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NEURAL CORRELATES OF SPONTANEOUS BEHAVIOURS IN THE
VENTRAL PREMOTOR CORTEX OF THE FREELY BEHAVING
MONKEY

CORRELATI NEURALI DI COMPORTAMENTI SPONTANEI NELLA
CORTECCIA PREMOTORIA VENTRALE DELLA SCIMMIA IN
LIBERTA' DI MOVIMENTO

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ABSTRACT - English

Classical neurophysiological studies allowed us to get fundamental insight on the neural underpinnings of motor and cognitive functions of the premotor system. However, traditional approaches suffer from several limitations, mostly arising from the tethered neural recording approaches: these methodologies require physical-restrained monkeys to make possible the connection of intracortical electrodes via cables to the amplifiers and recording devices. Thus, monkeys can only perform structured tasks characterized by fairly simple, repeated actions and movements in artificial conditions, which could affect the ecological validity of the conclusions and the range of behaviors that can be investigated. For this reason, advances in neurotechnologies have led to the development of wireless recording and stimulation systems, which promise to expand the information obtained with classical methodologies by recording the same neuronal cells' activity continuously, for long periods of time and in different ethologically relevant conditions. In this study, using a wireless recording system synchronized with a multicamera system, we recorded the neural activity of the ventral premotor cortex (specifically, of area F4) in one monkey during two experimental sessions in which it was totally unrestrained, so it could freely move within the experimental environment (the NeuroEthoRoom), interact with some enrichment items and perform spontaneous behaviors. We built an ethogram of observable behaviors performed by the monkey and found significative neural activation during some of them. Moreover, by means of a hierarchical aligned cluster analysis (HACA), we verified the possibility of inferring which behavior the monkey was performing at a given moment by means of neural activity readout. Although preliminary, these findings open new ways to investigate the neural basis of natural primates' behaviors.

ABSTRACT – Italiano

Gli studi neurofisiologici classici hanno permesso di ottenere informazioni fondamentali sulle basi neurali delle funzioni motorie e cognitive del sistema premotorio. Tuttavia, gli approcci tradizionali soffrono di diverse limitazioni per lo più derivanti dall'utilizzo di sistemi di registrazione via cavo: queste metodologie richiedono la restrizione dei movimenti della scimmia per rendere possibile la connessione degli elettrodi intracorticali, tramite cavi, agli amplificatori e ai dispositivi di registrazione. Pertanto, le scimmie possono svolgere solo compiti strutturati, caratterizzati da azioni e movimenti piuttosto semplici e ripetuti in condizioni artificiali, che potrebbero influenzare la validità ecologica delle conclusioni e la gamma di comportamenti che possono essere indagati. Per questo motivo, i progressi nelle neuro-tecnologie hanno portato allo sviluppo di sistemi di registrazione e stimolazione wireless, che promettono di espandere le informazioni ottenute con metodologie classiche registrando l'attività delle stesse cellule neuronali in modo continuo, per lunghi periodi di tempo e in diverse condizioni etologicamente rilevanti. In questo studio, utilizzando un sistema di registrazione wireless sincronizzato con un sistema multicamera, abbiamo registrato l'attività neurale della corteccia premotoria ventrale (nello specifico, dell'area F4) in una scimmia durante due sessioni sperimentali in cui era totalmente libera di muoversi all'interno dell'ambiente sperimentale (la NeuroEthoRoom), interagire con alcuni arricchimenti ed eseguire comportamenti spontanei. Abbiamo costruito un etogramma dei comportamenti osservabili eseguiti dalla scimmia e riscontrato un'attivazione neurale significativa durante alcuni di essi. Una hierarchical aligned clustered analysis (HACA) ha mostrato la possibilità di inferire quale comportamento stesse eseguendo la scimmia in un dato momento in base alle attivazioni neurali. Sebbene preliminari, questi risultati suggeriscono nuovi approcci per studiare le basi neurali dei comportamenti naturali dei primati.

1. INTRODUCTION

For so long neuroscience has been aspiring to explain how human brain activity causes behaviour, and the results of neurophysiological studies on animals, especially on non-human primates, have provided pioneering information about this issue. Thanks to Woolsey's studies (1952), we have been able to learn that our brain contains a somatotopic representation of our body and of the movements that we can perform with it. Subsequent studies on motor mapping with intracortical microstimulation (ICMS) have revealed that there is not only a single map, and that in large cortical territories local spots on the maps represent not just simple movements but complex, multi-joint goal-directed actions, such as reach-to-grasp or defensive behaviors (Graziano et al. 2002, 2005).

Studies on motor cortex have also found that Premotor cortex (BA6) differs from the Primary Motor cortex (BA4) citoarchitectonically and functionally (Gentilucci et al., 1988; Rizzolatti et al., 1998); the former contains neurons with more complex properties than the motor neurons of the Primary Motor cortex, and it has been suggested its involvement in higher order functions, such as sensory-motor transformations (Rizzolatti and Luppino, 2001), goal-directed actions coding (Rizzolatti et al., 1988a; Gentilucci et al. 1988; Rizzolatti et al., 1988b), action recognition (Gallese et al., 1996; Umiltà et al., 2001) and decision making (Pardo-Vasquez et al., 2008). Specifically, in Premotor Cortex neurons have been found that encode the goal of the actions instead of the single movements constituting them. For example, in F5 there are neurons that encode grasping actions (Rizzolatti et al., 1988a) regardless of the specific sequence of movements required to grasp the object (Umiltà et al. 2008), whereas in F4 there are neurons that encode reaching actions toward objects but not general extension of the arm for different purposes (Gentilucci et al. 1988; Rizzolatti et al., 1988b).

Areas of the ventral Premotor cortex are related to specific parietal regions, with which they form frontoparietal circuits underlying sensory-motor transformations allowing to convert sensory input, such as the visual ones, in to appropriate motor plan to achieve behavioural goals. Area F4 is reciprocally connected with the area VIP, forming the reaching circuit that transforms the spatial location of objects into the appropriate motor parameters of the arm to reach them. Area F5, together with area AIP, constitutes the grasping circuit, which commute the physical characteristics of objects, such as shape and size, in the correct hand posture and motor pattern to grasp them.

Even if classical neurophysiological studies allowed us to get fundamental insight on the neural underpinning of motor and cognitive functions of the premotor system, promoting the development of the most recent approaches to neuroprosthetics (Bouton et al., 2016), a deep and ecological valid comprehension of the brain-behaviour relationship, capable to open up new translational perspective and research avenues, implies to verify the robustness and generalization of this knowledge to unconstrained, natural behaviors. For this reason, advances in neurotechnology have led to the development of wireless recording and stimulation systems, which promise to expand the information obtained with classical methodologies by recording the same neuronal cells' activity continuously, for long periods of time and in different ethologically relevant conditions, which could not be studied otherwise: sleep (Jackson et al., 2007; Dragoi et al., 2020), motor activity (Berger et al., 2020), walking (Capogrosso et al., 2016) or social behaviour and interaction (Roy and Wang, 2016) are just some of the most recent examples of spontaneous and natural behaviors that underwent neurophysiological investigation.

For a long period of time, students have accepted the idea that a single motor map exists in the motor cortex: distal movements were deemed to be represented in BA4, while proximal and axial movements in BA6 (Woolsey et al. 1952).

Further studies on PMv have showed that distal movements are represented also in BA6 (Rizzolatti et al., 1981a), and neurons in the rostral part of BA6 are activated with tactile stimulation of distal parts of the body (Rizzolatti et al., 1981b). Moreover, microstimulation experiments (Gentilucci, 1988) have demonstrated that distal movements are represented both near the central sulcus and near the arcuate sulcus, while proximal movements are represented both in F4 and in the rostral portion of F1. However, unlike F1 where the representations of parts of the body are clearly segregated, PMv shows partially overlapping representations of different effectors. These findings demonstrate the existence of two independent motor maps in primates' motor cortex, but they also showed that even within the PMv there is a more complex organization of movement representation than that in primary motor cortex. In fact, electrical stimulations of area F4 frequently evokes arm movements associated to mouth, neck or face movements, while electrical stimulations in F1 recruits typically a single joint. The work of the Michael Graziano's group (2002, 2005) further extended this evidence by exploring cortical motor maps with increased duration of the intracortical microstimulations: from no more than 50/100 msec to time interval approximating the duration of ethologically-relevant behaviours (500 msec or longer). In this way, they discovered a map of actions in PMv, where "ethologically relevant behaviors" appears to be the organizational principle. For example, long-trains ICMS evoked multi-joint actions such as closing the hand in a grip while bringing the hand to the mouth and opening the mouth, extending the hand away from the body with the grip opened as for preparing to grasp an object or bringing the hand

inward to a region just in front of the chest while shaping the fingers as for manipulating an object (Graziano and Aflalo, 2007) (Figure 2).

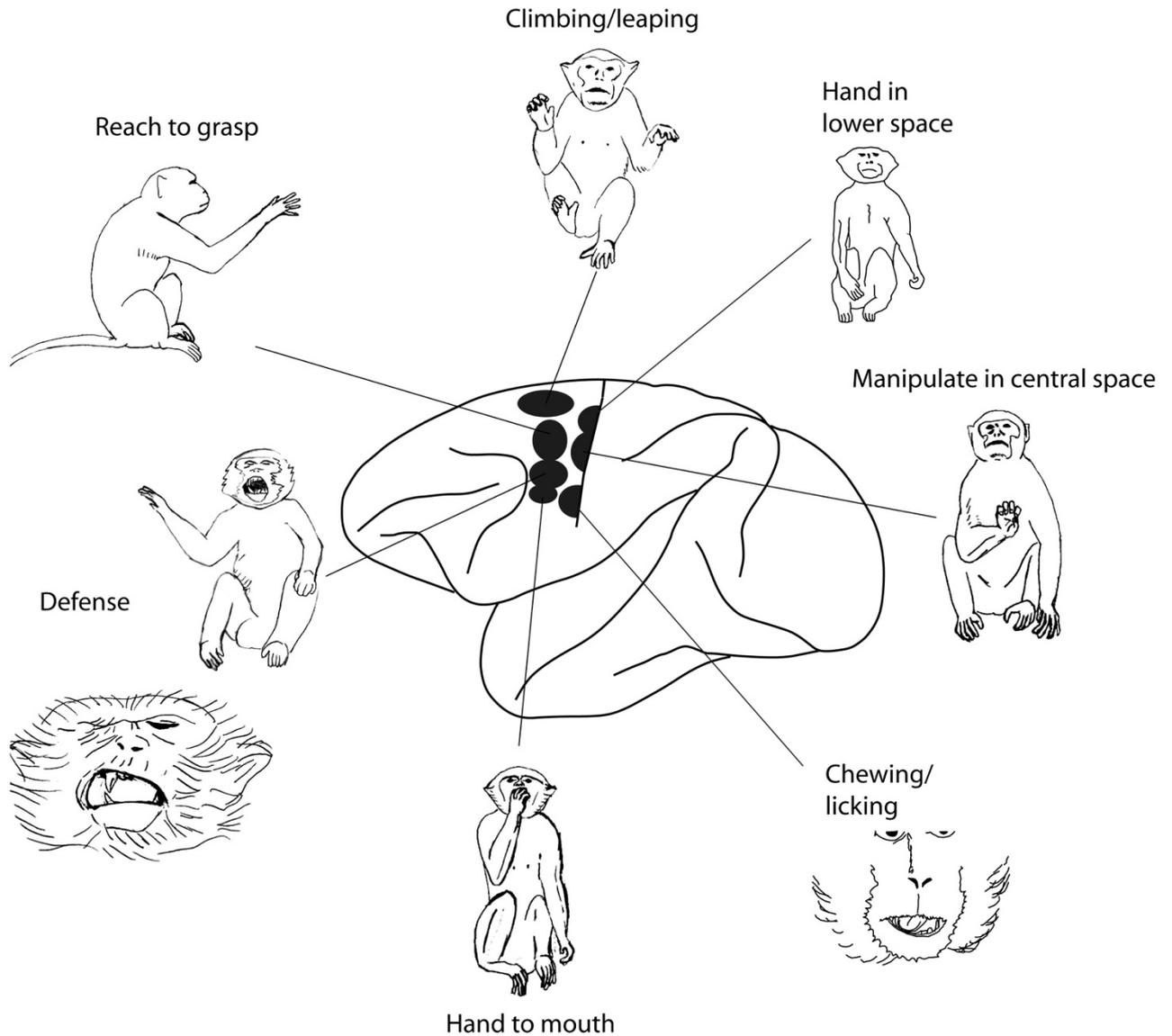


Figure 2. Action map in the motor cortex. Actions evoked by electrical stimulation on the "behaviorally relevant timescale of 0.5s" (Graziano and Aflalo, 2007)

Furthermore, in PMv some cells show somatosensory properties, particularly in F4; comparing motor maps with somatosensory maps, it has been discovered that in the first one is mainly represented arm, while in the second one is mainly represented face because

many F4 neurons are activated both by face stimulation and arm movements (Gentilucci et al., 1988).

These differences between the motor outputs of F1 and the more complex ones of BA6, suggest that the motor areas belonging to BA6 are not only involved in motor control but also in higher order functions, for instance sensory-motor transformations, goal-directed action coding, decision making about actions' execution and action recognition (Rizzolatti and Luppino 2001).

Specifically, a functional distinction based on their “extrinsic connections” (Rizzolatti and Luppino, 2001) has been made among BA6 sectors: posterior motor areas (F1-F5) are mainly linked with parietal regions, so they are defined “parieto-dependent” motor areas, while anterior areas (F6 and F7) are mainly connected with prefrontal cortex, so they are called “prefronto-dependent” motor areas. Prefronto-dependent areas, due to contextual and motivational information coming from prefrontal cortex, may determine whether and when a potential motor action becomes an effective one (Rizzolatti and Luppino, 2001). Parieto-dependent areas receive sensory inputs from different parietal areas and use them for sensory-motor transformations, for potential motor actions generation, for action recognition and for peripersonal space representation; F1, F3 and part of F2, in order to perform sensory motor transformation, mainly exploit somatosensory information, while F4, F5, F6 and the rostro-ventral part of F2 use both somatosensory and visual information (Rizzolatti and Luppino, 2001).

Since PMv receives inputs from visual (Markowitsch et al., 1987), parietal (Borra et al., 2008) and prefrontal (Lu et al., 1994) cortices and send outputs to motor areas (Luppino et al., 1993) and consistent with the fact that PMv neuronal activity is modulated during the execution of sensorimotor tasks (Rizzolatti and Luppino, 2001), it has been hypothesized that PMv may be involved in evaluating sensory information in order to make

behavioral decisions (Pardo-Vasquez et al. 2008). Several studies have shown the activation of ventral premotor neurons during somatosensory (Romo et al., 2004), visual (Pardo-Vasquez et al., 2008) and auditory (Lemus et al., 2009) discrimination tasks both in response to sensory stimulation and during the entire process (mnemonic recall of the first stimulus, evaluation and comparison between first and second stimulus and decision report) which leads to a perceptual decision. Thus, these findings have suggested that PMv play a role in decision making process.

1.1.1. Functional properties of ventral premotor neurons

Area F5 is located in the rostral part of PMv and represents mouth and hands movements (Rizzolatti et al. 1988a): the dorsal part of F5 hosts a representation of the hand, while its ventral portion shows a representation of the mouth, but there is also a part in which these two representations overlap. However, the peculiarity of this area is the presence of neurons that code the goal of the actions, not the single movements: for example, it has been discovered that neurons can fire when the subject grasps with the mouth and even with the right and the left hand (Rizzolatti et al. 1988a), suggesting that these neurons code the goal of the motor act “grasping” (i.e. taking possession of an object) regardless of the single movements necessary to achieve it. The hypothesis of the existence of neurons encoding motor goals rather than movement sequences has been confirmed by Umiltà and coworkers (2008). These authors demonstrated that F5 neurons discharge during grasping actions independently from the specific extension or flexion movement of the hand: indeed, in monkeys trained to use normal pliers (used by flexing the fingers to grab an object) and inverse pliers (used by extending the fingers to grab an object), F5 neurons could code “grasping” regardless of the effector and even the movement pattern activated to take possession of the target. In addition to grasping neurons, have also been found

neuronal classes with selectivity for different motor goals, for example tearing, breaking and holding actions (Rizzolatti et al.1988a). Furthermore, within each category there are some neurons that specify the mode of executing the action: among grasping neurons, for example, some fire specifically during precision grip, others during whole hand prehension or finger prehension.

The above-mentioned neurons are classified as purely motor, but it has been shown that in F5 there are also two other different categories of visuomotor neurons: canonical neurons, which are mostly located in F5p, and mirror neurons, histed mostly in F5c. Canonical neurons respond to the presentation of three-dimensional stimuli as well as during the preparation and execution of reaching-grasping actions directed to them (Murata et. al 1997), suggesting that these neurons transform the intrinsic physical features of an object (size, shape, weight) into the appropriate motor repertoire required for grasping it. Mirror-neurons, instead, fire during the execution of an action and during the observation of the same (or a similar) action performed by another subject, suggesting they may play a role in action recognition (Gallese et al.,1996). Finally, more recent studies (Bonini et al. 2014) have shown that mirror and canonical neurons are often located in the same part of F5 and that, rather than being categorically distinct neuronal classes, the “canonical” and “mirror” properties can often apply even to the same single neuron.

Conversely, F4 is located in the caudal part of PMv cortex, it contains the representation of neck, trunk, face and arms, and can control axial and proximal movements, as revealed by intracortical microstimulation studies (Maranesi et al. 2012). In 1996, Fogassi and coworkers have found that 55% of 539 neurons recorded in area F4 discharged when the monkey performed different movements: 28.4% of them fired during neck and upper trunk movements, 24.4% discharged during different types of goal-directed

arm movements such as reaching and bringing to the mouth, and 9.7% of them responded to mouth and face movements; finally, 33% of these neurons did not represent exclusively one of the previously mentioned movements but fired during two of them, for instance, some neurons discharged when the monkey brought the hand to the mouth but also when it opened the mouth, while others were activated during “neck and trunk orienting movements” (Fogassi et al., 1996a) and even during reaching movements. However, only 72 of the above-mentioned neurons were purely motor: in fact the remaining ones were also activated by somatosensory stimuli, visual stimuli or both. In fact, in F4 there are three classes of neurons: somatosensory neurons (30%), visual neurons (14%) and finally a third class of cells, called bimodal neurons (56%) because they respond both to tactile and visual stimulation (Fogassi et al., 1996b). Nonetheless, unlike classical visual neurons, bimodal neurons do not respond to common visual stimulation, they are activated by tridimensional stimuli moved around the subject in its peripersonal space (Rizzolatti et al., 1981c). For example, a neuron with a tactile receptive field on the cheek discharged when the cheek is touched but also when something is approaching this body part.

Moreover, it has been demonstrated that the visual receptive field of peripersonal neurons is typically constituted by the extension in depth of the tactile receptive field into the peripersonal space for about 30-40 cm. For this reason, it has been proposed that these two receptive fields are in register. Tactile receptive fields are located on the neck, trunk, face and arms (Figure 3), they are large and mostly contralateral to the hemisphere from which the neuron is recorded.

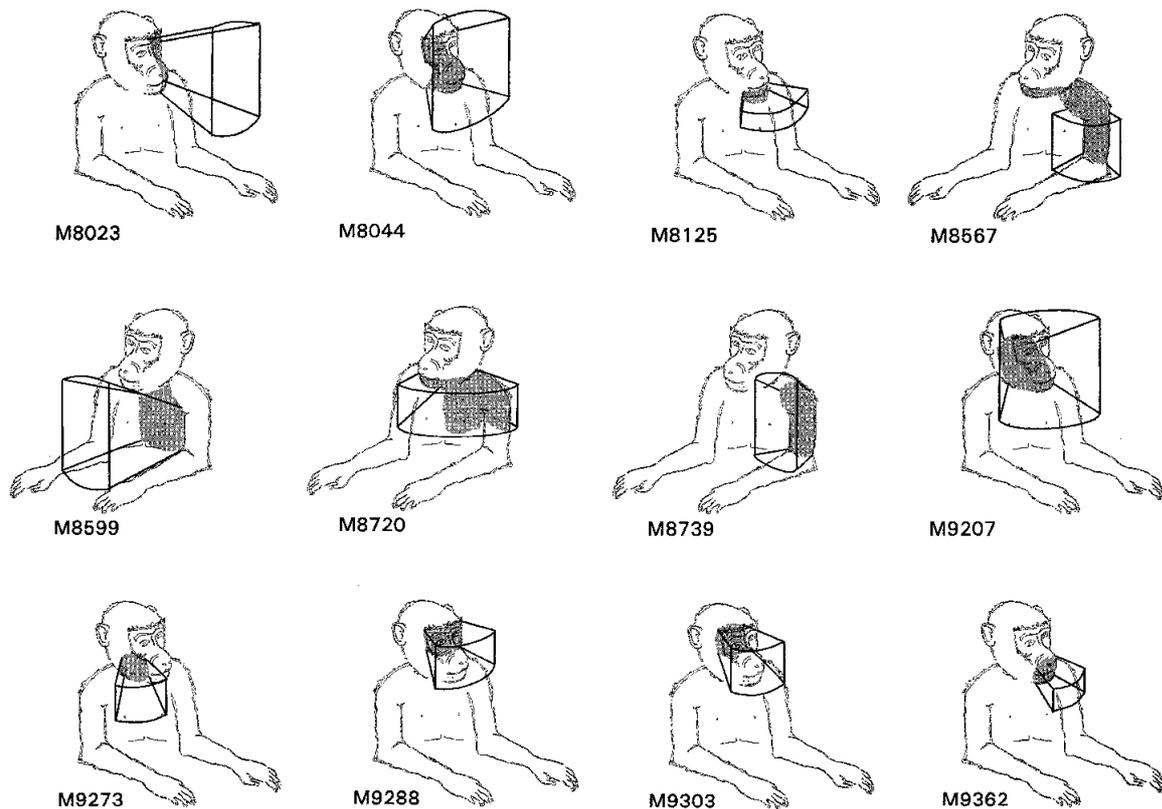


Figure 3. Examples of tactile and visual receptive fields of F4 bimodal neurons. (Fogassi et al., 1996b)

As far as the visual fields are concerned, in this area they are mostly coded in somato-centered coordinates, which implies that their position is independent from eye position and gaze direction, but it changes when the position of the body part where the tactile receptive field is located changes. Indeed, only 10% of the neurons have retinocentric visual receptive fields (Fogassi et. al. 1996a). Another interesting characteristic is that neurons with tactile RF on the head, have the visual RF in the upper portion of peripersonal space and they are activated by reaching actions toward the upper space, while neurons that have “*peripersonal field*” around the body, discharge during reaching movements toward the lower space. Moreover, it has been detected that neurons with peripersonal field on the right side of the body respond to reaching movement toward the right side of the space, while neurons with peripersonal field on the left side discharged in response to reaching movement toward the left side of the space. Thus, “*the receptive field location*

and the effective movements were organized in F4 in terms of functional relations” (Rizzolatti and Gentilucci, 1988b).

In addition to these types of neurons, in F4 there are also trimodal neurons: they respond to visual, tactile and auditory stimulation and their auditory receptive fields are in register with the visual and tactile ones (Graziano et al., 1999). Finally, Gentilucci (1988) has found a small group of F4 neurons with “complex properties”: unlike classical bimodal neurons they discharge when visual stimuli withdraw from the subject, but they are also activated by reaching movements, particularly by fast arm extension towards an object (Gentilucci et al., 1988).

In the light of physiological and functional characteristics of F4, it has been hypothesized that sensory inputs are involved in the organization of movements; in fact, in order to avoid an object coming toward the face it’s essential knowing its position relative to this body part. Thanks to the presence of neurons with visual RFs anchored to tactile RFs, F4 could localize the stimulus when the face is not yet stimulated by it and generate the appropriate movement to avoid it. Thus, F4 may use sensory inputs to localize a target in the personal or peripersonal space and to recruit neurons that control the appropriate movements (Fogassi et al., 1996a).

1.1.2 Parieto-frontal circuits

Different studies revealed that posterior motor areas (F1-F5) are mainly connected with parietal regions, while anterior motor areas (F6 and F7) receive their main inputs from the prefrontal cortex. Thus, each one of the parieto-dependent motor areas receive sensory inputs from one specific area of the parietal lobe, creating different segregated parieto-frontal circuits (Figure 4) that act in parallel and perform specific sensorimotor

transformations, which are necessary to generate “potential motor actions” (Rizzolatti et al. 2002).

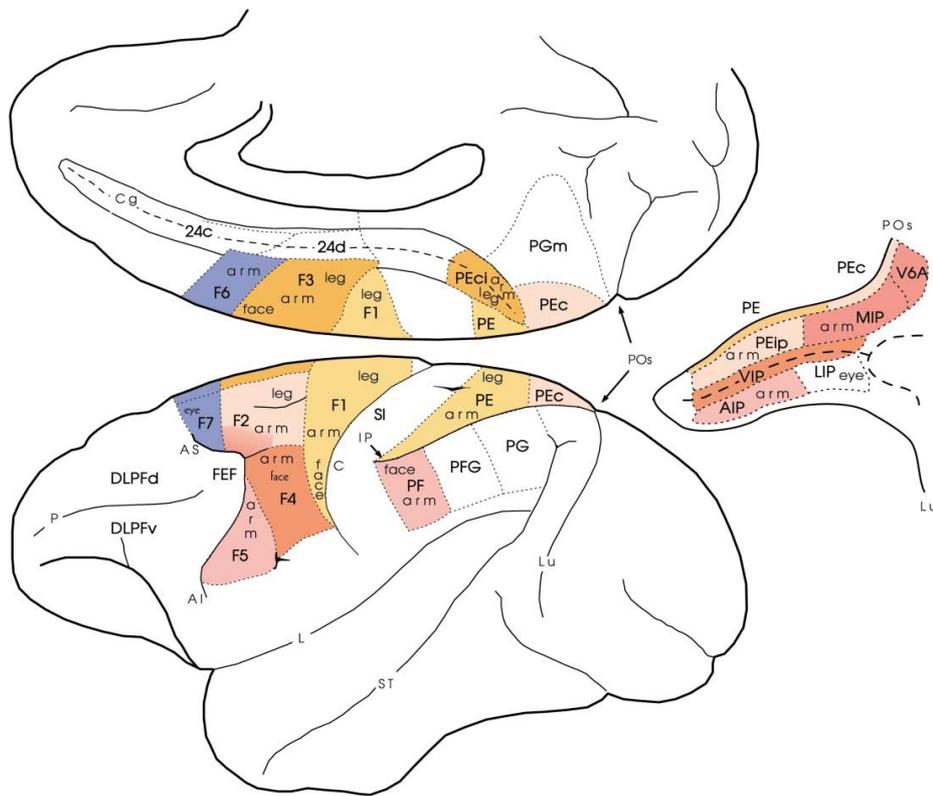


Figure 4. Mesial and Lateral Views of the monkey brain showing the parcellation of the motor cortex, posterior parietal and cingulate cortices. Fronto-parietal circuits are represented by illustrating related areas with the same color. (Rizzolatti and Luppino, 2001)

Specifically, area F5 is mutually connected with AIP and, together, they form the grasping circuit which transforms physical properties of objects, such as size, shape and weight into the appropriate motor plan to interact with them; indeed, area F4 is reciprocally related to area VIP, constituting the reaching circuit, which transforms extrinsic characteristics of objects, such as their position in the space around the subject, in the correct motor plan to reach them (Matelli and Luppino, 2001; Rizzolatti and Matelli, 2003). Furthermore, this circuit is also involved in defensive mechanism and in space perception.

VIP is located in the ventral portion of IPS and receives inputs from both somatosensory and visual (MT, MST) areas (Colby et al., 1993; Bremmer et al., 2001, 2002). Accordingly, VIP contains neurons that respond to visual stimulation but also bimodal neurons (Duhamel et al., 1991; Colby et al. 1993) that discharge both during visual and tactile stimulation and trimodal neurons which respond also to auditory stimulation (Schlack et al., 2005). Likewise those of F4, VIP bimodal neurons have visual receptive fields in register with tactile receptive fields, the first ones are coded in somato-centered coordinates and extend in depth within the peripersonal space. There is no evidence that VIP bimodal neurons discharged during movement execution; however, despite the fact that VIP is not a motor area, microstimulation of this area, performed by using 100-150 μ A electrical current, evokes facial and arms movements similar to those elicited by stimulating F4 (Graziano and Cooke, 2006). Moreover, lesional studies has shown that damages of area F4 and area VIP determine similar impairments: lesions of area VIP produce neglect for contralesional peripersonal hemispace (Rizzolatti and Matelli, 2003), while lesions of area F4 determine contralesional neglect for peripersonal space and even reaching deficits (Rizzolatti et al., 1983). These findings suggest that VIP-F4 circuit can bind together visual and somatosensory information and consequently can map the space in motor terms. However, sensory-motor transformations are not only involved in reaching movements but also in avoiding ones and in defensive actions.

During the microstimulation of the area VIP in monkeys defensive-like movements have been evoked: monkey blinks and squints, lifts its upper lip, retract its head, folds the ears against the head, lift its shoulders, and makes different arm movements (Graziano and Cooke, 2003). The same behavioral repertoire is evoked stimulating area F4. These findings suggest that the F4-VIP network could be involved in defensive and obstacle avoidance behaviours, specifically VIP may play a role in constructing a representation of

the environment head-centered, whereas, F4 may be more involved in generating defensive and avoidance actions. Thus, the F4-VIP network might be implicated in the protection of peripersonal space, especially the space around head and arms (Cléry et al.,2015).

1.1.3. Strength and weakness of non-human primate neurophysiology

One of the main purposes of neuroscience is to investigate the neural underpinnings of behavior and non-human primates have been and remain a crucial model to access single cell mechanism underlying perceptual, motor and cognitive functions that cannot be tackled with non invasive techniques in human subjects (Roelfsema and Treue 2014 Neuron; Buffalo et al. 2019 PNAS). However, traditional approaches suffer from several limitations. Classical methods mostly involve head-restrained monkeys sitting in a primate chair: they are limited in moving their upper limbs and cannot explore the environment or freely interact with other subjects because of the primate chair. Most of these limitation comes from the tethered neural recording approaches: because monkey are extremely manipulative and destructive animals, constraints are required not only to provide the necessary experimental control (e.g. of eye/head movements), but also to make possible the connection of the electrodes implanted in monkeys' brain via cables to the amplifiers and recording devices. Due to the physical restraints, monkeys can only perform behavioral tasks characterized by relatively limited, often stereotyped and repeated actions and movements, sometimes requiring prior cognitive processing to make decisions in rather artificial conditions, which could affect the ecological validity of the conclusions reached on the neural substrates of the investigated behaviors. Thus, we can conclude that constrained experimental conditions provide the methodological advantage of observing controlled movements, however they are likely to differ too much from natural behavior (Jackson et al., 2007). In fact, classical neurophysiological studies are characterized by

high experimental control that allow to isolate specific variables and to limit confounding factors, offering considerable internal validity; however, they suffer of poor ecological validity. For example, “*stereotyped motion imposes correlations between movement parameters, which could lead to spurious relationships being mistaken for neural coding* (Fetz 1992; Todorov 2000)” (Jackson et al., 2007).

In some cases, it becomes necessary the implementation of freely moving conditions since certain behaviors cannot be investigated under constrained conditions, which may nonetheless remain indispensable for other types of research. For example, discoveries such as the “*place cells*” could not have been possible if rats had been immobilized. However, while freely moving studies on small mammals, such as rats or mice, can be performed even with tethered recording systems; in contrast, studies on non-human primates under freely moving conditions cannot be realized using this type of setup because monkey are able to remove and damage cables and devices.

To overcome the limitations of traditional approaches, advances in technology have led to the development of wireless recording system. This type of neurotechnology allows investigating natural and ecologically relevant behaviors in freely moving animals and allow researchers to improve the reliability of the collected data. For instance, different from structured task, freely moving conditions together with wireless recording system allow the correlations between normal behaviours, including sleep, and neurons’ activity that can be followed long-term. Thus, even if unconstrained paradigms make it difficult to quantify the animal’s behaviour, they are necessary to understand the natural motor control. (Jackson et al., 2007).

1.2. Wireless neurophysiology: new paradigms from small mammals to non-human primates

Wireless technology has already been successfully used in research on several animal species, for instance on bats (Yartsev and Ulanovsky 2013; Omer et al. 2018), rats (Grievens et al. 2020), non-human primates (Berger et al. 2020; Roy and Wang 2012) and even on insects (Harrison et al. 2011).

The discovery of place cells in rats' hippocampus (O'Keefe, 1971) has represented a pioneering result for studies on spatial cognition. However, because of the utilization of tethered setup rats have been able to navigate freely only along two axes, so the study has only investigated the encoding of bidimensional space. Indeed, Yartsev and Ulanovsky (2013), using a wireless neural-telemetry recording system, have been able to investigate the encoding of 3D volumetric space in bats' hippocampus when they were freely flying. Moreover, using wireless recording system, Omer and coworkers (2018) could demonstrate that in the Egyptian fruit bat hippocampus exists a different type of place cells, defined as *social-place cells* because they represent the position of conspecific in allocentric coordinates, revealing the potential of freely-moving neurophysiology to shed light on complex behavioural processes like social interaction.

Concerning non-human primates, there are still few studies carried out in freely moving animals with wireless neural recording system. In 2007, Jackson and coworkers compared the data collected by using a wireless system with those obtained by using a tethered system in order to determine whether results obtained under constrained conditions generalize to freely moving conditions. Therefore, they recorded from the primary motor cortex neurons while monkeys (*Macaca nemestrina*) performed a torque-tracking task, while they freely behaved and during sleep. In each of awake conditions they

found correlations between neural activations and EMG. Even if the single cell-muscle correlations detected under unconstrained behavior did not correspond to those during task performance, concerning the population activity the average strength of cell-muscle correlations has been related to the preferred direction of the single neuron during the task (Jackson et al., 2007). So, the results obtained by the use of these two different types of methodologies partially overlap, but the utilization of wireless recording system in unrestrained conditions is important to extend the data obtained under classical restrained experimental conditions: the ultimate goal of neuroscientific studies is, indeed, to understand the brain-behavior relationship in conditions that are as close as possible to the ecological ones.

A fundamental advantage provided by the utilization of wireless recording techniques is the possibility of observing natural behaviors that animals do not exhibit under classical, constrained laboratory conditions. For instance, marmosets which are arboreal and highly social New World monkeys make a wide use of vocalizations to communicate with each other; however, when marmosets are restrained in a primate chair, they show an inhibition in vocal behavior. In 2012, Roy and Wang implemented a wireless multi-channel single-unit recording technique to sample cortical neurons' activity from freely roaming and vocalizing marmosets in a plexiglass cage (Roy and Wang, 2012), demonstrating the applicability of wireless neural recordings in freely moving marmoset as it allowed uninterrupted experimental sessions in which the marmosets produce vocalizations continuously.

Wireless technology has also allowed investigating motor control as well. Besides the earlier work of Jackson and coworkers (2007), Berger et. al have used wireless recording technology to investigate goal-directed movements and movement planning in

unrestrained monkeys. They developed an experimental environment called “the Reach Cage” that was equipped with a visuo-haptic interaction system (MaCaQuE) where trained monkeys performed controlled visually-guided reaching movements to a target with instructed delay; importantly, differently from previous studies of reaching actions, the targets could be within immediate monkey’s reach or beyond the immediately reachable space. Using video-based motion capture they measured three-dimensional wrist trajectories during task performance and, simultaneously, they sampled single unit activity in three cortical areas (parietal reach region PRR, dorsal premotor cortex PMd, and primary motor cortex M1) from a monkey performing reach and walk-and-reach movements (Berger et al., 2020). The researchers have presented the Reach Cage because it allows studying whole-body freely moving monkeys but at the same time it allows the monkey to perform structured tasks and with a good level of control, likewise in restrained monkey experiments. Thanks to this methodology they have demonstrated that premotor and parietal cortical activity contain information not only about the position of targets located in the peripersonal space but also of “*walk-and-reach*” targets located far away from the subject during movement planning (Berger et al., 2020).

In the light of these findings it is deemed that wireless recording technique can be used to replace the tethered systems (Fan et al., 2011) and in some cases it is necessary to do this replacement in order to study specific behaviors.

Finally, another crucial aspect of wireless neurotechnology concerns the possibility of using it not only to record brain activity but even to stimulate it; this opportunity represents a turning point in the translational research because it allows the implementation of brain-machine interfaces (BMI) capable of restoring motor functions in patients with motor impairments. In 2016, Capogrosso and coworkers implemented a wireless control

system which connected the online neural decoding of extension and flexion motor signals recorded by an intracortical microelectrode array implanted in the leg area of motor cortex with a stimulation system located in the spinal cord that generated these movements in real-time. This brain-spine interface was implanted in monkeys with spinal cord injury and could restore locomotion of the surgically paralyzed leg. Moreover, the components of this system were approved for further studies on human patients with motor impairments, leading to clinical application in spinal lesioned patients.

2. AIMS OF THE STUDY

Although some wireless neural recording studies have already been carried out, none of them have ever tested non-human primates in totally free behavioural conditions. In fact, contrary to studies on rodents that let them navigating freely within the experimental environment (Mimica et al., 2018; Grieves et al., 2020), in non-human primate studies monkeys are trained to perform well-structure tasks so they cannot decide on their behavior (Jackson et al., 2007; Berger et al., 2020), or they are confined into too small cages to allow monkeys to express a large variety of their ethologically-relevant behaviours (Berger et al., 2020; Roy and Wang, 2012).

This study, by means of a wireless recording system synchronized with a multicamera system, aims to provide a preliminary overview of the behaviors a monkey can perform during freely-moving sessions and their possible neural correlates focusing on the ventral premotor cortex (area F4).

3. MATERIALS AND METHODS

3.1 Subjects and surgery

The subject of the study is one male *Macaca mulatta* (13 Kg). First, the monkey was trained with positive reinforcement to spontaneously sit in a primate chair from its home cage, and then it was habituated to enter the experimental environment, called “NeuroEthoRoom” (NER), and to freely undertake a variety of spontaneous activities for about thirty minutes. Finally, the monkey returned into the primate chair to be brought back to its home cage.

When the training was completed, the monkey underwent surgical procedures in deep anesthesia and aseptic conditions for the implantation of a head-holder (not employed for the present study) and a recording chamber, for the subsequent implantation of the chronic electrodes and the connectors to the preamplifiers.

All experimental protocols complied with the European (Directive 2010/63/EU) and national (D.lgs 26/2014) laws on the protection of animals used for scientific purposes, they were approved by the Veterinarian Animal Care and Use Committee of the University of Parma (Prot. 52/OPBA/2018) and authorized by the Italian Ministry of Health (Aut. Min. 802/2018-PR).

3.2 Apparatus and Paradigm

This study did not entail the use of a specific behavioural paradigm or task: the monkey could move freely and perform spontaneous actions based on the objects and enrichment items provided in the NER during two experimental sessions.

The NER (Figure 5) is a custom-made, plexiglass structure (W x H x D: 208 x 205 x 181cm). One of the four side walls of the NER was endowed with two large doors allowing the experimenter to enter for preparing the environment before each session and to clean it up afterwards. Each door was equipped with two smaller vertical sliding

apertures, in front of which the monkey's chair had to be placed in order to allow it to move into the NER at the beginning of each session. Inside the NER different enrichment items were placed: a wooden structure (Figure 6) where to climb and sit on, a rope by which the monkey can reach the upper level of the cage and a dangling drilled plastic ball filled with fruit to manipulate.

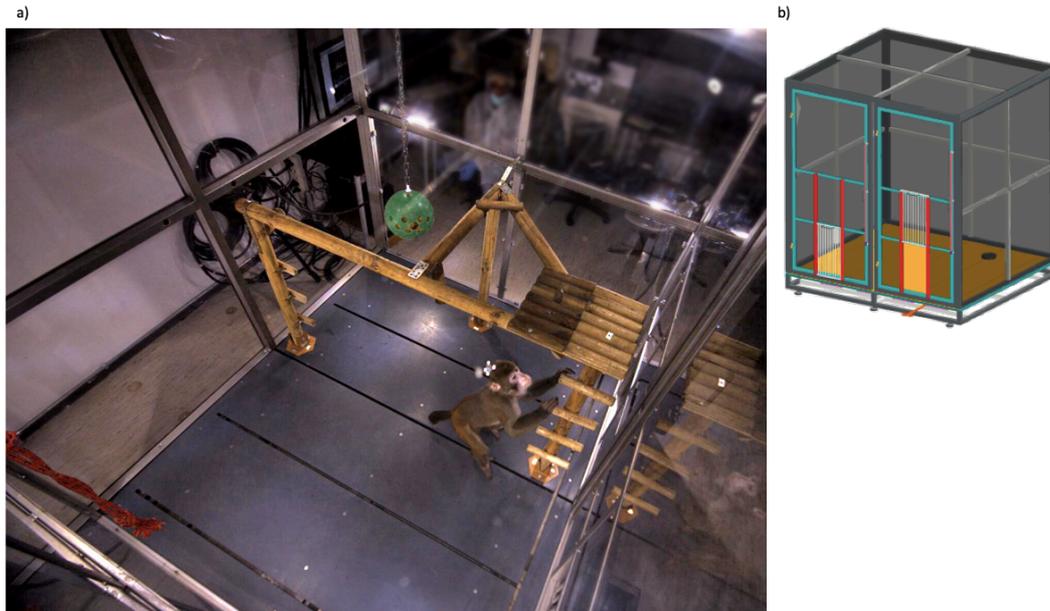


Figure 6. a) Monkey within the NER during an experimental session. In this picture is clearly represented the experimental environment and its equipment: the wooden structure, the rope, the plastic ball filled with fruit; b) external view of the NER: the two large doors are highlighted in green, the two sliding doors are highlighted in red.

This study analyzed two experimental sessions, one lasting 27 minutes and the other lasts 13 minutes. During the experimental sessions, the monkey could pick up the food from the ball or forage on the floor to eat its small food morsels; furthermore, the experimenter gave it some water to drink by introducing a syringe through some openings located at different sites of the NER.

Monkeys' behaviors were recorded by using a system of eight high-resolution cameras mounted on movable arms attached to the four corners of the room, at two different height levels. We use Dual Gigabit Ethernet Machine vision cameras (mvBlueCOUGAR-XD, Matrix Vision) with a resolution of 1936×1214 at up to 164

frames per second, set to 50Hz. The cameras are equipped with a global shutter with sensor size 1/2" format (5.86µm pixel), a manual C-Mount Lenses with 5 mm focal length (CCTV Lens, KowaOptical Products Co.,Ltd) and LEDs ring lights. Each camera has two RJ-45 Gigabit Ethernet connectors with screw-locking and two Industry standard 12-pin locking connectors to provide transmission of images and signals to the Windows computer, and to synchronize all cameras through a synchronization box connected to both cameras and computer.

3.3 Ethogram definition

In order to construct the ethogram, we observed many times the video recordings of several experimental sessions within the *NER*.

Firstly, we distinguished between *point events* and *state events*, the former indicating instantaneous events, while the latter indicating events with a certain duration. For example, we considered the grasping actions as point event because they are immediate, while manipulation actions were defined as state events because they have a relevant duration.

Next, we categorized in general terms the most important actions performed during the sessions: walk, grasp with mouth and grasp with hand, climb, rest, yawn, scratch and object manipulation. However, since only a small number of F4 neurons have bilateral receptive fields (Gentilucci et al., 1988) we decided to differentiate left hand actions and right hand actions.

Finally, we chose to consider the grasping actions performed during the plastic ball manipulation as different categories from the others grasping actions, in order to assess if different context have an influence on neuronal responses.

<i>BEHAVIOR</i>	<i>TYPE OF EVENT</i>	<i>OPERATIONAL DESCRIPTION</i>
<i>Reward liquid</i>	Point event	Monkey receives passively liquid reward with syringe, directly in the mouth.
<i>Grasp food frontal Left</i>	Point event	Monkey grasps food pieces with the left hand
<i>Grasp food frontal Right</i>	Point event	Monkey grasps food pieces with the right hand
<i>Grasp food supine Left</i>	Point event	Monkey grasps food pieces with the left hand “supine”: with palm of the hand up.
<i>Grasp food supine Right</i>	Point event	Monkey grasps food pieces with the right hand “supine”: with palm of the hand up.
<i>Active food to the mouth</i>	Point event	Monkey actively places the food into the mouth.
<i>Object manipulation</i>	State event	Monkey manipulate the plastic ball filled with fruit.
<i>Grasp food frontal Left Ball</i>	Point event	Monkey grasps food pieces within the ball with the left hand
<i>Grasp food frontal Right Ball</i>	Point event	Monkey grasps food pieces within the ball with the right hand
<i>Grasp food supine Left Ball</i>	Point event	Monkey grasps food pieces within the ball with the left hand “supine”: with palm of the hand up.
<i>Grasp food supine Right Ball</i>	Point event	Monkey grasps food pieces within the ball with the right hand “supine”: with palm of the hand up.
<i>Grasp with mouth</i>	Point event	Monkey directly grasps and eats food with its mouth (it doesn't pick it up with hands).
<i>Walk</i>	State event	Monkey walks along the floor and on the wooden structure.
<i>Climb</i>	State event	Monkey climbs to a different cage level (i.e. from the floor to the wooden structure, or from the wooden structure to the ceiling).
<i>Rest</i>	State event	Monkey sits on the surface of the wooden structure and stop to do other activities.
<i>Scratch</i>	Point event	Monkey scratches itself.
<i>Yawn</i>	Point event	Monkey yawns.

Table 1. *The ethogram*

3.4 Behavioral analysis

Behavioral analysis was realized by means video recorded sessions. Behavioral scoring was performed by using BORIS (Behavioral Observation Research Interactive Software) project (Friard and Gamba, 2016), a free and open-source event logging software for video and audio recordings.

To create a project on Boris we set the ethogram, defining the types of behavior as “point events” or “state events”, and decided a keyboard key associated to every behavior in order to log the behavioral events during the observations. To be more accurate in behaviors’ logging we used the frame-by-frame mode which consent to slow down the video recordings in order to capture the exact moment in which an action happened, or started and ended in case of state events. To increase accuracy and the trustworthiness of behavioral scoring, the video recordings were observed many times by different people independently and then it was calculated the inter-rater reliability using the Cohen’s kappa statistic.

Finally, we generated one output for each observation that contained all the behaviors with their start and stop and their duration or with the exact time in which they happened in case of point events (Figure 8).

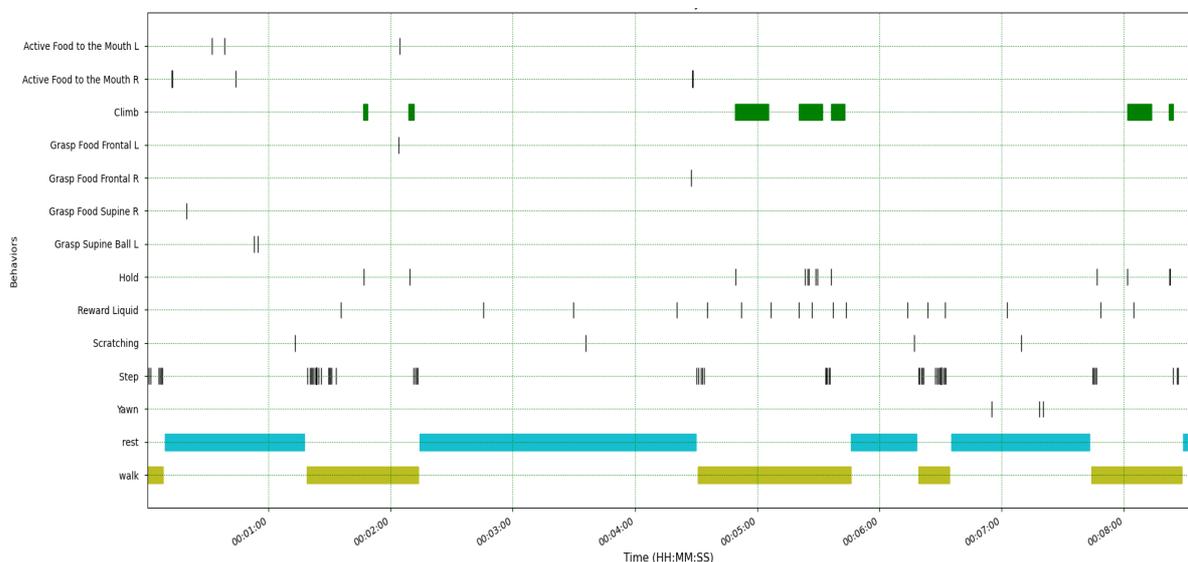


Figure 7. An example of Boris’ output where we can see how many times all the events observed during the session happened and their duration (in case of state events).

3.5 Neural recordings

Neuronal recordings were performed by means of semi chronic arrays of 8 linear silicon probes with 16 recording channels per shaft (shaft length 8 mm, shaft width 140 μm , shaft thickness 100 μm). Probes were implanted into the area F4 of the left hemisphere, the implantation sites were estimated by using MRI brain pictures as frame of reference, referred to stereotaxic coordinates for intraoperative identification of the implantation sites, together with visual identification of the anatomical landmarks of cortical sulci.

The probe shaft was attached to a highly flexible polyimide (PI)-based ribbon cable with a zero-insertion-force (ZIF) connector that was electrically connected through the connectors (OMNETICS A79025) to data logging devices (Deuteron Technologies Ltd, Israel). Specifically, we used the RatLog64 (Figure 7) which is a small, lightweight neural logger for recording neural activity from freely-moving animals.

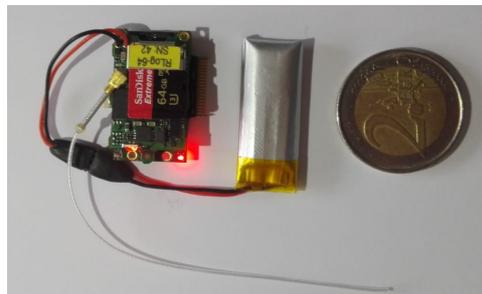


Figure 8. Deuteron neural logger connected to its battery

RatLog64 allows one to record neural signals from two sets of 32 channels with a digital high-pass filter with low frequency limit (Hz) set to 0.16 and a conversion rate of 32000 samples per second on each channel, thereby enabling to sample both LFP and single/multi-unit activity. Signals are amplified, digitized and stored in a MicroSD memory card (64 GB), thus preventing any possible transmission error. The device is powered by a small external battery (two hours maximum duration) connected via a short cable. Once the logger device was linked to the electrode arrays into the chamber, all the components were sealed with a cover on top of the chamber.

The logger communicates with a computer by means of a transceiver, which is connected via USB to the host Windows computer. This transceiver has four BNC connectors for digital inputs and one BNC for digital outputs; it allows to synchronize static equipment in the lab to the wireless logger. Specifically, in this study, a BNC cable is connected to the 8 cameras controlled through SIMI Motion software. The logger was controlled from the Windows computer, where we had a general control panel for monitoring the logger and synchronizing transceiver, in addition to the possibility to manipulate some recording parameters (for example, both the upper and lower cutoff frequencies). In addition, the Logger has a magnetic on/off switch, so that if there is a problem during a recording session (system crash or malfunction) when the device is sealed in the chamber, there is no need to physically touch the animal and remove all the components from the head to turn the logger on/off; instead, all that needs to be done is to attract the monkey near the bars of the front door and bring the magnet close to the chamber.

All formal signal analyses were performed off-line with fully automated software, Mountainsort (Chung et al. 2017), using a -3.0 standard deviations of the signal-to-noise ratio of each channel as threshold for detecting units. To distinguish between single-units and multiunits we used the noise overlap, whose value can vary between 0 and 1, considering as single only those units with a value below 0.10. Single unit isolation was further verified using standard criteria (ISI distribution, refractory period >1 ms, and absence of cross-correlated firing with time-lag of ≈ 0 relative to other isolated units to avoid oversampling). Possible artifacts were removed, and all the remaining waveforms that could not be classified as single units formed the multiunit activity.

3.6 Data analysis

3.6.1 Single units and multi units analyses

We took into account only behaviors that occurred at least seven times during each session; for this reason, we decided to group the eight different types of grasping (grasp food frontal left, grasp food supine left, grasp food frontal left ball, grasp food supine left ball, grasp food frontal right, grasp food supine right, grasp food frontal right ball, grasp food supine right ball) in two categories: grasp food with the left hand and grasp food with the right hand. Next, on the basis of this criterion, we selected nine events: active food to the mouth left, active food to the mouth right, grasp food with mouth, reward liquid, grasp food left, grasp food right, climb, walk and rest.

To visualize the waveforms we plotted the firing rate of each SUA and MUA in a 4s lasting interval, binned in 200 bins of 20 ms, and smoothed it with Gaussian smoothing ($\sigma=3$). Single units and multiunit activity were analyzed by means of a paired samples t-test between the average firing rate during the epoch in which the event occurred and the average firing rate during the baseline epoch, in order to determine if there is a significant difference in neural activity between the two epochs of interest.

The significant criterion for the analysis was $p<0.05$.

3.6.2 Definition of epochs of interest

Cells activity was analyzed in relation to the onset of behavioral events of interest defined according to the ethogram described above. First, we distinguished the behavioral categories according to the primary effector (or set of effectors) used, namely: whole body actions (climbing, walking, rest), hand actions (grasping food frontal/supine left, grasping food frontal/supine right, object manipulation and scratching) and mouth actions (active food to the mouth left, active food to the mouth right, reward liquid and grasping food with mouth).

Next, we defined the following epochs of interest: 1) baseline, from 1,5 s to 500 ms before the event and 2) movement, from the alignment point (onset of the behavior) to 1 s after this event, in case of body and hand actions events; regarding mouth actions, we chose 1) baseline, from 2 s to 1 s before the event and 2) movement, from 500 ms before to 500 ms after the event.

3.6.3 Population analysis

We calculated the average firing rate per bin of neurons recorded during the two experimental sessions and normalized it. Then, we plotted it with the standard error bar in order to visualize the waveform of the average cell response in relation to the events of interest. We took into account a 4 second interval subdivided in 20 ms bins, aligned the neuronal activity on the events of interest and smoothed the plot with a Gaussian smoothing ($\sigma= 3$).

3.6.4 Hierarchical aligned cluster analysis (HACA)

To evidence the relationship between the neural activity and the behaviors performed by the monkey and to verify the possibility of inferring which behavior it was performing at a given moment starting from the observation of the neural activations, we performed a hierarchical aligned cluster analysis (HACA) (Zhou et al., 2013). HACA is an “*unsupervised hierarchical bottom-up framework*” that finds a segregation of a time series into m disjointed parts; each one of these parts is included in one of k clusters. To perform the clustering, HACA combines kernel k-means with the generalized dynamic time alignment kernel (Zhou et al., 2013).

We chose a period of time of 1s in which searching for the behaviors, within which a behavior is considered in relation with the pattern of activation.

We first performed principal component analysis to reduce the dimensionality of our dataset and extract the variables for the HACA. Then, we performed HACA using

Euclidean distance as similarity criterion. This algorithm requires the number of clusters k and the length of patterns (Zhou et al., 2013): we decide to extract 6 clusters and we set 400 ms as minimum and 1 s as maximum length for each pattern. The HACA performs a temporal clustering on the basis of the similarity between variables during the time series of 3:30 minutes.

We took into account the following behaviors: active food to the mouth left and right, grasp with mouth, climb, walk, rest, reward liquid and object manipulation; we decided to exclude grasp food left and right because of their temporal proximity with the active food to the mouth, in fact the temporal distance between these two behaviors is inferior than 400 ms (the minimum length of patterns) so the HACA can't distinguish between these ones.

Finally, we plotted the clusters on the cartesian axes, where x corresponds to the first PC (PC1) and y to the second PC (PC2), while the pattern-behavior matching was plotted by means of histogram, where x corresponds to the behaviors and y to the percentual of occurrence of each behavior (normalized for the number of occurrences of each one) during each activation pattern.

4. RESULTS

4.1 Single unit and multiunit activity

We isolated 11 single units and 128 multi units. The activity of each unit was analyzed by means of a paired t-test comparing the average firing rate during a baseline epoch and the epoch of interest identifying a specific behavior. According to the criteria described in the Methods section, we have been able to analyze: “active food to the mouth-left” (63 trials total), “active food to the mouth-right” (35 trials total), “grasp food with mouth” (50 trials), “reward liquid” (46 trials), “grasp food-left” (34 trials), “grasp food-right” (32 trials), “climb” (34 trials), “walk” (32 trials) and “rest” (17 trials).

The plots below (figure 12) show examples of the recorded units: the first one (a) selectively discharged when the monkey placed the food in its mouth, regardless of the hand it used; the second (b) is a multiunit that significantly discharged only when the monkey uses the right hand to places the food in its mouth.

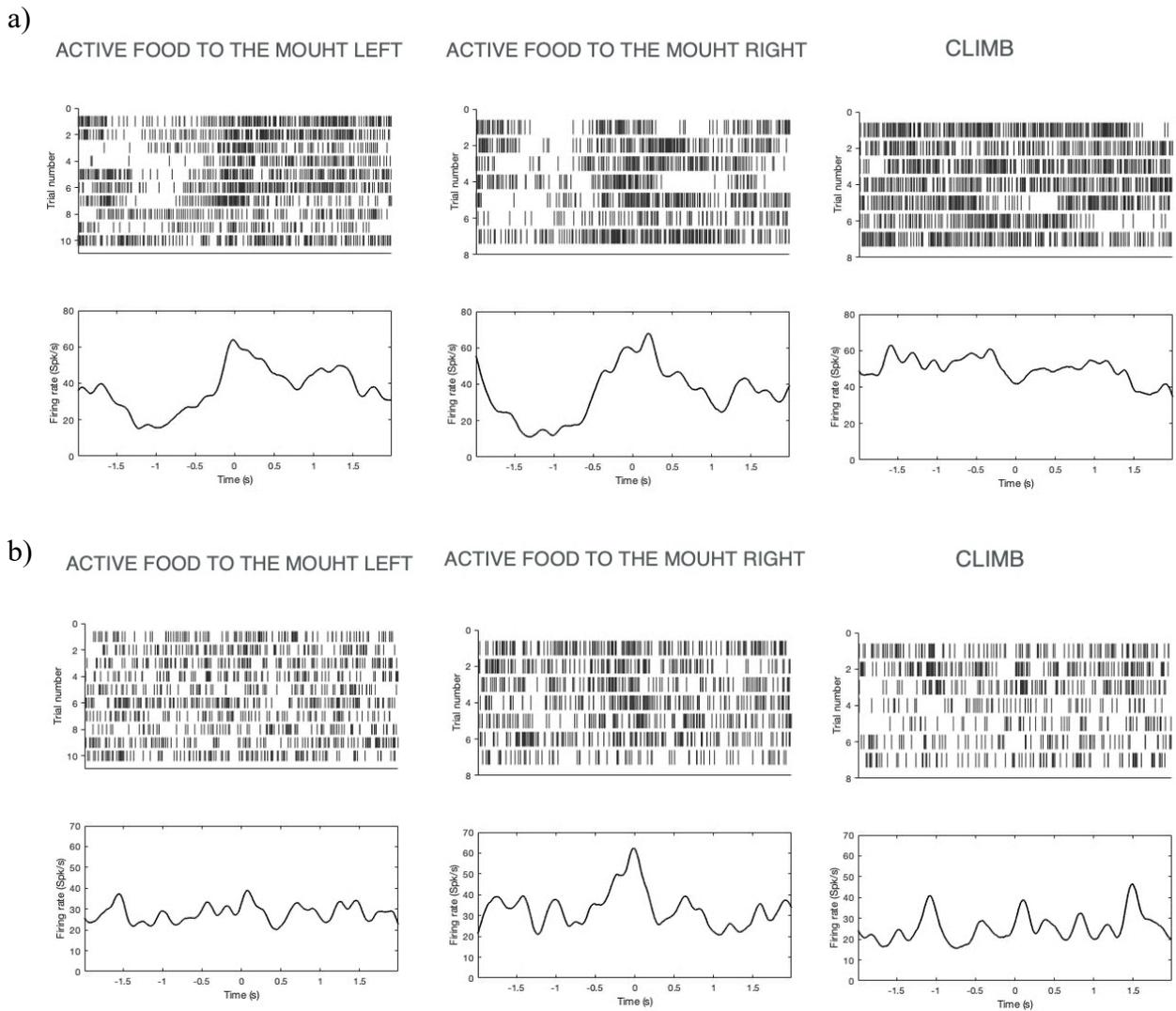


Figure 9. a) plots of the firing rate of one single unit during three different behaviors: this neuron significantly discharge during “active food to the mouth left” ($t= 18,3, p= 0,02$) and “active food to the mouth right” ($t= 31,4, p= 0,0173$), while it is not significantly activated during the “climbing”; b) plots of the firing rate of one multi unit during the precedent mentioned behaviors: in this case the unit discharge significantly only when the monkey places the food in its mouth with the right hand ($t= 15,04, p= 0,0264$).

The results (figure 10) reveal that most of units are facilitated during action execution, except for “walking”, during which there is a greater number of suppressed units, and for “rest”, during which the number of facilitations and inhibitions are balanced. A sizeable fraction of units responds during mouth actions and whole body actions: for example, 49 units are activated when the monkey receives a liquid reward from the experimenter (it drinks water from a syringe) and 26 units respond during climbing

behavior. Conversely, we can notice that a minor number of units are activated during hand actions, for instance only 10 units discharge when the monkey grasps some food with the right (contralateral) hand.

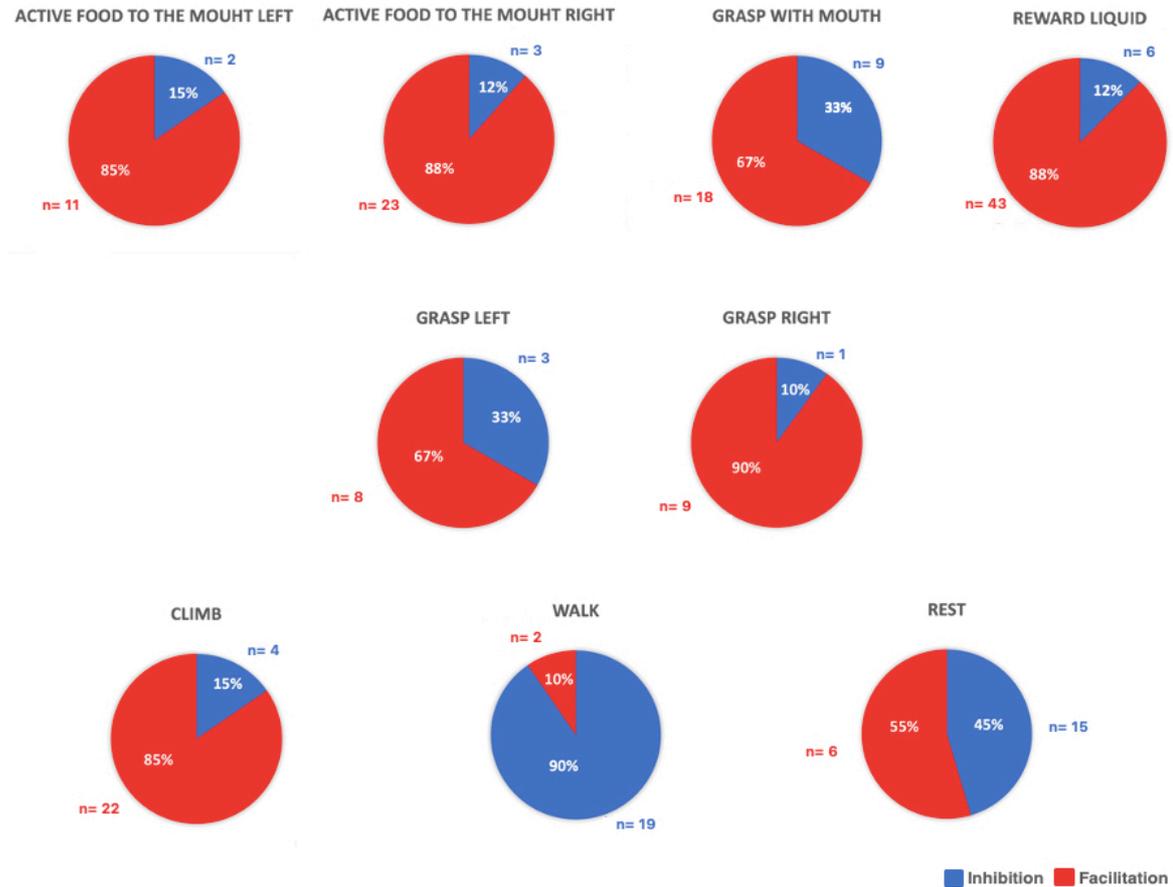


Figure 10. Pie charts that sum up how many neurons show significant facilitations or inhibitions for each behavior.

4.2 Population activity

Population activity shows different responses depending on the effector used to perform the action. Thus, we distinguished behaviors in three different clusters: mouth actions, hand actions and whole-body actions. We decided to include active food to the mouth in the first group because we aligned the activity to the moment in which the food comes into contact with the mouth.

The plots below (figure 11) show that, during mouth actions, the population discharges about 500 ms before the onset of the alignment, with a variable dynamic following this event depending on the condition. During the other two clusters of actions, the population shows a broader tuning or no tuning at all, modulating its activity mostly after the alignment event.

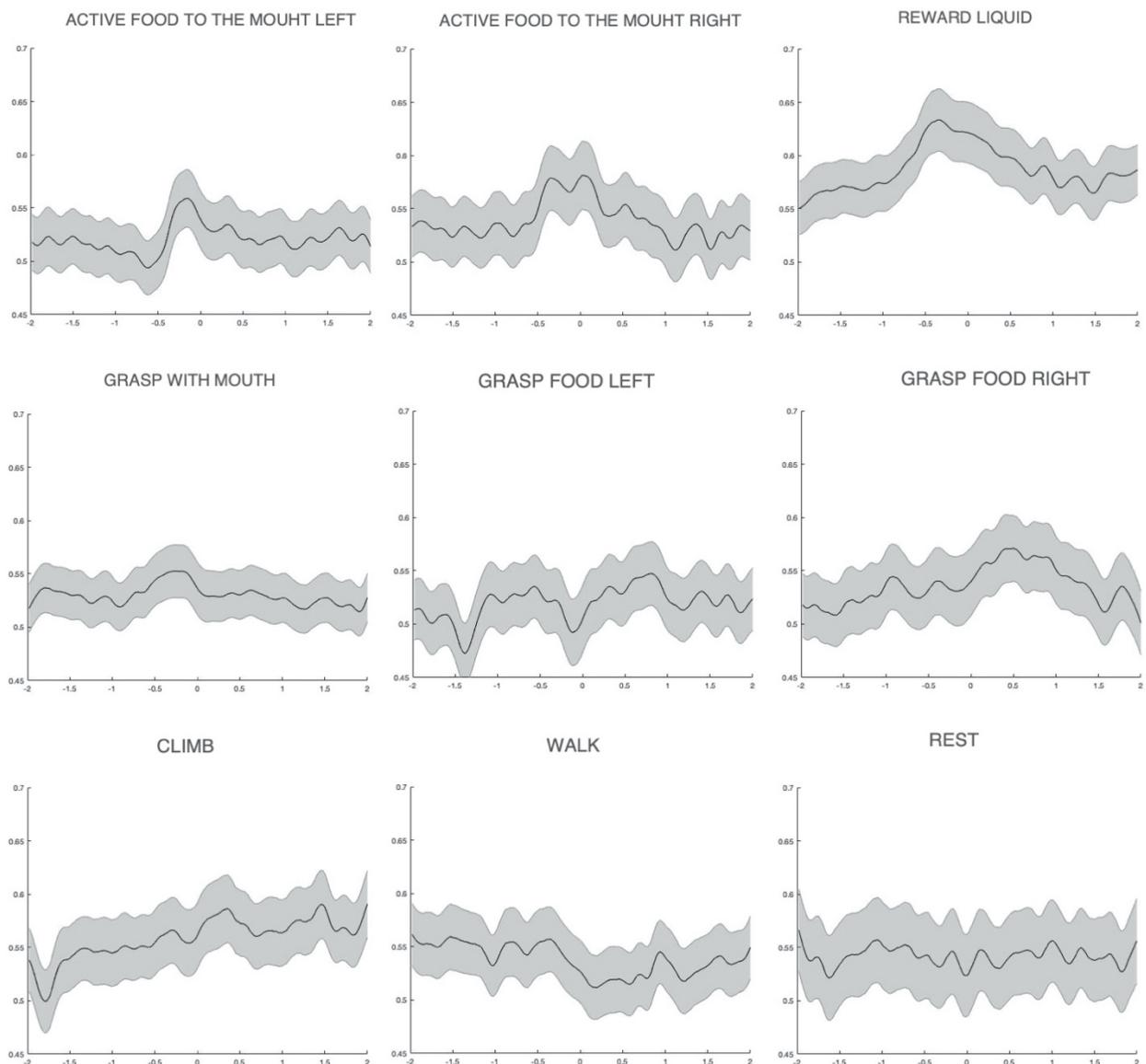


Figure 11. Plots of population activity during mouth actions, hand actions and whole-body actions. Gray shaded regions represent the standard error.

4.3 Unsupervised segmentation of neural activity

We performed a neural decoding analysis of one experimental session by means of HACA. As shown in the figure below (Figure 12), we found 6 clusters, represented by different colors. For each time point along the session it is possible to observe which cluster is activated and to assess which behavior the monkey was most likely performing at that time. A null hypothesis would predict equal probability for every behavior at any time point of each cluster, obviously depending on the relative frequency of each specific behavior. In contrast, it clearly emerges that, for example, “cluster 1” (red) corresponds to manipulative activities directed to the fruit ball; “cluster 2” (blue) was associated with a prevalence of climbing behaviours, “cluster 3” (green) indicated rest, “cluster 4” (pink) was associated in most cases with the manipulation of the fruit ball, “cluster 5” (black) and “cluster 6” (azure) indicated “active food to the mouth” with right hand.

a)

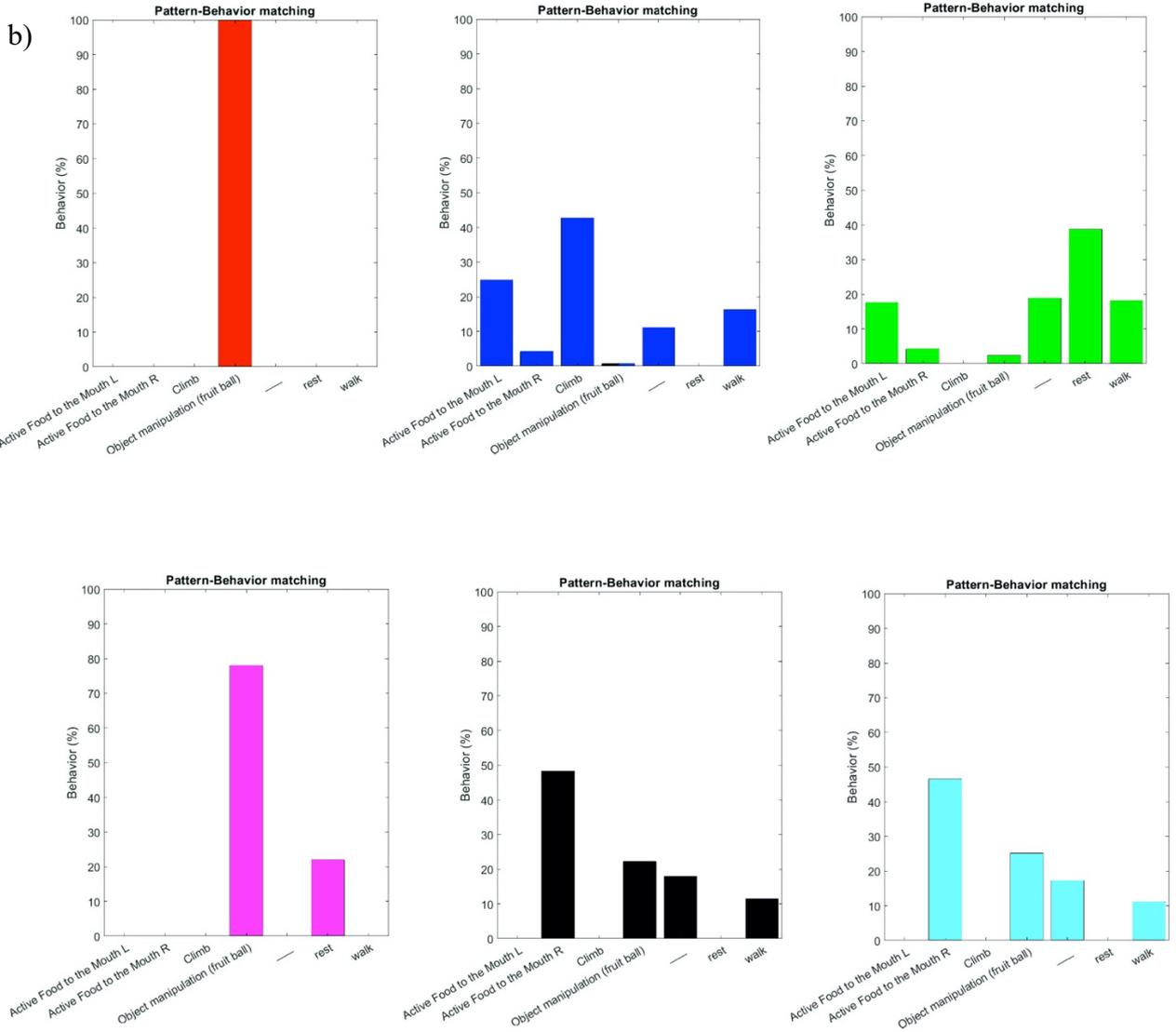
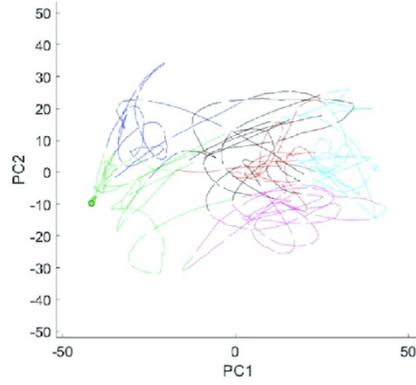


Figure 12. The plot represents six clusters, extract by the HACA, represented with six different colors. The histograms represent each one the percentual of occurrence of behaviors that the HACA has found in the period of time of 1 s during each cluster activation. The color of the histograms matches the color of the cluster represented in (a).

5. DISCUSSION

The goal of this study was twofold: first, it aims to provide a preliminary overview of the behaviors a monkey can perform during freely-moving sessions; second, it aims to explore the possible neural correlates of natural behaviors focusing on the ventral premotor cortex (area F4). To these purposes, we adopted a wireless data-logging system synchronized with a multicamera system to investigate, in parallel, monkey's behavior and associated neural activity. We implemented a large experimental environment, the NeuroEthoRoom (NER), in which the animal can freely move and interact with the enrichment items placed inside the NER. This experimental setup allowed us to observe spontaneous behaviors as those the animal could execute in natural conditions, for example when foraging on the ground, climbing or walking.

As mentioned in the previous chapters, we have categorized the most important actions performed during the session. However, while some actions were performed many times by the monkey (for instance, walking, taking liquid reward, putting actively the food in its mouth), others showed a little number of occurrences, such as climbing. Since some behaviors occurred few times, we could only use nine actions out of the seventeen identified in the ethogram in order to perform the data analysis. Moreover, for the same reason, we could not differentiate some behavioral specificities: for example, we had to consider "grasping actions" in general, even if during the sessions we observed the monkey using different hand configurations and wrist rotations while grasping, depending on the food location (e.g. the monkey grasped with the palm of the hand up to take the food out from the fruit ball, whereas when the food was located on a surface the monkey grab it with pronation of the palm). Future studies should certainly balance the number of actions' occurrences in order to better differentiating behaviors characterized by important and remarkable variability in the way they are executed. To this purpose, it could be useful

providing the monkey with solid food morsels (pieces of fresh or dried fruit) in different location of the cage, to stimulate it to perform different types of grasping. Moreover, manipulation behaviors could be increased by providing new objects, to be explore in order to obtain rewards from them. Finally, more complex behaviors, like social ones, could be investigated by putting two conspecifics within the NER, enriching the ethogram with intersubjective actions.

By simultaneously recording neural activity, we also aimed to describe the neural correlates of these behaviors in the premotor cortex, specifically area F4, which is widely investigated because of the presence of polymodal neurons (Fogassi et al., 1996; Graziano et al., 1998). In this study, we focused exclusively on possible motor properties of F4 neurons during unconstrained behavior, and found results partially consistent with those of previous neurophysiological studies; even if these are classical studies under constrained condition, they investigated some actions we can consider similar to those the monkey performs in freely-moving conditions, such as grasping and mouth actions. We found that most units respond during mouth actions (taking liquid reward from a syringe, grasping food with mouth, taking food with mouth from hand), while only a small number of units discharged during hand actions (grasping). These results are consistent with those obtained in previous recording and microstimulation studies in head-fixed monkeys, which have demonstrated that F4 contains the representation of neck, trunk, face and arms, and can control axial and proximal movements (Fogassi et al.,1996; Maranesi et al. 2012). Moreover, F4, in its most rostral portion (near the border with the area F5), also contains a small group of neurons that represent distal movements, likewise those of F5 (Gentilucci et al., 1988). The overlapping between the present results and the previous ones corroborates the reliability of wireless recording system in investigating the neural underpinnings of behaviors. Nonetheless, the major advantage of this technique consists

in the possibility to extend this investigation to more complex behaviors performed in highly variable contextual, postural and attentional conditions, as typically happens in daily life situations.

In fact, in our study we also took into account whole body actions (walking, climbing, rest) and found that a fair amount of units are activated especially during climbing and walking behaviors. We could hypothesize that the discharge of these units during climbing and walking behaviors is an anticipatory activation for an impending action, that the monkey voluntarily plans to get a reward. Indeed, when it climbs the rope or begins to walk, it does so with the purpose to get the food morsel it aims to eat. Future studies should also look at the correlations between neuronal activity and behavior by considering the forthcoming goal actually achieved by the monkey as an additional explanatory factor for ongoing neuronal activity.

Most interestingly, HACA results suggests the feasibility of predicting a wider range of unconstrained, ethologically classified behaviors by applying decoding algorithm to the neural activity readout. Of course, the main limitation of this algorithm is due to the need of presetting the number of clusters to focus on; however, other studies has demonstrated the robustness of decoding analysis in predicting ongoing behaviors and postures of freely moving animals (Mimica et al., 2018). In order to improve the accuracy of this technique, it could be used a machine learning approach, whose major advantage consists in the fact that researchers have to do significantly less assumptions about the decoded variables (Glaser et al., 2020); in this way, we could train the system to recognize the behaviors.

The present findings appear to be particularly promising in the context of the increasing interest for wireless recording technologies, which have already shown their potential in several neuroscientific fields, allowing the researchers not only to confirm data

collected in the past under classical and more artificial experimental conditions (Berger et al., 2020) but also to extend their investigations to more complex and natural behaviors, which could not be studied otherwise and appear to be particularly rich and articulated among primates: sleep (Jackson et al., 2007; Dragoi et al., 2020), motor activity (Berger et al., 2020), walking (Capogrosso et al., 2016) or social behaviour and interaction (Roy and Wang, 2016) are just some of the most recent examples of spontaneous and natural behaviors that underwent neurophysiological investigation.

To conclude, we can identify this study as a proof of concept of a novel behavioral decoding approach in freely-moving primates. Future, more extensive studies with greater amount of data will likely enable the decoding of a large variety of complex behaviors starting from neuronal activity readout.

7. REFERENCES

Berger, M., Agha, N. S., & Gail, A. (2020). Wireless recording from unrestrained monkeys reveals motor goal encoding beyond immediate reach in frontoparietal cortex. *ELife*, 9, e51322.

<https://doi.org/10.7554/eLife.51322>

Bezerra, B. M., & Souto, A. (2008). Structure and Usage of the Vocal Repertoire of *Callithrix jacchus*. *International Journal of Primatology*, 29(3), 671.

<https://doi.org/10.1007/s10764-008-9250-0>

Bonini, L., Maranesi, M., Livi, A., Bruni, S., Fogassi, L., Holzhammer, T., Paul, O., Ruther, P. (2014c). Application of floating silicon-based linear multielectrode arrays for acute recording of single neuron activity in awake behaving monkeys. *Biomed Tech.*, 59(4), 273–281.

<https://doi.org/10.1515/bmt-2012-0099>

Bonini, L., Maranesi, M., Livi, A., Fogassi, L., & Rizzolatti, G. (2014). Space-Dependent Representation of Objects and Other's Action in Monkey Ventral Premotor Grasping Neurons. *The Journal of Neuroscience*, 34(11), 4108. <https://doi.org/10.1523/JNEUROSCI.4187-13.2014>

Bouton, C. E., Shaikhouni, A., Annetta, N. V., Bockbrader, M. A., Friedenber, D. A., Nielson, D. M., Sharma, G., Sederberg, P. B., Glenn, B. C., Mysiw, W. J., Morgan, A. G., Deogaonkar, M., & Rezai, A. R. (2016). Restoring cortical control of functional movement in a human with quadriplegia. *Nature*, 533(7602), 247–250. <https://doi.org/10.1038/nature17435>

Bremmer, F., Klam, F., Duhamel, J.-R., Ben Hamed, S., & Graf, W. (2002). Visual-vestibular interactive responses in the macaque ventral intraparietal area (VIP): Visual-vestibular interaction in primate parietal cortex. *European Journal of Neuroscience*, 16(8), 1569–1586.

<https://doi.org/10.1046/j.1460-9568.2002.02206.x>

Bremmer, F., Schlack, A., Duhamel, J.-R., Graf, W., & Fink, G. R. (2001). Space Coding in Primate Posterior Parietal Cortex. *NeuroImage*, 14(1), S46–S51.

<https://doi.org/10.1006/nimg.2001.0817>

Buffalo, E. A., Movshon, J. A., & Wurtz, R. H. (2019). From basic brain research to treating human brain disorders. *Proceedings of the National Academy of Sciences*, 116(52), 26167–26172. <https://doi.org/10.1073/pnas.1919895116>

Capogrosso, M., Milekovic, T., Borton, D., Wagner, F., Moraud, E. M., Mignardot, J.-B., Buse, N., Gandar, J., Barraud, Q., Xing, D., Rey, E., Duis, S., Jianzhong, Y., Ko, W. K. D., Li, Q., Detemple, P., Denison, T., Micera, S., Bezdard, E., ... Courtine, G. (2016). A brain–spine interface alleviating gait deficits after spinal cord injury in primates. *Nature*, 539(7628), 284–288. <https://doi.org/10.1038/nature20118>

Cléry, J., Guipponi, O., Wardak, C., & Ben Hamed, S. (2015). Neuronal bases of peripersonal and extrapersonal spaces, their plasticity and their dynamics: knowns and unknowns. *Neuropsychologia*, 70, 313–326. <https://doi.org/10.1016/j.neuropsychologia.2014.10.022>

Colby, C. L., Duhamel, J.-R., and Goldberg, M. E. (1993) Ventral intraparietal area of the macaque: anatomic location and visual response properties, 1. *Neurophysiol.* 69,902-914. <https://doi.org/10.1152/jn.1993.69.3.902>

Cooke, D. F., Taylor, C. S. R., Moore, T., & Graziano, M. S. A. (2003). Complex movements evoked by microstimulation of the ventral intraparietal area. *Proceedings of the National Academy of Sciences*, 100(10), 6163. <https://doi.org/10.1073/pnas.1031751100>

Duhamel, J.-R., Colby, C. L., & Goldberg, M. E. (1991). Congruent representations of visual and somatosensory space in single neurons of monkey ventral intra-parietal cortex (area VIP). In *Brain and space*. (pagg. 223–236). Oxford University Press.

Eliades, S. J., & Wang, X. (2008). Chronic multi-electrode neural recording in free-roaming monkeys. *Journal of neuroscience methods*, 172(2), 201–214. <https://doi.org/10.1016/j.jneumeth.2008.04.029>

Fan, D., Rich, D., Holtzman, T., Ruther, P., Dalley, J. W., Lopez, A., Rossi, M. A., Barter, J. W., Salas-Meza, D., Herwik, S., Holzhammer, T., Morizio, J., & Yin, H. H. (2011). A Wireless Multi-Channel Recording System for Freely Behaving Mice and Rats. *PLoS ONE*, 6(7), e22033. <https://doi.org/10.1371/journal.pone.0022033>

Fogassi L., Gallese V., Fadiga L., Rizzolatti G. (1996a). Space Coding in Inferior Premotor Cortex (Area F4): Facts and Speculations. *Neural Bases of Motor Behaviour*, 99-120. https://doi.org/10.1007/978-94-017-2403-6_4

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Matelli, M., & Rizzolatti, G. (1996b). Coding of peripersonal space in inferior premotor cortex (area F4). *Journal of neurophysiology*, 76(1), 141–157. <https://doi.org/10.1152/jn.1996.76.1.141>

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7(11), 1325–1330. <https://doi.org/10.1111/2041-210X.12584>

Fritsch, G., and Hitzig, E. (1870). Uber die elektrische Erregbarkeit des Grosshirns. *Arch. f. Anat., Physiol und wissenschaftl. Mediz.*, Leipzig, 300–332.

Gallese, V., Fadiga, L., Fogassi, L., & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain*, 119(2), 593–609. <https://doi.org/10.1093/brain/119.2.593>

Gentilucci, M., Fogassi, L., Luppino, G., Matelli, M., Camarda, R., & Rizzolatti, G. (1988a). Functional organization of inferior area 6 in the macaque monkey. *Experimental Brain Research*, 71(3), 475–490. <https://doi.org/10.1007/BF00248741>

Glaser, J. I., Benjamin, A. S., Chowdhury, R. H., Perich, M. G., Miller, L. E., & Kording, K. P. (2020). Machine Learning for Neural Decoding. *eneuro*, 7(4), ENEURO.0506-19.2020. <https://doi.org/10.1523/ENEURO.0506-19.2020>

Graziano, M. S., & Aflalo, T. N. (2007). Mapping behavioral repertoire onto the cortex. *Neuron*, 56(2), 239–251. <https://doi.org/10.1016/j.neuron.2007.09.013>

Graziano, M. S., & Cooke, D. F. (2006). Parieto-frontal interactions, personal space, and defensive behavior. *Neuropsychologia*, 44(6), 845–859.

<https://doi.org/10.1016/j.neuropsychologia.2005.09.009>

Graziano, M. S., Aflalo, T. N., & Cooke, D. F. (2005). Arm movements evoked by electrical stimulation in the motor cortex of monkeys. *Journal of neurophysiology*, 94(6), 4209–4223. <https://doi.org/10.1152/jn.01303.2004>

Graziano, M. S., Reiss, L. A., & Gross, C. G. (1999). A neuronal representation of the location of nearby sounds. *Nature*, 397(6718), 428–430. <https://doi.org/10.1038/17115>

Graziano, M. S., Taylor, C. S., & Moore, T. (2002). Complex movements evoked by microstimulation of precentral cortex. *Neuron*, 34(5), 841–851. [https://doi.org/10.1016/s0896-6273\(02\)00698-0](https://doi.org/10.1016/s0896-6273(02)00698-0)

Grieves, R. M., Jedidi-Ayoub, S., Mishchanchuk, K., Liu, A., Renaudineau, S., & Jeffery, K. J. (2020). The place-cell representation of volumetric space in rats. *Nature Communications*, 11(1), 789. <https://doi.org/10.1038/s41467-020-14611-7>

Harrison, R. R., Fotowat, H., Chan, R., Kier, R. J., Olberg, R., Leonardo, A., & Gabbiani, F. (2011). Wireless Neural/EMG Telemetry Systems for Small Freely Moving Animals. *IEEE transactions on biomedical circuits and systems*, 5(2), 103–111. <https://doi.org/10.1109/TBCAS.2011.2131140>

Herwik, S., Paul O., Ruther P. (2011). Ultrathin silicon chips of arbitrary shape by etching before grinding. *Microelectromech Syst.*, 20(4), 791–793. <https://doi.org/10.1109/JMEMS.2011.2148159>.

Jackson, A., Mavoori, J., & Fetzi, E. E. (2007). Correlations Between the Same Motor Cortex Cells and Arm Muscles During a Trained Task, Free Behavior, and Natural Sleep in the Macaque Monkey. *Journal of Neurophysiology*, 97(1), 360–374. <https://doi.org/10.1152/jn.00710.2006>

Lemus, L., Hernandez, A., & Romo, R. (2009). Neural encoding of auditory discrimination in ventral premotor cortex. *Proceedings of the National Academy of Sciences*, 106(34), 14640–14645. <https://doi.org/10.1073/pnas.0907505106>

Matelli, M., & Luppino, G. (2001). Parietofrontal Circuits for Action and Space Perception in the Macaque Monkey. *NeuroImage*, 14, S27–S32. <https://doi.org/10.1006/nimg.2001.0835>

Milton, R., Shahidi, N., & Dragoi, V. (2020). Dynamic states of population activity in prefrontal cortical networks of freely-moving macaque. *Nature Communications*, 11(1), 1948. <https://doi.org/10.1038/s41467-020-15803-x>

Mimica, B., Dunn, B. A., Tombaz, T., Bojja, V. P. T. N. C. S., & Whitlock, J. R. (2018). Efficient cortical coding of 3D posture in freely behaving rats. *Science*, 362(6414), 584–589. <https://doi.org/10.1126/science.aau2013>

O'Keefe J. (1976). Place units in the hippocampus of the freely moving rat. *Experimental neurology*, 51(1), 78–109. [https://doi.org/10.1016/0014-4886\(76\)90055-8](https://doi.org/10.1016/0014-4886(76)90055-8)

Omer, D. B., Maimon, S. R., Las, L., & Ulanovsky, N. (2018). Social place-cells in the bat hippocampus. *Science*, 359(6372), 218–224. <https://doi.org/10.1126/science.aao3474>

Pardo-Vazquez, J. L., Leboran, V., & Acuna, C. (2008). Neural Correlates of Decisions and Their Outcomes in the Ventral Premotor Cortex. *Journal of Neuroscience*, 28(47), 12396–12408. <https://doi.org/10.1523/JNEUROSCI.3396-08.2008>

Rizzolatti, G., Gentilucci, M. (1988b). Motor and visual-motor function of the premotor cortex. *Neurobiology of Neocortex*, 269-284.

Rizzolatti G., Fogassi L., Gallese V. (2002). Motor and cognitive functions of the ventral premotor cortex. *Current Opinion in Neurobiology*, 12(2), 149-154. [https://doi.org/10.1016/S0959-4388\(02\)00308-2](https://doi.org/10.1016/S0959-4388(02)00308-2).

Rizzolatti, G., & Luppino, G. (2001). The cortical motor system. *Neuron*, 31(6), 889–901. [https://doi.org/10.1016/s0896-6273\(01\)00423-8](https://doi.org/10.1016/s0896-6273(01)00423-8)

Rizzolatti, G., & Matelli, M. (2003). Two different streams form the dorsal visual system: Anatomy and functions. *Experimental Brain Research*, 153(2), 146–157. <https://doi.org/10.1007/s00221-003-1588-0>

Rizzolatti, G., Cattaneo, L., Fabbri-Destro, M., & Rozzi, S. (2014). Cortical Mechanisms Underlying the Organization of Goal-Directed Actions and Mirror Neuron-Based Action Understanding. *Physiological Reviews*, 94(2), 655–706. <https://doi.org/10.1152/physrev.00009.2013>

Rizzolatti, G., Matelli, M., & Pavesi, G. (1983). Deficits in attention and movement following the removal of postarcuate (area 6) and prearcuate (area 8) cortex in macaque monkeys. *Brain : a journal of neurology*, 106 (Pt 3), 655–673. <https://doi.org/10.1093/brain/106.3.655>

Rizzolatti, G., Scandolara, C., Matelli, M., & Gentilucci, M. (1981b). Afferent properties of periarculate neurons in macaque monkeys. II. Visual responses. *Behavioural brain research*, 2(2), 147–163. [https://doi.org/10.1016/0166-4328\(81\)90053-x](https://doi.org/10.1016/0166-4328(81)90053-x)

Rizzolatti, G.; Scandolara, C.; Gentilucci, M.; and Camarda, R. (1981a). Response properties and behavioral modulation of "mouth" neurons of the postarcuate cortex (area 6) in macaque monkeys. *Brain Research*, 255, 421-424.

Roelfsema, P. R., & Treue, S. (2014). Basic Neuroscience Research with Nonhuman Primates: A Small but Indispensable Component of Biomedical Research. *Neuron*, 82(6), 1200–1204. <https://doi.org/10.1016/j.neuron.2014.06.003>

Romo, R., Hernández, A., & Zainos, A. (2004). Neuronal correlates of a perceptual decision in ventral premotor cortex. *Neuron*, 41(1), 165–173. [https://doi.org/10.1016/s0896-6273\(03\)00817-1](https://doi.org/10.1016/s0896-6273(03)00817-1)

Roy, S., & Wang, X. (2012). Wireless multi-channel single unit recording in freely moving and vocalizing primates. *Journal of Neuroscience Methods*, 203(1), 28–40. <https://doi.org/10.1016/j.jneumeth.2011.09.004>

Roy, S., Zhao, L., & Wang, X. (2016). Distinct Neural Activities in Premotor Cortex during Natural Vocal Behaviors in a New World Primate, the Common Marmoset (*Callithrix jacchus*). *Journal of Neuroscience*, 36(48), 12168–12179. <https://doi.org/10.1523/JNEUROSCI.1646-16.2016>

Schlack, A., Sterbing-D'Angelo, S. J., Hartung, K., Hoffmann, K. P., & Bremmer, F. (2005). Multisensory space representations in the macaque ventral intraparietal area. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 25(18), 4616–4625. <https://doi.org/10.1523/JNEUROSCI.0455-05.2005>

Tanji, J., Shima, K., & Mushiake, H. (1996). Multiple cortical motor areas and temporal sequencing of movements. *Cognitive Brain Research*, 5, 117–122. [https://doi.org/10.1016/S0926-6410\(96\)00047-X](https://doi.org/10.1016/S0926-6410(96)00047-X).

Umilta, M. A., Escola, L., Intskirveli, I., Grammont, F., Rochat, M., Caruana, F., Jezzini, A., Gallese, V., & Rizzolatti, G. (2008). When pliers become fingers in the monkey motor system. *Proceedings of the National Academy of Sciences*, 105(6), 2209–2213. <https://doi.org/10.1073/pnas.0705985105>

Umilta, M. A., Kohler, E., Gallese, V., Fogassi, L., Fadiga, L., Keysers, C., & Rizzolatti, G. (2001). I Know What You Are Doing: A Neurophysiological Study. *Neuron*, 31, 155-165. [https://doi.org/10.1016/s0896-6273\(01\)00337-3](https://doi.org/10.1016/s0896-6273(01)00337-3)

Woolsey, C.N.; Settlage, P.H.; Meyer, D.R.; Sencer, W.; Pinto Hamuy, T.; and Travis, A.M. (1952). Patterns of localization in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. *Res. Pub/. Ass. Nerv. Ment. Dis.* 30: 238-264.

Yartsev, M. M., & Ulanovsky, N. (2013). Representation of Three-Dimensional Space in the Hippocampus of Flying Bats. *Science*, 340(6130), 367–372. <https://doi.org/10.1126/science.1235338>

Yartsev, M. M., & Ulanovsky, N. (2013). Representation of three-dimensional space in the hippocampus of flying bats. *Science (New York, N.Y.)*, 340(6130), 367–372. <https://doi.org/10.1126/science.1235338>

Zhou, F., De la Torre, F., & Hodgins, J. K. (2013). Hierarchical Aligned Cluster Analysis for Temporal Clustering of Human Motion. *IEEE transactions on pattern analysis and machine intelligence*, 35(3), 582–596. <https://doi.org/10.1109/TPAMI.2012.137>