



UNIVERSITÀ DI PARMA

CORSO DI LAUREA MAGISTRALE IN PSICOBIOLOGIA E
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**Linkage of multiple visual and motor properties in single neuron
activity of monkey's anterior intraparietal area**

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ANNO ACCADEMICO 2018- 2019

*A mio padre e mia madre
a cui voglio molto bene.*

ABSTRACT – English

Neural coding of objects and actions involves many parieto-frontal areas including the anterior intraparietal area (AIP). Many studies show that neurons of this area become active not only during action execution but also during the observation of other's actions both when performed live in front of the monkey and when presented in a videotaped format. Nonetheless, no study has ever investigated the possible linkage between these multiple visual and motor properties, preventing to understand the underlying coding principles. To address this issue, we investigated AIP neuron activity during a large set of execution and action observation tasks. We recorded 134 neurons showing a wide variety of functional properties, with most of them responding during action execution and/or object presentation, and most of them also discharged during observation of actions. Among these latter, most encode both live and filmed actions, with a wide variety of mixed relationship with their motor properties. By means of an unsupervised clustering of functional properties at the population level, we have been able to show that the underlying coding principle of AIP neurons emphasize the relevance of the context in terms of space in which object and actions are presented and the agent who is expected to act in that context.

ABSTRACT – Italiano

L'elaborazione neurale di azioni osservate svolte da altri coinvolge diverse aree parieto-frontali, tra cui l'area intraparietale anteriore (AIP). Diversi studi mostrano che neuroni di quest'area si attivano non solo durante l'esecuzione dell'azione ma anche durante la visione di azioni svolte da altri sia presentate in un contesto live che in formato video. Ciò nonostante, nessuno studio ha mai indagato la possibile connessione tra queste multiple proprietà visive e motorie, prevenendo la comprensione dei sottostanti principi di codifica. Per affrontare questa questione, abbiamo investigato l'attività dei neuroni in AIP durante un ampio set di task di esecuzione e osservazione di azioni. Abbiamo registrato l'attività di 134 neuroni che hanno mostrato un'ampia varietà di proprietà funzionali, tra cui la maggior parte comprendevano risposte sia durante l'epoca di presentazione dell'oggetto che durante la presentazione di azioni. Tra queste ultime, la maggior parte codificava sia azioni live che video, con un'ampia varietà di relazioni miste con le loro proprietà motorie. Mediante l'utilizzo di una tecnica che permetteva il raggruppamento in modo non supervisionato di proprietà funzionali a livello di popolazione, siamo stati in grado di mostrare che i principi di codifica sottostanti i neuroni di AIP enfatizzano la rilevanza del contesto in termini di spazio dove l'oggetto e l'azione vengono presentati, e l'agente che dovrebbe agire in tale contesto.

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1 INTRODUCTION

In the last forty years, theoretical ideas and experimental data deeply changed the view of the motor system. Perception and action have been considered for a long time as two serially organized steps of processing, with the former relying on sensory brain areas and the latter implemented by the motor cortex. In this view, cognition would emerge as an intermediate step of information processing performed by associative cortical areas. While the classic view separates motor, sensory and cognitive functions, the latest scientific discoveries show that sensory and motor information even at single cell level shares the same representational format, suggesting that many perceptual and cognitive abilities can stem from the motor domain. Cortical motor regions have a crucial role in perception, especially when sensory information is required for acting.

In his influential theory of visual perception, James Gibson (1979) that the visual system automatically extracts the constellation of potential actions (termed “affordances”) we can perform on an object whose image impinges on the retina. Among the different possible affordances of an object, the one that will prevail and will be more likely turned into an overtly executed action depends upon the contextual situation, the goals and intentions of the perceiver. For example, a cup might afford grasping of its handle or of its body if one expects it contains a hot or cold drink, respectively. In addition, it might also afford grasping of its top, if it is empty and the agent wants simply to move it away. In all these cases, two types of parallel processing of the object take place: its semantic description, provided by higher order cortical visual areas, and a

pragmatic description, which includes the extraction of its various affordances and micro-affordances (Ellis and Tucker, 2000), and their possible translation into action (Jeannerod et al., 1995).

This can happen thanks to sensorimotor loops (Cisek, 2007; Cisek & Kalaska, 2010), or “perception-action” cycles (Fuster, 2015), that have been strongly supported by scientific discoveries of the last few decades. Here, perception and action are intimately interrelated and these two aspects cannot be clearly distinguished neither in the anatomical/spatial nor in the temporal domain. Scientists have found classes of neurons that can become active in response to external sensory information, providing an automatic translation of sensory stimuli into their potential behavioral meaning, and hence generating representations of several potential motor actions (Cisek, 2007). These neurons have been found in both agranular frontal cortex and the granular parietal cortex, which are subdivided in many anatomically and physiologically different areas strongly interconnected with each other.

Goodale and Milner (1992) suggested to distinguish the “ventral stream” linking primary visual cortex to the inferotemporal regions, which would be responsible for object recognition, from the “dorsal stream” ending in the posterior parietal region, deemed to play a crucial role in the sensorimotor transformations for visually guided object-directed actions. Based on clinical, functional and anatomical data, Rizzolatti and Matelli (2003) proposed to further subdivide the dorsal stream into two distinct functional systems, formed by partially segregated cortical pathways: the dorso-dorsal (d-d) and the ventro-dorsal (v-d) stream. According to their proposal, the d-d stream would correspond to the dorsal stream as previously defined by Milner and Goodale, exploiting sensory information for the control of reaching movements in space, while the v-d stream would

be specifically involved in sensorimotor transformation for grasping, space perception and action recognition. Thus, also within the originally defined dorsal stream, there is a subsystem, the v-d stream, which might play a role in perceptual functions.

Non-human primates, and particularly macaques, are the most suitable experimental model for obtaining highly detailed anatomical and functional information about the neural pathways and mechanisms underlying sensory, motor, and cognitive functions (see, e.g., Passingham, 2009; Sereno and Tootell, 2005) because of their close phylogenetic proximity to humans. Based on comparative observations, it is largely acknowledged that the monkey and human brains share a common plan in their anatomical and functional organization of cortical areas (Caminiti et al., 2015; Geyer et al., 2000; Mantini et al., 2013; Orban et al., 2004).

1.1 THE MOTOR SYSTEM

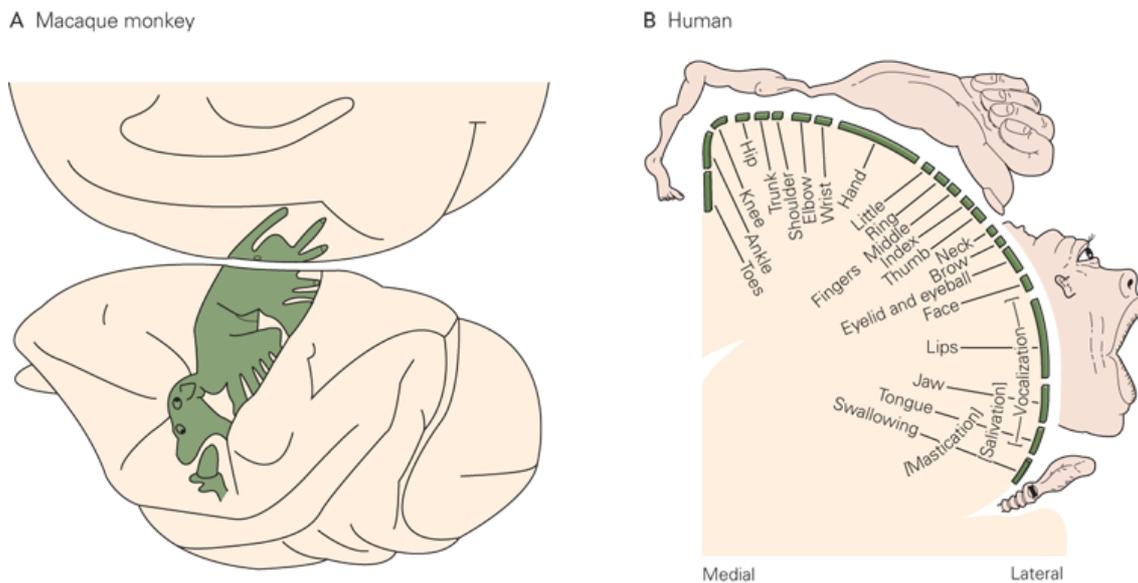


Figure 1 Somatotopic organization of the motor cortex. A) Side view of somatotopic organization of motor areas in the macaque monkey. B) Somatotopic organization in human motor cortex from a coronal section. *Figure from Principles of Neural Science, 5th edition.*

In 1860 Fritsch and Hitzig conducted an experiment that had a major influence on the emergence of the classic view on brain function and motor control. They used electrodes to apply weak electric currents to specific sites in the frontal cortex of the dog, showing that these currents evoked different movement relatively to the cortical location of the stimulation (Fritsch & Hitzig , 1870). Soon afterwards Woolsey identified a similar cortical motor map in monkeys showing that the cortical motor representation is highly somatotopic (Figure 1). These organization is conserved along phylogeny (Woolsey , Settlage, Meyer, & Spencer , 1952) up to human, where the canadian neurosurgeon Wilder Penfield mapped the cortex in awake patients during surgeries showing the same somatotopic map of the body. He also reported that motor cortex stimulation could lead to movement activation or inhibition as well as to muscle relaxation. These experiments

indicated that a primary role of this cortical area consists in the generation of movement. Hence, this brain region was named motor cortex.

Based on their anatomical specificities two main areas form the human motor cortex: the primary motor area (M1), which corresponds to Brodmann's area 4 (BA 4), and the complex formed by the premotor (PM) and supplementary motor (hSMA) areas, together corresponding to Brodmann's area 6 (BA 6). The laminar organization of BA 4 lacks the granular layer IV, which is also very poor in BA 6. In other areas of the neocortex layer IV is of particular interest because it receives a robust pathway from the thalamus, the earliest stage of the sensory relay to the cortex (García-Cabezas & Barbàs, 2015).

The best studied cortical motor output is the pyramidal tract, which originates in cortical layer V of precentral, parietal, and cingulate cortices. Beside contributing to the corticospinal tract, M1 neurons can end not only on spinal interneurons in the intermediate region of the spinal cord, but also on the ventral horn of the spinal cord (lamina IX) where they make contact with spinal motor neurons, directly. These neurons project monosynaptically to the motor neuronal pools dedicated to the control of the digits, hand, and wrist muscles rather than pools for more proximal parts of the arm. Supplementary motor area, and the cingulate motor areas contributing to the spinal cord only project to the intermediate laminae, thus modulating interneurons and circuits of the spinal cord (Dum & Strick, 2002)

Both areas BA 4 and BA 6 have their homologues in the monkey. Over the years architectonic studies demonstrated that BA 6 in both human and monkey is not homogeneous but consist in several distinct areas comprising a wide range of different somatotopic body movement representations. These subareas have specific connections

among themselves and with the rest of the cortex. Based on its topographic localization, architectonic feature and connections, the monkey's BA 6 has been divided in 3 main regions: mesial, dorsal and ventral, each of which has been further distinguished in its rostral and caudal portion (see Figure 2) (Matelli et al., 1985, Matelli et al., 1991, Schmahmann 1997).

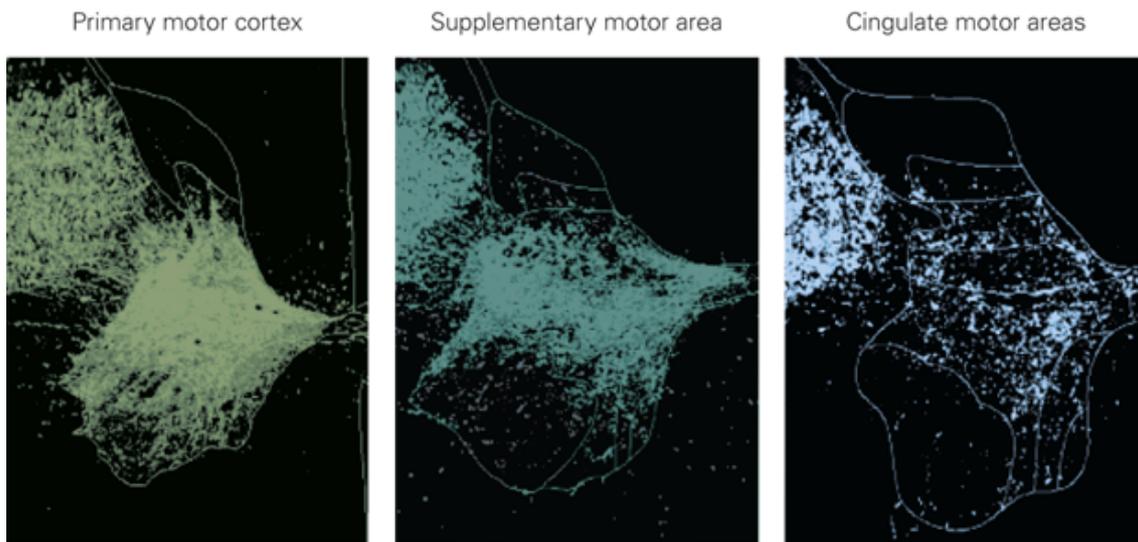


Figure 2. Fibers from the SNC projecting to the spinal cord. The axon of corticospinal fibres from the primary motor cortex, supplementary motor area and cingulate motor area terminate on interneuronal networks in the intermediate laminae (VI, VII, VIII). Only the primary motor cortex contains neurons whose axons terminate directly on the most ventral and lateral part of the ventral horn (lamina IX). The dense cluster of labelled axons adjacent to the horn in each section are the corticospinal axons descending in the dorsolateral funiculus before entering the spinal intermediate and ventral laminae. *Figure from Principles of Neural Science, 5th edition.*

The mesial regions (pre-SMA and SMA) F6-F3, the dorsal regions (PMd) F7-F2, and the ventral region (PMv) F5-F4 exhibit different somatotopic map and electrophysiological properties: short train stimulation in F3 shows a complete representation of body movements whereas long train stimulation in F6 shows slow and

complex movements related exclusively to the arm. The most caudal portion of PMd, F2, produces a complete somatotopic representation following intracortical stimulation (Raos et al., 2002). On the contrary, F7 shows no excitability in its rostral portion. In PMv, F4, caudal, and F5, more rostral, respond to stimulation showing arm, hand and mouth movements (Gentilucci et al, 1988; Gentilucci et al., 1989).

The multiplicity of cortical motor areas would be redundant if one admit they only play a role in the initiation or coordination of muscle activity. Indeed, studies on single neurons shows that this is not the case: these areas have unique properties and interact to perform diverse operation to plan, select and generate actions appropriate to external and internal context. At the single cell level, sensory stimuli such object spatial position, object features, and even others' observed movements elicit different responses. To assess the role of each area it has been necessary to assess the system in which it participates by analysing its neuronal responses and anatomical connections as well as perturbing it to analyse their influence in behaviour.

1.2 ANATOMO-FUNCTIONAL CHARACTERIZATION OF THE LATERAL GRASPING NETWORK

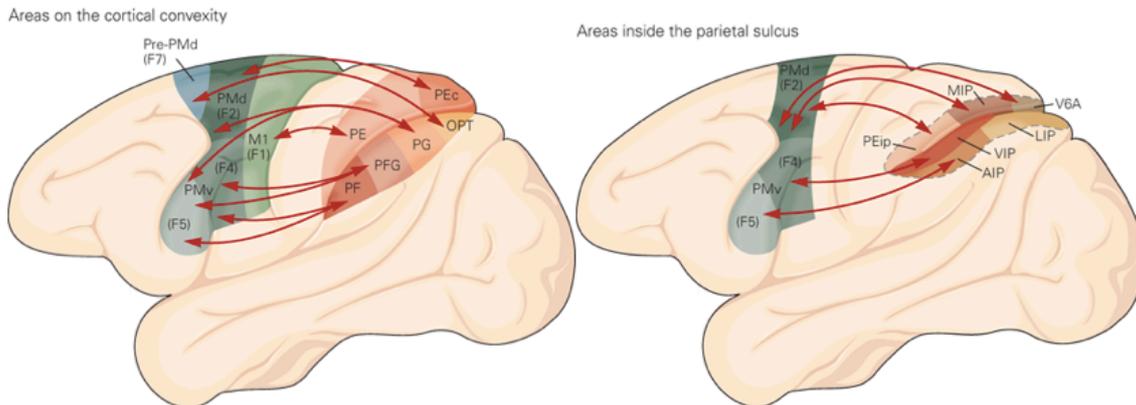


Figure 3. Sketch of parieto-frontal connections of the macaque's brain. The figure shows lateral views of a macaque brain. Motor cortex has been represented with different tones of green to highlight the cytoarchitectonic organization of this region. Similarly, cytoarchitectonic subdivisions of the parietal cortex have been indicated in tones of red. PMd, dorsal premotor; PMv, ventral premotor; MIP, medial intraparietal area; LIP, lateral intraparietal area; VIP, ventral intraparietal area; AIP, anterior intraparietal area. All the remaining acronyms defined as in (Pandya & Seltzer, 1982). *Figure from Principles of Neural Science, 5th edition.*

Neurophysiological studies in the monkey have revealed that the anterior intraparietal area (AIP) (Sakata et al., 1995; Baumann et al., 2009), the posterior parietal area V6A (Fattori et al., 2012; Fattori et al., 2015), the ventral premotor area F5 (Murata et al., 1997; Raos et al., 2006; Fluett et al., 2010; Bonini et al., 2014b; Vargas-Irwin et al., 2015) and the ventro-rostral portion of the dorsal premotor area F2 (Raos et al., 2004; Vargas-Irwin et al., 2015) host visuomotor neurons that discharge during both the visual presentation of target objects and the execution of reaching-grasping actions. These areas form a rich

set of parieto-frontal circuits (Figure 3) that underlie the visuomotor transformations of objects properties into the most appropriate motor acts to interact with them (Maranesi et al., 2014a).

Studies on the forelimb field of PMv (areas F4 and F5) and of the adjacent ventro-rostral sector of the dorsal premotor area F2 (F2vr) demonstrated the existence of several classes of neurons with distinct functional roles. The most obvious type of cells one would expect to find in a motor area are “purely motor neurons” (Figure 4, Unit A): they respond during the execution of hand, hand-and-mouth or arm motor acts, and are deemed to code “motor goals”, such as the direction of reaching (Kakei et al., 2001) or the features of graspable objects (Rizzolatti et al., 1988; Raos et al., 2006; Bonini et al., 2009), even regardless of the specific movement sequence required to attain the goal (Umiltà et al., 2008). In addition, it has been shown that hand-related neurons can also specify the type of grip to be employed for grasping (Rizzolatti et al., 1988; Raos et al., 2004; Raos et al., 2006; Bonini et al., 2009). Interestingly, besides purely motor neurons, many PM cells show bimodal or multimodal properties (Kohler, 2002). Single cell recording in both F5 and AIP shows the existence of “canonical neurons” (Figure 4, Unit B), a class of neurons responding to the visual presentation of objects and during the motor phase to grasp it (Murata et al., 1997; Sakata et al., 1995; Raos et al., 2006). These areas are tightly anatomically connected (Borra et al., 2008) and deemed to create visuomotor transformations underlying object grasping: the simple sight of a visually presented object produces the activation of the neuronal motor repertoire suitable to take possession of it (object affordance). Furthermore, reversible inactivation of both AIP (Gallese et al., 1994) and F5 (Fogassi et al., 2001) significantly impair visually guided object grasping, providing causal evidence of a critical role of

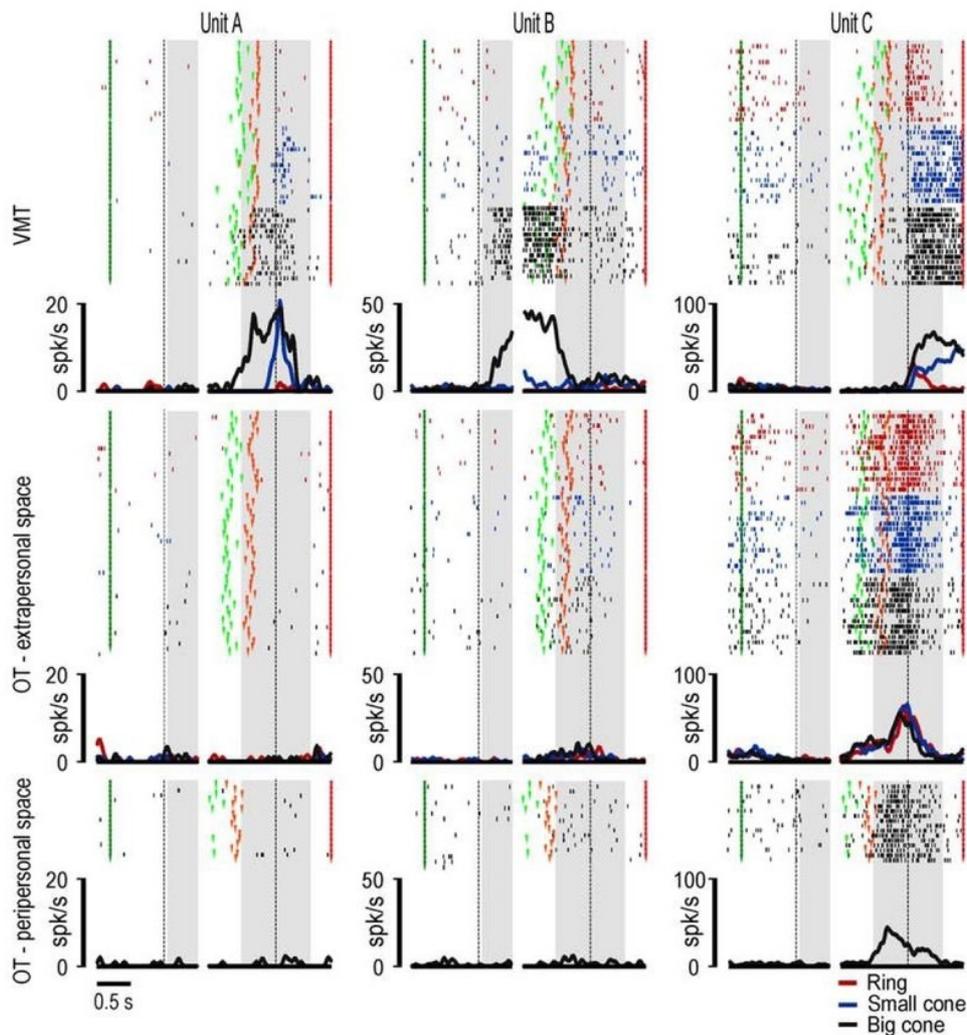


Figure 4. Area F5 single unit examples. Example of a purely motor neuron (Unit A), a canonical neuron (Unit B), and a mirror neuron (Unit C). For each neuron, the gap in the histogram and rastergram is used to indicate that the activity on its left side has been aligned on object presentation (first vertical dashed line in the left panel), whereas that on its right side is aligned on the pulling onset (second vertical dashed line in the right panel) of the same trial. The gray shaded areas represent the time windows used for statistical analysis of neuronal response. Markers: dark green, cue sound onset; light green, cue sound offset (go signal); orange, detachment of the hand from the starting position (reaching onset); red, reward delivery at the end of the trial. The same markers have been used to identify the behavioral event of interest of both the visuomotor and observation tasks. Figure and caption from (Bonini et al., 2014).

the AIP-F5 circuits in transforming the visual representation of object physical properties into the appropriate potential motor plan for interacting with them.

Recent studies with simultaneous recording from area AIP, F5 and F1 provide an elegant dynamic pictures of the visuomotor transformation process (Figure 5) (Schaffelhofer & Scherberger, 2016). They further support the idea that causal functional relationships between these nodes of the network causes sensory information in AIP to be turned into planned actions in area F5, which finally recruits the “common motor output” by means of its direct projections to the spinal cord (Borra et al., 2010) and, via its direct link with the primary motor area F1 (Kraskov et al., 2011; Schaffelhofer & Scherberger, 2016).

Another class of cell largely described in parietal and frontal areas is constituted by the so called “mirror neurons” (MNs, Figure 4, Unit C). The regions in which they have been found includes area F5 (di Pellegrino et al, 1992; Gallese et al, 1996; Rizzolatti et al, 1996), the inferior parietal areas PFG (Bonini et al, 2009; Fogassi et al, 2005), AIP (Maeda et al, 2015; Pani et al, 2014), PMd (Cisek and Kalaska 2004; Tkach et al, 2007), the medial frontal cortex, in particular area F6 (Yoshida et al. 2011; Livi et al, 2019) and anterior cingulate cortex (Mukamel et al, 2010; Yoshida et al, 2011).

Neurons with mirror properties have also been described in the primary motor cortex (Dushanova and Donoghue 2010; Tkach et al, 2007; Vigneswaran et al, 2013), and convergent anatomical (Borra et al, 2011; Gerbella et al, 2013) and functional evidence (Nelissen et al, 2011) suggests that even the ventrolateral prefrontal cortex (VLPF) may host neurons with mirror-like properties (Rozzi and Fogassi, 2017). MNs encode motor actions both when they are actively performed by the monkey and when

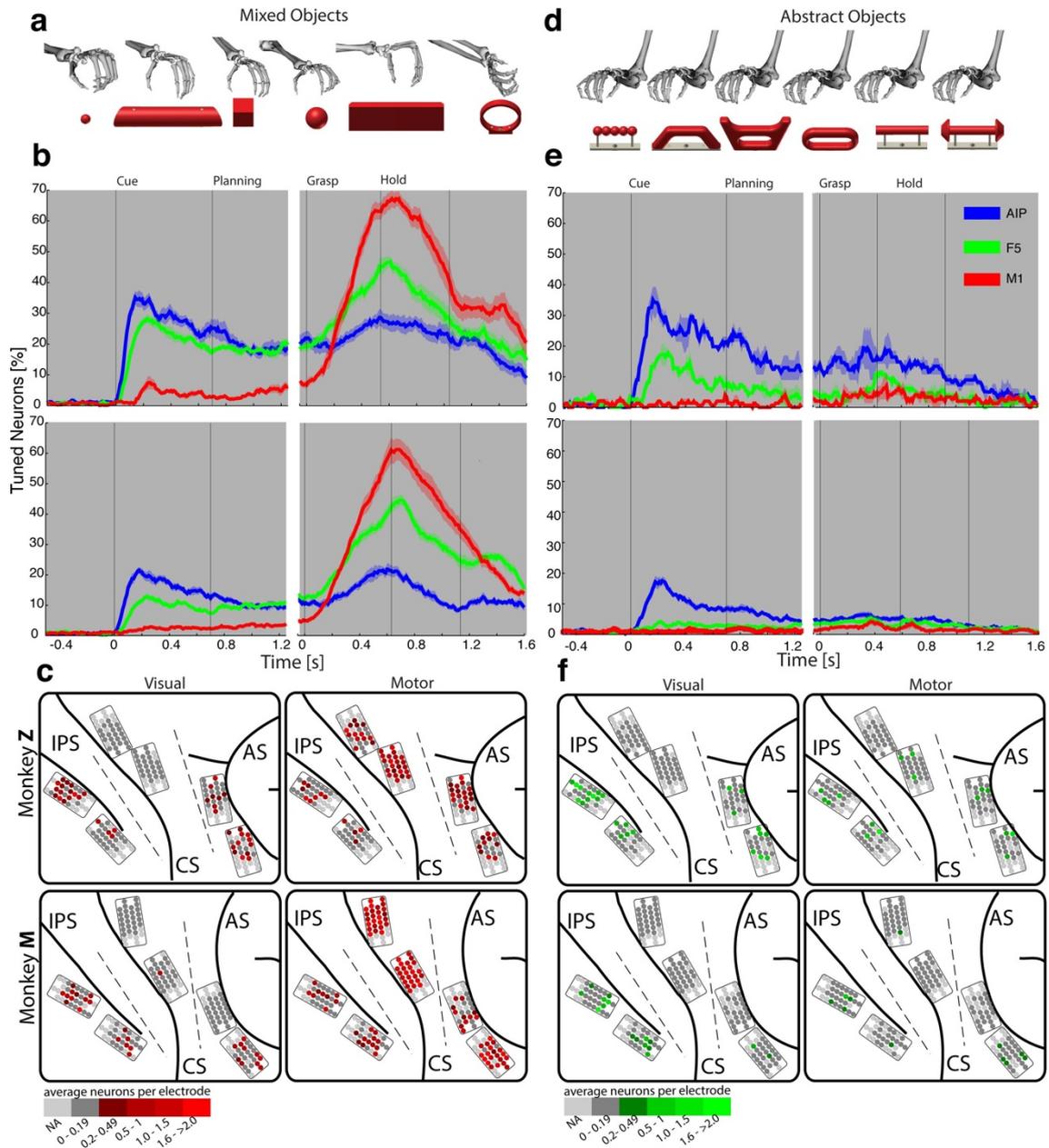


Figure 5. Visual processing of object shapes. a) Object presented b) Percentage of tuned neurons c) Tuned neurons (shades of red) were mapped to their recording location during the visual ($t = 0.16$ s after object presentation) and motor phase ($t = 0.7$ s after movement onset) d) Object presented. e) Similar to b, but for the abstract objects set. (f) Similar to c, but showing the map of tuned neurons (shades of green) with respect to the abstract object set. For b, e: Data is doubly aligned on cue onset and on the grasp (go) signal. Shades represent standard error from mean (s.e.m.) across recording sessions. For c, f: The number of tuned neurons per channel averaged across all recording sessions and visualized in shades of green and red for the abstract and mixed objects, respectively. (Schaffelhofer, Scherberger 2016).

they are observed, done by another agent (Gallese et al. 1996; Rizzolatti et al. 1996). MNs play a role in monitoring one's own actions in addition to those of others (Maranesi et al., 2015).

Visual information can reach F5 both through direct and indirect pathways (Ferrari et al., 2009): the classical direct pathway involves the inferior parietal areas AIP (Borra et al., 2008) and PFG (Rozzi et al., 2005), whereas the indirect pathways, which are still poorly investigated, may involve the VLPF and the MFC, which are anatomically linked with both inferior parietal and temporal cortices the superior temporal sulcus (STS) (Borra et al., 2011; Gerbella et al., 2013; Luppino et al., 1990; Luppino et al., 1993) which host neurons responsive to biological movements (Barraclough et al, 2009; Jellema and Perrett 2006; Perrett et al, 1989).

Recent findings (Bonini et al., 2014) showed even a more complex picture with the two types of visuomotor neurons, canonical and MNs, being not completely separated. Area F5 hosts two types of neurons with canonical properties: most of them fit with the classical description of “canonical neurons”, showing object presentation responses strictly constrained to the monkey's peripersonal (pragmatic) space, likely playing a role in the transformation of the “graspability” of an object into the appropriate motor plan to interact with it (Fogassi et al., 2001), whereas others, called ‘canonical-mirror’ neurons, can respond to the presentation of objects in the peripersonal space, but virtually all of them also show a response to action observation, even in the extrapersonal space, suggesting they might generate object-triggered, predictive representations of the upcoming action of the observed agent.

Neurons endowed with a given functional property (e.g., discharging during

grasping) does not necessarily play a relevant functional role in that specific function (e.g., controlling grasping execution). Indeed, the anatomical location and connections of neurons with similar functional properties enable them to play (or not to play) a role in a given function (Bonini, 2017). For example, grasping neurons of area F5p do play a causal role in the control of visually guided hand actions, whereas those of F5c (and hence MNs) do not (Fogassi et al., 2001).

2 THE ANTERIOR INTRAPARIETAL AREA IN THE PARIETO-FRONTAL MOTOR SYSTEM

Cortex of the intraparietal sulcus is highly involved in sensorimotor functions. Specifically, this cortex is involved in forming of intentions and cognitive plans for specific types of movements (Andersen and Buneo, 2002). Different subregions or areas of the intraparietal sulcus are involved in the planning of eye movements, grasping movements, reaching, and defensive forelimb and head movements. Sensory guidance is based on somatosensory, visual, and, to a lesser extent, auditory inputs. Important outputs are to motor and premotor areas of the frontal lobe. The cortex of the intraparietal sulcus of macaque monkeys has been divided into a number of areas. Along the lateral (caudal) bank of the sulcus, the anterior intraparietal area, AIP (Sakata and Taira, 1994), the lateral intraparietal area, LIP (Andersen et al., 1985), and the caudal interparietal area, CIP (Sakata and Taira, 1994) form a rostrocaudal sequence, with more rostral areas more dominated by somatosensory inputs, and more caudal areas by more direct visual inputs (see Stepniewska et al., 2005).

AIP, the most anterior area of the lateral bank of the intraparietal sulcus, is important in planning and organizing grasping movements of the hand (Gallese et al., 1994; Rizzolatti et al., 1997). Important outputs are to ventral premotor cortex, a motor area also important in hand movement control (Luppino et al., 1999). Neurons in AIP appear to code for hand movements that are specific to the perceived task (Baumann et al., 2009). Grasping behavior is abnormal after the inactivation of AIP (Gallese et al., 1994). Many

studies also highlight aAIP neuronal properties of discharging also during the vision of reaching-grasping actions performed by others.

In this chapter I will first highlight AIP's role in transforming visual information of graspable object in motor outputs then its properties of encoding one's own and others action.

2.1 SENSORIMOTOR TRANSFORMATION AND OBJECT AFFORDANCE:

ANATOMICAL, FUNCTIONAL AND NEUROPSYCHOLOGICAL EVIDENCE

The process of pictorially representing a graspable object in visual brain areas and, simultaneously, in its pragmatic description thus activating in areas of the v-d stream is more specifically called visuomotor transformation. Such process is at the basis of object grasping which is one of the most frequently performed and highly specialized behavior in primates (Jeannerod et al., 1995; Macfarlane and Graziano, 2009). One of the most challenging aspects in the control of grasping is the configuration of the hand according to the object features during the reaching phase (Jeannerod et al., 1995).

In the last decades, several studies on both humans and monkeys have been carried out in order to identify and describe the cortical mechanisms underlying such complex transformations. The aim of most of these studies is clarifying the role of areas of the ventral-dorsal stream, particularly of the anterior intraparietal area (AIP) and ventral premotor area F5, and of the areas connected with them. Anatomical studies investigate the connection between areas that share different aspects of the same sensory stimuli and their anatomical properties. Electrophysiology studies investigate the functional properties within each area. Pharmacological inactivation of the area probe the possible functional role of the identified functional properties by suddenly inhibiting neuronal activity to study behavioral changes occurring after the blocking.

Anatomical studies based on tracers injections in AIP have shown that this area is linked to many others through monosynaptic connections. In particular, they showed that area AIP forms an anatomico-functional module with the ventral premotor area F5 which

is the premotor area crucially involved in object grasping is ventral premotor area F5 (Luppino et al., 1999; Borra et al., 2008).

Luppino and coworkers showed that area F5 consists of three subareas: F5 anterior (F5a), F5 posterior (F5p) and F5 convexity (F5c). Their location is shown in Figure 6. F5a and F5p are located on the posterior bank of the inferior arcuate sulcus whereas F5c lies on the cortical convexity. Of these sub-areas, F5c and F5p have a typical agranular structure, while F5a appears to be a transition area towards the prefrontal cortex, showing some of the granular layer IV (Belmalih et al. 2009).

The whole area receives its main inputs from the inferior parietal lobule (IPL), mainly from areas AIP, PF, PFG and SII and send connections to M1 and to the spinal cord but F5 sub-areas show also specific different connections. F5p is the sub-area sending most projections to the spinal cord. F5a is the only sub-area receiving a rich input from the ventrolateral prefrontal cortex (areas 46v and 12r) and the frontal operculum (Gerbella et al. 2011). Furthermore, F5a is virtually not connected with the primary motor cortex and the spinal cord.

Moving more dorsally within SA (superior arcuate), F5p fades in area F2. The rostral part of area F2 (F2vr) appears to be involved in control of wrist rotation (Raos et al. 2004). This area sends projections to M1, the brainstem and the spinal cord (Dum and Strick 2002). F2vr is linked to parietal areas (MIP, V6A, PFG, and MST) (Matelli et al. 1998; Gamberini et al. 2009). It also receives connections from the prefrontal cortex mainly originating from the dorsal part of area 46 (46d) (Luppino et al. 2003).

Based on these connections, it is possible to hypothesize that F2vr is involved not only in grasping but also in reaching (Gerbella et al., 2017). F5 and F2vr are connected with one another as well as with other premotor areas. They also receive important

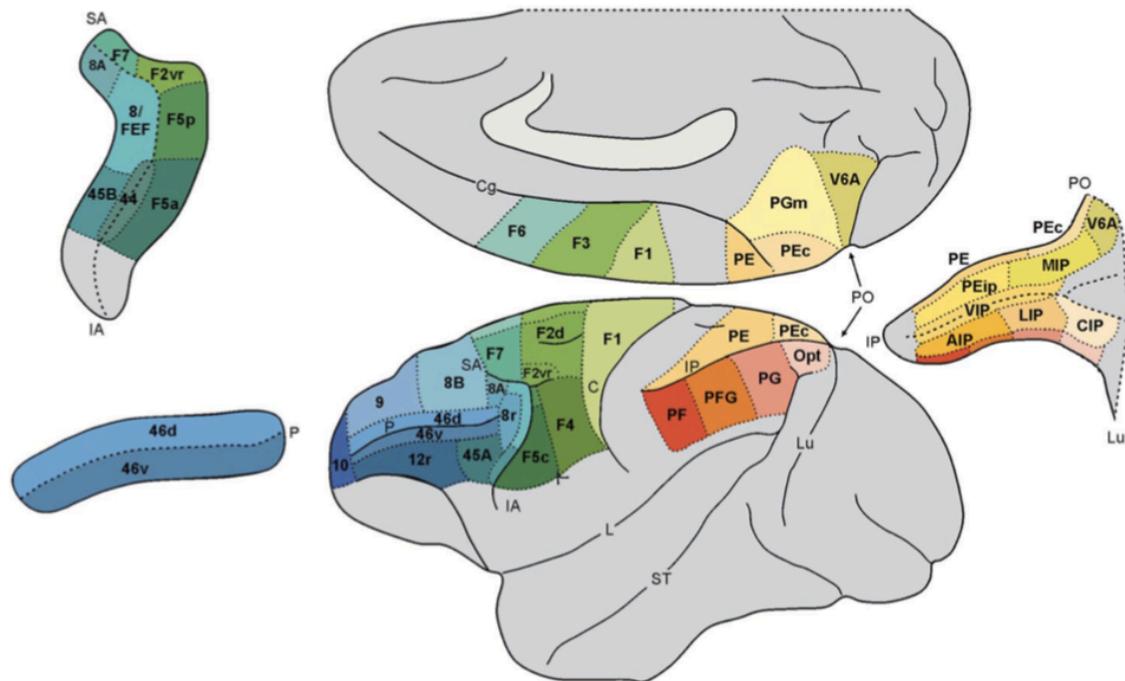


Figure 6. Lateral and mesial views of the macaque brain showing parcellation of the frontal and posterior parietal cortex. The prefrontal cortex is subdivided according to Carmichael and Price (1994), except for its caudo-ventral part (Gerbella et al. 2007). Agranular frontal areas are classified according to Matelli et al. (1991) and Belmalih et al. (2009). The parietal areas are named according to Pandya and Seltzer (1982). The areas located within the arcuate and the principal sulci are shown in an unfolded view of the sulci in the left side, areas located within the intraparietal sulcus are shown in the right part of the figure. Dashed lines indicate the architectonic borders. C central sulcus, Cg cingulate sulcus, IA inferior arcuate, L lateral fissure, Lu lunate sulcus, P principal sulcus, PO parieto occipital sulcus, SA superior arcuate, ST superior temporal

connections from area F6. From a cytoarchitectonic point of view, area F6 belongs to the agranular frontal cortex. This area lacks direct connections with the primary motor cortex and the spinal cord (Dum and Strick 2002; Luppino et al. 1993). F6 is linked with the prefrontal cortex and in particular with the intermediate part of both the ventral and dorsal

portions of area 46. In the parietal lobe, it is more strongly connected with areas PG and PFG and has weak connections with AIP and V6A (Gamberini et al. 2009; Luppino et al. 1993).

Historically, the parietal area considered to be the crucial node for the organization of grasping movement was area AIP (Taira et al., 1990; Sakata et al., 1995; Murata et al., 2000). From the early '90s, Sakata and colleagues have investigated monkey parietal cortex by means of a paradigm designed to study neuronal activity while the monkey had to observe and subsequently grasp or non-grasp objects of different size and shape in the light or in the dark, in separate sessions (Taira et al., 1990; Sakata et al., 1995; Murata et al., 2000). The authors were able to describe two types of visually-modulated neurons: “visual-dominant” neurons, which discharged during grasping in the light but not in the dark, and “visual-motor” neurons, which fired also during grasping in the dark, although weaker compared with the same action performed in the light. Within both these two populations of neurons, they further subdivided neurons in “object-type” or “non-object-type,” depending on whether or not they responded to object presentation during the fixation task. Interestingly, the discharge of many object-type neurons exhibited the same preference for a given object (or set of objects) during both object fixation and grasping. This finding suggests that object-type neurons play a crucial role in the visuomotor transformation of object affordances in the most appropriate hand shape for grasping. Their response and the preserved object selectivity, also during trials in which the monkey did not perform any action, further indicate that the neural mechanisms for the extraction of object affordances rely on the monkey motor possibilities, but not necessarily on its actual execution of a grasping action. Therefore, also the dorsal pathway (in particular the ventro-dorsal stream), appears to play a role in object perception. Other studies

showed that the adjacent area PFG also contains neurons encoding grasping movements (Hyva 1981; Rozzi et al. 2008; Bonini et al.,2010 ; Bonini et al.,2011). Further parietal areas involved in grasping are: area V6A and, in particular, its dorsal part (V6Ad; Fattori et al. 2015), and area MIP, connected to F2vr, which has been described involved in control of reaching movements (Andersen et al. 1997). AIP, MIP, and V6A are strongly interconnected to one another, whereas PFG is strongly connected only with the adjacent AIP. Interestingly, AIP and PFG also receive input from higher-order visual areas of the temporal cortex (Borra et al. 2008). A crucial issue of the reaching-grasping model is the fact that an object could be grasped in several ways. The chosen grip depends on the object's visual properties, but also on the object's meaning and on the agent's goal.

Based on anatomical data, Arbib and Mundhenk (2005) proposed a model suggesting that the prefrontal information on object semantics and the goals of the individual directly influence AIP area. The ventral prefrontal cortex is richly connected with the inferior parietal lobule. More specifically, recent studies showed that the prefrontal area 12r is mostly linked with AIP, while 46v area is linked with both AIP and PFG. Areas 12r and 46v could be subdivided into three vertical strips, caudo-rostrally located (shaded colors in Figure 7). The most caudal strip which include also FEF and SEF is mainly connected with LIP and subcortical centers (i.e., the intermediate and superficial layers of the superior colliculus) involved in control of eye movements (Gerbella et al. 2010, 2013; Borra et al. 2015; Saleem et al. 2014). The most rostral strip 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 (Gerbella et al. 2013, 2016; Saleem et al. 2014; Borra et al. 2017).

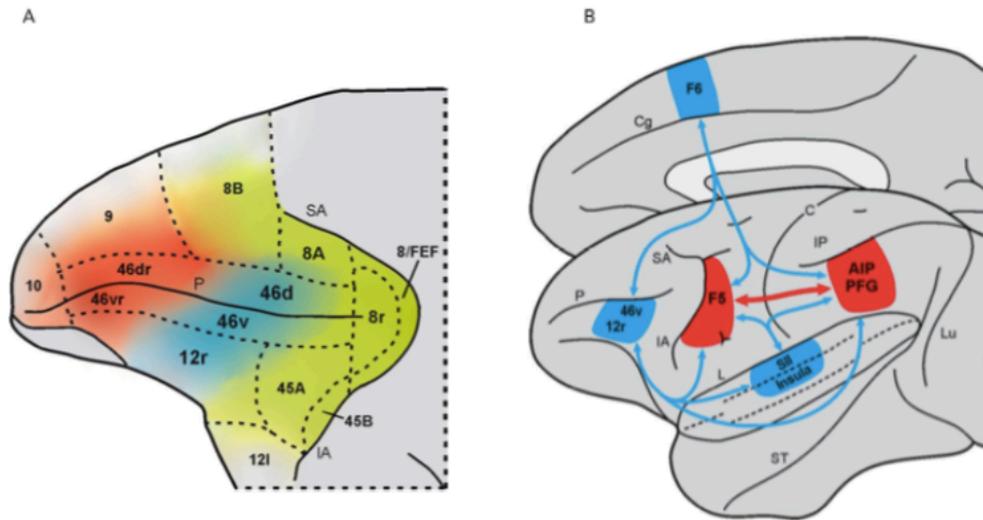


Figure 7. Lateral and mesial view of the extended lateral grasping network. A) View of the monkey prefrontal cortex. Dashed lines indicate the architectonic borders. The coloured shadings delimit three groups of areas. Green: areas mostly connected to oculomotor cortical and subcortical centers (Gerbella et al. 2010, 2013; Borra et al. 2015; Saleem et al. 2014); blue: areas mostly connected to skeletomotor cortical and subcortical centers (Borra et al. 2011, 2014; Gerbella et al. 2013, 2016; Saleem et al. 2014); red: areas mostly showing intrinsic prefrontal connections (Borra et al. 2011; Gerbella et al. 2013; Saleem et al. 2014) Lateral and mesial views of the macaque brain showing the connections between the two main nodes (red) and the additional ones (blue) forming the extended object-grasping network. Abbreviations as in Figure 6.

On the basis of these findings Gerbella et al., (2017) postulate that while AIP and PFG describe several possible affordances, the input coming from the frontal lobe selects only one of them. Affordance selection based on the overarching goal could mostly rely on the input coming from 46v, more strongly connected with the parietal and premotor cortex. Regardless of how they are selected, these affordances activate F5 motor

representations. These representations remain potential until an appropriate signal arrives through F6. Once the appropriate affordance is selected, there are other factors that may modulate hand shaping and grip force during grasping. These are the visually detectable physical properties of the object (e.g., texture, slipperiness, etc.) and other properties related to previous experience with the same object (e.g., weight). Such information on the physical properties of the object can be provided by the connections of the basic AIP-F5 circuit with the SII region. In fact, this region contains neurons that respond well to active movement, especially during object grasping, and others whose sensitivity to cutaneous inputs changes with hand conformation, being tuned to the orientation of the object, and of the gratings indented into the finger pad during object grasping (Bensmaia et al., 2008). Note also that, as recently demonstrated, SII is endowed with visually responsive neurons besides the well-known neurons responsive to somatosensory stimuli (Hihara et al. 2015). Considering these properties and SII connections with areas 12r, 46v and F5, it is conceivable that SII could represent a fundamental source of sensory information on object physical properties. Recent studies shown that SII hosts neurons motor properties firing during active hand and mouth movements (Taoka et al. 2013; Ishida et al. 2013). Ishida et al. (2013) proposed that the motor activity of these SII hand-related neurons could be an efference copy of motor commands from F5, used to make a prediction of the sensory consequences of the movement, and comparing them with the actual sensory feedback. In turn, SII could be a source of somatosensory feedback information used in F5, AIP, and PFG for continuously monitoring and updating grasping motor acts.

2.2 ENCODING OF OBSERVED GRASPABLE OBJECTS

Nowadays is well established the involvement of AIP in a parietofrontal circuit dedicated to visuomotor transformations for grasping. In the AIP there are grasping- and manipulation-related neurons, which often have both motor and visual properties. Some neurons are defined as purely motor neurons, discharging during active movements performed in the light and in the dark, thus without the visual presence of the object or one's own reaching-grasping visual feedback (Figure 8, lower panel). Among these neurons, some discharge more vigorously when the action is visible (Figure 8, middle panel). Many visually responsive neurons can be active while passively observing objects (Figure 8, upper panel). Other physiological studies have also revealed that neurons in AIP show grasping activity that integrate motor signals and the 3-D structure (Schaffelhofer and Sherberger 2016, Theys et al., 2015; Murata, Gallese, Luppino, Kaseda, & Sakata, 2000; Murata et al., 1997; Sakata, Taira, Murata, & Mine, 1995; Taira, Mine, Georgopoulos, Murata, & Sakata, 1990), and even the 2-D contours of object images (Romero, Van Dromme, & Janssen, 2012).

Some of these neurons show less activity during movement in the dark than in the light, suggesting that they encode visual representations. A portion of them respond to viewing an object per se (visual-motor object-type neurons), but the remaining neurons do not respond to viewing an object per se (visual-motor non-object-type neurons). Visual responses of the latter type are concerned with the image of the hand during the action (Maeda et al., 2015).

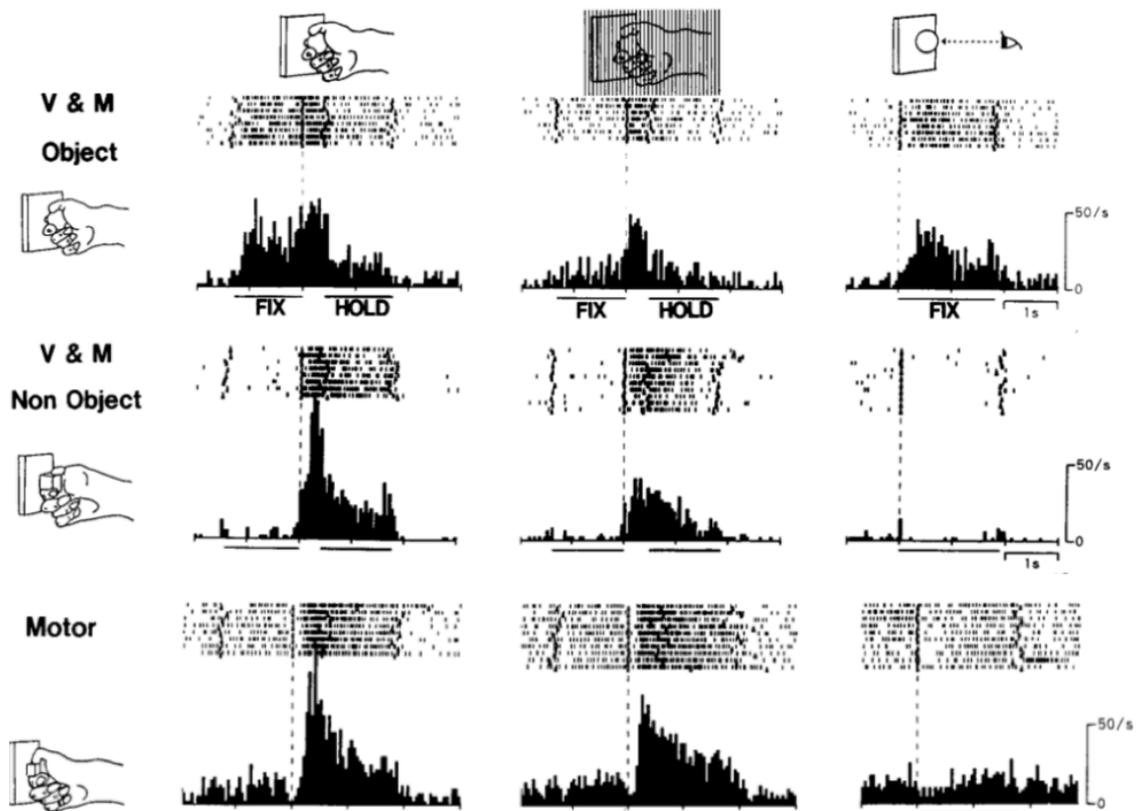


Figure 8. Typical motor neurons in AIP. Each row represents one neuron. The neuron represented in the first upper row discharges during the manipulation in the light and in the dark, and also during the object fixation epoch. The second row represents a visuomotor non object type neuron discharging during manipulation epochs only but with a clear preference for the manipulation in the light. The last row represents a motor neuron discharging similarly during grasping in the light and grasping in the dark epoch but not during object fixation epoch (Murata et al., 2000).

It is widely believed that these visual responses in AIP represent an important stage in the visual extraction of object features that can be used to plan the appropriate grip. Many AIP neurons only become active when the hand starts to move toward the object (Murata et al., 2000) during grasping in the light. This increase in activity while the monkey receives visual information about its own hand moving toward the object has been related to the visual analysis of the shape of the hand or to the interaction between

the moving hand and the object to be grasped: during visually guided grasping, AIP neurons may monitor the grip aperture of the hand to adjust it to the dimensions of the object. Many of these show a wide range of selectivity for the physical properties of the object such as shape, size, and orientation (Figure 9 and Figure 10). An area with similar functional properties has also been identified in the human intraparietal sulcus and referred to as putative human AIP (Binkofsky et al., 1998; Tunik et al., 2005; Frey et al., 2006).

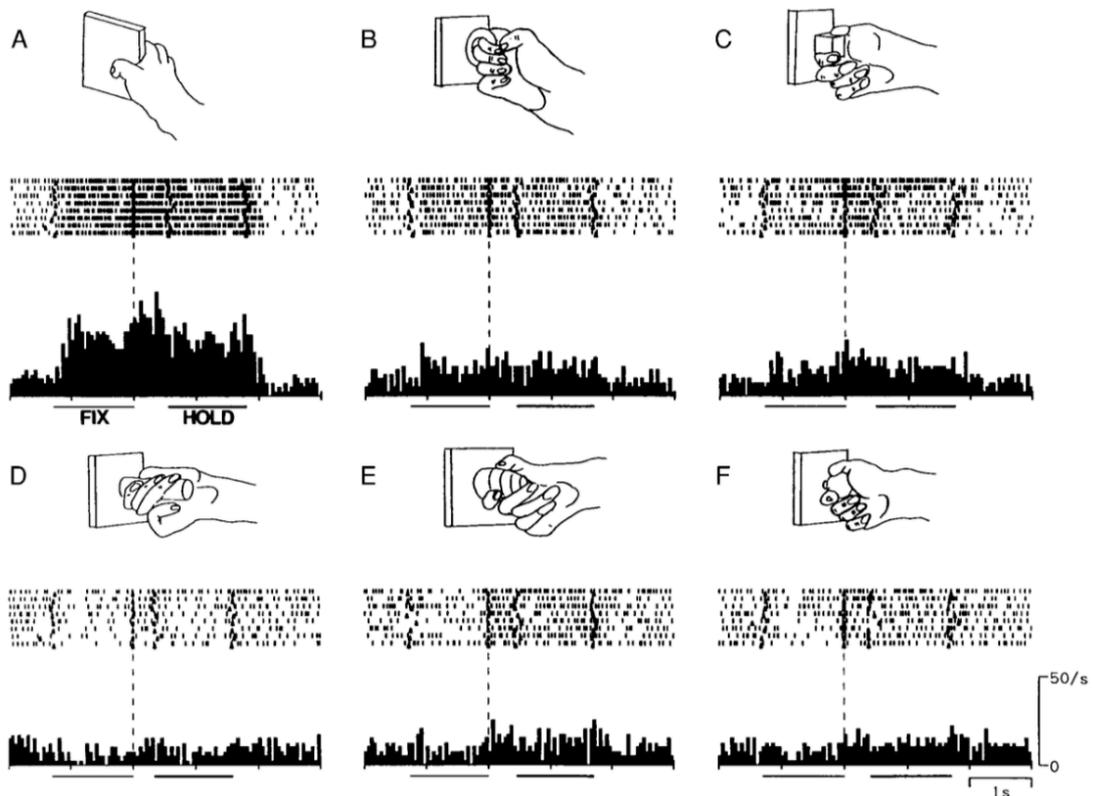


Figure 9. Highly selective activity of an object-type visual-motor neuron for 6 different objects. Each of the raster and histograms shows the activity levels during the manipulation of medium-sized objects. Illustration above each raster indicates the objects and the type of handgrip. A: vertical plate; B: vertical ring; C: cube; D: cylinder; E: cone; F: sphere. Conventions of raster and histogram are the same as for the manipulation task shown in Figure 7. Neurons showed strong preference for the vertical plate (Murata et al., 2000).

AIP motor neurons respond to object orientation and grip type, either in the movement preparation or in the active grasping phase (Maeda et al., 2000, Baumann et al. 2009, Fluet et al. 2010). Baumann et al. (2009) recorded single neurons in area AIP of monkeys performing a delayed grasping task (Figure 10, Figure 11).

During this task, monkeys were presented with a handle (target object) in different orientations, and a colored LED (cue signal), which instructed the animal to subsequently perform a power or a precision grip. Results showed that AIP neurons could represent both the handle orientation and the instructed grip type immediately after the presentation of the visual stimulus, indicating that AIP neurons can process object features in a context-dependent fashion. The neural response during cue was dominated by the spatial object feature. Orientation-selective neurons outnumbered the grip type-selective ones more than twofold during cue. In addition, 71% of all grip type-selective neurons were also selective for the object feature orientation during cue.

A modified version of the task (cue separation task) enabled to study neuronal responses also when information on object orientation and the required grip type were separately presented. In particular, when the target object was presented first, visuomotor neurons became active regardless of the preference for power or precision grip that they exhibited in the delayed grasping task. In contrast, when the cue was presented first (and the object was not yet visible), this information was only weakly represented in area AIP, while it was strongly encoded thereafter, when the target object was revealed (Figure 11).

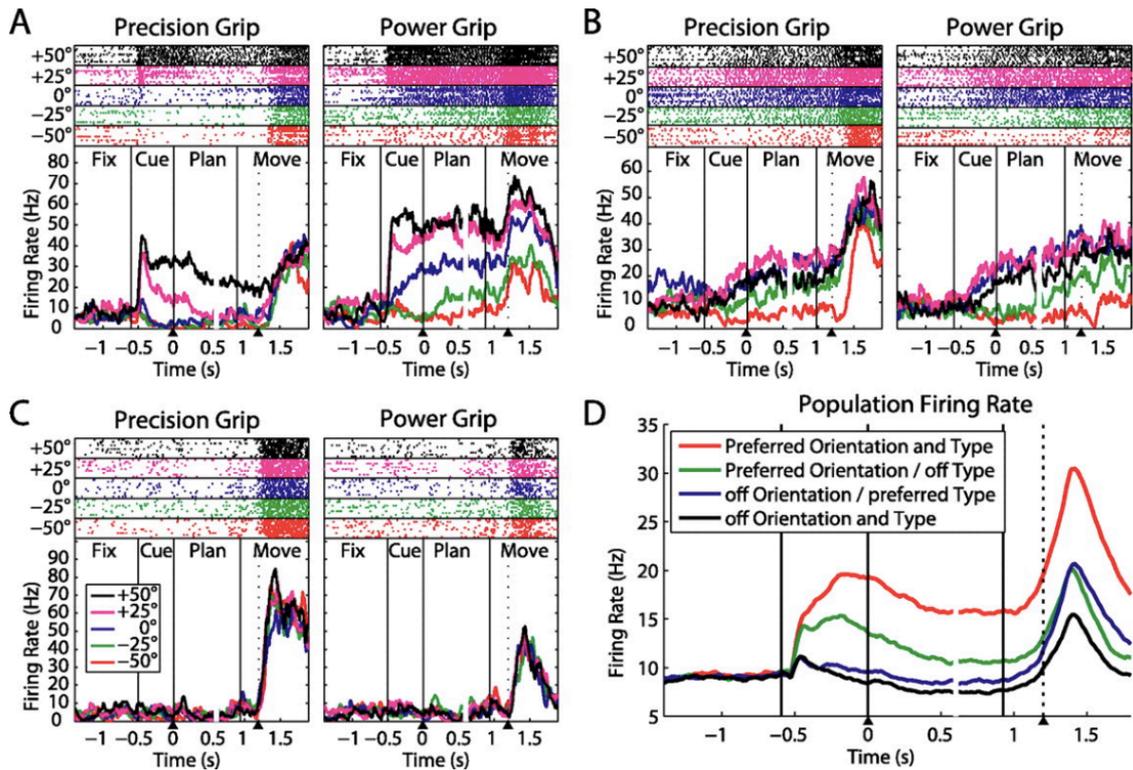


Figure 10. Three example neurons with different tuning onsets. For each neuron, precision grip trials are shown on the left panel and power grips on the right panel. Different colors indicate various handle orientations, for which spike rasters (on top) and averaged firing rates (at bottom) are shown individually. The dotted line within the movement epoch indicates the release of the hand rest button (movement start). All trials are aligned to both the end of the cue epoch and the start of the movement (arrow heads below); gaps in the curves (at ~ 0.6 s) indicate the realignment. A, Neuron that exhibits tuning for the handle orientation and the instructed grip type starting in the cue period and extending until movement execution. B, Neuron with tuning for the handle orientation starting in cue, but with significant grip type modulation only during movement execution. C, Neuron showing no response during cue presentation and movement planning, but with a strong selectivity for precision grips during movement execution without significant orientation tuning. D, Population firing rate across all 571 neurons for each combination of the cells' preferred and nonpreferred grip type and orientation (Baumann et al., 2009).

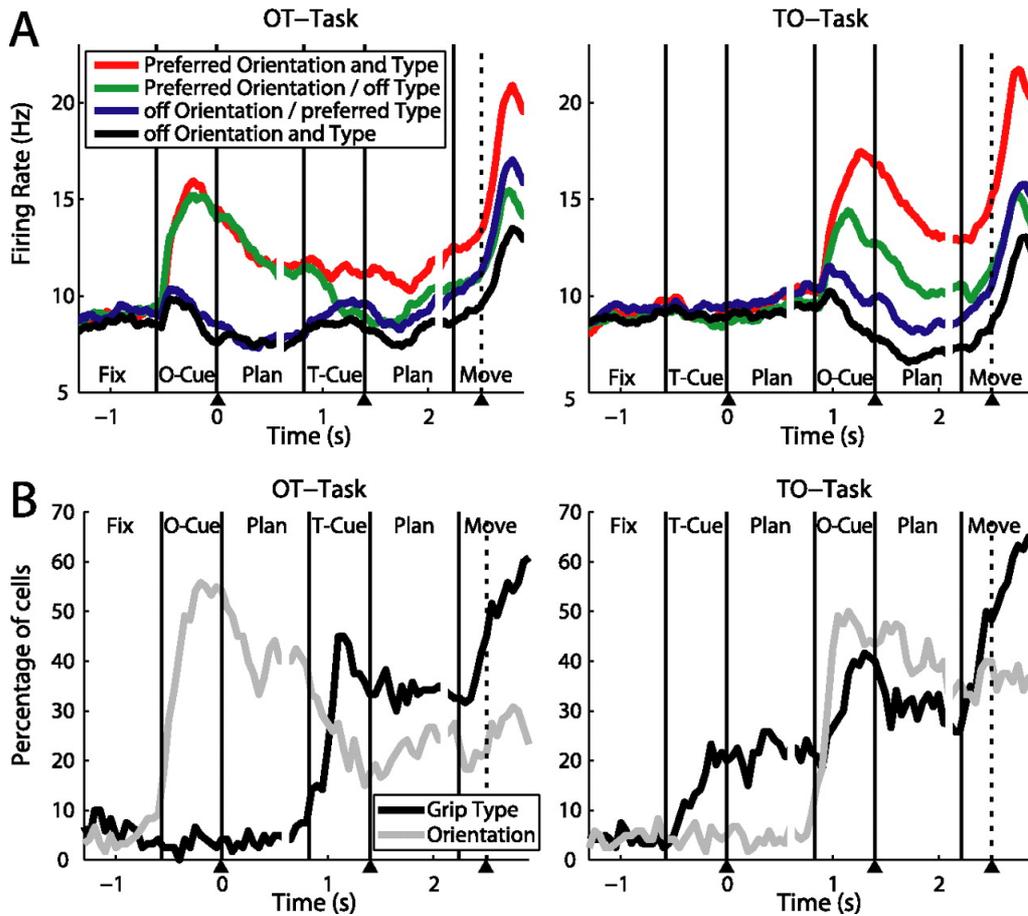


Figure 11. Population analysis of the cue separation task. A, Population firing rates in the cue separation task ($N = 120$) in which object was presented first, and on the right panel, trials in which first was presented the object's orientation and then the type of grip (instructed by the color of a led). B, Fraction of cells that were significantly tuned by grip type and orientation in the course of the two tasks (Baumann et al., 2009).

These findings indicate that, besides transforming object properties into the appropriate grip type, AIP motor neurons can also encode abstract information provided by any visual stimulus previously associated with a specific grip. However, both object- and context-driven transformations of visual information into an appropriate motor representation of a hand grip require that the object to be grasped be visible in front of

the monkey. Thus, area AIP does not simply associate contextual visual stimuli with motor representations but plays an active role in the processing of a pragmatic description of observed objects. Interestingly, even human fMRI studies showed that area AIP can activate during both the recognition and construction of three-dimensional shapes in the absence of visual guidance, but not during mental imagery of the same processes (Jancke et al., 2001), where overt sensory input and motor output are absent: this finding clearly supports the idea that the physical presence of the object is crucial for triggering area AIP neurons activity. Furthermore, TMS of the posterior parietal cortex in humans (Reichenbach, Bresciani, Peer, Bühlhoff, & Thielscher, 2011; Chib, Krutky, Lynch, & Mussa-Ivaldi, 2009; Desmurget et al., 1999) interferes with the on-line control of visually guided reaches.

Taken together, all these data strongly indicate the crucial role of AIP in visuomotor transformation for visually- and somatosensory-guided manipulation of objects.

2.3 ENCODING OF SELF AND OTHER'S OBSERVED ACTION

As I previously introduced, mirror neurons are a fascinating class of cells originally discovered in area F5 and, subsequently, in the inferior parietal lobule (IPL) of the macaque, which fire when animals manipulate objects in a certain way and when they observe others performing a similar action (Fogassi et al., 2005; Rizzolatti, Fogassi, & Gallese, 2001; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti, Fadiga, Gallese, & Fogassi, 1996; Di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). Hence, they represent “action” in an agent-shared motor format, encoding motor goals both when these are attained by the subject or by another agent (Figure 12). Several regions in the primates' brain host neurons that encode both one's own and others' actions (Giese & Rizzolatti, 2015; Bonini, 2017; Bruni et al., 2018), hence generating a putatively agent-invariant code for actions (Sinigaglia & Rizzolatti, 2011), which is deemed to be at the basis of several cognitive functions and social interaction skills (Bonini & Ferrari, 2011; Rizzolatti & Sinigaglia, 2016). Neurons with mirror properties have also been reported in other animal species and may subserve a large variety of socio-cognitive functions (Bonini & Ferrari, 2011). They can exploit contextual information to generate predictive representations of what others are going to do (Fogassi et al., 2005; Bonini et al., 2010; Maranesi et al., 2014). Most of these neurons have been also shown to be strongly influenced by specific contextual factors, such as space (Caggiano et al., 2009; Bonini et al., 2014b), value (Caggiano et al., 2012), social stimuli (Yoshida et al., 2012) and non-social visual or auditory cues (Kohler 2002; Cisek & Kalaska, 2004; Fogassi et al., 2005;

Bonini et al., 2010; Fluet et al., 2010; Maranesi et al., 2014) leading to many hypotheses on their possible role in daily life situations.

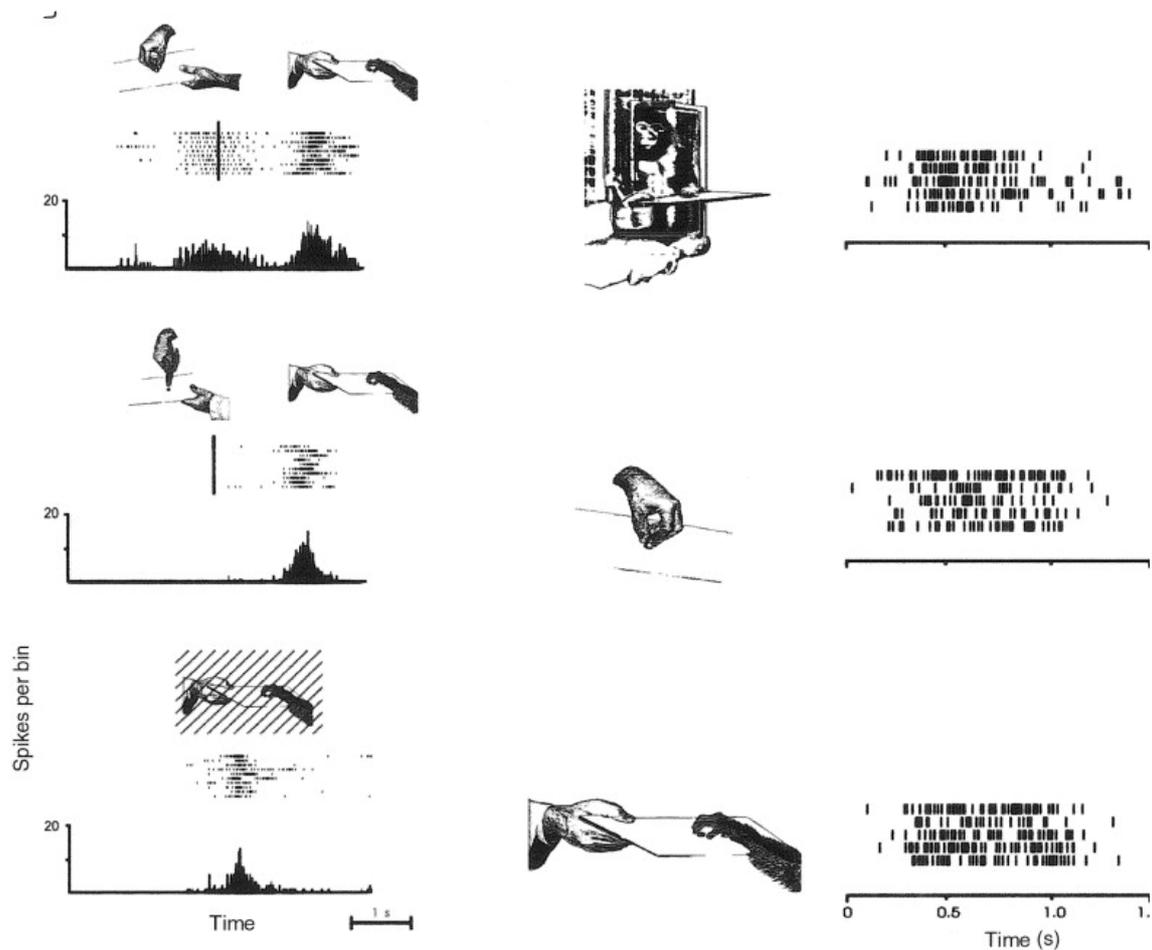


Figure 12. Visual and motor responses of a MN. In the upper part of each panel the behavioral context in which the neuron was studied is shown. In the lower part of each panel a series of consecutive rasters and the relative peristimulus response histograms are shown.

Pani et al. (2014), observed that most of AIP's MNs can also be activated by pure visual stimulation with a video of the same grasping action. The majority of these neurons

were also activated by videos showing an isolated hand on a scrambled background and even by a shape entering or appearing within the visual field (see Figure 13).

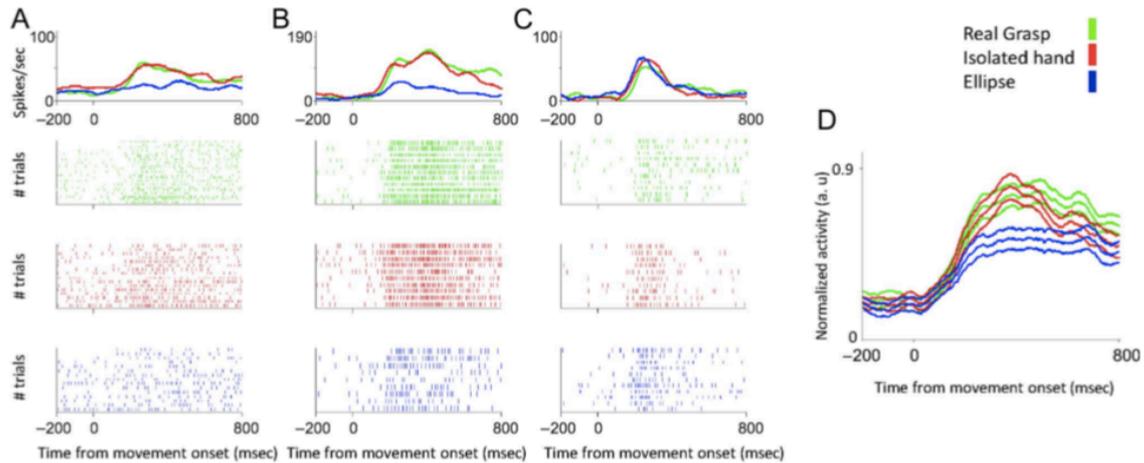


Figure 13. Effector specificity of grasping observation activity in AIP. Spike density function and raster plots during passive observation of videos of the real grasping action (green), an isolated hand (red), and an ellipse (blue), aligned on the onset of the movement. (A) Example neuron responding selectively to the vision of the moving hand (isolated hand and real grasping) but not to the vision of the ellipse. (B) Example neuron responding strongly to the vision of the moving hand (isolated hand and real grasping) and less to the vision of the ellipse. (C) Example neuron equally responding to the vision of the real grasping, isolated hand, and ellipse. (D) Normalized average firing rates of the GO neurons ($n = 51$, mean \pm SEM, in spikes/sec) during passive observation of videos of the real grasping action (green), an isolated hand (red), and an ellipse (blue), aligned to the movement onset. (Pani et al., 2014).

Lanzilotto et al. (2019), showed that AIP neurons encode the identity of specific observed manipulative actions, whether it is conveyed by either mostly static (body shape) or dynamic (body motion) information (Figure 14). Visual selectivity for observed manipulative action identity was stronger in pAIP, where it was associated with motor selectivity for the grip type as well as to high sensitivity to visual feedback of the monkey's own hand during grasping execution. The rostrocaudal increase in preference

for the visual encoding of manual actions of self and others parallels a rostrocaudal increase in anatomical connectivity with temporal areas of the ventral visual stream, oculomotor regions and prefrontal cortex, which may provide visual and contextual information relevant for manipulative action processing. These results revise current models of action observation network in the macaque, indicating that pAIP constitutes a parietal hub for routing information about observed manipulative actions identity to the other parietal, premotor and prefrontal nodes of the network (Bonini 2017; Rozzi and Fogassi 2017).

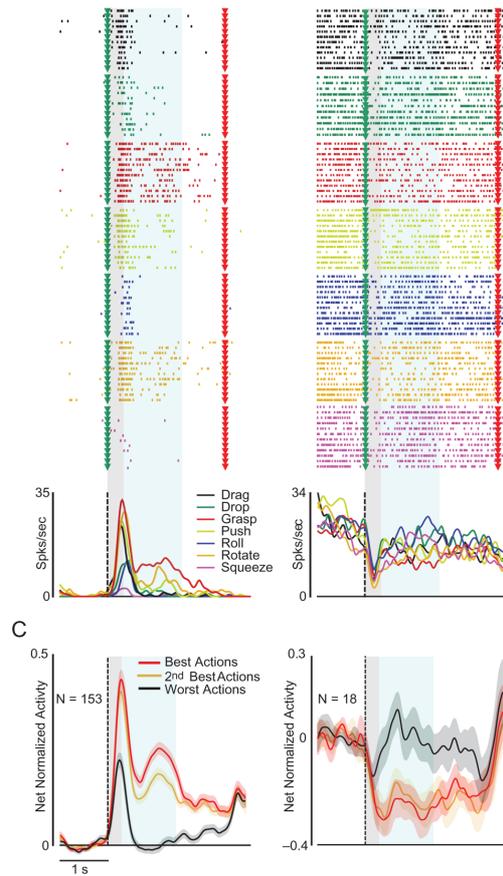


Figure 14. Observed manipulative actions selectivity of single neurons in AIP. Single neuron examples, population activity and tuning properties of AIP units in the observation task in which videos of different grasping actions (Lanzilotto, 2019).

Most extant studies of mirroring mechanisms, in both humans and monkeys, have focused on the observation of others' action (Kilner and Lemon 2013). Nevertheless, in everyday life, we certainly have more frequent opportunities to observe our own actions than those of others. Indeed, from birth, the motion of our own hands constitutes an extremely engaging stimulus (White et al., 1964) and a crucial source of information for driving sensorimotor development (Held and Bauer 1967; Van der Meer et al., 1995). This has led many authors to propose that infants' early experience with their own actions is crucial in order to endow originally motor neurons with mirror properties (Casile et al.,

2011; Del Giudice et al., 2009; Press et al., 2011 ; Tkach et al., 2008). If this is the case, although technical and ethical problems prevent researchers from verifying this hypothesis by recording single-neuron activity in newborn primates, it is reasonable to expect that own-hand visual feedback is a stimulus that retains a particular relevance for MNs discharge even in adulthood, and as a consequence, neurons in the lateral-grasping network could be sensitive to the sight of one's own actions in addition to those of others.

As previously mentioned, single-neuron studies on area AIP (Sakata et al., 1995) have reported the existence of a particular class of neurons called “visual dominant non-object type neurons,” which exhibited the intriguing property of firing when the monkey grasped an object in the light, but not in the dark (Figure 15). Importantly, these neurons did not fire when the object was simply visually presented, suggesting that they specifically contribute to the visuomotor processing of own-hand actions.

Maeda et al., (2015) demonstrated that a set of AIP neurons fitting with the original description of visual dominant non-object type neurons, in addition to responding during the monkey's grasping act in the light but not in the dark, discharged when the monkey observed its own action presented on a screen from a subjective viewpoint, even when the target object was artificially removed with video-editing techniques. Thus, at least some AIP neurons can specifically process visual information related to own-hand actions (Figure 16).

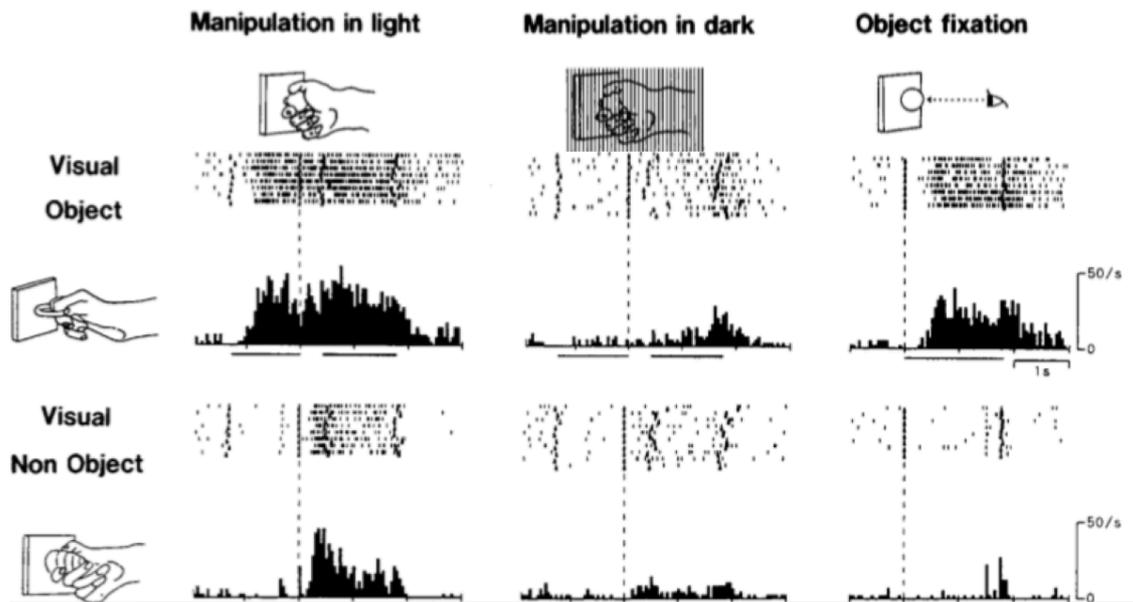


Figure 15. Two examples of different visual neurons: visual dominant object (above) and non-object (below) type neurons. Note that neuronal discharge is strictly related to the monkey’s own hand (Murata et al., 2000).

Similar conclusions come from a more recent study carried out on area F5 (Maranesi et al., 2015).

These findings suggest that at least some of the MNs in the adult monkey can process similarly the visual information coming from both its own and another’s hand, thus supporting previous models in which MNs are deemed to play a role in monitoring one’s own actions (Bonaiuto and Arbib 2010) as well as in predicting those of others (Keysers and Gazzola 2014). Although a small percentage (4.7%) of neurons specifically tuned to the observation of another’s action (i.e., “mirror-like” neurons, devoid of any response during grasping execution) have also been described since the original report (Gallese et al., 1996), similar cells seem to be more frequent in area F6 and adjacent cingulate (9.5%) cortex (Yoshida et al., 2011; Livi et al. 2019).

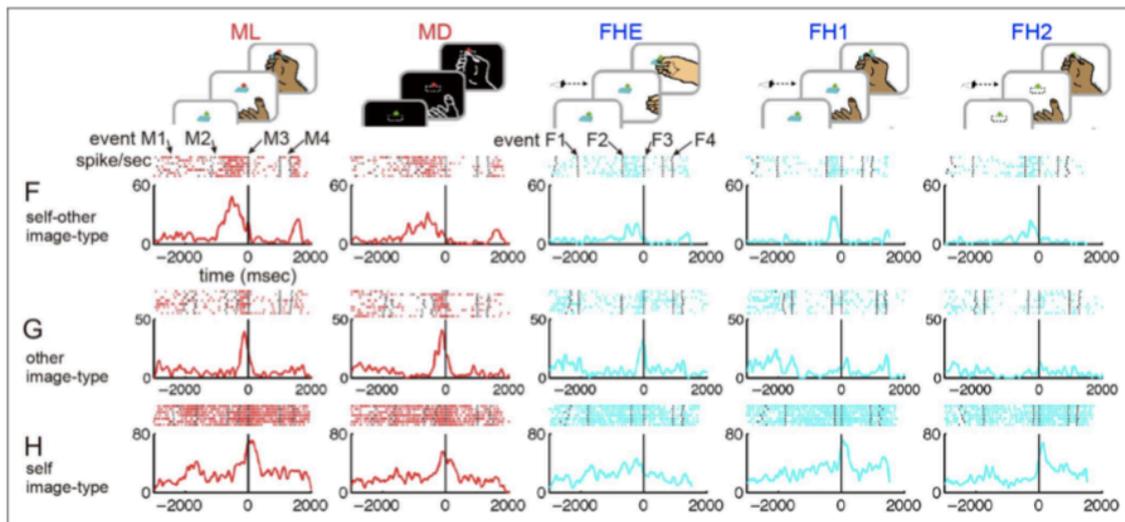


Figure 16. Three examples of hand manipulation-related neurons. These neurons respond to the monkey's own and/or the experimenter's hand image shown in red rasters, light-blue rasters respectively (Maeda et al., 2015).

The findings so far reviewed support the idea that all the regions of the extended cortical grasping network host neurons specifically involved in the processing of one's own or others' actions, besides those with mirror properties. On the one hand, the presence of neurons modulated by hand visual feedback could be the vestigial sign of their ontogenetic origin from individuals' visuomotor experience with their own actions. On the other, this property can retain an important role even in adulthood, constituting a developmentally preserved resource of neural plasticity for the fine-tuning of MNs visual properties as a consequence of sensorimotor learning (Casile and Giese 2006; Engel et al., 2012; Oztop et al., 2013; Wiggett et al., 2012).

3 AIMS OF THE STUDY

Although many properties of area AIP were described by the existing literature, settings and tasks were often very different each other, allowing a fragmented description of AIP functional properties. In order to compare AIP neuronal discharge in different contexts and with different objects (possible target for the monkey or for the experimenter) we presented the monkeys with different execution and action observation conditions.

More specifically this experiment test the agent encoding, the space-specificity modulating discharge in single neuron level during action observation and their ability to generalize even during the action observation of video showing reaching-grasping actions.

4 MATERIALS AND METHODS

4.1 SUBJECT AND SURGERIES

Experiments were carried out on two *Macaca mulatta*, one female (Mk1, 4 kg) and one male (Mk2, 7 kg). Before recordings, monkeys were habituated to sit in a primate chair and to interact with the experimenters. Then, they were trained to perform a visuomotor tasks (VMT) and observation tasks performed live (OTp and OTe) or video shown (OTv) as described below.

When the training was completed, each animal underwent surgical procedures for the construction of a head implant including a head holder and a chamber for single-unit recordings and microstimulation. Each animal was anesthetized (ketamine hydrochloride, 5 mg/kg intramuscular [i.m.] and medetomidine hydrochloride, 0.1 mg/kg i.m., repeatedly administered during the surgery) and placed in a stereotaxic apparatus. Under aseptic conditions, an incision was made in the scalp, the skull was trephined over the target region, and the dura was opened to expose the IPL convexity. Chronical implantation sites were chosen by using anatomical landmarks and MRI brain pictures as frame of reference, referred in terms of stereotaxic coordinates and location of anatomical landmarks such as the IPS, the lateral fissure (LF), and the superior temporal sulcus (STS).

During all surgeries, hydration of the monkey was maintained with continuous infusion of saline solution. A heating pad stabilized the monkey's body temperature

throughout the surgical procedure. Heart rate, respiratory depth, and body temperature were continuously monitored. Analgesics were administered intra- and postoperatively. Upon recovery from anesthesia, the animal was returned to its home cage and closely monitored until complete recovery

Dexamethasone and prophylactic broad-spectrum antibiotics were administered pre- and postoperatively, its administration was continued for 1 week after the surgery.

All experimental protocols complied with the European law on the humane care and use of laboratory animals (directives 86/609/EEC, 2003/65/CE, and 2010/63/EU), they were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 78/12 17/07/2012) and authorized by the Italian Ministry of Health (D.M. 294/2012-C, 11/12/2012).

4.2 APPARATUS AND BEHAVIORAL PARADIGM

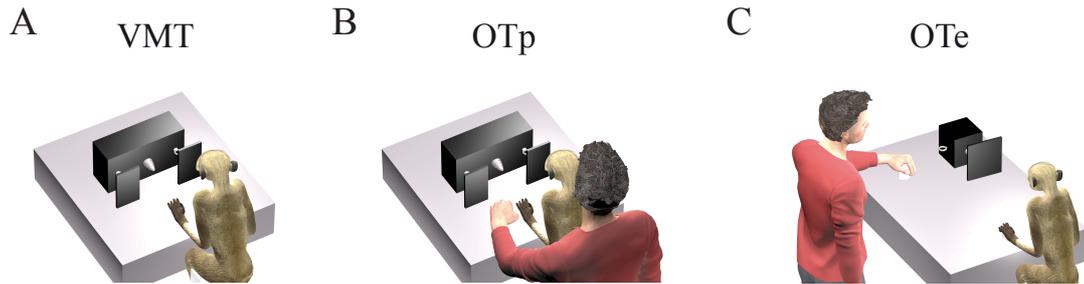


Figure 17. Setup configurations for the behavioral tasks. The behavioral setup allowed three different configurations for performing the VMT (a), the OTp (b) and the OTe tasks (c).

Both monkeys were trained to perform, in different blocks, 1) the visuomotor task (VMT, Figure 17a), 2) an Observation Task carried out in the monkey peripersonal space (OTp, Figure 17b), 3) an Observation Task carried out in the monkey extrapersonal space (OTe, Figure 17c), 4) a video Observation Task showing static and dynamic videos of the same grasping action (OTv, Figure 19).

In the VMT, in the OTp and OTe tasks, the monkey was seated on a primate chair in front of a box, shown in Figure 17d from the monkey's point of view, while during OTv the monkey chair was rotated by 180°, to face a video monitor (1920x1080, 60Hz). The monitor was located at a distance of 57cm from the monkeys' face, and the area of the video encompass (13.04° x 9.85°) of the visual field respectively in the horizontal and vertical dimension. All the tasks were performed within the same session.

The custom-made box was divided horizontally into two sectors by a half-mirror: the upper sector contained a small black tube with a white light-emitting diode (LED) that could project a spot of light on the half-mirror surface; the lower sector contained a sliding plane hosting three different objects. When the LED was turned on (in complete darkness), the half-mirror reflected the spot of light so that it appeared to the monkey as located in the lower sector (fixation point), in the exact position of the center of mass of the not-yet- visible target object. The objects – a ring, a small cone and a big cone – were chosen because they afforded three different grip types, hook grip (in which the index finger enters the ring), side grip (performed by opposing the thumb and the lateral surface of the index finger), whole-hand prehension (achieved by opposing all the fingers to the palm). All tasks were performed by the monkey or experimenter with the hand contralateral to the recorded hemisphere.

Objects were presented, one at a time during different experimental trials, through a 7 cm opening located on the monkey's sagittal plane at a reaching distance from its hand starting position. A stripe of white LEDs located on the lower sector of the box allowed to illuminate it during specific phases of the task. Note that, because of the half-mirror, the fixation point remained visible in the middle of the object even when the lower sector of the box was illuminated.

The VMT task included three basic conditions, as illustrated in Figure 18: grasping in the light, grasping in the dark and a no-go condition. Each of them started

when the monkey held its hand on a fixed starting position, after a variable intertrial period ranging from 1 to 1.5 seconds from the end of the previous trial.

In each task were collected 12 correctly performed trials with each object for each condition. The reward was automatically delivered (pressure reward delivery system, Crist Instruments, Hagerstown, MD) were all the constrains of the trial were respected.

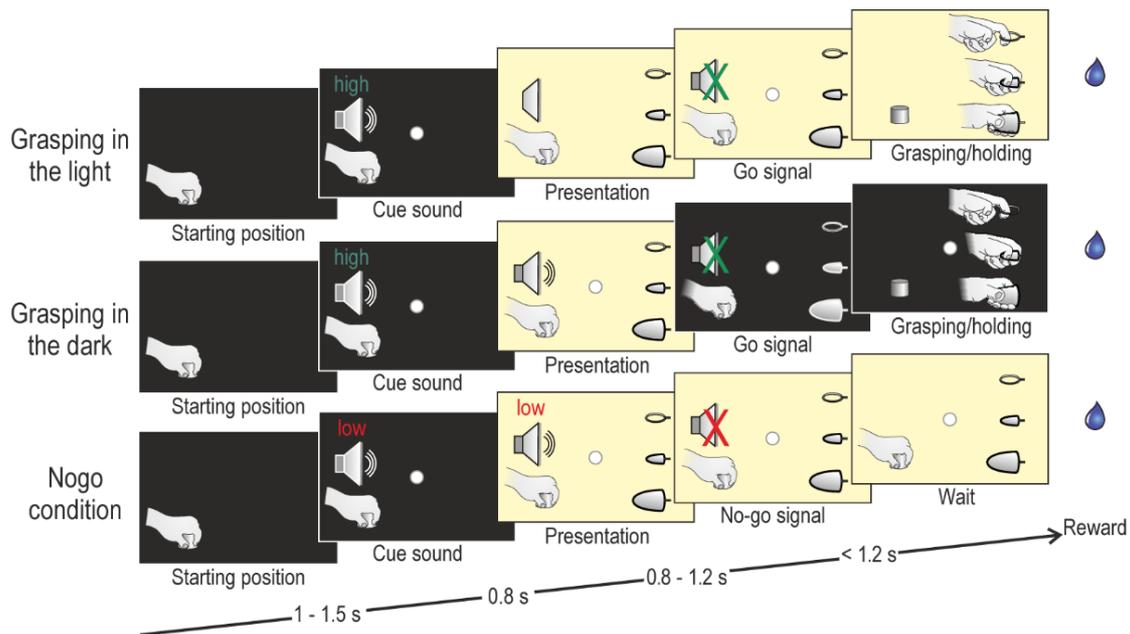


Figure 18 Temporal sequence of task events. The first upper line shows the Grasping in the light condition, the line below shows the Grasping in the dark while the least line shows the No-go condition.

1. *Grasping in the light* (Figure 18, upper line). The fixation point was presented and the monkey was required to start fixating it within 1.2 s. Fixation onset resulted in the presentation of a cue sound (a pure high tone constituted by a 1200 Hz sine wave), which instructed the monkey to grasp the subsequently presented object (go-cue). After 0.8s the lower sector of the box was illuminated and one of the objects became visible. Then, after a variable time lag (0.8-1.2s), the sound ceased

- (go-signal), and the monkey had to reach, grasp and pull the object within 1.2s, keeping the object pulled for at least 0.8 s.
2. *Grasping in the dark* (Figure 18, middle line). The temporal sequence of events in this condition was identical to that of grasping in the light. However, when the cue sound (the same high tone as in grasping in the light) ceased (go-signal), the light inside the box was automatically switched off and the monkey performed the subsequent motor acts in darkness, without seeing its own hand. The fixation point was visible for the entire duration of each trial, providing a spatial guidance for reaching the object position in the absence of visual feedback. In this paradigm, grasping in the light and grasping in the dark trials were identical and unpredictable until the occurrence of the go signal, to ensure that action planning was the same in both conditions and the only difference between them was the presence/absence of visual feedback from the acting hand and the target object.
 3. *No-go condition* (Figure 18 lower line). The temporal sequence of events was the same as in the go conditions, but a different cue sound (a pure low tone constituted by a 300 Hz sine wave), instructed the monkey to remain still and continue fixating the object for 1.2 s in order to receive the reward.

In both the Observation Tasks performed live (OTp and OTe), the apparatus, the target objects and all the task stages were the same as in the VMT task. Each Observation Task was carried out in different block of trials. In OTp, an experimenter stands on the back of the animal and performs the task. The monkey had to remain still with its hand on the initial position and to maintain fixation while the experimenter performed the task. Experimenter's hand was placed in a manipulandum 10 cm next to the monkey's hand

(Figure 17e). The OTp task included the two basic conditions which were the same as VMT task: grasping in the light and no-go trials (Figure 18).

In the OTe, the experimenter performed the task while the animal looked at the scene from a lateral viewpoint, and the monkey could not reach the object target of the experimenter action. The OTe was shown in the hemifield contralateral to the recorded hemisphere. Indeed, the upper part of the apparatus containing the sliding plane with the objects could be switched on the left or right side (Bonini et al., 2014).

In the Video observation task (OTv, Figure 19) videos of object alone (a small ball or a big ball), of grasping hand with or without (pantomime) the object or a static frame of the same video (grasping or pantomime) were randomly presented. Similarly to the VMT, the monkey had to gaze at the fixation point while holding its hand on a fixed starting position for 1 s. Then, the video stimulus started and lasted 2 s.

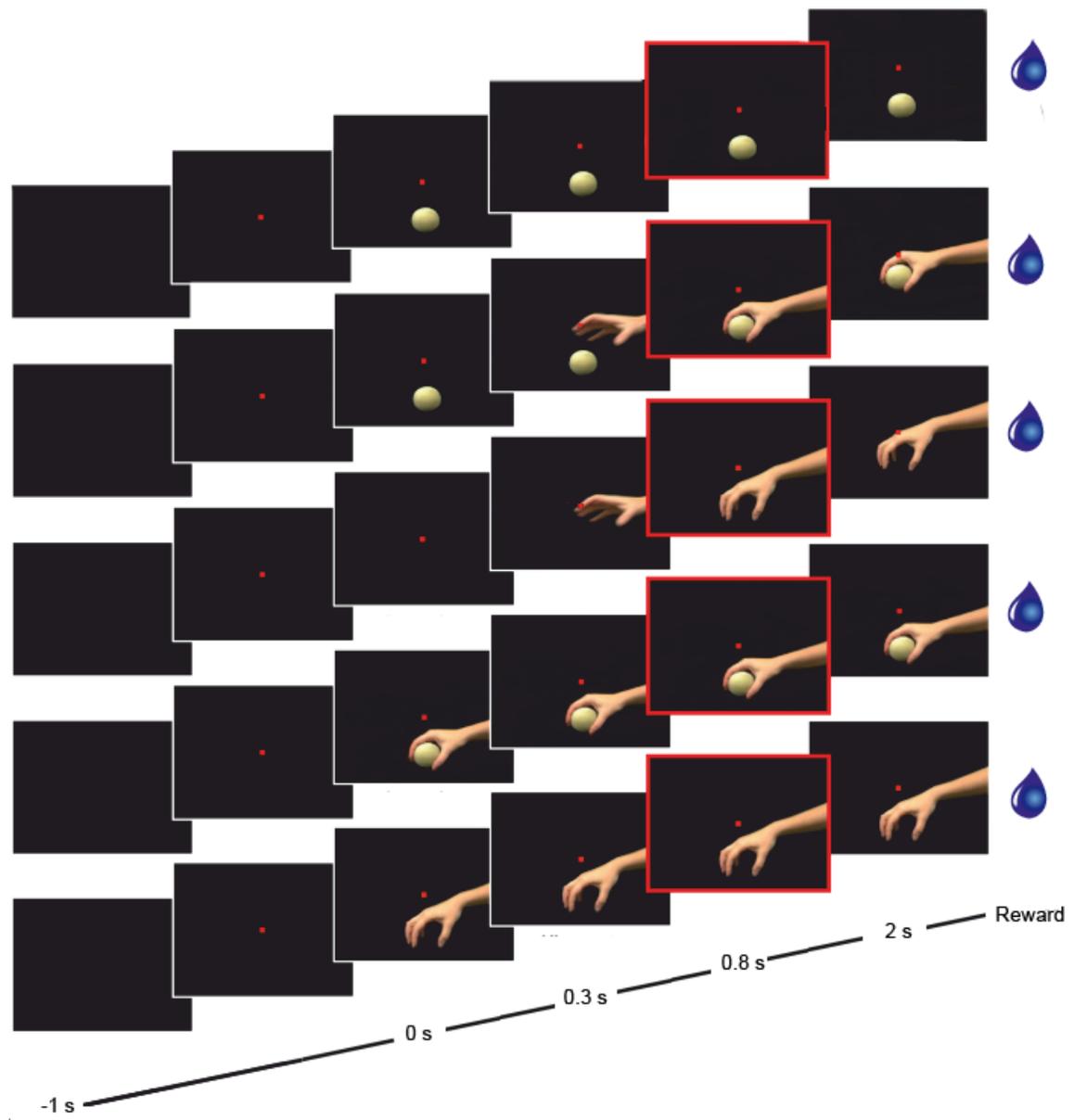


Figure 19. Frames sequences of video observation task. The frame highlighted in red highlighted the hand-object interaction (grasping conditions) or the equivalent frame in the pantomime conditions.

The phases of the all the tasks were automatically controlled and monitored by LabView-based software, enabling the interruption of the trial if the monkey broke fixation, made an incorrect movement or did not respect the task temporal constraints

described above. In all these cases, no reward was delivered. After correct completion of a trial, the monkey was automatically rewarded with the same amount of juice in all conditions (pressure reward delivery system, Crist Instruments, Hagerstown, MD).

4.3 RECORDING OF BEHAVIORAL EVENTS

Distinct contact sensitive devices (Crist Instruments) were used to detect when the monkey (grounded) touched with the hand the metal surface of the starting position or one of the target objects. To signal the onset and tonic phase of object pulling, an additional device was connected to the switch located behind each object. Each of these devices provided a TTL signal, which was used by the LabView-based software to monitor the monkey's performance and to control the generation and presentation of the behavioral paradigm's auditory and visual cue signals.

Eye position was monitored in parallel with neuronal activity with an eye tracking system consisting of a 50Hz CCD video camera provided with an infrared filter and two spots of infrared light. Analog signal related to horizontal and vertical eye position was fed to a computer equipped with dedicated software, enabling calibration and basic processing of eye position signals. The monkey was required to maintain its gaze on the fixation point (tolerance radius 5°) throughout the task, and the eye position signal was monitored by the same LabView-based software dedicated to the control of the behavioral paradigm.

The same software also generated different digital output signals associated with auditory and visual stimuli, the target object presented in each trial, the reward delivery and possible errors made by the monkey during the task (i.e., when the monkey broke fixation). These signals, together with the TTL signals related to the main behavioral events described above, were fed to the Omniplex system, subsequently used to construct the response histograms and the data files for statistical analysis.

4.4 RECORDING TECHNIQUES AND DEFINITION OF EPOCH OF INTERESTS

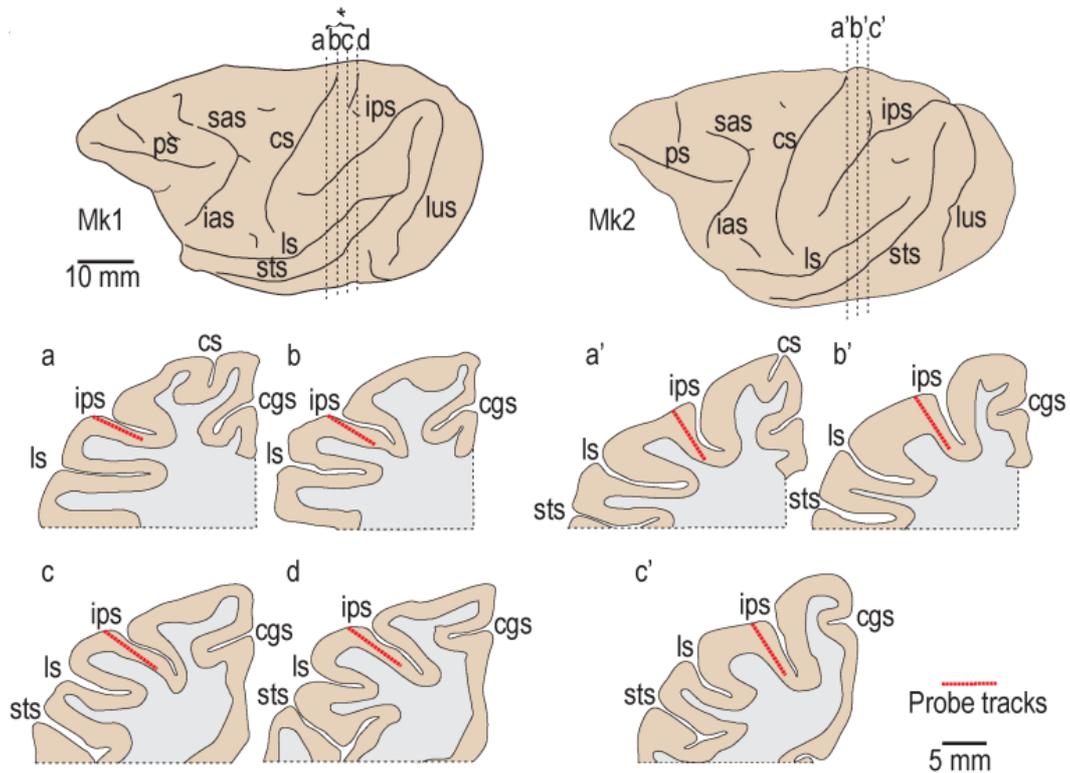


Figure 20. Reconstruction of probes locations along the intraparietal sulcus of Mk1 and Mk2. Vertical dashed lines indicate the position of each probe's track, illustrated in the coronal sections below (a-d for Mk1 and a'-c' for Mk2). Cgs, cingulate gyrus; cs, central sulcus; ias, inferior arcuate sulcus; ips, inferior parietal sulcus; ls, lateral sulcus; lus, lunate sulcus; ps, principal sulcus; sas, superior arcuate sulcus; sts, superior temporal sulcus.

Probes were implanted by estimating the angle of penetration with MRI-based reconstruction of the outline of the intraparietal sulcus at the selected site of insertion (Figure 20). Neuronal recordings were performed by means of chronically implanted arrays of linear silicon probes (see Figure 21) with 32 recording channels per shaft (Herwik, Paul, & Ruther, 2011; Barz, Paul, & Ruther, 2014).

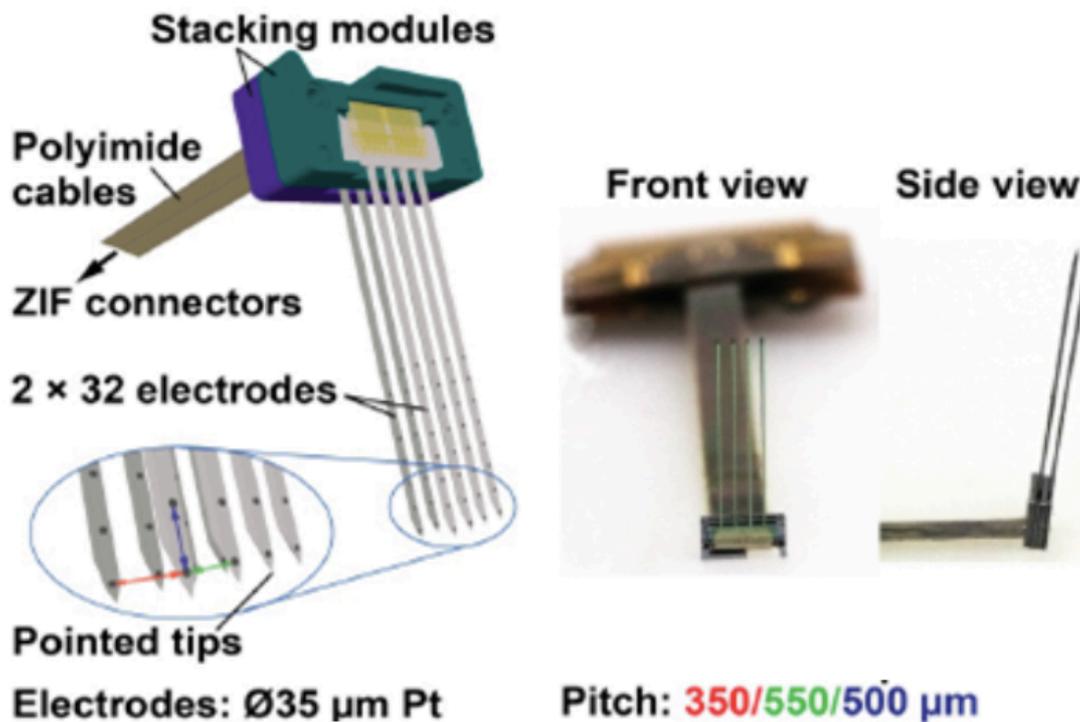


Figure 21. Schematic drawing, features and photograph of a 3d probe. The schematic drawing on the upper part of the figure and the photograph on the bottom illustrate the assembly of 2 2D probes in a 3D configuration. These probes have been implanted in the rostral part of F6 in the left hemisphere of M1 (N=1) and in the right hemisphere of M2 (N=2). For more details on the assembly concept see Barz et al., 2014.

The signal of 128 channels was simultaneously amplified and sampled at 30 kHz with four 32-channel Intan amplifier boards (Intan Technologies, Los Angeles, CA, USA), controlled in parallel via the electrophysiology platform Open Ephys (<http://open-ephys.org/>). All formal signal analyses were performed off-line with fully automated software (Mountain sorter, Chung et al. 2017), using a 2.5 standard deviation negative threshold relative to the signal-to-noise ratio of each channel and distinguishing units into single and multi-units. Single unit isolation was further verified using standard criteria (ISI distribution, refractory period > 1 ms, and absence of cross-correlated firing with

time-lag of ≈ 0 relative to other isolated units to avoid oversampling), possible artifacts were removed, and all the remaining waveforms that could not be classified as single units formed the multiunit activity.

Single neuron activity in the VMT was analyzed in relation to the digital signals related to the main behavioral events, by considering the following epochs of interest: 1) baseline, 500 ms before object presentation; 2) object presentation, from 0 to 500 ms after switching the light on; 3) premovement, 500 ms before reaching-grasping onset (detachment of monkey's hand from the starting position); 4a) reaching-grasping in high sound trials, from reaching onset to pulling onset (of variable duration, calculated on a trial-by-trial basis); 4b) non-reaching grasping in low sound trials were defined as 500ms after the sound off (no-go signal); 5) object holding, from pulling onset to 500 ms after this event. Note that during baseline the monkey kept its hand on the starting position, was staring at the fixation point and was already aware of whether the ongoing trial was a go or a no-go trial: these features enabled us to assess possible variation in neural discharge specifically linked with the subsequent task stages within the ongoing behavioral set. In the OT epochs of interest were defined under the same criteria and conditions. During OTv epochs were defined as follow: 1) baseline 1000ms after the MK gaze toward the red fixation point located in the center of the screen 2) video presentation 1000ms in which the visual stimulus was presented. This allowed us to include the hand shaping, the reaching toward the object, the hand closing to the object (grasping) as well as the object holding.

4.5 SINGLE UNITS ANALYSIS

The raw signals were high-pass filtered offline (300Hz). Single units were then isolated using principal component and template matching techniques provided by dedicated offline sorting software (Plexon) (Bonini et al., 2014). After identification of well-isolated single units we classified neurons significantly activated during specific epoch of interest and task context, depending on the experiment.

We first tested movement-related response during grasping in the light and grasping in the dark, separately, with two 3x4 repeated measures ANOVAs (factors: Object and Epoch). We classified as ‘motor’ all neurons showing, at least during the grasping-in-the-dark condition, a significant main effect of the factor Epoch and/or an interaction between the two factors Object and Epoch, whose discharge differed from baseline during at least one of the three movement-related epochs (premovement, reaching-grasping, pulling) relative to baseline for at least one of the three target objects (Bonferroni post-hoc tests).

In both OTp and OTe the same 3x4 repeated measure ANOVA was applied to test neuronal response during the experimenter’s action. In OTv, a 2x2x2x2 repeated measures ANOVA (factors: Conditions, Object, Action and Epoch) was used for stimuli including the hand (grasping and pantomime). VMT,

We also assessed possible responses to object presentation relative to baseline in VMT, OTp and OTe by considering go and no-go conditions, with a 2 x 3 x 2 repeated measures ANOVA (factors: Condition, Object and Epoch). In OTv we performed a 2x2 repeated measure ANOVA (factors: Object and Epoch) to data collected during only

object video. Concerning all the observation tasks and the analyses on object presentation in all the tasks, only neurons showing at least a significant effect of the factor Epoch, alone or in interaction with one or both of the other factors, were classified as visually triggered (Bonferroni post-hoc tests).

All the analyses described above, allowed us to test the object/grip selectivity of each neuron, depending on the epoch considered (i.e. object presentation and movement epochs respectively): the object that evoked the strongest response (in terms of absolute firing rate) during object presentation/movement epochs was selected as the best one, whereas the one evoking the weakest response in the same epochs was selected as the worst.

The significant criterion for all the analyses was $p < 0.05$.

4.6 HIERARCHICAL CLUSTERED ANALYSIS

To evidence the possible relationship among neural representation of tasks and conditions we performed a hierarchical cluster analysis. Given a certain neural population of N units, firing rates of all units were calculated binning the spiking activity and averaging it across trials. We created a firing rate matrix F with N rows and $c \cdot t$ columns (where c is the number of conditions and t the number of time points per condition within the epoch of interest). Then, we computed the Mahalanobis linkage distances (Matlab function: `manova1`) between the activities in the N -dimensional state space of all possible pairs of conditions in the epoch of interest. Since the Mahalanobis distance between any pair of arbitrarily selected conditions increases linearly as a function of the number of units in the population (see Figure S1), the resulting matrix of distances was normalized dividing it by N . Finally, normalized distance matrix was used to create a hierarchical cluster tree based on the average linkage criterion (Matlab function: `manovacluster`), presenting the cluster solutions in the form of dendrograms. While building the dendrograms, we sorted the leafs within a branch on the basis of their average distance to nearest branches (Matlab function: `optimalleaforder`).

4.7 POPULATION ANALYSIS

Population analyses were carried out taking into account single-neuron responses expressed in terms of mean activity, and normalized across all the compared conditions, and analyzed with 2x4 (factors: object, epoch) repeated measures ANOVAs (as described in the main text of the results chapter).

5 RESULTS

We isolated a total of 134 single neurons with unsupervised offline sorting package (Chung et al. 2017). Based on VMT responses (see Methods), we classified neurons as motor related (32/134, 23.9%), visual related (22/134, 16.4%), visuomotor (67/134, 50%), and task unrelated (13/134, 9.7%). Next, we will describe the properties of these distinct neuronal categories defined according to previous studies (Murata et al. 2000; Lanzilotto et al. 2016).

Motor-related neurons recorded during the VMT

Neurons were classified as motor-related based on their firing properties in the VMT discharge at least during grasping in the dark condition but not during object presentation. Most of them (N=20, 62.5%) were facilitated, increasing their discharge during grasping, whereas others (N=12, 37.5%) showed suppressed activity.

Figure 22 and 23 shows examples of facilitated (Figure 22A) and suppressed (Figure 23) neurons tested in the VMT. Neuron 1 is a typical grip-selective neuron, strongly discharging when the monkey grasped the small or the big cone, but not the ring. The similarity of neuron response during grasping in the light and in the dark ensures that its response cannot be accounted for by vision of monkey's own hand or the target object. Neuron 2, instead, shows the typical response profile of suppressed motor-related neurons, with strong reduction of spontaneous activity during grasping in the light and in

the dark, regardless of the type of target object. Population activity of neurons selected based on their properties during grasping in the dark have been analyzed in

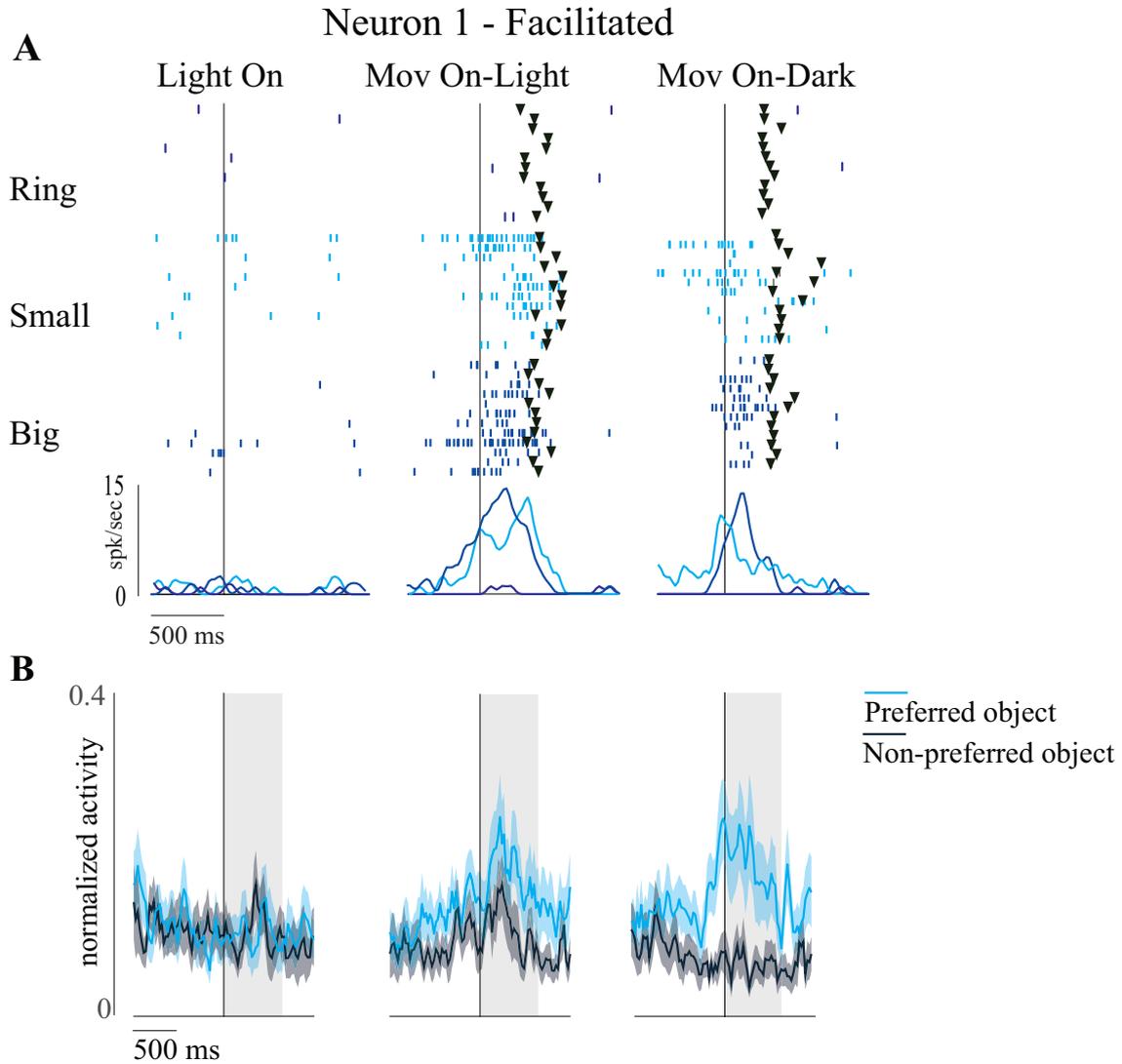


Figure 22. Example neuron and population activity of motor-related neurons. (A) Example of a facilitated motor-related neuron. **(B)** Population activity of facilitated motor-related neurons (N=20). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Shaded regions along each population activity indicates ± 1 ES. Gray shaded regions in population plots indicate epochs considered for statistical analysis.

grasping in the light with a 2x4 repeated measures ANOVA (factors: object, epoch) ($F(3, 57) = 7.7367$, $p = 0.0002$), followed by Fisher post-hoc test, revealing significant discharge during grasping epoch in the light and in the dark compared to both baseline and object presentation ($p < 0.01$ for all the comparisons) and grip selectivity ($p < 0.01$ for all the comparisons).

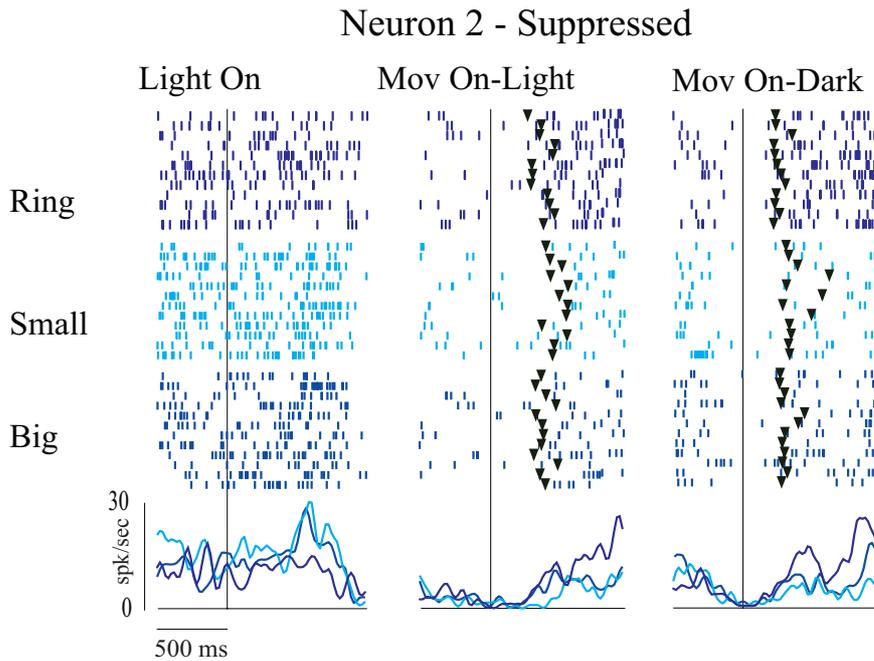


Figure 23. Example of a suppressed motor-related neuron. In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Triangular markers indicate the object pulling onset.

Visual-related neurons

Neurons discharging only during object presentation or grasping in the light (but not in the dark) were classified as visual-related. Among these neurons, 3 (13.64%) discharged only during object presentation, 9 (40.91%) only during grasping in the light and 10 (45.45%) during both object presentation and grasping execution but only in the light.

Figure 24 and 25 shows examples of visual neurons tested during grasping in the light and in the dark. Neuron 3 showed selectivity for the ring object, both during object

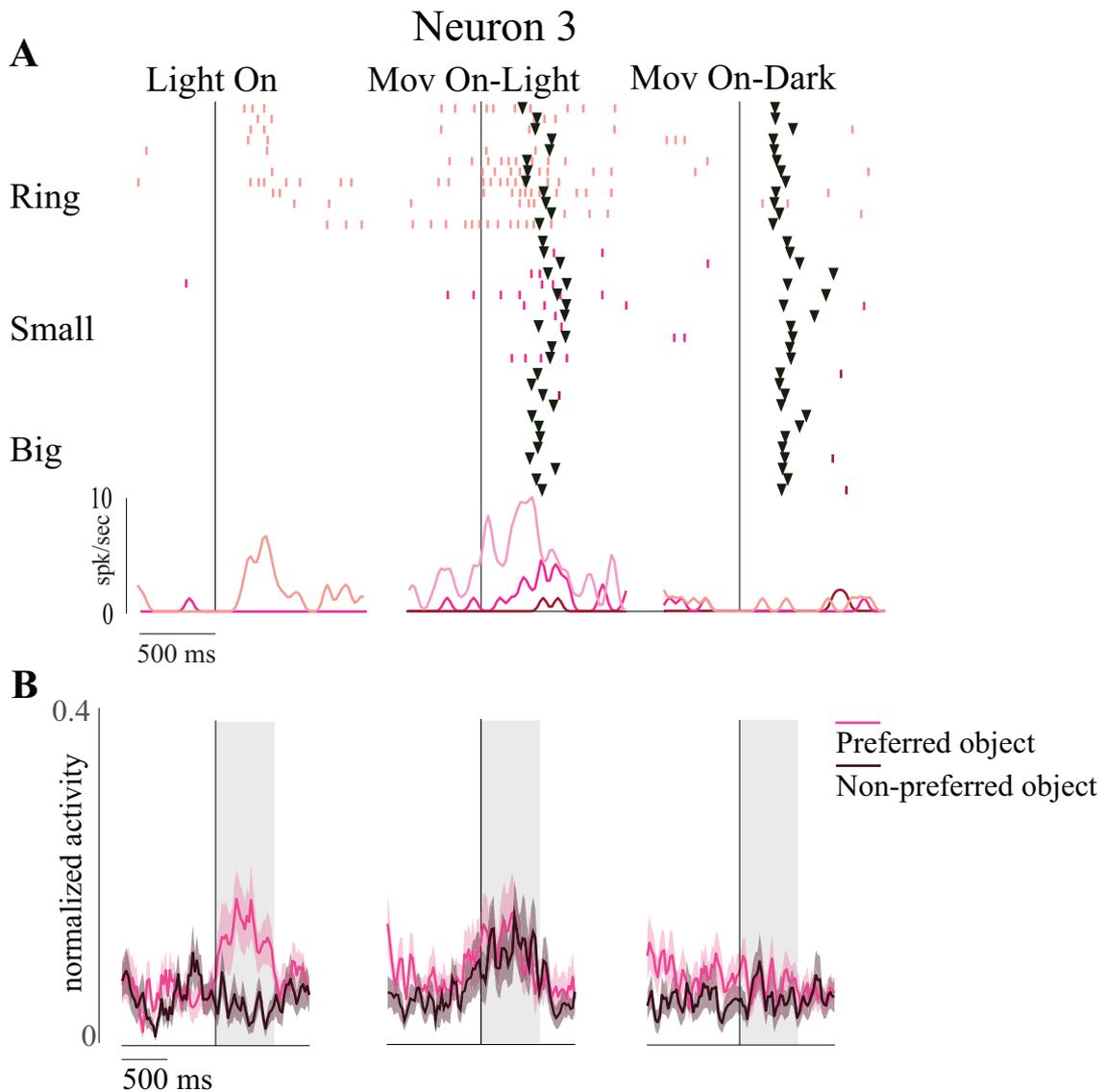


Figure 24. Example neuron and population activity of visual-related neurons. (A) Example of a facilitated visual-related neuron during both object presentation and grasping execution in the light only. **(B)** Population activity of visual-related neurons (N=22). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Shaded regions along each population activity indicates ± 1 ES. Gray shaded regions in population plots indicate epochs considered for statistical analysis.

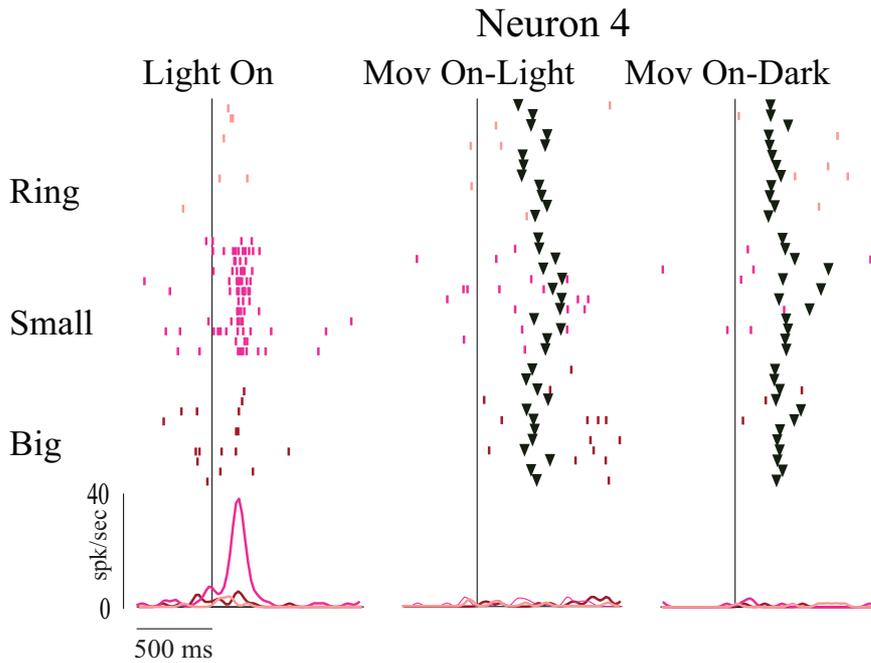


Figure 25. Example of a visual-related neuron discharging only during object presentation. In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Triangular markers indicate the object pulling onset.

presentation and grasping (in the light). Neuron 4 showed selectivity for the small cone only during object presentation.

As for motor neurons, we performed a repeated-measures ANOVA to population discharge during object presentation and grasping of preferred and non-preferred object defined based on neuronal activity during object presentation ($F(3, 63) = 5.36$, $p = 0.00236$). Fisher *post hoc* tests indicate that population activity, which is not significant during grasping in the dark ($p = 0.06$), is significantly modulated during grasping in the light ($p < 0.01$ for both preferred and not preferred object) though with no selectivity for the grip ($p > 0.036$ for both comparisons), but with selectivity during object observation ($p < 0.01$).

Visuomotor neurons

The majority of the recorded neurons were classified as visuomotor neurons, discharging both during grasping in the dark and object presentation epoch. Based on their modulation during grasping in the dark, most of them were facilitated visuomotor neurons (N=50, 74.63%), whereas some (N=17, 25.37%) showed suppressed discharge. Examples of these two types of neurons are shown in figure 26A and 27, respectively. Neuron 5 showed enhanced and object-selective activity during the object presentation epoch and grasping in the light, with a preference for the big object. This same preference was also observed during grasping in the dark. Example neuron 6 showed sustained activation for all objects from object presentation to grasping initiation, with no stable preference for any object during task unfolding. Population activity of neurons selected based on their properties during object presentation have been analyzed during the subsequent execution of grasping in the light and in the dark with a 2x4 repeated measures ANOVA (factors: object, epoch) ($F(3, 147) = 18.279, p < 0.001$), followed by Fisher post-hoc test, revealing significant discharge ($p < 0.01$) and grip selectivity ($p < 0.01$) during both object presentation and grasping in the light and in the dark.

Neuron 7 is a typical example of inhibited visuomotor neuron. While during object presentation epoch it is suppressed and did not show any differential activity among all objects, it showed a significant inhibition also during grasping of the small cone, in the light, and of all objects, particularly the small cone, during grasping in the dark. Population activity of visuomotor neurons selected based on their properties during object presentation have been analyzed as for facilitated neurons with a 2x4 repeated measures ANOVA (factors: object, epoch) ($F(3, 48)=17,739, p < 0.001$), followed by Fisher post-

hoc test, revealing significant suppression of population discharge ($p < 0.001$) during both object presentation and grasping in the light and in the dark, but no significant effect of the factor object.

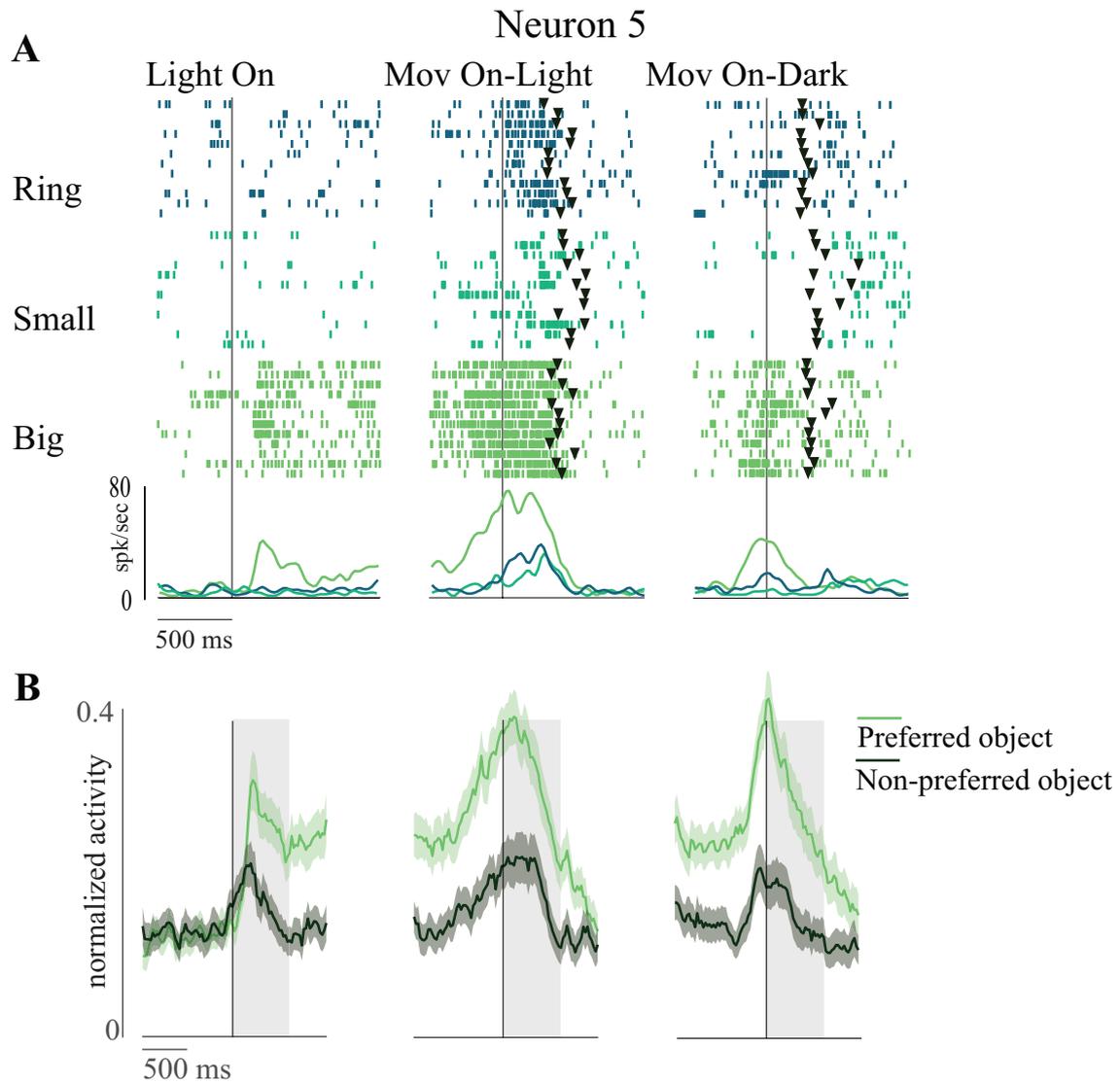


Figure 26. Example neurons and population activity of facilitated visuomotor neurons. (A) Example of visuomotor neuron showing object selectivity. **(B)** Population activity of facilitated visuomotor neurons (N=50). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Shaded regions along each population activity indicates ± 1 ES. Gray shaded regions in population plots indicate epochs considered for statistical analysis.

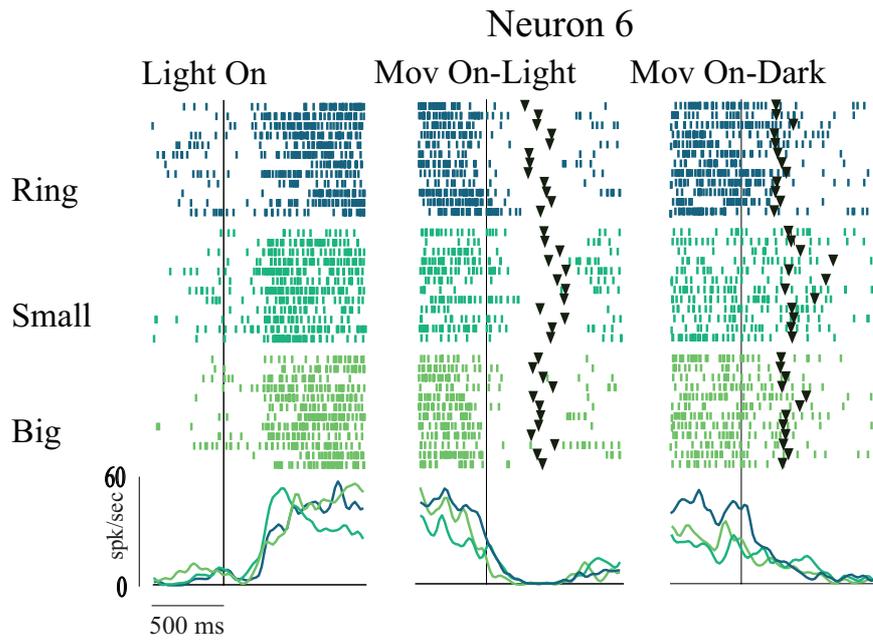


Figure 27. Example of non-selective visuomotor neuron. Triangular markers indicate the object pulling onset. In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Triangular markers indicate the object pulling onset.

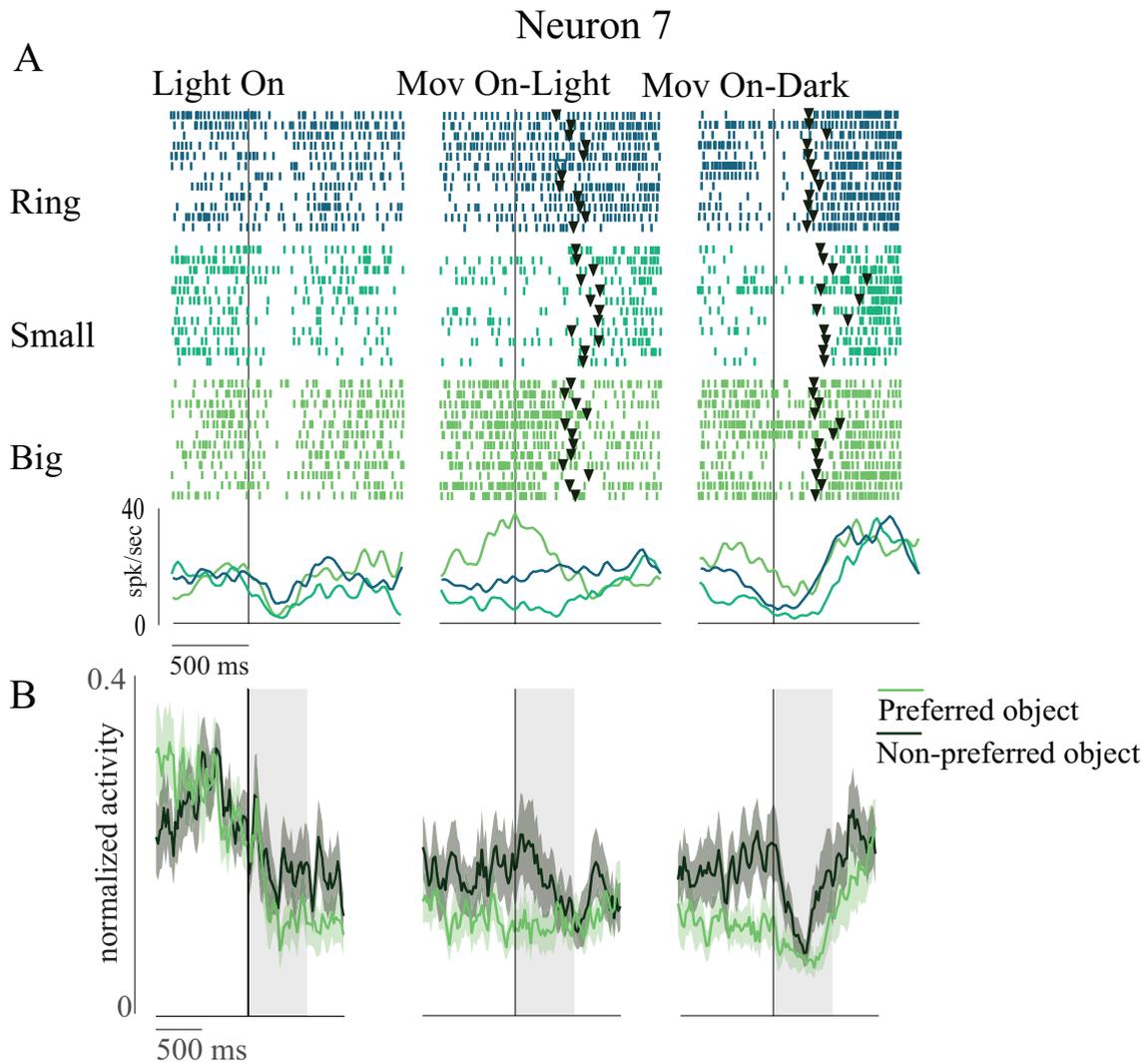


Figure 28. Example neurons and population activity of inhibited visuomotor neurons. (A) Example of inhibited visuomotor neuron. **(B)** Population activity of inhibited visuomotor neurons (N=17). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Shaded regions along each population activity indicates ± 1 ES. Gray shaded regions in population plots indicate epochs considered for statistical analysis.

Interestingly, the classical property ascribed to area AIP reported above were only part of those investigated with the set of tasks employed in this study, allowing us to clarify the neuronal properties of the various type of cells during observation of the task performed by another agent in different conditions. Virtually all of the recorded neurons (125/134, 93.29%) responded also in at least one of the additional observation tasks with which they were tested (see Table 1).

		VMT non related	Motor related	Purely Visual	Visuomotor	Tot.
LIVE	OTp	1	2	1	5	9
	OTe	3	3	5	0	11
	Both	3	5	6	9	23
OTv		0	2	2	0	4
LIVE + OTv		1	17	7	53	78
NO OBS		5	3	1	0	9
		13	32	22	67	134

Table 1. Table showing the distribution of response of all neurons (N=134) among all tasks.

Based on their response in OTp, OTe and OTv we could assess various functional categories of neurons but, among them, neurons in the most represented shows significantly activity in all visual tasks (78/134, 50.2%).

Examples of neuron with complex canonical-mirror properties (Bonini et al. 2014) are shown in figure 29 and 30. Neuron 8 shows enhanced activity during object presentation and grasping execution epochs of both OTp and OTe. Neuron 9 was significantly activated during object presentation epoch in both tasks but showed an increased discharge during the vision of grasping in OTe whereas it was significantly inhibited during vision of grasping action in OTp. On the contrary, Neuron 10 was

significantly activated during grasping execution epoch in both tasks but exhibited an increased discharge during the object presentation epoch in OTp and a significant inhibition during object presentation epoch in OTe.

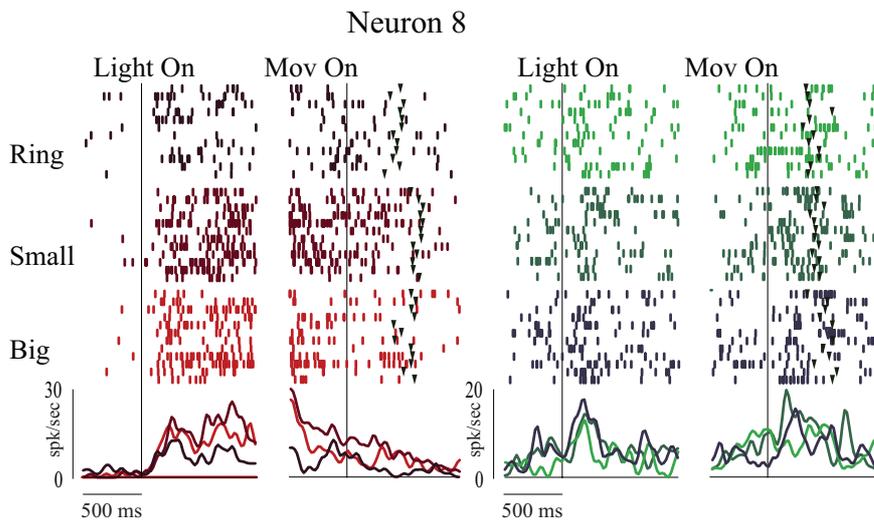


Figure 29. Examples of canonical-mirror neuron responding in both epochs of both tasks. Left side show neurons tested during OTp (red) and OTe (green). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Triangular markers indicate the object pulling onset.

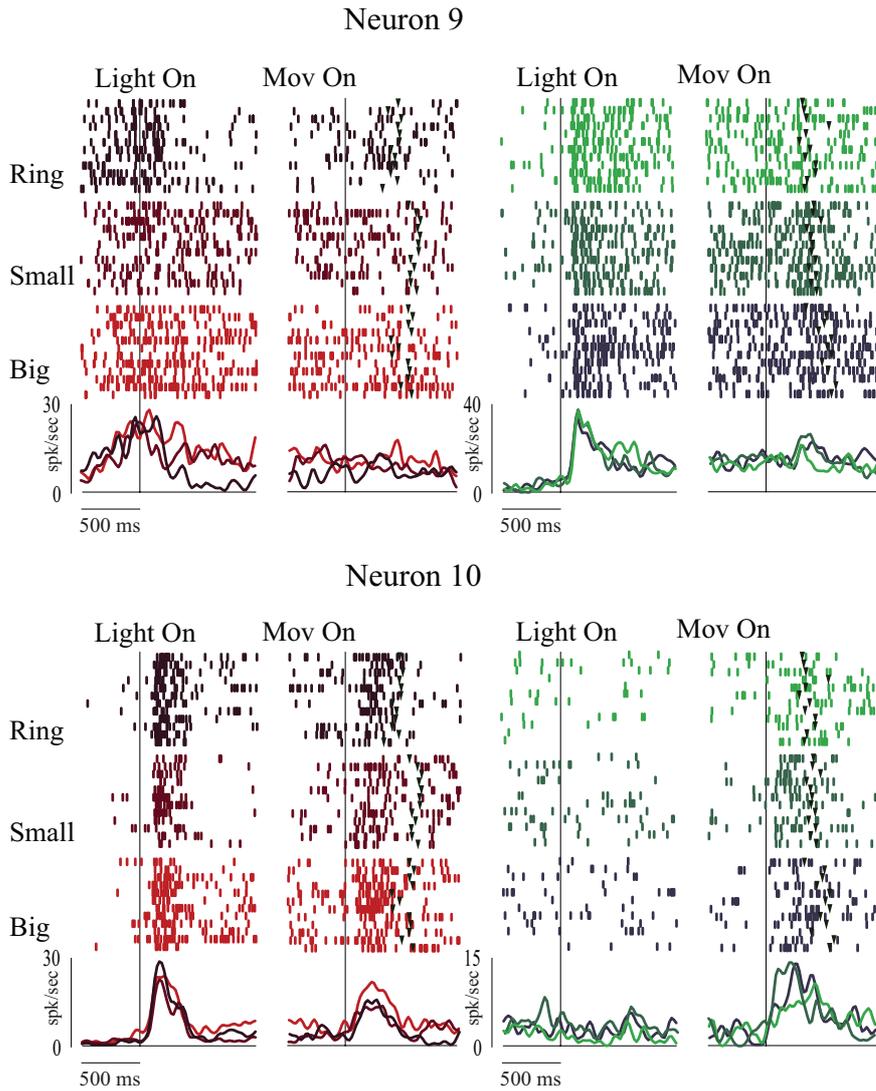


Figure 30. Examples of canonical-mirror neurons with complex properties. Left side show neurons tested during OTp (red) and OTe (green). In each condition vertical bars show the activity aligned when the light is turned on (Light On), and the manipulandum release (Mov On-Light, Mov On-Dark). Triangular markers indicate the object pulling onset.

Linkage of AIP motor and visual information processing in multiple tasks.

The combination of multiple tasks clearly creates a large variety of possible functional properties. Examples are shown in figure 31 and 32. Neuron 11 is a typical example of visuomotor neuron based on its properties in the VMT, discharging for both objects during grasping in the light and grasping in the dark but not during no-go trials. During OTp and OTe, it discharged significantly during grasping epochs in both tasks but preferentially in OTp. During observation of videotaped actions, it significantly discharged during both action and pantomime. This neuron clearly shows preference for action executed in the peripersonal space only in live context. On the contrary, Neuron 12 showed preferential activity to the vision of action outside the monkey peripersonal space. This previously assessed visuomotor neuron discharged similarly for both object in peripersonal and extrapersonal space, but showing no modulation during active grasping.

Neuron 11

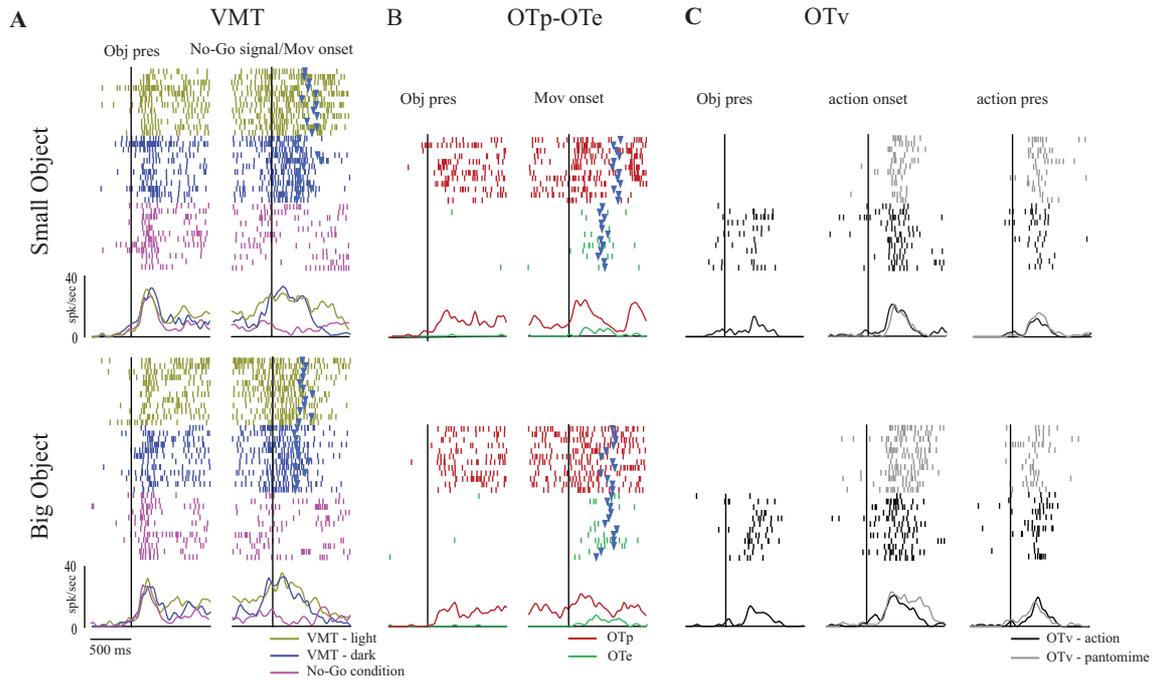


Figure 31. Example of visuomotor neuron (based on VMT classification) tested during VMT (A), OTp and OTe (B), and during OTv (C). Within each task category, rasters and histograms are aligned on the object presentation (left) and movement onset (right) in the VMT, OTp and OTe, and on the presentation of stimuli in OTv. Triangular markers indicate the object pulling onset.

Based on their discharge during grasping epoch in OTe, neurons discharging in all the OT task were subsequently divided in facilitated and suppressed populations. Since it is clearly difficult to capture a general relationship between the multiple functional properties characterized with a so large set of tasks, we employed hierarchical cluster analysis by computing the Mahalanobis distances between each condition of interest in trials where small and big objects were the target in the complete neural state space and presenting the clusters solutions for different epochs as dendrograms (figure 33). During baseline epoch, the linkage distances among tasks (run in blocks and hence known to the

Neuron 12

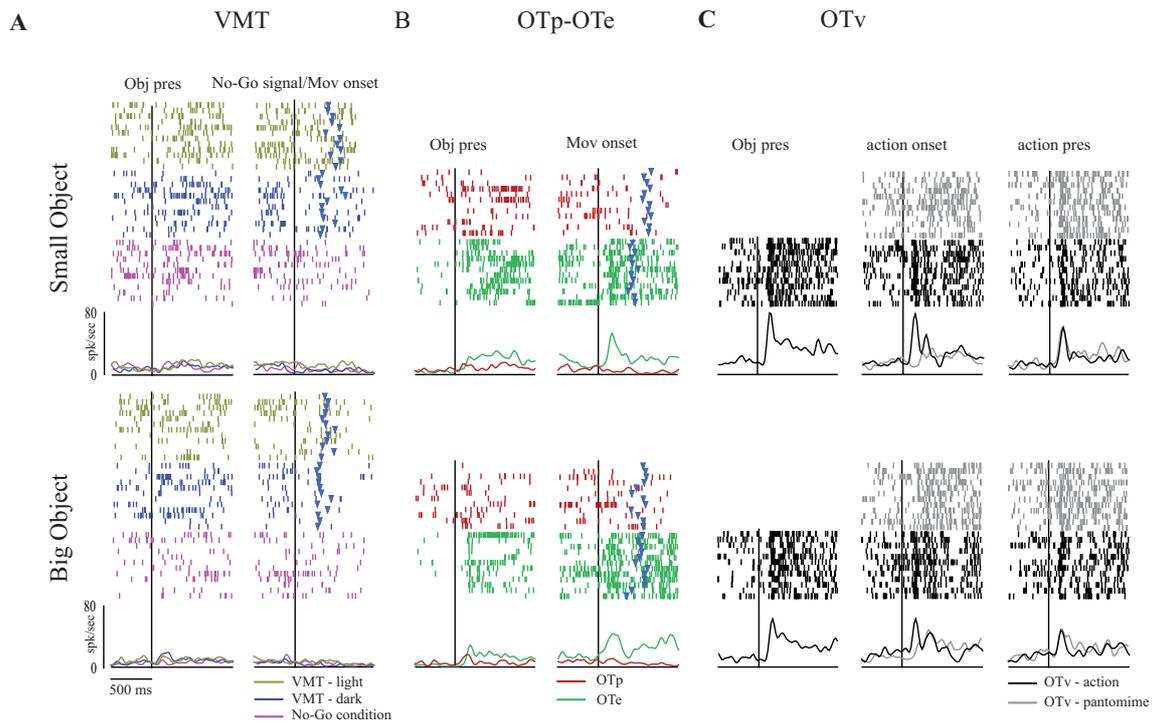


Figure 32. Example of visuomotor neuron (based on VMT classification) tested during VMT (A), OTe and OTe (B), and during OTv (C). Within each task category, rasters and histograms are aligned on the object presentation (left) and movement onset (right) in the VMT, OTe and OTe, and on the presentation of stimuli in OTv. Triangular markers indicate the object pulling onset.

monkey) are greater than those between conditions within each task (unknown to the monkey before cue sound presentation). At a first level, population activity separates task contexts depending on the (near/far) space in which the agent will act. At a second level, within near space, population activity segregates agent (monkey/experimenter), while within far space, live action observation is different from videotaped action.

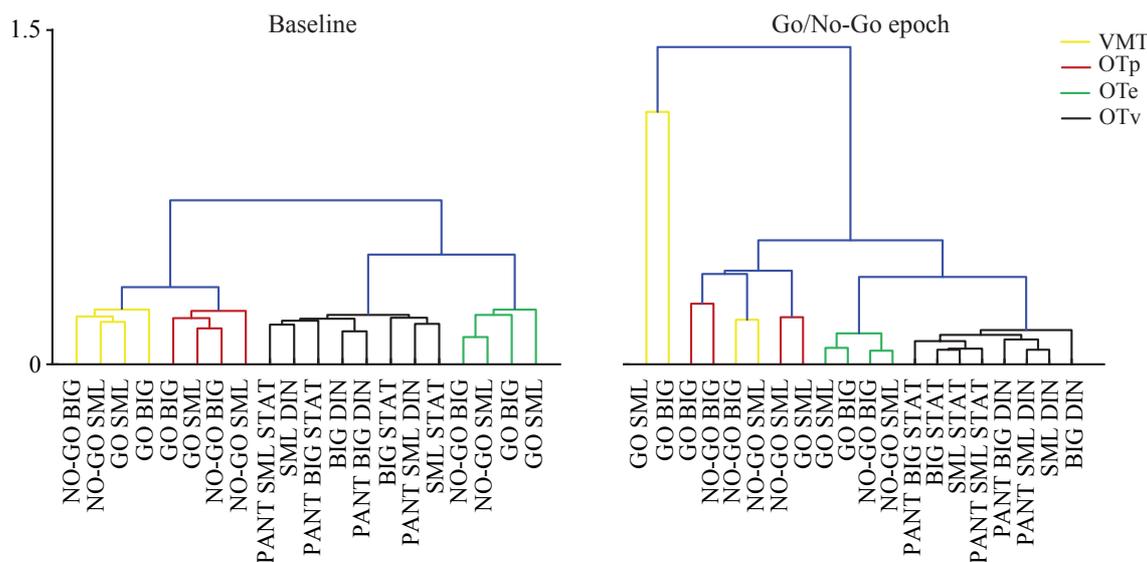


Figure 33. Hierarchical cluster analysis of the AIP population. Dendrograms representing Mahalanobis distance of AIP population during the baseline computed in the 500ms before the cue sound onsed (left) and the go/no-go epoch computed 500ms after the go/no-go signal.

In contrast, considering Go/No-Go epoch (500 ms after the go-no go signal), at a first level monkey's action execution segregates with respects to all the remaining conditions. At a second level population activity still separates task contexts depending on the (near/far) space in which the agent will act. Within peripersonal space, OTp tasks segregates more for different target object condition, while within extrapersonal space, live action observation is still different from videotaped action.

Functional properties of AIP neurons during observation of live and filmed actions

Most of the recorded neurons (N=101, 75.4%) responded during action observation performed in the extrapersonal space. Among these, some responded only in OTe (40/101, 39.6%), a few only in OTv (15/101, 14.9%), and almost half (46/101, 45.5%) responded to both action observation conditions in OTe and OTv.

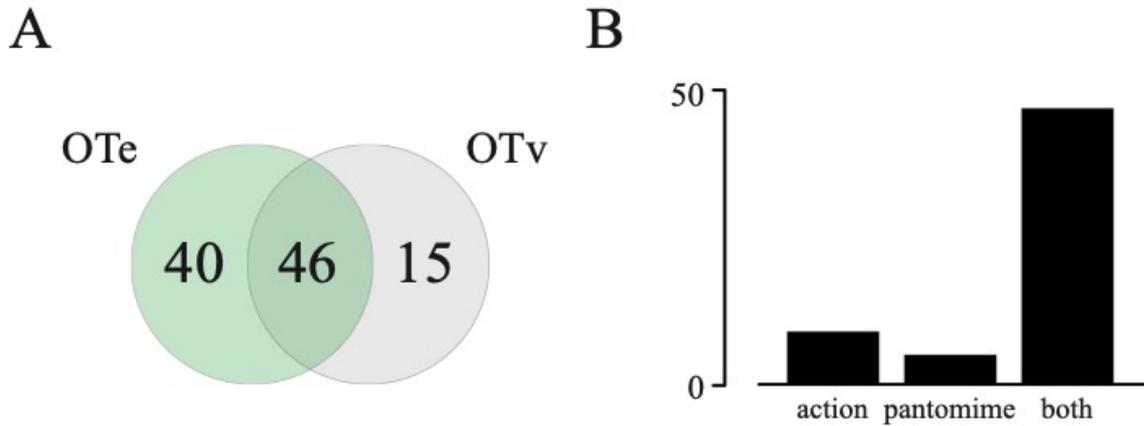


Figure 34. Distribution of neurons showing mirror properties in at least one of the two OTe and OTv tasks. A) Green region contains number of neurons significantly activated in grasping epochs during OTe only, gray region contains number of neurons significantly activated during grasping epoch during OTv only, middle region contains number of neurons significantly activated during grasping epoch in both tasks.

The majority of neurons active during OTv (47/61, 77%) discharge both to transitive and pantomimed actions, thus suggesting that the majority of them generalize action observation activity in OTv regardless from the presence of the target object.

Population activity of facilitated neurons in OT and VMT did not differ among conditions in excited neurons, thus suggesting a high generalization capacity. Differently, population activity of suppressed neurons in OT significantly differed between VMT and OT: while during motor execution in VMT such neurons showed increased activity, their discharge in OTp was suppressed, thus suggesting different functional properties among these two classes of cells.

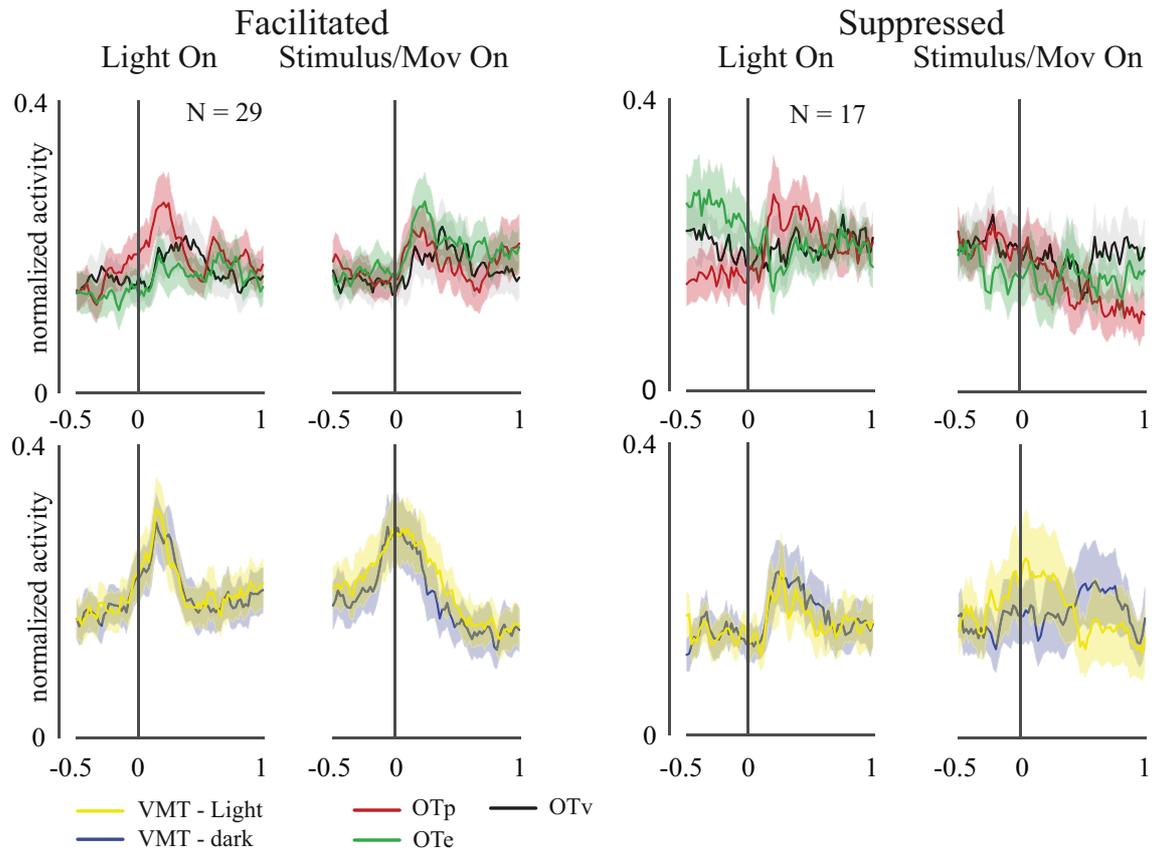


Figure 35. Time course and intensity of population activity of facilitated (left; N=29) and suppressed activity (right; N= 17) of AIP neurons discharging during both OTe and OTv. Within each task category vertical bars show population activity aligned when the light is turned on (Light On), and the manipulandum release or to the first frame of video visual stimuli (Mov On, Stimulus On). Population activity during preferred object is shown in light blue color, during non-preferred object is shown in dark blue color. Shaded regions along each population activity indicates ± 1 ES.

6 DISCUSSION

In the present study, we investigated the functional properties of single neurons of area AIP. Each isolated neuron was tested for its motor and visual responses by using a set of tasks specifically designed to investigate the motor and visual properties of AIP neurons. Noteworthy, in previous studies these tasks were only used separately to test visuomotor processing of objects (Murata et al., 2000; Scheffelhofer and Scherberger 2016) or others' actions (Pani et al., 2014; Maeda et al., 2015), whereas here we employed all of them to test the same single neurons' activity, allowing us to investigate the possible relationship among such a variety of functional properties. I will first discuss the main results obtained with the VMT and then how they relate with those reported by analyzing neuronal properties with the observation tasks.

The proportion of neurons showing motor-related discharge in the absence of visual responses to object is similar to that reported by previous studies (Murata et al., 2000). Given the chronic recording approach employed, we could provide an unbiased estimate of the relative proportion of facilitated and suppressed neurons. It is clear that, as previously reported in PMv (Kraskov et al. 2009), also AIP hosts a relevant set of neurons with suppressed discharge. In general, facilitated neurons did also show grip selectivity of their motor response, which has been evaluated in the dark in order to exclude that it could be accounted for by vision of the object (Lanzilotto et al., 2016), whereas observations carried out on the response properties of suppressed neurons (not sufficiently numerous to perform population analyses) suggest a modest or absent grip selectivity.

We considered as visual related those neurons encoding visually presented objects and/or grasping execution only in the light, hence devoid of truly motor activity. Only a few of these neurons encoded just the visually presented object, whereas the majority responded also or exclusively to the sight of monkey's own hand reaching for and grasping the object. This finding is in line with previous studies (Maeda et al. 2015; Pani et al. 2014) suggesting a role of AIP neurons in monitoring self-actions. Interestingly, although object selectivity was clearly present during target presentation, this selectivity was not maintained during subsequent grasping in the light. The lack of sustained visual-to-motor discharge and selectivity suggests that the same cells could map independently object representation and own actions' visual feedback rather than contributing to a visuomotor transformation.

Visuomotor neurons responded during both object presentation and grasping execution in the dark, supporting their role in object processing and motor control (Schaffelhofer et al., 2015). Importantly, in contrast to visual neurons, visuomotor neurons display a sustained activity and object selectivity from the visual presentation of the target to grasping execution. As reported from several previous studies in both parietal (Murata 2000; Sakata 1995; Taira 1990) and premotor (Fluet et al., 2010; Murata et al., 1997; Raos et al., 2006; Theys et al., 2012; Vargas-Irwin et al., 2015) areas, this property is deemed to be a hallmark of visuomotor transformation. Indeed, in AIP, the reversible inactivation of these type of neurons produce impairments in visually guided grasping (Gallese et al. 1994). A minority of visuomotor neurons also showed suppressed discharge during grasping execution, as previously observed for motor related neurons, and the same effect is evident during object presentation phase, although with weak object selectivity. This finding support the idea that the major contribution to the output of area

AIP in terms of visuomotor transformation is played by facilitated cells, whereas the suppressed ones may play a role in modulating the local circuitries.

Linkage of multiple visual and motor properties in AIP neurons

By evaluating the relationship of neuronal properties among all the observation tasks using as a template the findings reported with the VMT, we found that AIP neurons show a wide variety of behaviors. First, neurons classified as visuomotor with the VMT also showed some visual response to live or filmed actions, and most often to both: none of the visuomotor neurons was non responsive in the action observation tasks. Nonetheless, also most of the neurons classified as motor with the VMT showed responsiveness to the observation tasks, and half of them to both live and filmed actions. It is interesting to consider that solely based on a motor task, these latter neurons would have been considered purely motor neurons, whereas we showed that they can map others' observed actions as well, thus they have to be classified as mirror neurons (Gallese et al., 1996; Rizzolatti et al., 1996). Furthermore, neurons that would have been classified as canonical (Rizzolatti and Kalaska, 2012) based on the VMT had to be considered as canonical-mirror when tested in the observation tasks (Bonini et al. 2014).

In order to explore the possible relationship of neuronal coding properties among the different tested conditions we employed hierarchical cluster analysis of AIP neuronal population. The results highlight, during baseline period, the presence of a clear segregation between peripersonal and extrapersonal space and, within peripersonal space, execution conditions segregates with respect to observation conditions. Furthermore, within extrapersonal space, the block of videotaped actions segregates with respect to live action observation. In the subsequent Go/No-go epoch, we found a clear-cut segregation

of the go condition in the VMT with respect to all other conditions. Among these latter, is maintained the segregation between peripersonal and extrapersonal spaces and, within extrapersonal space, the separation between live and videotaped actions. Although no previous study has ever compared at the same time filmed and live actions, and visuomotor properties, a recent work on presupplementary motor area (Albertini et al. 2019) has reported that space and agent constitute two critical segregated factors by the activity of premotor neurons as well, suggesting that the presence of stimuli within or outside an observer peripersonal space is a critical factor to trigger the activity of neurons in multiple nodes of the cortical motor system.

AIP neuronal properties during vision of live and filmed actions

Focusing our attention on neurons responding during observation of actions in the extrapersonal space, we could compare the discharge of the same set of neurons during the observation of live and filmed actions. Previous studies showed that AIP neurons could encode both live (Maeda et al. 2015) and filmed (Pani et al. 2015; Lanzilotto et al. 2019) actions, but no direct comparison has ever been performed on the same set of neurons. Here we showed that the majority of the neurons discharge during both task, suggesting a high generalization capacity of AIP neurons between different presentation format of observed action stimuli. Furthermore, among neurons responsive to video stimuli it is clearly sufficient the presence of a moving hand (in the absence of a target) to evoke neuronal discharge. The presence of a response to pantomimed action has been recently demonstrated to be a hallmark of ventral and dorsal premotor neurons with mirror

properties (Murata et al., 1997; Raos et al., 2006; Fluet et al., 2010; Bonini et al., 2014; Vargas-Irwin et al., 2015).

Considering only neurons discharging during both live and filmed actions in the extrapersonal space, population analyses showed that the various conditions evoke similar neuronal discharge, and that both neurons with facilitated and suppressed responses in the extrapersonal space show clear facilitated response during both target presentation and action observation in the motor task, supporting the view that neurons contributing to planning and execution of grasping actions can undergo a suppression in their activity during action observation, likely balancing and preventing unwanted motor output during action observation, as originally proposed for PMv (Kraskov et al. 2009).

6.1 CONCLUSIONS

The present study highlights that, by leveraging a larger variety of motor and visual tasks relative to previous studies, motor actions of self and others and potential target objects in the near or far space can be encoded in a wide variety of multiple formats by AIP neurons. Unbiased clustering of neural data revealed that the main dimensions encoded by AIP neuronal activity are the space sector in which stimuli occur and the potential agents that can act on them. Neuronal categories with various type of responses to graspable objects retain different functional relevance when tested in action observation tasks: visuomotor neurons seems to be capable to shift or remap their functional properties depending on the context to subserve visuomotor transformation for grasping during executive context or to predict other's action in different spaces when observing others.

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RINGRAZIAMENTI

La realizzazione e la stesura di questa tesi è stata possibile grazie alla guida e all'aiuto di MM, e LB.

L'esperienza formativa svolta all'interno del Bonini's lab è stata un'occasione di crescita formativa d'eccellenza. I miei ringraziamenti vanno a tutti coloro che hanno avuto la pazienza di rispondere alle mie innumerevoli domande e di discutere nonostante l'im maturità scientifica che può mostrare una mente all'inizio della sua formazione professionale.

Vorrei brevemente ricordare, in particolare con CGF, DA, ML e MG alcuni tra i risolti e non risolti principali temi di discussione dell'anno 2018: vantaggio evolutivo della decussazione e delle differenze interemisferiche, la diversa forma del potenziale d'azione registrato da fibra e da soma, una possibile differente caratterizzazione funzionale tra strati, il circuito AIP-F5, l'albero nella foresta, il pendolo come sistema dinamico, la luna ed Einstein e le non dimostrazioni pratiche su come approcciare alle nuove fasi di training.

Un grazie speciale a tutto il lab e in particolare a ML per le stimolanti discussioni e per avermi sempre incoraggiato a perseguire i miei sogni.

