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Neural representations of self and others' actions in the monkey putamen nucleus

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ABSTRACT

It is widely accepted that the cortical motor system plays a pivotal role not only in motor control, but also in a variety of advanced perceptual, cognitive, and social functions as well. When observing the actions of others, an extended Action Observation Network (AON) encompassing cortical regions devoted to action planning and execution becomes active. Anatomical data in monkeys demonstrated that areas of the AON send convergent projections to overlapping territories of the putamen nucleus in the basal ganglia, suggesting that this nucleus may not only contribute to the selection of one's own action, but also to the representation of the action of others.

To address this issue, we recorded neuronal activity from 573 single-units in the putamen nucleus of two male rhesus macaques (*Macaca mulatta*) during a Mutual Action Task in which each animal and an experimenter facing the same device in a shared operational space in between them, took turns based on learned contextual cues to grasp or observe the other agent grasping and lifting the same multi-affordance object, which could be grasped either with a precision grip (PG) or with a whole hand prehension (WH).

Most of the recorded neurons (n = 301) exhibited a modulation during the reaching-grasping period of the task performed by the animals, most often with an increase of their firing rate with respect to the baseline. Almost 40% of the motor-related units (n = 120) showed a significantly different discharge between the two examined grip types, with a similar number of neurons exhibiting a preference for PG and WH prehension.

Amongst those neurons whose activity was modulated during the reaching-grasping epoch of the task, we found that the great majority encoded selectively monkeys' own action (self-type, n = 212), a smaller fraction was active only during action observation (other-type, n = 66), whereas the remaining discharged in both conditions (self-other type, n = 89). During active movement facilitated neurons prevailed over suppressed ones, whereas in observation trials we found a balanced number of excited an inhibited cells. Among self-other type neurons, the majority exhibited a "classical" MN activity, being facilitated during both action execution and observation (FF-type, n = 41), however we also recorded a sizeable fraction of cells being consistently suppressed during both conditions (SS-type, n = 26), or that showed opposite discharge patterns depending on which subject was performing the action (FS- and SF-type).

Our findings constitute one of the first empirical demonstrations of the existence of putamen neurons specifically modulated by others' observed actions, supporting the hypothesis of an involvement of the basal ganglia in the AON and indicating the need to causally investigate its overall modulatory impact on the functioning of the cortical AON.

1. INTRODUCTION

The brain plays a crucial role in guiding an organism's interactions with the outside world in order to achieve specific goals. Over the course of evolution, mammals have developed neural circuits that enabled them to adapt their voluntary movements to various environmental conditions, helping them to find food, sexual partners, and to avoid predators. Such a behavioral flexibility maximized mammals' chances of survival. In primates, humans in particular, it has been a fundamental driving force for the development of abilities to transform the external world, profoundly altering the environment and life on our planet.

An organism's repertoire of motor behaviors can be conceived as a hierarchically organized set of levels of control, ranging from the lower level of muscle synergies at the periphery, to the highest level of intentional future plans for goal-directed actions, in the telencephalon. At the lower level, certain muscle groups can be controlled through rhythmic movements as well as reflex mechanisms. Rhythmic movements can also undergo voluntarily control by higher motor centers, which influence the selection and activation of circuits in the spinal cord and in the brain stem defined as "central pattern generators". Reflexes, eventually, are rather stereotypical responses to specific environmental stimuli, generated by simple neural circuits in the spinal cord and in the brain stem, which can adapt to changes occurring in an individual's behavioral goal or the current context but cannot be controlled voluntarily.

Voluntary movements are distinct from reflexes and basic locomotor rhythms in several ways, primarily because they are intentional and oriented to specific behavioral goals. Moreover, voluntary actions involve making choices among multiple alternatives, and their effectiveness improves with experience: indeed, the motor system constantly updates its movement repertoire, making the neural control of voluntary behavior by far more complex than simply generating spatio-temporally organized sequences of muscle activations. Movement is the outcome of extensive processes involving different brain systems that constantly interact in a perception-action cycle, coordinated with the crucial involvement of subcortical systems, like the cerebellum and the basal ganglia.

1.1 The cortical motor system

1.1.1 Neuroanatomy of the frontal motor cortex

For centuries, it was believed that the human cerebral cortex was responsible solely for conscious and higher-order cognitive functions. However, in the mid-19th century, the English neurologist John Hughlings Jackson (1874) proposed that a specific region of the cerebral cortex, situated rostrally to the central sulcus, played a significant role in initiating movement, likewise demonstrated in animal models by Fritsch and Hitzig (1870). Indeed, these researchers observed that electrical stimulation of a specific area of the cortex in anesthetized dogs triggered movements in corresponding regions of the opposite half of the body. Additionally, they found that this cortical area contained a systematically organized motor map of the contralateral half of the body

and was crucial for voluntary control of those body parts, thus confirming its causal role in movement (Fritsch & Hitzig, 2009). Nonetheless, until the latter half of the 1900s, with advancements in anesthesia and aseptic surgical techniques enabling direct experimental research on the cerebral cortex of awake human subjects, conclusive evidence was lacking. Thanks to the pioneering studies of Penfield and Boldrey (1937) by means of electrical stimulation of human patients' cortex during awake surgeries (Penfield & Boldrey, 1937), the original experimental evidence obtained in dogs and other animal models could be extended to humans, demonstrating that a well-defined region of the cerebral cortex rostral to the central sulcus is dedicated to motor function.

Shortly after, Woolsey identified a similar cortical map in monkeys, revealing a highly somatotopic representation of motor functions (*Figure 1*). This organization remained consistent across species, including humans (Woolsey et al., 1952), as showed by the Canadian neurosurgeon Wilder Penfield who demonstrated the presence of a somatotopic map in human patients through electrical stimulation of the cortex during awake surgeries. In addition, he noted that the stimulation of the motor cortex could trigger or inhibit movement, as well as induce muscle relaxation. These experiments established the crucial role of this cortical area in generating movement and led to its designation as the motor cortex.

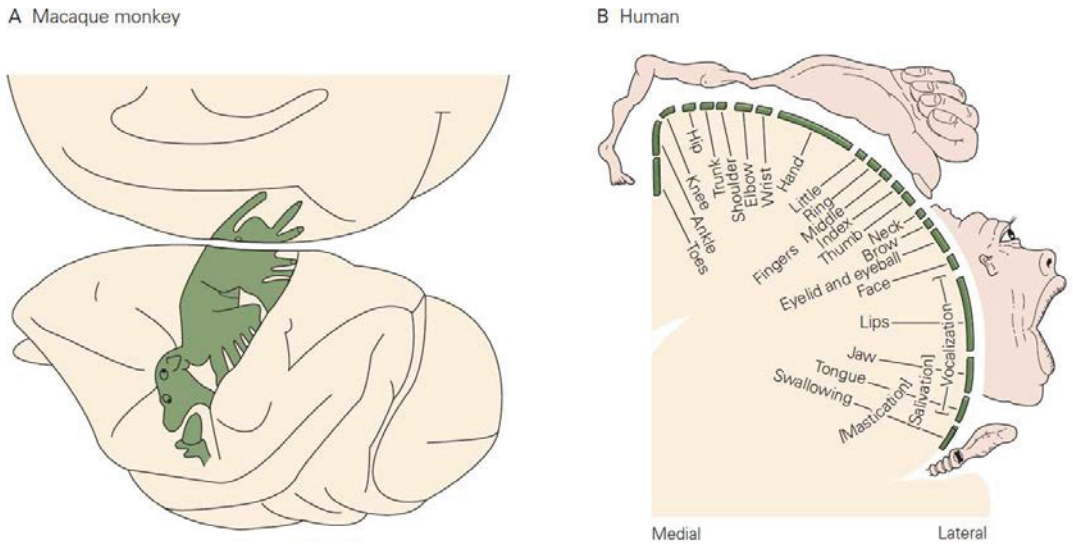

Figure 1 | Somatotopic organization of the motor cortex. (A) Lateral and mesial view of somatotopic organization of motor areas in monkey brain. **(B)** Somatotopic organization in human motor cortex from a coronal section. *Figure from Kandel et al., 2021.*

In parallel, classical histological investigations conducted at the beginning of the 20th century by Korbinian Brodman and Alfred Campbell revealed that the primate motor cortex, also known as the precentral gyrus in primates, could be subdivided into two distinct regions based on their different cytoarchitecture. These areas are referred to as the primary motor cortex (Brodmann area 4) and the premotor cortex (Brodmann area 6). Further research led by Woolsey and his colleagues allowed for a more

detailed subdivision of the premotor cortex into two parts: a medial portion, known as the supplementary motor area, and a lateral portion, as shown in *Figure 2A*. Subsequently, these areas were further explored, leading to the identification of smaller functional subdivisions within the premotor cortex and supplementary motor area, which had already been observed in non-human primates (*Figure 2B*).

Currently, in non-human primates' maps of the precentral cortex, Brodmann area 4 (primary motor cortex) roughly corresponds to F1 area, while Brodmann area 6 is further divided into three main regions: mesial, dorsal and ventral. Each of these regions has been additionally categorized into its rostral and caudal segments on the basis of their distinct cytoarchitectonic features and electrophysiological characteristics (Matelli et al., 1985, 1991): the mesial regions F3 and F6, the dorsal regions F2 and F7, and the ventral regions F4 and F5.

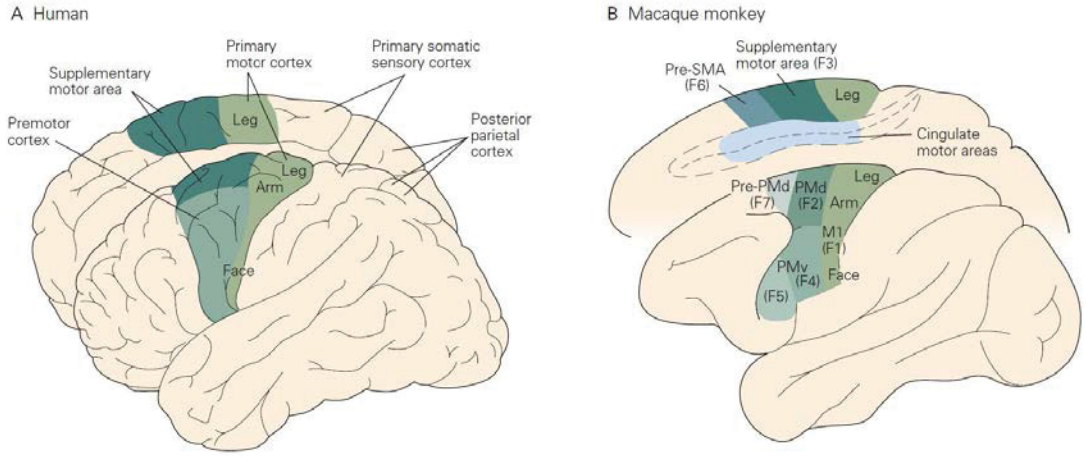

Figure 2 | Somatotopic organization of motor areas. (A) Lateral and mesial view of Human brain. **(B)** Lateral and mesial view of Macaque brain. *Figure from Kandel et al., 2021.*

Intracortical stimulations of these areas demonstrated that with brief trains of electrical pulses, F3 exhibits a complete representation of contralateral body movements (Luppino et al., 1991), whereas prolonged stimulation of F6 induces slow and complex movements of the face, arm and hand (Lanzilotto et al., 2016). Notably, the most caudal portion of PMd (area F2) hosts a roughly somatotopic representation of the hand, wrist and forearm along the rostro-caudal direction (Raos et al., 2003), whereas the rostral area F7 is poorly excitable, particularly in its rostral portion and is deemed to play a role in distributing attention by contributing to eye movements (Luppino et al., 1991). In the PMv, both F4 and F5, when stimulated, give rise to movements involving the arm, hand, and mouth, arranged in a rough dorso-lateral direction (Gentilucci et al., 1988, 1989).

The presence of multiple cortical motor areas may appear redundant if their unique purpose were to initiate or coordinate muscle activity. However, it is important to highlight that neurons in these areas possess specific features and collaborate with each other to support various functions, such as selecting,

planning, and generating motor actions optimized for the specific context in which they are performed. Therefore, to gain a comprehensive understanding of the motor system, it is essential to combine the study of local neuronal properties with that of their anatomical connections.

1.1.2 Anatomy and physiology of parieto-frontal networks

Each area of the motor cortex establishes connections both with other motor regions (referred to as intrinsic connections) and with cortical areas beyond the agranular frontal cortex (extrinsic connections), as depicted in *Figure 3*. Additionally, these motor areas send projections to subcortical centers and the spinal cord (referred to as descending projections).

Recent studies have revealed significant distinctions in connectivity between the posterior (F2, F3, F4 and F5) and anterior (F6 and F7) premotor areas (Rizzolatti & Luppino, 2001). The formers receive rich parietal projections and are directly connected to the primary motor cortex (F1), with the exception of the most rostral portion of F5, called F5a, which exhibits a more prefronto-dependent pattern of connections (Gerbella et al., 2011); in contrast, anterior premotor areas are richly connected with prefrontal and cingulate cortices and do not send projections to F1, but rather have extensive connections with posterior premotor areas.

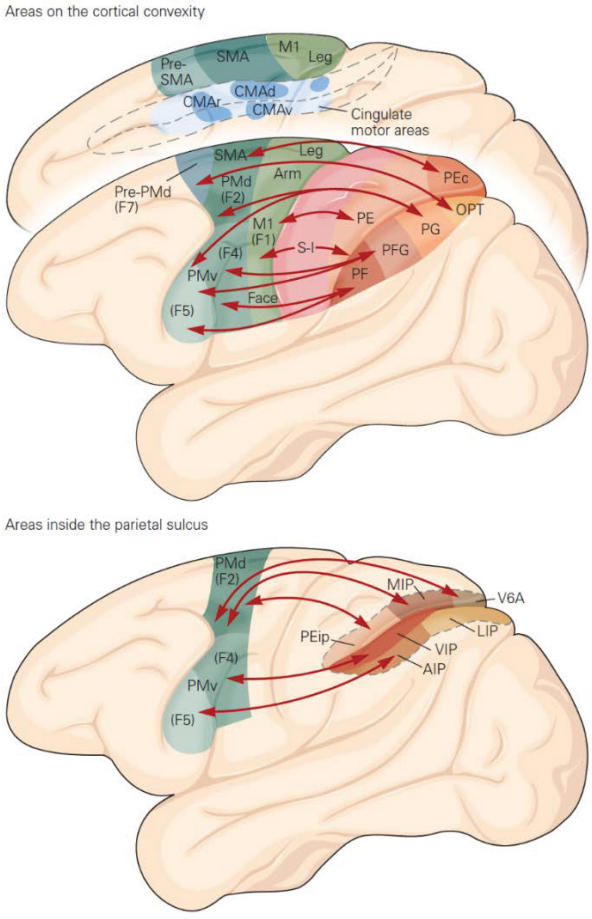

Figure 3 | Sketch of fronto-parietal reciprocal connections supporting voluntary movement. (A) The figure shows the lateral perspective of the Macaque brain. The motor cortex is visually highlighted using different shades of green to emphasize its

cytoarchitectonic structure. Similarly, the cytoarchitectonic divisions of the parietal cortex are indicated with varying shades of red. PMd, dorsal premotor; PMv, ventral premotor; MIP, medial intraparietal area; LIP, lateral intraparietal area; VIP, ventral intraparietal area; AIP, anterior intraparietal area. All the remaining acronyms are defined as per the specifications outlined in Pandya and Seltzer work (Pandya & Seltzer, 1982). *Figure from Kandel et al., 2021.*

An analogous division between posterior parieto-dependent areas and anterior prefronto-dependent ones, can be observed at the level of the descending efferent projections: F2, F3 and certain portions of F4 and F5 contribute with the primary motor cortex (F1) to generate the cortico-spinal tract, whereas F6 and F7 do not establish direct connections with the spinal cord indicating that, unlike the posterior areas, their influence on movement planning and control is more indirect and mediated by subcortical pathways and other premotor areas. Notably, fibers originating from F1 terminate in the intermediate region of the spinal cord as well as in lamina IX, where motor neurons are located; descending projections from the other posterior premotor regions (F2 and F5) predominantly target the intermediate spinal cord region, not the motor neurons. The former are involved in fine-tuning distal movements, while the latter contribute to a more global control of movement.

The main function of the parietal lobe revolves around processing sensory information for guiding the motor behavior, based on the transformation of sensory into motor signals (*sensorimotor transformations*). This process is supported by robust and mutual connections with the premotor cortex (Borra & Luppino, 2017), which differently involves the two main subdivisions of the posterior parietal cortex (PPC): the superior (SPL) and inferior (IPL) parietal lobules, divided by the intraparietal sulcus (IP), as depicted in *Figure 4*.

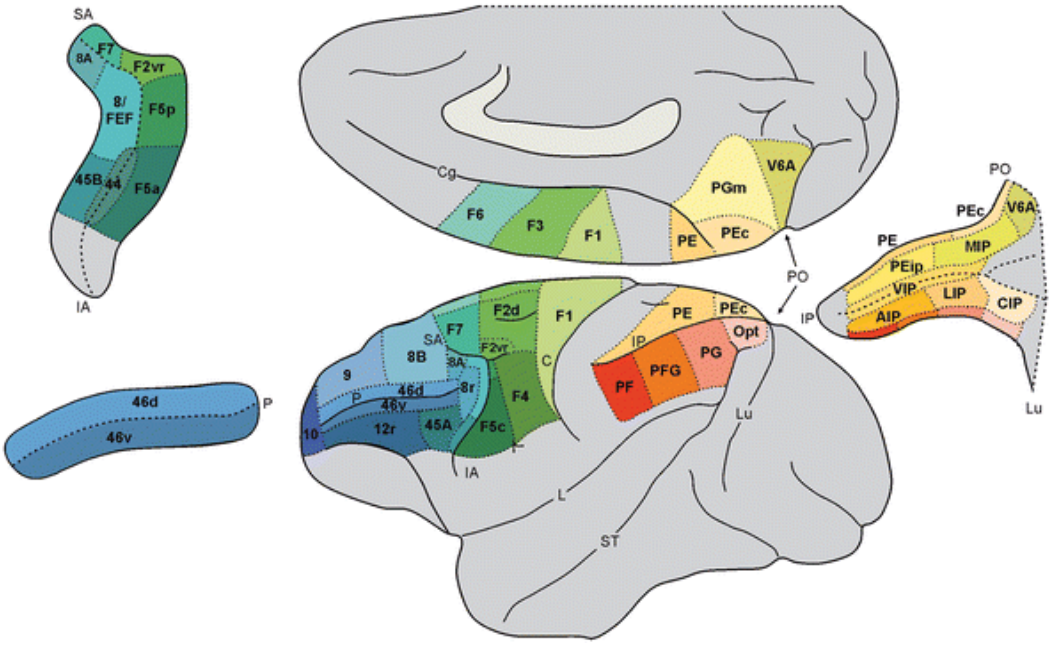

Figure 4 | Lateral and mesial views of the macaque brain showing parcellation of the frontal and posterior parietal cortex. The prefrontal cortex is subdivided according to Carmichael and Price (1994), except for its caudo-ventral part (Gerbella et al., 2007). Agranular frontal areas are classified according to Matelli et al. (1991) and Belmalih et al. (2009). The parietal areas are named

according to Pandya and Seltzer (1982). The areas located within the arcuate and the principal sulci are shown in an unfolded view of the sulci in the left part of the figure, and the areas located within the intraparietal sulcus in the right part of the figure. *Dashed lines* indicate the architectonic borders. C, central sulcus; Cg, cingulate sulcus; IA, inferior arcuate; L, lateral fissure; Lu, lunate fissure; P, principal sulcus; PO, parieto-occipital sulcus; SA, superior arcuate; ST, superior temporal sulcus.

The PPC is predominantly dedicated to the analysis of various high order sensory information, particularly with an emphasis on visual information. One primary objective of this form of processing is to use sensory inputs to guide motor actions, achieved through the integration of sensory and motor signals via robust and reciprocal connections with the motor cortex (including the primary motor and premotor areas) (Rizzolatti et al., 1998; Caminiti et al., 2015), as depicted in *Figure 5*. This integration of sensory and motor information constitutes the basis of a neural mechanisms in which automatically processed sensory inputs trigger the activation of representation of "potential actions" within the motor cortex (Cisek, 2007; Cisek & Kalaska, 2010; Rizzolatti & Luppino, 2001).

This specific anatomical segregation implies a corresponding functional specialization. In fact, based on physiological studies, the primary motor area F1 receives inputs from area PE (area 5), which is a somatosensory region dedicated to the processing of proprioceptive information. This data suggest that the role of PE-F1 circuit is to provide F1 with information concerning the precise position of body parts necessary for movement control (Borra & Luppino, 2017).

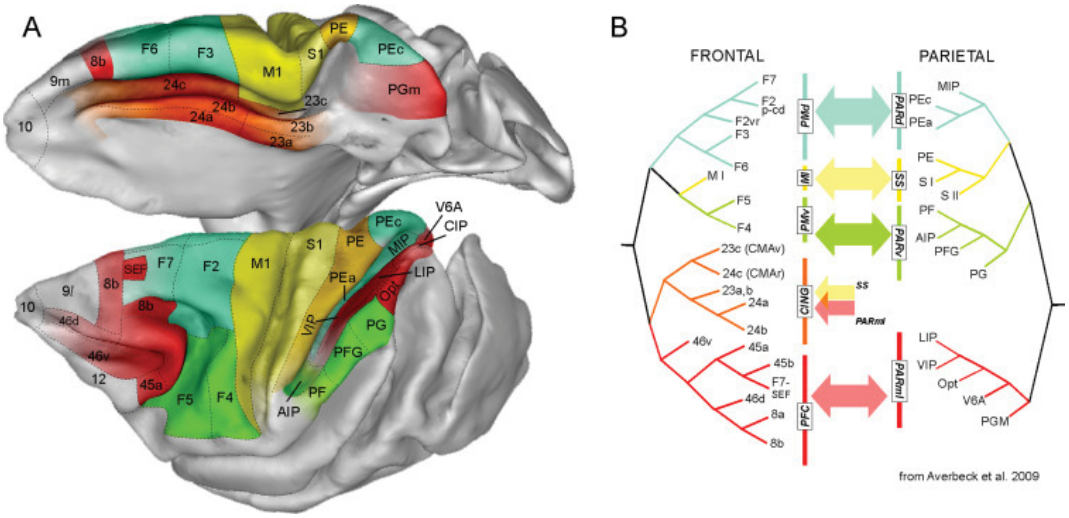

Figure 5 | Parieto-frontal processing streams in macaque monkey. (A) Parcellation of parietal and frontal areas based on parieto-frontal connectivity. Similar colors in parietal and frontal cortex identify cortico-cortically connected areas. Sulci are opened to show approximate location and extent of cortical areas buried in their walls **(B)** Cluster of parietal and frontal areas based on their cortico-cortical input. Different clusters are shown in different colors according to the dominant connections (arrows) of each parietal cluster with frontal clusters. The color of each cluster corresponds to the color of the cortical areas belonging to that clusters, as shown in (A). *Figure from Caminiti et al., 2015*.

The dorsal premotor area F2 establishes reciprocal connections with the medial intraparietal area MIP (specifically F2vr), area PE and area PEip (specifically F2d). These circuits appear to be involved in

monitoring and controlling arm movements during the transport phase towards objects (reaching movements) on the basis of somatosensory and visual information (Bakola et al., 2010).

Regarding the ventral premotor cortex, area F4 establishes a direct link with the ventral intraparietal area VIP, in addition to areas PF and PFG (Rozzi et al., 2006), which are involved in the control of arm and hand movements directed to make or avoid contact with looming or moving objects (Yokochi et al., 2003), or with the subject's own body, in the animal's peripersonal space (Graziano & Gross, 1993). In contrast, the ventral premotor area F5 exhibits reciprocal connections with the anterior intraparietal area AIP (Borra et al. 2008), and research demonstrated that the AIP-F5 pathway plays a crucial role in facilitating the visuomotor transformations necessary for organizing grasping movements; in fact, reversible inactivation of both areas causes altered hand shaping during visually-guided grasping (Gallese et al. 1994; Fogassi et al. 2001).

Lastly, the circuit consisting of the lateral intraparietal area LIP and the frontal eye fields (FEF), is responsible for the control of rapid eye movements aimed at directing the fovea to a specific target (Andersen & Cui, 2009).

1.1.3 The cortical mirror neuron network

The discovery of mirror neurons (MNs) - a group of visuomotor neurons that activate both when performing specific purposeful motor actions (like grasping and object) and when observing another individual performing the same actions – contributed to the development of a new view on the general role of the motor system. In fact, it is now widely believed that parieto-frontal circuits are not limited to the mere motor control of actions but also play a role in various perceptual and cognitive processes that include object (Murata et al., 1997) and space coding (Fogassi et al., 1996; Graziano et al., 1994), imitation (Iacoboni et al., 1999), motor learning (Buccino et al., 2004) and interindividual communication (Mooney, 2014; Prather et al., 2008).

Neurons exhibiting mirror-like properties were first detected in the macaque premotor area F5 (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996). Subsequently, neurophysiological studies identified neurons with mirror properties in a network of interconnected brain regions, encompassing the ventral premotor area F5, known as "cortical mirror network" (*Figure 6*). These regions include the inferior parietal lobule (Bonini et al., 2010; Fogassi et al., 2005), in particular area AIP (Lanzilotto et al., 2019; Maeda et al., 2015; Pani et al., 2014), the dorsal premotor cortex (Cisek & Kalaska, 2004; Papadourakis & Raos, 2017; Tkach et al., 2007), and the medial frontal cortex (MFC), specifically the pre-supplementary motor (pre-SMA) and anterior cingulate cortex (Livi et al., 2019; Mukamel et al., 2010; Yoshida et al., 2011). In addition, neurons with mirror properties have been observed also in the primary motor cortex (Dushanova & Donoghue, 2010; Tkach et al., 2007; Vigneswaran et al., 2013). Furthermore, both anatomical (Borra et al., 2011; Gerbella et al., 2013) and functional evidence (Nelissen et al., 2011) suggest that even the ventrolateral

prefrontal cortex (VLPF) may host neurons exhibiting mirror-like properties (Bonini, 2017; Simone et al., 2017).

The cortical regions that form this network do not operate in isolation, but they work in concert with subcortical nodes such as the cerebellum and the basal ganglia (BG). Although the presence of neurons with mirror properties in both these structures has not been proved yet, numerous pieces of evidence suggest that these structures, particularly the BG, play a role in the *extended MN network* (Alegre et al., 2010; Bonini, 2017; Bonini et al., 2022; Caligiore et al., 2013; Gerbella et al., 2016; Kessler et al., 2006).

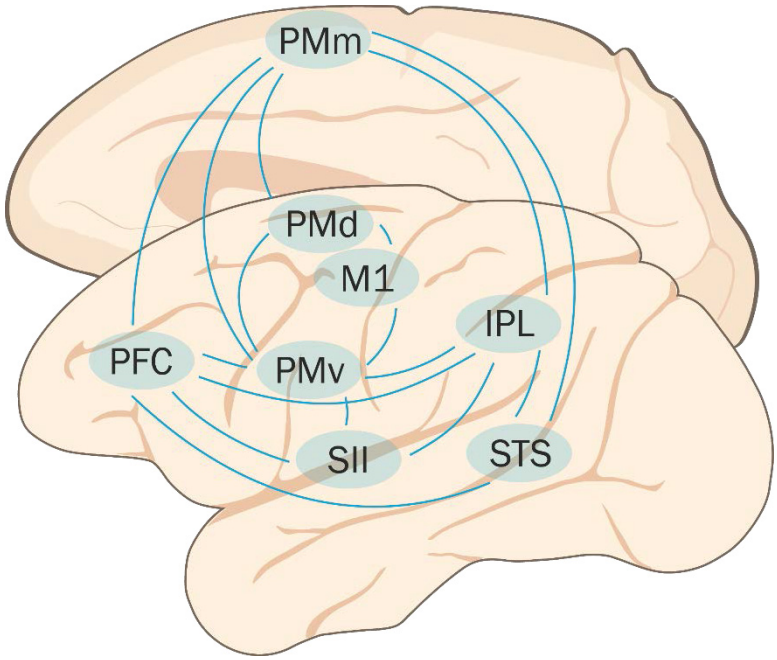

Figure 6 | Mirror networks in the primate brain. Lateral and mesial view showing the organization of the primate sensorimotor MN networks based on macaque neuroanatomical studies on areas in which neurons with mirror properties have been found. IPL, inferior parietal lobule; PMd, dorsal premotor cortex; PMm, mesial premotor cortex; PMv, ventral premotor cortex; M1, primary motor cortex; PFC, prefrontal cortex; SII, secondary somatosensory cortex; STS, superior temporal sulcus. *Figure modified from Bonini et al., 2022.*

Thirty years after the discovery of MNs, there is still an intense debate regarding their origin and functions (Bonini et al., 2022; Casile et al., 2011; Cook et al., 2014). According to the initial hypothesis, they were proposed as a possible neural basis for action recognition and others' intention understanding by automatically matching the visual description of other's observed actions with the corresponding motor representations within the observer's repertoire of motor actions (Gallese et al., 1996; Rizzolatti and Sinigallia, 2006). However, more recent perspective propose their potential involvement in anticipating and predicting others' behavior in order to select appropriate motor responses to observed actions during social interactions (Bonini et al., 2022; Bonini & Ferrari, 2011; Orban et al., 2021; Pomper et al., 2023).

1.1.4 Mirror neurons coding of one's own and others' actions

Despite the question about the functional role of MNs still lacks a definitive answer, at present, several authors concur regarding the significance of early experiences of infants with their own actions, which serve as a crucial source of information for shaping sensorimotor development by potentially endowing originally motor neurons with mirror properties (Casile et al., 2011; Del Giudice et al., 2009; Press et al., 2011; Tkach et al., 2008). Until single unit recording during early stages of development is available, the ontogeny of MNs will continue to be an open issue. However, it is reasonable to assume that visual feedback from one's own hand remains a particularly relevant stimulus for the discharge of MNs in adulthood, making them (or some of them) capable to exhibit sensitivity to the sight of one's own actions in addition to those of others (Bonini, 2017).

On one hand, Sakata and his research team observed a specific class of neurons termed "*visual dominant non-object type neurons*" in area AIP that only fired when the monkey grasped an object in full light but not in the darkness or when the object was merely visually presented, suggesting their specialized role in processing the visual feedback of monkey's own hand during grasping actions (Sakata et al., 1995). Subsequent studies (Maeda et al., 2015; Pani et al., 2014) have shown that inferior parietal (mostly AIP) neurons can also become active when a monkey observes another's hand grasping an object from a subjective point of view, even if the target object is digitally deleted, demonstrating that these cells exhibit specific tuning to own-hand visual feedback. Similarly, Maranesi and colleagues tested MNs in area F5 when monkeys grasped and object and while they were observing an experimenter grasping the same object, in the same spatial position, from a subjective viewpoint. Crucially, action execution response was tested during both grasping in the dark and in the light, showing generally stronger discharge during grasping in the light compared to dark (Maranesi et al., 2015). These results indicate a particular sensitivity of MNs to the sight of monkey's own hand, even when contrasted with grasping neurons lacking mirror properties simultaneously recorded at the same cortical sites. Furthermore, the increased activity of MNs induced by visual feedback from the monkey's own hand positively correlated with their net activity during action observation.

Additionally, Yoshida and colleagues identified a group of neurons in the macaque frontal cortex that actively monitored and responded to errors made by other individuals during social interactions, suggesting a potential role in learning from others' behavior and a contribution to the subsequent control of one's own behavior (Yoshida et al., 2012). This aligns with previous models concerning the possible role of MNs in monitoring one's own actions (Bonaiuto & Arbib, 2010) and predicting those of others (Keysers & Gazzola, 2014). Altogether, these studies collectively suggest the existence of a substrate that can process visual information from both one's own and another individual's action in a similar manner supporting the idea that MNs play a crucial role in action monitoring and social learning.

1.1.5 Regulation of motor resonance phenomena: cortical and sub-cortical mechanisms

The MN network serves the purpose of not only monitoring an individual's own action, but also mapping the actions of others onto one's own motor representations. This unique ability raises an important question: how does the brain prevent us from automatically imitating the actions we observe, if our motor system is – at least partially – recruited as when we actively plan and perform our own actions?

Despite possessing motor properties, MNs do not seem to have a primary motor function. Indeed, several studies in human and monkeys evidence the absence of EMG activity during action observation (Kraskov et al., 2009). Thus, as a matter of fact, the output of MNs must be decoupled from the motor output. This can occur via cortical as well as subcortical mechanisms.

First, the motor response of MNs may represent an efference copy, rather than an efferent signal, that the extended MN network makes available for carrying out perceptual, cognitive, and even social functions (Bonini, 2017): this hypothesis derives from neurophysiological studies in songbirds showing that audio-vocal mirror neurons in birds' premotor-like structures are causally related to a projection from vocal centers, and take part in perceptual and learning phenomena within a circuits involving a basal-ganglia-like structure (Mooney, 2014; Prather et al., 2008). Notably, there is compelling evidence indicating that the avian counterpart of the BG (referred to as area X) receives inputs from a specific subset of projection neurons situated within the telencephalic nucleus HVC, which is recognized for its crucial role in normal song perception and learning and for housing auditory-vocal MNs (*Figure 7*).

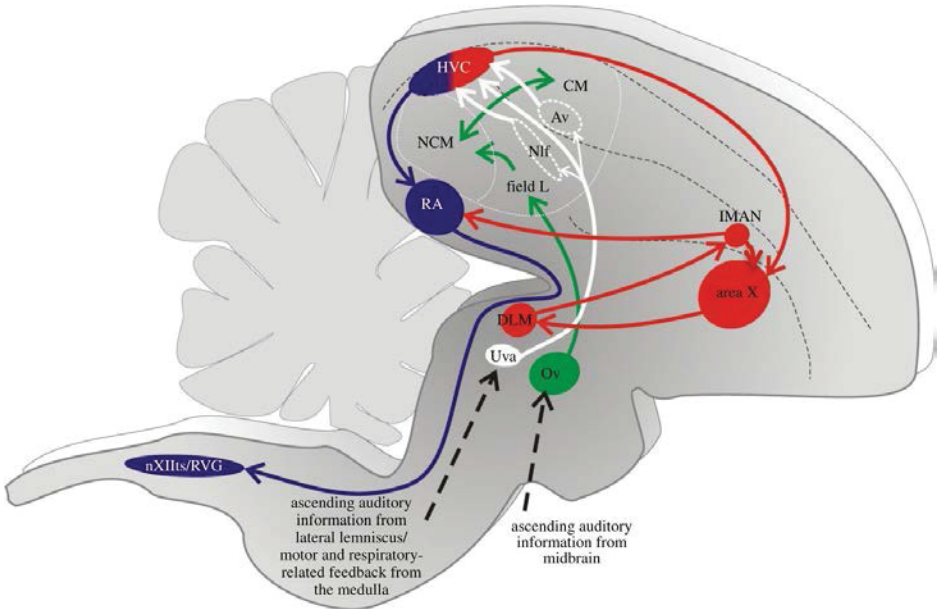

Figure 7 | A schematic of the song system emphasizing HVC and its connections. This parasagittal view of the songbird brain shows the song motor pathway (blue) and anterior forebrain pathway (red), the ascending auditory pathways (green) and the auditory inputs to HVC (white). At the microscopic level, HVCX nad HVCA cells are randomly intermingled within HVC. Av, nucleus avalanche; CM, caudal mesopallium; DLM, medial part of the dorsolateral thalamic nucleus; HVC, abbreviation used as proper name; LMAN, lateral magnocellular nucleus of the anterior nidopallium; NCM, caudomedial nidopallium; NIf, nucleus interface; OV, nucleus ovoidalis; RA, robust nucleus of the arcopallium; Uva, nucleus uvaeformis; VRG, ventral respiratory group; nXIIts, tracheosyringeal division of the huypoglossal nucleus. *Figure from Mooney (2014)*.

However, the specific mechanisms enabling the decoupling of MNs motor response from actual motor output, in primates, remain a topic of ongoing investigation, but could be formalized in a high probability to find neurons with mirror properties among corticostriatal, rather than corticospinal, cells.

Interestingly, evidence have been provided for a possible gating mechanism at the cortical level. In fact, classical MNs exhibit activity both when an action is executed and when it is observed. However, among those MNs whose axons contribute to the pyramidal tract, known as pyramidal tract neurons (PTNs) identified by antidromic electrical stimulation of their axons at the level of the bulbar pyramids, there are two distinct activation patterns during action observation. Some neurons display the "classical" MN activation pattern, known as *facilitated MNs*, increasing their firing both when actions are executed and observed (*Figure 8A-H*). However, in area F5 (Kraskov et al., 2009) and M1 (Vigneswaran et al., 2013), other MNs exhibit a different pattern: they increase their firing during action execution but show suppressed activity when observing actions, termed *suppressed MNs* (*Figure 8K-R*).

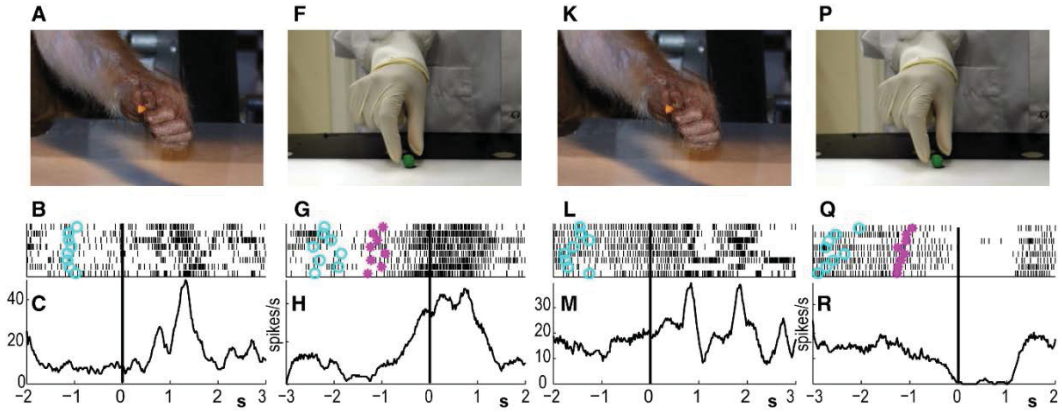

Figure 8 | Examples of two different types of response in F5 PTNs. (A and K) Photo of monkey grasping a piece of food in a precision grip; **(F and P)** photo of experimenter grasping a piece of food in precision grip; **(B and L)** raster plots for two PTNs during self-grasp aligned to cue for onset of reach-to-grasp movement (indicated by black vertical lines); data from ten successive trials are shown. **(G and Q**) raster plots for two PTNs during ten trials of mirror testing aligned to the moment of contact of the experimenter's hand with the target object (indicated by black vertical lines). Light-blue circles on each trial indicate beginning of baseline interval for each trial (experimenter's hand motionless in full view of monkey), and magenta asterisks indicate beginning of experimenter's movement toward the object. **(C, H, M, and R**) Average firing rates based on rasters above (spikes/s). *Figure from Kraskov et al., 2009*.

Kraskov and colleagues also described a category of F5 neurons they referred to as *non-mirror PTNs*. Interestingly, this last class of cells displayed the same suppression pattern observed in suppression MNs during action observation, although this phenomenon was not formally analyzed by the authors. These findings led to an important conclusion: regardless of whether PTNs neurons in area F5 are classified as "mirror" or not, they generally decrease their overall corticospinal output when observing actions. Vigneswaran and colleagues (2013) extended these observations to the area M1, which plays a fundamental role in movement production, demonstrating the existence of facilitation and suppression MNs there as well and concluding that there is a consistent "disfacilitation" in corticospinal output from M1 during action observation.

Although direct evidence of this assumption is still lacking, indirect evidence supports this conclusion. In fact, deoxyglucose labeling studies showed that when grasping is physically performed, there is an increase in glucose consumption in the spinal forelimb representation on the same side as the limb used for the action. However, when grasping is merely observed, there is a bilateral reduction in glucose utilization within the same spinal region. This is likely attributed to a decreased corticospinal signal, as proposed by Nudo and Maserton in 1986, which strengthens the idea that, while present, the overall corticospinal output during action observation does not reach the threshold required to trigger overt movements (Nudo & Masterton, 1986; Schieber, 2011).

Furthermore, new findings from recent studies on monkeys conducted in various parietal and frontal brain regions suggest that the typical MNs may display spike shapes that are considered associated with inhibitory interneurons (INs), thus implying the possible existence of "mirror interneurons" (Ferroni et al., 2021), which could contribute to actively inhibit other (maybe primarily motor) neurons during action observation. Consequently, a diverse range of cell types seems to play a role in encoding information related to the actions of others within brain regions primarily devoted to the processing of one's own actions, both with cortical and subcortical mechanisms.

Indeed, subcortical mechanisms might also play a role in disconnecting the activity of cortical neurons from the actual motor output. For instance, it is conceivable that certain spinal interneurons could locally inhibit spinal motor neurons during motor preparation, action observation and imagery processes (Fetz et al., 2002). Similarly, there is substantial evidence indicating that cortico-basal ganglia-thalamo cortical loops are involved in suppressing undesired movements. From an anatomical perspective, corticostriatal neurons (CSNs) in layer 2/3 and the upper layer 5 of M1 often project to other cortical regions (referred to as intra-telencephalic neurons or ITNs; Reiner et al., 2010), while it has been addressed that, at least in rodents, some PTNs selectively project to the striatum (Cowan & Wilson, 1994; Molnár & Cheung, 2006; Shepherd, 2013). Furthermore, impairments in the cortico-basal ganglia-thalamo cortical circuits appear to affect PTNs more than ITNs (Pasquereau & Turner, 2011). This implies that PTNs are particularly sensitive to the facilitation or disfacilitation effects brought about by BG activity.

Building on these considerations, it can be hypothesized that specific INs and CSNs with mirror properties in both the primary motor and premotor cortices might receive efference copies from PTNs (Ferroni et al., 2021), as suggested by the findings of research on audiovocal MNs in songbirds (Mooney, 2014), and play a role in refining the response of PTNs and CSNs endowed with mirror properties. INs might participate in shaping the response of PTNs and CSNs with mirror properties due to the influence of additional sensory-driven-feed-forward signals. This phenomenon could explain the overall decreased (or completely suppressed) premotor activity recorded during action observation (Bonini et al., 2014). Furthermore, CSNs MNs may actively suppress PTNs output during action observation by recruiting D_2-expressing striatal neurons in the indirect pathway (*Figure 9*). If this were the case, local injections of D_2 receptor agonists should

selectively alter the activity of striatal neurons exhibiting action observation responses, ultimately resulting in an overall reduction in thalamic facilitation of PTNs during action observation (Bonini, 2017).

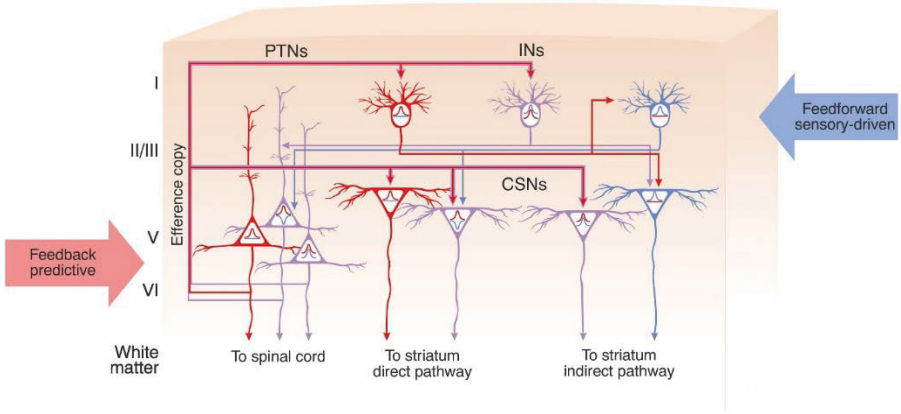

Figure 9 | Schematic of the established and hypothesized anatomo-functional classes of cells and local circuitries of the mirror mechanism. Predictive feedback signals from prefrontal and mesial frontal regions (red arrow) contribute to the selection and activation of pyramidal tract neurons (PTNs; upward-pointing triangles) and corticostriatal neurons (CSNs; downward-pointing triangles) for action execution. The curves inside each neuron illustrate the discharge modulation during action execution (red) and observation (light blue). PTNs can display purely motor (red) or mirror (purple) properties, and the latter exhibit either a facilitated or a suppressed response during action observation (Jerjian et al., 2020; Kraskov et al., 2009). Efference copies of PTNs' output may be fed to interneurons (INs) with mirror properties and CSNs. INs may contribute to inhibitory sculpting of the response of PTNs and CSNs endowed with mirror properties, as a result of the contribution of additional sensory-driven feed-forward signals (light-blue arrow), which may explain the overall reduced (or even suppressed) premotor activity typically recorded during action observation. In addition, CSNs MNs may contribute to the selectively suppression of PTN output during action observation by recruiting D_2-expressing striatal neurons of the indirect pathway, thereby functionally decoupling mirror activity from the descending motor output and contributing to the selection of potential motor responses afforded by the observation of actions performed by others (Orban et al., 2021). *Figure from Bonini et al., 2022*.

The evidence collected thus far suggests the need to further investigate the presence of neurons with responses to others' observed actions in the BG, to verify the hypothesized cortico-subcortical mechanisms just described.

1.1.6 From agent-shared to agent-based coding of other's action

The first criteria used to designate neurons as "mirror neurons" according to the pioneering studies of the '90s (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996) were quite restrictive. In particular, a neuron could be labeled as "mirror" if (1) it responded selectively to others' actions (not to visually presented objects, non-biological movements, or tool actions); (2) it activated during action execution in the dark; (3) it displayed a clear relationship between visual and motor responses. For many years, the focus was on precise alignment between visually and motorically coded actions, suggesting that individual neurons faithfully *mirrored* observed actions onto the neural substrates devoted to encoding one's own actions. However, recent studies challenged this view, showing that congruence in the discharge of mirror neurons mostly arises from neuronal populations rather than individual neurons' activity (Mazurek et al., 2018; Papadourakis & Raos, 2019), tweaking the concept of an exact "mirror" representation of observed actions.

Subsequent studies expanded the original criteria, highlighting agent-based coding over agent-shared coding (Livi et al., 2019). Specifically, they emphasized neural selectivity for information related to others. Despite being overlooked for years, a notable fraction of F5 neurons (i.e. ~20% of the neurons responding during action observation, see Gallese et al., 1996) respond during others' action observation without displaying any motor response during action execution. These "other-related" neurons have been found in various brain areas and animal species (Livi et al., 2019; Mukamel et al., 2010; Viaro et al., 2021; Yoshida et al., 2011) and generally reside in brain structures primarily focused on self-related information processing, often coexisting with neurons selective for the self or exhibiting self-other properties (Bonini et al., 2022).

The function of neurons coding other's actions (regardless of whether other-related coding are matched or not with self-related ones) is still not clear, but it has been suggested that other-selective neurons may play a role in social learning and planning proper behavioral responses to others' actions across various social domains and contexts (Bonini et al., 2022).

1.2 The basal ganglia

Anatomical data on macaques (Gerbella et al., 2016) demonstrated that the above-mentioned areas belonging to the cortical MN network (i.e, area AIP/PFG, PMv, and VLPF) do not operate in isolation, but send convergent projections to specific regions of the putamen, the cortico-recipients BG nucleus recognized for its well-established motor functions (*Figure 10*). While the existence of single-neuron responses related to the observation of other's actions in the BG has not been reported so far, these anatomical observations, along with indirect functional evidence in humans (Alegre et al., 2010; Errante & Fogassi, 2020; Kessler et al., 2006), strongly support the idea that the BG should be considered part of an extended cortico-subcortical MN network (Caligiore et al. 2013; Bonini, 2017; Bonini et al., 2022).

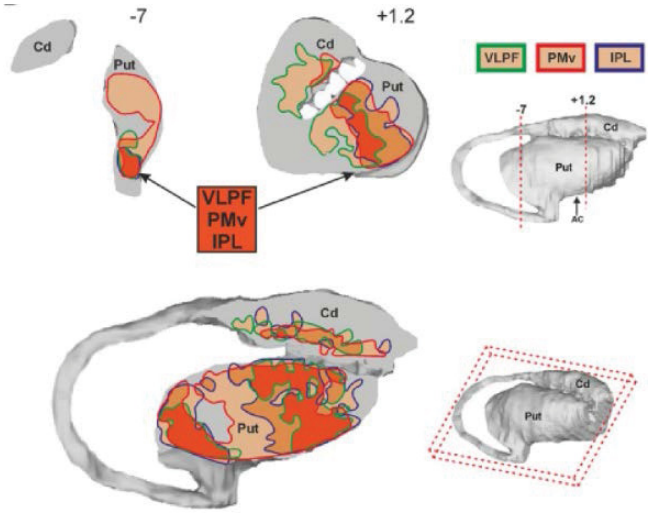

Figure 10 | Territories of the BG receiving projections from the areas belonging to the cortical MN network, namely PMv (indicated by red borders), IPL (marked with blue borders) and VLPF (outlined in green borders). The varying shades of orange represent regions within the BG where to or even all three of these distinct sources of corticostriatal projections overlap. The coordinates (-7 and +1.2) mentioned in the top right panel denote the anteroposterior positions of the two BG slices depicted on the left. Put, putamen; Cd, caudate nucleus. *Figure from Gerbella et al., 2015*.

1.2.1 Structural and functional architecture of the basal ganglia

The BG complex refers to a system of symmetrical, interconnected subcortical nuclei traditionally known for their involvement in motor control, as well as in motor learning, executive functions, and emotions (Grillner & Robertson, 2016; Ward et al., 2013). The term *basal ganglia*, in its strictest sense, refers to a pair of nuclei situated deep within the cerebral hemispheres, namely, the striatum, composed by the caudate and putamen nuclei, and the globus pallidus, divided into an internal and external segment. Additional related nuclei belonging to the system include the subthalamic nucleus (STN) and the substantia nigra (SN) (*Figure 10*). These nuclei are traditionally classified into *input* nuclei, *output* nuclei and *intrinsic* nuclei, according to their main connections with cortical and subcortical areas or the thalamus.

The striatum and STN serve as input stations, receiving incoming signals directly from cortical, nigral and thalamic structures. On the other hand, the external segment of the globus pallidus (GPe) and the substantia nigra pars compacta (SNc) are intrinsic nuclei, functioning as intermediate points in the information-processing pathway. Finally, the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) act as output nuclei, projecting signals outside the BG, primarily to the thalamus (ventral nuclei). From there, the information is relayed back to the cortex, forming interconnected loops known as the cortico-basal ganglia-thalamo-cortical circuits (Alexander et al., 1986; Parent & Hazrati, 1995) (*Figure 11*).

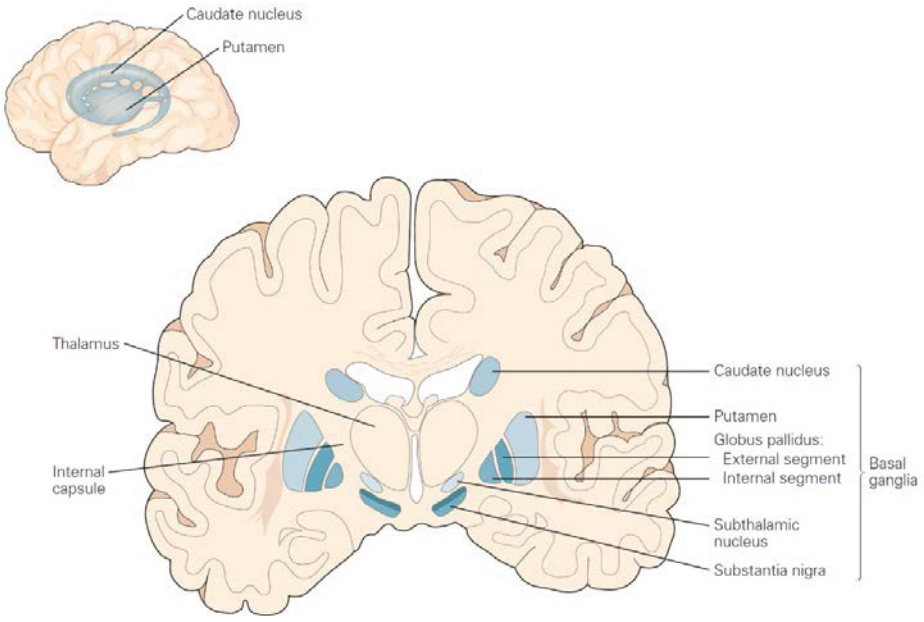

Figure 11 | The basal ganglia nuclei and surrounding structures. The top-left image depicts a parasagittal cross-section of the brain, illustrating the rostrocaudal extent of the caudate nucleus and putamen. The bottom-right image is a coronal brain section, displaying the arrangement of various nuclei. *Figure from Kandel et al., 2021*.

Some signals are also directed towards brainstem motor centers responsible for tasks like eye movements, spatial orientation, or posture. These signals reach the thalamus via additional pathways, often in the form of efference copies (Grillner & Robertson, 2016). It is important to note that these nuclei lack direct connections to the spinal cord. While most cortical regions contribute sending inputs to the basal ganglia, thalamic feedback primarily targets frontal cortical areas, including the prefrontal, premotor and supplementary motor regions (Alexander & Crutcher, 1990).

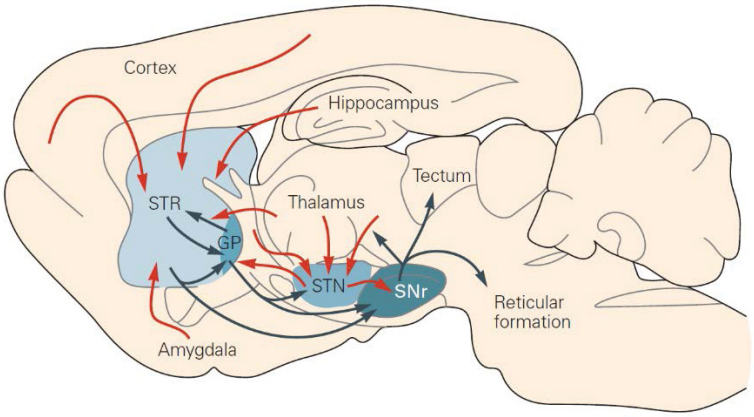

Figure 12 | The principal input, intrinsic and output connections of the mammalian basal ganglia. The primary input nuclei consist of the striatum (STR), subthalamic nucleus (STN) and the substantia nigra pars compacta (not displayed in the image). These nuclei directly receive inputs from various sources, including the thalamus, cerebral cortex, and limbic structures, such as the amygdala and hippocampus. Conversely, the main output nuclei are the substantia nigra pars reticulate (SNr) and the internal globus pallidus/entopeduncular nucleus (not depicted). The external globus pallidus (GPe) is categorized as an intrinsic nucleus because it primarily connects with other BG nuclei. This depiction is presented in a sagittal representation of the rodent brain, with red and dark gray arrows indicating excitatory and inhibitory connections, respectively. *Figure from Kandel et al., 2021*.

The main and largest nucleus of the BG is the striatum. The functional divisions within this nucleus are primarily determined by the organization of input connections, particularly from the cerebral cortex. In each of its distinct functional territories, there is a striking similarity in cellular structure. Across all these regions, the principal cell type consists of inhibitory γ-aminobutyric acid GABAergic medium spiny neurons (MSNs), constituting over 90% of all neurons (Tepper & Bolam, 2004). Furthermore, in all these functionally defined areas, such neurons can be categorized into two groups based on their relative expression of neuroactive peptides (substance P and dynorphin versus enkephalin) or the presence of D_1 and D_2 dopamine receptors, which are thought to positively and negatively regulate cyclic adenosine monophosphate signaling within these cells (Albin et al., 1989). These distinct populations play varying roles in different outputs from the striatum. Apart from their long-range inhibitory connections to other BG nuclei, MSNs also project local collaterals to neighboring cells (Lanciego et al., 2012). The coexistence of GABAergic and peptidergic neurotransmission within the same region leads to local interactions involving mutual inhibition and excitation. The remaining 5% to 10% of neurons in the striatum are exclusively GABAergic and cholinergic interneurons, which can be differentiated on their neurochemical, electrophysiological and sometimes

morphological characteristics (Kawaguchi et al., 1995; Kita, 1993; Kubota et al., 1993; Kubota & Kawaguchi, 1993).

The traditional interpretation of the functional organization of the BG was proposed in the late 1980s by Roger Albin and his research team (Albin et al., 1989) (*Figure 13A*). According to this model, signals originating from the cortex are distributed to two sets of medium spiny output neurons within the striatum. One group of neurons, which contain substance P and predominantly express D_1 dopamine receptors, establishes direct inhibitory connections with the BG output nuclei (the GPi and SNpr), forming what they termed the *direct pathway*. In contrast, another group of striatal neurons, containing enkephalin and mainly expressing D_2 dopamine receptors, cause a facilitatory modulation of the output nuclei by inhibiting the GPe that otherwise keeps tonically inhibited the STN: the resulting increase in GPi and SNpr activity promoted by STN, inhibits action execution, and represents the final effect of the *indirect pathway*. The model proposed that the output of the BG reflects a balance determined by cortical inputs between these inhibitory and excitatory final effects on the output structures, namely the GPi and the SNr. In conclusion, according to this conceptual framework, the direct pathway would promote the execution of specific behaviors, whereas the indirect pathway would prevent the execution of competing actions. Despite the linearity of this classical conception, recent anatomical observations have revealed that the internal structure of the BG is more intricate than initially envisioned (*Figure 13B*).

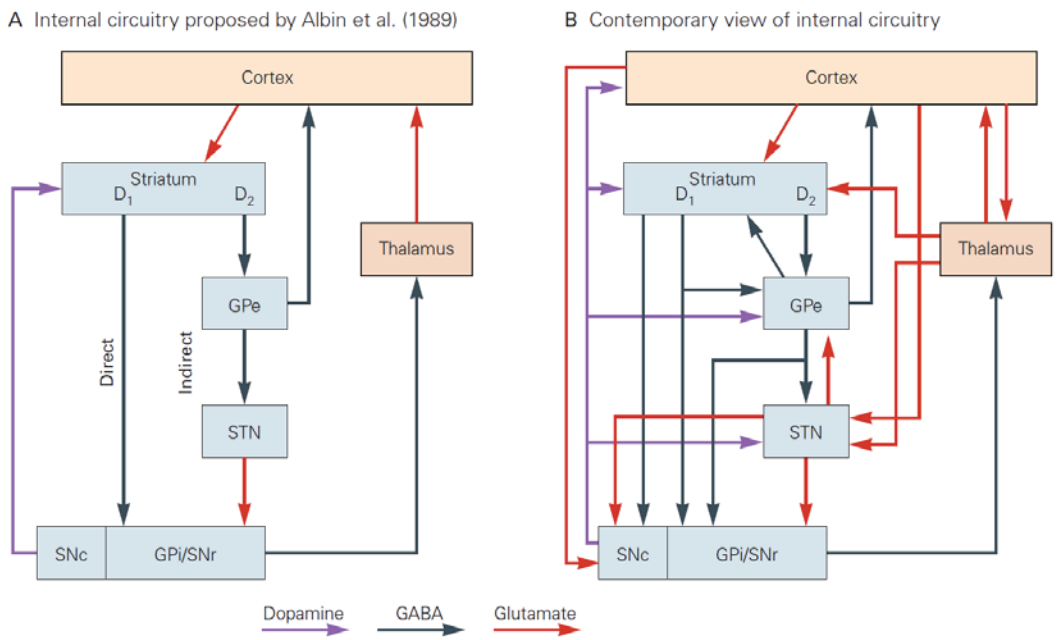

Figure 13 | Intrinsic connections within the basal ganglia. (A) According to the influential concept introduced by Albin and colleagues, the BG output is governed by a balance between two pathways originating from the striatum. The fist pathway involves a direct connection to the output nuclei, specifically the GPi and the SNr, which promotes certain behaviors. The second pathway is an indirect route from the striatum to the output nuclei, passing through the GPe and STN, which tents to suppress behavior. This equilibrium between direct and indirect projections was believed to be regulated by dopaminergic signals from the SNc acting on D_1 and D_2 receptors that are distribute differentially. **(B)** Recent anatomical investigations have unveiled a notably more intricate organization within the BG, making less straightforward to predict how inputs are transformed to generate specific outputs. *Figure from Kandel et al., 2021*.

The key findings have been that: (1) MSNs of the direct pathway also send collateral inputs to the GPe (Fujiyama et al., 2011; Kawaguchi et al., 1990; Wu et al., 2000); (2) neurons in the GPe establish direct connections with the output nuclei, in addition to their traditional indirect connections to the STN, often forming branching connections with all three structures (Mallet et al., 2012); (3) the GPe also projects back to the striatum and to regions outside the BG (Parent & Parent, 2002); (4) the STN, in addition to its feedforward connections to the two BG output nuclei (GPi and SNr), also projects back to the GPe (Carpenter et al., 1981; Sato et al., 2000); (5) significant inputs to the STN originate from both cortical and subcortical structures external to the BG (Polyakova et al., 2020).

Consequently, the subthalamus is now recognized as a major input structure of the BG rather than simply serving as a relay in the intrinsic indirect projection. Given this complex organization of the BG, it is no longer feasible to intuitively predict how a specific input might be transformed by the BG to generate a particular output. Therefore, computational modeling of the internal circuitry of the BG has gained increasing importance.

Despite the intricate nature of the intrinsic circuitry within the BG, there is a systematic topographical organization in the connections between its components. While certain projections are highly targeted, such as the striato-nigral projections, others are more spread out, like the subthalamo-nigral projections. A substantial decrease in the number of neurons in afferent structures, the striatum, and the output nuclei indicates a significant condensation of information as it undergoes processing within the BG.

The organizational principle proposed by Alexander and colleagues in 1986 (Alexander et al., 1986) is grounded in spatial arrangements related to input projections, intrinsic connections and outputs within the BG. The connections between the cerebral cortex and the BG can be conceptualized as a series of parallel, reentrant, and partly isolated pathways referred to as cortico-striato-nigro-thalamo-cortical loops (*Figure 14*). Consequently, a significant portion of projections originating from different functional areas of the cerebral cortex (e.g., limbic, associative, sensorimotor) selectively connects with specific regions of the BG input nuclei. This regional segregation is consistently maintained in the forward projections across the internal circuitry. Focused output signals originating from functional territories represented within the BG output nuclei are subsequently relayed through appropriate thalamic pathways back to the cortical regions that initially provided the input signals.

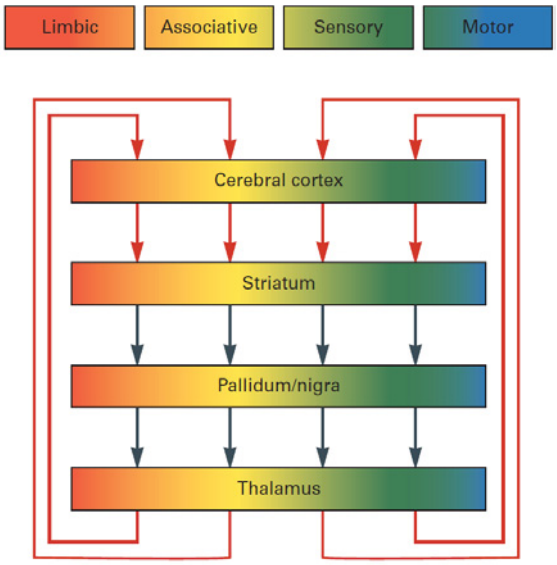

Figure 14 | Connections between the basal ganglia and cerebral cortex. The relationship between the cerebral cortex and the BG can be conceptualized as a set of parallel pathways that are predominantly isolated from each other. The functional regions found within the cerebral cortex remain distinct as they traverse through the BG and thalamic relays. However, within each of these pathways, there are specific points in the cortex, BG and thalamus, where the activity within the pathway can be influenced or altered by signals coming from sources external to that loop. Excitatory connections are indicated by red arrows; inhibitory connections are represented by dark gray arrows. *Figure from Kandel et al., 2021*.

Thanks to these multiple, parallel loops and reentering circuits, the BG constitute a common foundational architectural and functional structure responsible for: (1) the selection and enhancement of prefrontal-striato-pallidal activity during the execution and learning of new activities and tasks, constituting the *goal-directed system*; (2) the reinforcement learning mechanism that forms habitual responses, allowing them to be automatically executed by the motor circuit, known as the *habit system*; (3) the ability to halt an ongoing activity and transition to a new one if needed, which is primarily mediated by the cortical circuit involving the inferior frontal cortex and the STN (Lanciego et al., 2012).

1.2.2 Clinical dysfunctions and functional hypotheses

Studies examining the functional significance of the BG have predominantly focused on the typical symptoms that arise when these structures are affected by disease, including impairments in both motor and non-motor functions.

In a study conducted by McCairn and colleagues, it was observed that injection of bicuculline (a GABA A receptor antagonist that binds to the β subunit) into the motor putamen of monkeys resulted in localized, brief muscle jerks resembling tics (McCairn et al., 2009). This was accompanied by an increase in neuronal activity in the GPe, a significant reduction in activity in GPi, and synchronized firing in M1 coinciding with the occurrence of these jerks. These findings highlight a direct link between the initiation of movement and the decrease in GPi output activity (Lanciego et al., 2012). In addition, when bicuculline was injected into specific subregions of the striatum, GPe and STN, it led to various behavioral abnormalities, including dyskinesias,

stereotypies, and hyperactivity. These effects were particularly pronounced when bicuculline was administered into the postero-lateral (motor) segment, associative, and limbic regions (Francois, 2004; Karachi et al., 2009; Worbe et al., 2009), suggesting that specific lesions of primate BG might be involved in the development of various neurological and neuropsychological disorders (Bostan et al., 2018). For example, disturbances affecting the striatum can lead to disruptions in the regulation of behavior, as observed in conditions such as Autism Spectrum Disorders (Estes et al., 2011), Attention/Hyperactivity Disorders (Durston et al., 2011; Emond et al., 2009); Tourette Syndrome (Peterson et al., 2003), and Obsessive-Compulsive Disorders (Milad & Rauch, 2012). On the other hand, there is also evidence suggesting that heightened dopaminergic activity in the limbic region of the BG may play a role in mediating the positive symptoms of Schizophrenia (Inta et al., 2011; Simpson et al., 2010).

Maintaining normal cortical functioning necessitates a precise regulation of neuronal excitability within each BG nucleus, a process governed by the intricate organization of the striatum thanks to the significant role played by dopaminergic transmission (Wichmann & Dostrovsky, 2011). In humans, motor deficits resulting from BG dysfunction encompass a diverse range of clinical manifestations, spanning from reduced movement (hypokinesia) to excessive, involuntary movements (hyperkinesia). The underlying cause for both extremes on this spectrum can be explained by considering a disruption in the balance of corticostriatal activity, which can be attributed to changes in the functioning of specific subpopulations of MSNs (Chesselet & Delfs, 1996; DeLong, 1990).

The most well-known hypokinetic disorder is Parkinson's disease (PD), a clinical condition characterized by its primary symptoms, including resting tremors, muscle stiffness, slow movements (bradykinesia) and postural instability. These symptoms are attributed to a depletion of dopamine (DA) resulting from the degeneration of DA-producing cells in SNc, which disrupts the self-stabilizing loops within the BG, thus preventing their compensatory function. As a consequence, there is an increased activity in the classical indirect pathway and reduced facilitation of the direct circuit neurons. This imbalance leads to increased output from the GPi and SNr, resulting in excessive inhibition of the thalamo-cortical pathway and brainstem motor centers. Consequently, the likelihood of initiating movement decreases with the progression of the disease (Obeso et al., 2000, 2008).

To address these BG-related motor dysfunctions, modulation of this circuitry is considered a promising therapeutic strategy (Kravitz et al., 2010). In fact, optogenetic studies demonstrated that activating the direct pathway, successfully mitigated deficits in a mouse model of PD, suggesting a potential avenue for improving BG-related motor impairments. In contrast, hyperkinetic disorders, exemplified by conditions like Huntington's disease, levodopa-induced dyskinesia, or hemiballismus, involve an abundance of uncontrolled and sudden ballistic movements that disrupt the usual course of voluntary actions. These irregularities can be attributed to specific dysfunction of striatal neurons that project to the output nuclei, resulting in an excess of abnormal movements (Albin et al., 1989; Galvan et al., 2012).

Among the numerous functions of the BG, more intricate functions believed to be influenced by their activity encompass procedural (Packard & Knowlton, 2002) and working memory (Monchi et al., 2006). Additionally, BG are implicated in habit formation (Yin & Knowlton, 2006), perception (Brown et al., 1997), attention shifting (Ravizza & Ivry, 2001), decision making (Balleine et al., 2007), as well as diverse forms of implicit learning, including motor skills (Doyon et al., 2009; Hikosaka et al., 2002), category learning (Moustafa and Gluck, 2011), and reward-related learning (Tanaka et al., 2016).

The numerous reciprocal connections established by these nuclei with the principal cortical nodes of the MN network suggested that BG might play a role in the action-observation process as well (Caligiore et al., 2013). The authors contended that examining the specific contributions of these subcortical regions could enhance our understanding of crucial aspects of other's action observation. This includes factors like the impact of the observer's motor experience, the different levels at which an observed action can be represented, and the development of action recognition abilities.

Indirect support to this hypothesis comes from recent research examining the involvement of the BG in the process of observing actions in human subjects. Kessler et al. (2006) utilized whole-head magnetoencephalography (MEG) to investigate the timing of long-range synchronization among cortical networks during an imitation task involving both biological and non-biological movements (Kessler et al., 2006). Subjects were required to perform an imitation task in which they observed a biological or non-biological movement of two fingers. The results showed that BG activation started earlier in the processing of biological motion, implying a potential role of these nuclei in selecting appropriate motor programs to match the observed stimuli.

In another study, Alegre et al. (2010) recorded local field potentials (LFPs) from the STN in patients with Parkinson's disease and reported that when observing movements, there were changes in the beta oscillatory activity, marked by a bilateral reduction in STN power and cortico-STN coherence. These changes were in line with those typically observed during the actual execution of movements, although of smaller amplitude.

Further indirect evidence comes from a functional magnetic resonance imaging (fMRI) study by Ge et al. (2018) in which participants were presented with hand actions from both first-person and third-person perspectives. They found similar activations not only in cortical regions of the MN network, but also in various parts of the BG and of the limbic system, including the putamen, the insula and the hippocampus. Similar results were gained in another recent fMRI investigation in which the authors described a notable shared activation in subcortical structures during both the execution and observation of object manipulations (Errante & Fogassi, 2020). This shared activation was particularly evident in bilateral GP and the left STN.

1.2.3 The putamen nucleus

The putamen (Put), in conjunction with the globus pallidus (GP), includes the lentiform nucleus and, when considered with the caudate nucleus, it constitutes the striatum (*Figure 15*) (Ghandili & Munakomi, 2023).

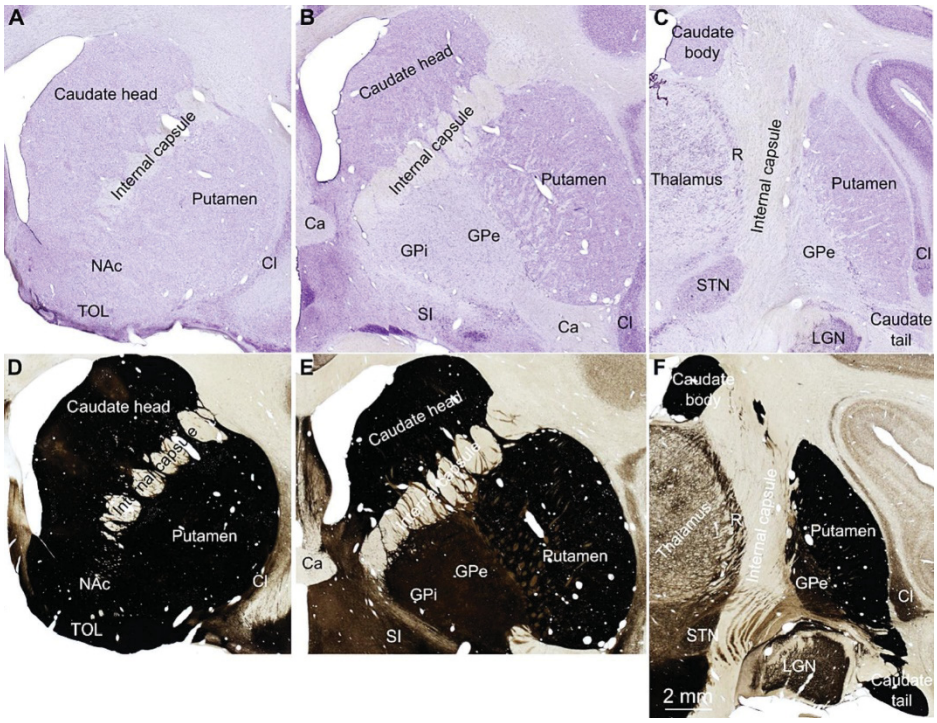

Figure 15 | Architecture of the primate striatum. (A, B, C) Coronal sections of the macaque (*Macaca fascicularis*) brain through the striatum from rostral (A) to caudal (C) stained with Nissl technique. **(D, E, F)** Coronal sections of the macaque brain through the striatum from rostral (D) to caudal (F) stained with AChE technique. Ca, anterior commissure; Cl, claustrum; GPe, globus pallidus external part; GPi, globus pallidus internal segment; LGN, lateral geniculate nucleus of the thalamus; NAc, nucleus accumbens; R, reticular nucleus of the thalamus; SI, substantia innominate; STN, subthalamic nucleus; TOL, olfactory tubercule. *Figure from* del Rey & García-Cabezas, 2023.

Among the various nuclei within the BG, the putamen, in conjunction with the caudate nucleus, serves as the entry point for the BG circuit. Nevertheless, much of its functional characteristics remain largely uncharted territory. By employing immunohistochemical markers, researchers have further divided the striatum into striosomes – which are mainly influenced by limbic projections – and matrix compartments that receive inputs from motor and sensory cortical regions, as well as thalamo-striatal afferents (Fujiyama et al., 2011).

From a connectivity perspective, the putamen receives organized projections from several cortical sensorimotor areas, such as the primary motor cortex (M1), supplementary motor area (SMA), caudal and rostral cingulate motor areas (CMAc/r), and premotor regions (PM). Traditionally, the thalamus was thought to function just as a relay of information of the BG output to the cortex. However, McFarland and Haber's (2000) retrograde tracing experiment revealed projections from the ventrolateral complex, specifically VLo, VPo, and VA nuclei, to various regions in the putamen. This finding suggests that these thalamic nuclei may directly influence striatal activity. In addition, it has been proposed that neurons in the centromedian-

parafascicular (CM-Pf) nuclear complex also project to the putamen, thus providing striatal neurons with information about behaviorally relevant sensory events (Matsumoto et al., 2001).

The information from the putamen are directed towards the output nuclei GPi and SNr, which, in turn, form the principal pathways connecting the BG with the upper motor neurons hosted by the cortex and brainstem. The path leading to the motor cortex is relayed through the VA and VL nuclei situated within the lateral thalamus, which subsequently project directly to the motor regions in the frontal cortex (Holsapple et al., 1991; Kurata, 1994; Matelli et al., 1989; Matelli & Luppino, 1996).

In a connectivity study aimed at investigating to what extent corticostriatal input zones from motor cortical areas of the macaque overlap in the putamen, it has been demonstrated that corticostriatal projections originating from the forelimb representations of F1 and supplementary motor area (SMA) remain largely separated and are primarily distributed in the lateral and dorsomedial regions of the putamen, in that respective order, with a zone of overlap in the medial region (Takada et al., 1998), as depicted in *Figure 16*.

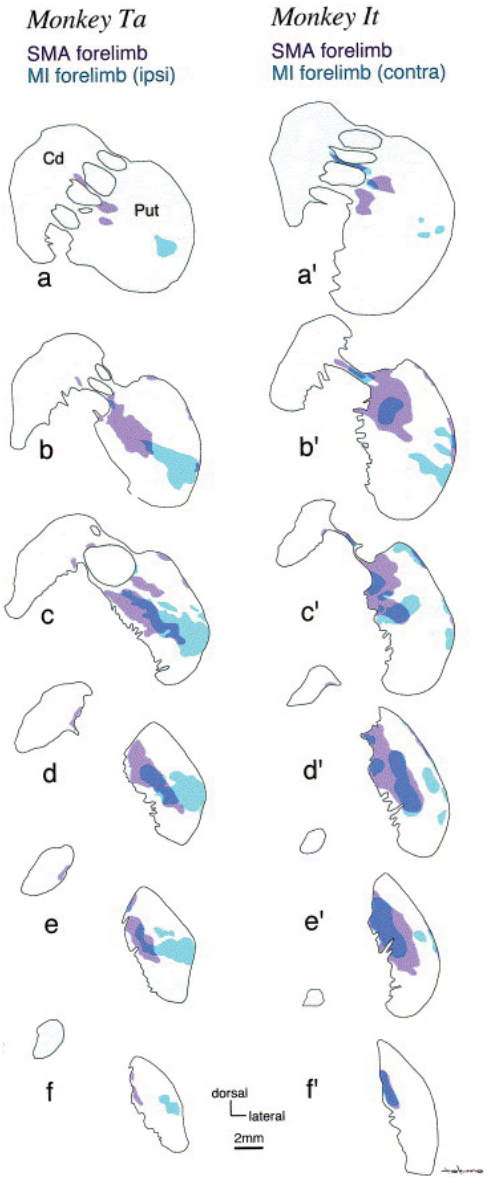

Figure 16 | Distribution patterns in the striatum of anterogradely labeled terminal zones after paired injections of BDA and WGA-HRP, respectively, into the forelimb region of SMA or the ipsilateral F1 (Monkey Ta; a-f), and into the forelimb region of SMA or contralateral F1 (Monkey It; a'-f'). Superimposed in each case are six representative sets of adjacent coronal sections through the caudal 2/3 extent of the striatum ipsilateral to the SMA injection. Purple areas represent terminal zones labeled from SMA; cyan areas represent terminal zones labeled from M1; blue areas represent the field of overlap of the two terminal zones. Cd, caudate nucleus; Put, putamen. *Figure from Takada et al., 1998.*

Subsequent studies using retrograde tracing methods (McFarland & Haber, 2000), delved further into the organization of motor inputs to the putamen. In particular, they highlighted an overlap of M1 and SMA terminals in the central zone that runs from the middle to the side, but not between those originating from M1 and PM.

Somatotopy appears to be consistently maintained throughout the motor loop, ensuring that signals related to different body parts primarily target specific regions within each nucleus. In the putamen, projections from distinct sensorimotor areas follow a similar arrangement, with a dorsolateral region devoted to the hindlimb, a ventromedial sector related to orofacial region, and a forelimb zone positioned in between (Flaherty & Graybiel, 1993; Künzle, 1975; Takada et al., 1998). Corticostriatal fibers originating from regions related to both proximal and distal body parts terminate in the dorsomedial and ventrolateral portions of the putamen, respectively (Tokuno et al., 1999). This organization results in the existence of two distinct but partially overlapping sets of somatotopic representations within the putamen: one in the area that receives inputs from M1 and another in the zone that receives input from SMA (*Figure 17*). This arrangement was then confirmed through electrophysiological investigations that examined putamen projection neurons orthodomically activated in response to stimulation applied to the forelimb region of the presumed cortical areas of origin (Nambu et al., 2002).

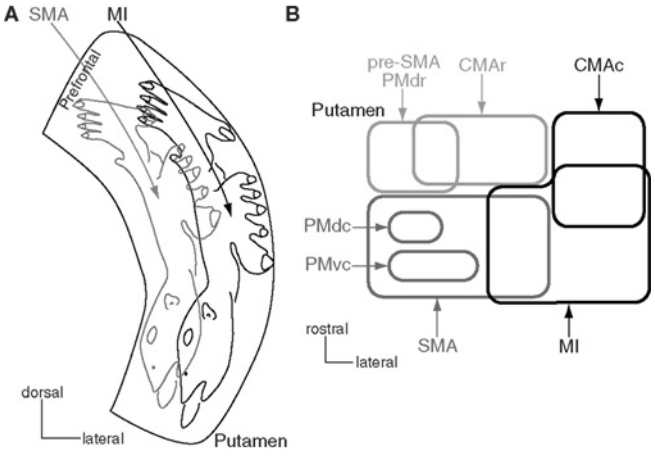

Figure 17 | Somatotopy of the putamen. (A) Somatotopy of the putamen is schematically shown in a frontal section. In the caudal aspect of the putamen, the lateral part receives somatotopic inputs from M1, and the medial part from SMA. The Somatotopy in the SMA territory is located dorsomedially to that in the M1 territory. Projections from the orofacial, forelimb and hindlimb regions of the M1 and SMA converge in the mediolateral central zone in the putamen. The most dorsomedial part receives inputs from the frontal cortex. **(B)** Input from motor cortices to the putamen is schematically shown in a horizontal section. CMAc and CMAr, caudal and rostral parts of the cingulate motor area; PMdc, PMdr, and PMvc, caudal part of dorsal premotor cortex, rostral part of dorsal premotor cortex, and caudal part of ventral premotor cortex. *Figure from Nambu et al., 2011*.

Similarly, research in humans indicates the presence of an organized pattern of corticostriatal connections. Specific circuits linking the cortex to distinct parts of the putamen, including its posterior (related to sensorimotor functions), anterior (linked to associative functions), and ventral (associated with limbic functions) compartments, have been documented (Lehéricy et al., 2004). Within the sensorimotor areas of the putamen, a somatotopic organization akin to that observed in monkeys has been identified, with the representation of the leg positioned dorsally, the face in the ventral region, and the arm in the intermediate one (Delmaire et al., 2005). The presence of a segregation of the represented effector within the BG has become clinically relevant with the increasing of the surgical procedures like ablation or deep brain stimulation (DBS). These procedures necessitate precise targeting of specific subcortical regions within the sensorimotor loop to improve dysfunctions while avoiding side effects linked to interference with non-motor circuits responsible for associative or emotional processing (Romanelli et al., 2005).

Regarding the functional features of this subcortical structure, Crutcher and DeLong (Crutcher & DeLong, 1984) observed that many putamen neurons displayed activity patterns associated with active movements of specific body parts and even responded to passive somatosensory stimuli, often in a highly specific manner. They also noted a higher proportion of cells linked to the proximal compared to the distal portion of the arm, aligning with the idea that the BG play a significant role in controlling proximal musculature and posture. Recent single-neuron recordings in the caudal putamen have identified a subset of neurons with activity related to saccadic eye movements, suggesting a potential role for this structure in oculomotor control (Phillips & Everling, 2012).

Alexander and DeLong (Alexander & DeLong, 1985) discovered *striatal microexcitable zones* (SMZs), i.e., distinct areas within the monkey putamen whose microstimulation led to the initiation of corresponding bodily movements. Notably, the induced motor responses consistently occurred in the opposite side of the body relative to the site of intrastriatal stimulation, occasionally affecting both sides for axial and orofacial movements. In addition, in the putamen (similarly to the GP) the initiation of neuronal activity associated with stimulus-triggered movements occurred after the activity in cortical motor regions. This observation hinted at the possibility that the BG might be receiving a corollary discharge, or efference copy, from the cortex. It is worth noting that microstimulation proved to be significantly more effective in inducing body movements when applied to the lateral part of the putamen, which receives inputs from areas M1, as compared to the medial portion, which receives inputs from SMA (Nambu et al., 2002).

Putamen neuronal activity is influenced by various aspects of motor behavior. Cells in the dorsolateral region of this structure are associated with movement initiation (Kimura, 1990). Conversely, neurons in the dorsomedial region exhibit responses related to pre-movement activity in tasks involving cues (Gardiner & Nelson, 1992). The SMA region, compared to area M1, contains more neurons displaying preparatory activity dependent on task instructions, with changes in firing rate following the instructions.

These neurons were also located more rostrally and medially compared to those exclusively responsive to the movement (Alexander & Crutcher, 1990).

Electrophysiological studies in macaques showed that putamen neurons that become active during voluntary movements can exhibit specificity towards various factors, that may include location of the target in space along the horizontal plane, and the movement's direction (regardless of the specific muscle patterns involved) (Alexander, 1987; Liles, 1985). In addition, some neurons may show preference for variables related to movement dynamics, such as for slow and gradual movements over fast and "ballistic" ones (Crutcher & DeLong, 1984). These findings indicate that the BG potentially play a role in controlling movement direction and adjusting movement parameters, such as speed and amplitude. This role aligns with the functions of the SMA, which serves as the primary cortical region sending projections to the striatum in the motor circuit for movement planning and control.

Kimura (1986, 1990), put forward a hypothesis suggesting the potential involvement of a subgroup of putamen neurons termed "type IIa cells" in the initiation of movements. More precisely, these neurons were expected to exhibit brief burst of activity related to the choice of a previously learned arm or orofacial movement prompted by contextual sensory cues. This activity was thought to contribute to the creation of a context-specific representation of stimuli-responses association. Subsequently, in 2003, Ueda and Kimura made a discovery regarding a significant portion of dorsomedial putamen neurons, revealing their selectivity for preprogrammed combinations of movements and direction of the initial movement (Ueda & Kimura, 2003). They proposed that the interplay of these characteristics might have a role in shaping the visuospatial and temporal organization of movements.

A likely role for the putamen in guiding the movement and the interactions with proximal objects derives from an experiment conducted from Graziano and Gross (1993), who identified a category of neurons that exhibited dual responsiveness to both visual and somatosensory stimuli (such as gentle touch, joint motion or deep muscle pressure). These neurons show features like those of area F4 peripersonal neurons, and appeared to establish a visual spatial map surrounding the monkey's body organized in accordance with the somatotopic representation of the nucleus. Remarkably, the characteristics of these neurons closely resembled those observed in cortical regions associated with the *cortical reaching network*, including areas like 6, 7b, and VIP. In light of these findings, the authors proposed the possibility that the putamen might be integrated into a connected network responsible for representing the peripersonal space in somatotopically defined coordinates.

Similar to what has been suggested for the other BG nuclei, it appears that the functional role of the putamen may extend beyond mere motor functions. Specifically, its posterior portion is believed to give an important contribution to sensorimotor functions, while the anterior part likely plays a role in various non-motor aspects of cognition (Ell et al., 2004). Moreover, the putamen might not only be involved in representing context-specific behaviors, but also in their acquisition. This includes the stimulus-responses

(Horvitz, 2009), as well as stimulus-category (Cincotta & Seger, 2007) associations. Furthermore, it may also play a role in the flexible development of new habitual behaviors (Yin & Knowlton, 2006).

Several lines of evidence involving monkey and human studies suggest that putamen significantly contributes to reward-related functions. Muranishi and collaborators (2011) conducted an experiment involving the injection of muscimol into the monkey's putamen, discovering that selective inactivation of this region interfered with the animal's ability to choose actions based on past reward experiences. Cromwell and Schultz (2003) conducted experiments where they recorded the activity of striatal neurons in macaque monkeys during a task that involved spatial memory and observed that some of these neurons exhibited activity related to the task during the preparation and execution of movements, immediately before and after the delivery of juice rewards. Notably, about half of these neurons showed different levels of response based on the anticipated magnitude of the reward to be received. In a separate study using fMRI, McClure et al. (2003) found a connection between the activity in the putamen and errors in predicting the timing of rewards. They observed that putamen activation increased when juice rewards were unexpectedly delivered and decreased when they were unexpectedly withheld, indicating its involvement in reward-related processes.

Finally, this nucleus appears to have a relevant role in memory functions. Research conducted on human subjects has suggested that the anterior part of the putamen contributes to the maintenance of working memory (Voytek & Knight, 2010), with its activation being influenced by the memory load (Chang et al., 2007). In addition, the left putamen consistently comes into play in the encoding of verbal episodic memories (Ystad et al., 2010). Neuroimaging studies employing set-shifting tasks (Rubia et al., 2006), reveal increased putamen activity during trials requiring a shift in mental sets, suggesting its involvement in the capacity to adapt and flexibly update strategic responses. Monchi et al., (2006) also reported that the caudoventral part of the putamen is engaged when executing non-routine actions guided by self-determined novel strategies, in contrast to the rostro-dorsal portion, which is active during preparatory phases and the sequencing of finger movement sequencing.

1.3 Aims of the study

Data from existing literature support the significant involvement of cortical motor regions in a wide array of advanced perceptual, cognitive, and even social functions, suggesting that the motor functions should be viewed as an extensive domain characterized by potential interactions with the surrounding environment, including inanimate objects and other agents. Furthermore, it is known that the cortical motor system, including higher-order premotor areas, is closely connected to the subcortical BG complex, which seems to have a crucial role in modulating the activity of these regions. While it is undoubtedly that the cortico-BG network is essential for proximal motor control, movement initiation and postural regulations – as evidenced by clinical dysfunctions –, the potential contributions of these nuclei to the regulation of distal manual

actions, such as the selection of different grip types, and socio-cognitive and perceptual functions in primates – such as the observation of others' action – have received limited investigation so far.

Therefore, the current study aims at exploring the functional characteristics of the putamen through single-cell recording during a *Mutual Action Task* specifically designed to investigate neuronal responses during the visual presentation of a graspable object with two different possible grip types, and the observation of the same actions performed by another individual in a shared operational space.

2. MATERIALS AND METHODS

2.1 Experimental subjects

This study involved two purpose-bred, socially housed, adult male monkeys (*Macaca mulatta*, 9 and 12 Kg). Training procedures were carried out by means of operant conditioning with positive reinforcement and step-by-step shaping methods. To encourage desired behaviors that matched or resembled the target behavior, liquid rewards like fruit juice or water were provided, while undesired behaviors were gradually extinguished. At the end of each training session, fresh pieces of fruit and pellet mush were given to strengthen cooperation and create an as much as possible positive experience for the animals. Training sessions were carried out daily to establish a predictable routine and help the animals to become accustomed to it.

The monkeys were initially taught to interact and collaborate with the researchers and then familiarized with the process of entering and sitting in a primate chair that was later moved from the animal facility to the laboratory. Following this initial phase, they received specialized training for the main experimental task in the lab, which will be detailed in the upcoming section. The task was subdivided into simpler stages, with each stage introduced only after the monkey had fully learned the previous one, gradually leading to the mastery of the entire task.

In preparation for neural data collection, the monkeys underwent a surgical procedure for the implantation of a recording chamber over the region of interest, leaving the skull intact. The recording probes were implanted during subsequent surgeries. Each surgery was conducted in stereotaxic and aseptic conditions, under general anesthesia 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 monitored with a multiparametric monitor. Finally, to ensure the animals remained hydrated, a constant intravenous infusion of saline solution was provided, and vitamin A gel was used to prevent eye dryness during anesthesia. Monkeys received analgesics, broad-spectrum antibiotics, and anti-inflammatory drugs both during and after surgical procedures.

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, and were authorized by the Italian Ministry of Health.

2.2 Behavioral paradigm and recordings

The monkeys were progressively trained to perform the *Mutual Action Task (MAT)*, designed to enable investigation of neural properties during action execution and observation.

In the initial phase of the task, each monkey sat in its primate chair positioned at one end of a table, with the experimenter sitting in front of it on the opposite side, taking on the role of a collaborative partner. Both subjects shared an operational space containing a multi-affordance object that could be reached and manipulated by both individuals (*Figure 18*).

Figure 18 | Schematic drawing of setup configuration for the *MAT*. On the left, figure displaying an execution trial. On the right, figure of an observation trial.

The object, positioned 16 cm away from the initial hand position of each subject, was created using 3D printing technology and crafted from polylactic acid (PLA) material. It consisted of a cylindrical body with a diameter of 5.5 cm and a height of 6 cm, atop which a parallelepiped measuring 4.5 × 4.5 × 1.5 cm was inserted. This device offered two distinct grasping options: a precision grip (PG), achieved by pinching the thumb and index finger together against the central parallelepiped, or a whole hand prehension (WH), accomplished by wrapping the entire hand around the main cylindrical body. The specific type of grip required for each trial was indicated by a visual cue displayed on a rectangular OLED screen (2 × 1 cm) embedded on the upper surface of the parallelepiped: an empty square symbolized the PG, whereas a full white square indicated the WH prehension. Both components of the object were encircled by distinct metallic plates, ensuring that each grip activated a specific capacitive circuit, which, in turn, triggered a TTL signal for recording and storage the main phase of grasping alongside other behavioral and task-related event, as well as to provide a reliable and immediate control of the correct execution of the instructed grip (*Figure 19*).

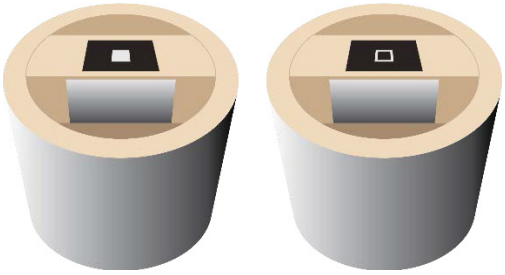

Figure 19 | Object description. On the left, the OLED display depicts a full square (*whole hand prehension* cue). On the right, the OLED display features an empty square (*precision grip* cue).

Each monkey, trained separately to perform the task with an experimenter, had to respond to a specific auditory cue (either a high- or low-pitched tone) in an opposite manner. Specifically, the pure high-pitched tone (a 1200 Hz sine wave) instructed MK1 to execute the action and MK2 to remain still, while the action was performed by the experimenter; conversely, the pure low-pitched tone (a 300 Hz sine wave), instructed MK2 to execute the action and MK1 to remain still, while the action was performed by the experimenter.

In each trial (see *Figure 20*), the task started in complete darkness, with both subjects sitting in their initial positions, pressing a button with the right hand. After a 1-second interval, the cue sound was presented (*Sound onset*), providing an opposite instruction (*Go* vs *No-Go*) to the two subjects. Following 770 ms from the Sound onset, and OLED screen illuminated (*OLED onset*) displaying either an empty or a full square. Subsequently, 730 ms after the OLED onset, the environmental light was turned on, making the entire object visible (*Light onset*). After an additional 570 ms time lag, the sound ceased (*Go signal*) and the subject who received the Go instruction was required to release the button, reach and grasp the object using the specified type of prehension within 1 second, and finally hold the object for at least 500 ms to get the reward.

Whenever the monkey successfully completed all the steps of an execution trial or remained motionless during the entire duration of a partner's trial – a fixed quantity of liquid reward was dispensed automatically; otherwise, the trial was aborted. An experimental trial was considered as correct only if both participants behaved correctly. Potential errors that could result in the trial being excluded from analysis included: (1) "*wrong start*", consisting in early release of the button during a trial, no later than 300 ms prior to the end of the Go/No go cue sound, suggesting the intention to abort the trial, (2) "*false start*", consisting in releasing the start button during the last 300 ms of the sound before its end, suggesting the attempt to speed up/anticipate action initiation, (3) "*omitted start*", consisting in keeping the hand still even after a Go signal, (4) "*out of time*", consisting in reaching and contacting the object after more than 1 second after the Go signal, (5) "*no lift*", consisting in omission of the lifting of the object after having correctly grasped it, (6) "*wrong grip*", consisting in timely executed grasping but performed with the incorrect type of grip, (7) "*no hold up*", consisting in releasing the object less than 500 ms after lifting or (8) "*others*", consisting in rare occurrences of other incorrect motor actions, such as releasing the button after the Go signal but not touching the object.

In half of the trials, the light remained on until the task was completed (*Light condition*), while in the other half it was switched off immediately after the monkey released the button, so that the action was executed in darkness (*Dark condition*). This latter condition was carried out to ensure that any possible motor-related responses could not be attributed to the visual feedback from the monkey's moving hand.

For each animal, we collected 15 trials for each of the 8 conditions (2 grip types × 2 agents × 2 visual feedbacks), for a total of 120 correctly performed trials for each session.

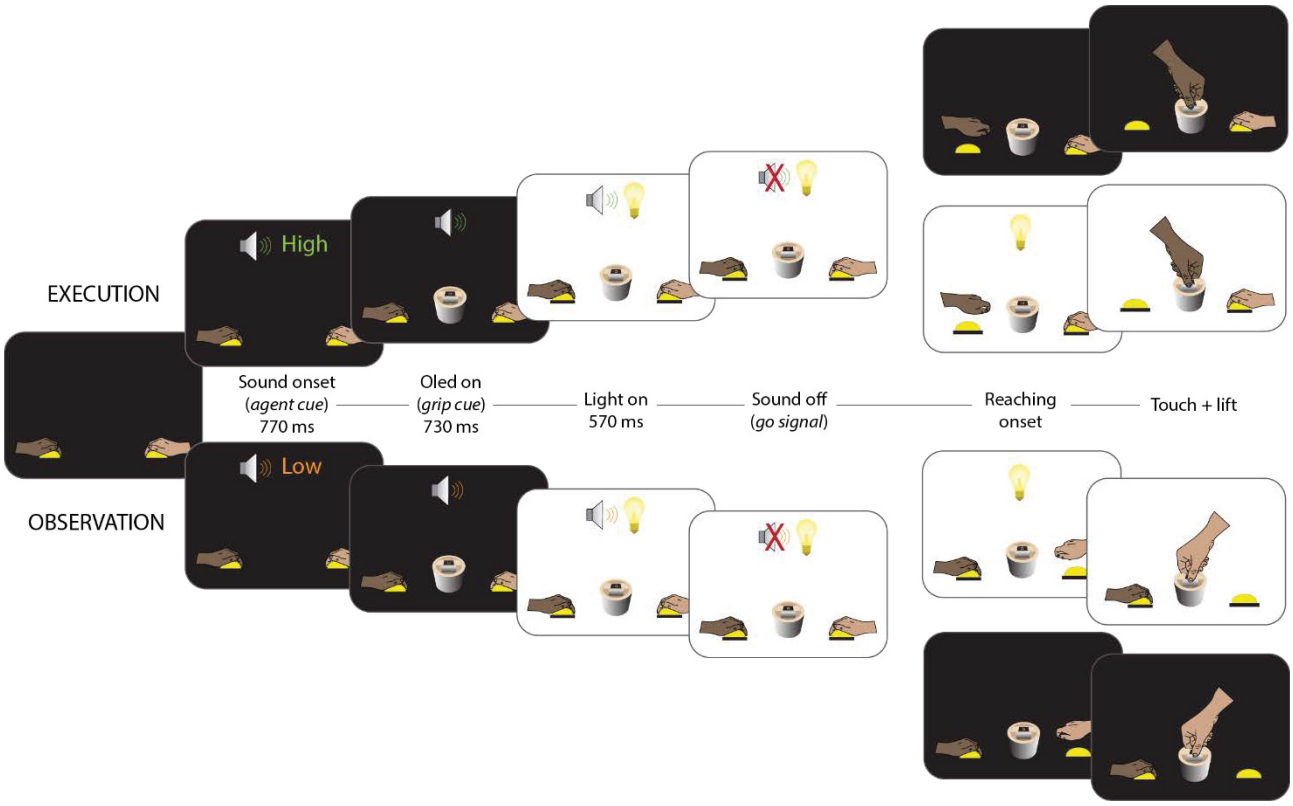

Figure 20 | Temporal sequence of task events.

Behavioral events were detected using distinct contact-sensitive devices that signaled the detachment of each subject's hand from the button, the contact with the object (depending on the type of prehension) and the object lifting. These interactions generated specific TTL signals, which were sent to a PC equipped with a dedicated LabView-based software that monitored the behavioral performance of the subjects and controlled the digital output signals related to the auditory and visual cues, light switching on and turning off, and reward delivery. All input and output signals were recorded and saved synchronized with the neural data for subsequent statistical analysis.

2.3 Neural recordings

In order to collect neuronal signals, each monkey was implanted with a biocompatible plastic recording chamber (4.5 × 5 × 2.5 cm for MK1; 2.8 × 3.3 × 2.5 for MK2; *Figure 21*) endowed with a grid of parallel grooves (width 2 mm, inter-groove distance 3 mm) for housing the Omnetics connector blocks (up to 8 for MK1 and up to 4 for MK2). These connectors interfaced the multielectrode contacts and the headstages (Deuteron Technologies Ltd) for wireless data logging.

The recording chambers were crafted by cutting and shaping them based on a 3D reconstruction of the monkeys' cranial structure realized with the 3D Slicer software, using data obtained from 7T Magnetic Resonance images.

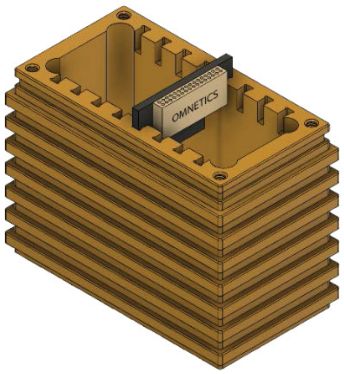

Figure 21 | Design of the recording chambers. Chamber body for MK1 implant.

After complete recovery from the placement of the chambers, different insertion procedures were carried out to implant the recording probes in the two animals. In MK1, we used 32 channel linear multielectrode silicon probes (ATLAS Neuroengineering, Iridium Oxide (IrOx) with recording sites spaced apart 250 μm, see Barz et al., 2017), chronically implanted through a small craniotomy performed within the chamber, with most of the surrounding bone left intact. Each probe was 24 mm long and had a rectangular section of 30 μm width and 100 μm thickness, featuring an average impedance in the range of 0.23 to 0.29 MΩ. Notably, all the probes were outfitted with a pointy tip feature, which served to minimize tissue dimpling when the probes were inserted, thereby facilitating penetration. The probe's shaft was connected to a highly flexible polyimide-based ribbon cable and an Omnetics connector, allowing electrical connection to the recording data logging devices. A collagen-based dural regeneration matrix (DuraGen® Plus) was placed around the insertion sites, and liquid dental cement was then poured into the recording chamber, which rapidly solidified to secure the probes in place and prevent any contamination.

Five distinct linear probes were implanted, using a dedicated insertion device, in two different surgeries, with a two-month interval between them. The first two implanted probes were removed during the second procedure prior to the insertion of the other three probes. The insertion sites for these probes were determined based on stereotaxic coordinates obtained from MRI images of the specific regions of interest. The angle of penetration was fixed at 90° (vertical), taking into account the deviation that might occur in the probe's trajectory as it passed through the tissue. This deviation was primarily due to the pointy tip angle and has been calculated through in vitro experiments with agarose, a suitable material for simulating the mechanical properties of brain tissue because of its poroelastic characteristics (Pomfret et al., 2013). These tests revealed that the probe exhibited a deflection of 0.05-0.12 mm per millimeter of penetration, which translated to a systematic deviation of 1.2-2.9 mm of the tip along the entire 24 mm length of the probe. Importantly, this deviation consistently occurred in the opposite direction to the tip's beveling, and this information was taken into account during the surgical procedures to ensure the correct placement of the recording shaft.

In MK2 we used a different implantation approach. Following the placement of the recording chamber on the skull, a 13 mm layer of dental cement was created by pouring liquid cement into the chamber and leaving it solidify. Subsequently, in two separate surgical procedures performed 4 months away from each other, four polyimide guide tubes, each measuring 36 mm in length (outer diameter 820 μm; inner diameter 760 μm) were permanently implanted by drilling vertical holes through the cement and the bone layers with a stereotaxically manipulated drill bit to ensure straight trajectory: the guide tubes served as permanent guides, allowing for the precise, repeated insertion of two individual linear probes to the desired depths, preventing any deviation of the probes. The probes used were linear pig-tailed Plexon V-Probe endowed with 32 Platinum Iridium (Pt/Ir) channels along the shaft with 200 μm inter-electrode spacing; the probe total length was 45 mm and was surrounded by a reinforcement tube tied to the ground (37 mm long, 640 μm dia). Probes were inserted in the awake animal through the previously implanted guide tubes, approximately on a weekly base, and fixed in a semi-chronic manner by means of Kwik-Cast™ silicone sealant.

After connecting the logger device to the electrode arrays, all the components were enclosed with a cover atop the recording chamber. A digital bandpass filter was employed with upper and lower cut-off frequencies set at 2 and 7000 Hz, respectively, allowing for the sampling of both local field potentials (LFPs) and single/multi-unit activity. The conversion rate on each channel was 32000 Hz and the acquired signals subsequently underwent amplification, digitization, and were locally stored on a MicroSD memory card (64 GB) to mitigate potential transmission errors. The logger established communication with a PC through a transceiver featuring four BNC connectors for digital inputs and one for digital outputs, which was connected to the host computer via USB.

2.4 Tracers injections and histological procedures

In MK2, retrograde neural tracers were injected in the putaminal zones where the last neural activity was recorded. Tracers were slowly pressure injected through a stainless steel 31-gauge beveled needle attached through a polyethylene tube to a Hamilton syringe (Reno, NV) whose tip was lowered 4.5 mm below the end of the implanted guide tubes. At the level of the rostral guide tube, we injected Fast Blue (FB, 3% in distilled water, Drilling Plastics GmbH, Breuberg, Germany), whereas at the level of the caudal guide tube we injected cholera toxin B subunit, conjugated with Alexa 488 (CTB-g, 1% in phosphate-buffered saline; Molecular Probes).

The precise positioning of the probe tips was subsequently verified through a post-mortem examination of both monkeys' brains. After 28 days of survival period for tracers transport in MK2 and after the end of the experiments in MK1, the animals underwent deep anesthesia induced by ketamine and medetomidine, followed by a lethal dose of sodium thiopental. Subsequently, they were perfused through the left cardiac ventricle in sequential stages with approximately 2 liters of saline (over 10 minutes), 5 liters

of 3.5% formaldehyde (over 30 minutes), and 3 liters of 5% glycerol (over 20 minutes). All perfusion solutions were prepared in 0.1 M phosphate buffer at pH 7.4.

The brains were then coronally blocked on a stereotaxic apparatus, extracted from the skulls, and placed in 10% buffered glycerol for 3 days, followed by 20% buffered glycerol for 4 days. Finally, the brains were frozen and cut into coronal sections of 60 μm of thickness. In MK2, in which FB and CTB were injected, one section of each five was mounted, air-dried and quickly cover-slipped for fluorescence microscopy. For both monkeys, two sets of the sections of each brain were subjected to staining using the Nissl method (0.1 thionin in 0.1 M acetate buffer at pH 3.7).

2.5 Spike sorting and data analysis

All formal signal analyses were carried out offline. Single units were identified using dedicate offline sorting software (Offline Sorter™ by Plexon Inc) by imposing a 3 standard deviations threshold from the signal to noise on the band-pass filtered signal (4 pole Bessel filter, 300 – 7000 Hz) for waveform detection, and then sorting units with template matching. For each isolated unit, we confirmed the temporal stability of its isolation throughout the task by projecting its spikes in the 3D space defined by the first two principal components and the acquisition time throughout the entire session. We excluded from our analyses only noisy or non-physiologically plausible waveforms, but not those belonging to units with very low firing rates or numbers of spikes, thereby achieving an unbiased and comprehensive overview of the neuronal properties and firing features of single units in the studied area, given its largely unknown functional properties.

For recordings carried out from the same implanted probe across multiple days, we verified whether and when waveforms with the same spike shape, interspike interval, and response profile during the task could be detected across multiple days from the same (or adjacent) channel. To avoid resampling biases, neurons consistently identified in the same or adjacent channels based on the above-mentioned features were considered only once in the dataset, using the signal-to-noise ratio (i.e. the amplitude of the averaged waveform for the unit relative to the threshold of each session) as a selection criterion.

The task was specifically designed to enable the evaluation of sensory and motor responses linked to the planning and execution of the monkeys' own actions as well as to the observation of the experimenter's actions. In this context, all analyses of neuronal activity were synchronized with task or behavioral events. The current project focuses exclusively on the evaluation of motor responses. Thus, leveraging digital signals associated with the main behavioral events, we identified distinct epochs of interest: (1) *baseline epoch*, ranging from 600 to 100 ms before the cue sound presentation; (2) *pre-movement,* corresponding to the 300 ms interval before the hand detaches from the starting button; (3) *reaching-grasping,* corresponding to the interval ranging from the detachment of the hand from the starting button to target object contact; and (4) *lifting-holding*, ranging from object contact to 300 ms after this event. We ensured to analyze only the period starting with the beginning of the reaching action and ending 300 ms after the contact with the object. This

timeframe encompassed the initial lifting and holding phase while excluding any potential responses associated with reward in order to prevent potential misattribution of the neurons' discharge (Ikemoto et al., 2015; Nougaret & Ravel, 2015).

We classified neurons exhibiting significant activation during the motor epochs of the task relative to the baseline epoch during both task execution in the light and in the dark. For this purpose, we used a 2×4 repeated-measures ANOVA (factors: Grip and Epoch) to analyze the data of each condition (Light and Dark), separately. Neurons were classified as motor-related if they discharged differently during any of the task epochs relative to baseline because of a main effect of the factor Epoch, an interaction of the factors Grip and Epoch, or both ($p < 0.05$, Tuckey corrected). Modulations could be either positive (facilitated neurons) or negative (suppressed neurons). All the neurons showing, in addition, a significant main effect of the factor Grip or its interaction with the factor Epoch ($p < 0.05$, Tuckey corrected), were considered as grip-selective motor-related neurons. The same approach was employed to assess neuronal responses during action observation.

To build heat maps and calculate population responses, for each neuron we first calculated its baseline firing rate for action execution (EXE) and observation (OBS), separately. Then, we computed the net normalized activity of each unit by subtracting its baseline activity in a given condition from its firing rate of each bin. Next, we soft-normalized the resulting net activity vector by dividing each data point by the absolute maximum across all conditions plus 5 spk/s. This constant factor's impact is greater the lower is the neuron's firing rate, allowing to weight the contribution of neurons with highly different firing rate in a population.

All analyses related to the monkeys' execution trials were conducted exclusively for the dark condition. Conversely, neuronal responses during the monkeys' observation trials were examined solely for trials in the Light Condition, in which the monkeys could visually observe the experimenter's action.

To assess how information about the agent was represented by SOT neurons, we employed the Neural Decoding Toolbox (Meyers, 2013). Specifically, we assessed the decoding accuracy of a Poisson naive Bayes classifier trained and tested to classify different variables, that is, execution or observation. For each neuron, data were first converted from raster format into binned format. Specifically, we created binned data that contained the average firing rate in 100-ms bins sampled at 20-ms intervals for each trial (data point). We obtained a population of binned data characterized by a number of data points corresponding to the number of trials per conditions (i.e. $30 \times 2 = 60$ data-points for self/other decoding) in an N-dimensional space (where N is the total number of neurons considered for each analysis). Next, we randomly grouped all the available data points into a number of splits corresponding to the number of data points per condition, with each split containing a "pseudo-population", that is, a population of neurons that could be partially recorded separately but treated as if they were recorded simultaneously. Before sending the data to the classifier, we pre-selected those features (neurons) that showed a difference between conditions with $p <$

0.5. Subsequently, the classifier was trained using all but one of the splits of the data and then tested on the remaining one. This procedure was repeated as many times as the number of splits (i.e., 30 in the case of self-other decoding), leaving out a different test split each time. As a measure of classification performance, we used the classification accuracy (Quian Quiroga & Panzeri, 2009), defined as the percentage of trials correctly attributed to the trained condition during test.

3. RESULTS

We recorded single neuron activity during 33 recording sessions (19 for MK1 and 14 for MK2) from the putamen nucleus of both monkeys. We characterized the behavioral performance of both animals during all the sessions from which neuronal recordings were carried out and, at the end of the experiments, we histologically verified the location of the electrodes tracks in both animals and, in MK2, we could also inject neuronal tracers to verify the connectional fingerprint of the physiologically investigated sector.

3.1 Anatomical characterization of the recorded regions

Histological reconstruction of the electrodes' track in each of the recorded animals confirmed that all the penetrations and hence the neuronal recordings were carried out in the putamen nucleus. *Figure 22A* shows the example of an electrode's track in MK2.

Preliminary analyses of cortical labelling following tracers' injections in MK2 showed that the investigated region in the putamen nucleus receives cortico-striatal projections frontal motor, cingulate motor, and superior parietal areas. Specifically, very dense labeling involved areas F1 and F3 (*Figure 22B*); relatively reach labeling was found in the ventral premotor cortex, especially in the subdivisions of area F5 (F5a, F5p and F5c, *Figure 22C*), whereas labeling in the dorsal premotor cortex was more sparsely observed. In the cingulate sulcus, dense labeling involved areas 24c and, less densely, area 24d. In the parietal cortex, labeled cells were observed mostly in area PE, but also in S1. Furthermore, a cluster of labeled cells was observed in the dysgranular insula. Virtually no labelled cells were observed in more rostral premotor and prefrontal cortical regions, confirming that the recordings were confined to a putaminal sector linked with sensorimotor, essentially motor and premotor, areas (*Figure 22D*).

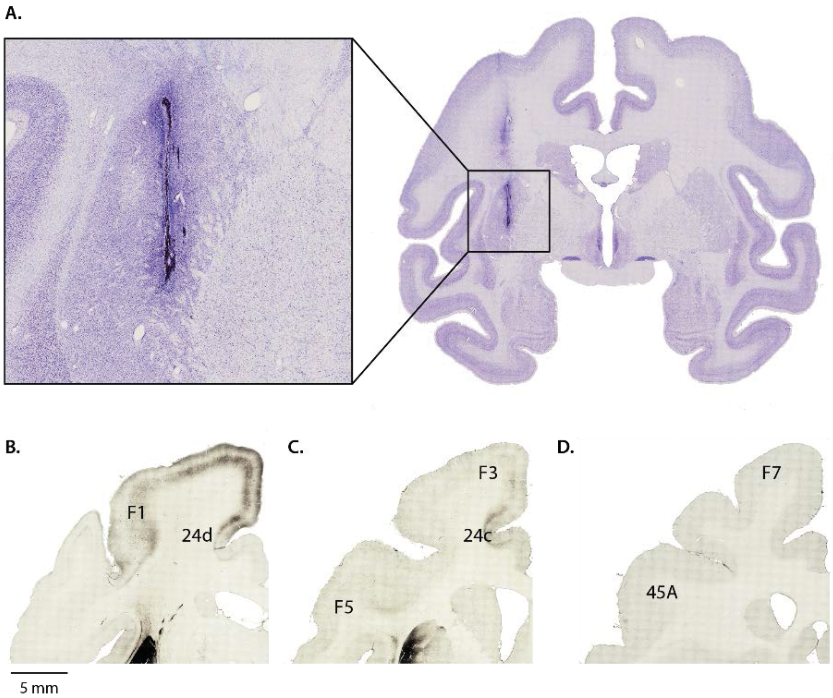

Figure 22| (A) Nissl-stained coronal section of MK2's brain. The blue line in the white matter corresponds to the track of one of the implanted guide tubes and that in the putamen is a gliosis attributable to the electrode penetration for recordings and the needle penetration for injecting FB (yellow deposit). **(B) Immuno-stained section for Alexa 488** showing marked cells in F1 and F3. **(C) Immuno-stained section for Alexa 488** showing labeled cells in F5, F3 and 24c. **(D) Immuno-stained section for Alexa 488** showing absence of marked cells in rostral premotor areas.

3.2 Behavioral data and neuronal responses during task execution

We recorded 573 single units from the putamen nucleus of the two monkeys (428 from MK1 and 145 from MK2) during the *MAT*. These neurons were collected in parallel with the digital signals related to the main behavioral task events and met all the specified criteria for well-isolated single neurons, with no resampling of the same neuron across subsequent recording days with the same implanted probe (see par. 2.5 in Materials and Methods section).

3.2.1 Task execution and error analysis

The monkeys underwent positive reinforcement training to execute the *MAT* with an experimenter acting as a partner (see par. 2.1 in Materials and Methods). Once the task was learnt, the average success rate during the recording sessions, calculated by considering successful trials relative to the total number of trials in a session, was on average 83% for MK1 and 87% for MK2, with single sessions peaking up to 95% and 97% in MK1 and MK2, respectively, indicating an overall robust acquisition of the fundamental task rules by both animals.

Figure 23 reports the frequency of the various types of errors made by each animal across all the recording sessions (see par. 2.2 in Materials and Methods section) and the average time spent to perform the different phases of the task for precision grip (PG) and whole hand grip (WH) trials. Both monkeys performed some errors in the initial stages of the trials, consisting of either aborting the trial as soon as the Go/No-Go cue was presented (*Wrong start*) or releasing the button before the Go signal (*False start*), in the apparent attempt to anticipate the end of the sound. These errors were more frequently made by MK2, who in turn rarely omitted to perform the action (*Omitted start*) or to perform it too slow (*Out of time*) with respect to MK1, emphasizing the presence of an interindividual difference in the propensity of the two animals to perform the task. Besides this difference, a remarkable similarity emerges between the two animals in the generally low number of errors occurring after the action onset and concerning the interaction with the target object, such as *Wrong grip* and *No-hold-up* errors (*Figure 23A*). These findings demonstrate a similar and high degree of accuracy in task performance and compliance with the task instructions for both animals.

The type of errors and the interindividual differences so far described allow to predict a faster reactivity and higher speed in action execution of MK2 relative to MK1, which are confirmed by the analysis of the reaction and action execution time (*Figure 23B*). Nonetheless, it is evident the substantial similarity in the performance of the different stages of task execution in the two animals. Indeed, the reaction time does

not differ between trials instructed with the PG or WH cue in both animals, while PG trials, which require a higher degree of motor control than WH trials, are associated with longer reaching time (although significantly longer only in MK2) and, most importantly, longer grasping/lifting time in both animals (MK1, $p < 0.001$; MK2, $p < 0.001$).

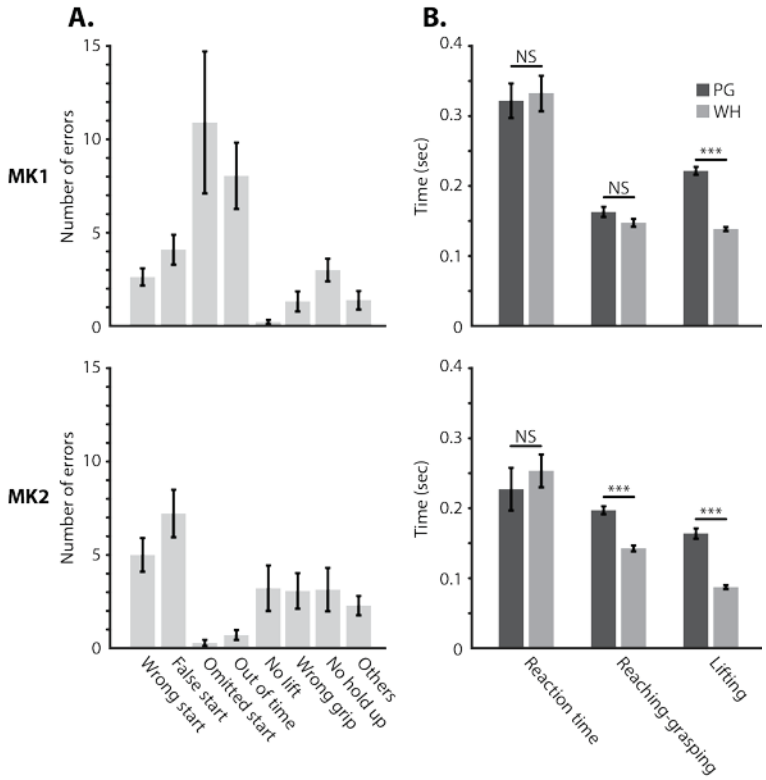

Figure 23| (A) Classification of error types. Each bar represents the average number of errors (± 1StdEr) for each type of error. **(B) Average** (± 1StdEr) **reaction time and execution time for the different motor phases of the MAT** (see Materials and Methods). ***p < 0.001.

3.2.2 Single neuron functional properties

Considering execution trials of the *MAT*, we found that 301 out of 573 recorded neurons (52%) showed motor-related responses (see par. 2.5 in Materials and Methods section). Most of the motor-related cells were facilitated (*n* = 194, 64%), increasing their discharge during different phases of action execution: some increased their firing relative to the baseline already during the *pre-movement* epoch (*n* = 83), and most during *reaching-grasping* (*n* = 140) and/or *lifting-holding* epochs (*n* = 138). The remaining motor-related cells (*n* = 107, 36%), exhibited a significant suppression of their discharge, more uniformly distributed across the different phases of the task, from *pre-movement* (*n* = 52) to *reaching-grasping* and *lifting-holding* (*n* = 61 in each of these phases) (*Figure 24A*).

Examples of facilitated and suppressed motor-related neurons are shown in *Figure 24B*. Neuron 1 exhibits an increase of its baseline activity after the presentation of the object, which further increases during reaching-grasping and peaks slightly before the contact of the hand with the target, with a similar discharge intensity regardless of the grip type; neuron 2 exemplifies the pattern of a suppressed motor-related cell

exhibiting a considerable reduction in its activity relative to baseline before movement onset, with a peak in suppression right after the contact with the target, regardless of the grip type used.

At the population level (*Figure 24C*), we found that both facilitated and suppressed neurons exhibited a similar timing in their peak of (facilitated or suppressed) activity after movement onset, approximately corresponding with hand-target contact. However, suppressed neurons' activity exhibits an earlier and more progressive modulation starting shortly after the start of the trial, when the cue sound is presented, relative to facilitated neurons' response, which is more phasic and time-locked to the Go signal.

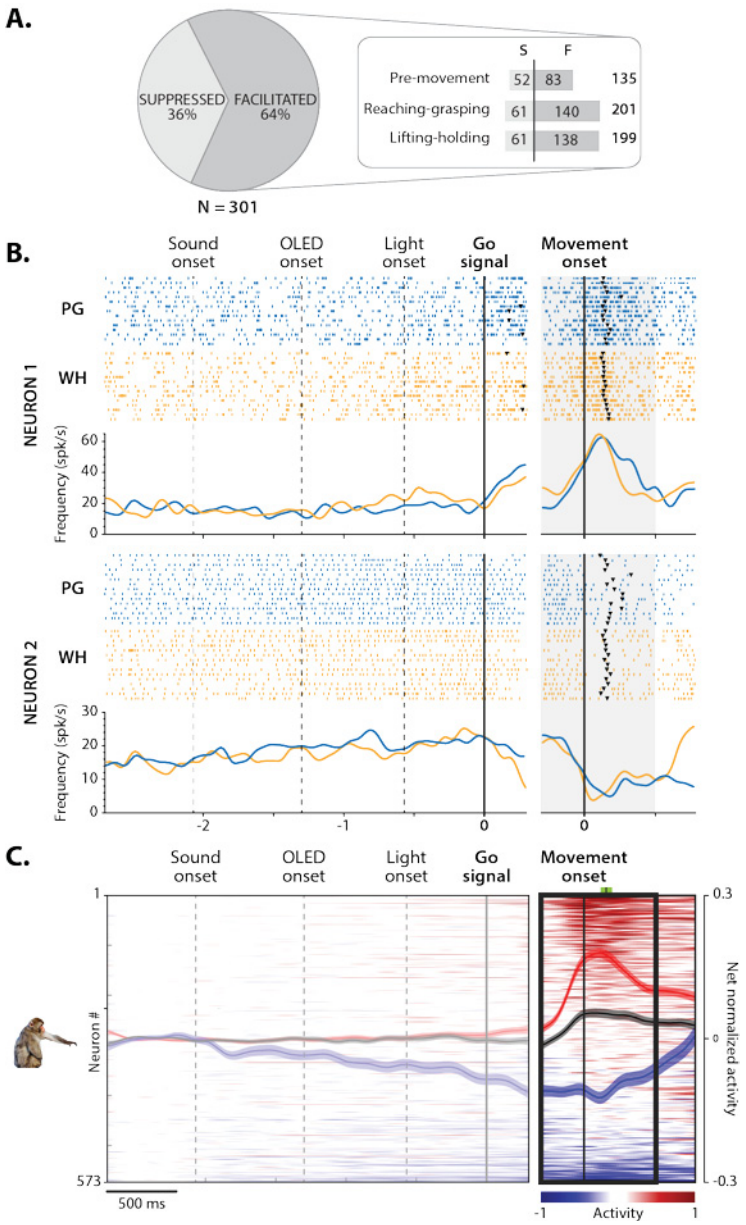

Figure 24| (A) Classification of motor-related neurons. On the left, pie chart displaying the percentage of facilitated (F) and suppressed (S) motor-related units responding throughout the motor period. On the right, number of F and S neurons modulated during the three motor epochs. **(B) Examples of F and S neurons during action execution**. In the first portion of each panel (before the gap), neuronal activity is aligned (vertical black line) to the Go signal [-2.7 to 0 s]; in the second panel (after the gap) it is aligned to Movement onset [-300; +800 ms]. Black triangular markers correspond to the contact with the object. **(C) Heat map and averaged population activity of putaminal neurons recorded during action execution.** The average response and standard error of facilitated, suppressed, and non-motor-related neurons is shown by red, blue and grey lines, respectively. Population activity is superimposed

on the hea tmap of each individual units' net normalized activity (ranging theoretically between -1 and 1), ordered by the magnitude of their modulation around the Movement onset [-300; + 500 ms]. Vertical black bars at the center of the green shaded area depict the average ± standard deviation of contact with the object.

3.2.3 Grip selectivity during action execution

As summarized in *Figure 25A*, 40% of the motor-related units (*n* = 120) showed a significantly different discharge between the two tested grip types, with a remarkably similar number of neurons exhibiting a preference for PG (*n* = 59, 49%) and WH (*n* = 61, 51%). The sign of the modulation of grip-selective neurons has been assessed by calculating the average firing rate of each cell across all the three motor epochs considered together with respect to the baseline. The direction of the modulation of this subset of neurons reflects the one reported for the whole population of motor-related neurons: most of the grip-selective units were facilitated (*n* = 99, 82.5%), while the remaining 21 grip-selective neurons (17.5%) were significantly suppressed.

Figure 25B displays two examples of grip-selective neurons. Neuron 3 exhibits a similar modulation of its activity for both grip types during the pre-movement period and a significant excitation after the release of the button, only for PG trials. On the contrary, neuron 4 represents the example of a unit with an opposite selectivity, displaying a sharp enhancement of its activity before movement onset during WH trials.

The activity of grip-selective facilitated neurons starts to rise after the go signal, almost 200 ms before the release of the starting button, with a peak of activity in correspondence of the contact of the hand with the object. Suppressed units, instead, display a gradual decline shortly after the start of the trial, with a sustained minimum of suppressed activity between the Go signal and object grasping (*Figure 25C*).

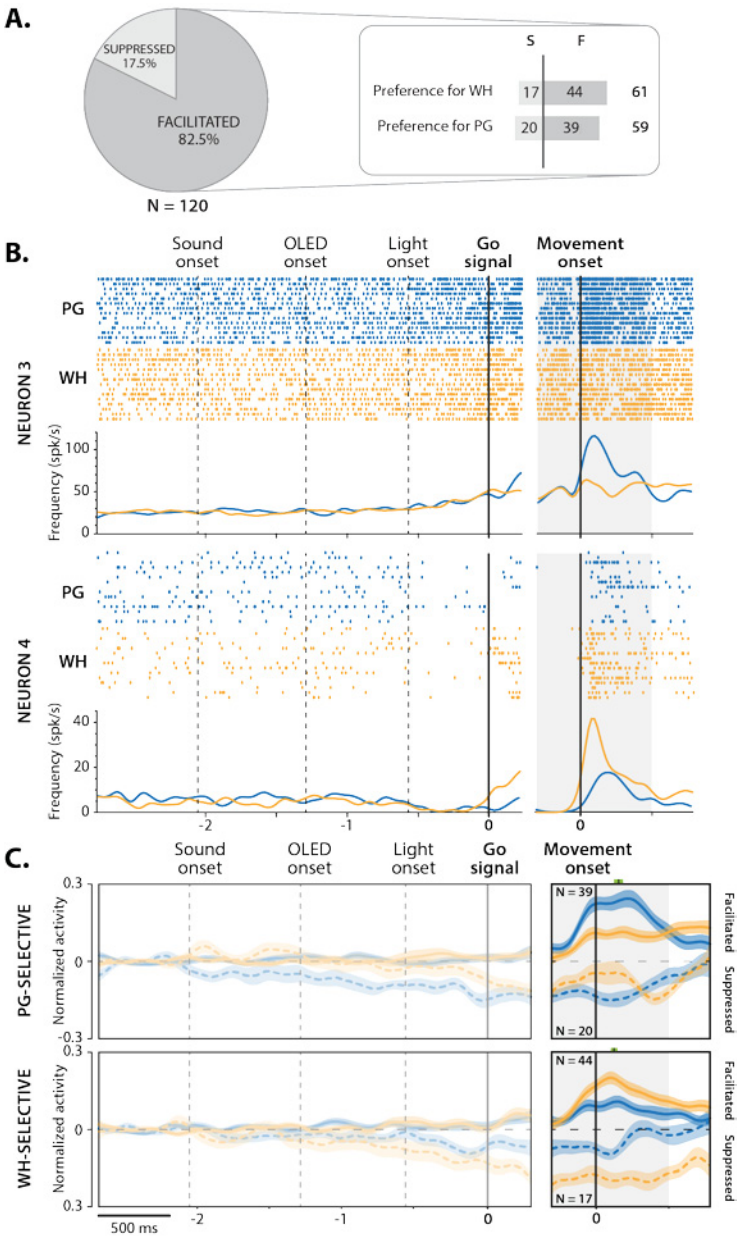

Figure 25 | (A) Classification of grip-selective neurons. On the left, pie chart displaying the percentage of facilitated (F) and suppressed (S) grip-selective units responding throughout the motor period. On the right, number of facilitated (F) and suppressed (S) neurons modulated during action execution for separate grips. **(B) Examples of grip-selective neurons during action execution**. In the first portion of each panel (before the gap), neuronal activity is aligned (vertical black line) to the Go signal [-2.7 to 0 s]; in the second panel (after the gap) it is aligned to Movement onset [-300; +800 ms]. Black triangular markers correspond to the contact with the object. **(C) Population activity of grip-selective neurons during PG and WH trials.** Vertical black bars at the center of the green shaded area depict the average ± standard deviation of contact with the object.

To better investigate the distinction between the two types of prehension, we applied a nonlinear dimensionality-reduction technique (Uniform Manifold Approximation and Projection, UMAP; McInnes et al., 2018) to analyze how the spiking activity of all recoded neurons (including facilitated and suppressed) is related to the type of grip required to perform the reaching-grasping action. The results revealed an absence of segregation between the two grips both during baseline and pre-movement epochs. However, a notable and distinct separation between the clusters becomes evident during the reaching-grasping epoch and persists throughout the lifting-holding period (*Figure 26*). These findings suggest that the spiking activity in

this sector of the putamen is not influenced by the dichotomic instruction concerning the type of grip to perform prior to movement initiation.

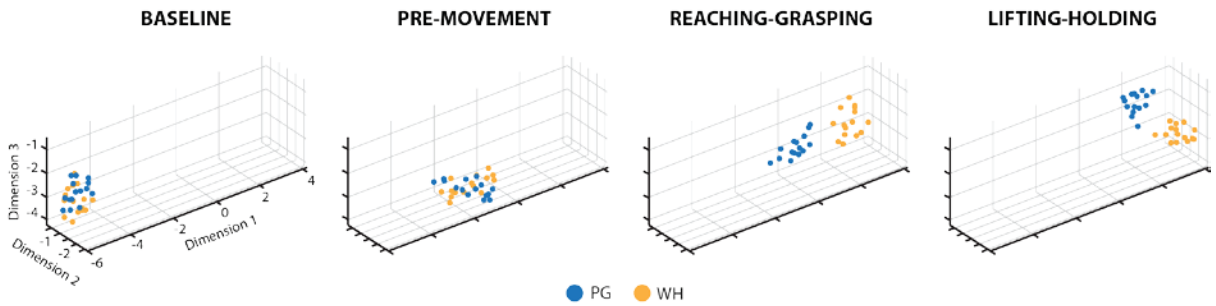

Figure 26 | Population coding of grip and epoch. 3D visualization of the distribution of mean firing rates associated with different epochs and grip types obtained applying a non-linear dimensionality reduction method (UMAP) to the population activity.

3.3 Modulation for self and other's action

3.3.1 Single neuron functional properties

Half of the trials of the *MAT* were performed by the experimenter while the monkey remained still with its hand on the initial position. Thus, we could examine changes in neuronal activity during the observation of the task performed by the experimenter, focusing in particular on the epochs of experimenter's action execution.

Of the 301 neurons previously identified as motor-related, more than 2/3 (n = 212, 70%) did not exhibit any response during action observation, being therefore labeled as *self-type* neurons (ST), while the remaining 89 cells (30%) responded during both action execution and the observation of other's action, being therefore classified as *self-other type* neurons (SOT). An additional set of cells that did not respond when the monkey actively performed the action were selectively modulated during other's action observation trials only, being labeled as *other-type* (OT) neurons (n = 66) (*Figure 27A*).

Examples of each of these classes of neurons are illustrated in *Figure 27B*. Neuron 5 is the example of a ST cell exhibiting facilitated response only during the trials performed by the monkey, and no modulation during the observation of the action performed by the experimenter. On the contrary, neuron 6 exemplifies an OT neurons, which exclusively encodes the observed action being unmodulated during the execution of reaching-grasping actions by the monkey. Finally, neuron 7 is a SOT neuron exhibiting a facilitation for both executed and observed action.

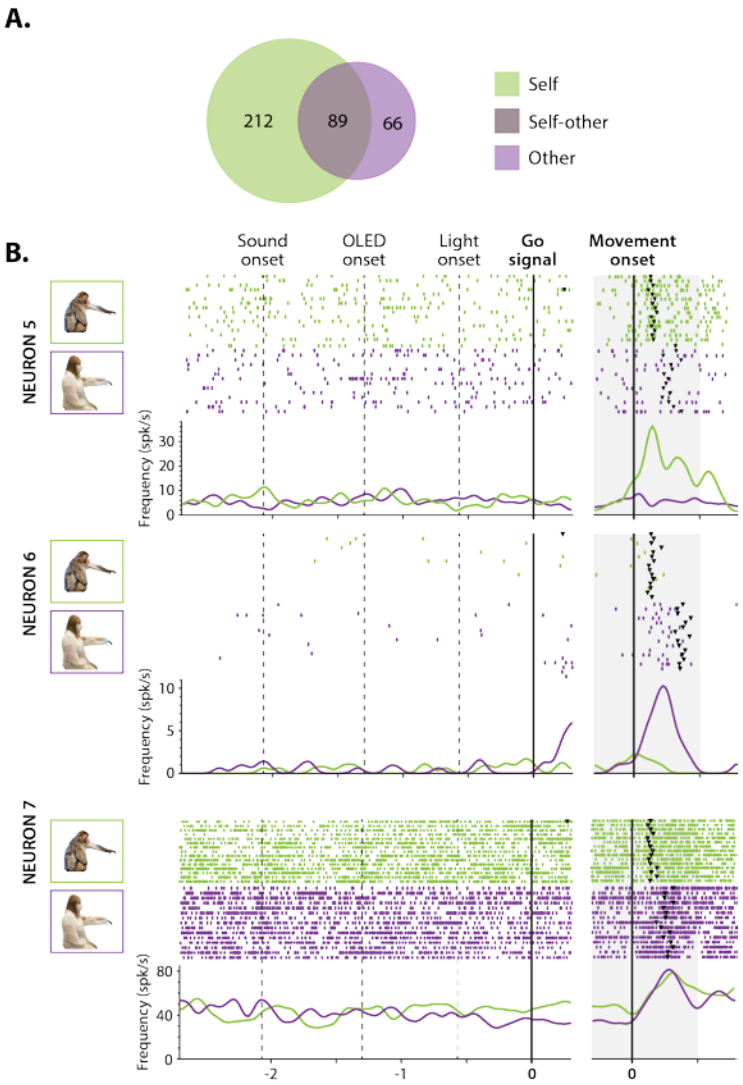

Figure 27 | (A) Classification of motor-related neurons. Venn diagram displaying the number of self-type, other-type and self-other type neurons. **(B) Examples of motor- and other-related neurons**. In the first portion of each panel (before the gap), neuronal activity is aligned (vertical black line) to the Go signal [-2.7 to 0 s]; in the second panel (after the gap) it is aligned to Movement onset [-300; +800 ms]. Black triangular markers correspond to the contact with the object. The activity of each neuron refers to that linked to the best grip.

Overall, considering the functional properties of all the recorded cells characterized by means of the *MAT*, we found that 155 out of the 573 recorded neurons (27%) showed a response for other's action (SOT and OT neurons). The number of facilitated and suppressed cells during the different phases of action observation was well-balanced (F = 81, 52%; S = 74, 48%): some neurons increased their discharge during the *pre-movement* epoch (*n* = 10), some during the *reaching-grasping* epoch (*n* = 17) and most during the *lifting/holding* epoch (*n* = 24). On the contrary, the remaining other-related cells exhibited the strongest suppression in the *pre-movement* (*n* = 22), and a more distributed inhibition during the *reaching-grasping* (*n* = 16) and *lifting-holding* (*n* = 18) periods (*Figure 28A*).

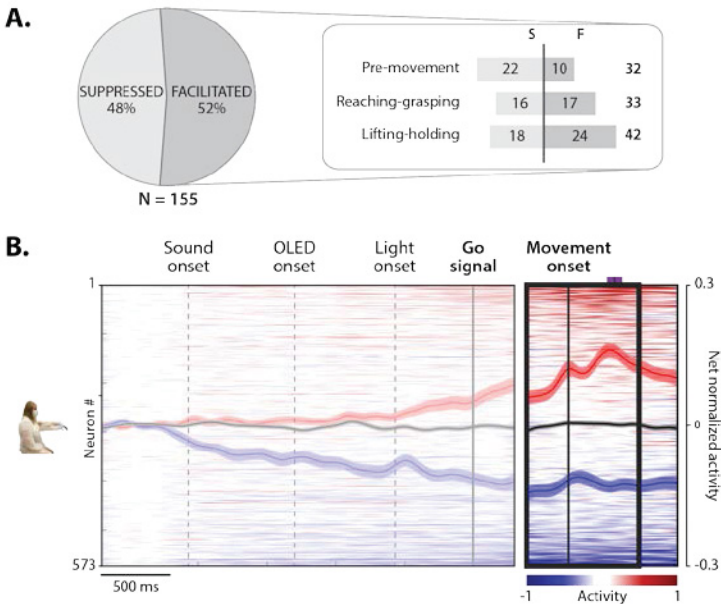

Figure 28 | (A) Classification of other-related neurons. On the left, pie chart displaying the percentage of facilitated (F) and suppressed (S) other-related units responding throughout the motor period. On the right, number of F and S neurons modulated during the three motor epochs. **(B) Functional fingerprint of putaminal neurons during action observation.** The average response and standard error of facilitated, suppressed, and non-action-related neurons is shown by red, blue and grey lines, respectively. Population activity is superimposed on the heat map of each individual units' net normalized activity (ranging theoretically between -1 and 1), ordered by the magnitude of their modulation around the experimenter's movement onset [-300; + 500 ms]. Vertical black bars at the center of the green and purple shaded area depict the average ± standard deviation of contact with the object for execution and observation trials, respectively.

Differently from what has been observed in the execution condition, the facilitated units during action observation reached their peak of activity in correspondence of the contact with the object. Conversely, suppressed neurons displayed a decrease of their discharge, particularly pronounced during the observation condition, following the beginning of the trial (*Figure 28B*).

Among neurons responding to the observed action, about 19% of them displayed a significantly different modulation when observing actions performed with different types of grip (*n* = 13 out of 66 OT neurons and *n* = 34 out of 89 SOT neurons). However, when comparing visual and motor selectivity of SOT neurons, only 21 cells resulted to be grip-selective during both execution and observation and, among them, only five neurons maintained a congruent preference for the same grip type in the visual and motor mode, whereas the remaining 16 cells exhibited an opposite selectivity.

Considering the subpopulation of all those neurons responding at least to the executed action (n = 301), we found that 48 out of 89 SOT neurons (54%) showed a significant discharge difference between PG and WH trials in the execution condition, being classified as grip-selective, in contrast to only 72 out of 212 (34%) of ST neurons ($\chi^2 = 10.4$, $p < 0.05$), indicating a more widespread motor selectivity for the grip type among neurons responding also to others' observed actions.

In order to address the impact of monkey's hand visual feedback on the motor discharge of ST and SOT putaminal neurons, we tested all the recorded cells both when the action was performed in complete darkness and in full light condition, randomizing the two types of trials in each recording session, thereby

enabling us to compare single neuron discharge between the two conditions. To do so, for each recorded neuron we first identified the grip associated to the greater modulation when the action was executed in complete darkness and compared its response during the same type of grip performed in the light (as well as during the observation of the same type of grip performed by the experimenter). We calculated the absolute difference between the average firing rate recorded throughout all three motor epochs considered together and the baseline for that specific grip condition in order to take into account the modulation of both facilitated and suppressed units. We found that the great majority of ST neurons (*n* = 178, 84%) did not exhibit significantly different discharge between grasping in the dark and in the light; 23 neurons (11%) discharged significantly stronger during grasping in the dark and only 11 cells (5%) exhibit a preference for grasping in the light. Interestingly, the subset of neurons discharging also during the observation of the experimenter's action exhibits an even more marked lack of preference for the grasping in the light condition: indeed, most SOT neurons (*n* = 80, 90%) did not discharge differently between the two conditions and all the remaining 9 cells exhibit a preference for grasping in the dark. Thus, these findings suggest that the availability of monkey's own hand visual feedback does not significantly impact on putaminal neurons motor discharge, even in the case of those neurons that exhibit a response during the passive observation of others' action.

3.3.2 Agent selectivity

The results presented so far suggest a considerable distinction of neuronal activity related to self and other's action in the macaque putamen. To directly explore this issue, we first assessed the population activity of agent-selective (ST and OT) neurons during execution and observation trials, distinguishing facilitated and suppressed units (*Figure 29*). Noteworthily, among ST neurons, facilitated cells largely prevailed with respect to suppressed ones. In addition, OT cells show a balanced number of facilitated and suppressed units relative to ST neurons ($\chi^2 = 9.47$, $p < 0.05$).

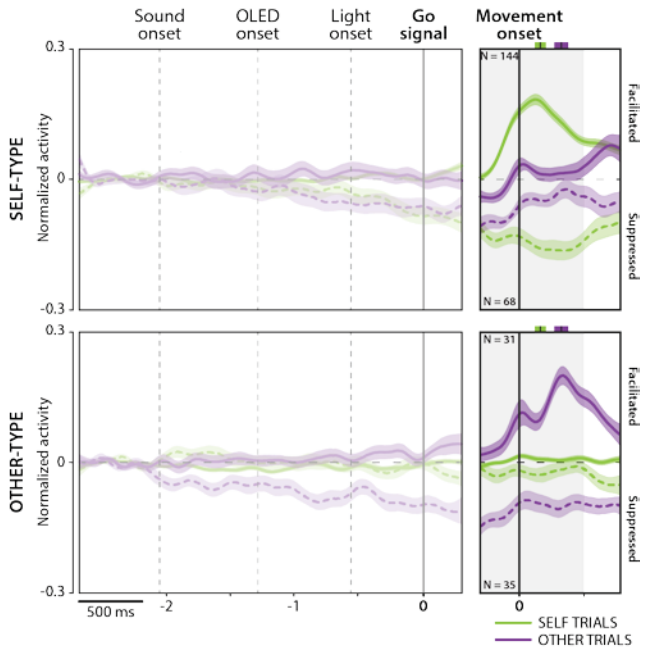

Figure 29 | Population activity of agent-selective neurons during execution and observation trials. Vertical black bars at the center of the green shaded area depict the average ± standard deviation of contact with the object. Solid and dashed polyline indicate population activity for facilitated and suppressed neuronal populations, respectively.

Next, we wanted to better investigate the pattern of discharge of those neurons exhibiting both a motor and visual response during task execution and observation, so putatively showing agent-shared coding. Interestingly, among the 89 SOT neurons, less than half (*n* = 41, 46%), were facilitated during both task execution and observation (facilitated-facilitated, *FF*), but exhibited a stronger and earlier discharge during task execution relative to task observation; another set of neurons (*n* = 26, 29%) consistently displayed suppression (suppressed-suppressed, *SS*) in both contexts (*Figure 30*). The remaining 25% of the neurons exhibited opposite modulation in the two contexts, being significantly excited during active movement but inhibited during action observation (facilitated-suppressed, *FS, n* = 13, 15%), or vice versa (suppressed-facilitated, *SF, n* = 9, 10%), thus being agent-selective.

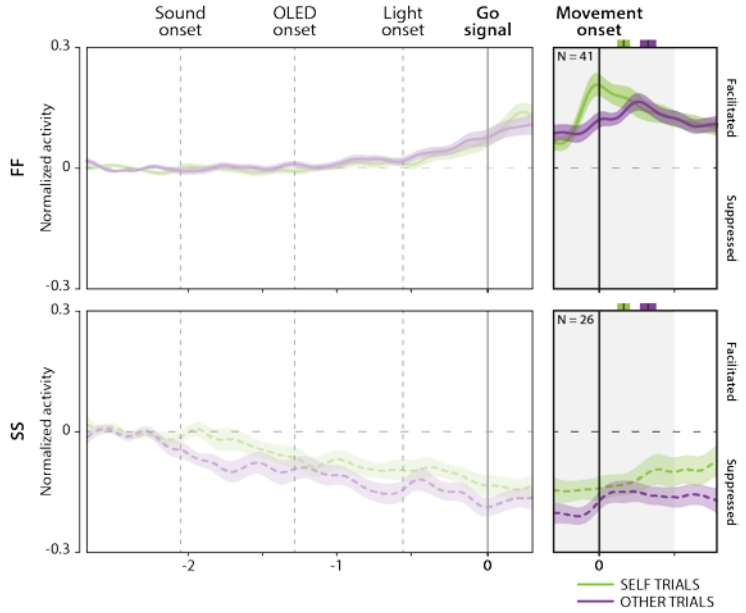

Figure 30 | Population activity of self-other neurons during execution and observation trials. Vertical black bars at the center of the green shaded area depict the average ± standard deviation of contact with the object during execution trials. Vertical black bars at the center of the purple shaded area depict the average ± standard deviation of contact with the object during observation trials.

In order to assess the magnitude of information about the acting agent carried by these neuronal subpopulation, we trained a classifier to distinguish self- from other's action based on the activity of SOT neurons recorded during own-action execution and other-action observation: by testing the decoding performance using the entire neural population of SOT neurons (see par. 2.5 in Materials and Methods), we found that agent decoding accuracy raises considerably even before movement onset (*Figure 31A*). Interestingly, a similar decoding accuracy is achieved also by focusing only on FF-SOT neurons (*Figure 31B*), indicating that despite an apparently shared coding of executed and observed actions, also those neurons apparently carry agent-specific signals enabling to discriminate who is performing the action. No clear

distinction between self and other's action can be assessed by SS-SOT neurons: the classification accuracy of the decoder barely reaches 80% only after movement onset.

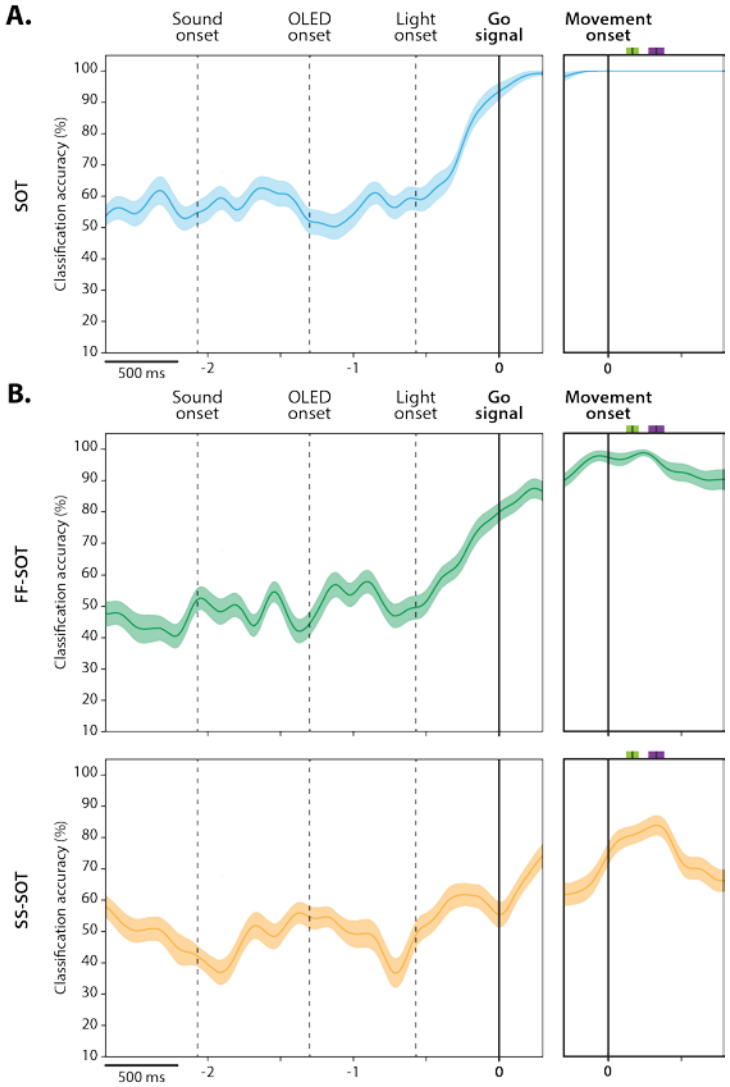

Figure 31 | Classification accuracy about agent during self- and other's trials. (A) Classification accuracy about agent during execution and observation trials decoded from SOT neuronal population activity. **(B)** Classification accuracy about agent during execution and observation trials decoded from FF- and SS-SOT neuronal population activity.

4. DISCUSSION

A growing body of evidence supports a significant role played by areas within the cortical motor system not only in action planning and execution, but also in a wide array of perceptual, cognitive, and social functions. It is widely accepted that regions dedicated to motor control also contribute to the processing of others' observed actions, forming an extended Action Observation Network (AON) (Bonini et al., 2022). Recent anatomical data have revealed a tight anatomo-functional connectivity between cortical nodes of the AON and specific regions of the putamen nucleus in the basal ganglia (Gerbella et al., 2016), suggesting that this structure may constitute a potential additional, subcortical node of the AON (Bonini, 2017). However, the functional characteristics of putamen neurons are still largely unknown, both in terms of their functional properties during action execution (e.g. the capacity to encode distal features of manual actions such as the grip type) and during the observation of actions performed by others.

Here, we showed that during a Mutual Action Task in which the monkey and an experimenter took turns to perform a manipulative grasping action on the same device in a shared space, many neurons in the putamen nucleus can code reaching-grasping actions performed by the monkey, sometime exhibiting selectivity for the type of grip; other neurons specifically encode the experimenter's action passively observed by the monkey, and another subpopulation is modulated during both the execution of monkey's own action and the observation of the experimenter's action. In the next paragraphs, these different response properties and their interaction at the single neuron level will be discussed in comparative terms with respect to the well-established properties of neurons in the cortical regions of the AON that send convergent projection to this striatal region.

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

According to the robust evidence of sensorimotor processing of contextual cues instructing how (Bonini et al., 2012; Buchwald & Scherberger, 2021; Raos et al., 2004, 2006; Sakata et al., 1995) and when (Livi et al., 2019; Maranesi et al., 2014; Mazurek et al., 2018) to grasp objects in cortical areas of the AON, we designed the task to evaluate the possible contribution of putaminal neurons in this function. Indeed, during the instructive cue phase, the type of sound that was presented instructed the monkey to plan and subsequently perform the action, or to refrain from acting while the experimenter did the action in front of it. Previous studies showed that neurons belonging to parietal and premotor areas projecting to the putamen exhibit very weak or no response to auditory instructive cues (Albertini et al., 2020; Bonini et al., 2014; Bruni et al., 2015; Lanzilotto et al., 2019, 2020; Livi et al., 2019). Likewise, we did not find any evidence of significant coding of the instructive cue sound, neither at the single neuron level nor in population decoding analyses. More surprisingly, we did not find clear evidence of agent or object coding neither during the presentation

of visual cues instructing the type of grip to be performed, nor the target object. Both these types of information have been shown to strongly modulate neurons in both the parietal and premotor areas (Bonini et al., 2012; Buchwald & Scherberger, 2021; Raos et al., 2004, 2006; Sakata et al., 1995). It may be argued that presenting a variety of objects with an unpredictable variety of sizes and shapes increases the need of neuronal tuning to encode these variables. However, previous studies on the same parieto-frontal regions used color codes to instruct monkeys about how to grasp the same manipulandum, with either a PG or WH grip as in the current study, and found consistent modulation in both single neuron and population activity in both parietal (Baumann et al., 2009) and premotor (Fluet et al., 2010) regions known to send consistent projections to the investigated sectors of the putamen nucleus (Gerbella et al., 2016), as also confirmed by the observation derived from tracers injection in the current experiment. Thus, it seems that this sector of the putamen is not particularly sensitive to visuomotor information about visually presented objects or specific instructions related to the distal component of manual actions. This was rather unexpected given previous evidence of set-related activity in putaminal neurons for arm movements toward different directions (Alexander, 1987; Kunimatsu et al., 2019). A possible interpretation of this discrepancy is that putaminal neurons' discharge reflect some specificities of the hand-shape for object grasping derived from cortico-striatal projection, but its local processes does not enhance this distal selectivity, possibly favoring broader action selection mechanisms such as those involved in arm-reaching direction.

Based on the above-mentioned observations on the cue periods, here we focused the analysis on the rich set of properties reported during action execution. Similarly to what was found in premotor area F5, more than half of the recorded neurons showed a modulation of their discharge during some phases of the grasping actions performed by the monkeys, with a peak of activity after movement initiation (Ferroni et al., 2021). However, differently from premotor areas this region is linked with, putaminal neurons do not exhibit modulation in response to visual instructions distinguishing the type of grip required to perform the action, and the selectivity emerges only after movement onset. This suggests that when the object is presented, AIP generates an early signal indicating object selectivity, closely coordinated with F5, which then transmits the information to putaminal neurons which, in turn, select the appropriate cortical motor plan by projecting back to the cortex, which details the distal shape of the fingers to adapt to the object features.

A recent work by Ferroni and colleagues (2021) studied the functional properties of neurons recorded from three of the crucial nodes of the AON that are known to have reciprocal connections with the basal ganglia (BG): premotor areas F5 and F6, and AIP. The experimental paradigm employed by the authors of this research was broadly similar to the one employed in the current study, allowing for a direct comparison between our findings and those collected in the cited cortical areas that are source of projection to the putamen (Borra et al., 2021; Gerbella et al., 2016) and therefore casting light on the possible similarities and differences in the cortical and BG properties. Consistent with observations in the AIP and F5 areas, where a majority of the recorded neurons displayed facilitated activity during action execution, we found that more

than half of putaminal neurons were facilitated during the monkey's active movements, reflecting the glutamatergic excitatory input coming from the cortex. This differs from the findings in F6, where suppressed neurons were predominant. Notably, the fraction of units significantly facilitated during the motor period was higher than that of the suppressed units, with most of the cells exhibiting a facilitation during the two epochs of active movement, that are those requiring the higher degree of motor control.

However, the effect these modulations exert on motor output requires further investigations. It is presumable to assume that we recorded the activity of MSNs, which represent the 90% of the population of striatal neurons (Tepper & Bolam, 2004). Depending on the receptors these neurons express, the outcome on movement is different: the increased discharge of D_1-expressing MSNs, which project to the GPi and SNpr, results in a facilitation of movement; on the contrary, the increased discharge of D_2-expressing MSNs results in a disinhibition of the activity of STN, which promotes the activity of GPi and SNpr, thereby inhibiting movement. Future studies should undertake direct chemical perturbation of putaminal neurons' activity with agonists/antagonists of these specific dopamine receptors in order to offer causal evidence and gain deeper understanding of the properties of neurons belonging to the direct or indirect pathway.

We also found units exhibiting a suppression during action execution, whose inhibition might be the result of a local mechanism in which neurons projecting to GPi or GPe simultaneously suppress the activity of nearby neurons, given that the overwhelming majority of afferences to the putamen are excitatory and should produce facilitation rather than inhibition.

4.2 Encoding one's own and other's action

Most of the recorded neurons were active during either monkeys' own movement (*self-type*), the observation of the experimenter's action (*other-type*), or both (*self-other type*); only a smaller fraction of cells did not show any action-related modulation.

Several evidence supports the genuinely other-related response of these neurons. First, although we did not monitor EMG activity during the observation task, leaving open the possibility that the modulation of SOT and OT neurons might be a result of the monkey's covert movement, we simultaneously recorded from multiple neurons, including many ST cells that exhibited a clear preparatory and motor-related discharge but remained inactive during action observation. Second, these neurons did not exhibit any response to the cue instructing which grip type had to be executed nor during object presentation, excluding any modulation related to own eye movements that the monkey must perform in order to be able to select the appropriate action to execute. In addition, similarly to what has been assessed in premotor and parietal areas (Caggiano et al., 2009; Ferroni et al., 2021; Maranesi et al., 2015), neurons responding to others' action exhibit a different tuning to the different phases of the task with respect to self-related neurons, with the peak of activity occurring right before the contact of the hand with the object.

To date, neurons encoding others' action have been found in a variety of brain areas, in particular in mesial frontal areas (Livi et al., 2019; Ninomiya et al., 2020; Yoshida et al., 2011). Among those neurons showing a modulation for others' action, we found that more that 40% of them did not respond during monkeys' own movement, being specifically activated by the observed action. Interestingly, 55 of them were recorded in MK1 (55 out of 428, 13%), whereas the remaining 11 OT cells were recorded in MK2 (11 out of 145, 7.5%). Despite the similarity of these percentages (χ^2 = 2.945, p = 0.08), further anatomo-functional analyses should scrutinize if, at least in MK1 where a larger and more anterior region of the putamen was explored, OT neurons are more markedly present in those regions of the putamen that receive projections from the cortical areas in which the dichotomic coding of self- and other's action is more pronounced compared to the lateral AIP-F5 circuit.

4.2.1 Regulation of motor resonance

Another notable result of the current study consists in the identification of neurons that exhibited a modulation during both action execution and observation. It is important to note that not all these neurons possessed exclusively "classical" mirror-like properties characterized by a sharp increase in firing rate during both conditions (FF), although such neurons were more abundant (49%). In addition, a considerable fraction of units (29%) consistently displayed suppression (SS), while others exhibited opposite changes in activity during the two considered conditions (FS and SF).

Kraskov and colleagues (2009) previously demonstrated the presence of mirror-like activity in F5 pyramidal tract neurons (PTNs) and were the first to note the existence of suppressed MN activity during action observation. They described the presence of a noteworthy fraction of units (56%) exhibiting a suppressed pattern of discharge during action observation, being excited during action execution (FS). Soon after, the existence of neurons with reduced activity during action observation has also been reported in M1, where the 20% of recorded neurons was consistently suppressed during the observation of the experimenter's grasping movement and facilitated during the monkeys' own movement (Kraskov et al., 2014; Vigneswaran et al., 2013).

The authors proposed that, while the facilitated ventral premotor output is commonly associated with the "motor resonance" phenomenon (Cattaneo et al., 2009; Fadiga et al., 1995; Montagna et al., 2005; Rizzolatti & Luppino, 2001; Strafella & Paus, 2000), the inhibitory mirror-like activity in PTNs might constitute one of the mechanisms contributing to the disfacilitation of motoneurons that subserve the suppression of unwanted self-movement during the observation of others' actions. However, it is assumable that the suppression during action observation is not part of an intrinsic mechanism that "disfacilitates" PTNs' output, but the result of a disfacilitation resulting by an activation of D_2-expressing neurons of the BG by means of corticostriatal SOT neurons that, via the thalamo-cortical projections of the indirect pathway, inhibits movement. If this were the case, local injections of D_2 receptor agonists should selectively affect the activity

of striatal neurons displaying responses during action observation relative to ST neurons. This, in turn, would lead to a general decrease in thalamic facilitation of cortical PTNs during action observation.

Finally, the activations we observed when manipulating the visual feedback of the action suggest that the response of putaminal neurons to other's action might not be comparable to those observed in the cortex, where information about biological movement arrives to parietal and premotor neurons that exhibit a facilitation when the action is performed in full light (Albertini et al., 2021; Maeda et al., 2015; Maranesi et al., 2015; Raos et al., 2004; Sakata et al., 1995). Indeed, the modulations we recorded during action observation may be related to an inhibitory control of movement due to the fact that the other is interacting with the same target the monkeys have access to. This is supported by the fact that almost all SOT neurons do not exhibit a significantly different response when the action is executed in full light or in dark condition, suggesting that the information that is conveyed by the BG is the byproduct of a sensory-motor integration already processed by the cortex.

4.2.2 Conclusions

In summary, the present findings constitute one of the first empirical demonstrations of the existence of neurons specifically modulated by others' observed actions in the putamen nucleus of the macaque. These results strengthens the hypothesis, consistent with previous human studies (Alegre et al., 2010; Kessler et al., 2006), suggesting the involvement of this subcortical nucleus in the AON. They also and highlight the need of thoroughly investigating its overall modulatory impact on the functioning of the AON cortical nodes and exploring its possible functional role.

It would be interesting to further explore the firing properties of OT neurons when the experimenter's action is performed in the dark to probe the hypothesis that an endogenous process linked to the dynamic reconstruction of what the other is doing activates neurons responding to other's action. It is assumable that their activation relies on a pragmatic coding mechanism that exploits information about other's action to select or inhibit the agent's potential actions. We therefore may hypothesize that by interposing a barrier between the monkey and the target object when the experimenter grasps the object, these neurons decrease their activity because the mechanisms necessary to withhold an action conflicting with the other's action become unnecessary.

In addition, future studies in which putaminal neurons' activity is chemically perturbed are necessary to offer causal evidence and gain deeper understanding of the potential mechanisms by which cortical and subcortical mechanisms collaborate to decouple cortical motor representations from motor output, preventing the automatic re-enactment of observed actions by the observer.

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