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SEEING AND ACTING BEHIND A BARRIER: IMPACT ON THE RESPONSE OF MONKEY PUTAMEN NEURONS

VEDERE ED AGIRE ATTRAVERSO UNA BARRIERA: IMPATTO SULLA RISPOSTA DEI NEURONI DEL PUTAMEN DEL MACACO

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ABSTRACT (ENG)

The putamen is a well-known nucleus for its major role in mediating basal ganglia (BG) motor functions and for being the main input station of the motor loop, receiving and modulating cortical signals before sending them back to either promote or inhibit a movement. Recent investigations have shown that the putamen receives convergent projections from the most of the cortical regions that form the mirror neuron (MN) network for hand action, and that all these areas show a comparable activation during the observation of hand actions, supporting the idea that BG were involved in an extended MN network, contributing to decouple MN activity from the motor output, allowing it to be exploitable for high-order perceptual, cognitive, and even social functions.

In the current study, we recorded neuronal activity from 258 single-units in the macaque putamen nucleus during a Mutal Action Task (MAT) a paradigm in which the animal and the experimenter act on the same object in a shared operational space or, by interposing a transparent barrier between the object and one of the agents, they act – and observe the action – being able to see the other agent without the possibility of interacting. This paradigm allowing us to investigate not only the possible presence of mirror response, but also how these responses could vary by changing the operational distance within the actor and the observer.

We found neurons responsive to action execution, action observation, or both. The latter category was the most represented and made up of units whose firing rate could increase or decrease in both conditions, or flipped the modulation sign. In general, excited neurons prevailed during the action and inhibited neurons prevailed during the observation. Another finding of the present study was the presence of neurons modulated by the presence or the absence of the barrier, both when the animal performed the action, and when he observed it.

Despite preliminary, our findings represent one of the first empirical demonstrations of the existence of neurons in the putamen that are specifically modulated by the other's actions, suggesting that putaminal neurons may encode the distance of potential actions in an operational framework.

ABSTRACT (ITA)

Il putamen è un nucleo ben noto per il suo principale ruolo di mediatore delle funzioni motorie dei gangli della base (BG), costituisce inoltre la principale stazione di input del loop motorio, ricevendo e modulando i segnali corticali, per poi rimandarli indietro al fine di facilitare o inibire un movimento. Recenti studi hanno dimostrato il putamen riceve proiezioni convergenti dalla molte delle aree corticali che formano il mirror neuron (MN) network per le azioni manuali, e che tutte queste regioni mostrano un'attivazione comparabile con quella del putamen durante l'osservazione di azioni manuali, supportando l'ipotesi del coinvolgimento dei BG nel MN network esteso, i quali contribuirebbero a disaccoppiare l'attività dei MN dall'output motorio, al fine di utilizzarla per le funzioni percettive, cognitive e sociali di ordine superiore.

Nel presente studio, abbiamo registrato l'attività neuronale di 258 single-units nel putamen del macaco durante il Mutal Action Task (MAT), un paradigma in cui l'animale e lo sperimentatore agiscono sullo stesso oggetto in uno spazio operativo condiviso o, interponendo una barriera trasparente tra l'oggetto e uno degli agenti, agiscono – e osservano l'azione – potendo vedere l'altro agente senza possibilità di interazione. Questo paradigma ci ha permesso di indagare non solo la possibile presenza di risposte mirror, ma anche l'eventuale variazione di queste risposte cambiando la distanza operativa tra l'attore e l'osservatore.

Abbiamo trovato dei neuroni che rispondono all'esecuzione dell'azione, all'osservazione dell'azione o ad entrambe. Quest'ultima categoria era la più rappresentata e costituita da neuroni il cui firing rate aumentava o diminuiva in entrambe le condizioni, oppure c'era un inversione del segno della modulazione. In generale, i neuroni eccitati prevalevano durante l'azione e i neuroni inibiti prevalevano durante l'osservazione. Un altro risultato di questo studio è l'aver trovato dei neuroni modulati dalla presenza o dall'assenza della barriera, sia quando l'animale eseguiva l'azione, che quando la osservava.

Sebbene preliminari, questi risultati rappresentano una delle prime dimostrazioni empiriche dell'esistenza di neuroni nel putamen specificamente modulati dalle azioni dell'altro, suggerendo che questi neuroni potrebbero codificare la distanza delle azioni potenziali da un punto di vista operativo.

1. INTRODUCTION

1.1 The cortical motor system

1.1.1 Neuroanatomy of the frontal motor cortex

The frontal motor cortex is the final common cortical area where intention are turned into action (Capaday et al., 2013). Located in the precentral gyrus, it consists in a portion of agranular cortex classically subdivided into two architectonic areas, namely area 4 and area 6 (Brodmann, 1925). In accordance with this classification, area 4, located rostrally to the central dimple, would form a single large functional area (primary motor cortex or M1), and area 6, anterior to the area 4, was considered a secondary motor area (called premotor cortex) (Woolsey et al., 1952).

One of the first attempts to map the monkey motor cortex was represented by the Woolsey's simiunculus, obtained by the cortical application of stimulating electrodes: depending on the location of the stimulated cortical area, different body part movements were evoked. By adopting this technique two somatotopic maps were found, corresponding to area M1 and a supplementary motor area (SMA): the former, contains a representation of the entire contralateral body (with the hindlimbs located medially, the forelimbs and the head located ventrally), the latter, located more mesially, hosts a less detailed body representation extending along the caudo-rostral axis (figure 1). In the same years, another group (Penfield and Welch., 1951) applied direct electrical stimulation of the cortical surface on human patients undergoing brain surgeries, obtaining the homunculus, a very similar map corresponding to the one found in the monkey. In these maps the representation of movements follows the principle of innervation density: there is a magnification of the bodily areas that require a finer control of the movements and that are, therefore, more densely innervated. Furthermore, this artificial stimulation studies were consistent with the previous Jackson's (1870) clinical observations on epileptic patients: he noted, in fact, that in the epileptic seizure there is a focal onset of activity limited to a specific body part that subsequently propagates according to a systematic path that suggested the presence of a correspondingly organized motor map.

A more recent series of anatomic and functional studies revealed that this view of the agranular frontal cortex is over simplified, since Brodmann's area 4 and area 6 are not only distinct

from each other, but area 6 is further composed of a variety of anatomo-functional areas with different afferent and efferent connections and playing different functional roles in motor control (Luppino and Rizzolatti, 2000). A new parcellation of the motor cortex was proposed, in which the whole area 4 corresponds to the primary motor cortex (called F1), and area 6 would consist of a mosaic of areas that can be subdivided into three groups: mesial (F3, F6), dorsal (F7, F2) and ventral (F5, F4) areas (Matelli et al., 1991, 1985). The most anterior areas within each subdivision (F6, F7 and F5) stronger link with the prefrontal cortex, whereas the caudal areas (F3, F2 and F4) are more strongly connected with parietal areas (Rizzolatti and Luppino, 2001) (Figure 1).

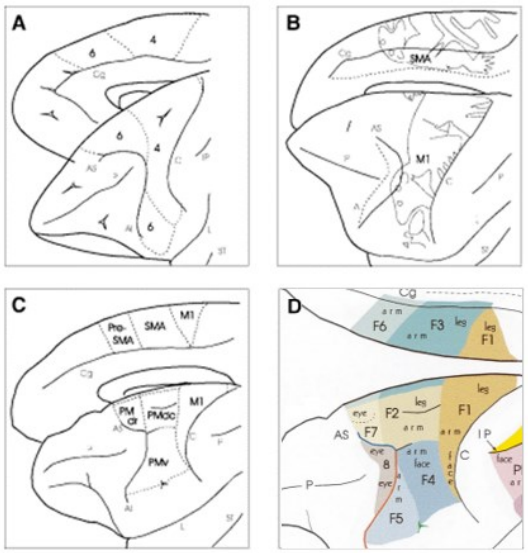


FIGURE 1 | Comparative view of some of the proposed subdivisions of the agranular frontal cortex of the monkey. A: cytoarchitectonic map of Brodmann (1); B: functional map of Woolsey et al. (1952); C: modern, functional subdivision; D: histochemical and cytoarchitectonic map of Matelli et al. (1985, 1991). AI, inferior arcuate sulcus; AS, superior arcuate sulcus; C, central sulcus; Cg, cingulate sulcus; F1–F7, agranular frontal areas; IP, intraparietal sulcus; L, lateral fissure; M1, primary motor cortex; P, principal sulcus; PMdc, dorsal premotor cortex, caudal; PMdr, dorsal premotor cortex, rostral; PMv, ventral premotor cortex; SMA, supplementary motor area; ST, superior temporal sulcus. Panel A, B, C from Luppino and Rizzolatti (2000); panel D from Rizzolatti at al. (1998).

Concerning the cortico-spinal projections - which form the corticospinal tract – all the caudal motor areas (F1, F2, F3 and part of F4 and F5) project to the intermediate medullary section where interneurons are located, but only F1 projects also on the ventral horns, which contains motoneurons (Porter and Lemon, 1993). These data indicate that F1 is the motor area with the most strong and direct access to the motor neuron pools of the spinal cord, contributing to exploit innate synergies organized at the spinal level and thereby determining the fine morphology of the movement; the others caudal motor areas, instead, can contribute to the global form of movement, by projecting to

the spinal interneurons, and by their influence on F1 as well. The organization of the rostral motor areas is quite different because both F6 and F7 are neither connected with the spinal cord nor with F1: these areas cannot control movement directly, but can control it indirectly through their subcortical projections (since they project to various parts of the brain stem and to the basal ganglia), and to the caudal motor areas (Luppino and Rizzolatti, 2000).

Intracortical microstimulation studies have demonstrated that the motor cortex contains many functional motor representations. Area F1 somatotopy basically corresponds to that of area 4: low intensity stimulation on this cortical region evokes sharp and immediate single joint movements of the entire body. Actually, this area plays a major role in segmenting actions planned by other motor areas into elementary movements, and is unique among the motor areas in controlling independent finger movements (Porter and Lemon, 1993). Recording and inactivation studies have shown that F1 neurons encodes movement parameters such as force (Evarts, 1968) and direction (Georgopoulos et al., 1986). As F1, F3 is electrically excitable with low-intensity currents and contains a complete body movement representation: evoked movements mainly involve proximal and axial muscles and, typically, a combination of different joints. In contrast, F6 is weakly excitable, and its motor responses typically consist of slow and complex movements restricted to the arm (Luppino et al., 1991), although more recent studies also showed evidence of electrically evoked movements of the fingers (Lanzilotto et al., 2016). Area F2 is excitable with a low-threshold current and has a rough somatotopic organization, with leg and arm representation located dorsal and ventral to the superior precentral dimple, respectively (Fogassi et al., 1999; Raos et al., 2003). Much less is known about F7: its dorsal part contains the supplementary eye field (SEF), that is richly connected with the frontal eye field (FEF) (Schlag and Schlag-Rey, 1987). The remaining part of F7 is scarcely excitable and has been subject of few functional studies. Area F4 contains a representation of arm, neck, and face movements (Gentilucci et al., 1989), and long-train stimulation evokes more complex movements as defensive movements, manipulation, grasping and mouthing, depending on specific cortical location (Graziano et al., 2002). Finally, F5 contains a movement representation of the hand and the mouth (Rizzolatti et al., 1988), associated with a variety of sensory responses (Maranesi et al. 2012).

1.1.2 Parieto-frontal circuits for the coding of executed and observed movements

The frontal motor cortex allows mammals to plan and control voluntary movement by flexibly adapting to incoming sensory information thanks to a rich set of projections of the parietal cortex to the frontal lobe. Anatomo-functional evidence shows that, like the motor cortex, the posterior parietal cortex is composed by a multiplicity of independent regions, dealing with different aspect of sensory information and with specific effectors. Each of these regions appears to be linked preferentially with a frontal motor area, forming a series of relatively segregated, parallel parieto-frontal circuits, which differentially contribute to specific sensory-motor transformations (i.e. the transformation of sensory description of stimuli into a motor plan to interact with them) (Borra et al., 2017; Rizzolatti et al., 1998) (Figure 2).

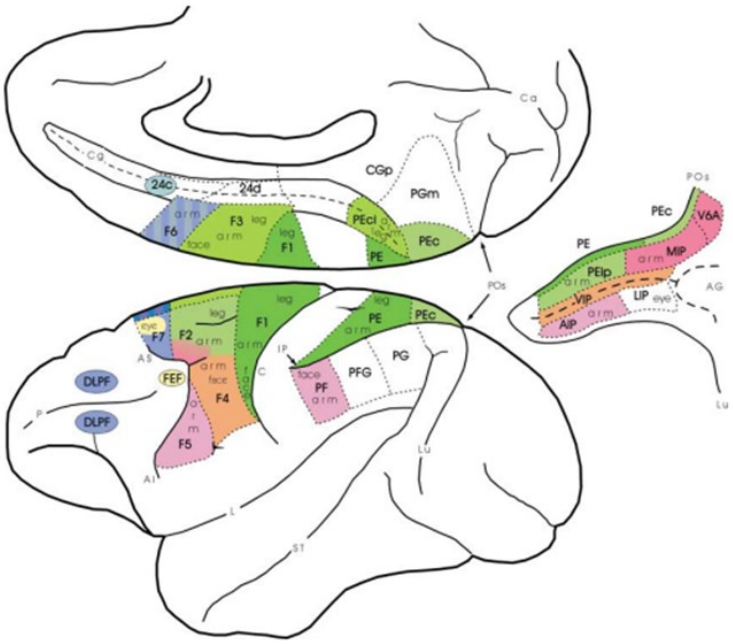


FIGURE 2 | Mesial and lateral views of the monkey brain showing the parcellation of the parieto-frontal motor system, including frontal, posterior parietal, and cingulate cortices. The areas located within the intraparietal sulcus are shown in an unfolded view of the sulcus in the right part of the figure. The posterior motor areas and the parietal areas that are the source of their major cortical afferents are indicated with the same color. The anterior areas are indicated in blue. AI, inferior arcuate sulcus; AS, superior arcuate sulcus; C, central sulcus; Cg, cingulate sulcus; DLPFd, dorsolateral prefrontal cortex, dorsal; DLPFv, dorsolateral prefrontal cortex, ventral; L, lateral fissure; Lu, lunate sulcus; P, principal sulcus; POs, parieto-occipital sulcus; ST, superior temporal sulcus. Figure from Luppino & Rizzolatti (2000).

One of these circuits, which connects the anterior intraparietal (AIP) area to the area F5, is crucial for visuomotor transformations for grasping. In this circuit, the coding of the objects' visual features (such as size, shape, and orientation) is associated with motor plans suitable for interacting

with the observed objects (Jeannerod et al., 1995). This process has been described as “affordances extraction” (see, e.g., Fagg and Arbib, 1998) or, more recently, it has been included in the so called “affordance competition” (Cisek, 2007). According to Gibson’s classical view (1979), affordances are all the motor possibilities that an object offers to an individual, depending on the motor capabilities of the observer. Both areas AIP and F5, contain a variety of cell types, ranging from purely motor neurons, discharging exclusively during active movements of the hand and the wrist (Rizzolatti et al., 1988; Umiltà et al., 2008) to visuomotor neurons, which fire during the static presentation of a graspable object and also when the object is grasped. This neural activity can be interpreted as the correlate of visuomotor transformations subserving object affordance extraction (Cisek, 2007). The classical view emphasized the visuo-motor correspondence between the visually and motorically coded object: for example, if a neuron respond to visually presented small objects it should selectively respond during precision grip, whereas if a neuron encode large object or a ring, it should respond preferentially during whole-hand prehension or hook-grip, respectively (Taira et al., 1990; Sakata et al., 1995; Murata et al., 2000). Today, it is known that this is not the case, and population activity of neurons with poorly matched visual and motor selectivity transform dynamically the visual features of object into potential motor plans for grasping them along the AIP-F5-F1 pathway, which can ultimately lead to the execution of one of the afforded grip types (Schaffelhoefer and Scherberger, 2016).

In addition to neurons responding to visually presented objects, area F5 and AIP also contains neurons responding during the observation of others’ action, known as "mirror neurons" (MNs) (Di Pellegrino et al., 1992; Gallese et al., 1996). The visuomotor congruence between MN discharge during observed and executed motor acts has been initially described (Gallese et al., 1996) as varying from very strict (same grip type encoded during action execution and observation) to broader (same type of action, e.g. grasping, but with variable selectivity for the type of grip). To date, thanks to more controlled experiments we know that the probability to observed a strictly congruent match between MN discharge during executed and observed actions basically depends on the number of tested grip types, and it essentially correspond to chance level, indicating that the congruence does not appear to be an essential feature of MN visuomotor discharge (Papadourakis and Raos, 2017).

Furthermore, it is important to note that visuomotor neurons cannot be considered as functionally distinct categories depending on the type of visual stimuli to which they respond to, as classically assumed (see Kandel et al., 2021): in fact, visual responses to objects ("canonical") and actions ("mirror") are not anatomically segregated in different sectors of areas F5, and "canonical" and "mirror" properties frequently coexist in the same single neuron (Bonini et al., 2014; Livi et al., 2019).

Neurons with mirror properties have been identified also in others cortical areas, each receiving visual information thanks to direct or indirect links with the superior temporal sulcus (STS), where the processing of visual signals primarily takes place (Barraclough et al., 2009; Jellema and Perrett 2006; Perrett et al., 1989). This *extended MN network*, include AIP (Maeda et al 2015; Pani et al 2014; Lanzilotto et al., 2019), PFG (Bonini et al., 2010; Fogassi et al., 2005), F1 (Dushanova and Donoghue 2010; Tkach et al., 2007; Vigneswaran et al., 2013), the dorsal premotor cortex (Cisek and Kalaska 2004; Tkach et al., 2007; Papadourakis and Raos 2019), the medial frontal cortex (in particular the anterior cingulate cortex and the pre-SMA) (Mukamel et al 2010; Yoshida et al., 2011; Livi et al., 2019) and even the ventrolateral prefrontal cortex (Borra et al., 2011; Gerbella et al., 2013; Nelissen et al., 2011; Simone et al., 2017) (Figure 3).

Since MNs discovery, there have been many controversies about their possible functional role: it was firstly hypothesized that MNs were the neural substrate for recognizing (or "understanding") other's actions, through an automatic match between their visual description with corresponding motor representations belonging to the observer's motor repertoire (Gallese et al., 1996; Rizzolatti and Sinigaglia, 2010), but this view has been often criticized (e.g. Cook et al. 2014; Hickok, 2013). One of the most currently accepted view is that the mirror mechanism could contribute to imitative behaviors and the recognition of speech, especially in noisy environment (Heyes and Catmur, 2021), but most recent hypotheses also raised the issue of a possible role of mirror mechanisms in the preparation of interactive response to others, proposing to consider the mechanisms exactly as the one more extensively explored for object affordance (Orban et al. 2021; Bonini et al. 2022).

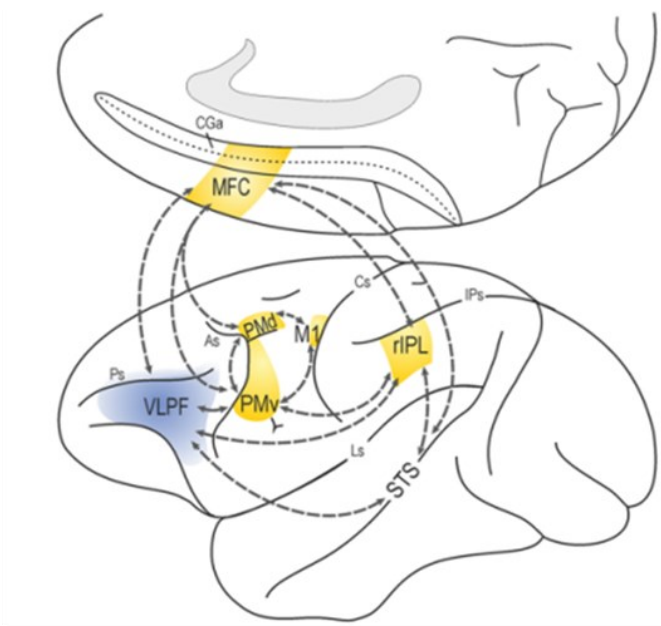


FIGURE 3 | The extended MN network. The cortical MN network includes a set of areas in which the presence of single neurons with mirror properties has been directly demonstrated (yellow) as well as other regions (the ventrolateral prefrontal areas 12 and 46) in which the presence of MNs is supported by anatomical evidence but not yet directly demonstrated (blue). The arrows represent the main anatomical connection between these areas. As, arcuate sulcus; Cs, central sulcus; CGa, anterior cingulate gyrus; Ips, intraparietal sulcus; Ls, lateral sulcus; Ps, principal sulcus. M1, hand sector of the primary motor cortex; MFC, medial frontal cortex; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; rIPL, rostral inferior parietal lobule; STS, superior temporal sulcus; VLPF, ventrolateral prefrontal cortex. Figure from Bonini (2016).

An interesting line of research in the mirror neuron literature focused on neurons whose axons contribute to the pyramidal tract. Two types of mirror responses were found in these cells: during action execution, most corticospinal MSs increased their discharge, but during the observation of others' action about half of them showed an increased firing (facilitation MNs) whereas the remaining half, both in F5 (Kraskov et al., 2009) and M1 (Vigneswaran et al., 2013), were inhibited (suppression MNs). This mechanism could contribute to suppress unwanted motor output during action observation; however, it is important to note that MNs, despite showing genuine motor properties, do not appear to have a direct motor role. Indeed, F5 sector hosting neurons with mirror properties is the poorly linked with the spinal cord relative to other functionally similar and adjacent PMv regions (e.g. Fogassi et al., 1996; Fogassi et al., 2001; Maranesi et al., 2012), and can influence only indirectly, through corticotectal projections (He et al., 1993) or via its connection with M1 (Kraskov et al., 2011; Schmidlin et al., 2008), motor execution. This indirect motor role, and the presence of neural mechanisms enabling to prevent motor output during action observation (Kraskov et al. 2014), have suggested that the motor properties of MNs may be the result of an efference copy of motor signals that, decoupled from the motor output (via cortical and, possibly, subcortical mechanisms), could make these representations available for perceptual, cognitive, and even social functions (Bonini, 2017; Bonini et al., 2022).

1.1.3 Space dependent representation of objects and actions

Since the pioneering studies on the frontal motor cortex of non-human primates it was observed that, quite surprisingly, a robust deficit following ablation of post-arcuate cortex was a neglect of the contralateral space, which could improve with time after the lesion but always leaving clear extinction for contralaterally-presented stimuli (Rizzolatti et al., 1983). Thus, the premotor organization of actions is strictly space-constrained, and the capacity to process and attend stimuli in the surrounding, operative space, appears to depend on the possibility to plan actions directed to them.

From a phenomenological point of view, our surrounding space is perceived as unitary, but experimental evidence suggests that the brain generates multiple representations of space, with specific cortical regions mostly contributed to the processing of peripersonal space (PPS) - which directly surrounds us and on which we can act - and other contributing to extrapersonal space (EPS) - the space that is far away from us and that cannot be directly acted upon by the body (Cléry et al., 2015).

Several neuropsychological studies showed a double dissociation between PPS and EPS in primates. Rizzolatti and colleagues (1983) carried out a study on the frontal cortex of macaques in which they performed a unilaterally surgical ablation of the post-arcuate cortex (area 6) or – as a control – the FEF (area 8). After the post-arcuate lesion monkeys exhibited a deficit limited to the PPS, that included a failure to grasp food with the mouth when it was presented contralaterally to the lesion, a reluctance to use the contralateral hand and a severe hemi-inattention in both the somatosensory and visual modalities. Even when motor and attentional deficits improved, a preference for the use of the ipsilateral hand was maintained and the phenomenon of the extinction occurred (i.e. when two stimuli were presented, a preference for the more ipsilateral on the injured side was expressed). Conversely, when area 8 was ablated, there was a reduction in eye movements toward the space sector contralateral to the lesion and neglect of the contralateral hemispace, but no somatosensory deficit was found, and the visual neglect was more pronounced for far space than for near space. An analogue dissociation was found in human neurological patients with neglect, who could exhibit a selective deficit for the PPS (e.g., Berti & Frassinetti, 2000; Halligan & Marshall,

1991) or only for EPS (e.g., Cowey et al., 1994). Furthermore, neuroimaging studies confirm the role of a dorsal network in near space coding in humans, which include the left dorsal occipital cortex, the left intraparietal cortex, and the left ventral premotor cortex, as well as the complementary role of a ventral network in far space processing, including the ventral occipital cortex bilaterally and the right medial temporal cortex (Aimola et al., 2013; Weiss et al., 2000).

To investigate space dependent coding of objects and actions at single neuron level, a series of neurophysiological studies have been conducted on the parieto-premotor circuits in the monkey, with a particular attention to area F4. This region contains neurons discharging when a monkey performs a reaching act, with a directional preference and a selectivity for the goal (e.g. they activate themselves if the movement is a reach to grasp, but not if the same movement is made to push away) (Gentilucci et al., 1988). In addiction, F4 contains also neurons with somatosensory, visual and bimodal (or trimodal) properties: somatosensory neurons discharge during tactile stimulation of the face, neck, arms or hands (Rizzolatti et al., 1981); visual neurons respond to stimuli moved toward the monkey and bimodal neurons are activated by both tactile and visual – and often even acoustic – stimuli (Graziano et al., 1999). This latter type of neurons respond when the experimenter touches, for example, the monkey's cheek and also when a tridimensional object is approached toward the same body part (Figure 4) .

The visual response is present only when the stimulus moves within the spatial volume surrounding the tactile receptive field (RF) of the neuron (Fogassi et al., 1996b). Bimodal neurons' visual RFs respond to visual stimuli in somatocentric (but usually not retinocentric) coordinates, because their location is independent from eye position relative to the observed object, hence it appears to be "anchored" to the tactile RFs (Fogassi et al., 1996a; Graziano et al., 1994).

F4 participates in a circuit that transforms extrinsic characteristics of an object, such as its position in space, into the correct motor plan to reach it, thanks to its mutual connection with VIP, an area located in the intraparietal sulcus that contains neurons with similar sensorimotor properties (Matelli and Luppino, 2001; Rizzolatti and Matelli, 2003). This area contains a majority of visual neurons – since it receives information from other visual cortices, such as MT/MST (Ungerleider and Desimone, 1986) – but also somatosensory, bimodal and trimodal neurons, and seems to be

involved in the generation of a multisensory, head-centered representation of nearby space (Colby et al. 1993; Duhamel et al., 1997; Duhamel et al. 1998; Schlack et al., 2005).

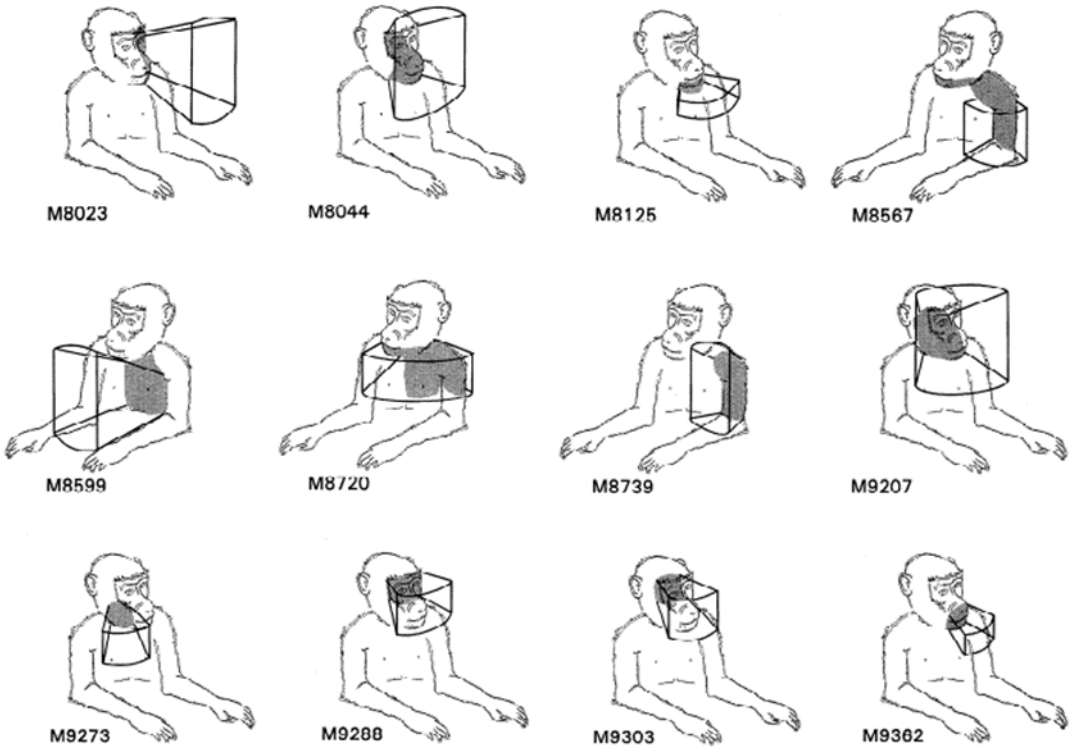


FIGURE 4 | Different types of tactile and visual RFs of F4 bimodal neurons. Shadowed areas: tactile RFs. Solids around different body parts: visual RFs. Neurons M8023-M8I25 and neurons M9207-M9362 were recorded from the left hemisphere; neurons M8567-M8739 were recorded from the right hemisphere. Figure from Fogassi et al. (1996).

There are several factors other than proximity which can affect PPS (see Bufacchi and Iannetti, 2018): the stimulus approach speed (Fogassi et al., 1996) or the use of a tool (Iriki et al., 1996). Furthermore, the influence of social information on PPS processing has been extensively studied in literature (Di Pellegrino & Làdavas, 2015): some neurons, for instance, have been shown to respond to visuo-tactile stimulation not only in one's own PPS but also in the PPS of others.

The influence on the MN response of space sector in which an object target was presented has been investigated by Caggiano and colleagues (2009): they recorded neural activity in area F5 and found MNs with a different modulation depending on the location in space of the observed motor acts relative to the monkey. Half of the recorded neurons encoded observed action depending on the space sector in which it was performed, half showing a preference for PPS, the other half for the EPS. Although some neurons encoded space in metric terms, many seems to reflect an operational

framework, changing their properties depending on whether the monkey was allow to interact with the object or it was prevented from doing it by interposing a transparent plastic barriers that left the metric distance unchanged but made pragmatically inaccessible the target object. Subsequent study that dichotomized in a more radical way the PPS and the EPS showed that MNs have a general preference for the PPS, especially when other's action were observed from a subjective viewpoint (Maranesi et al. 2017). Similar space-constrained coding was reported for graspable object as well: indeed, when presented with an object behind a transparent plastic barrier, most of object-related neurons of the area F5 discharged weakly or not at all. This finding clearly demonstrates that neuronal responses to objects are dependent on the monkey's possibility to interact with them (Bonini et al., 2014), suggesting the physical objects and other's actions could rely on similar sensorimotor coding principles, whose shared role might be to prepare potential motor actions of the observer toward the physical or social targets in the outside world.

1.2 Action execution and observation in the cortico-basal ganglia circuits

The cortical areas forming the mirror circuit do not work in isolation from sub-cortical structures. Anatomical studies have shown that most of the areas constituting crucial nodes of the cortical MN network for hand actions – i.e., area AIP/PFG, PMv, VLPF (Gerbella et al., 2015) and F6 (Albertini et al., 2020) – send convergent projections to specific sectors of the putamen, a nucleus of the basal ganglia (BG) known for its motor properties (Figure 5). Functional evidences in humans have also investigated the possible involvement of BG in MN network: Kessler and others (2006) conducted a MEG study showing that, in an imitation task, BG activation started early during the observation of biological movements, with a behavioral advantage also in the execution; Alegre and colleagues (2010) recorded local field potentials from the subthalamic nucleus (STN) of patients with Parkinson Disease and reported that movement observation was accompanied by changes of the beta oscillatory activity consistent with those observed during movement execution. Finally, fMRI studies found comparable activations in key areas of the MN network and in subcortical regions, included the putamen nucleus, during the observation of hand actions (Ge et al., 2018) and showed significant

shared activation during the execution and observation of object manipulations in bilateral globus pallidus (GP) and left STN (Errante and Fogassi, 2020).

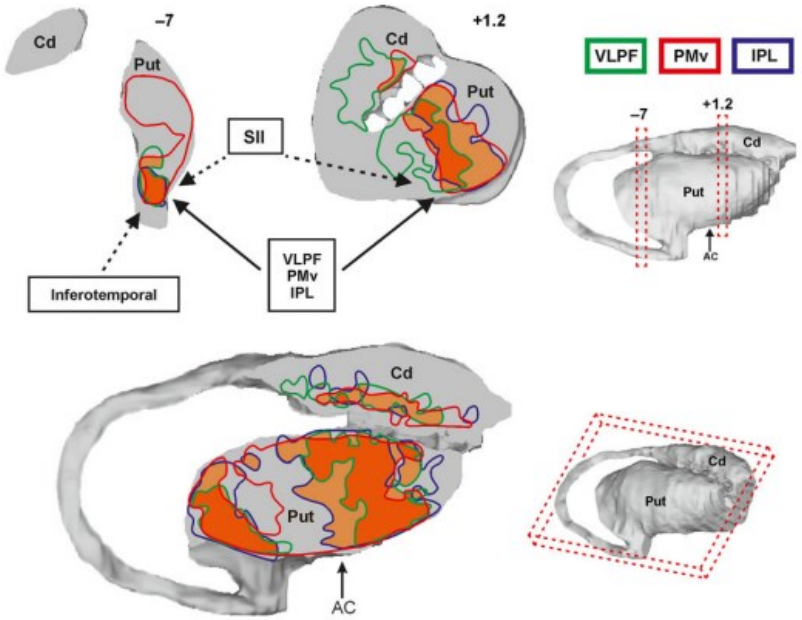


FIGURE 5 | Composite views of the distribution of the striatal focal projections from VLPF (green lines), PMv (red lines), and IPL (blue lines) hand-related areas obtained by warping the focal projections observed in each individual case to template 1-mm-thick coronal and 2-mm-thick oblique sections. The sections were taken at the levels indicated in the 3D reconstructions of the striatum shown in the right part of the figure. Overlap of the focal projections from 3 and 2 regions is shown in darker and lighter orange, respectively. In the upper part, arrows indicate the sources of projections to the 2 input channels. Dashed arrows indicate possible additional source of projections. AC, anterior commissure; Cd, caudate nucleus; IPL, inferior parietal lobule; PMv, ventral premotor cortex; Put, putamen nucleus; SII, VLPF, ventrolateral prefrontal cortex. Figure from Gerbella et al. (2015).

Caligiore et al. (2013) suggested a role for the BG in the action-observation mechanism, arguing that an analysis of their specific contribution could help to understand the neural basis of some important facets of other's action processing –regarding, for example, the influence of the observer's motor experience, the multiple levels at which an observed action can be represented, and the acquisition of action recognition abilities. Furthermore, Bonini (2017) proposed that the BG could contribute to the mirror network by providing a mechanism for decoupling MNs activity from the motor input, i.e., preventing us from the automatic enactment of observed actions. These data addressed the hypothesis of a possible inclusion of the BG in an extended cortico-subcortical MN network, although no study has directly demonstrated the presence of MNs in these subcortical nuclei so far.

1.2.1 Cortical projection to the basal ganglia

The BG complex is a group of interconnected subcortical structures traditionally studied for their involvement in motor control, now also known for their role in a wide variety of functions related to behavior, cognition and emotions (Ward et al., 2013). The term *basal ganglia*, in the strictest sense, refers to a group of nuclei located in the depth of the cerebral hemispheres. These include the striatum, - composed of the caudate nucleus, the putamen and the nucleus accumbens - the globus pallidus, which is in turn subdivided into an internal (GPi) and an external (GPe) segment, the subthalamic nucleus (STN), and the substantia nigra – subdivided in the pars compacta (SNc) and pars reticulata (SNr) (Figure 6) (Lanciego et al., 2012). Such structures can be categorized according to their main connections with cortical and subcortical areas: the striatum is considered as the input station, receiving afferents mainly from cortical, thalamic and nigral sources; GPe, SNc and STN are intrinsic nuclei, with different modulatory functions, that impact on the functioning of relaying the incoming information to the output nuclei, namely the GPi and SNr. The output nuclei project to the ventral nuclei of the thalamus which in turn project back to the cortex (mainly the frontal lobe), forming a cortico – BG – thalamo - cortical loop (Alexander et al., 1986; Parent and Hazrati, 1995). The release of dopamine from the SNc to the striatum is required for proper functioning of the system; indeed, its depletion is associated to symptoms typical of Parkinson's disease (Haber, 2014; Rice et al., 2011).

The striatum is a major site of synaptic plasticity in the BG, receiving excitatory glutamatergic afferents from the cortex and the thalamus as well as extensive innervation from midbrain dopamine neurons (Bolam et al., 2000; Gerdeman et al., 2003; Gerfen, 2000). This region is unique in its complete lack of glutamatergic neurons, instead, the majority of striatal neurons are GABAergic projection neurons (called medium-sized spiny neurons, MSNs) and the others neural types are interneurons that use GABA or acetylcholine as neurotransmitters. The latter modulate the activity of the former, and are aspiny neurons with smooth dendrites. Striatal interneurons can be further classified – primarily on the basis of data obtained from studies on rodents – into four groups depending on their morphological and neurochemical profile (Kawaguchi et al. 1995; Kreitzer and Malenka, 2008; Lanciego et al., 2012; Yelnik, 2002).

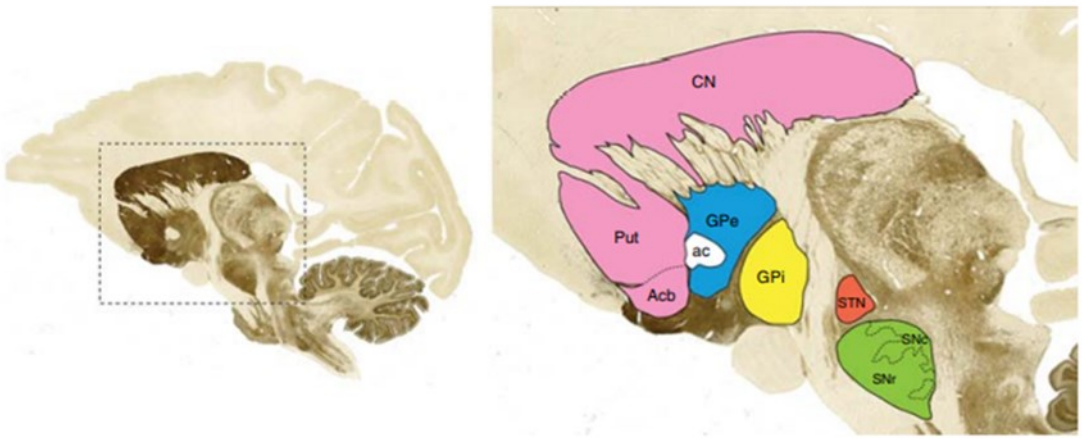


FIGURE 6 | Basal ganglia nuclei. Parasagittal section through the monkey brain (stained with the acetylcholinesterase method) showing the localization and boundaries of all major components of the basal ganglia system. Ac, anterior commissure; Acb, nucleus accumbens; CN, caudate nucelus; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; Put, putamen nucleus; SNc, subtantia nigra pars compacta; SNr, subtantia nigra pars reticulata; STN, subthalamic nucleus. Figure from Lanciego et al. (2012).

There is a class of cholinergic neurons with a large soma – identified in rats (Bolam et al., 1984; Phelps et al., 1985) and in primates (DiFiglia, 1987; DiFiglia and Carey, 1986), which are supposed to correspond to the physiologically defined tonically active neurons, that show a spontaneous pattern of continuous and constant activity (Apicella et al., 1991; Kimura et al., 1984); another class is composed of GABAergic neurons containing parvalbumin, called Fast-Spiking Interneurons for their electrophysiological properties found in rodents (Tepper et al., 2004; Wilson, 2007) and also identified in non human primates based on their spike properties (Yamada et al., 2016); the last two classes include GABAergic neurons, containing calretinin or nitric oxide, called low-threshold spiking neurons (Kawaguchi, 1993). However, the majority of striatal neurons are MSNs, which have high spine density and low spontaneous firing rates, probably corresponding to the so called phasically active neurons in monkeys (Apicella, 2002; Wilson, 1993). This class of neuron were divided into two subcategories based on their axonal targets and expression of peptides and receptors, in murine models: MSNs that innervate the output nuclei (GPi and SNr) express the dopamine receptor subtype 1 (D_1R), the neuropeptides substance P and dinorphine; MSNs that innervate GPe, express the dopamine receptor subtype 2 (D_2R) and the neuropeptide enkephalin (Albin et al., 1989; Cepeda et al., 2008). Although these classifications are based mainly on rodents, recent studies have shown that they are quite generalizable to human and non-human primates (Bernàcer et al., 2012;

Del Rey et al., 2022), with a substantial difference in their quantitative distribution: the population of interneurons appears higher in the primate striatum (6–26%) (Bernàcer et al., 2012; Deng et al., 2010; Graveland and DiFiglia, 1985; Krienen et al., 2020; Roberts et al., 1996), including the human striatum (10–26%) (Bernàcer et al., 2012; Bernàcer et al., 2007; Graveland et al., 1985; Krienen et al., 2020; Oorschot, 2013; Roberts et al., 1996), than in the rodent striatum (3-5 %) (Deng et al., 2010; Graveland and Di Figlia, 1985; Krienen et al., 2020; Oorschot, 2013).

The striatum can be further subdivided into two isolated compartments: the striosomes, irregulars patches showing weak acetylcholinesterase (AChE) activity, immersed within a background of more intense AChE staining, called matrix (Figure 7). The MSNs situated in the striosomes project preferentially to the SNr and receive from limibic and prelimbic cortical areas, basolateral amygdala and ventral part of the SNc, whereas the striatofugal neurons situated in the matrix innervate the GPi, the GPe, the SNr and receive from motor and sensory cortical areas (with a topographic organization), from the thalamus and from dopaminergic neurons of the SNc (Fujiyama et al. 2011; Graybiel and Ragsdale, 1978; Prensa et al., 1999; Gimenez-Amaya and Graybiel , 1990).

Furthermore, the striatum receives input from the entire cerebral cortex, and from several subcortical structures such as the thalamus, the substantia nigra, the amygdaloid complex and the dorsal rafe nucleus (Lanciego et al., 2012). Both ipsilateral and contralateral cortex send to the striatum glutamatergic projections that make synapses with the spines of the MSNs: cortical limbic areas innervating the striosomal compartment (Gerfen 1984), and bulk of corticostriatal projections spread through the matrix compartment with topographically organized projections (Selemon and Goldman-Rakic, 1985). The thalamus also provides a glutamatergic input projecting to all types of striatal neurons, with synapses on both spines and dendritic shafts (Powell and Cowan, 1956; Raju et al., 2006; Sadikot et al., 1990). The nigrostriatal system originates from the SNc and represents the main source of dopaminergic innervation, to which are also added the retrorubral field and the ventral tegmental area. Dopaminergic neurons of the ventral part of SNc enter the SNr and give rise to a topographically organized nigrostriatal projection, that is much denser in the matrix than in

the striosome and exerts a facilitatory effect on the D_1R neurons and an inhibitory effect on the D_2R neurons (Haber et al. 2000; Lynd-Balta and Haber 1994; Lanciego et al., 2012).

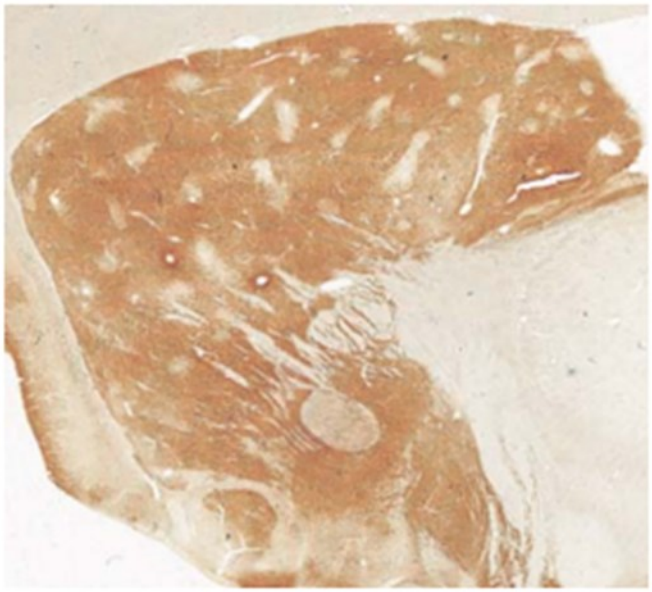


FIGURE 7 | Striatal compartments. Although the striatum appears as a rather homogeneous structure, several histochemical and immunohistochemical stains evidence the presence of two compartments named striosomes and matrix. The photomicrograph is taken from a parasagittal section through the primate striatum. The immunohistochemical detection of the calcium-binding protein calbindin reveals the presence of a number of patchy areas with weak calbindin stain (striosomes) immersed within a background showing higher calbindin stain (matrix). Figure from Lanciego et al. (2012).

The nigrostriatal pathway is characterized by a very high axonal arborization: in primates a single dopaminergic neuron makes synapses with about 1 million striatal neurons, from which it follows that dopamine exerts a great modulatory effect through this projection. Finally, the amygdaloid complex and the rafe nucleus provide to the striatum glutamatergic (Ragsdale and Graybiel, 1988) and serotoninergic projections, respectively (Andén et al., 1966; Bobillier et al., 1976; Szabo, 1980).

As for the intrinsic nuclei, the GPe contains large and sparse GABAergic neurons and is immunopositive to parvalbumin (all these properties are shared with the GPi, with which it is reciprocally connected), it receives GABAergic afferences from the D_2R striatal neurons and it is also connected with the STN from which it receives glutammatergic projections. The STN, located rostrally to the SNc, receive GABAaergic afferences from the GPe, glutammatergic afferences from the cortex (F1, premotor cortices, frontal eye fields) (Nambu et al., 2002; Nambu et al., 2004) and from the thalamus, and dopaminergic input from the SNc (part of the nigro-extrastriatal projection system) (Rommelfanger and Wichmann 2010). Finally, the SNc provides dopaminergic input to BG along with areas A8 and A10 (Dahlstrom and Fuxe, 1964). The degeneration of these neurons and the resulting dopamine deficiency is characteristic of Parkinson's disease (Lanciego et al., 2012).

The output nuclei, (GPi and SNr) are both nuclei composed of GABAergic neurons with a high firing rate, which discharge tonically to inhibit their targets. They receive two types of input: a GABAergic input from the striatal D_1R neurons, and a glutamatergic input from the STN, and then project to the thalamus and to the brainstem (Lanciego et al., 2012).

It is possible to distinguish two main routes in the BG circuitry, on the basis of the type of striatofugal projection: the direct pathway, which connects the striatum directly to the output nuclei, and the indirect pathway, which links the striatum to output nuclei via intrinsic nuclei. Cortical activation results in a glutamate release to striatal MSNs, which project directly to the output nuclei: since MSNs are GABAergic cells, they have an inhibitory effect on the GABAergic neurons in the SNr and GPe, causing a pause in their tonic pattern of firing. Once the SNr is inhibited, the thalamic glutamatergic neurons—which receive SNr input and project to the cortex—are also disinhibited. The cascade of events along the direct pathway facilitates action onset. In contrast, the activation of striato-pallidal MSNs, which project indirectly to the output nuclei via the intrinsic nuclei, inhibits the GPe's GABAergic neurons, resulting in disinhibition of the STN's glutamatergic neurons and, in turn, in an increased discharge of SNr GABAergic neurons projecting to the thalamus, thereby increasing motor inhibition (Albin et al., 1989; De Long, 1990). In addition to these two pathways, there is a hyperdirect pathway in which frontal afferents directly facilitate STN neurons causing an increased activation of the GPi and hence enhanced inhibition of the motor plans (Figure 8) (Nambu et al. 1996, 2002a).

1.2.1.1 The direct and the indirect pathways: classical model and current concepts

The direct and indirect pathways have opposing functional effects on BG output: the former promotes initiation and execution of body movements by disinhibiting the appropriate motor networks, while the latter is likely engaged in the suppression of competing behavioral patterns. Comparisons between the cyclostome and mammalian BG intrinsic organization show consistent similarities, indicating that it appears to be a successful arrangement that has remained relatively unchanged during evolution (Grillner and Robertson, 2016).

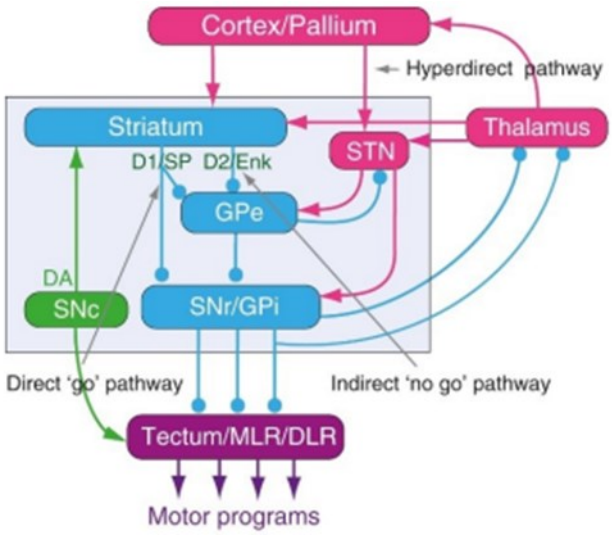


FIGURE 8 | Schematic representation of the BG main pathways. Excitatory glutamatergic neurons are represented in red and GABAergic structures in blue color. The dopamine input from the SNc to striatum and brainstem centers is represented in green color. Figure from Grillner & Robinson (2016).

MSNs of the direct and indirect pathways are distinguished by the differential expression of dopamine receptors in addition to their distinct projections: D_1 receptors are expressed by MSNs belonging to the direct pathway, whereas D_2 receptors are expressed by MSNs of the indirect pathway (Gerfen and Surmeier, 2011; Gerfen et al., 1990). According to the BG's canonical functional model, they work as a "go through" station in the motor loop, receiving cortical signals, modulating them, and then sends out to the cortex to either promote or inhibit a movement. Historically, two opposing views have been proposed regarding how signals from various areas are managed (Nambu, 2011). The parallel processing hypothesis (Alexander and Crutcher, 1990; Hoover and Strick, 1993) states that afferent inputs from different sources are kept separate and processed independently in different sites of the BG, whereas the information convergence hypothesis (Chevalier and Deniau, 1990) states that different signals are "funneled" and integrated in the same site. A more recent perspective, influenced by the emergence of new empirical data, suggests that both theories can be true to some extent: cortical influences originating in regions with similar functions tend to converge, as opposed to those originating in areas that are not functionally related.

GB-cortical circuits are not limited to the selection and control of motor planes, but also include the limbic and associative cortices: based on the relationship with different projection areas

and the involvement of these regions, it is possible to identify different domains – sensorimotor, associative, limbic – that originates in the cortex, runs via a region of the BG, and returns back via the thalamus to form distinct multiple re-entrant loops that interact with different parts of the brain, including the areas of the MN network (Figure 9) (Alexander et al., 1986; Humphries and Prescott, 2010; Middleton and Strick, 2000; Romanelli et al., 2005).

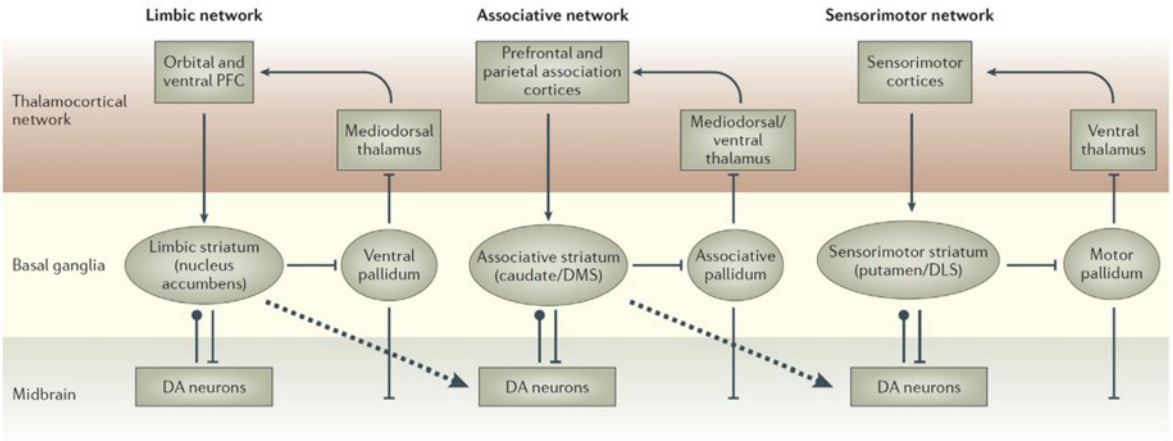


FIGURE 9 | The three main cortico-striatal loop. Standard arrows indicate excitatory glutamate connections. Flat arrowheads indicate inhibitory GABA connections. Dot arrowheads indicate dopaminergic connections whereas dashed arrows indicate cross-loop connections. Figure from Yin and Knowlton (2006).

Concerning the sensorimotor loop, it connects putamen with F1, premotor cortices, and SMA (Romanelli et al., 2005; Tang et al., 2007) and is clearly implicated in the selection of final sensorimotor mappings based on the current context (Alexander et al., 1986; Romanelli et al., 2005; Yin and Knowlton, 2006).

Bonini (2017) supports the idea that cortico-BG-thalamo-cortical loops play an important role in the MN-network, preventing the automatic executions of unwanted movements during action observation, by a mechanism for decoupling MNs activity from the motor input. According to this hypothesis, ITNs of primary and premotor cortex neurons with mirror properties, through their preferentially facilitatory connections with striatal neurons of the indirect pathway and/or with STN neurons of the hyperdirect pathway, would promote a reduction of thalamo-cortical activity contributing to suppressing unwanted motor output during observation of others' action. Recent studies investigating the BG involvement in action-observation processes in human subjects

provided some indirect evidence in support of this hypothesis (Alegre et al., 2010; Fogassi and Errante, 2020; Ge et al., 2018; Kessler et al., 2006). Furthermore, as shown from comparative studies on songbirds, the avian homologous of the BG (area X) is innervated by a subpopulation of projection neurons situated in the telencephalic nucleus HVC, which is known to be crucial for normal song perception and learning and host a highly precise audio-vocal mirroring mechanism (Prather et al., 2008; Mooney, 2014). These findings support the theory that putaminal cells and corticostriatal neurons may play an important role in the exploiting observed action for social learning and interaction (Bonini, 2017; Bonini et al., 2022).

1.2.2 The putamen nucleus: anatomical and functional organization

In primates, the putamen nucleus is the main input station of the "motor loop" and plays a major role in mediating the motor functions of the BG. It receives projections from sensory and motor cortices (Künzle 1977; Jones et al. 1977), particularly the premotor areas (Künzle 1978). Despite its main motor role, this nucleus can be subdivided into different territories: dorsal, ventral and rostral putamen, which are implicated in the motor, limbic and associative loops depending on the areas from which they receive. Each putaminal sector mostly sends back to the same cortical areas from which it receives, via the GPi or the SNr, through the thalamus (Alexander et al., 1990; Parent and Hazrati, 1995).

The macaque putamen is somatotopically organized (figure 10): neurons related to movements of body parts are segregated and each body part is represented over a large anteroposterior extent of the nucleus. Leg neurons are located in the dorsolateral putamen, orofacial neurons in its ventromedial portion, whereas arm-related neurons are located between the leg and orofacial areas (Crutcher and DeLong, 1984a; Liles 1979). Alexander and DeLong (1985) discovered striatal microexcitable zones (SMZs), which are discrete regions of the putamen whose microstimulation elicited the movement of a single body part.

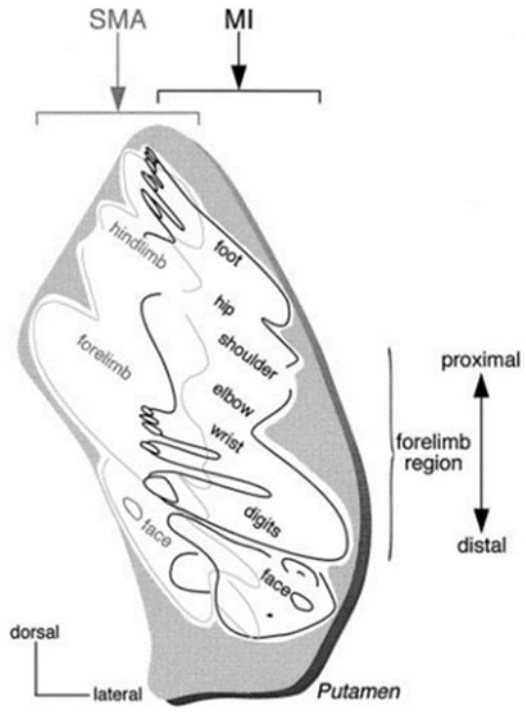


FIGURE 10 | Somatotopic organization of the putamen according to cortical motor inputs from the MI and SMA. Drawing in black lines represent somatotopically arranged cortical inputs from the MI, whereas those in gray lines roughly represent somatotopic inputs from the SMA. Inputs from both the MI and SMA converge onto the mediolateral central part of the Put. There is a distal-proximal somatotopic organization in the MI- and MI+SMA-recipient forelimb regions. Figure from Nambu et al. (2002).

Evoked motor responses appeared to be consistently contralateral to the striatal stimulation site, except for axial and orofacial movements, which appeared to be bilateral. Furthermore, in a tracer study striatal projections from three subregions of the forelimb representation area of F1 were examined: the distal and proximal subregions in the anterior bank of the central sulcus (distal and proximal-bank subregions) and the proximal subregion in the surface of the precentral gyrus (proximal-surface subregion). It was found that the distribution areas of fibers from the distal, proximal-bank and proximal-surface subregions were located from ventrolateral to dorsomedial putamen and were largely segregated from one another (Tokuno et al., 1999). Several studies also reported that laterally and medially located neurons firing differently in relation to distinct aspects of motor behavior: simple movement activate lateral portions of the putamen, while complex movements are related to activity in medial ones, which could be due to different cortical inputs of these regions (F1 and SMA, respectively) (Alexander and Crutcher 1990a,b; Liles, 1983, Nambu, 2002). Alexander and Crutcher (1990b), also showed that putamen neurons manifesting preparatory activity, were located more rostrally and medially than those showing movement-related activity only. These differences could be ascribed to distinct cortical motor inputs since neurons showing preparatory activity are most prominent in the SMA than in the F1.

From a connectional point of view, neuroanatomical studies using intracortical microstimulation and anterograde tracing injections in the frontal cortices have shown that corticostriatal input zones from hindlimb, forelimb, and orofacial representations of M1 and SMA were arranged from dorsal to ventral within the putamen, but "hotspots" of dense labeling from M1 were located predominantly in the lateral putamen, whereas those from the SMA were in its medial section. On the other hand, corticostriatal afferents from forelimb representations areas of the premotor cortex were distributed mainly in the dorsomedial aspect of the putamen. In addition, the corticostriatal input zones from M1 and SMA partly overlapped in the mediolateral central sector of the putamen, while the corticostriatal input zone from the premotor cortex largely overlapped that from the SMA, but not from the M1 (Takada et al., 1998).

Nambu and colleagues (2002b) chronically implanted stimulating electrodes in the distal and proximal parts of the forelimb representation of monkeys F1 and SMA. They found neuron responding exclusively to F1 stimulation (F1-recipient zone) and those responding exclusively to SMA stimulation (SMA-recipient zone) distributed predominantly in the ventrolateral and dorsomedial portion of the caudal putamen, respectively. They found also neurons responding to stimulation in both F1 and SMA, located in the intermediate sector between the M1 and SMA-recipient zones (F1+SMA recipient zone). Movements of the forelimb were readily elicited by microstimulation in the MI-recipient zone, less frequently in the F1 + SMA-recipient zone, and rarely in the SMA-recipient zone. The activity pattern of F1 + SMA-recipient neurons was of an intermediate type. Miyachi and colleagues (2006) injected retrograde trans-synaptic rabies virus into hindlimb, proximal and distal forelimb, and orofacial representations of macaque F1 and observed two distinct set of labeling in the striatum: one in the dorsal putamen, and the other in the ventral striatum (ventromedial putamen and nucleus accumbens). The labeling of the dorsal striatum was somatotopically organized, with a distribution pattern that corresponded to that of the corticostriatal inputs, whereas the distribution pattern of the ventral striatal labeling was essentially the same in all cases, suggesting that the cortico-basal ganglia motor loop involving the F1 and the putamen, appears as a somatotopically organized closed loop, while the ventral ("limbic") striatum provides divergent multisynaptic inputs to the entire F1. The motor territory of the putamen also receives a

somatotopically organized input from the motor thalamus and centromedian and parafascicular nuclei, which are reciprocally connected with motor cortices (McFarland and Haber, 2000).

Concerning the functional properties of the putamen, data from the existing literature report that putamen neurons change their activity in relation to movements of the limbs and other body parts (Crutcher and DeLong 1984a), and a series of studies has shown that neurons in this nucleus have patterns of activity that are closely related to various parameters of active movements of the arm, such as direction (Crutcher and DeLong 1984b), velocity (DeLong and Strict, 1974), and amplitude (Georgopoulos et al., 1986) of movement, as well as the force (Liles, 1983) exerted during muscle contraction. Crutcher and DeLong (1984b) noted that a vast majority of the neural responses in the putamen nucleus occurs after the first EMG change, according with the view that the basal ganglia are more involved in the execution or facilitation of movement than in the timing of its initiation (Anderson and Horak 1981; Aldridge et al. 1980; Hallett and Khoshbin 1980). Graziano and Gross (1993) found bimodal neurons in monkey putamen face and arm representation areas, which responded to both somatosensory (light cutaneous stimulation, joint movement or deep muscle pressure) and visual (objects near the tactile receptive field, without any type of selectivity for shape or color) stimuli; they found also purely visual or purely somatosensory neurons. The putamen nucleus may also play a role in oculomotor control, how suggested by a single neuron recoding study that found a subset of neurons, in the caudal part of the nucleus, with saccade-related activity (Phillips and Everling, 2012).

1.3 Aims of the study

Data from the literature have provided compelling evidence in support of a major involvement of cortical motor areas in a broad range of advanced perceptual, cognitive, and even social functions, suggesting that the motor function should be considered as a largescale domain, shaped around the concept of potential interactions – with the surrounding environment, objects, and other agents – and provided with blurred boundaries, extending well beyond the mere movement execution. It is also known that the cortical motor system, including high-order premotor regions, has a tight anatomo-

functional connectivity with the subcortical complex of the BG, which appears to play a key modulatory role on the activity of such regions. Although the cortico-BG network is clearly required for motor control, as made evident by its clinical dysfunctions, possible contributions of the BG to the control of manual actions and motor-based socio-cognitive and perceptual functions in primates have been poorly investigated.

The present study aims at exploring the functional properties of the putamen by means of single cell recording during a mutual action task specifically designed to study neuronal responses during (1) the visual presentation of objects, (2) first-person execution of grasping actions, and (3) the observation of the same actions performed by another individual in a shared (or not shared) operational space.

This approach could shed light on the neuronal mechanisms underlying the involvement of the putamen in the representation of objects' affordances, PPS, and the monitoring of others' behavior – probing the hypothesis that action selection and inhibition is supported by the BG system in individual as well as social contexts.

2. MATERIALS AND METHODS

2.1 Experimental subjects

This study involved one purpose-bred, socially housed, adult male monkey (Macaca mulatta, 9 kg). All experimental procedures were conducted in agreement with the European (Directive 2010/63/EU) and Italian (D.lgs 26/2014) legislation for the protection of animals used for scientific purposes, received the approval of the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 52/OPBA/2018), and were authorized by the Italian Ministry of Health (Aut. Min. 802/2018-PR).

Training procedures were carried out by means of operant conditioning with positive reinforcement and step-by-step response shaping methods. Liquid reward (fruit juice or water) was used to reinforce specific behavioral responses matching or resembling the target one, while unwanted behaviors were gradually extinguished by the lack of positive outcomes. At the end of each session, fresh fruit pieces were administered to strengthen collaboration and make of the session an as much as possible positive experience for the animal. Training sessions were conducted on a daily basis, in order to ensure continuity and help the animals to get accustomed to a predictable routine.

The monkeys were initially habituated to enter and sit in a primate chair, that was then transported from the animal enclosure to the laboratory, and to interact and cooperate with the experimenters. Afterwards, they were specifically trained on the main experimental task whose description will be provided in the following section. The task was subdivided into easier steps, each chained to the previous one only after complete acquisition of both of them, until the animal was able to master the whole task.

In preparation for neural acquisitions, the monkeys underwent a surgical procedure for the implantation of a recording chamber, through which recording probes could be inserted during subsequent surgeries. Every surgery was performed in stereotaxic and aseptic conditions, under general anaesthesia induced by intramuscular injection of ketamine (5 mg/kg) and medetomidine hydrochloride (0.05 mg/kg) and maintained with 2% isofluorane vaporized in 100% oxygen. The monkey's vital parameters were constantly controlled with a multiparametric monitor. Hydration of

the animal was guaranteed with continuous 29 intravenous infusion of saline solution, and vitamin A gel was used to ensure eye hydration during anaesthesia. The monkey received analgesics, broad-spectrum antibiotics, and anti-inflammatory medicines both during and after surgical procedures.

2.2 Behavioral paradigm and recordings

The monkeys were progressively trained to perform the Mutual Action Task (MAT), whose behavioral paradigm was designed to enable investigation of neuronal properties during action execution and observation, as well as during visual encoding of objects presented in the animal's PPS.

At the beginning of the task, the monkey sat in its primate chair at one end of a table, just in front of the experimenter, who was seated on the opposite side and acted as a partner. They faced a shared operational space containing a multi-affordance device that could be reached and manipulated by both subjects (Figure 11A, B).

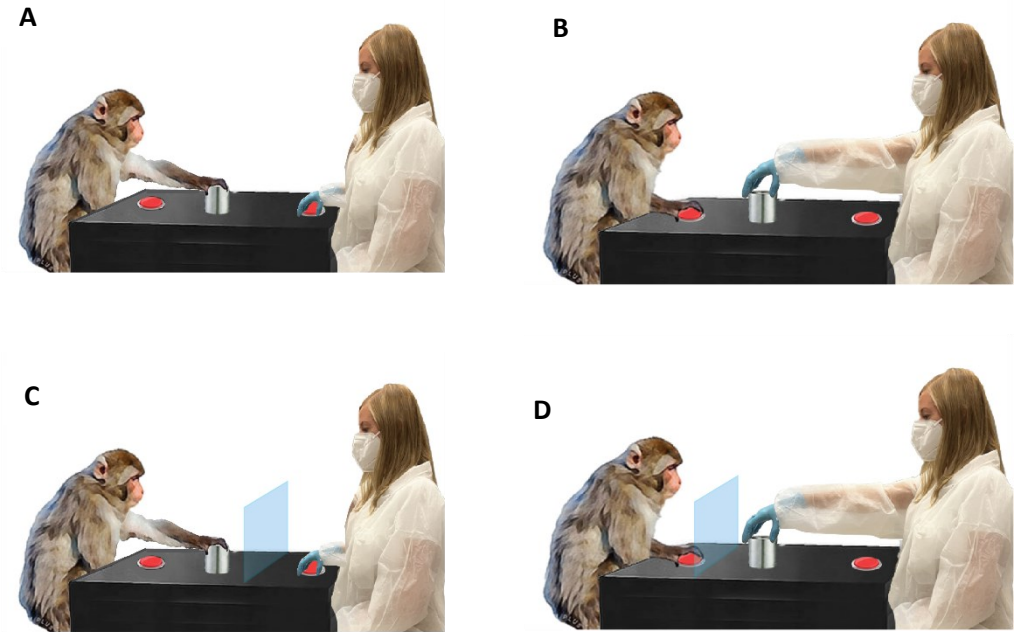


FIGURE 11 | Schematic drawing of setup configuration for the behavioral task. A, Social condition (Execution); B, Social condition (Observation); C, Barrier condition (Execution); D, Barrier Condition (Observation).

The object, which was located at a distance of 16 cm from each agent's hand starting point, was 3D-printed in a polylactic acid (PLA) material and was formed by a cylindric body (diameter 5.5 cm, height 6 cm) on top of which a parallelepiped (4.5x1.5x1.5 cm) was inserted. It afforded two different types of prehension, either a precision grip (PG), by using the thumb and index finger opposed to the central parallelepiped, or a whole hand prehension (WH), when the whole hand was wrapped around the main cylinder's body. The kind of grip required in each trial was specified by a visual cue that appeared on a rectangular oled screen (2x1 cm) inserted on the top side of the parallelepiped: an empty square cued the WH, whereas a full white square cued the PG. Both solids composing the object were surrounded by metallic plates, so that each grip resulted in closing of a capacitive circuit that triggered a TTL signal to be recorded and stored together with the other behavioral and task events (Figure 12).

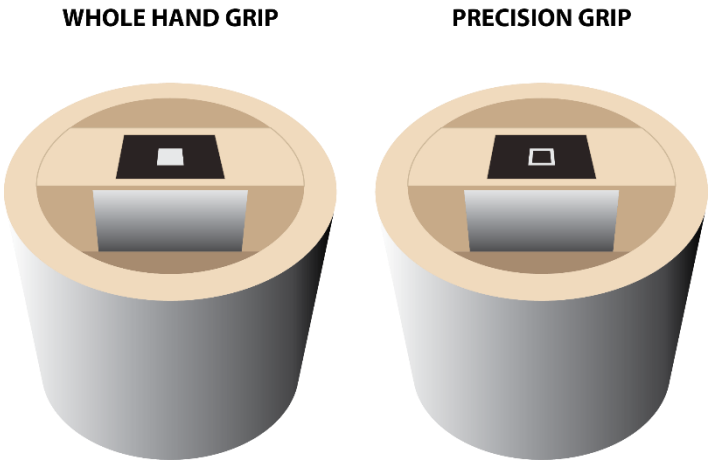


FIGURE 12 | Object description. On the left, oled showing an empty square (precision grip cue). On the right, oled with a full square (whole hand prehension cue).

Each trial (Figure 13) started in complete darkness, with both subjects seated still in the starting position, pressing a manipulandum with the right (dominant) hand. After 1 second, either a high (1200 hz) or a low (300 hz) pure sine wave tone was presented (Sound onset), instructing each subject in an opposite way (Go vs. No-Go): specifically, the high tone instructed the monkey to Go and the experimenter to remain still, whereas the low tone instructed the monkey to remain still while the experimenter was performing the action (Agent Condition). After 770 ms from the sound onset, the oled screen was turned on (Oled onset), showing either the empty or the full square – i.e., the visual cue related to the PG or the WH, respectively. Next, 730 ms after Oled onset, an environmental

light was turned on making the whole object visible (Light on). After 570 ms, the sound ceased (Go/No-go signal) and the subject that received the Go signal had to release the manipulandum to reach the object, grasp it with the specified prehension type within 1 s (Grip Condition), and hold it up for at least 500 ms (Up-keep) to get the reward.

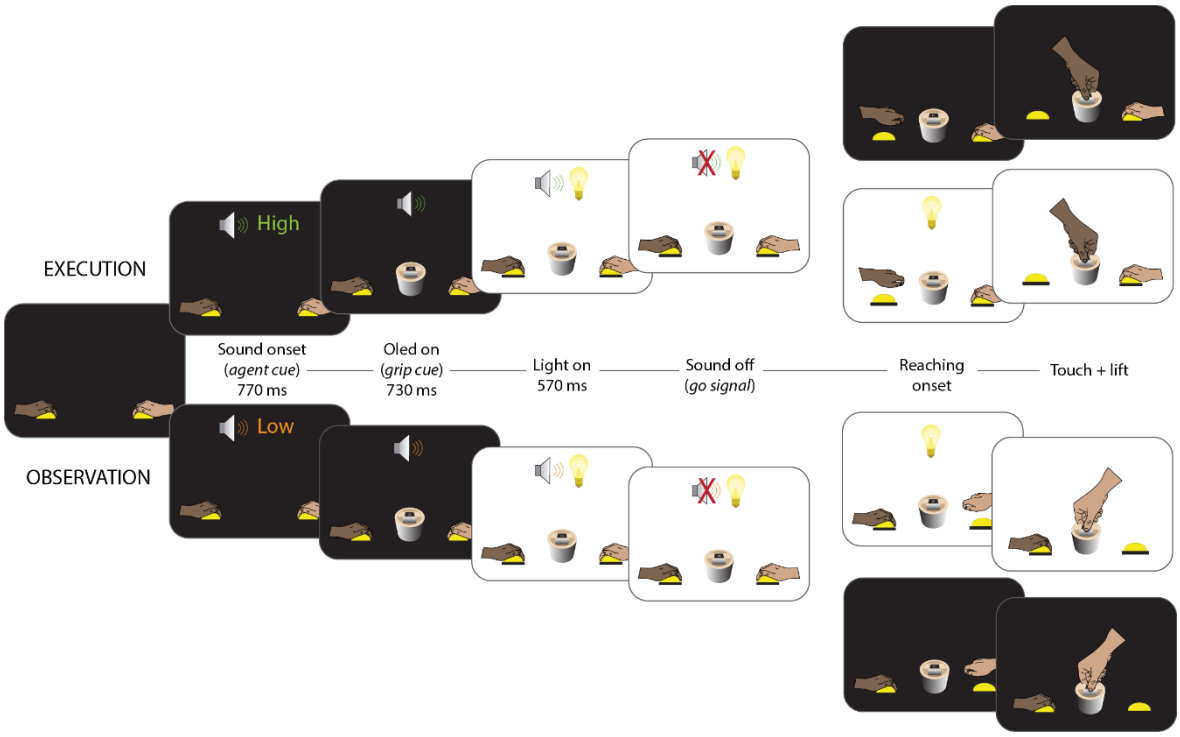


FIGURE 13 | Temporal sequence of events (Social Condition).

Whenever the monkey managed to successfully perform every step of the sequence – including remaining still during Go trials of the partner –, liquid reward was automatically administered in a fixed amount, otherwise the trial was aborted. Each experimental trial was considered correct only if both agents succeeded. Possible errors leading to exclusion of the trial from analyses included starting before sound end, failing in discriminating the auditory cue – i.e., leaving the manipulandum during the other's trials, or staying still during one's own –, performing the wrong type of grip, and not holding the object up enough.

In half of the trials the light remained on until the completion of the task (Light Condition), whereas in the remaining half it was turned off as soon as the manipulandum was released, requiring the subject to execute the action in the dark (Dark Condition): these conditions (Visual feedback) ensured that potential motor responses recorded from putamen neurons could be considered as

actually motor, and did not depend on visual feedback from the moving hand. We collected 15 trials for each of the 8 conditions (2 grip types x 2 agents x 2 visual feedback), for a total of 120 correct trials per session.

The task was also carried out with an additional condition (Barrier Condition) in which the temporal sequence of events remained the same, but all trials were performed in full light, and a transparent sliding barrier was interposed between one of the subjects and the target object, thus preventing potential interactions with the target of one's own or the other's action (figure 11C, D).

Detection of behavioral events was allowed by distinct contact sensitive devices signaling hand-target interaction – object touch (specific for prehension type), object lifting, and positioning of the barriers – with the generation of TTL signals that were fed to a PC equipped with a dedicated LabView-based software. The software was used to monitor the subjects' performance but also to control digital output signals associated with the auditory and visual cues, lighting settings, and reward administration – or withdrawal, in case of errors committed by one or both subjects during the trial. All input and output signals were recorded and stored in parallel with the neural data and synchronized to them, and were subsequently used to align the neural signal for statistical analysis.

2.3 Neural data acquisitions

To be able to acquire neural responses, the monkeys was implanted with a biocompatible plastic recording chamber (45x50x25 mm; Figure 14) housing on its top a removable lid to protect a batch of parallel grooves (width 2 mm, inter-groove distance 1 mm) that served as slots for up to 8 connectors blocks (Omnetics Connector Corporation). The connectors interfaced the multielectrode contacts with the headstages, in turn linked with the neural acquisition system.

The chamber was cut and shaped on a 3D reconstruction of the animal's cranial theca realized with the 3D Slicer software, starting from 7T magnetic resonance images previously acquired. After complete recovery from the positioning of the chamber, secured to the skull with titanium bone screws and dental cement, briefer surgeries were performed to implant individual, linear multielectrode probes through a small craniotomy performed within the 34 chamber, leaving intact most of the remaining bone. Collagen-based dural regeneration matrix (DuraGen) was placed around

the insertion sites, and liquid dental cement was poured in the recording chamber; it rapidly solidified securing the probes and preventing contamination.

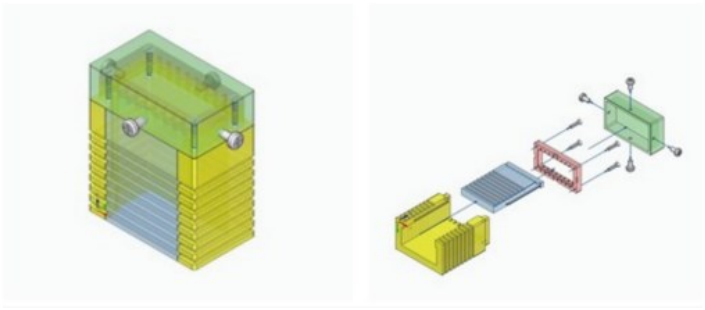


FIGURE 14 | Design of the recording chamber. On the left, chamber assembly. On the right, chamber exploded view. Yellow: main body. Blue: sliding panel. Pink: constituent with grooves. Green: removable lid.

Neural recordings were performed by means of individual, chronically implanted linear silicon probes (ATLAS Neuroengineering; Figure 15) endowed with 32 Iridium Oxide (IrOx) recording sites arranged along the shaft (Barz et al., 2017) with an intersite distance of 250 μm. Each probe had a total length of 24 mm, it was 30 μm large and 100 μm thick, and electrodes had an average impedance ranging from 0,23 to 0,29 MΩ. All probes were equipped with the pointy tip feature, which significantly reduces any tissue dimpling during probe insertion, thereby facilitating the penetration. The shaft was attached to a highly flexible polyimide-based ribbon cable with a zero-insertion-force (ZIF) connector that could be electrically connected to the recording data logging devices.

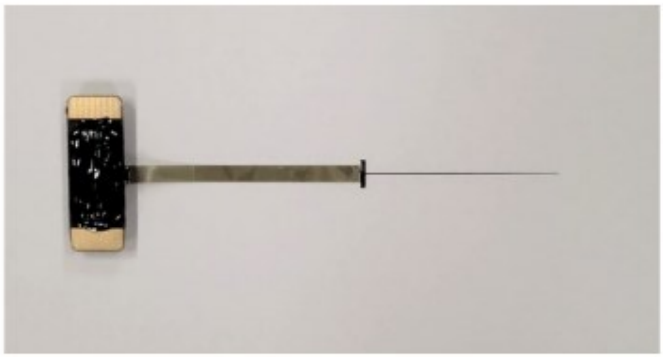


FIGURE 15 | Linear silicon probe. From the left: connector, ribbon cable, recording shaft.

Five different linear probes were implanted, using a dedicated insertion device, in two different surgeries, performed two months away from each other. The two probes that were implanted first were removed during the second operation.

The sites of insertion were established in terms of stereotaxic coordinates of the region of interest obtained on the basis of the MRI images. Penetration angle was set to 90° (vertical), considering the deviation in the probe trajectory while passing through the tissue because of the pointy tip angle and calculated following in vitro experiments with agarose, a viable simulation of the brain tissue mechanic features because of its poroelasticity properties (Pomfret et al., 2013). These tests allowed the detection of a probe deflection of 0.05-0.12 mm per millimeter (which translates into 1.2-2.9 mm of systematic deviation along 24 mm probe length), always in the opposite direction with respect to the tip's beveling, that was accordingly considered during the surgeries.

Neural signals were recorded using a small, lightweight neural logger (Deuteron Technologies Ltd) that allows the acquisition of neural data from freely-moving animals. In this experiment, although the monkey's movement was limited by the primate chair, the head was unrestrained and all upper body movements were allowed. Specifically, for the first implant (64 channels) a RatLog64 (figure 16) was used, whereas a RatLog128 was employed for the second implant (96 channel). The device is powered by a small external battery (two hours maximum duration) to which it is connected via a short cable, and it is equipped with a magnetic on/off switch to ensure the possibility of prompt response in the event of system crash or malfunctioning during a recording session.

Once the logger device was linked to the electrode arrays, all the components were sealed with a cover on top of the recording chamber. A digital bandpass filter with upper and lower cut-off frequencies set at 2 and 7000 Hz, respectively, and a conversion rate of 32000 Hz on each channel was applied, allowing us to sample both local field potentials (LFPs) and single/multi-unit activity. Signals were amplified, digitized, and locally stored in a MicroSD memory card (64 GB) to prevent possible transmission errors. The logger communicated with a computer through a transceiver with 4 BNC connectors for digital inputs and one for digital outputs, which was connected to the host computer via USB.

FIGURE 16 | Deuteron neural logger (RatLog64).

2.4 Spike sorting and neural data analysis

All formal signal analyses were performed offline with a fully automated sorting software, Mountainsort (Chung et al., 2017), setting -3.0 standard deviations of the signal-to-noise ratio of each channel as a threshold for detecting units. The main criterion used to distinguish between single- (SUA) from multi-unit activity (MUA) was the noise overlap, which represents the fraction of points overlapping with the noise cluster, i.e., estimates the fraction of ‘‘noise events’’ (above-threshold events not associated with well-isolated clusters) in a waveform cluster. In most of the recording sessions, the noise overlap distribution is bimodal, with putative single units associated with values below 0.15 and putative multi-units with values above 0.3. Thus, we considered as well-isolated single units those with noise overlap values lower than 0.15. We excluded from analyses all possible artifacts (i.e., units with very noisy waveforms), but not units having a very low firing rate/number of spikes, since our goal was to provide an as much as possible unbiased and inclusive overview of the neuronal behavior of an area whose functional properties are mostly unknown yet, rather than investigating the responses of a specific functional category of neurons.

To avoid oversampling, units that were consistently found in the same channel among different recording sessions were only considered once for further analyses, except when they displayed a significantly different activity pattern – in terms of spike shape, ISI, and response properties.

We first computed the baseline firing rate for each individual neuron, corresponding to an interval of 500 ms preceding 100 ms the cue-sound presentation (Sound On). We then computed the net normalized activity of each unit: its baseline activity in a given condition was subtracted from the firing rate of each bin, and the resulting net activity vector was soft-normalized dividing each data point by the absolute maximum across all conditions + 5 spk/s; this latter constant factor was used to reduce more the overall net normalized activity of neurons with lower firing rate. Net normalized activity obtained in this way thus ranged (theoretically) between -1 and 1 and was used to produce heatmaps showing individual neurons' firing rate in a comparable form during task-unfolding periods.

Since the task was conceived in order to enable separate assessment of sensory and motor responses – both related to the planning and execution of the monkey's own action and to the observation of the experimenter's – for all analyses neuronal activity was aligned to a specific task-related or behavioral event and only evaluated within a precise time interval around it. The selected period for the sensory epoch included the 2.1 s preceding the Go signal (sound off), whereas the one used to assess motor responses started with movement onset (release of the manipulandum) and ended after 750 ms, in order to exclude the reward-related responses (Figure 17).

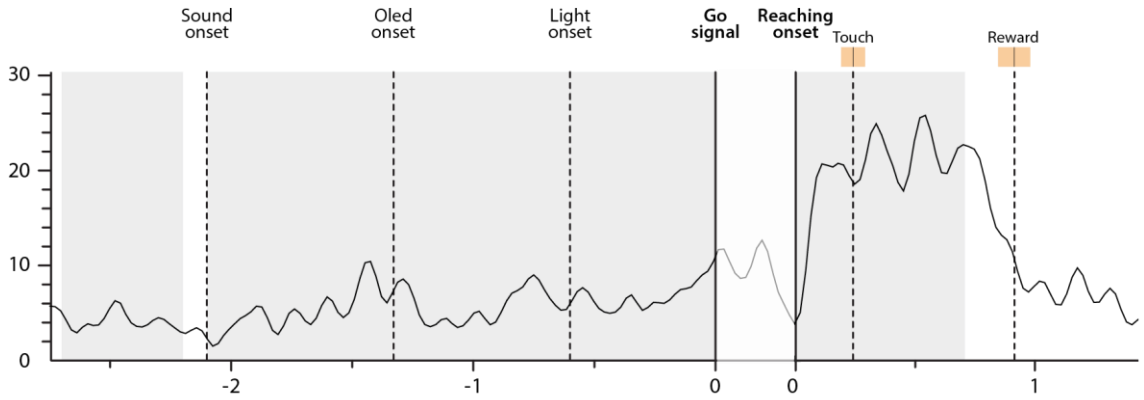


FIGURE 17 | Reference periods used to analyze task-related neural activity. Baseline: 500 ms interval preceding 100 ms the Sound onset; Sensory Epoch: 2.1 s interval corresponding to the sound duration, aligned to the Sound off; Motor Epoch: 750 ms interval aligned to the Reaching onset, including the object touch, but excluding the reward (Yellow marks represents averages and standard deviations of touch and reward).

In this work, all the analysis, have been performed by merging PG and WH trials, regardless of possible grip selectivity; the four experimental conditions here considered where Social-Execution, Social-Observation, Barrier-Execution and Barrier-Observation (2 agents x 2 task).

Neurons were classified as either facilitated or suppressed depending on the sign of the average modulation shown during the selected time interval. To test whether the neurons' facilitated or suppressed response was statistically significant, we compared the baseline activity with each bin of the entire period of interest (either the sensory or the motor period) applying a one-tailed sliding t test (window = 200 ms, step = 20 ms, $p < 0.05$, uncorrected). We considered as significantly modulated all the neurons with at least five consecutive significant bins, whereas neurons that did never meet this criterion were classified as non-significantly modulated. The statistical criterion here adopted is much more permissive than conventional epoch-based approaches, which – especially given the considerable number of epochs of our task – would have strongly biased the results and would have been too restrictive for the purpose of our investigation.

We further classified neurons based on the sign of their average modulation in Social and Barrier conditions (considering Execution and Observation separately), in the following way: neurons facilitated in Social and Barrier conditions (FF), neurons suppressed in both conditions (SS), neurons facilitated in the social condition and suppressed in the barrier condition (FS), neurons suppressed in the social condition and facilitated in the barrier condition (SF).

We also investigated the possible presence of neurons specifically modulated by the presence of the transparent barrier, i.e., units that responded differently to the Social vs. Barrier condition (considering separately, Execution and Observation). To test this hypothesis, we applied a two-tailed sliding t test (window = 200 ms, step = 20 ms, $p < 0.025$, uncorrected) to the subset of neurons significantly modulated with respect to the baseline in both Social and Barrier conditions. These comparisons were performed only for the motor epoch – since in sensory epoch the neuronal activity appeared to be set related and there were no evident modulations – and for FF and SS neuronal classes, which turned out to be the most represented.

In order to quantitatively assess the degree of preference expressed by single neurons for Social or Barrier condition (separately for execution and observation trials) a preference index (PI) was calculated with an identical procedure for each epoch and agent: $PI = (R_s - R_b) / (R_s + R_b)$, where R_s and R_b are the average responses of the neuron in the Social and Barrier conditions, respectively. The PI values range from - 1 (complete selectivity for barrier condition) to 1 (complete selectivity

for social condition), and a value of zero corresponds to identical discharges in the barrier and social conditions. To describe the distribution of PIs in neuronal population, this index was calculated also for those neurons showing no statistically significant differences between the two conditions.

Finally, we investigated the possible presence of neurons specifically modulated by subject performing the action, i.e., units that responded differently to Execution vs. Observation, considering Social and Barrier task separately. To test this hypothesis, we applied the same analysis described above, only for neurons modulated in motor epoch and which resulted to be facilitated (FF) or suppressed (SS) in both execution and observation conditions. The PI was calculated as follows: PI = $(R_e - R_o) / (R_e + R_o)$, where R_e and R_o are the average responses of the neuron in Execution and Observation, respectively.

2.5 Histological procedures

At the end of the study, the animal was deeply anesthetized with an overdose of sodium thiopental and perfused through the left cardiac ventricle consecutively with saline (about 2 L in 10 min), 3.5% formaldehyde (5 L in 30 min), and 5% glycerol (3 L in 20 min), all prepared in 0.1 M phosphate buffer, pH 7.4. The brain was then blocked coronally on a stereotaxic apparatus, removed from the skull, and placed in 10% buffered glycerol for 3 days and 20% buffered glycerol for 4 days. Finally, it 36 was cut frozen into coronal sections of 60 μm thickness, two series of which were stained with the Nissl method (0.1% thionin in 0.1 M acetate buffer, pH 3.7).

3. RESULTS

The recordings have been carried out in the putamen nucleus and histologically localized post-mortem in coronal, Nissl-stained sections of the monkey's brain (Figure 18).

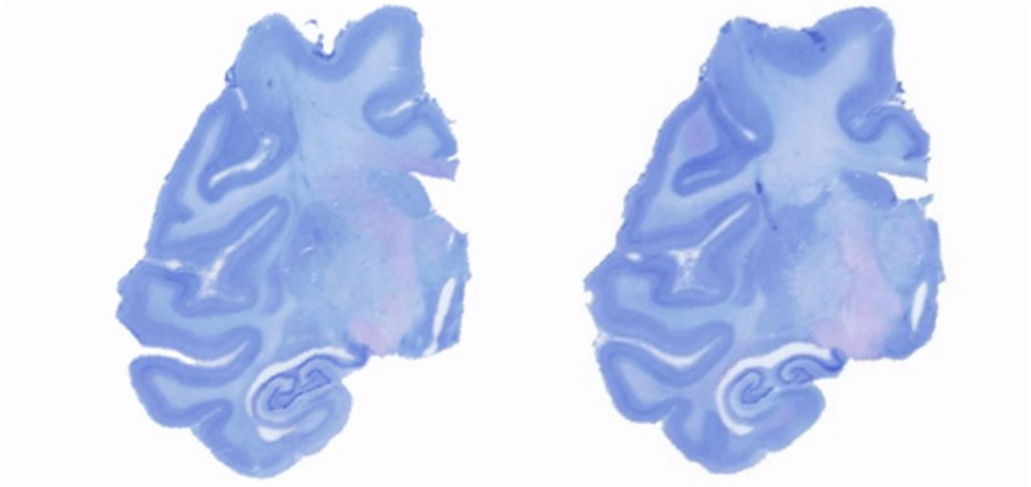


FIGURE 18 | Nissl-stained coronal sections of the monkey's brain showing probes' tracks in the left putamen nucleus.

3.1 Neuronal properties

The final dataset included 258 single units recorded during the Mutual Action Task, collected during 19 recording sessions and fulfilling all established criteria for single neuron identification.

Units were classified as modulated if their activity was either significantly facilitated or suppressed (regardless of the sign of such modulation) during the selected time intervals (sensory epoch and motor epoch) of any of the four condition. During Social task, we found that in monkey's Execution conditions 165 neurons (64%) showed task-related activity during the sensory period and 178 neurons (69%) during the motor period, whereas in Observation condition 155 neurons (60%) were modulated during the sensory period and 129 neurons (50 %) were modulated during the motor period.

When the Barrier was present, in Execution condition we found 178 neurons (69%) modulated during the sensory period and 68 neurons (65%) modulated during the motor period, whereas in Observation trials we found 150 neurons (58%) modulated during the sensory period and

126 neurons (49%) modulated during the motor period. Most units were responsive during both periods, whereas a smaller fraction of cells only displayed sensory-related activity, motor-related activity, or no significant modulation at all. In general, the fraction of neurons belonging to each category remained similar to the same condition carried out without the barrier.

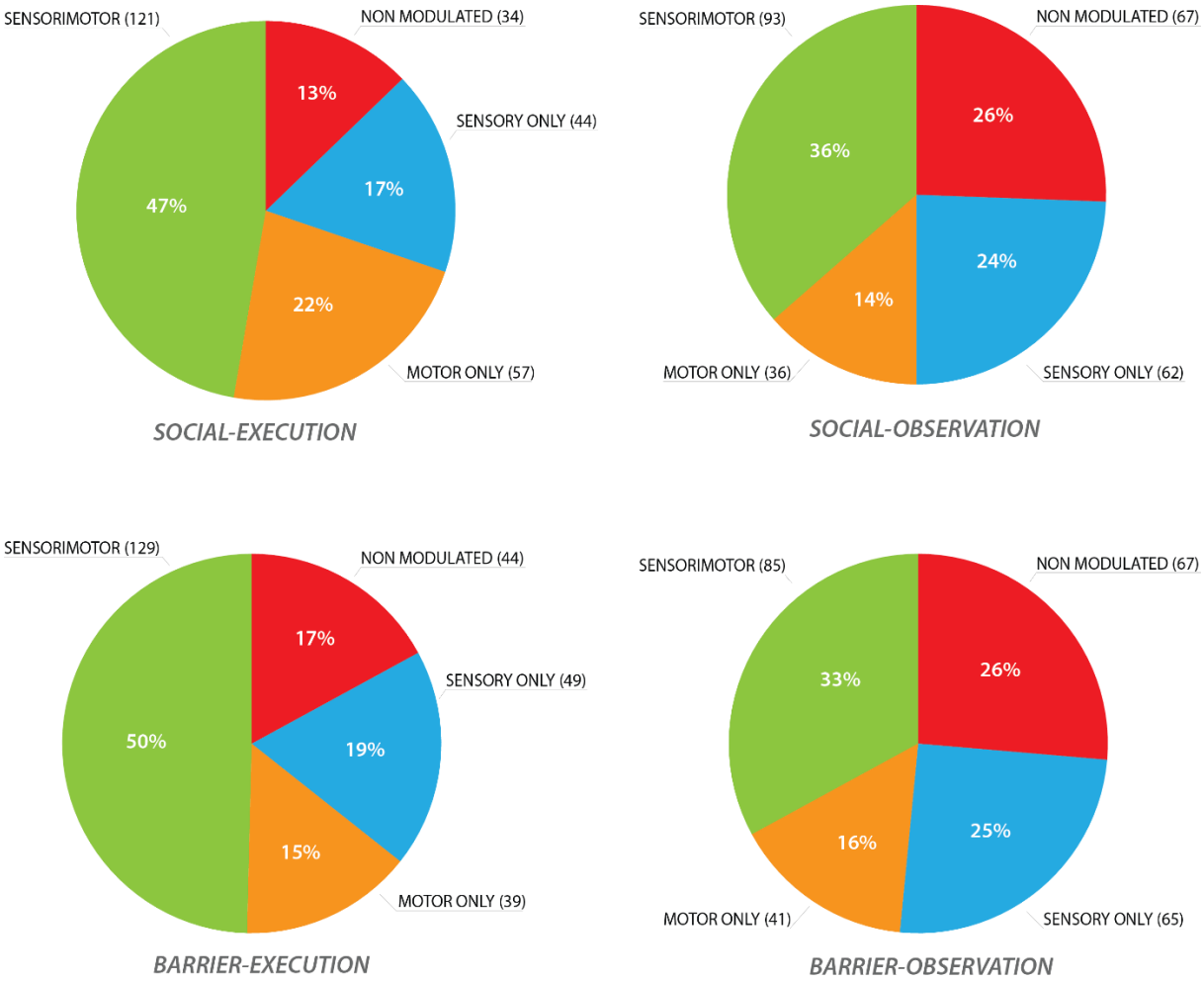


FIGURE 19 | Percentage of task-related units responding during the sensory period (blue section), the motor period (orange section), or both (green section).

3.1.1 Population analysis

3.1.1.1 Responses during sensory epoch

In Figure 20, histograms on the right of each panel report the percentage of neurons that were significantly facilitated (red bars), suppressed (blue bars), or non-modulated (grey bars), during at least five consecutive 200 ms bins (slit forward in steps of 20 ms) of the sensory period (2.1 s from Sound onset to Go signal), separately for the four conditions. The mean firing rate and standard error

of each class of neurons (facilitated and suppressed) during the whole task unfolding period is shown by red and blue lines, respectively, while the black one represents the average activity of all recorded cells, superimposed to the heatmap of individual units' normalized activity.

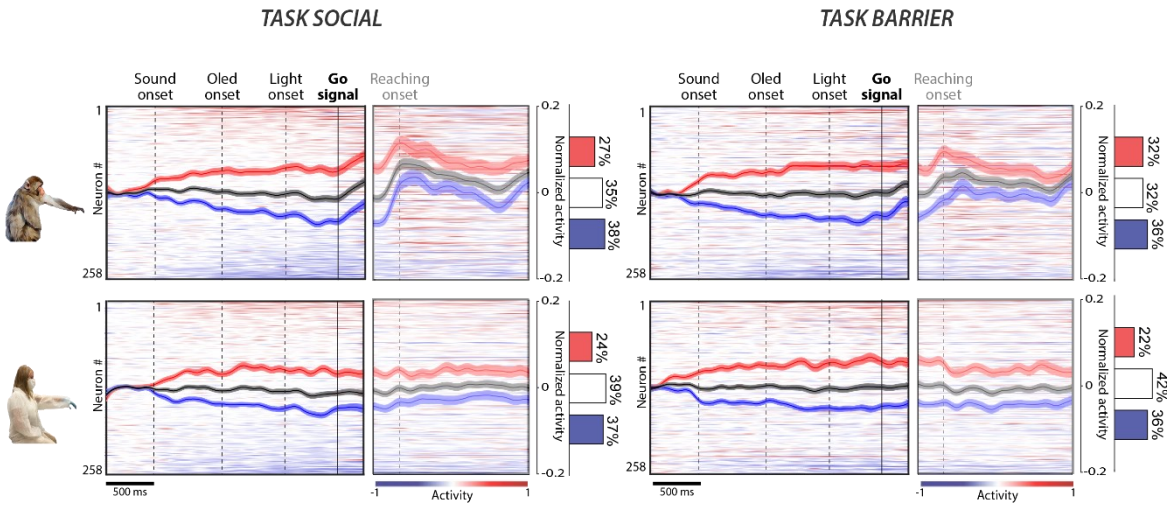


FIGURE 20 | Functional fingerprint of putaminal neurons during the sensory interval. The four panels show the heatmaps of all recorded neurons during the Social (left) and Barrier (right) tasks, in Execution (above) and Observation (below) contiditions. Each line represents one cell (its average neuronal activity over the task unfolding period in the 15 trials of the selected condition) and, for Social task cells are ordered (from top to bottom) based on the magnitude of their activity with respect to baseline (red, facilitated; blue, suppressed) in the interval of 2.1 s before the Go signal, independently for Execution and Observation. Cells in Barrier task have the same index order of those in Social task. Each cell's baseline activity was calculated in a period of 500 ms preceding the Go cue. Black lines represent the averaged response of each population as a whole. The histograms on the right indicate the percentage of facilitated (red), suppressed (blue), and nonsignificant (grey) neurons. Dashed vertical lines represent significant sensory events with fixed timing – Sound onset (go cue), Oled onset (grip cue), and Light onset (object presentation) – and the main reference events to which neuronal activity was aligned – Go signal (sound off) and Reaching onset (the release of the manipulandum).

In Social-Execution condition (Fig. 20), 70 neurons (27%) were classified as facilitated, 98 (38%) as suppressed, and 90 (35%) as non-modulated. In Observation trials, 62 units (24%) were facilitated, while the suppressed ones were 96 (37%), and the non-responsive ones 101 (39%).

During Barrier-Execution condition, there were 82 (32 %) facilitated neurons, 94 (36%) suppressed neurons and 82 (32%) not significantly modulated neurons, whereas in observation trials 56 neurons (22 %) were classified as facilitated, 94 (36%) as suppressed, and 108 (42 %) as non-responsive.

Comparing Execution and Observation conditions, in the latter there were many more non-modulated than in the former, and less facilitated ones. Otherwise, there were not many differences in the fractions of modulated neurons between Social and Barrier tasks concerning the sensory period.

3.1.1.2 Responses during motor epoch

Modulation of neural activity during the 750 ms epoch included in the motor interval (starting with reaching onset) was analyzed as well and it is summarized in Figure 21.

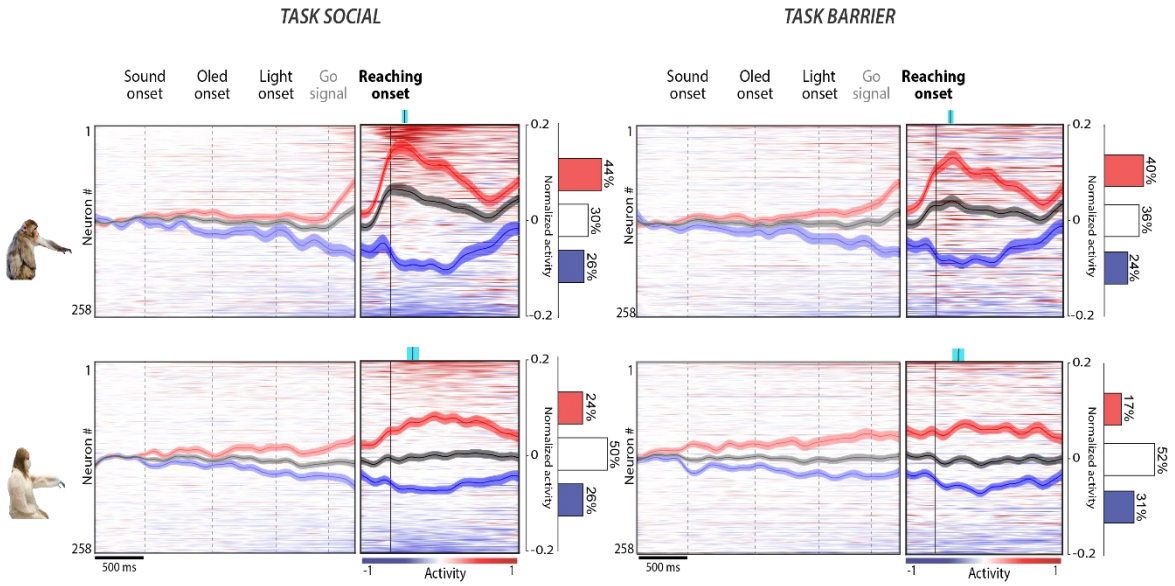


FIGURE 21 | Functional fingerprint of putaminal neurons during the motor interval. The two panels show the heatmaps of all recorded neurons during the movement period of the MAT. Neuronal activity was calculated in the interval of 750 ms after reaching onset. Light blue marks represent average ± SD of the touch movement. All other conventions as in Fig. 21.

In the Social-Execution condition, we found that 113 neurons (44%) were significantly facilitated, 67 (26 %) were suppressed, and 78 (30%) were non-modulated. During Social-Observation, 64 neurons (24%) were classified as facilitated, 65 (26 %) as suppressed, and the remaining half was classified as non-responsive. There was a moderate increase in the number of non-modulated neurons from the Execution to the Observation trials, a notable decrease in the fraction of facilitated ones and a slight increase of the inhibited ones.

During the Barrier-Execution condition, we found that 104 neurons (40 %) were significantly facilitated, 62 (24%) were suppressed, and 92 (36 %) were non-modulated. During the Barrier-Observation, 41 neurons (17 %) were classified as facilitated, 79 (31 %) as suppressed, and 133 (52 %) as non-responsive.

With respect to the Social task, the presence of the barrier did not appear to markedly influence the percentages of neurons facilitated, inhibited or non-modulated, but at population level, the barrier reduces the magnitude of the response in both Execution and Observation conditions.

3.1.2 Reaction times (RTs) during Social and Barrier tasks

Figure 22 shows the averages monkey and experimenter RTs during Social and Barrier tasks, separately for monkey and experimenter execution trials. We calculated the time lag between Sound Off and Reaching Onset (RT1), Reaching Onset and object Touch (RT2) and between Sound Off and object Touch (RT1+RT2) of in the Social and Barrier conditions.

After the Go Signal, monkey took an average of 322.70 ms (SD = 153.70) to release the manipulandum during Social task, whereas during the Barrier task (mean = 257.00 ms; SD = 166.40) it was significantly more rapid ($p < 0.05$, Cohen d = 0.4). Experimenter RT1 during Barrier task (mean= 377.70 ms, SD = 70.20) was significantly slower compared to the Social task (mean = 367.80 ms, SD= 61.10 ms), but the effect size was negligible ($p < 0.05$, Cohen d = 0.14).

From the Reaching Onset to the object Touch moment, the monkey was significantly slower during the Barrier task (mean= 180.30 ms, SD= 36.00) with respect to the Social task (mean= 155.60 ms, SD= 34.50) and the dimension of the effect was large ($p< 0.05$, cohen d = 0.7). As for RT1, experimenter RT2 was slower in Barrier task (mean = 317.7 ms, SD = 66.20) than in Social task (mean = 309.6, SD = 63.70), but again with a negligible effect size ($p< 0.05$, cohen d= 0.1).

Moreover, during Social task monkey was slower to start the reaching movement and then faster to touch the object, taking a total of 477.90 ms (SD = 154.50) to perform the entire reaching act from the Sound Off. Otherwise, during Barrier task it was faster to release the manipulandum after the Go Signal, and slower to reach the object (mean = 437.40 ms, SD = 169.30). Compared with monkey, experimenter was slower to start moving and reach the object and he was overall slower during Barrier (mean= 694.80 ms , SD = 92.60) than in Social task (mean = 677.30 ms, SD = 63.40). Both mean differences between Social and Barrier RTs considering the entire period from Sound Off to Touch were significant ($p < 0.05$), but the magnitude of these differences was negligible (cohen d = 0.2).

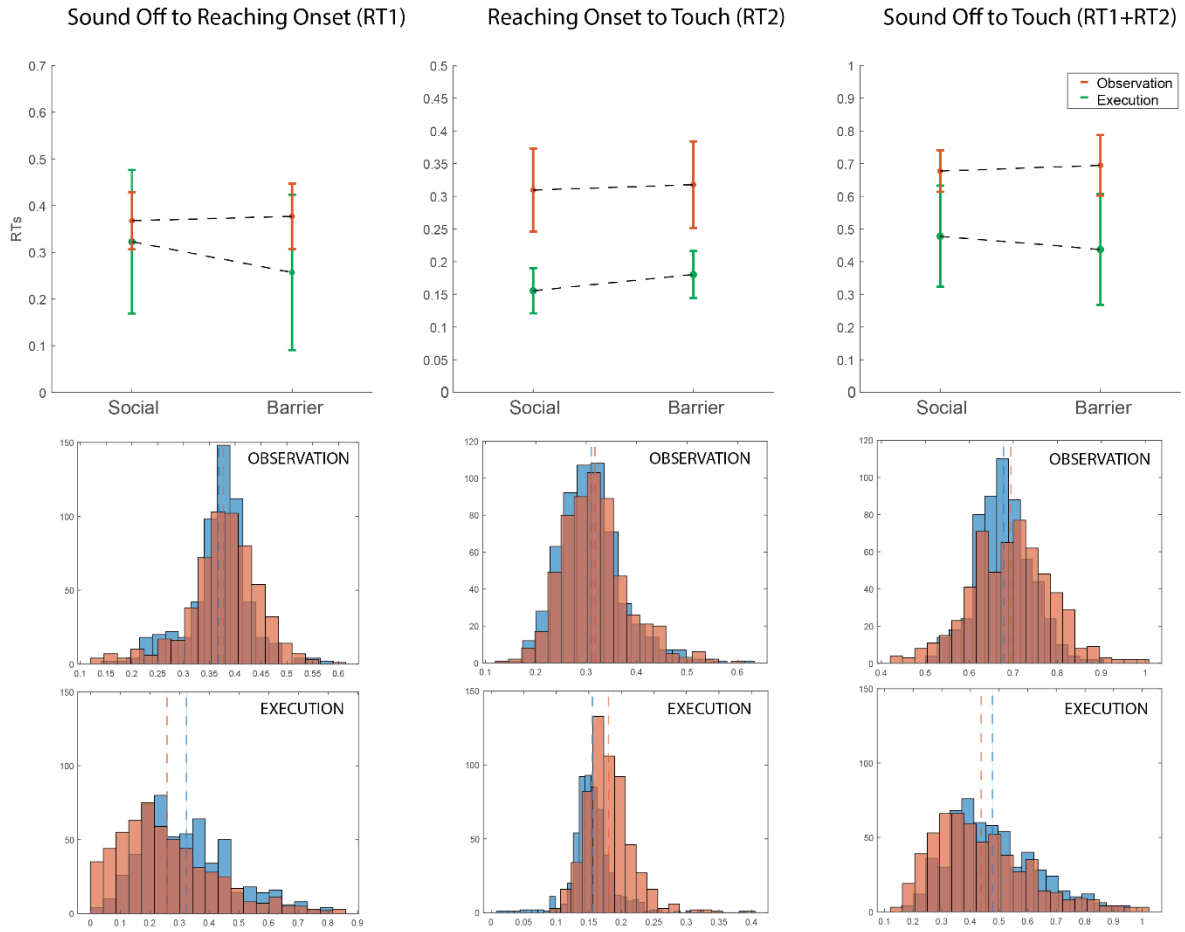


FIGURE 22 | Averages monkey (Execution) and experimenter (Observation) RTs during Social and Barrier tasks. First row contains plots of means and standard deviations of the monkey and experimenter RTs during Social and Barrier tasks. Histograms in second and third rows shows the RTs distributions in Social (blue bars) and Barrier (orange bars) tasks, separately for Execution (third row) and Observation (second row); dashed lines represents mean RTs. RT1 was calculated as the difference between the Reaching Onset (the release of the manipulandum) and the Sound Off (the Go Signal); RT2 was calculated as the difference between the Touch (the moment in which monkey or experimenter touch the object) and the Reaching Onset. RT1+RT2 represents the entire periods of time between the Sound Off and the Touch.

3.1.3 Modulation in Social and Barrier tasks

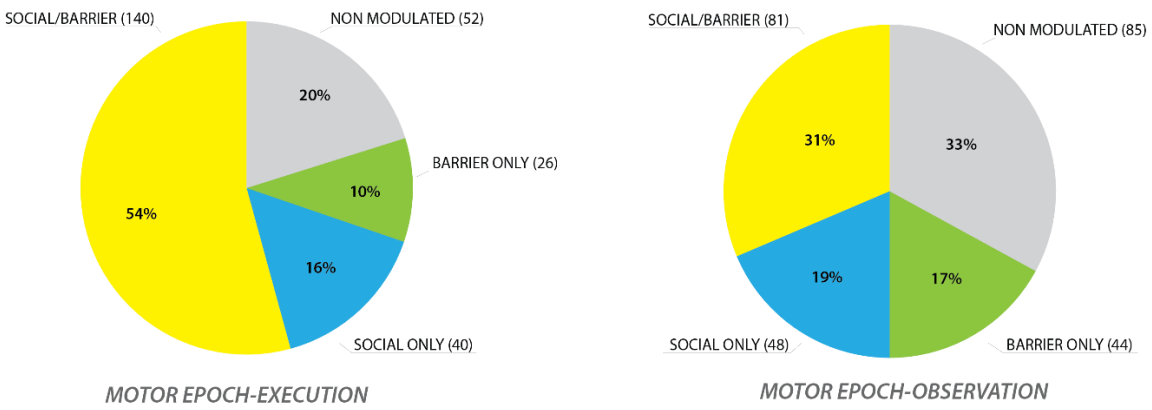


FIGURE 23 | Numbers of neurons responsive during Social and Barrier tasks. Pie charts shows percentages of neurons responding only during the Social task (blue section), only during the Barrier task (green section), or during both tasks (yellow section).

Figure 23 shows the relative numbers of units responding to Social, Barrier or both tasks, during Execution and Observation trials. During the Execution trials the majority of the units were responsive for both conditions, whereas a smaller proportion of cells were only active in the Social condition, in the Barrier condition, or showed no significant modulation at all. With respect to the Execution trials, in the Observation trials there was an increase of non modulated units, especially during the motor epoch. Figure 24 illustrates classification of neurons resulted to be active during both Social and Barrier tasks – during motor epoch – based on the sign of their average modulation in each task, separately for Execution and Observation trials.

In the Execution trials we found 140 units responsive during both Social and Barrier tasks. Among these, most units were FF-type (N = 81, 58%), 42 (30%) were SS-type and only a very small number of neurons were FS (N = 9, 6.5 %) or SF-type (N= 8, 5.5 %). In the Observation trials there were 81 units activated in both tasks: 29 (36%) were FF-type, 37 (46%) were SS-type, 9 (11%) were FS-type and 6 (7%) were SF-type. We further analyzed the two most represented classes of FF and SS-type.

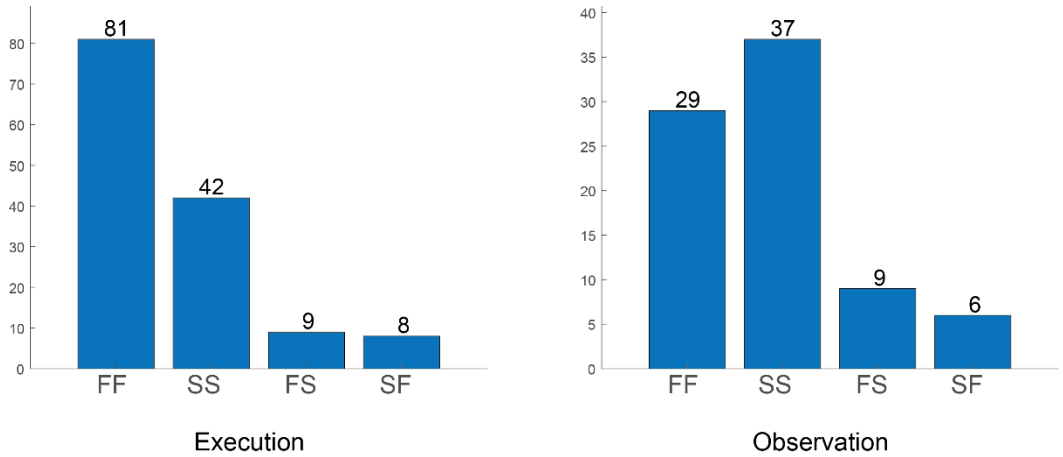


FIGURE 24 | Classification of neurons active in both Social and Barrier tasks based on the sign of modulations in each task. Each bar represents the number of units. Left, Execution trials; right, Observation trials.

3.1.2.2 FF-type neurons

FF-TYPE NEURONS

	Social > Barrier	Barrier > Social	Social = Barrier	TOT
Exe	**17** (21%)	**8** (10%)	**56** (69%)	**81**
	25 (31%)			
Obs	**6** (21%)	**5** (17%)	**18** (62%)	**29**
	11 (38%)			

TABLE 1 | Behavior of "Social-Barrier" neurons during motor epoch in Execution and Observation conditions (FF -type). First and second columns represents the numbers of neurons that were significantly more facilitated in Social (first column) or Barrier (second column) task. Third column contains the number of neurons facilitated in the same way in both tasks. In the last column there was the total number of FF-neurons. First row, execution; second row, observation. Note: Values that refer to the number of neurons appear in bold.

As shown in table 1, we found 81 and 29 FF-type neurons during Execution, and Observation trials, respectively. In Execution, there were 25 (31%) neurons that discriminate between Social and Barrier tasks: most of them (N = 8, 21%) were more excited during Social task, and the remaining 10% (N = 8) were more facilitated in Barrier task. More than half of neurons (N = 56, 69%) are equally facilitated in both tasks. During observation, as in execution trials, we found that more than half of units (N = 18, 62%) did not discriminate between Social and Barrier tasks. Concerning neurons that discriminate between the two tasks (N = 11, 38%), 6 (21%) units had a stronger facilitation during Social task and 5 (17%) were more facilitated during Barrier task. Figure 25 illustrates some examples of "Social-Barrier" FF-type neurons.

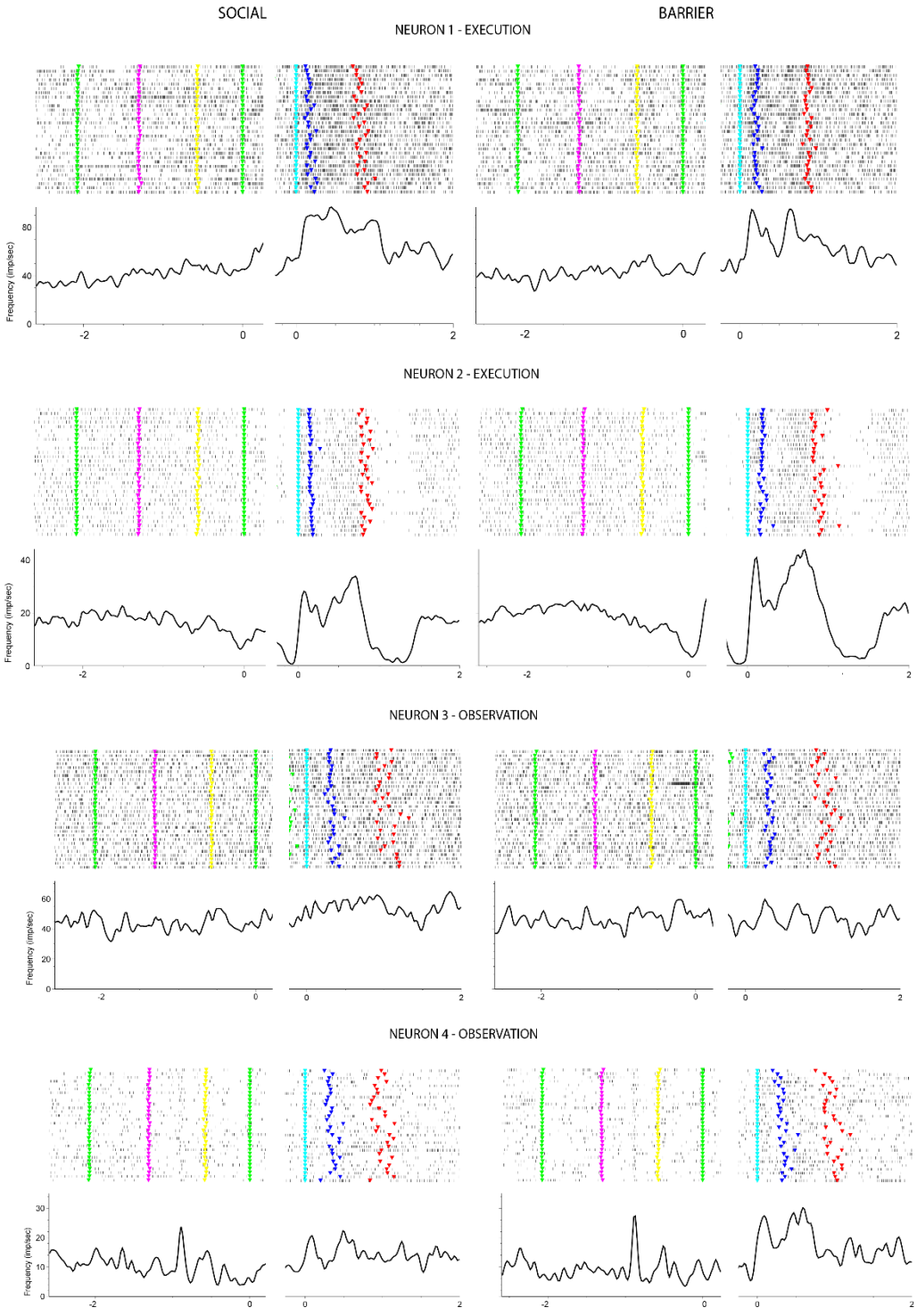


FIGURE 25 | Examples of FF-type "Social-Barrier" neurons. Triangular markers correspond to sound onset and sound offset (green markers), oled onset (violet markers), light onset (yellow onset), movement onset (light blue markers), touch (blue markers), and reward delivery (red markers). In the left portion of each raster neuronal activity was aligned to the sound offset, whereas in the right one it was aligned to movement onset. First row, a neuron significantly more facilitated during Social task in execution; second row, a neuron significantly more facilitated during Barrier task in execution; third row, a neuron significantly more facilitated during Social task in observation; fourth row, a neuron significantly more facilitated in Barrier task in observation.

Histograms in figure 26 shows the distribution of task PIs calculated on FF-type neurons for execution (mean = 0.06, SD = 0.30) and observation (mean= 0.09, SD = 0.38) trials. Boxplots in

same figure shows that the 50% of the PI values were comprised from -0.07 to 0.22 (median= 0.05) in execution, and from -0.12 and 0.28 (median= 0.12) in observation. PIs of execution condition had a quite symmetric distribution, whereas PIs distribution of observation trials displayed a slight preference for the Social task.

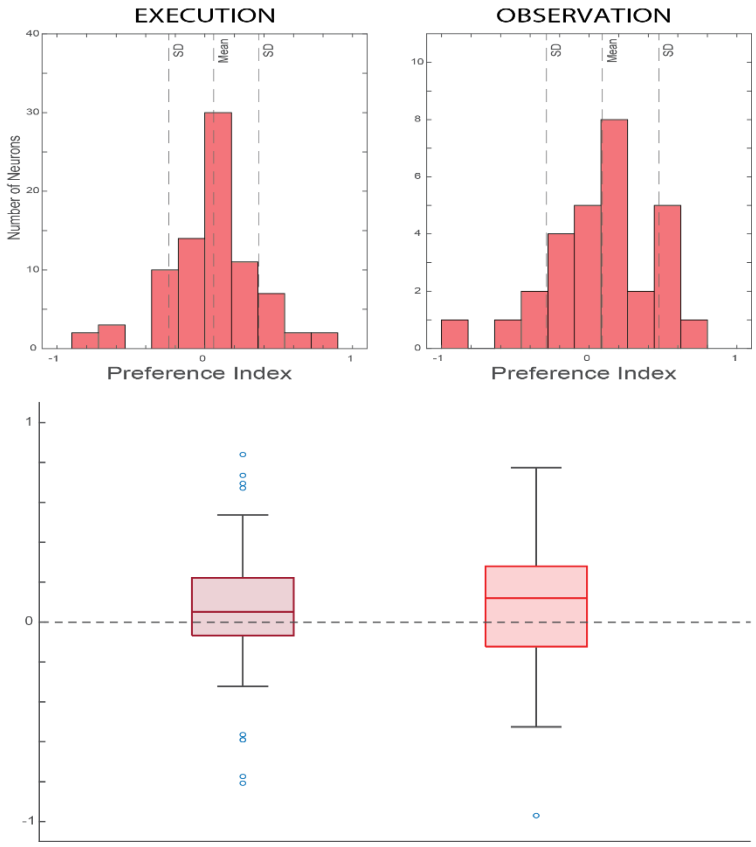


FIGURE 26 | Distributions of task PIs, in execution and observation trials (FF- type neurons). Histograms on the top shows distribution of the PIs: each bar represents the number of neurons and dashed lines represents mean and standard deviation. On the bottom, boxplot distribution of PIs. PIs values of 1 indicate a preference for Social task; -1 values indicate a preference for Barrier task; 0 values indicate an absence of any preference. Left, execution; Right, observation.

3.1.2.3 SS-type neurons

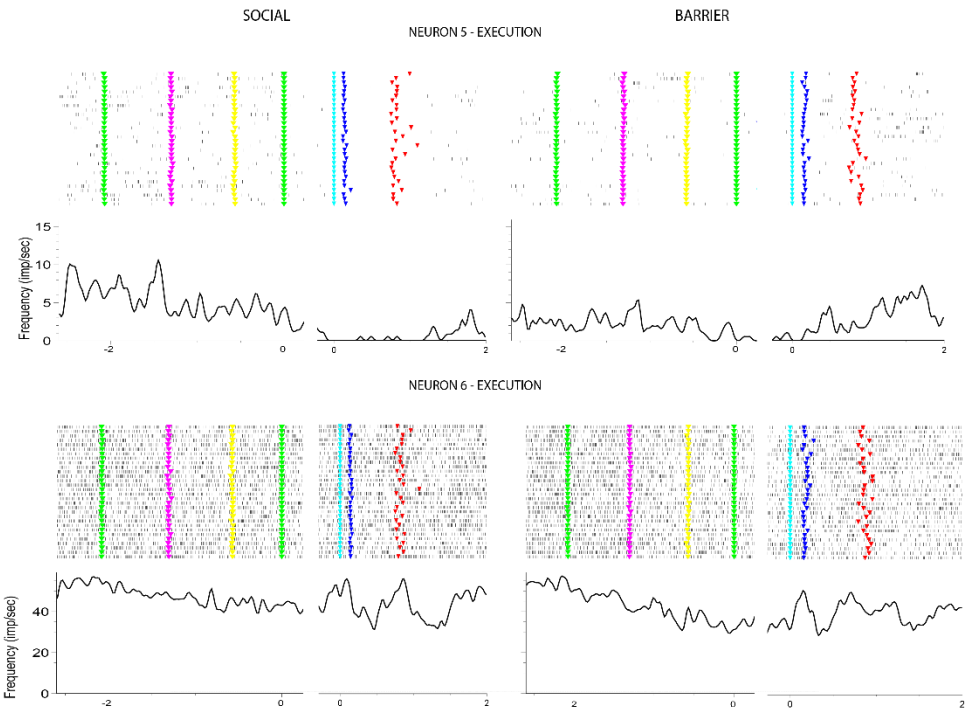
Table 2 shows the behavior of SS-type neurons during execution (N = 42) and observation trials (N = 37).

SS-TYPE NEURONS

	Social > Barrier	Barrier > Social	Social = Barrier	TOT
Exe	**4** (10%)	**6** (14%)	**32** (76%)	**42**
	10 (24%)			
Obs	**7** (19%)	**2** (5%)	**28** (76%)	**37**
	9 (24%)			

TABLE 2 | Behavior of "Social-Barrier" neurons during motor epoch in Execution and Observation conditions (SS-type). First and second columns represents the numbers of neurons that were significantly more inhibited in Social (first column) or Barrier (second column) tasks. Third column contains the number of neurons inhibited in the same way in both condition. In the last column there was the total number of SS-neurons. All other conventions as in table 1.

During monkey execution, 10 (24 %) units showed a significantly difference between Social and Barrier tasks: 4 (10 %) neuron were more inhibited in Social task and 6 (14%) neurons showed a higher inhibition in Barrier task. More than half of the units (N = 32, 76%) did not significantly discriminate between the two tasks, as for the previously described FF-type. During monkey observation we found the same percentage of neurons that did not discriminate between Social and Barrier tasks (N = 28, 76 %) than in execution. Among the units that are more inhibited in one of the two tasks (N = 9, 24%), 7 (19%) neurons were more inhibited in Social task, and only 2 (5%) had a higher inhibition in Barrier task. Figure 27 illustrates some examples of "Social-Barrier" FF-type neurons.



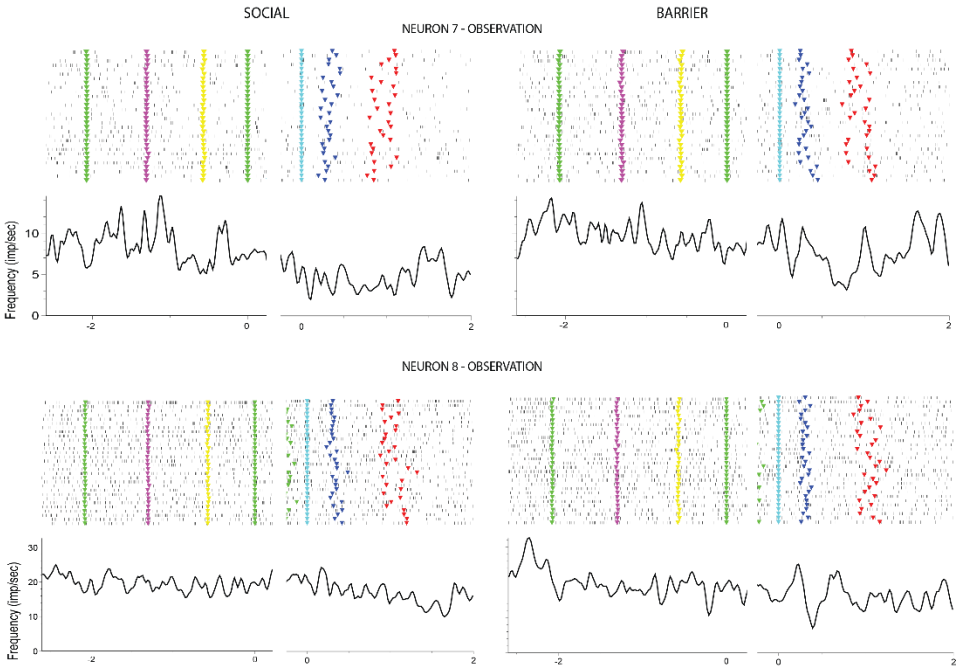


FIGURE 27 | Examples of SS-type "Social-Barrier" neurons. First row, a neuron significantly more inhibited during Social task in execution; second row, a neuron significantly more inhibited during Barrier task in execution; third row, a neuron significantly more inhibited during Social task in observation; fourth row, a neuron significantly more inhibited in Barrier task in observation. All other convention as in figure 25.

Histograms in figure 28 shows the distribution of task PIs calculated on SS-type neurons for execution (mean = -0.05, SD = 0.38) and observation (mean= 0.06, SD = 0.33) trials. Boxplots in same figure shows that the 50% of the PI values were comprised from -0.27 to 0.19 (median= - 0.04) in execution, and from -0.12 and 0.16 (median= 0.10) in observation. PIs distributions do not seem to show any particular preference towards Social or Barrier tasks.

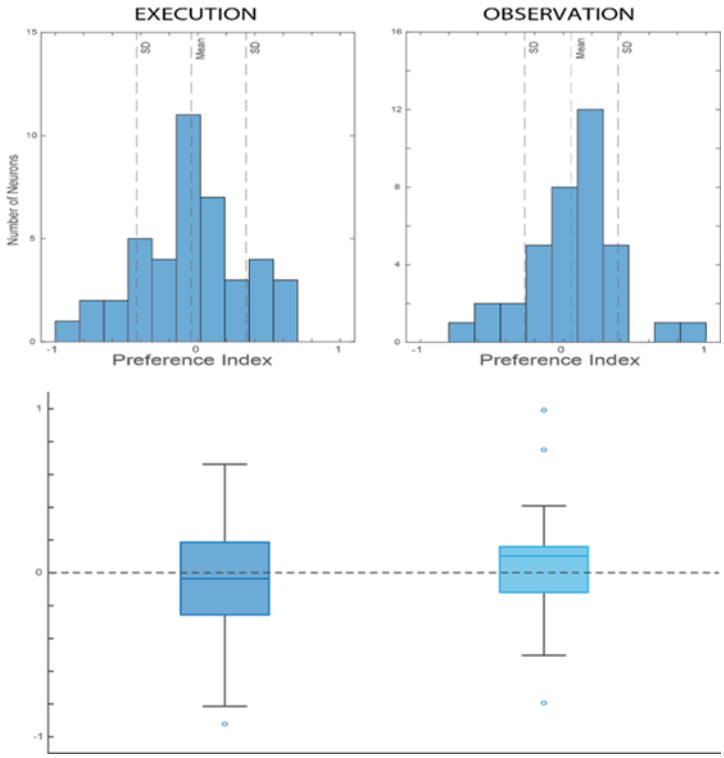


FIGURE 28 | Distributions of social-barrier PIs, in execution and observation trials (SS- type neurons). Histograms on the top shows distribution of the PIs: each bar represents the number of neurons and dashed lines represents mean and standard deviation. On the bottom, boxplot distribution of PIs. PIs values of 1 indicate a preference for Social task; 1 values indicate a preference for Barrier task; 0 values indicate an absence of any preference. Left, execution; Right, observation.

3.1.4 Modulation for self and others' actions

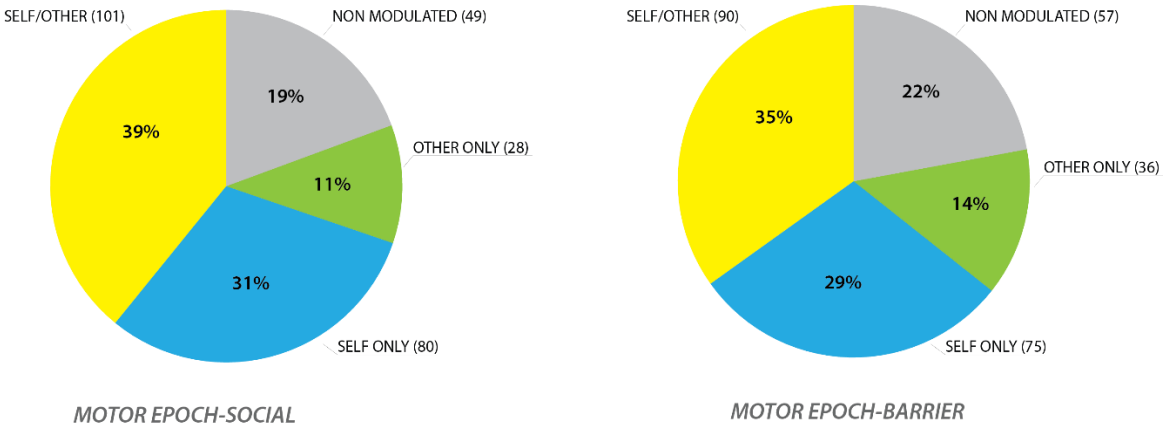


FIGURE 29 | Numbers of neurons responsive during Execution and Observation tasks. Pie charts shows percentages of neurons responding only during the Execution ("Self only", blue section), only during Observation ("Other only", green section), or during both tasks ("Self-Other", yellow section).

Figure 29 shows the relative numbers of units responding to Execution, Observation or both agent conditions, during Social and Barrier tasks. During both tasks the majority of the units were responsive for both agent conditions, and there was a great proportion of neurons responsive during

execution trials, whereas a smaller proportion of cells were only active during observation trials. There was also a fraction of units that showed no significant modulation at all. With respect to the Execution trials, in the Observation ones there was an increase of non modulated units, especially during motor epoch. Figure 30 shows numbers of FF-type, SS-type, FS-type and SF-type neurons that were active during both Execution and Observation trials (motor epoch), separately for Social and Barrier tasks.

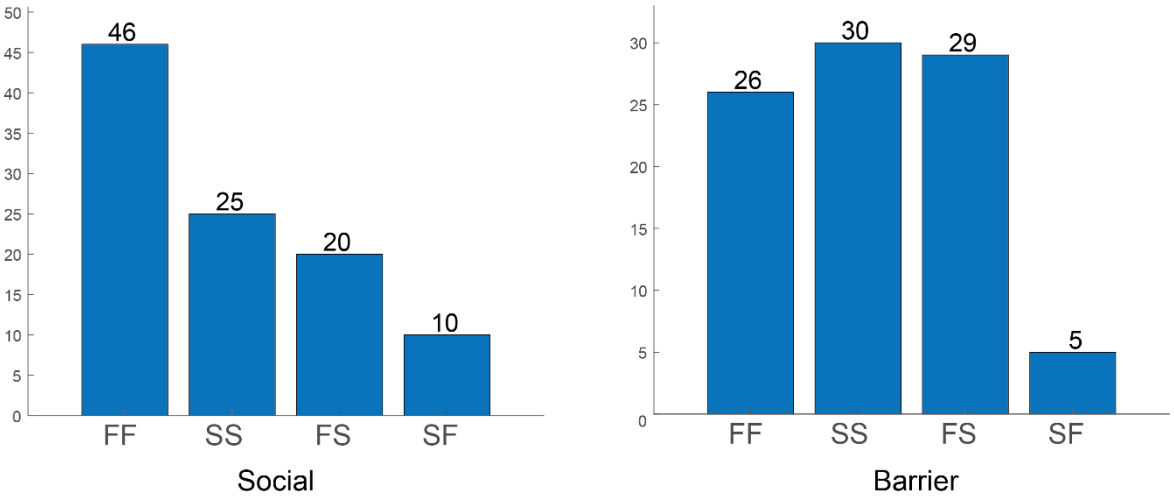


FIGURE 30 | Classification of neurons active in both agent conditions based on the sign of modulations in each condition. Each bar represents the number of units. Left, Social task; right, Barrier task.

During Social task we found 101 units responsive in both execution and observation conditions. Among these, most units were FF-type (N = 46, 45%), 25 (25 %) were classified as SS-type, 20 (20 %) were classified as FS-type and 10 as SF-type (10 %). During Barrier task there were 90 units activated in both conditions: 26 (29 %) were FF-type, 30 (33 %) were SS-type, 29 (32 %) were FS-type and 5 (6 %) were SF-type.

3.1.3.1 FF-type neurons

Table 3 summarizes the behavior of FF-type "Self-Other" neurons recorded in motor epochs (i.e. neurons that responding both during Execution and Observation), separately for Social and Barrier tasks.

FF-TYPE NEURONS

	Self > Other	Other > Self	Self = Other	TOT
Social	**25** (55%)	**7** (15%)	**14** (30%)	**46**
	32 (70%)			
Barrier	**18** (69%)	**5** (19%)	**3** (12%)	**26**
	23 (88%)			

TABLE 3 | Behavior of "Self-Other" neurons during motor epoch in Social and Barrier tasks (FF- type). First and second columns represents the numbers of neurons that were significantly more facilitated in Execution (first column) or Observation (second column) condition. Third column contains the number of neurons facilitated in the same way in both conditions. In the last column there was the total number of FF-neurons. First row, Social; second row, Barrier. All other conventions as in table 1.

We found, respectively, 46 and 26 FF-type neurons during execution and observation trials. Most neurons modulated in Social task discriminate between execution and observation conditions (N = 32, 70%): about a half of the total (N = 25, 55 %) was more facilitated during Execution trials, and the other 7 units (15%) had a stronger facilitation during the Observation. We also found 14 (30%) neurons facilitated in both Execution and Observation trials, that didn't have a significant difference in their responses. During Barrier task the overall number of FF-neurons is lower and, similar to the Social task, the majority of the units had a significantly different response for execution and observation trials (23, 88 %): 18 (69%) units had a stronger facilitation during execution trials and 5 (19 %) were more excited during observation trials. Finally, we found 3 (12%) neuron equally active in both execution and observation conditions. Figure 31 shows some examples of "Self-Other" FF-type neurons.

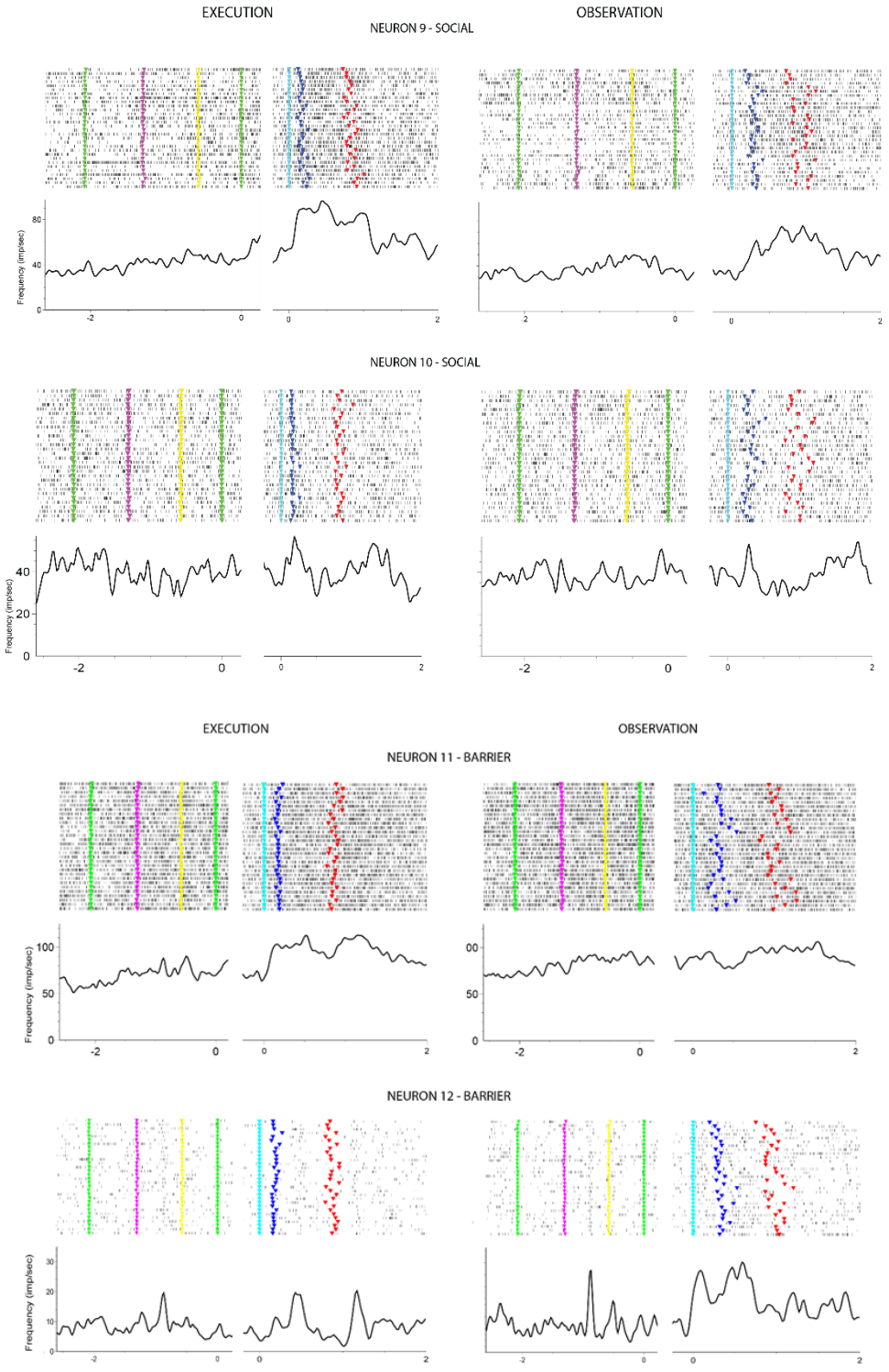


FIGURE 31 | Examples of FF-type "Self-Other" neurons. First row, a neuron significantly more facilitated in execution during Social task; second row, a neuron equally facilitated in execution and observation during the Social task; third row, a neuron significantly more facilitated in execution during Barrier task; fourth row, a neuron significantly more facilitated in observation during Barrier task. All other conventions as in figure 25.

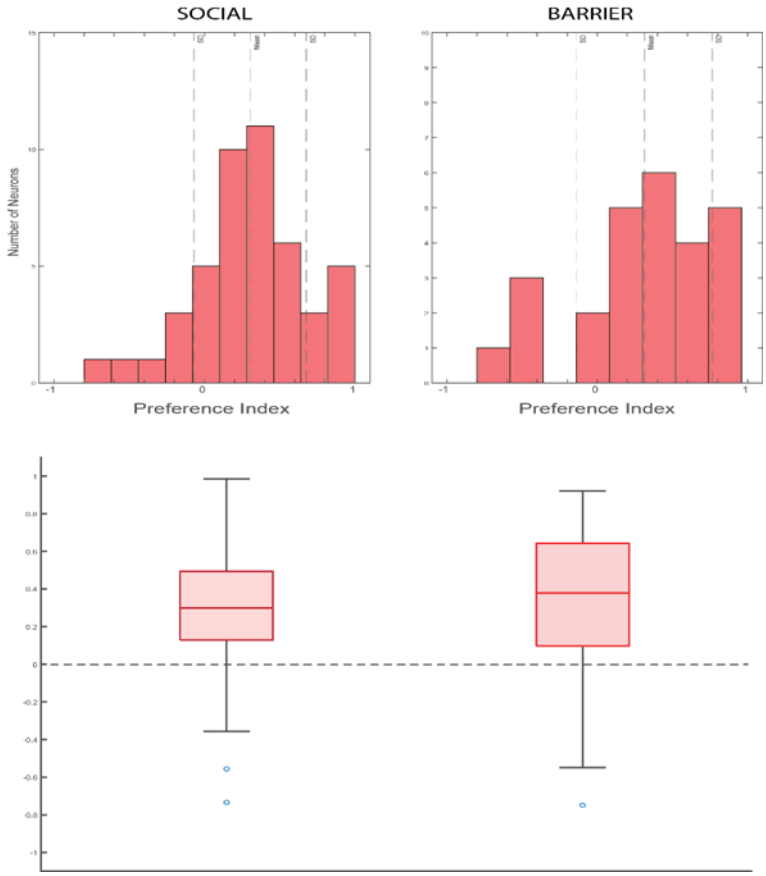


FIGURE 32 | Distributions of agent PIs, in Social and Barrier tasks (FF- type neurons). Histograms on the top shows distribution of the PIs: each bar represents the number of neurons and dashed lines represents mean and standard deviation. On the bottom, boxplot distribution of PIs. PIs values of 1 indicate a preference for execution; -1 values indicate a preference for observation; 0 values indicate an absence of any preference. Left, Social; Right, Barrier.

Histograms in figure 32 shows the distribution of agent PIs calculated on FF-type neurons in Social (mean= 0.30, sd= 0.37) and Barrier (mean= 0.31, sd= 0.45) tasks. Boxplots shows that the 50% of the PI values were comprised from 0.13 to 0.49 (median= 0.30) in Social task, and from 0.10 to 0.64 (median= 0.38) in Barrier task. It appears that among Self-Other FF-neurons the preferred condition was the execution, for both Social and Barrier tasks.

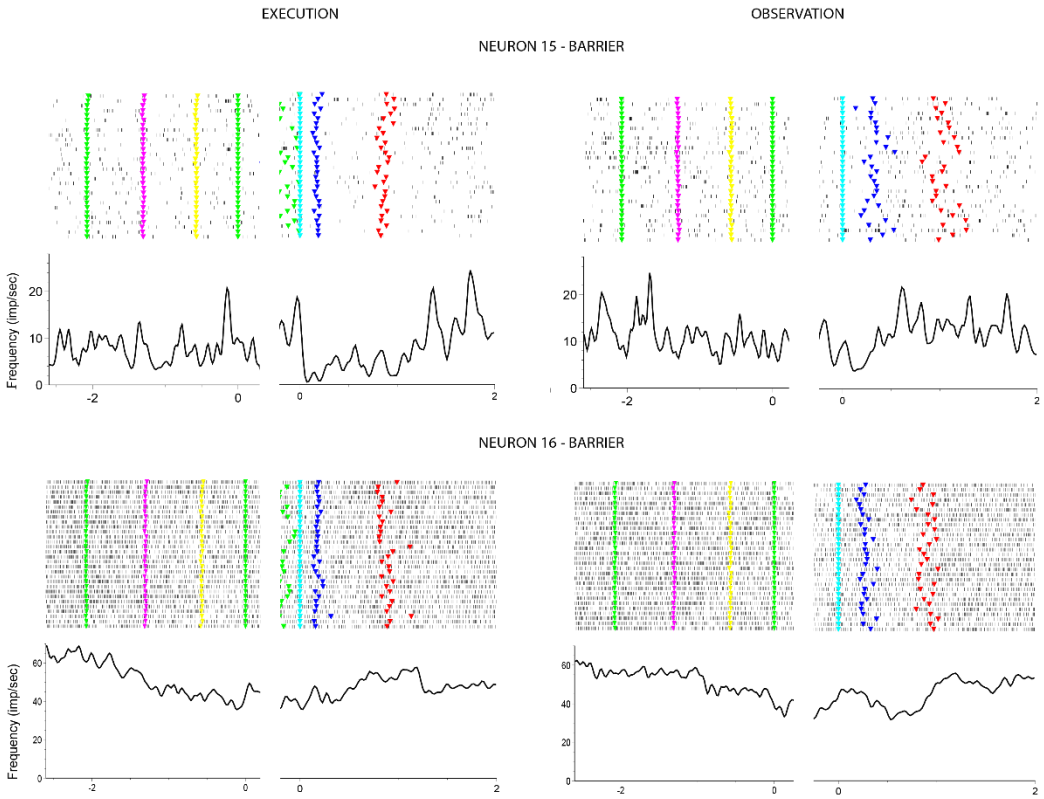
3.1.3.2 SS- type neurons

SS-TYPE NEURONS

	Self > Other	Other > Self	Self = Other	TOT
Social	**2** (8%)	**8** (32%)	**15** (60%)	**25**
	10 (40%)			
Barrier	**12** (40%)	**10** (33%)	**8** (27%)	**30**
	22 (73%)			

TABLE 4 | Behavior of "Self-Other" neurons during motor epoch in Social and Barrier tasks (SS -type). First and second columns represents the numbers of neurons that were significantly more inhibited in execution (first column) or observation (second column) condition. Third column contains the number of neurons inhibited in the same way in both conditions. In the last column there was the total number of SS-neurons. All other conventions as in table 1.

As shown in table 4, in Social condition we found 44 "Self-Other" SS-type neurons and 25 in Barrier condition. During Social task, we found 10 (40%) neurons that discriminate between execution and observation: most of them (N = 8, 32%) showed a higher inhibition during other condition, and the remaining 8% (N = 2) were more inhibited in self condition. More than half of neurons (N = 15, 60%) are equally inhibited in both execution and observation trials. During Barrier task we found that most units (N = 22, 73%) discriminated between execution and observation conditions: 12 (40%) units were more inhibited in execution condition and 10 (33%) units had a higher inhibition in observation condition.



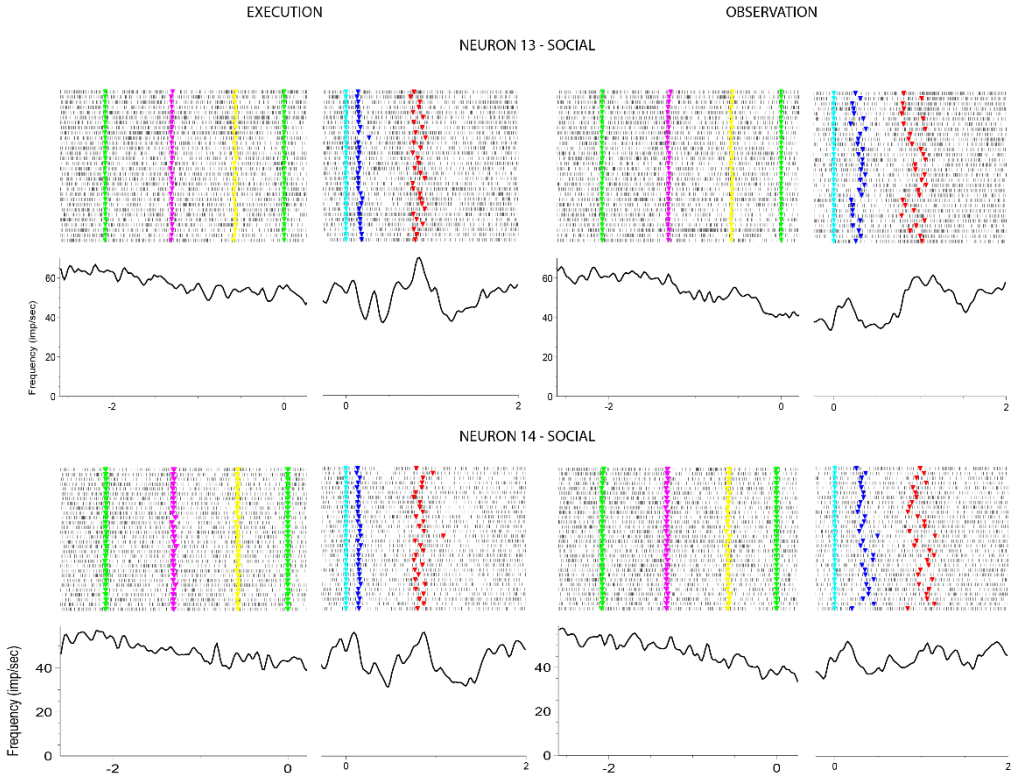


FIGURE 33 | Examples of SS-type "Self-Other" neurons. First row, a neuron significantly more inhibited in observation during Social task; second row, a neuron equally inhibited in execution and observation during the Social task; third row, a neuron significantly more inhibited in execution during Barrier task; fourth row, a neuron significantly more inhibited in observation during Barrier task. All other conventions as in figure 25.

The remaining 27% (N= 8) were inhibited in a not significantly different way in Execution and Observation conditions. Figure 33 shows some example of "Self-Other" SS-type neurons.

Histograms in figure 34 shows the distribution of agent PIs calculated on SS-type neurons in Social (mean= -0.13, sd= 0.38) and Barrier (mean= 0.04, sd= 0.37) tasks. Boxplots shows that the 50% of the PI values were comprised from -0.46 to 0.11 (median= -0.04) in Social task, and from -0.70 to 0.28 (median= 0.07) in Barrier condition. The distribution of PIs of the Social task was asymmetric indicating a moderate preference for the observation condition, whereas PIs distribution in Barrier task was more symmetric and showed a slighter preference for execution condition.

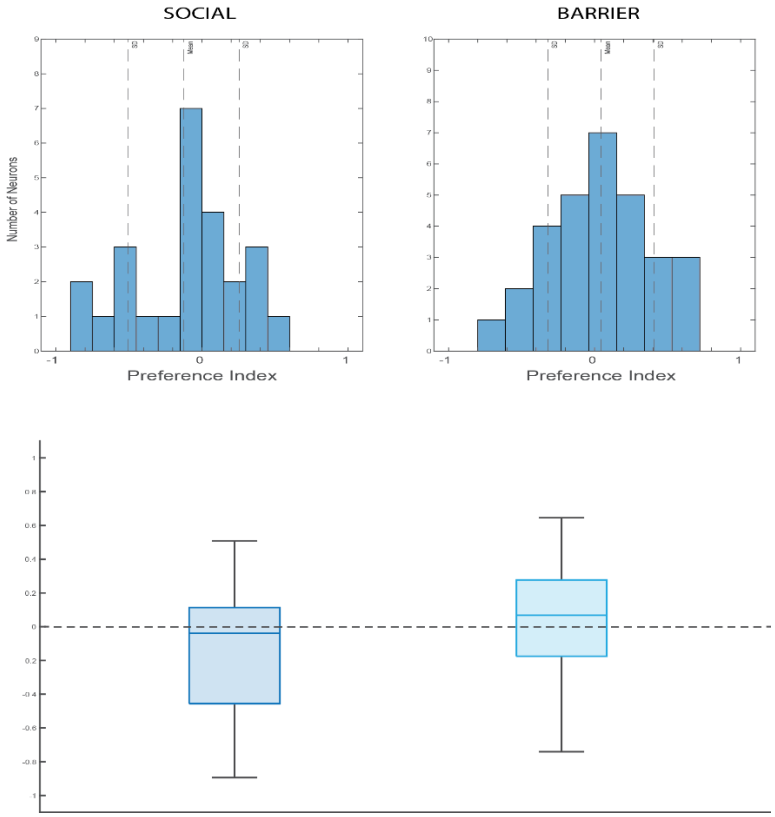


FIGURE 34 | Distributions of agent PIs, in Social and Barrier tasks (SS- type neurons). Histograms on the top shows distribution of the PIs: each bar represents the number of neurons and dashed lines represents mean and standard deviation. On the bottom, boxplot distribution of PIs. PIs values of 1 indicate a preference for execution condition; -1 values indicate a preference for observation condition; 0 values indicate an absence of any preference. Left, Social; Right, Barrier.

4. DISCUSSION

In the present study, we investigated the functional properties of single neurons in the putamen nucleus, well known for its major role in mediating motor function of the BG and for being the "go through" station in the motor loop, receiving and modulating cortical signals, in order to send them back to either promote or inhibit a movement. More recent data have shown that the putamen nucleus receives convergent projections from most cortical areas belonging to MN network for hand actions – i.e. area AIP/PFG, PMv, VLPF (Gerbella et al., 2015) and F6 (Albertini et al., 2022) –, and that these areas exhibit a comparable activation during the observation of hand actions (e.g. Ge et al., 2018), supporting the motor decoupling hypothesis and therefore BG involvement in the mirror system (Bonini, 2017).

To test the possible presence of mirror properties in the putamen neurons, we used the MAT, a paradigm in which the monkey and the experimenter act on the same object in a shared operational space or, by interposing a transparent barrier between the object and one of the agents, they act – and observing the action – being able to see the other agent but without the possibility of interacting. This additional condition allowed us to study not only the responses of neurons to the action execution and action observation, but also how these responses can vary by changing the operational distance within the actor and the observer.

4.1 From the processing of contextual information to the selection of an appropriate behavioral response

We found that most of the recorded neurons showed some modulation during the task-unfolded period, most often during both sensory epoch and the motor one, in at least one of the four considered conditions (2 Agent x 2 Task).

During the sensory cue period – for both Social and Barrier task – , the fraction of units significantly inhibited was higher than that of the facilitated ones, especially in the experimenter's execution trials, even though in this latter condition a smaller amount of units was classified as task-responsive. Clearly, due to the length of the selected interval and the inclusion of several behaviorally

relevant events, such as the Go/No-Go cue, grip cue, object presentation, and Go/No Go signal, a fraction of the neurons modulating their firing rate throughout the sensory epoch may be represented by false-positive occurrences. Further studies will be needed to identify which stimuli specifically modulated single neuron activity: according to previous studies using different behavioral paradigms, visual stimuli exert the most powerful and reliable source of modulation of the putaminal neurons (e.g. Nougaret and Ravel, 2015; Romero et al, 2008; Vicente et al., 2012), moreover, in areas that are source of anatomical projection to the putamen, auditory instructive cues only weakly modulate the neuronal discharge (Albertini et al., 2020; Bonini et al., 2014; Bruni et al., 2015; Lanzilotto et al., 2016; Lanzilotto et al., 2020; Livi et al., 2019). Overall, the activation of putaminal neurons during the instructive phases of the task would be consistent with the widely recognized role of BG in action selection (e.g., of the type of grip that must be performed) and inhibition (required when the monkey had to observe the experimenter's action), supporting the hypothesis that striatal neurons may receive behaviorally relevant sensory information about the current context (Matsumoto et al., 2001; Gerbella et al., 2016), resulting in instruction-dependent preparatory activity that influences the selection and generation of the cortical action plan (e.g., Alexander and Crutcher, 1990; Apicella, 2017).

4.2 Encoding one's own and other's actions

After the instructive cue phase, based on the type of the sound that was presented, the monkey or the experimenters had to release the manipulandum and perform a reaching-grasping act. We ensured to analyze only the period, from the beginning of the reaching act, that always included the touch of the object, but that excluded the possible reward-related responses that have already been found in previous studies (e.g. Nougaret and Ravel, 2015; Vicente et al., 2012).

Most neurons were active during either the monkey's own movement ("Self-type"), observation of the experimenter's action ("Other-type"), or both of these conditions ("Self-Other type"); only a small fraction did not show any action-related modulation. Interestingly, the largest class was represented by Self-Other neurons, which accounted for nearly half of all recorded units.

During monkey's execution trials, there were most facilitated neurons, whereas during action observation trials most neurons turned to be non-modulated and the inhibited neurons prevailed to the facilitated ones. Using a paradigm broadly similar to that used here and a similar data analysis approach, Ferroni and colleagues (2021) investigated the behavior of neurons in cortical areas directly connected with the putamen nucleus and belonging to MN network (Gerbella et al., 2015; Albertini et al., 2022) and demonstrated that in AIP and F5, more than half of the neurons were facilitated during active monkey movement, whereas suppressed neurons predominated in F6. In action observation trials, as in putaminal neurons, the overall modulation was lower than in action execution trials in all investigated areas; additionally, the number of excited and inhibited units was balanced in both AIP and F5, unlike in F6, where suppressed cells prevailed.

Another noteworthy finding of the current study is that, among those neurons that were modulated during both action execution and observation, we found not only cells with "classical" mirror-like properties (i.e., showing facilitation during both conditions; FF-type), but also a great fraction of units that were consistently suppressed (SS-type), and others that displayed opposite changes in activity in the two considered conditions (FS-type and SF-type). Examples of mirror-like neurons exhibiting diminished activity during action observation have been previously described in F5 pyramidal tract neurons (Kraskov et al., 2009) and in M1 (Vigneswaran et al., 2013; Kraskov et al., 2014). The authors hypothesized that this inhibitory "mirror-like" activity could be involved in the disfacilitation of motoneurons that serve the suppression of unwanted movement during observation of other's actions, and that it could be directly transmitted to other regions of the motor network, including its subcortical components, such as the BG, whose critical role in action inhibition is widely recognized. Cortical and subcortical mechanisms are likely to work together to decouple cortical motor representations from motor output, preventing the observer from automatically re-enacting the observed action (Bonini, 2017). Direct chemical perturbation of putaminal neuron activity with dopamine receptor agonist/antagonist is a necessary step in future studies to provide causal evidence and a deeper understanding of these possible mechanisms.

4.3 Spatial-dependent representation of self and others actions in the putamen nucleus

Similarly to few previous works (Bonini et al., 2014; Caggiano et al., 2009), we implemented an additional condition to the MAT paradigm, in which the setting was identical except the presence of a transparent plastic barrier interposed either between the object and the monkey (i.e. the monkey observed the action performed by the experimenter outside its operational space), or between the object and the experimenter (i.e. an execution condition in which the monkey acted in an operational space that did not include the experimenter). The latter condition was not present in previous studies, moreover, unlike Caggiano and colleagues (2009), we have not investigated the metric representation of space, but only the operational one, which turns out appears to be the main frame of reference in F5 (Bonini et al., 2014). In addition, compared to Bonini and colleagues (2014) paradigm, we did not investigate the no-go condition.

In the present study, most neurons were active during both tasks (Social-Barrier type), moreover, there were also neurons responding exclusively during one of the two tasks (Social type and Barrier type). During observation trials, with respect to execution ones, the fraction of Social-Barrier neurons decreased and there was an increase of Social and Barrier units. Population analysis showed that during execution and observation in Barrier task, there was an increase of non modulated units and, interestingly, only during observation there was an increase of inhibited neurons.

Considering only Self-Other type neurons during the Social task, about half of them were FF-type. Otherwise, when the barrier was added, one third of the neurons was SS-type, another third was FS, there was also a great fraction of FF-type units as well. Applying the same classification to Social-Barrier type-neurons, in monkey's execution trials, more than half of the units were facilitated in both tasks, and another smaller fraction was suppressed in each of them. During the observation trials the neuronal behavior was inverted, since the majority of the units were always suppressed and a smaller fraction was facilitated in both tasks.

Since the most represented neuronal categories was FF and SS-type, we considered only these two latter classes to compare and the modulation during monkey execution and observation of the Self-Other neurons and compare the firing rates during Social and Barrier task of the Social-

Barrier neurons. We found that most Self-Other neurons increased or decreased their firing rate between execution and observation, both during the Social task and during the Barrier task, and most of them discharged more during the execution trials. Among Social-Barrier neurons, the greater fraction of units didn't significantly change their discharge between Social and Barrier task, and a smaller fraction had an higher response in either task. Interestingly, during observation trials the preferred task was the Social one.

These finding may suggest that in the putamen nucleus there are neurons differently modulated by the location of an observed action with respect to the observer's operational space, similarly to previous finding on F5 mirror neurons (Bonini et al., 2014; Caggiano et al., 2009). Clearly, it would also be necessary to more specifically analyze the response to the presentation of the object, and check other factors such as the metric distance and the angle of the experimenter's position with respect to the monkey. Another interesting finding was the presence of neurons differently modulated by the presence or absence of the barrier also during the execution trials, where the unique difference was related to the location of the experimenter inside or outside the monkey's operating space and consequently, the possibility of interacting.

4.4 Conclusions

Despite their preliminary nature, our findings represent one of the first empirical demonstrations of the existence of neurons in the putamen that are specifically modulated by the other's actions. This work supports the hypothesis of an involvement of this subcortical nucleus in the MN network (Bonini, 2017), which appears to be supported by anatomical (Albertini et al., 2022; Gerbella et al., 2015) and human studies (Alegre et al., 2010; Errante and Fogassi, 2020; Ge et al., 2018; Kessler et al., 2006), and highlights the need to investigate its overall modulatory activity on the functioning of the action-observation cortical nodes, as well as its possible functional role. A second finding of this study is that putaminal neurons, as F5 mirror neurons (Bonini et al., 2014; Caggiano et al., 2009) was also modulated by the presence or absence of a transparent barrier located among the object and the

monkey, or the object and the experimenter, suggesting that these neurons may encode the distance of object and actions in an operational framework.

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