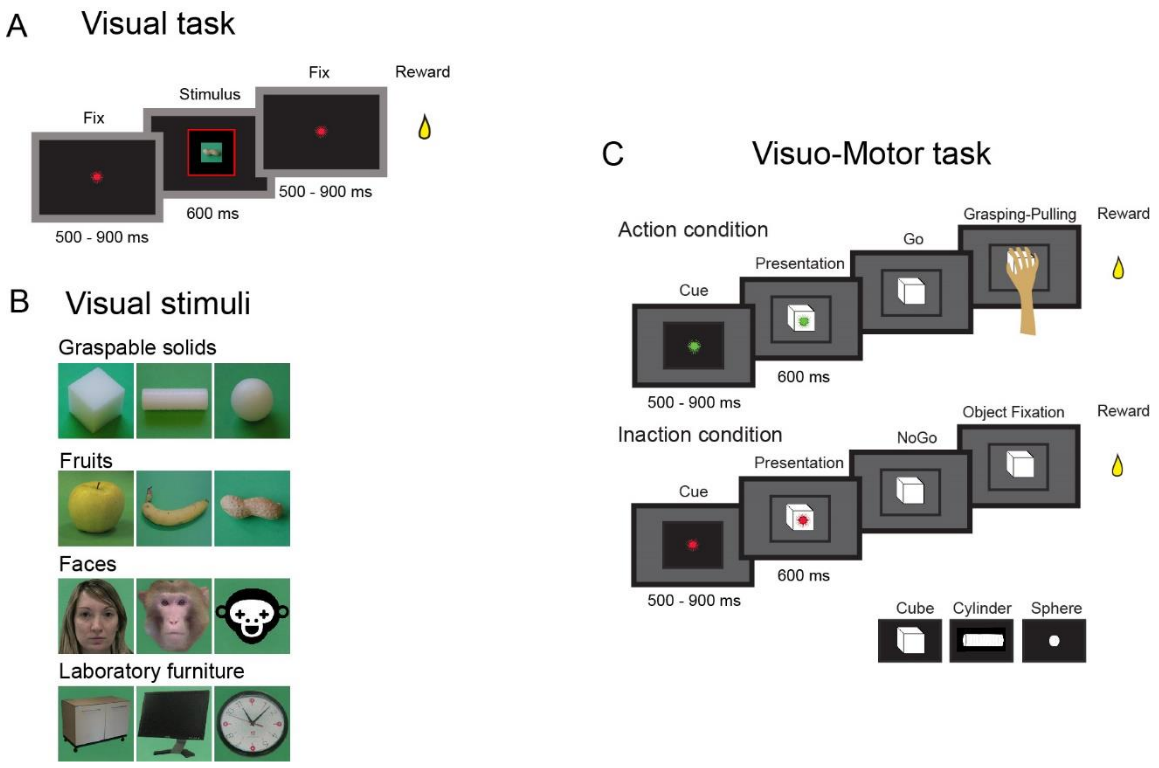


Figure 1. A Temporal sequence of events in the Visual task; **B** stimuli presented in the visual task; **C** Temporal sequence of events in the Visuo-Motor task.

b) Visuo-Motor task

The Visuo-Motor task corresponds to that described in (Simone *et al.*, 2015). Briefly, the task consisted of two basic conditions: Action and Inaction (Figure 1C). Each trial started with the monkeys' hand on a starting position. Then one of the two instructing cues (green = Action condition; red = Inaction condition) turned on and projected onto the closed box door. In both conditions, the monkeys had to maintain fixation within a 6°x6° fixation window centered on the instructing cue for a randomized time interval (500 - 1100 ms). Then the box door opened allowing the monkey to see one of three objects. The objects were a small sphere (diameter 1 cm), a large cube (side 2 cm) and a cylinder (length 4 cm, diameter 1.5 cm, horizontally oriented).

In the Action condition, during object presentation, the monkeys had to maintain fixation and the green cue was still on, projected onto the object. After a randomized time (700 to 1100 ms) the green cue turned off (Go signal), instructing the monkeys to reach for, grasp the object and pull it. If the monkeys correctly performed a trial, a drop of liquid reward was delivered at the end of it. In the Inaction condition, the monkeys were instructed by a red cue whose extinction required the monkeys to keep fixating the object for 600 ms. After correct completion of the trial, the monkey was rewarded as in the Action condition. The order of presentation of both objects and conditions was randomized.

Data analysis:

a) Visual task

In the visual task, we recorded neural activity for at least 120 successful trials, 10 for each stimulus. For the statistical analysis, two epochs were defined: 1) Baseline: 500 ms preceding stimulus presentation, during which the monkey was looking at the fixation point; 2) Stimulus: the first 500 ms of image presentation.

Single-neuron responses were statistically evaluated by means of a 2X12 ANOVA for repeated measures (Factors: Epochs, Stimuli, $p<0.01$) followed by Newman-Keuls post hoc tests. A neuron was considered visually responsive when the 2X12 ANOVA revealed: 1) a significant Main effect Epoch and/or 2) a significant Interaction effect, in which the Post-hoc test showed a significant difference between at least one stimulus epoch of one image and its baseline.Visually responsive neurons were classified as selective when the 2X12 ANOVA revealed a significant Interaction effect and the post-hoc test showed a significant difference among the activity recorded in the stimulus epoch of one stimulus and that of 1) its baseline and 2) the stimulus epoch of at least another stimulus. Neurons were classified as unselective when the statistical test revealed a significant Main effect Epoch and/or a significant Interaction effect, and the post-hoc test did not show any difference among the activities recorded in the stimulus epoch of the 12 stimuli.

b) Comparison of neuron response in the Visual and Visuo-Motor tasks

In the Visuo-Motor task, the neural activity was recorded for at least 60 successful trials (30 per condition, 10 for each object). For statistical analysis of the neural activity, we defined nine epochs (see Simone *et al.*, 2015):

1) Baseline: from 750 ms to 250 ms before the onset of the instructing cue; *2*) Pre-cue: 250 ms preceding the onset of the instructing cue; 3) Cue: 250 ms following the onset of the instructing cue); 4) Pre-presentation: 500 ms preceding the opening of the box door; 5) Presentation: 500 ms following door opening (object presentation); 6) Set: 250 ms before the offset of the instructing cue; 7) Go/NoGo, from the offset of the instructing cue to the release of the hand starting position (Action condition) or 250 ms following the offset of the instructing cue (Inaction condition); 8) Grasping-Pulling/Object fixation: from 250 ms before to 250 ms after the Pulling onset (Action condition) or a time period ranging from 250 ms to 500 ms after the offset of the instructing cue (Inaction condition); 9) Reward: 500 ms following reward delivery.

Single-neuron responses were statistically evaluated by means of a 9X2 ANOVA for repeated measures (Factors: Epoch, Condition, $p<0.01$) followed by Newman–Keuls post hoc tests. In this study, neurons were considered as visually responsive when there was a significant main effect Epoch ($p<0.01$), and the following post-hoc test showed a significant difference between the presentation and the baseline epochs.

The comparison between the Visual and the Visuo-Motor tasks was performed on those neurons considered as visually responsive in at least one task (according to the criteria defined in the previous sections) and whose neural activity was recorded in 90 successful trials, 10 for each stimulus (Cube, Cylinder and Sphere) in each condition (Visual, Action and Inaction). Single-neuron responses were statistically evaluated by means of a 3X3 ANOVA for repeated measures (Factors: Conditions, Stimuli, $p<0.01$) followed by Newman-Keuls post hoc tests.

Since the Visual and Visuo-Motor tasks were acquired in different blocks, to ensure that baseline activity was not changed across tasks, we compared the activity of each neuron during the period preceding the stimuli presentation of both tasks. To this aim we conducted a t-test between the baseline epochs of the Visual task and the pre-presentation epoch of the Inaction condition of the Visuo-Motor task. Note that in these two epochs the monkey had to keep fixation on the same red fixation point and was not required to program any movement. We discarded all neurons showing a significant difference between these two epochs.

Computation of depth of tuning, selectivity and category indexes

To quantify the degree and depth of selectivity of the neurons for the images used in the Visual task, we calculated two indexes: the depth of tuning index (d_i), measuring the difference between the maximal and the minimal response normalized to the cell's maximal response, and the selectivity index (s_i), quantifying the extent to which activity in all non-preferred stimuli deviates from the maximal activity for the preferred stimulus. Each index was computed for each neuron across the twelve stimuli employed. These indexes, previously employed for assessing the neuronal directional tuning (Moody *et al.*, 1998), are defined as follow:

$$d_i = \frac{i_{max} - i_{min}}{i_{max}}$$

$$s_i = \frac{k - \left(\frac{\sum_{n=1,k} i_n}{i_{max}}\right)}{k-1}$$

where k is the number of stimuli; and i_{min} and i_{max} are, respectively, the minimum and maximum responses of the i^{th} neuron across the twelve different stimuli and are calculated based on the peak of discharge observed in the stimulus epoch.

Population analyses

In order to characterize the time course and the discharge rate of different neuronal populations, the neuronal activity of each population was aligned with the beginning of Stimulus epoch (Visual task) or the actual objects appearance (Visuo-Motor task). The population activity was computed as follows. The mean single neuron activity over trials, in terms of firing rate, was calculated for each 20 ms bin in the different conditions. The average Baseline (Visual task) or Pre-presentation epoch (Visuo-Motor task) activity was then subtracted from the mean single neuron activity over trials for each bin. Each neuron contributed one entry to each data set. In the Visual task, each bin represents the average activity recorded in the 12 stimuli of the number of neurons included in the population. Due to mechanical timing of door opening in the Visuo-Motor task, the object became visible 38 ms after door opening. Since population analysis was calculated in bins of 20 ms, we aligned population activity in the Action and Inaction conditions two bins (40 ms) after door opening.

In order to assess whether the population activity recorded in the Stimulus epoch of the Visual task statistically differed among the 12 presented stimuli, we conducted a One-way ANOVA, Tukey-Kramer criterion, $p<0.01$ (Matlab functions: anova1, multcompare). Similarly, we compared the population activity recorded in the Stimulus epoch of the Visual task and in the Presentation epoch of the Visuo-Motor task by means of a One-way ANOVA, Tukey-Kramer criterion, $p< 0.01$ (Matlab functions: anova1, multcompare).

Anatomical reconstruction of the neuronal properties

The recording region was reconstructed based on the location (in stereotaxic coordinates) of the penetrations on the MRI scans of the brain of both investigated monkeys, as described in (Simone *et al.*, 2015, 2017). Penetration depth, as reported by the protocol, was matched with its location with respect to the sulci.

3.3 Results

This study had two main aims: the first was to describe and map the responses of VLPF neurons to the observation of a set of visual stimuli, with the only requirement for the monkey to simply fixate the image, the second was to assess whether prefrontal neurons respond differently to real objects or to their pictures and activate differently when these objects are passively observed or are target of an action. Accordingly, we will first present the results obtained in the Visual task, and subsequently those derived from the comparison of neural responses recorded with the Visual and the Visuo-Motor tasks.

We recorded 1607 neurons from VLPF during performance of the Visual task.

The 2x12 ANOVA for repeated measures (Factors: Epochs, Stimuli, $p<0.01$, see Methods)

reveals, that, of these neurons, 863 were visually responsive. The majority was active during the observation of all stimuli, while 93 neurons (10.8% of visually responsive neurons, Figure 2A) had an interaction effect, showing some type of preference for one or more stimuli (selective neurons, see Methods). Figure 2A shows also the number of neurons selective for 1, 2, 3 or more stimuli. It is clear that most of them (n=58, 62%) responds only to one stimulus.

Figure 2B, C, D depicts examples of three visually responsive neurons. Figure 2B shows the discharge of an unselective neuron, responding equally well to all the presented stimuli (see Methods). In Figure 2C, a neuron responding only to the presentation of a peanut is shown. With respect to image onset, the discharge begins at about 100 ms, peaks at about 200 ms, and ends before image offset. Figure 2D shows the response of a neuron selective for two stimuli (Cylinder and Banana). Note that there is no significant difference between them. The discharge for both stimuli begins and peaks shortly after image onset, and declines just after image offset. Interestingly, although the two stimuli belong to different categories, they share common features, namely shape and orientation.

Table 1 shows the number of neurons grouped on the basis of their best response to the presented stimuli. One can appreciate that, although all stimuli are coded by the selective neurons, some stimuli are coded by a higher number of neurons. However, it is also clear that the best neural responses fall almost equally within the different semantic categories set a priori in our experiment of stimuli. (χ^2 (3) = 6.4659, p-value = 0.09102). In addition, note that the neuron selectivity for specific stimuli does not seems to correlate with the difference in peak-firing rate or in the timing of peak discharge (Supplementary figure 1).

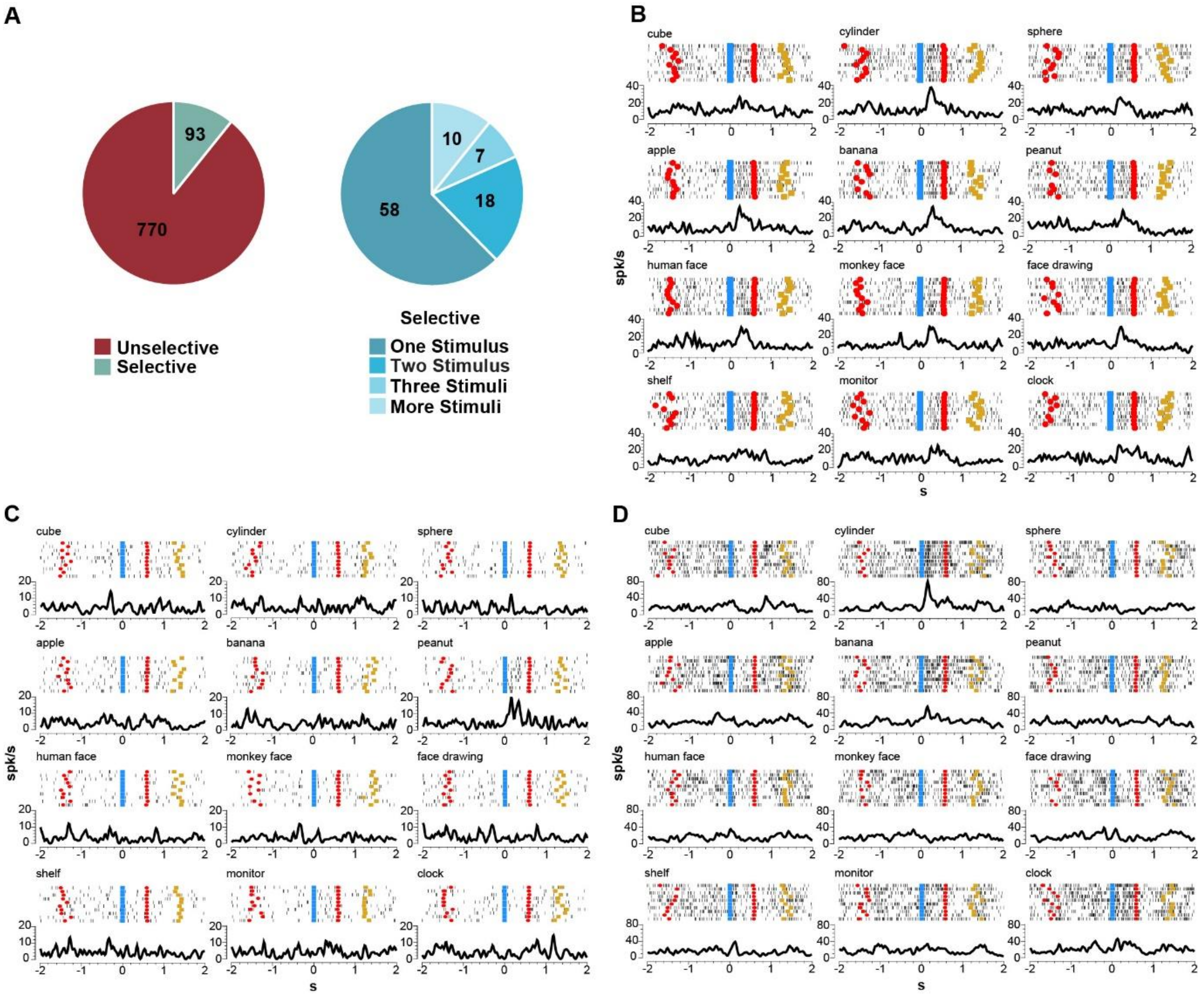


Figure 2. Selective and Unselective neurons. **A** Pie plots showing the proportion of Visual Neurons classified as Selective or Unselective (left) and the number of neurons selective for 1, 2, 3 or more stimuli (right). **B** Example of neuron responding to all the presented stimuli. **C** Neuron responding exclusively during the observation of a Peanut. **D** Neuron responding to the observation of Cylinder and Banana. The neuronal activity represented by rasters and histograms is aligned on the beginning of stimulus presentation. In each raster, cyan squares indicate the beginning of stimulus; red circles indicate the switching on of the red laser light; yellow squares indicate reward delivery. Abscissae: time (s); Ordinates: firing rate (spikes/s).

Stimulus	n. neurons	Stimulus	n. neurons	Stimulus	n. neurons	Stimulus	n. neurons
Cube	2	Apple	6	Human face	7	Shelf	7
Cylinder	13	Banana	13	Monkey face	7	Clock	7
Sphere	5	Peanut	12	Face drawing	3	Monitor	11
Solids	20	Fruits	31	Faces	17	Furniture	25

Table 1. Number of selective neurons grouped on the basis of their best response to each stimulus. The best response is the highest among the coded stimuli.

Concerning the possibility of neurons to categorize, among those responding to more than one stimulus, none discharge during observation of the three stimuli belonging to one pre-set semantic category and not to those belonging to the other three categories; 8 neurons respond to two of the three stimuli belonging to only one semantic category.

In order to quantify the stimulus selectivity of the neurons, we calculated two indexes (see Methods). The depth of tuning index (d_i) indicates how sharply each neuron codes the best stimulus with respect to the worst, and the selectivity index (s_i) quantifies the extent to which activity in all non-preferred stimuli deviates from the maximal activity for the preferred stimulus, indicating how much each neuron is tuned to one stimulus. Figure 3 shows the results of the calculation of depth of tuning (d_i) and selectivity (s_i) indexes for selective and unselective neurons. The average d_i is higher (0.72 ± 0.16) for selective with respect to unselective neurons (0.53 ± 0.17), indicating a larger difference in discharge between the best and worst stimuli in the selective neurons. As far as s_i is concerned, its average is clearly higher for selective neurons (0.49 ± 0.15 vs 0.31 ± 0.12).

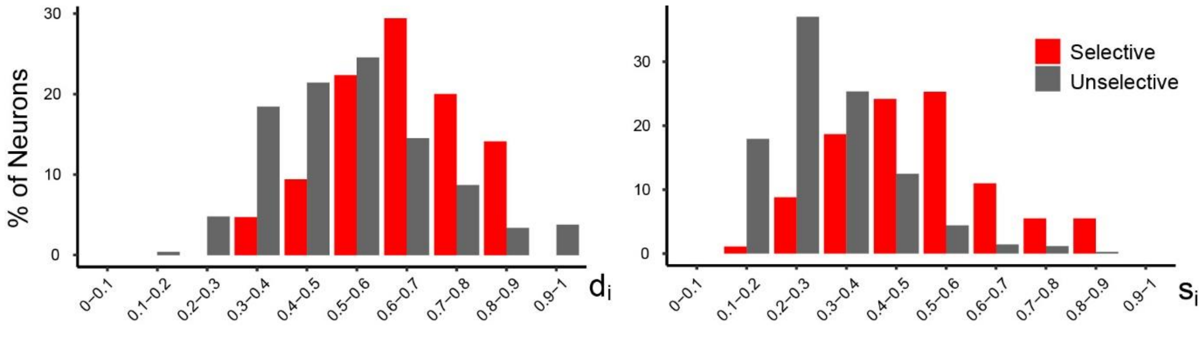


Figure 3. Histograms showing the distribution in percentage of selective (red) and unselective (gray) neurons based on d_i (**A**) or s_i (**B**). The value of each bin in the abscissa corresponds to a range of 0.1 for each index.

Population analyses

Population analyses were conducted on selective and unselective neurons. Figure 4A shows that the average discharge intensity, among selective neurons, is higher for those stimuli that are encoded by a higher number of neurons (One way ANOVA, $p<0.01$). On the contrary, statistical analysis did not show any difference in discharge in unselective neurons (Fig.4B, One way ANOVA: n.s.).

Figure 4C shows the comparison in the time course of the discharge, averaged among all stimuli, between the two populations. The beginning of the response is the same for both curves, while the maximal raising slope (calculated as the maximum of the derivative) and the peak of activity is slightly earlier (20 ms) for the population of unselective neurons (80 vs.100 and 120 vs. 140 ms after stimulus onset, respectively). Then, after an initial rapid decrease in the discharge similar in both populations, the two profiles have a different time course. While the population of unselective neurons returns to baseline level before stimulus offset, that of selective neurons presents a sustained discharge falling to baseline level only during the second fixation epoch. Note that both populations present a second minor peak after stimulus offset, in coincidence with the second appearance of the fixation point. The statistical comparison between the two population discharges shows that they start differing 180 ms after stimulus onset, just after peak of discharge. Differential discharge ends 140 ms after stimulus offset (Fig. 4D).

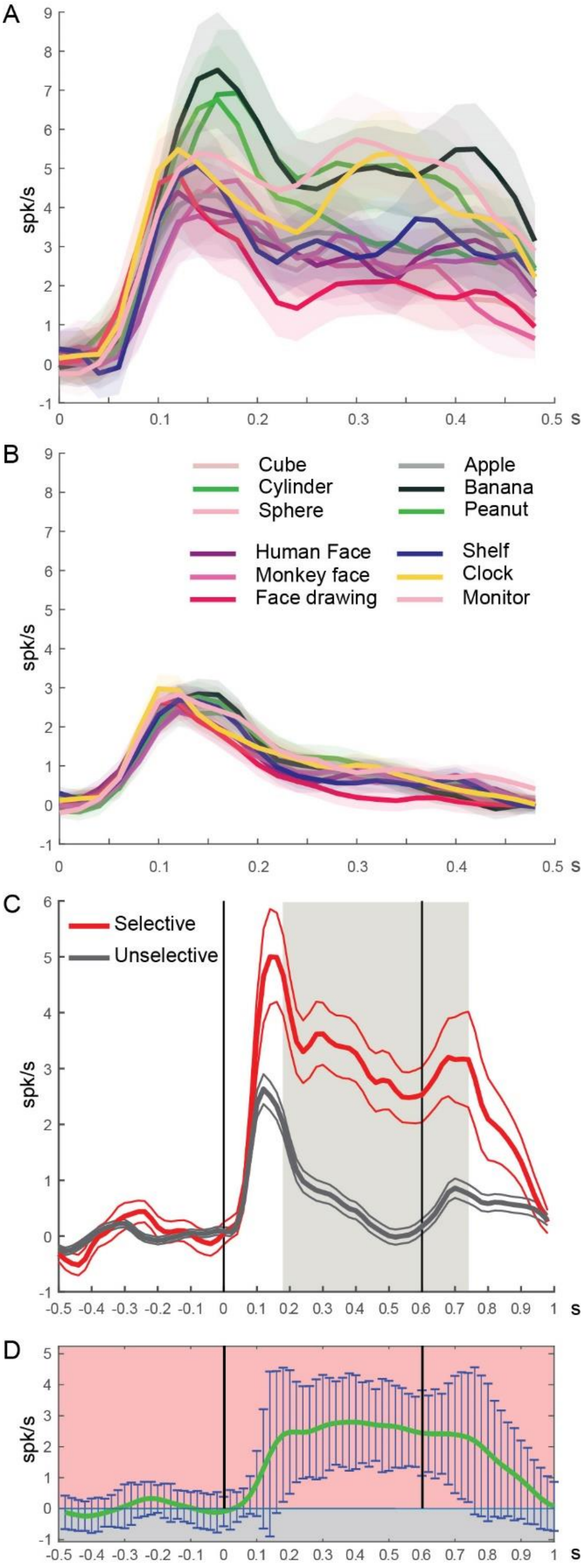


Figure 4. Temporal profile of the net mean activity of the populations of selective (A) and unselective (B) neurons during the observation of visual stimuli. The different colored lines indicate the populations net mean activity for each stimulus, the shaded colored contours represent the standard errors. The activity is aligned (0) with the stimulus onset.

C. Temporal profile of the net mean activity of the populations of selective (red) and unselective (gray) neurons in the visual task, averaged among the 12 stimuli. The thick lines indicate the populations average activity, the thin lines represent the standard errors. The shaded area represents the temporal span in which the two activities differ significantly. D. Temporal profile of the net differential activity calculated as subtraction between the average activities of Selective and Unselective populations. Error bars indicate three times the standard error for each 20 ms bin. The vertical lines indicate the onset and offset of stimulus presentation. Abscissae: time (s); Ordinates: firing rate (spikes/s).

Localization of selective neurons

Figure 5 depicts the distribution of neurons showing selective discharge for visual stimuli belonging to different categories in the two monkeys. Note that in both monkeys the selective neurons are localized over a wide region likely corresponding to areas 46, 12 and 45. In monkey 2 there is an additional cluster of neurons close to oculomotor areas. No evident segregation is apparent among the various classes of stimuli.

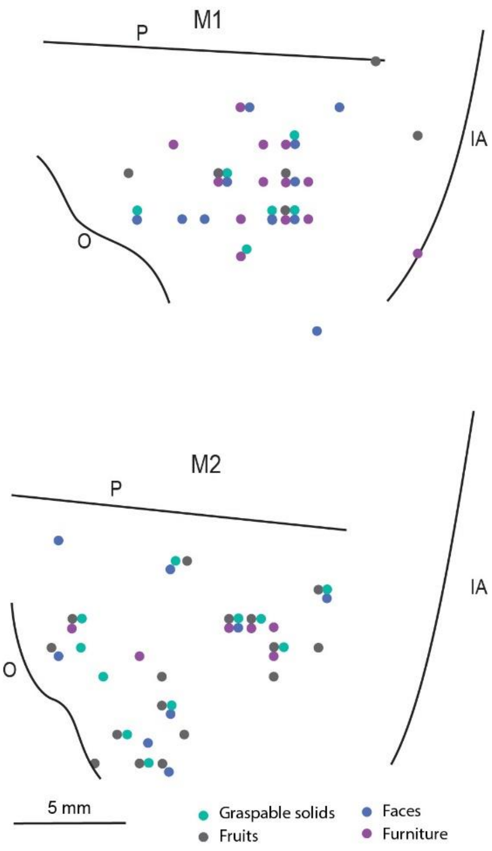


Figure 5. Distribution of penetrations containing neurons with selective discharge for visual stimuli belonging to different categories in the recorded region of the two monkeys (M1 and M2). IA, inferior arcuate sulcus; O, orbital reflection; P, principal sulcus.

Comparison between responses to objects observation in the Visual and Visuo-Motor tasks.

Single neurons responses

The responses of 492 neurons tested in both Visual and Visuo-Motor tasks were analyzed using a 3X3 ANOVA for repeated measures (factors: condition and object, $p < 0.01$) in order to verify the effect on neural discharge of the context in which the stimuli were observed and of the type of object. Figure 6A shows the distribution of neurons according to the results of this statistic analysis. Briefly, about half of the analyzed neurons (n= 242) had a significant condition effect, 11% (n= 56) had a significant object effect and only a minority of neurons (4%, n=21) had an interaction effect; 229 neurons (46.5%) had no significant effect.

The majority of neurons with main effect condition (n=182, 75%) showed a significant difference between one condition and the other two (Fig. 6B). Among them, we recognized three first categories based on the condition better coded than the other two. The most represented category is that formed by neurons discharging during object presentation in the Action condition (n=66). An example of neuron belonging to this category is shown in Figure 7A. The neuron strongly activates during object observation in the Action condition, has a much weaker activation in the Inaction condition, and does not respond at all in the Visual condition. The neuron does not show any object selectivity. Among the neurons preferring the Action condition, 4 showed object preference and 7 resulted also active during movement execution (according to the criteria adopted by Simone et al., 2015). A second category is formed by neurons responding best to object presentation in the purely Visual condition (n=38). A third category is formed by neurons responding best to object presentation in the Inaction condition (n=18).

Then, we could recognize three further categories formed by neurons coding two conditions better than the remaining one. The first is formed by neurons that respond better in the Visuo-Motor task than in the Visual task (n=49). The second contains neurons that respond in the Action and purely Visual condition better than in the Inaction condition (n=8). The third is formed by neurons responding in the Inaction and Visual condition better than in the Action condition (n=3).

The remaining 60 neuron (25%) showed a significant difference only between two conditions, the other comparisons being not significant.

Neurons showing condition effect can be classified in a different way, based on object dimensionality (2D vs 3D)/task complexity (Visuo-Motor vs Visual). Thus, we calculated the number of neurons falling within this macro-category. We found that 85 out of 242 neurons (35%) belong to it. On the contrary, 105 neurons (43%) show a significant difference between Action and Inaction conditions, thus clearly do not code the considered aspects. The remaining 52 neurons do not fit this categorization.

Neurons showing an object and/or an interaction effect in the 3X3 ANOVA have been categorized based on their best response. This categorization shows that 39 neurons have a highest discharge for the cylinder, 11 for the cube and 10 for the sphere. Note that four neurons had the same best discharge for two objects. Figure 7B shows an example of a neuron selective for the cylinder in all conditions. The response for the other two objects is much weaker or absent.

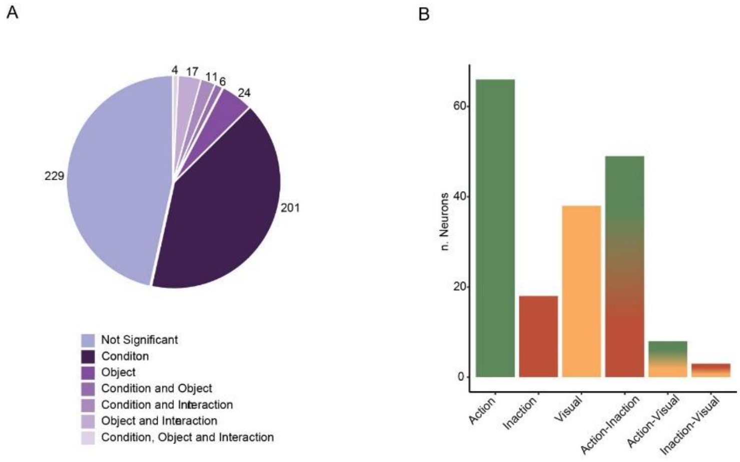


Figure 6. A Pie plot showing the distribution of neurons according to the results of statistical analysis (3X3 ANOVA for repeated measures; factors: Condition and Object). **B** Histograms representing the distribution of neurons showing differential activity during object observation in the Action, Inaction and Visual conditions. Single-coloured histograms indicate neurons responding better in one condition than in the other two; double coloured histograms indicate neurons responding equally well in two conditions and better than the remaining one (see text for the detailed description of each category).

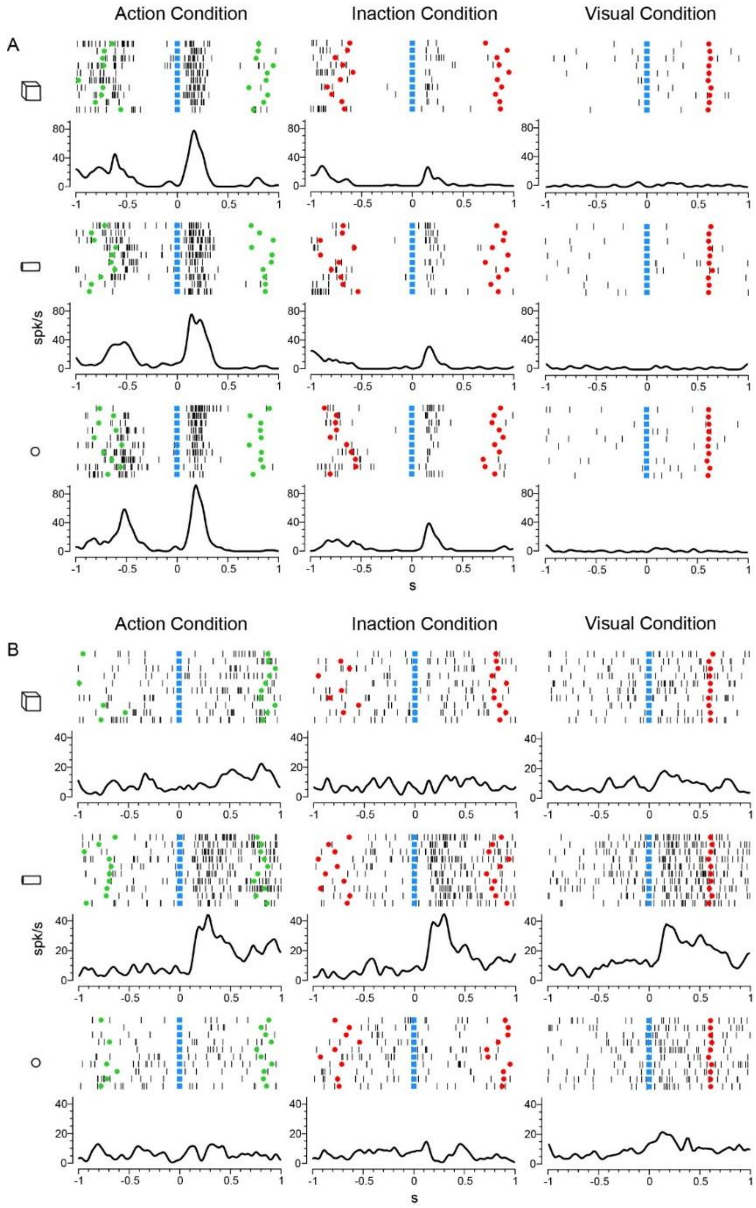


Figure 7A. Example of VLPF neuron discharging strongest during object presentation in the Action condition. B. Example of VLPF neuron responding selectively to the observation of the cylinder in all conditions. For both neurons the activity is aligned on the beginning of stimulus presentation. Green circles indicate the switching on of the green laser light. Other conventions as in figure 2.

Population response

Figure 8 shows the time course of the population responses of the neurons recorded in the three conditions. The population of neurons with a significant condition effect (Fig. 8A) shows the highest discharge for objects observation during the Action condition, an intermediate discharge for the Inaction condition, and the weakest response for the Visual condition. The statistical analysis reveals a significant difference among conditions and that the Action condition has a stronger response than the Visual one (one way ANOVA, Tukey-Kramer criterion, $p<0.01$). The plot of the time course of the differential activity between pairs of conditions confirms a significant difference only between the Action and the Visual condition in two bins preceding the peak of the activity in the Action condition (Fig. 8 A2).

The population of neurons without a condition effect (Fig. 8B) shows a similar trend, although the discharge in the Action and Inaction condition is quite similar. The one way ANOVA (Tukey-Kramer criterion, $p<0.01$) does not reveal any significant difference among conditions. The plot of the time course of the differential activity between pairs of conditions does not reveal any significant difference.

Looking at the time course of the two populations, it is worth to make two further considerations. First, the peak of activity is higher in the condition dependent population; second, while in the non-condition dependent population the timing of peak in the three conditions is perfectly aligned (160 ms after object appearance), in the condition dependent population, in Action and Inaction condition, the peak occurs before that of the Visual condition (140 vs 180 ms after object appearance).

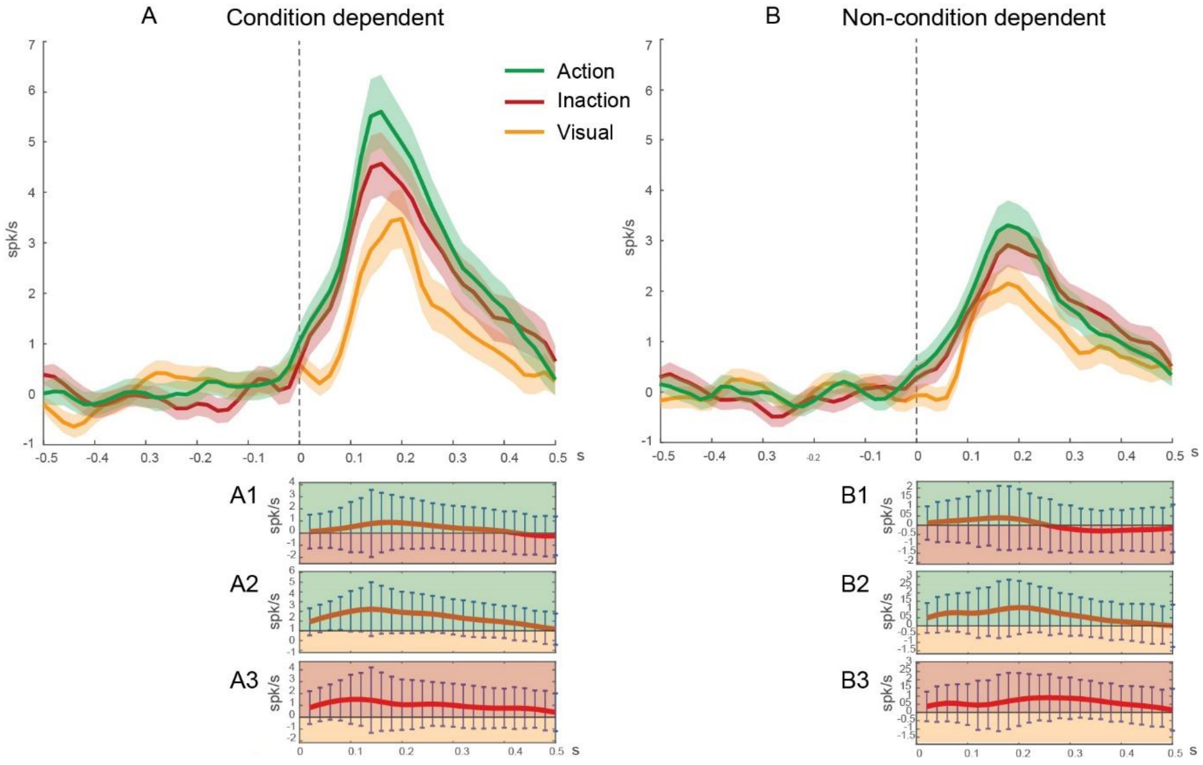


Figure 8. Temporal profile of the net mean activity of the populations of Condition dependent (**A**) and Non condition-dependent (**B**) neurons during the observation of visual stimuli in the Action, Inaction and Visual conditions. The colored lines indicate the population net mean activity for each condition, the colored shaded contours their standard errors. The activity is aligned (0) with the stimulus onset. Temporal profiles of the net differential activity calculated as Action minus Inaction (**A1**, **B1**); Action minus Visual (**A2, B2**); Inaction minus Visual (**A3, B3**) of the condition dependent and non-condition dependent populations. Other conventions as in Fig. 4.

Localization of neurons selective for the Action, Inaction and Visual conditions

Figure 9 depicts the distribution of neurons showing selectivity for one of the conditions in the two monkeys. The two maps are different in terms of amount of neurons showing selectivity. Even the map of the monkey showing the largest number of neurons (M2) does not show any clear segregation between the three types of selective neurons. Indeed, they are localized over a wide region covering areas 46, 12 and 45.

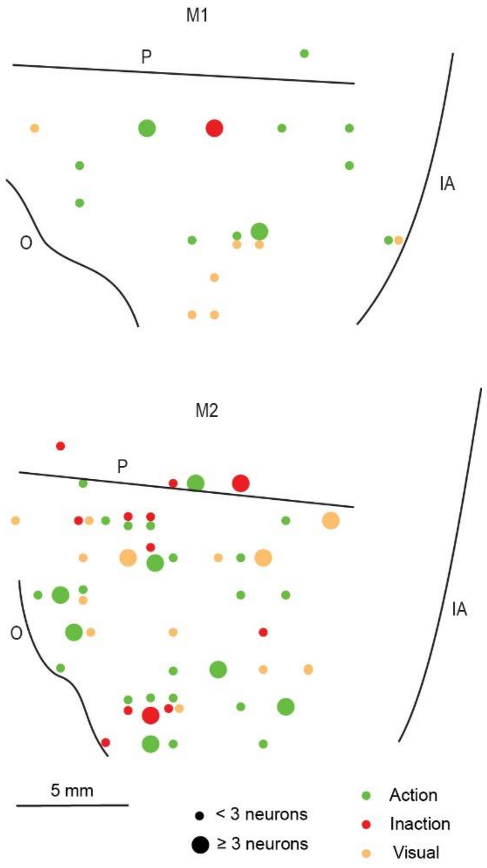


Figure 9. Distribution of neurons showing selectivity for one of the conditions in the recorded region of the two monkeys (M1 and M2). Different size of the circles indicate different numerosity of neurons. IA, inferior arcuate sulcus; O, orbital reflection; P, principal sulcus.

3.4 Discussion

The results of the first part of the present study, aimed to describe the VLPF neurons responses to different types of passively presented visual stimuli, show that: a) about half of the recorded neurons respond to visual stimuli; about 10% of them show also stimulus selectivity, the majority being selective for only one stimulus; b) the main categories of the used stimuli are equally represented; c) the time course of the discharge of the populations of both selective and unselective neurons presents a peak at about 130 ms after stimulus onset, but only selective neurons show a prolonged discharge for the whole duration of the stimulation; d) visually responding neurons are widely distributed within the recorded region, covering areas 45A, 46v, 12, sparing only FEF.

The results of the second part of the study, concerning the comparison between neuronal responses to objects observation in the passive observation task (Visual condition) and in the visuo-motor task (Action and Inaction conditions) show that: a) about half of the tested neurons show a differential discharge between conditions; b) the most represented category is that of neurons responding best during the Action condition; c) the time course of the population response shows the highest activation in the Action condition, an intermediate activation in the Inaction condition and the lowest activation in the Visual condition; d) there is no specific anatomical segregation within the recorded region for neurons responding best to a single condition.

Visual selectivity in VLPF neurons

The presence of selective neurons in VLPF is in good agreement with the literature on the passive response of prefrontal cortex to visual stimuli, reporting that the percentage of neurons showing visual specificity is quite limited. It is noteworthy that in most cases neurons were tested with a restricted number of stimuli (O Scalaidhe, Wilson, Goldman-Rakic, *et al.*, 1999; Riley *et al.*, 2016; Wilson, O’Scalaidhe, *et al.*, 1993). On the other hand, it has also been reported that in VLPF there are neurons with very specific responses to complex stimuli such as faces either static, or dynamic in combination with vocalizations (Diehl and Romanski, 2014; Ó Scalaidhe *et al.*, 1997; O Scalaidhe, Wilson, Goldman-Rakic, *et al.*, 1999; Romanski and Averbeck, 2005; Romanski and Diehl, 2011). In our study, we employed a set of stimuli larger than those previously employed in studies on prefrontal cortex. This allowed us to show that most of the employed stimuli are specifically coded by VLPF neurons. Very likely, by using an even larger set of stimuli, a higher number of selective neurons could have emerged.

The fact that in our study neurons responding to faces were not so represented as in previous works, could partly depend on the wider region we recorded. In addition, previous studies showing responses to faces actually used only faces as stimulus (Romanski and Diehl, 2011), so we do not know if these neurons could have responded to other visual stimuli, too.

Some studies demonstrated the capacity of VLPF neurons of categorizing visual stimuli (Freedman *et al.*, 2001). This appears in contrast with our findings, showing that most selective neurons respond to only one stimulus, and that the stimuli coded by neurons responding to more than one stimulus belong to different categories. A possible explanation of the lack of categorical generalization is that we concentrated on specific semantic categories, excluding other possible stimuli. Another possibility could be related to the task we employed, that did not require to perform any active operation referred to the observed stimuli, as done in other studies.

An interesting finding emerging from our study is that in VLPF it is possible to elicit neuronal responses using passive stimulation. This probably reveals the contribution of inferotemporal and posterior parietal input to prefrontal cortex. This input represents the first step in the intrinsic prefrontal processing. Indeed, it has been shown that when presentation of visual stimuli is compared between a passive observation task and a task in which the stimulus is an instruction for a subsequent response, there are neurons whose visual response does not change and others that do show a response only in the active condition (Constantinidis and Qi, 2018; Duncan, 2001; Genovesio *et al.*, 2014; Miller and Cohen, 2001).

The visually selective neurons found in the present study are evenly distributed in the recorded region, thus encompassing areas 45A, 46v and 12. No segregation was detected for either specific stimuli or categories. This finding is quite in agreement with the literature in which the same cortical sector was explored (Ó Scalaidhe *et al.*, 1997; Rainer and Miller, 2000; Saga *et al.*, 2011). Thus, it looks as if the incoming temporal and parietal visual input reaches prefrontal cortex without a specific organizational pattern, such as, for example, similar shapes, categories or affordances. Note, however, that the possibility that some type of organization of visual responses in VLPF does exist comes from the demonstration of the presence, in ventral and orbital prefrontal cortex, of three patches activated by face observation (Tsao *et al.*, 2008), in line with the patchy representation of faces in inferotemporal cortex.

Population analysis clearly reveals that both selective and unselective neurons have a first peak of response with a similar timing. This could be partly attributed to an initial attention-related response or to an activity related to a specific, crucial, phase of task unfolding (Desimone and Duncan, 1995; Miller and Cohen, 2001; Ninokura *et al.*, 2003, 2004; Saga *et* *al.*, 2011; Saito *et al.*, 2005; Tanji and Hoshi, 2008). These explanations could be completely valid for the second peak of discharge occurring after stimulus offset when the fixation point reappears. On the contrary, in the case of the first peak, the population of selective neurons has twice the discharge of that of unselective neurons. Thus, if the peak were related only to unspecific factors, or phases, one would expect exactly the same discharge intensity in both populations. On the contrary, it seems that selectivity is the main factor capable of explaining this difference. Furthermore, only in the population of selective neurons the response remains sustained for the whole presentation period.

Regarding the role of the sustained discharge, we can exclude that it can be attributed to memory, since in this period the stimulus is always visible. In addition, it cannot be due to some sort of preparation, since the monkeys have just to passively observe the visual stimuli. Another possible explanation for this discharge, is that it contains some sort of expectation of the next phase of the task (second fixation), since visual presentation lasts a fixed period of time. This seems unlikely because if this were the case the discharge should increase before stimulus offset. The interpretation we favor is that the sustained discharge represents a top-down activation of the temporal/parietal neurons that provided the visual specific information. This would serve to maintain attention on the target (Ekstrom *et al.*, 2008; Gregoriou *et al.*, 2014; Miller and Cohen, 2001; Paneri and Gregoriou, 2017; Roelfsema, 2006).

VLPF neurons responding to objects observation play a role in planning behavior

The second part of the study was aimed to verify whether the same types of objects presented in the Visual or Visuo-Motor task could elicit the same or different response in the VLPF recorded neurons.

This comparison shows that half of the tested neurons did show a different discharge among conditions. In order to better understand the type of coding of these neurons, we can first consider neurons showing specificity for only one condition. The majority of them has the best activation for the Action condition, followed by those preferring the Visual condition, while the minority activates best for the Inaction condition. The large prevalence of neurons preferring the Action condition suggests that the response occurring during visual presentation is clearly related to the action the monkey is going to perform on the object. This does not mean that the neuron must show a discharge during action execution (only 7 neurons show movement related discharge), but rather that the neuron is involved in a neuronal chain whose activation leads to action-goal achievement. Note that these neurons do not have a role in visuo-motor transformation, as it is also confirmed by the very low number of them having an object preference in the Action condition (n= 4). The absence of this role was already demonstrated in a previous work on movement related neurons of this same region using the same task (Simone *et al.*, 2015). A similar explanation can apply to the role of neurons preferring the Inaction condition, although in this case the effect is the opposite. In fact, the response to the object has the meaning of action withholding. Thus, very likely these neurons participate to the inhibition of neuronal chains activated by this same object. This role is in complete agreement with the well-established function of the prefrontal cortex to inhibit unwanted actions (see (Miller and Cohen, 2001b; Tanji and Hoshi, 2008; Miller and Cohen, 2001; Tanji and Hoshi, 2008). Concerning neurons preferring the Visual condition, the most likely explanation is that their discharge is related to the 2D features of the stimulus as provided by the inferotemporal cortex (see Orban, 2011). These neurons could have the role of emphasizing the inferotemporal-dependent object recognition for its possible use in abstract tasks (e.g. categorization, stimulus-reaction association).

A certain number of neurons respond equally well to two conditions and higher than the remaining one. Two categories are worth to be discussed. The first, larger category is made by neurons responding well to Action and Inaction conditions, their activation being weaker or absent in the Visual condition. The type of coding of these neurons could be related either to the 3D vs 2D features of the object or to task complexity (Visual vs Visuo-motor). The second category is formed by neurons responding stronger to the Action and the Visual condition, with a weak or absent discharge during Inaction condition. The type of coding of these neurons would be related to a situation in which action inhibition is not requested.

Overall, the various types of preference shown by single VLPF neurons indicate that several factors can influence the response to object observation. This is very likely related to the fact that this region on the one hand reflects visual inputs coming from high order visual cortex, on the other the possible meaning of these inputs when exploited for specific behaviors.

The population analysis provides a wider view on the function of the recorded region. Indeed, object observation in the Action condition produces the highest population response, followed by that in the Inaction condition. Object observation in the Visual condition elicits the weakest discharge. This suggests that the highest the behavioral relevance of the object, the strongest the prefrontal neuron response. This is in agreement with the literature showing higher neuronal responses when a visual abstract cue becomes associated to a specific behavioral response (Di Pellegrino and Wise, 1993; White and Wise, 1999), see (Duncan, 2001). Our data show that this concept applies also when the visual stimulus is a graspable object.

General conclusion

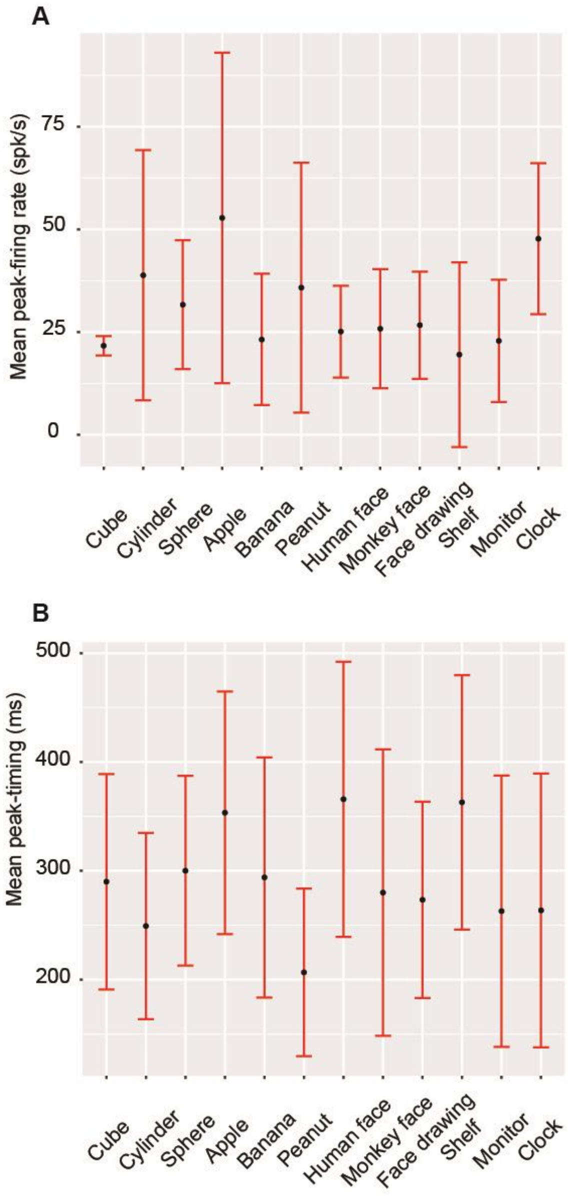
In this thesis, we explored two aspects of the neuronal mechanisms underlying the guidance of behavior based on the context. The first concerns the neural format of the prospective use of abstract rules and object features for guiding the instructed behavior. To this aim, we analyzed single neurons properties recorded during task unfolding and the temporal dynamics of specific VLPF populations. The second is related to whether and to what extent the visual response to object presentation is modulated when objects are target of goal directed actions.

The results obtained in the first study indicate that the mid-sector of VLPF is involved in the intentional coding of grasping actions, and allow to identify the format in which goals are encoded. In particular, the results of our decoding analyses indicate that a common type of neural coding is shared, at the population level, among the different phases of the task, supporting the idea that VLPF neurons actually encode the goals in a ‘pragmatic’ format. This suggests that the representation of the *goals of basic motor acts* (e.g. grasping, manipulating, etc.) provided by the parieto-premotor circuits can be exploited by prefrontal neurons for encoding at a more abstract level the *goals of complex sequential actions* (Chersi *et al.*, 2011; Fogassi *et al.*, 2005; Saito *et al.*, 2005; Shima *et al.*, 2006; see Rozzi and Fogassi, 2017; Tanji and Hoshi, 2008). This type of coding would also be crucial for the control of action unfolding, and for ending the motor programs when goals are achieved.

The results of the second study focused on the neuronal responses to visual presentation of several types of stimuli belonging to different categories, confirm the strong impact of inferotemporal cortex input on VLPF, in line with the data from neuroanatomical studies (Gerbella *et al.*, 2011; Webster *et al.*, 1994). The comparison between objects presentation in the passive Visual task and the active Visuo-Motor task show that visual responses to objects are often modulated by the task conditions in which the object is observed, with the strongest response when the object is target of an action. Thus, the connections between lateral prefrontal cortex and both posterior parietal and inferotemporal cortices can provide the former with both a pragmatic and a pictorial description of the object, so that VLPF, based on different types of instructions, can exploit this information to guide behavior.

Altogether, the data of the two studies indicate that VLPF neurons encode sensory stimuli (e.g. instructing cues and real objects) dependent of the current individual intention, and we propose that VLPF sensory-related responses are encoded at the neural level in terms of their behavioural outcome (pragmatic hypothesis).

Appendix: Supplementary Information (Study 1)



Supplementary Figure 1

Average peak-firing rate (a) and average peak timing (b) of neurons coding different stimuli based on their best response (see Table 1 for classification and numerosity). The black dots and the red bars indicate the average and standard deviation of peak (a) and timing (b), respectively. For each neuron, the peak firing rate was identified using a moving average (step=3bins, bin=20ms) of the activity recorded in the Stimulus epoch of the preferred stimulus.

References

Asaad, W.F., Rainer, G. and Miller, E.K. (1998), “Neural activity in the primate prefrontal cortex during associative learning”, *Neuron*, Neuron, Vol. 21 No. 6, pp. 1399–1407, doi: 10.1016/S0896-6273(00)80658-3.

Asaad, W.F., Rainer, G. and Miller, E.K. (2000), “Task-specific neural activity in the primate prefrontal cortex”, *Journal of Neurophysiology*, American Physiological Society Bethesda, MD, Vol. 84 No. 1, pp. 451–459, doi: 10.1152/jn.2000.84.1.451.

Averbeck, B.B., Chafee, M. V., Crowe, D.A. and Georgopoulos, A.P. (2002), “Parallel processing of serial movements in prefrontal cortex”, *Proceedings of the National Academy of Sciences of the United States of America*, Proc Natl Acad Sci U S A, Vol. 99 No. 20, pp. 13172–13177, doi: 10.1073/PNAS.162485599.

Averbeck, B.B., Sohn, J.-W. and Lee, D. (2006), “Activity in prefrontal cortex during dynamic selection of action sequences.”, *Nature Neuroscience*, Nature Publishing Group, Vol. 9 No. 2, pp. 276–82, doi: 10.1038/nn1634.

Badre, D. and D’Esposito, M. (2007), “Functional magnetic resonance imaging evidence for a hierarchical organization of the prefrontal cortex”, *Journal of Cognitive Neuroscience*, J Cogn Neurosci, Vol. 19 No. 12, pp. 2082–2099, doi: 10.1162/JOCN.2007.19.12.2082.

Badre, D. and D’Esposito, M. (2009), “Is the rostro-caudal axis of the frontal lobe hierarchical?”, *Nature Reviews Neuroscience*, Nat Rev Neurosci, September, doi: 10.1038/nrn2667.

Barbas, H. and Pandya, D.N. (1989), “Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey”, *Journal of Comparative Neurology*, John Wiley & Sons, Ltd, Vol. 286 No. 3, pp. 353–375.

Boch, R.A. and Goldberg, M.E. (1989), “Participation of prefrontal neurons in the preparation of visually guided eye movements in the rhesus monkey”, American Physiological

Society Bethesda, MD , Vol. 61 No. 5, doi: 10.1152/JN.1989.61.5.1064.

Borra, E., Belmalih, A., Calzavara, R., Gerbella, M., Murata, A., Rozzi, S. and Luppino, G. (2008), "Cortical connections of the macaque anterior intraparietal (AIP) area", *Cerebral Cortex*, Vol. 18 No. 5, pp. 1094–1111, doi: 10.1093/cercor/bhm146.

Borra, E., Gerbella, M., Rozzi, S. and Luppino, G. (2011), "Anatomical Evidence for the Involvement of the Macaque Ventrolateral Prefrontal Area 12r in Controlling Goal-Directed Actions", *Journal of Neuroscience*, J Neurosci, Vol. 31 No. 34, pp. 12351–12363, doi: 10.1523/jneurosci.1745-11.2011.

Borra, E., Gerbella, M., Rozzi, S. and Luppino, G. (2015), "Projections from caudal ventrolateral prefrontal areas to brainstem preoculomotor structures and to basal ganglia and cerebellar oculomotor loops in the macaque", *Cerebral Cortex*, Oxford University Press, Vol. 25 No. 3, pp. 748–764, doi: 10.1093/cercor/bht265.

Borra, E., Gerbella, M., Rozzi, S. and Luppino, G. (2017), "The macaque lateral grasping network: A neural substrate for generating purposeful hand actions", *Neuroscience & Biobehavioral Reviews*, Pergamon, Vol. 75, pp. 65–90, doi: 10.1016/J.NEUBIOREV.2017.01.017.

Borra, E., Gerbella, M., Rozzi, S., Tonelli, S. and Luppino, G. (2014), "Projections to the Superior Colliculus From Inferior Parietal, Ventral Premotor, and Ventrolateral Prefrontal Areas Involved in Controlling Goal-Directed Hand Actions in the Macaque.", *CEREBRAL CORTEX*, Vol. 24, pp. 1054–1065, doi: 10.1093/cercor/bhs392.

Boussaoud, D. and Wise, S.P. (1993), "Primate frontal cortex: neuronal activity following attentional versus intentional cues", *Experimental Brain Research*, Springer-Verlag, Vol. 95 No. 1, pp. 15–27, doi: 10.1007/BF00229650.

Bruce, C.J. and Goldberg, M.E. (1985), "Primate frontal eye fields. I. Single neurons discharging before saccades", *Journal of Neurophysiology*, J Neurophysiol, Vol. 53 No. 3, pp. 603–635, doi: 10.1152/JN.1985.53.3.603.

Bruni, S., Giorgetti, V., Bonini, L. and Fogassi, L. (2015), “Processing and Integration of Contextual Information in Monkey Ventrolateral Prefrontal Neurons during Selection and Execution of Goal-Directed Manipulative Actions.”, *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, Society for Neuroscience, Vol. 35 No. 34, pp. 11877–90, doi: 10.1523/JNEUROSCI.1938-15.2015.

Butter, C.M. (1969), “Perseveration in extinction and in discrimination reversal tasks following selective frontal ablations in Macaca mulatta”, *Physiology and Behavior*, Vol. 4 No. 2, pp. 163–171, doi: 10.1016/0031-9384(69)90075-4.

Carlén, M. (2017), “What constitutes the prefrontal cortex?”, *Science*, American Association for the Advancement of Science, 27 October, doi: 10.1126/science.aan8868.

Cavada, C. and Goldman-Rakic, P.S. (1989), “Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and limbic areas with the frontal lobe.”, *Journal of Comparative Neurology*, Vol. 287 No. 4, pp. 422–45.

Chersi, F., Ferrari, P.F. and Fogassi, L. (2011), “Neuronal chains for actions in the parietal lobe: a computational model”, *PloS One*, PLoS One, Vol. 6 No. 11, doi: 10.1371/JOURNAL.PONE.0027652.

Chugani, H.T., Phelps, M.E. and Mazziotta, J.C. (1987), “Positron emission tomography study of human brain functional development”, *Annals of Neurology*, John Wiley & Sons, Ltd, Vol. 22 No. 4, pp. 487–497, doi: 10.1002/ANA.410220408.

Cipolloni, P.B. and Pandya, D.N. (1999), “Cortical Connections of the Frontoparietal Opercular Areas in the Rhesus Monkey”, *J. Comp. Neurol*, Vol. 403, pp. 431–458, doi: 10.1002/(SICI)1096-9861(19990125)403:4.

Constantinidis, C. and Procyk, E. (2004), “The primate working memory networks”, *Cognitive, Affective & Behavioral Neuroscience*, Cogn Affect Behav Neurosci, Vol. 4 No. 4, pp. 444–465, doi: 10.3758/CABN.4.4.444.

Constantinidis, C. and Qi, X.L. (2018), “Representation of spatial and feature information in the monkey dorsal and ventral prefrontal cortex”, *Frontiers in Integrative Neuroscience,* Frontiers Media S.A., August, doi: 10.3389/fnint.2018.00031.

Corbetta, M. and Shulman, G.L. (2002), “Control of goal-directed and stimulus-driven attention in the brain”, *Nature Reviews Neuroscience*, Vol. 3 No. 3, pp. 201–215, doi: 10.1038/NRN755.

Desimone, R. and Duncan, J. (1995), “Neural mechanisms of selective visual attention”, *Annual Review of Neuroscience*, Annu Rev Neurosci, Vol. 18, pp. 193–222, doi: 10.1146/ANNUREV.NE.18.030195.001205.

Di Pellegrino, G. and Wise, S.P. (1993), “Effects of attention on visuomotor activity in the premotor and prefrontal cortex of a primate”, *Somatosensory & Motor Research*, Somatosens Mot Res, Vol. 10 No. 3, pp. 245–262, doi: 10.3109/08990229309028835.

Dias, R., Robbins, T.W. and Roberts, A.C. (1996), “Dissociation in prefrontal cortex of affective and attentional shifts”, Vol. 380 No. 6569, pp. 69–72.

Diehl, M.M. and Romanski, L.M. (2014), “Responses of Prefrontal Multisensory Neurons to Mismatching Faces and Vocalizations”, *Journal of Neuroscience*, Society for Neuroscience, Vol. 34 No. 34, pp. 11233–11243, doi: 10.1523/JNEUROSCI.5168-13.2014.

Duncan, J. (2001), “An adaptive coding model of neural function in prefrontal cortex.”, *Nature Reviews. Neuroscience*, Vol. 2 No. 11, pp. 820–9, doi: 10.1038/35097575.

Ekstrom, L.B., Roelfsema, P.R., Arsenault, J.T., Bonmassar, G. and Vanduffel, W. (2008), “Bottom-up dependent gating of frontal signals in early visual cortex”, *Science*, NIH Public Access, Vol. 321 No. 5887, pp. 414–417, doi: 10.1126/science.1153276.

Everling, S., Tinsley, C.J., Gaffan, D. and Duncan, J. (2002), “Filtering of neural signals by focused attention in the monkey prefrontal cortex”, *Nature Neuroscience*, Nat Neurosci, Vol. 5 No. 7, pp. 671–676, doi: 10.1038/NN874.

Fecteau, J.H., Munoz, D.P., 2003. “Exploring the consequences of the previous trial”. Nature Reviews Neuroscience 2003 4:6 4, 435–443. https://doi.org/10.1038/nrn1114.

Fogassi, L., Ferrari, P.F., Gesierich, B., Rozzi, S., Chersi, F. and Rizzolotti, G. (2005), “Neuroscience: Parietal lobe: From action organization to intention understanding”, *Science*, doi: 10.1126/science.1106138.

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Matelli, M. and Rizzolatti, G. (1996), “Coding of peripersonal space in inferior premotor cortex (area F4).”, *Journal of Neurophysiology*, Vol. 76 No. 1, pp. 141–57.

Freedman, D.J., Riesenhuber, M., Poggio, T. and Miller, E.K. (2001), “Categorical representation of visual stimuli in the primate prefrontal cortex”, *Science*, American Association for the Advancement of Science, Vol. 291 No. 5502, pp. 312–316, doi: 10.1126/science.291.5502.312.

Freedman, D.J., Riesenhuber, M., Poggio, T. and Miller, E.K. (2002), “Visual Categorization and the Primate Prefrontal Cortex: Neurophysiology and Behavior”, *Journal of Neurophysiology*, American Physiological SocietyBethesda, MD , Vol. 88 No. 2, pp. 929–941, doi: 10.1152/jn.2002.88.2.929.

Funahashi, S. (2014), “Saccade-related activity in the prefrontal cortex: its role in eye movement control and cognitive functions”, *Frontiers in Integrative Neuroscience*, Vol. 8 No. June, pp. 1–13, doi: 10.3389/fnint.2014.00054.

Funahashi, S. and Andreau, J.M. (2013), “Prefrontal cortex and neural mechanisms of executive function”, *Journal of Physiology Paris*, Vol. 107 No. 6, pp. 471–482, doi: 10.1016/J.JPHYSPARIS.2013.05.001.

Funahashi, S., Bruce, C.J. and Goldman-Rakic, P.S. (1989), “Mnemonic coding of visual space in the monkey’s dorsolateral prefrontal cortex”, *Journal of Neurophysiology*, J Neurophysiol, Vol. 61 No. 2, pp. 331–349, doi: 10.1152/JN.1989.61.2.331.

Funahashi, S., Bruce, C.J. and Goldman-Rakic, P.S. (1991), “Neuronal activity related to saccadic eye movements in the monkey’s dorsolateral prefrontal cortex”, *Journal of Neurophysiology*, J Neurophysiol, Vol. 65 No. 6, pp. 1464–1483, doi: 10.1152/JN.1991.65.6.1464.

Funahashi, S., Inoue, M. and Kubota, K. (1993), “Delay-related activity in the primate prefrontal cortex during sequential reaching tasks with delay”, *Neuroscience Research*, Vol. 18 No. 2, pp. 171–175, doi: 10.1016/0168-0102(93)90019-M.

Fuster, J.M. (1997), “Network memory”, *Trends in Neurosciences*, Vol. 20 No. 10, pp. 451–459, doi: 10.1016/S0166-2236(97)01128-4.

Fuster, J.M. (2008), *The Prefrontal Cortex*, fourth edi., Academic Press, London.

Fuster, J.M. and Alexander, G.E. (1971), “Neuron activity related to short-term memory”, *Science (New York, N.Y.)*, Science, Vol. 173 No. 3997, pp. 652–654, doi: 10.1126/SCIENCE.173.3997.652.

Genovesio, A. and Ferraina, S. (2014), “The influence of recent decisions on future goal selection”, *Philosophical Transactions of the Royal Society B*: Biological Sciences 369, 20130477. https://doi.org/10.1098/rstb.2013.0477doi: 10.1098/rstb.2013.0477.

Genovesio, A., Wise, S.P. and Passingham, R.E. (2014), “Prefrontal-parietal function: From foraging to foresight”, *Trends in Cognitive Sciences*, Vol. 18 No. 2, pp. 72–81, doi: 10.1016/J.TICS.2013.11.007.

Gerbella, M., Baccarini, M., Borra, E., Rozzi, S. and Luppino, G. (2014), “Amygdalar connections of the macaque areas 45A and 45B”, *Brain Structure and Function*, Springer Verlag, Vol. 219 No. 3, pp. 831–842, doi: 10.1007/S00429-013-0538-2.

Gerbella, M., Belmalih, A., Borra, E., Rozzi, S. and Luppino, G. (2007), “Multimodal architectonic subdivision of the caudal ventrolateral prefrontal cortex of the macaque monkey”, *Brain Structure and Function*, Brain Struct Funct, Vol. 212 No. 3–4, pp. 269–301, doi: 10.1007/s00429-007-0158-9.

Gerbella, M., Belmalih, A., Borra, E., Rozzi, S. and Luppino, G. (2010), “Cortical connections of the macaque caudal ventrolateral prefrontal areas 45A and 45B", Cereb Cortex, Vol. 20 No. 1, pp. 141–168.

Gerbella, M., Belmalih, A., Borra, E., Rozzi, S. and Luppino, G. (2011), "Cortical connections of the anterior (F5a) subdivision of the macaque ventral premotor area F5", *Brain Structure and Function*, Vol. 216 No. 1, pp. 43–65, doi: 10.1007/S00429-010-0293-6.

Gerbella, M., Borra, E., Mangiaracina, C., Rozzi, S. and Luppino, G. (2016), "Corticostriate projections from areas of the 'lateral grasping network': Evidence for multiple hand-related input channels", *Cerebral Cortex,* Oxford University Press, Vol. 26 No. 7, pp. 3096–3115, doi: 10.1093/cercor/bhv135.

Gerbella, M., Borra, E., Tonelli, S., Rozzi, S. and Luppino, G. (2013), "Connectional heterogeneity of the ventral part of the macaque area 46", *Cerebral Cortex*, Cereb Cortex, Vol. 23 No. 4, pp. 967–987, doi: 10.1093/cercor/bhs096.

Gerbella, M., Rozzi, S. and Rizzolatti, G. (2017), "The extended object-grasping network", Vol. 235, pp. 2903–2916, doi: 10.1007/s00221-017-5007-3.

Gilbert, C.D. and Sigman, M. (2007), "Brain States: Top-Down Influences in Sensory Processing", *Neuron*, Vol. 54 No. 5, pp. 677–696, doi: 10.1016/J.NEURON.2007.05.019.

Goldman-Rakic, P.S. (1990), "Cellular and circuit basis of working memory in prefrontal cortex of nonhuman primates", *Progress in Brain Research*, Prog Brain Res, Vol. 85 No. C, pp. 325–336, doi: 10.1016/S0079-6123(08)62688-6.

Goldman-Rakic, P.S. (1987), "Circuitry of Primate Prefrontal Cortex and Regulation of Behavior by Representational Memory", *Comprehensive Physiology*, John Wiley & Sons, Inc., pp. 373–417, doi: 10.1002/CPHY.CP010509.

Gregoriou, G.G., Rossi, A.F., Ungerleider, L.G. and Desimone, R. (2014), "Lesions of prefrontal cortex reduce attentional modulation of neuronal responses and synchrony in V4", *Nature Neuroscience*, NIH Public Access, Vol. 17 No. 7, p. 1003, doi:

10.1038/NN.3742.

Hoshi, E., Shima, K. and Tanji, J. (1998), "Task-dependent selectivity of movement-related neuronal activity in the primate prefrontal cortex", *Journal of Neurophysiology*, J Neurophysiol, Vol. 80 No. 6, pp. 3392–3397, doi: 10.1152/JN.1998.80.6.3392.

Hoshi, E., Shima, K. and Tanji, J. (2000), "Neuronal activity in the primate prefrontal cortex in the process of motor selection based on two behavioral rules", *Journal of Neurophysiology*, J Neurophysiol, Vol. 83 No. 4, pp. 2355–2373, doi: 10.1152/JN.2000.83.4.2355.

Howells, H., Simone, L., Borra, E., Fornia, L., Cerri, G. and Luppino, G. (2020), "Reproducing macaque lateral grasping and oculomotor networks using resting state functional connectivity and diffusion tractography", *Brain Structure and Function*, Springer Science and Business Media Deutschland GmbH, Vol. 225 No. 8, pp. 2533–2551, doi: 10.1007/s00429-020-02142-2.

Iaccarino, L., Chieffi, S. and Iavarone, A. (2014), "Utilization Behavior: What Is Known and What Has to Be Known?", doi: 10.1155/2014/297128.

Ichihara-Takeda, S. and Funahashi, S. (2007), "Activity of primate orbitofrontal and dorsolateral prefrontal neurons: task-related activity during an oculomotor delayed-response task", *Experimental Brain Research*, Exp Brain Res, Vol. 181 No. 3, pp. 409–425, doi: 10.1007/S00221-007-0941-0.

Katsuki, F., Saito, M. and Constantinidis, C. (2014), "Influence of monkey dorsolateral prefrontal and posterior parietal activity on behavioral choice during attention tasks", doi: 10.1111/ejn.12662.

Kobak, D., Brendel, W., Constantinidis, C., Feierstein, C.E., Kepecs, A., Mainen, Z.F., Qi, X.-L., *et al.* (2016), "Demixed principal component analysis of neural population data", *Elifesciences.Org*, doi: 10.7554/eLife.10989.001.

Koechlin, E., Ody, C. and Kouneiher, F. (2003), "The architecture of cognitive control in the human prefrontal cortex”, *Science*, American Association for the Advancement of Science , Vol. 302 No. 5648, pp. 1181–1185, doi: 10.1126/science.1088545.

Koechlin, E. and Summerfield, C. (2007), “An information theoretical approach to prefrontal executive function”, *Trends in Cognitive Sciences*, Trends Cogn Sci, Vol. 11 No. 6, pp. 229–235, doi: 10.1016/J.TICS.2007.04.005.

Kolk, S.M. and Rakic, P. (2022), “Development of prefrontal cortex”, *Neuropsychopharmacology*, Springer Nature, Vol. 47 No. 1, pp. 41–57, doi: 10.1038/S41386-021-01137-9.

Kubota, K. and Funahashi, S. (1982), “Direction-specific activities of dorsolateral prefrontal and motor cortex pyramidal tract neurons during visual tracking”, *Journal of Neurophysiology*, J Neurophysiol, Vol. 47 No. 3, pp. 362–376, doi: 10.1152/JN.1982.47.3.362.

Kusunoki, M., Sigala, N., Nili, H., Gaffan, D. and Duncan, J. (2010), “Target detection by opponent coding in monkey prefrontal cortex”, *Journal of Cognitive Neuroscience*, J Cogn Neurosci, Vol. 22 No. 4, pp. 751–760, doi: 10.1162/JOCN.2009.21216.

Levy, B.J. and Wagner, A.D. (2011), “Cognitive control and right ventrolateral prefrontal cortex: reflexive reorienting, motor inhibition, and action updating”, *Annals of the New York Academy of Sciences*, John Wiley & Sons, Ltd, Vol. 1224 No. 1, pp. 40–62, doi: 10.1111/J.1749-6632.2011.05958.X.

Levy, R. and Goldman-Rakic, P.S. (2000), “Segregation of working memory functions within the dorsolateral prefrontal cortex.”, *Experimental Brain Research*, Vol. 133 No. 1, pp. 23–32.

Lhermitte, F. (1986), “Human autonomy and the frontal lobes. Part II: Patient behavior in complex and social situations: The ‘environmental dependency syndrome’”, *Annals of Neurology*, John Wiley & Sons, Ltd, Vol. 19 No. 4, pp. 335–343, doi: 10.1002/ana.410190405.

Meyers, E.M. (2013), “The neural decoding toolbox”, *Frontiers in Neuroinformatics*, Frontiers, Vol. 7 No. APR, p. 8, doi: 10.3389/fninf.2013.00008.

Miller, E.K. (2000), “The prefontral cortex and cognitive control”, *Nature Reviews Neuroscience 2000 1:1*, Nature Publishing Group, Vol. 1 No. 1, pp. 59–65, doi: 10.1038/35036228.

Miller, E.K. and Cohen, J.D. (2001), “An integrative theory of prefrontal cortex function”, *Annual Review of Neuroscience*, Annu Rev Neurosci, Vol. 24, pp. 167–202, doi: 10.1146/ANNUREV.NEURO.24.1.167.

Miller, E.K., Erickson, C.A. and Desimone, R. (1996), “Neural Mechanisms of Visual Working Memory in Prefrontal Cortex of the Macaque”, *Journal of Neuroscience*, Society for Neuroscience, Vol. 16 No. 16, pp. 5154–5167, doi: 10.1523/JNEUROSCI.16-16-05154.1996.

Miller, E.K., Lundqvist, M. and Bastos, A.M. (2018), “Working Memory 2.0”, *Neuron*, Neuron, Vol. 100 No. 2, pp. 463–475, doi: 10.1016/J.NEURON.2018.09.023.

Miller, E.K., Nieder, A., Freedman, D.J. and Wallis, J.D. (2003), “Neural correlates of categories and concepts”, *Current Opinion in Neurobiology*, Elsevier Ltd, Vol. 13 No. 2, pp. 198–203, doi: 10.1016/S0959-4388(03)00037-0.

Moody, S.L., Wise, S.P., Di Pellegrino, G. and Zipser, D. (1998), “A model that accounts for activity in primate frontal cortex during a delayed matching-to-sample task.”, *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, Vol. 18 No. 1, pp. 399–410.

Muhammad, R., Wallis, J.D. and Miller, E.K. (2006), “A comparison of abstract rules in the prefrontal cortex, premotor cortex, inferior temporal cortex, and striatum”, *Journal of Cognitive Neuroscience*, Vol. 18 No. 6, pp. 974–989, doi: 10.1162/jocn.2006.18.6.974.

Murata, A., Fadiga, L., Fogassi, L., Gallese, V., Raos, V. and Rizzolatti, G. (1997), “Object representation in the ventral premotor cortex (Area F5) of the monkey”, *Journal of*

Neurophysiology, Vol. 78 No. 4, pp. 2226–2230, doi: 10.1152/jn.1997.78.4.2226.

Murata, A., Gallese, V., Luppino, G., Kaseda, M. and Sakata, H. (2000), “Selectivity for the shape, size, and orientation of objects for grasping in neurons of monkey parietal area AIP”, *Journal of Neurophysiology*, J Neurophysiol, Vol. 83 No. 5, pp. 2580–2601, doi: 10.1152/JN.2000.83.5.2580.

Nakahara, K., Hayashi, T., Konishi, S. and Miyashita, Y. (2002), “Functional MRI of macaque monkeys performing a cognitive set-shifting task”, *Science (New York, N.Y.)*, Science, Vol. 295 No. 5559, pp. 1532–1536, doi: 10.1126/SCIENCE.1067653.

Nee, D.E. and D’Esposito, M. (2016), “The hierarchical organization of the lateral prefrontal cortex”, *ELife*, Elife, Vol. 5 No. MARCH2016, doi: 10.7554/eLife.12112.

Nelissen, K., Borra, E., Gerbella, M., Rozzi, S., Luppino, G., Vanduffel, W., Rizzolatti, G., *et al.* (2011), “Action observation circuits in the macaque monkey cortex”, *Journal of Neuroscience*, J Neurosci, Vol. 31 No. 10, pp. 3743–3756, doi: 10.1523/JNEUROSCI.4803-10.2011.

Ninokura, Y., Mushiake, H. and Tanji, J. (2003), “Representation of the Temporal Order of Visual Objects in the Primate Lateral Prefrontal Cortex”, *Journal of Neurophysiology*, American Physiological SocietyBethesda, MD , Vol. 89 No. 5, pp. 2868–2873, doi: 10.1152/jn.00647.2002.

Ninokura, Y., Mushiake, H. and Tanji, J. (2004), “Integration of temporal order and object information in the monkey lateral prefrontal cortex”, *Journal of Neurophysiology*, J Neurophysiol, Vol. 91 No. 1, pp. 555–560, doi: 10.1152/JN.00694.2003.

Ninomiya, T., Noritake, A., Kobayashi, K. and Isoda, M. (2020), “A causal role for frontal cortico-cortical coordination in social action monitoring”, *Nature Communications*, Nat Commun, Vol. 11 No. 1, doi: 10.1038/s41467-020-19026-y.

O Scalaidhe, S.P., Wilson, F.A.W. and Goldman-Rakic, P.S. (1999), “Face-selective Neurons During Passive Viewing and Working Memory Performance of Rhesus Monkeys:

Evidence for Intrinsic Specialization of Neuronal Coding", *Cerebral Cortex,* Oxford Academic, Vol. 9 No. 5, pp. 459–475, doi: 10.1093/cercor/9.5.459.

Ó Scalaidhe, S.P., Wilson, F.A.W. and Goldman-Rakic, P.S. (1997), "Areal segregation of face-processing neurons in prefrontal cortex", *Science,* doi: 10.1126/science.278.5340.1135.

O Scalaidhe, S.P., Wilson, F.A.W.W., Goldman-Rakic, P.S., Ó Scalaidhe, S.P., Wilson, F.A.W.W., Goldman-Rakic, P.S., O Scalaidhe, S.P., *et al.* (1999), "Face-selective Neurons During Passive Viewing and Working Memory Performance of Rhesus Monkeys: Evidence for Intrinsic Specialization of Neuronal Coding", *Cerebral Cortex,* Oxford Academic, Vol. 9 No. 5, pp. 459–475, doi: 10.1093/cercor/9.5.459.

Orban, G.A. (2011), "The extraction of 3D shape in the visual system of human and nonhuman primates", *Annual Review of Neuroscience,* Annu Rev Neurosci, Vol. 34, pp. 361–368, doi: 10.1146/annurev-neuro-061010-113819.

Paneri, S. and Gregoriou, G.G. (2017), "Top-down control of visual attention by the prefrontal cortex. Functional specialization and long-range interactions", *Frontiers in Neuroscience,* Frontiers, September, doi: 10.3389/fnins.2017.00545.

Passingham, D. and Sakai, K. (2004), "The prefrontal cortex and working memory: Physiology and brain imaging", *Current Opinion in Neurobiology,* Curr Opin Neurobiol, Vol. 14 No. 2, pp. 163–168, doi: 10.1016/j.conb.2004.03.003.

Passingham, R.E. (1993), *The Frontal Lobe and Voluntary Action.,* Oxford University Press, Oxford.

Passingham, R.E., Toni, I. and Rushworth, M.F.S. (2000), "Specialisation within the prefrontal cortex: the ventral prefrontal cortex and associative learning", *Experimental Brain Research*, Exp Brain Res, Vol. 133 No. 1, pp. 103–113, doi:10.1007/S002210000405.

Paus, T. (1999), "Structural maturation of neural pathways in children and adolescents: In vivo study", *Science,* Vol. 283 No. 5409, pp. 1908–1911, doi: 10.1126/SCIENCE.283.5409.1908.

Petrides, M. (2005), "Lateral prefrontal cortex: architectonic and functional organization", *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Philos Trans R Soc Lond B Biol Sci, Vol. 360 No. 1456, pp. 781–795, doi: 10.1098/RSTB.2005.1631.

Petrides, M. and Pandya, D.N. (1984), "Projections to the frontal cortex from the posterior parietal region in the rhesus monkey.", *Journal of Comparative Neurology,* Vol. 228 No. 1, pp. 105–16.

Petrides, M. and Pandya, D.N. (1999), "Dorsolateral prefrontal cortex: Comparative cytoarchitectonic analysis"in the human and the macaque brain and corticocortical connection patterns", *European Journal of Neuroscience,* Eur J Neurosci, Vol. 11 No. 3, pp. 1011–1036, doi: 10.1046/j.1460-9568.1999.00518.x.

Petrides, M. and Pandya, D.N. (2002), "Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey", *European Journal of Neuroscience,* Vol. 16, pp. 291–310, doi: 10.1046/j.1460-9568.2002.02090.x.

Preuss, T.M. and Goldman-Rakic, P.S. (1989), "Connections of the ventral granular frontal cortex of macaques with perisylvian premotor and somatosensory areas: Anatomical evidence for somatic representation in primate frontal association cortex", *Journal of Comparative Neurology,* Vol. 282 No. 2, pp. 293–316, doi: 10.1002/cne.902820210.

Rainer, G. and Miller, E.K. (2000), "Effects of visual experience on the representation of objects in the prefrontal cortex.", *Neuron,* Vol. 27 No. 1, pp. 179–89.

Rainer, G., Rao, S.C. and Miller, E.K. (1999), "Prospective Coding for Objects in Primate Prefrontal Cortex", *Journal of Neuroscience,* Society for Neuroscience, Vol. 19 No. 13, pp. 5493–5505, doi: 10.1523/JNEUROSCI.19-13-05493.1999.

Raos, V., Umiltá, M.A., Murata, A., Fogassi, L. and Gallese, V. (2006), "Functional properties of grasping-related neurons in the ventral premotor area F5 of the macaque monkey", *Journal of Neurophysiology,* Vol. 95 No. 2, pp. 709–729, doi: 10.1152/jn.00463.2005.

Riley, M.R., Qi, X.-L. and Constantinidis, C. (2016), "Functional specialization of areas along the anterior–posterior axis of the primate prefrontal cortex", *Cerebral Cortex,* pp. 1–15, doi: 10.1093/cercor/bhw190.

Rizzolatti, G., Cattaneo, L., Fabbri-Destro, M. and Rozzi, S. (2014), "Cortical Mechanisms Underlying the Organization of Goal-Directed Actions and Mirror Neuron-Based Action Understanding", *Physiological Reviews,* Vol. 94 No. 2, pp. 655–706, doi: 10.1152/physrev.00009.2013.

Roelfsema, P.R. (2006), "Cortical algorithms for perceptual grouping", *Annual Review of Neuroscience,* Annual Reviews, July, doi: 10.1146/annurev.neuro.29.051605.112939.

Romanski, L.M. (2007), "Representation and integration of auditory and visual stimuli in the primate ventral lateral prefrontal cortex.", *Cerebral Cortex (New York, N.Y. : 1991),* NIH Public Access, Vol. 17 Suppl 1 No. Suppl 1, pp. i61-9, doi: 10.1093/cercor/bhm099.

Romanski, L.M. and Averbeck, B.B. (2005), "Neural representation of vocalizations in the primate ventrolateral prefrontal cortex", *Journal of Neurophysiology,* Vol. 93, pp. 734–747, doi: 10.1152/jn.00675.2004.

Romanski, L.M. and Diehl, M.M. (2011), "Neurons responsive to face-view in the primate ventrolateral prefrontal cortex", *Neuroscience,* NIH Public Access, Vol. 189, pp. 223–235, doi: 10.1016/j.neuroscience.2011.05.014.

Rozzi, S., Bimbi, M., Gravante, A., Simone, L. and Fogassi, L. (2021), "Visual response of ventrolateral prefrontal neurons and their behavior-related modulation", *Scientific Reports,* Nature Research, Vol. 11 No. 1, p. 10118, doi: 10.1038/s41598-021-89500-0.

Rozzi, S., Calzavara, R., Belmalih, A., Borra, E., Gregoriou, G.G., Matelli, M. and Luppino, G. (2006), "Cortical connections of the inferior parietal cortical convexity of the macaque monkey", *Cerebral Cortex*, Vol. 16 No. 10, pp. 1389–1417, doi: 10.1093/cercor/bhj076.

Rozzi, S. and Fogassi, L. (2017), "Neural coding for action execution and action observation in the prefrontal cortex and its role in the organization of socially driven behavior", *Frontiers in Neuroscience*, Vol. 11 No. SEP, pp. 1–9, doi: 10.3389/fnins.2017.00492.

Saga, Y., Iba, M., Tanji, J. and Hoshi, E. (2011), "Development of multidimensional representations of task phases in the lateral prefrontal cortex.", *Journal of Neuroscience*, J Neurosci, Vol. 31 No. 29, pp. 10648–65, doi: 10.1523/JNEUROSCI.0988-11.2011.

Saito, N., Mushiake, H., Sakamoto, K., Itoyama, Y. and Tanji, J. (2005), "Representation of Immediate and Final Behavioral Goals in the Monkey Prefrontal Cortex during an Instructed Delay Period", doi: 10.1093/cercor/bhi032.

Sakagami, M., Tsutsui, K.I., Lauwereyns, J., Koizumi, M., Kobayashi, S. and Hikosaka, O. (2001), "A Code for Behavioral Inhibition on the Basis of Color, But Not Motion, in Ventrolateral Prefrontal Cortex of Macaque Monkey", *Journal of Neuroscience*, Society for Neuroscience, Vol. 21 No. 13, pp. 4801–4808, doi: 10.1523/JNEUROSCI.21-13-04801.2001.

Saleem, K.S., Kondo, H. and Price, J.L. (2008), "Complementary circuits connecting the orbital and medial prefrontal networks with the temporal, insular, and opercular cortex in the macaque monkey", *The Journal of Comparative Neurology*, J Comp Neurol, Vol. 506 No. 4, pp. 659–693, doi: 10.1002/CNE.21577.

Saleem, K.S., Miller, B. and Price, J.L. (2014), "Subdivisions and connectional networks of the lateral prefrontal cortex in the macaque monkey", *Journal of Comparative Neurology*, Vol. 522 No. 7, pp. 1641–1690, doi: 10.1002/cne.23498.

Seger, C.A. and Miller, E.K. (2010), "Category learning in the brain", *Annual Review of Neuroscience*, doi: 10.1146/annurev.neuro.051508.135546.

Semendeferi, K., Lu, A., Schenker, N. and Damasio, H. (2002), "Humans and great apes share a large frontal cortex", *Nature Neuroscience 2002 5:3*, Nature Publishing Group, Vol. 5

No. 3, pp. 272–276, doi: 10.1038/nn814.

Shima, K., Isoda, M., Mushiake, H. and Tanji, J. (2006), “Categorization of behavioural sequences in the prefrontal cortex”, *Nature 2006 445:7125*, Nature Publishing Group, Vol. 445 No. 7125, pp. 315–318, doi: 10.1038/nature05470.

Simone, L., Bimbi, M., Rodà, F., Fogassi, L. and Rozzi, S. (2017), “Action observation activates neurons of the monkey ventrolateral prefrontal cortex”, *Nature Publishing Group*, doi: 10.1038/srep44378.

Simone, L., Rozzi, S., Bimbi, M. and Fogassi, L. (2015), “Movement-related activity during goal-directed hand actions in the monkey ventrolateral prefrontal cortex”, *European Journal of Neuroscience*, doi: 10.1111/ejn.13040.

Sowell, E.R., Thompson, P.M., Holmes, C.J., Jernigan, T.L. and Toga, A.W. (1999), “In vivo evidence for post-adolescent brain maturation in frontal and striatal regions”, *Nature Neuroscience 1999 2:10*, Nature Publishing Group, Vol. 2 No. 10, pp. 859–861, doi: 10.1038/13154.

Spaak, E., Watanabe, K., Funahashi, S. and Stokes, M.G. (2017), “Stable and Dynamic Coding for Working Memory in Primate Prefrontal Cortex”, doi: 10.1523/JNEUROSCI.3364-16.2017.

Tanila, H., Carlson, S., Linnankoski, I., Lindroos, F. and Kahila, H. (1992), “Functional properties of dorsolateral prefrontal cortical neurons in awake monkey”, *Behavioural Brain Research*, Behav Brain Res, Vol. 47 No. 2, pp. 169–180, doi: 10.1016/S0166-4328(05)80123-8.

Tanji, J. and Hoshi, E. (2001), “Behavioral planning in the prefrontal cortex”, *Current Opinion in Neurobiology*, Elsevier Ltd, Vol. 11 No. 2, pp. 164–170, doi: 10.1016/S0959-4388(00)00192-6.

Tanji, J. and Hoshi, E. (2008), “Role of the lateral prefrontal cortex in executive behavioral control”, *Physiological Reviews*, American Physiological Society, Vol. 88 No. 1, pp. 37–

57, doi: 10.1152/physrev.00014.2007.

Tsao, D.Y., Schweers, N., Moeller, S. and Freiwald, W.A. (2008), “Patches of face-selective cortex in the macaque frontal lobe.”, *Nature Neuroscience,* Vol. 11 No. 8, pp. 877–9, doi: 10.1038/nn.2158.

Walker, A.E. (1940), “A cytoarchitectural study of the prefrontal area of the macaque monkey”, *Journal of Comparative Neurology,* John Wiley & Sons, Ltd, Vol. 73 No. 1, pp. 59–86, doi: 10.1002/CNE.900730106.

Wallis, J.D., Anderson, K.C. and Miller, E.K. (2001), “Single neurons in prefrontal cortex encode abstract roles”, *Nature,* Nature Publishing Group, Vol. 411 No. 6840, pp. 953–956, doi: 10.1038/35082081.

Watanabe, M. (1996), “Reward expectancy in primate prefrental neurons”, *Nature 1996 382:6592,* Nature Publishing Group, Vol. 382 No. 6592, pp. 629–632, doi: 10.1038/382629a0.

Webster, M.J., Bachevalier, J. and Ungerleider, L.G. (1994), “Connections of inferior temporal areas TEO and TE with parietal and frontal cortex in macaque monkeys”, *Cerebral Cortex,* Vol. 4 No. 5, pp. 470–483, doi: 10.1093/cercor/4.5.470.

White, I.M. and Wise, S.P. (1999), “Rule-dependent neuronal activity in the prefrontal cortex”, *Experimental Brain Research 1999 126:3,* Springer, Vol. 126 No. 3, pp. 315–335, doi: 10.1007/S002210050740.

Wilson, F.A.W., Scalaidhe, S.P.. and Goldman-Rakic, P.S. (1993), “Dissociation of object and spatial processing domains in primate prefrontal cortex”, *Science (New York, N.Y.),* Science, Vol. 260 No. 5116, pp. 1955–1958, doi: 10.1126/SCIENCE.8316836.

Yamagata, T., Nakayama, Y., Tanji, J. and Hoshi, E. (2009), “Processing of visual signals for direct specification of motor targets and for conceptual representation of action targets in the dorsal and ventral premotor cortex.”, *Journal of Neurophysiology,* Vol. 102 No. 6, pp. 3280–94, doi: 10.1152/jn.00452.2009.

Yamagata, T., Nakayama, Y., Tanji, J. and Hoshi, E. (2012), “Distinct Information Representation and Processing for Goal-Directed Behavior in the Dorsolateral and Ventrolateral Prefrontal Cortex and the Dorsal Premotor Cortex”, *Journal of Neuroscience*, Vol. 32 No. 37, pp. 12934–12949, doi: 10.1523/JNEUROSCI.2398-12.2012.

Yeterian, E.H., Pandya, D.N., Tomaiuolo, F. and Petrides, M. (2012), “The cortical connectivity of the prefrontal cortex in the monkey brain.”, *Cortex*, Elsevier Srl, Vol. 48 No. 1, pp. 58–81, doi: 10.1016/j.cortex.2011.03.004.

Yoshida, K., Saito, N., Iriki, A. and Isoda, M. (2011), “Representation of others’ action by neurons in monkey medial frontal cortex”, *Current Biology : CB*, Elsevier Ltd, Vol. 21 No. 3, pp. 249–253, doi: 10.1016/j.cub.2011.01.004.