UNIVERSITÀ
DI PARMA

DIPARTIMENTO DI MEDICINA E CHIRURGIA

CORSO DI LAUREA MAGISTRALE IN PSICOBIOLOGIA E NEUROSCIENZE COGNITIVE

PROPERTIES OF PERIPERSONAL NEURONS OF THE VENTRAL PREMOTOR CORTEX IN FREELY MOVING MONKEYS

Proprietà dei neuroni peripersonali della corteccia premotoria ventrale in scimmie libere di muoversi

Relatore: Chiar.mo Prof. LUCA BONINI

Correlatrice: Chiar.ma Prof.ssa MONICA MARANESI

Laureanda:
CRISTINA PAGLIARA

ANNO ACCADEMICO 2021 - 2022

INDEX

ABSTRACT (ENG)

A representation of the space around us is essential for interacting properly with the outside world. The ventral premotor cortex plays a crucial role in this function; indeed, it is known that in macaques it hosts neurons encoding tactile stimuli applied to specific body parts and visual stimuli moving within the space near their tactile field thereby forming visuo-tactile place fields (PFs). Because of their specificity for stimuli in the space near the body, these neurons are named peripersonal neurons (PPNs). So far, however, studies on PPNs only focused on single neuron recordings in constrained animals passively receiving sensory stimulation. In the present study, we used unbiased multi-electrode recordings and systematic stimulation of the body surface and the peripersonal space to 1) replicate classical findings in constrained conditions and 2) explore the same neurons' properties during freely moving conditions. We analysed four sessions in two different monkeys. We recorded 127 neurons, 59% of which exhibited visuo-tactile properties (N=75), and 16 neurons fulfilled a rigorous definition of PPNs. Of these, we observed the visual PFs, emphasizing the interaction between space and the direction of movement of the stimulus. Finally, we performed a descriptive evaluation of the neural activity of two example neurons, which led us to hypothesise that part of the PPNs may change the localization of their visual PFs when moving from the constrained condition to the freely moving condition.

ABSTRACT (ITA)

Avere una rappresentazione dello spazio che ci circonda e degli stimoli che lo popolano è essenziale per interagire correttamente con il mondo esterno. La corteccia premotoria ventrale svolge un ruolo cruciale in questa funzione; è infatti noto che nei macachi essa ospiti neuroni che codificano stimoli tattili applicati a parti specifiche del corpo e stimoli visivi che si muovono nello spazio vicino al loro campo recettivo tattile, formando così campi recettivi visuo-tattili. A causa della loro specificità per gli stimoli nello spazio vicino al corpo, questi neuroni sono chiamati neuroni peripersonali. Finora, tuttavia, gli studi sui neuroni peripersonali si sono concentrati solo su registrazioni di singoli neuroni in animali costretti a ricevere passivamente stimoli sensoriali. Nel presente studio, abbiamo utilizzato registrazioni multi-elettrodo imparziali e una stimolazione sistematica della superficie corporea e dello spazio peripersonale per 1) replicare i risultati classici in condizione vincolata e 2) esplorare le proprietà degli stessi neuroni nella condizione di movimento libero. Abbiamo analizzato quattro sessioni in due scimmie diverse. Abbiamo registrato 127 neuroni, il 59% dei quali mostrava proprietà visuo-tattili (N=75) e 16 neuroni soddisfacevano una definizione rigorosa di neurone peripersonale. Di questi abbiamo osservato i campi recettivi visivi, enfatizzando l'interazione tra lo spazio e la direzione di movimento dello stimolo. Infine, grazie all'implementazione della tecnologia wireless, abbiamo svolto una valutazione descrittiva dell'attività neurale di due neuroni d'esempio la quale ci ha portato ad ipotizzare che parte dei neuroni peripersonali possa cambiare la localizzazione dei propri campi recettivi visivi passando dalla condizione vincolata a quella in libero movimento.

1. INTRODUCTION

The first observation of neurons responding to stimuli close to the body was made by Leinonen and Nyman (1979) in the parietal cortex (7b), but it was Rizzolatti and colleagues who described similar properties of some premotor neurons in 1981 and named them ‘peripersonal neurons’(Leinonen et al., 1979; Rizzolatti et al., 1981). These neurons are characterized by the fact that they are multimodal neurons encoding stimuli based on a reference frame anchored to the body parts (Graziano et al., 1994). Neurons with similar properties have been found in cortical and subcortical areas, such as the parietal area VIP (Colby et al., 1993) and the putamen nucleus (Graziano & Gross, 1993). The perceptive and somato-centric properties of these neurons and their localization in these particular motor structures led researchers to interpret them as a suitable substrate to form a map of the space near the body, enabling subject to coordinate movements in the space close to the body (Graziano et al., 1994). However, over the years, it emerged the idea that the notion of peripersonal space cannot refer to a well-delineated region of the external world with sharp and stable boundaries (Vignemont et al., 2020). Thus, the concept of peripersonal space assumed a wider meaning, being better defined as a space in which stimuli are more relevant because of their potential to enter in contact with the body (Vignemont et al., 2020); such potentiality is determined by stimuli characteristics (movement direction, speed, stimulus type) and from the motor possibilities of the subject.

1.1 Premotor cortex in monkeys: functional properties and functional roles

When peripersonal neurons were discovered (Rizzolatti et al., 1981) the authors investigated the whole posterior periarcuate area. This postarcuate cortex can be identified with Brodmann’s area 6, which corresponds to the cytoarchitectonically agranular isocortex lacking layer IV (Kurata, 2018). Its involvement in motor control was reported by Fulton in 1935, who referred to this area with the term “premotor cortex” (PM) (Fulton 1935). Subsequently, several other studies divided this area into a dorsal (PMd) and ventral (PMv) part relative to the spur of the arcuate sulcus. In turn, each of these sectors was split into other areas along the rostro-caudal direction: in the dorsal premotor cortex it was identified a rostral (F7) and a caudal (F2) subdivision, based on anatomical and physiological differences (Matelli et al., 1991); similarly, a rostro-caudal anatomo-functional border in PMv allowed researchers to subdivide it in areas F5 and F4 (Matelli et al., 1985). Belmalih et al. in 2009 also identified three architectonically distinct areas in the rostral PMv (F5), named F5a (antero-ventral), F5p (postero-dorsal) and F5c (adjacent post arcuate convexity cortex), possibly involved in different aspects of motor control and cognitive motor functions (Belmalih et al., 2009). Then, thanks to the study conducted by Maranesi and colleagues (2012), it turned out to be important to create another dorso-ventral anatomo-functional subdivision in caudal PMv (F4), defined on the basis of both the visual responses and the type of represented effector (Maranesi et al., 2012). Thus, this led to identify the two sectors in which F4 is divided, namely F4v (ventral) and F4d (dorsal).

1.1.1 Body maps: representation of bodily actions in space

Each area is distinguished from the others not only based on cytoarchitectonic features but also because it exhibits different functional roles and functional proprieties related to different body maps. Based on the literature, the functional roles of premotor cortex can be subdivided considering two circuits: one composed by areas F7 and F2, the other composed by PMv areas F4 and F5 (Matelli & Luppino, 2001), which appear to be more specifically involved in the motor coding of stimuli in the peripersonal space..

1.1.1.1 Intracortical microstimulation maps

Intracortical microstimulation (ICMS) is one of the main ways to interrogate the possible functional role of an area from a behavioural point of view. The short train ICMS mapping of the ventral premotor region evidences different excitability of the cythoarchitectonic areas F4 and F5, with F5 convexity poorly excitable compared to F4, which exhibits excitability thresholds not significantly different from those of the adjacent F1 sector (Maranesi et al., 2012; Schieber, 2001). The stimulation maps revealed a rough somatotopic organization, with brachio-manual movements and proximal and distal forelimb movements represented only in the dorsal portion of PMv (F4d, F5p) whereas lip, tongue, and jaw movements especially mapped in F4v and F5c. Other studies showed that in F5p hand movements were mostly represented, whereas, in the ventral part of F5 convexity (F5c) neurons coding mouth movements mostly predominate. On the other hand, intracortical microstimulation of the sector F5a in anesthetized monkeys is not effective in evoking body movements (Belmalih et al., 2009).

The application of long stimulation trains (500 ms), instead of evoking brief muscle twitches, results in complex movements aimed at ending in a specific posture with a dynamic that resembles that of ethologically relevant, goal-directed actions. The study by Graziano and coworkers in 2002 (see *Figure 1.1*) demonstrated that within the large arm and hand representation, the stimulation-evoked postures were organized across the cortex to form a map of hand positions in space: going from antero-ventral to postero-dorsal sites, the hand position moves from upper space to lower space and along the horizontal axis (rostro-caudal) hand positions were distributed from contralateral to ipsilateral space. The long stimulation trains applied to sites located in the hand region of the ventral premotor cortex elicit hand-to-mouth movements, whereas defensive movements (facial squint, head turn, arm and hand moving to a guarding posture) are evoked by stimulating the dorsal part of area F4 at the boundary with the ventro-rostral part of F2, named "polysensory zone" (PZ) (Graziano et al., 2002; Graziano & Cooke, 2006). In this zone, whose size and exact location vary among monkeys, most of the recorded neurons showed visual-tactile responses to nearby stimuli.

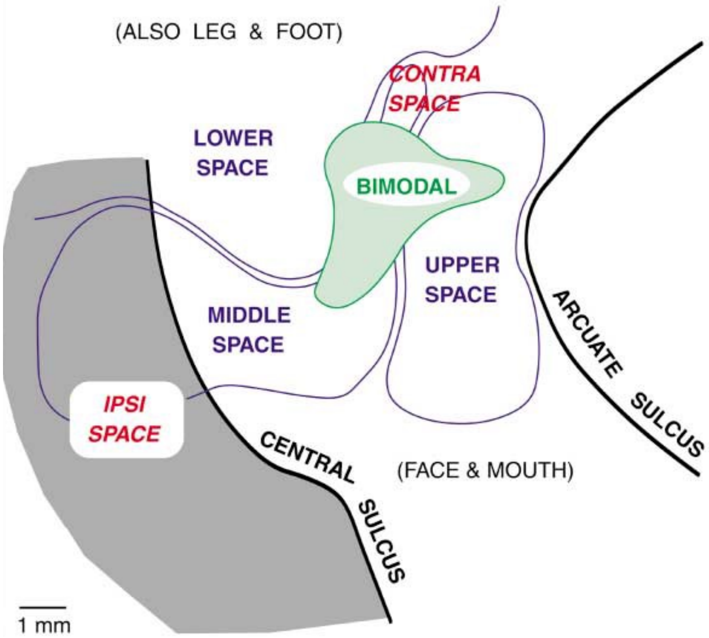


Figure 1.1 *– Organization of the precentral gyrus as determined by microstimulation. Blue lines show vertical axis of hand position map indicated by Upper Space, Middle Space, and Lower Space. Red shows horizontal axis of hand position map indicated by Contra Space and Ipsi Space. Green shows bimodal, visual-tactile zone from defensive postures were evoked. Shaded area to the left of the lip of the central sulcus represents the anterior bank of the sulcus (Graziano, 2002).*

1.1.1.2 Neuron properties maps

The functional proprieties of each premotor area are observed by recording neuronal activity during motor acts or stimuli presentation. More than one functional property can be found up to the single neuron level and, based on the specific response properties it exhibits, the neuron can be associated with a particular functional category, such as purely motor, peripersonal, canonical or mirror. As a proof that these categories cannot be considered as functionally segregated, more recent studies have demonstrated that 'canonical' and 'mirror' properties can often apply even to the same single neuron (Bonini et al., 2014).

Regarding motor responses, Maranesi and colleagues in 2012 showed that during brachio-manual motor acts – alone or associated with mouth responses – and axio-proximal movements, some F4d and F5c neurons are activated. In these areas most penetrations showed responses during goal-directed motor acts and, while in F5c more than half of the sites showed activity related to only one type of grip, in F4d most neurons did not show a neuronal grip selectivity. F4v, in turn, shows a high number of penetrations related to mouth simple movements, instead sites with responses related to mouth motor acts were more frequently found in area F5c (Maranesi et al., 2012).

Besides an activation during motor behaviours, many neurons respond to somatosensory stimuli. Somatosensory responses are generally characterized by typically large tactile fields, and different body parts are frequently represented in the same cortical site (Gentilucci et al., 1988; Maranesi et al., 2012; Rizzolatti et al., 1981). These tactile fields are arranged in a rough somatotopic manner: when electrode penetrations were made in the medial part of PMv, near the genu of the arcuate sulcus, the somatosensory receptive fields were usually located on the arm or hand, while when penetrations were made a few millimetres laterally, the tactile receptive fields were usually located on the face or inside the mouth (Gentilucci et al., 1988; Graziano et al., 1997). Then, Maranesi and coworkers in 2012 confirmed that cortical sites related to the lip and inner mouth are mainly localized in F4v, whereas tactile fields on the hand, arm, or neck/trunk are more frequently found in F4d (Maranesi et al., 2012). In the most rostral part of F4 and in F5 strongly responsive tactile neurons tend to decrease and are substituted by neurons less easily triggered by passive stimulation (Gentilucci et al., 1988).

Neurons in which visual responses are identified also activated during the monkey's active movements or during somatosensory stimulation, whereas a small percentage showed visual responses not associated with any motor response (Maranesi et al., 2012). In particular, the study of Maranesi and coworkers (2012) showed that visual responses were nearly absent in F4v, whereas they were highly represented in F5 and F4d (see *Figure 1.2*). Here the authors identified five different visual responses, according to the type of stimulus most effective in eliciting neuronal activity (Maranesi et al., 2012); among these, 'mirror' responses, 'object presentation' responses and 'peripersonal' responses (black points, red points, and blue points respectively in *Figure 1.2*). These latter were tested through the approaching or receding of a three-dimensional object to different monkey body parts in its reaching space. As shown in the lower part of the *Figure 1.2*, F4d contained the highest percentage of 'peripersonal' visual responses compared with the other premotor areas, and these responses manly co-localized with electrically excitable penetrations; in the F5 sector located in the posterior part of the inferior post-arcuate bank (F5p) the most frequently observed visual responses were those to visually presented objects, whereas responses to others' observed actions are more frequently found in the most rostral part of the recorded region, particularly in area F5c and F5a, which are poorly electrically excitable (Kurata, 2018; Maranesi et al., 2012).

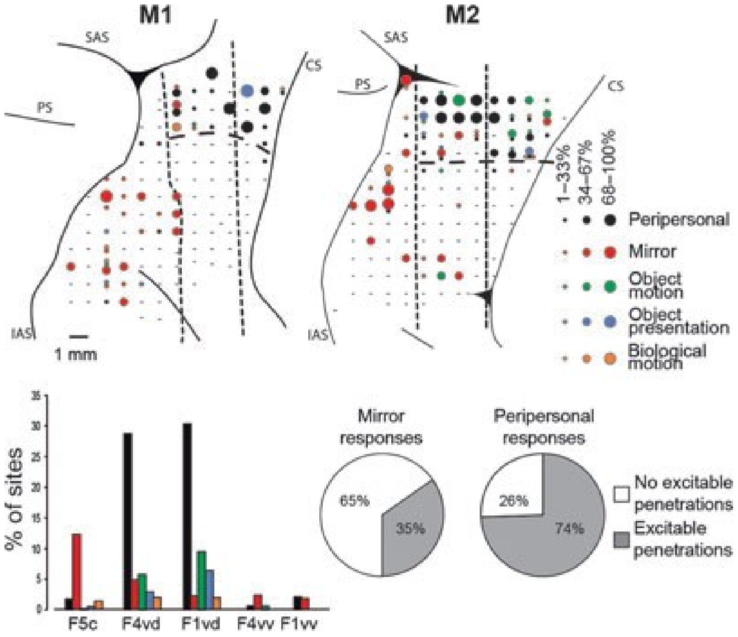


Figure 1.2 *– Top: distribution of the different types of visual properties in F1v, F4 and F5c regions. Bottom left: histograms showing the proportion of each type of visual property in each of the investigated sectors. Bottom right: pie charts representing the proportion of excitable (grey) vs. non-excitable (white) penetrations among those endowed with mirror and peripersonal responses (Maranesi et al., 2012).*

1.1.3 Functional properties of peripersonal neurons

Peripersonal neurons are characterized by at least two fundamental functional properties: a somatosensory response to the stimulation of a delimited region of the body surface, and a visual response to objects moving toward or around the somatosensory receptive field, thus being defined as bimodal neuron. In some cells, auditory stimulation coming from a specific region of the surrounding space may also be effective in triggering the neuron's response: Graziano and coworkers in 1999 demonstrated that around 40% of ventral premotor neurons respond to auditory stimulation coming from the same direction of the effective visual stimulus (Graziano et al., 1999). Peripersonal neurons are defined as multisensory neurons capable to encode the spatial position of a moving stimulus with respect to the body. The modulation of their discharge to auditory and/or visual stimuli is particularly strong at the distance of about 30 cm. In fact, for example in one of the first studies conducted by Gentilucci and colleagues, it was claimed that among the studied peripersonal neurons some responded only to stimuli very close to the animal's body (10 cm or less), while others were triggered by stimuli located more distantly, but always within the animal's reaching distance (Gentilucci et al., 1988). In another work, Fogassi and coworkers demonstrated that the 86% of studied neurons had visual receptive fields whose outer borders, measured from the animal's skin, extended in depth for about 40 cm (Fogassi et al., 1996); again, Graziano et al. in 1999 showed that the 59% of the studied neurons responded significantly better to closer acoustic stimuli (Graziano et al., 1999).

However, this boundary turned out to be much more plastic than it was initially described, thus paving the way for other interpretations less related to the concept of nearby space. For example, Graziano in 1997 introduced the evidence that peripersonal neurons did not show a sharp outer border of the visual receptive field; in fact, some described units gave a response at the onset of stimulus motion, when the stimulus was at its maximum distance, then their firing rate returned to baseline and then began to increase again when the stimulus had approached closer to the face (Graziano et al., 1997). Also, Fogassi in 1996 demonstrated that the extent in depth of most F4 visual receptive fields is modified from different stimulus velocities, not always in a linear fashion (Fogassi et al., 1996). Furthermore, Iriki et al. in 1996 conducted a study where they showed that during tool use the hand-visual receptive field was enlarged up to covering the tip of the rake (Iriki et al., 1996). In addition to this evidence, many other studies demonstrated that several factors are able to modify the visual receptive filed of peripersonal neurons, among which multimodal attention (Kandula et al., 2015), threat of the stimulus (Ruggiero et al., 2017), subject stress (Sambo & Iannetti, 2013). For this reason, the focus in time shifted from the concept of space to the concept of action preparation.

1.2 Peripersonal space frameworks

Since the first study conducted by Rizzolatti and coworkers in 1981, it was speculated that the information that peripersonal neurons convey has a praxic content, and that their possible function could be to prepare motor acts, thanks to their anticipatory response (Rizzolatti et al., 1981). Also, Fogassi and coworkers in 1996 proposed to think about the discharge in response to stimulus presentation in terms of an internal representation of a movement toward a particular spatial location (Fogassi et al., 1996). Within this wide definition, years of literature defined two specific action categories that could be prepared by peripersonal neurons: reaching and defensive actions (Vignemont et al., 2020). This double function is supported by several authors e.g., Cléry and coworkers in 2015 proposed a subdivision that identifies two distinct peripersonal networks: one constituted by projection from parietal area VIP to premotor area F4, and another composed by parietal area 7b and premotor region F5 (Cléry et al. 2015; Noel et al. 2021).

The involvement of these peripersonal neurons in the protection of the body from the external stimuli was introduced by Graziano through the study conducted in 2002. In this work, before electrical stimulation, the authors studied single neurons activity in F4d (PZ) and showed that most of them were visuo-tactile neurons. After that, they showed that the evoked movements, through long train ICMS, appeared to be spatially directed as if to protect the location of tactile receptive fields of the stimulated neurons (Graziano et al. 2002; Graziano e Cooke, 2006). To confirm the role of PZ in the coordination of defensive movements, the same authors in another study published in 2004 injected a stimulating drug (bicuculline) to increase neuronal activity or an inhibitory drug (muscimol) to reduce it (Cooke & Graziano, 2004). With bicuculline stimulation, neurons in PZ began to fire in intense spontaneous burst each of which was fallowed at short latency by the standard set of defensive-like movements (blinking, shifting the head away from sensory receptive filed, shrugging the shoulder) showed also during electrical stimulation, while when muscimol was injected into PZ the magnitude of the defensive reaction was approximately 30%

smaller. The concept of a zone around the body that constitutes safety margins was initially represented by Hediger (1955) with the term of ‘flight zone’ and it was assimilated with the concept of human ‘personal space’ (Dosey & Meisels, 1969; Hediger, 1955). Graziano speculated that the peripersonal visual receptive fields could be like bubbles of space anchored to the body surface that represents exactly the way through which a margin of safety around the body is maintained (Graziano & Cooke, 2006).

The second network 7b-AIP/F5 instead presents multiple properties; in these areas were discovered a variety of visual responses (like ‘canonical’ or ‘mirror’) linked to grasping behavior (Murata et al., 1997). These properties, especially in F5 neurons, remain connected to the reachable space; this was demonstrated by Caggiano and coworkers in 2009 (Caggiano et al., 2009) who noted that some ‘mirror’ neurons show these properties only inside the peripersonal space, and this tuning is increased when the space in which the observed action occurs is more categorically distinct (Maranesi et al. 2017). This circuit is interpretated as the component involved in the approaching behavior.

Ultimately, taking into account the two previous point of view, we could assume a flexible organization of peripersonal space that would depend on current task and environmental constraints (e.g., Bufacchi e Iannetti 2018). This system could have as purposes both the contact prediction of the stimuli with the body and the preparation of goal-directed behavior.

1.2.1 Visuo-tactile predictive mechanisms of peripersonal space

Dijkerman & Medendorp (2021) accurately described a predictive multisensory mechanism that could be used to anticipate the consequences of bodily contact with an external object as an important aspect of multimodal peripersonal space. This mechanism explains the anticipatory visual activation, described in parietal (Hyvärinen et al. 1974) and premotor (Fogassi et al., 1996) neurons, as an evaluation of the tactile consequences of visual stimuli. This estimate allows us to program appropriate actions and responses toward approaching or receding visual stimuli, considering all the information conveyed by this, like its spatial or temporal qualities, features (sharp, blunt, hard, soft), or even its affective value (pleasant, unpleasant). *Figure 1.3* shows the hypothetical neural basis of the visuo-tactile predictive system for coding peripersonal space proposed by Dijkerman & Medendorp (Dijkerman & Medendorp, 2021). Here, purely visual information is guided toward the posterior parietal/premotor cortex from the dorsal visual stream, which codes spatiotemporal visual input, and from ventral visual stream, which codes visual object characteristics and identity (Goodale & Milner, 1992). The affective content instead is conveyed to the parieto-frontal representation of peripersonal space by amygdala and periaqueductal grey (Mobbs et al. 2007; Zald 2003; Vieira, Pierzchajlo, e Mitchell 2020) for threat stimuli and by cerebellum for positive emotions (Schutter et al. 2009). The visuo-tactile binding, that allows the integration of visual and tactile representation, is achieved through repeated spatiotemporal coupling of spatiotemporal visual information (conveyed by visual dorsal stream) and spatiotemporal somatosensory input (conveyed by SI). This joining is the base of the multisensory integration thanks to which we have a predictive link between approaching visual stimuli and tactile judgements (Kandula et al. 2015). The authors suggest that the acuity and the emotional valence of predicted tactile consequences influence the extent of peripersonal space (Dijkerman & Medendorp, 2021).

The central role of impact prediction into the concept of peripersonal space was confirmed by a recent study conducted by Straka and colleagues in 2022. This work provides a normative model of impact prediction using Bayesian Decision Theory (Colombo & Seriès, 2012) – this is a mathematical model that

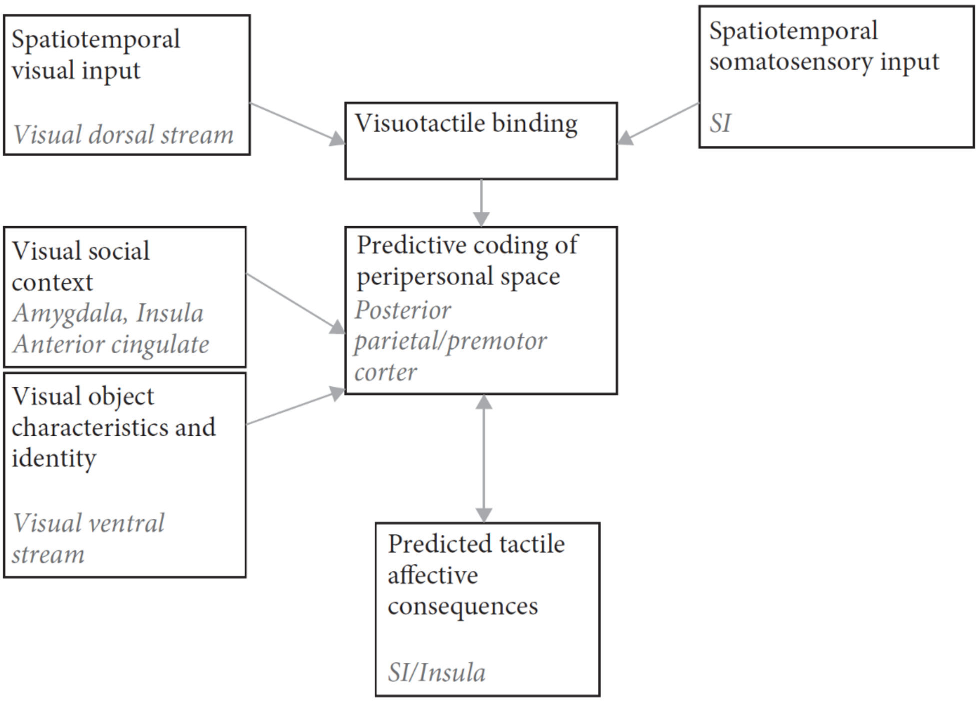


Figure 1.3 *– An overview of the visuo-tactile predictive system for coding peripersonal space. Central is a visuo-tactile representation of PPS in parieto-premotor areas. This develops through visuo-tactile spatiotemporal binding and is modulated by visual information about object characteristics involving ventral stream processing and visual social and affective cues (left). The visuo-tactile representation allows prediction of the bodily consequences of contact with the visual object/person (bottom), which is used to modulate PPS (Dijkerman & Medendorp, 2021).*

is statistically optimal at predicting future impact (taking into account loss/cost of a contact) – proceeding then to examine if the properties of this model are similar to those of the peripersonal neurons. The model was able to modify its boundaries between near and far space differently for different body parts, object speed, direction, and object value, confirming all the well-known peripersonal system characteristics. Furthermore, the study demonstrated that this normative model mapped out a graded boundary between near and far space (Straka et al. 2022).

1.2.2 Action field theory

A more inclusive theory that tries to explain both peripersonal neurons' functions, that is, the defensive and approaching functions, was proposed by Bufacchi and Iannetti (2018) and took the name of 'action field theory'. The scaffolding on which this model is built upon is represented by the 'affordance competition hypothesis', proposed by Cisek and Kalaska in 2010.

The mechanism suggested by the two authors goes against the traditional hierarchical perspective, proposing instead a model capable of explaining the way in which we can interact with a world full of action choices. According to this model, within our brain we can represent simultaneously all the possible actions that are coherent with the external environment; these representations use as substrate competing neuronal populations, localized into the parietal-premotor circuit (action specification). Through lateral inhibition, cells with different preferences for the potential parameters of movements inhibit each other, while cells with similar preferences mutually excite each other; the same mechanism is so responsible for defining the choices as well as for implementing the competition between them. Moreover, also attention and intention affect the process of action selection that progressively narrows the set of potential actions that will be selected (Cisek & Kalaska, 2010). All the behavioral and physiological responses that are modulated by the distance constitute a measure of the peripersonal space, for example the peripersonal neurons firing rate. This modulation, from the prospective of the affordance competition hypothesis, is read as an index of the action or set of actions relevance (or value) represented by the underling neuronal populations. The actions that, by definition, modify naturally their relevance based on the proximity of the stimulus are the ones aiming to create or avoid a contact between the stimulus and the body, that is proactive or defensive actions.

Following this track, Bufacchi and Iannetti defined the peripersonal space as “a set of continuous fields describing physiological or perceptual responses that reflect the behavioural relevance of actions aiming to create or avoid contact between objects and the body”. This definition introduced three new concepts that must be explained. The first being the concept of field, defined as a quantity that has a magnitude for each point in space and time (McMullin, 2002). This concept automatically implies into the definition of peripersonal space the fact that such space is not limited by sharp boundaries, is not an in-or-out space as it was – for the sake of simplicity – classically described, but includes all near and far space (Graziano et al., 1997) in which instead the action assumes a different behavioral relevance based on distance and other qualities (see *Figure 1.4*). The other point to stress is indeed the fact that the action relevance (that is the measurement of peripersonal space) is not exclusively modulated by the distance but also by many other factors playing a role in action selection, such as movement-related factors (speed, direction) and non-movement-related factors (size, value, environmental landscape). These environmental factors might affect action choices and thus peripersonal space fields: e.g., the receptive field of various visuo-tactile neurons changes when a barrier is placed in between the monkey and the stimulus (Bonini et

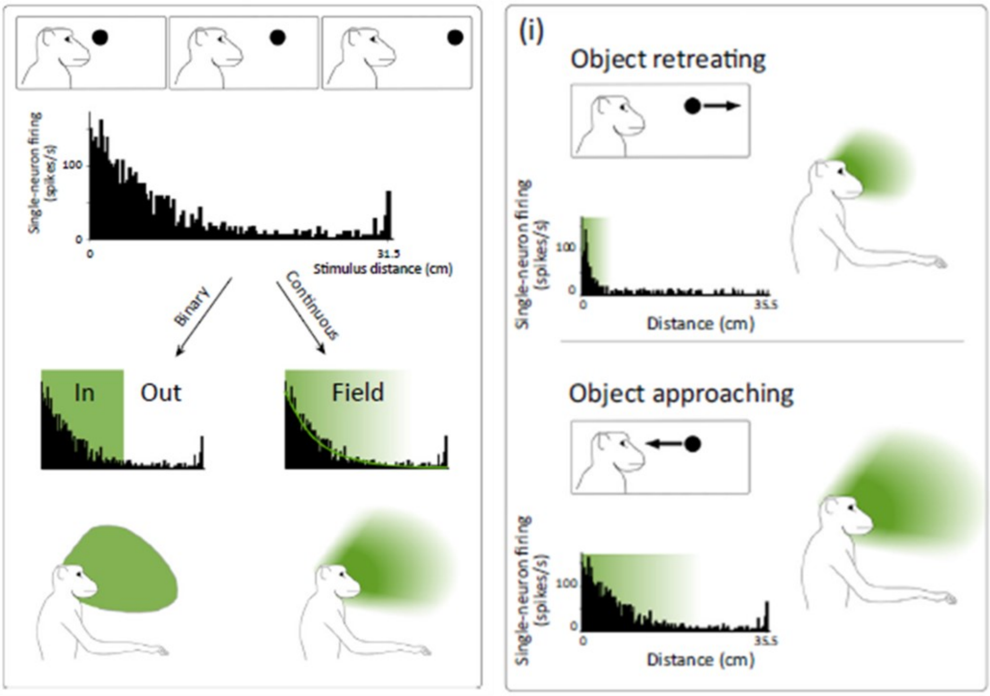


Figure 1.4 *– On the left: bimodal visuo-tactile neurons fire more when visual stimuli are close to their tactile receptive fields. The image shows the difference between a receptive field represented in terms of ‘in-or-out’ space and a graduated receptive field. On the right: additional factors modulate PPS-related measures. Although the magnitude of PPS-related measures is commonly affected by proximity to a body part, many other factors also affect these PPS measures, such as various types of motion (Bufacchi e Iannetti, 2018).*

al., 2014; Maranesi et al., 2012). Finally, defining peripersonal space as a set of fields shatters the idea of unity of peripersonal space, that instead is better described as a space composed of multiple graduated fields: each kind of measurement of a distance-modulated physiological or behavioral response (peripersonal neurons firing rate, blink reflex etc.) defines in fact a different space, which is bound to an action tailored for the external stimulus. This framework moves us definitively away from the classically favored implicit link between peripersonal concept and proximity. The peripersonal space fields are modulated by many separate variables, one of which is proximity (Bufacchi & Iannetti, 2018).

In conclusion, we must note that both the affordance competition hypothesis and the action field theory see the brain from an ethological point of view: behaviors and acts involve continuous sensorimotor interaction between the organism and its environment (Cisek & Kalaska, 2010). For this reason, the classic laboratory condition, in which the time is divided into a sequence of distinct events, each starting with a discrete stimulus and ending with a specific response, are not the better way to study and test this complex mechanism (Cisek & Kalaska, 2010).

1.3 Exploring peripersonal space in freely moving animals

Traditional neural recording systems are composed by implanted microelectrodes connected with cables to the amplifier and recording equipment: these require to keep the animal in constrained condition to make possible the recording of single cell activity (Lemon 1984). Indeed, typical experiments on non-human primates engage monkeys in physical restraint and head-fixed settings by using the so-called primate chairs. These experimental conditions allow the control of many parameters (such as head position and body and arm posture) increasing the results' internal validity, but at the same time they limit their ecological validity (Berger et al., 2020) and animal wellbeing. Therefore, although classical neurophysiology experiments provided insights into the underlying mechanisms of brain functioning, it is not clear if it is possible to extend these results to unconstrained natural behaviours, making it necessary to implement freely behaving experimental conditions, especially while investigating the mechanisms underlying motor functions; in fact, freely moving animals perform a large variety of whole-body behaviours, which were not previously possible to study.

Freely moving experiments by means of tethered recording systems has been successfully employed in small species such as rats (O'Keefe, 1976) and small primate species, for instance squirrel monkeys (Ludvig et al., 2004) or marmosets (Courellis et al., 2019; Nummela et al., 2017), however they cannot be implemented with larger non-human primates, which could easily remove and damage cables and devices. To overcome tethered system limitations, wireless recording systems have been implemented; thus, even if in unconstrained conditions it is more difficult to quantify animals' behaviours and confounding factors are more difficult to control, freely moving paradigms together with wireless recording systems allows to overcome traditional limits of non-human primate studies. In this manner researchers can investigate natural behaviours, address ecologically relevant questions, improve reliability and ecological validity of the data and apply the refinement principle (Russell, W. M. S., & Burch, R. L., 1959).

Successful wireless technology has already been implemented in various animal species such as rats (Grieves et al., 2020), bats (Omer et al., 2018; Yartsev & Ulanovsky, 2013) and non-human primates (Berger et al., 2020; Roy & Wang, 2012). Jackson and coworkers (2007) compared the data collected by using a wireless system with those obtained by using a classic constrained condition system; in this way they found that results obtained with the restrained and wireless methodologies partially overlapped (Jackson et al., 2007). In addition to confirming the data obtained under classical restrained experimental conditions, the wireless recording system must expand these data. This aim was achieved by Berger and colleagues (2020), which, thanks to the unconstrained setup, were able to study motor goal encoding even beyond the immediate reachable space and during ongoing walking movements (Berger et al., 2020).

AIMS

In the present study, we attempted to replicate the literature data regarding peripersonal neurons by recording the activity of single neurons in the ventral premotor area of two monkeys in the classical chair condition. To do so, however, we introduced the use of multi-electrode recordings, and we also sought to understand how the activity of the peripersonal neurons found in the constrained condition changed in the unconstrained situation by replicating constrained visual stimulation in the freely moving condition as well.

The study focused mainly on the unbiased search for peripersonal neurons (PPNs), distinguishing between neurons with bimodal visual-tactile responses, neurons with bimodal and proximal place fields (PFs), and neurons with bimodal and proximal PFs anchored to body parts (PPNs). After locating them, we studied their properties according to the space and direction of movement of visual stimuli and, finally, we tried to understand how these properties change in the unconstrained condition.

2. MATERIALS AND METHODS

2.1 Subject

The study was performed on two male macaque monkeys (*Macaca mulatta*), which will be referred to as M1 and M2 (13 kg and 15 kg respectively). Before recordings, the monkeys were habituated to sitting in a primate chair and interacting with the experimenters through a positive reinforcement training. Then, they were habituated to a visuo-tactile task (see paragraph 2.2.4 below), and after this training, the monkeys were familiarized with the NeuroEthoRoom (NER, see paragraph 2.2.1 below). Subsequently, a head fixation system and chronic multielectrode arrays were implanted, during different surgeries, under general anaesthesia and in aseptic conditions.

All experimental protocols complied with the European law on the humane care and use of laboratory animals (Directive 2010/63/EU). 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).

2.2 Apparatus and behavioural paradigm

2.2.1 NeuroEthoRoom

The environment for the neural recording was constituted by a custom-made, transparent Plexiglas enclosure called NeuroEthoRoom (NER - W x H x D: 208 x 205 x 181cm, see *Figure 2.1*). 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. In the first two phases of the experimental session, NER simply housed primate chair, where monkey received tactile and visual stimulations. Otherwise, in the last phase, thanks to a small vertical sliding openings placed on the door of the NER, the monkey was allowed to enter into the NER and freely moved into the environment. In this condition, the NER could be equipped with food and non-food enrichment items, such as a wooden structure where to climb on, some plastic handholds to move on the walls, and two or four hooks with food hanging on them.

A place like the NER makes it possible to observe a large variety of ethologically relevant behaviours in the macaques’ repertoire, which are otherwise impossible to investigate with conventional methods.

***Figure 2.1** – The NeuroEthoRoom (NER), a large Plexiglas enclosure where the sessions are recorded. On the left, a view from the outside, when the enclosure is empty; on the right, a view from the inside, while the monkey is freely moving around the enriched cage.*

2.2.2 Video monitoring system

A system of 8 video cameras were located at each corner of the NER: 4 in the upper part and 4 in the lower part. Dual Gigabit Ethernet Machine vision cameras (mvBlueCOUGAR-XD, Matrix Vision) with a resolution of 1936×1214 set to 50 frame-per-second acquisition rate were used. The cameras are equipped with a global shutter with sensor size 1/2” format (5.86 µm pixel), a manual C-Mount Lenses with 5 mm focal length (CCTV Lens, KowaOptical Products Co., Ldt) 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. They fed their signal to a computer with the dedicated software (Simi Motion - see paragraph 2.3.1 below), necessary for the video recording of the experimental sessions and for tracking the marker’s position.

2.2.3 Neural recording system

Neuronal recordings were performed by means of chronically implanted floating microelectrode arrays, each with 32 recording channels (FMA, Microprobes for Life Science, Gaithersburg, MD, USA). Probes were implanted into the ventral premotor cortex of the left hemisphere, using anatomical references, particularly the superior and inferior arcuate sulci and the central sulcus were taken as fundamental landmarks. Monkey M1 was implanted with 4 FMAs, while monkey M2 received 6 FMAs (see *Figure 2.2*).

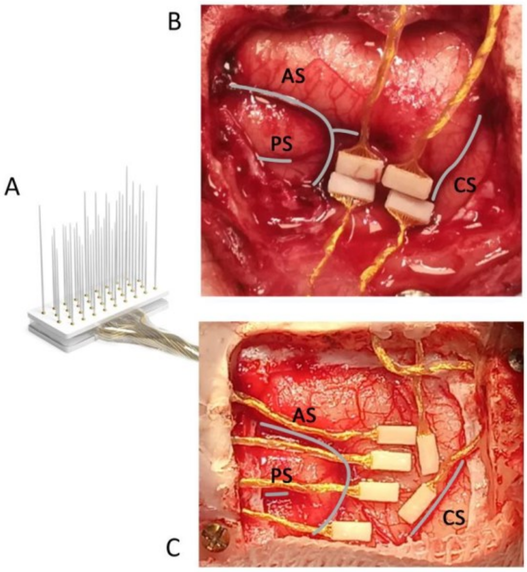


Figure 2.2 *– Floating microelectrode arrays (FMAs) implanted in macaques, M1 and M2. (A) Schematic representation of a FMA with 36-channels; (B) Image of microelectrode arrays placement in monkey M1 and, (C) monkey M2. Anatomical landmark descriptions: CS - central sulcus; AS - arcuate sulcus and PS - principal sulcus.*

During experimental session we simultaneously recorded from 128 channels with a wireless data logging system (Deuteron technologies, RatLog-128), set with a band-pass filter (2 - 7000 Hz) at a conversion rate of 32000 Hz for each channel, thus permitting to sample both local field potentials (LFPs) and single/multi-unit activity. Data were amplified, digitized, and stored locally, in a MicroSD memory card (64 GB). The device was powered by an external battery (3.7V) connected via a short cable. Once the logger device was linked to the electrode arrays into the chamber via micro-omnetics connectors, all the components were sealed with a cover on top of the chamber and switched on by magnet. The logger communicated with a computer through a transceiver with 4 BNC connectors for digital inputs and one for digital outputs, which was connected to the host computer via USB. To synchronize the recordings, a 50

Hz pulse signal of 4 V generated by a LabView-based program was sent simultaneously to the recording devices, namely, the logger and the 8 cameras.

2.2.4 Experimental protocol

During the first two phases of experiments, the head-fixed monkey was sitting in a primate chair at the centre of NER. The experimental session was divided into two different stages: the first provided for tactile stimulation whereas the second for visual stimulation.

Tactile stimulation of the facial skin was executed with a short stick whose extremity was equipped with a reflective sphere the diameter of 1 cm. In the experimental protocol the monkey's face was ideally divided into six sectors, as shown in *Figure 2.3*. Each sector, from 1 to 6, underwent 20 horizontal stimulations (10 from front to back and conversely) and 20 depth stimulation (10 from medial to lateral and conversely) while monkey's eyes were closed with small opaque goggles. Then, the same stimulations were repeated on the adjacent sector. Finally, the same protocol was applied to a surface covering two adjacent sectors. For M2 we had also upper body stimulation. At the end, were carried out two minutes of control stimulation consisting in a random movement of the stick near the monkey's face with goggles and other two minutes without goggles. During and after the tactile condition some kind of liquid reward was given to the monkey.

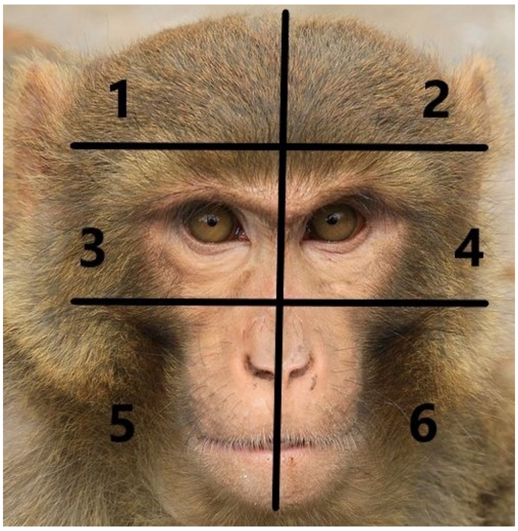


Figure 2.3 *– Tactile stimulation protocol. Sectors of monkey's face used to perform the tactile stimulation with uniform coverage of the skin surface.*

Visual stimulation was carried out with a long Plexiglas stick (length: 100 cm) with a 7 cm diameter sphere at the end for two of the included sessions, while a new stick with a 2 cm diameter sphere at the end was used for the other two sessions. The movements of the stick were traced thanks to reflective tape located below the sphere. During visual stimulation, the experimenter was outside the enclosure and performed 560 stimulations: 140 in horizontal direction, 140 in vertical direction and 280 in depth. All these stimulations were repeated for each hemifield – first contralateral hemifield (right), then ipsilateral hemifield (left) – with distances ranging from 10 cm to 70 cm and stimulation repeated every ten. The visual stimulation for each hemifield lasted approximately 20 minutes, after which a two-minute random visual stimulation started. After the stimulation of one hemifield, liquid reward was given to the monkey.

The latter phase of the experimental session, which started at the end of the visual stimulation, allowed the monkey to move freely in the NER, previously equipped. Before entering into the NER, while the monkey was sitting in the primate chair, the experimenter secured a device with the four (M2) or three (M1) retroreflective markers on the headpost. This allowed us to track the position and the orientation of the monkey's head. The tools used in this condition, in only one of the four experimental sessions considered, were: two or four hooks with food hanging on them and constantly refilled until the monkey had taken food from the hooks at least 10 times for hand, and a long, Plexiglas stick that was moved towards and away from the monkey. This was used to repropose the same kind of stimulation as the visual one but in in unconstrained condition. All this equipment were marked with a reflective tape. During this phase the monkey received food and liquid reward to enhance its exploration and movements in the environment. Overall, monkey was free to move into the NER for around 30 minutes and at the end, when the door was opened, the monkey spontaneously returned into the primate chair.

2.3 Data analysis

Our analyses were conducted following two parallel tracks: one was focused on head-fixed task and the other on a freely moving task. In both cases the animal's behaviour, the positions of monkey and stimuli and the neuronal activity were analysed by aligning the video monitoring system with the neural recording system. On the experimental session footage, we have worked with the software BORIS (Behavioural

Observation Research Interactive Software – see paragraph 2.3.2 below) and with the Simi Motion software (see paragraph 2.3.1 below), whereas for neural recording traces it was used MountainSort software first, and then customized codes in Matlab (MATLAB version R2020b - see paragraph 2.3.3 below). Our datasets include two experimental session per monkey.

2.3.1 Marker tracking

Simi Motion software (http://www.simi.com/en/) was used for data capture and analysis. The software detected and tracked all the reflective markers. This means that in the first two phases of the experimental session (tactile and visual tasks) sticks only were tracked, whereas in the last part the markers were located both on the monkey's head and stick.

By detecting the markers in time bins of 20 ms (corresponding to a rate of the video monitoring system of 50 Hz) simultaneously on eight cameras, it was possible to extract 3D-coordinates of instantaneous marker's position and then recreating their trajectory in 3D. In order to do this, after the video acquisition, we had to start automatic tracking into an interest's area (for example, face in tactile stimulation) so that Simi Motion could detects every contrasted signal identifying it as a marker. Then it was required to label all the markers retrieved (in the video) and to extract the 3D position of each stimulus. At the end of this work we obtained, for constrained tasks the 3D trajectory of the sticks, instead for freely moving condition the 3D movements of monkey and stimuli. Since in three of the four included sessions the stick reflective marker was located about 7 cm below the external surface of the sphere, here the coordinates of the stimuli were adjusted adding to all dimensions 7 cm with a maximum error of 3 cm.

2.3.2 Behavioural data analysis

BORIS (Behavioural Observation Research Interactive Software, http://www.boris.unito.it/) is a free, open-source event-logging software for video or audio coding and live observation of primates' behaviour (Friard & Gamba, 2016). We used it to create a correct link between neural data and certain behaviours. As a matter of fact, BORIS project file includes all information related to a set of observations, like the ethogram (list of behaviours), the independent variables and the subjects. The behaviour type can be considered as a point event (without duration) or a state event (with duration). It is possible to analyse each step in the video easily and with high accuracy because BORIS can be slowed down and switched on and off to a frame-by-frame mode. In this way, within the video of tactile-visual tasks, it was possible to identify each specific set of frames in which the monkey moves itself. These intervals, aligned with neural recording data, were removed in order to extract only the neural modulation induced by sticks. In freely moving condition BORIS software was used only to cut the moments in which monkeys showed some specific behaviours, like eating or sucking or touching the stick, and thus frames with steps or simple movements were maintained. In this last case the data were noisier but, given the variability of the behaviours, the presence of a regular neural modulation during the stick stimulation was considered enough to associate the two events.

2.3.3 Neural data analysis

All formal signal analyses were performed off-line with fully automated software (Mountain Sort, Chung et al., 2017), using a 3.0 standard deviation of the signal of each channel as threshold for detecting units. To distinguish single units from multi units we used the noise overlap, a parameter between 0 and 1, with units with a value below 0.15 considered as single (Chung et al., 2017). Single unit isolation was further verified using standard criteria: by visual inspection of the Inter Spike Interval distribution and the waveform shape. Furthermore, possible artefacts were removed and all the remaining waveforms that could not be classified as single units formed the multi-unit activity. In all the subsequent analyses, we considered only the single units.

2.3.3.1 Firing rate maps

For all analyses, three-dimensional volumetric firing rate maps were constructed adopting the approach used by Grieves et al., 2020 (Grieves et al., 2020). The stimulated space was divided into small cubes (voxels) of dimension 1x1x1 cm (Tactile) and 10x10x10 cm (Visual). The firing rate of a neuron in a certain voxel was calculated as the ratio between a weighted sum of the distance of the recorded spikes from the voxel centre and a weighted sum of the distance of the marker from the voxel centre (Equation 1):

$$f(x) = \frac{\sum_{i=1}^{n} g(S_i - x)}{\int_0^T g(y(t) - x)}$$

where x is the voxel centre, S_i is the position of every recorded spike, the period [0 T] is the recording session time period, y(t) is the position of the marker at time t. The weighting function g is a truncated Gaussian function, so as spikes and position data closer to a voxel's centre have more influence on that voxel's firing rate and data outside a certain threshold distance have no influence on the firing rate:

$$g(x) = e^{-0.5\left(\frac{\{x : x < d\}}{\sigma}\right)}$$

where d is the distance threshold of the truncated Gaussian, which was set to 2.5 voxels and σ is the standard deviation of the Gaussian, which was set to 1 voxel. If a voxel was stimulated less than 50 ms the voxel was considered unvisited.

2.3.3.2 Place field criteria

We defined a place field (PF) as a portion of space consisting of at least 27 consecutive voxels sharing at least one face where a neuron fires more (excitatory PF) or less (inhibitory PF) than a certain threshold relative to its mean firing rate. The volume in voxel corresponds in the visual task to a cube approximately 30x30x30 and instead in the tactile task, since a surface is being stimulated, the minimum surface defining a tactile PF is approximately 5x5. The threshold is at a percentage of the maximum firing rate of the neuron according to:

$$PF_{threshold} = \sqrt{{}^{1sec}\!/_{t_{mean}}}$$

+ 5 Spk/s, where the constant term is to reduce the variability of low-firing neurons. PFs defined as above can be visualized as convex hulls (*Figure 2.4*), and several features can be extracted, such as the position of their centroids.

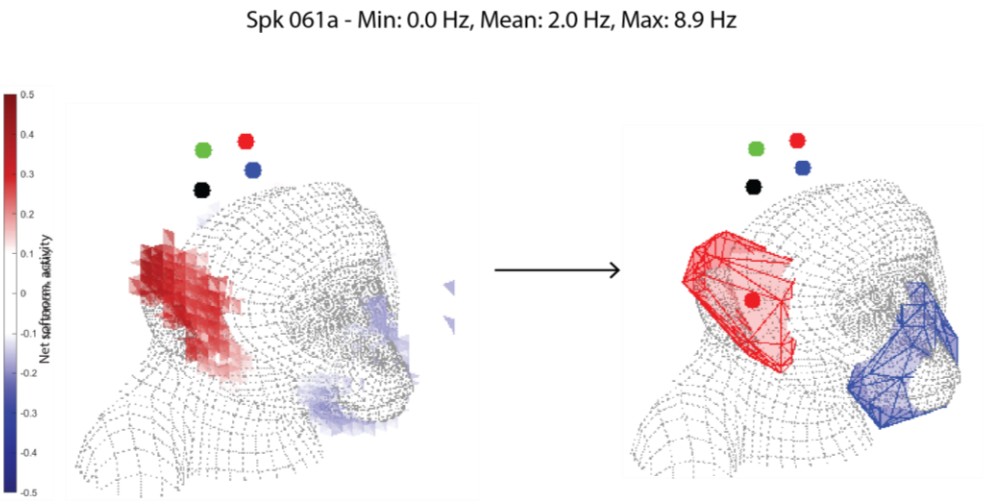


Figure 2.4 *– Illustrative image of the transition from Rate map to Place File (PF). On the left in red are the voxels in which the neuron fired more than 0.1 Spk/sec, in blue those in which it fired less than 0.1 Spk/sec. On the right the red polygon represents the excitatory PF, the blue polygon the inhibitory PF, according to the criteria established in the Materials and Methods 2.3.3.2.*

2.3.3.3 Peripersonal neurons definition

We defined a peripersonal neuron as a bimodal neuron (BN), i.e. with at least one tactile and one visual excitatory PF defined as above. The visual PFs assessed are derived from visual stimulation in the constrained condition, considering all the different PFs elicited by the 6 different directions of stick movement. In order to possess peripersonal properties, the tactile and visual PFs must be in a particular relationship: in fact, the visual PF must be in spatial proximity with the tactile one. We defined this spatial proximity by taking into account both (a) the distance between the visual and tactile voxels that form the neuron's PFs, and (b) the angle between the two vectors that connect the centre of the monkey's head with the centroids of the visual PFs and with the centroids of the tactile PFs. For criterion (a), we established a minimum distance of 15 cm between at least one voxel forming the tactile PF and one voxel forming the visual PF. For criterion (b), on the other hand, we included all PF pairs (tactile-visual) that created an angle of less than 45°. PF pairs that observe both criteria (a - b) are defined as Peripersonal and a neuron with at least one of these pairs is defined as a Peripersonal Neuron (PPN). Conversely, PF pairs that observe only criterion (a) are termed Proximal and a neuron with at least one of these pairs is termed a Proximal Neuron (PXN).

2.3.3.4 Trials definition

We defined the trials in which the visual stimulation in constrained condition is divided (Materials and Methods 2.2.4), and we created six types: Horizontal looming, Horizontal receding, Vertical looming, Vertical receding, Depth looming and Depth receding. For each of these trials the movement directions (Horizontal, Vertical and Depth) were evaluated taking, for each session, the corresponding time in which the stimulations were made. Instead, the trajectories (looming and receding) were calculated observing the first distance derivative: if it was positive the trial was a receding trial, while if it was negative the trial was a looming trial. In the freely moving condition, the stimulation trials were subdivided considering only the stimulation trajectories (looming and receding), given the lower precision of the stick movements in this phase.

3. RESULTS

3.1 General results of the constrained stimulation paradigm

Through the stimulation paradigm (Materials and Methods 2.2.4) we could systematically cover a large part of the available experimental space. In more detail, *Figure 3.1* shows clearly in which voxels the stick passed and how many times. The tactile stimulation (*Figure 3.1* **A**) covers the whole face of the monkey excluding eyes, covered by the goggles, and the areas behind the implanted recording system; t; the visual stimulation covers a large volume around the monkey's head with the stick moved along different direction and trajectories, up to 80 cm right- and leftward to the monkey on the horizontal plane (*Figure 3.1* **B**)), up to 50 cm above and below the monkey's head on the vertical plane, and about 80 cm in depth. To control for possible non-homogeneity in the coverage of the visual space, the place field criterion has been adjusted to make it proportional based on the voxel stimulation time for each modality (Materials and Methods 2.3.3.2).

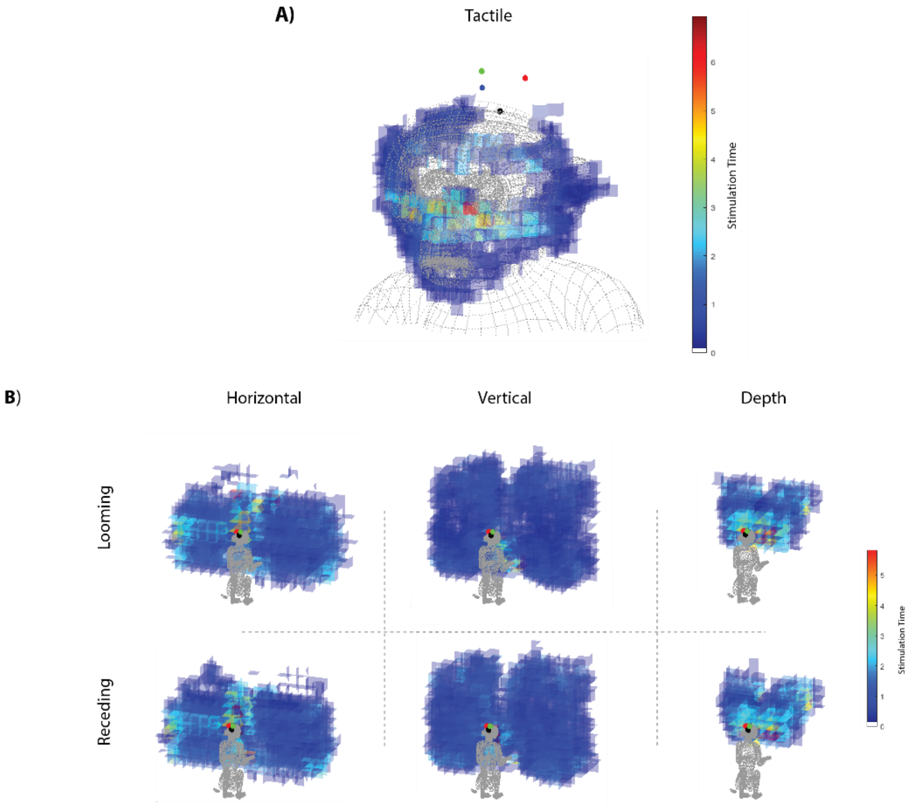


Figure 3.1 *– Calculation of the time spent by the stick in the voxels and visualization of the stimulated space.* ***A)*** *Stimulated space during the tactile stimulation;* ***B)*** *Stimulated space during the visual stimulation dividing by the six directions of movement. The color bar shows in white how voxels stimulated less than 50 ms (see Materials and Methods 2.3.3.1) are excluded from the analysis; all voxels shown in the figure were stimulated at least more than 50 ms.*

3.2 Bimodal, Proximal and Peripersonal neurons

For our analyses we used 4 experimental sessions, two per monkey, and in total we counted 127 Single Units (M1 – 83 SUA, M2 – 44 SUA). Considering only the excitatory PFs (rad polygons in *Figure 3.2 – Figure 3.3 – Figure 3.4* ***A***) obtained by analysing the different movement directions of the stick during the visual constrained stimulation (*Figure 3.2– Figure 3.3 – Figure 3.4* ***B***), only 21 (16.5%) of the recorded neurons did not show any tactile PFs or visual PF (see *Figure 3.5* ***A***). Among the neurons significantly

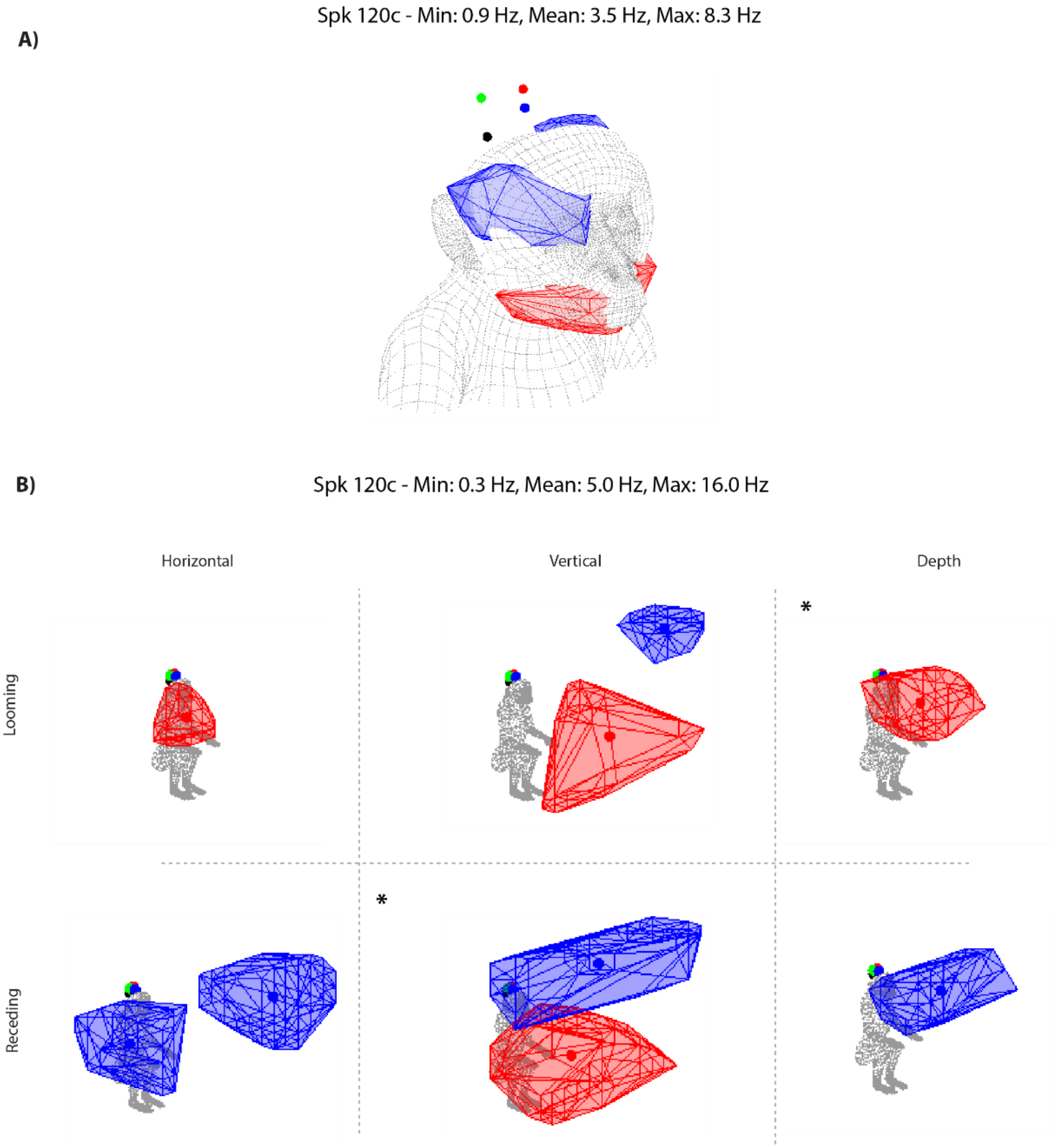


Figure 3.2 *– Example neuron with tactile and visual place fields (M2).* ***A.*** *Firing rate map revealing an excited and a suppressed body place field; Color code: red, excitatory PFs; blue, inhibitory PF.* ***B.*** *Firing rate map maps derived from the visual stimulation of the peripersonal space along different trajectories and directions. Color code as in A. Asterisks mark functional properties fitting with the operational definition of peripersonal neuron.*

modulated during tactile stimulation and showing at least 1 PF (N=94), 19 neurons had no visual field (15%); conversely, among the neurons significantly modulated by visual stimulation and showing at least 1 visual PF (N=87), 12 neurons had no tactile PF (9.4%). Importantly 75 neurons (59.0%) displayed both (at least one) tactile and visual field, and fit with the definition of Bimodal Neurons (BNs). Among these, most (N=57, 76%) exhibited only one tactile PF; in contrast, the 78.7% of the BNs presented multiple visual

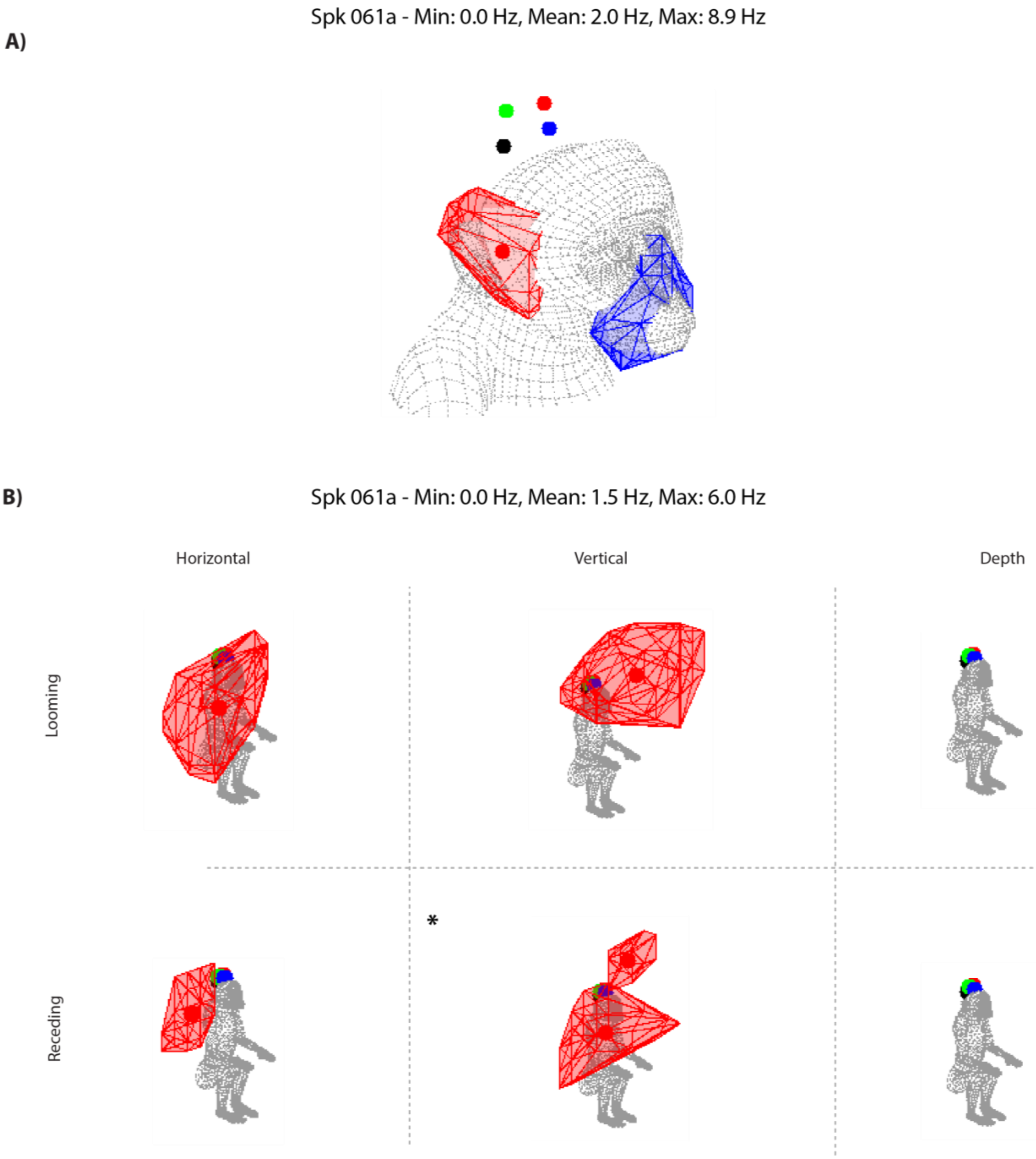


__Figure 3.3__ – Example neuron with tactile and visual place fields (M1). __A.__ Firing rate map revealing an excited and a suppressed body place field; Color code: red, excitatory PFs; blue, inhibitory PF. __B.__ Firing rate map maps derived from the visual stimulation of the peripersonal space along different trajectories and directions. Color code as in A. Asterisks mark functional properties fitting with the operational definition of peripersonal neuron.

receptive fields, from 2 to 7 (see *Figure 3.5* ***B)***. Once a criterion of spatial proximity between the tactile and visual RD is added to define Proximal neurons (PXNs, see Materials and Methods *2.3.3.3*), of the 75 BNs 35 (46.7%) fit with the definition of PXNs. Among PXNs, most (77.1%) showed only one tactile PF, and many also exhibit only one visual receptive fields (45.7%). Finally, neurons fitting with the operational

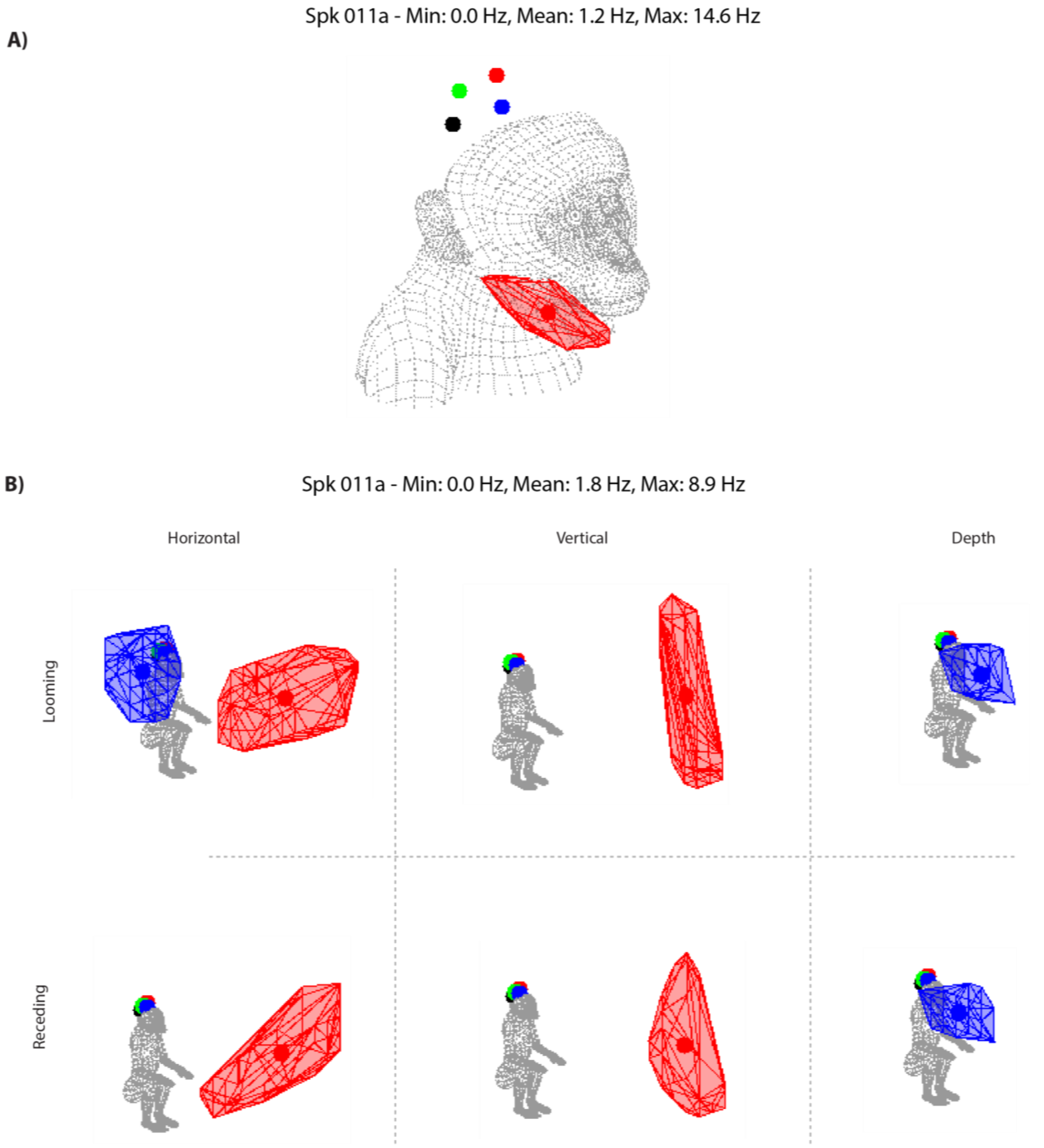
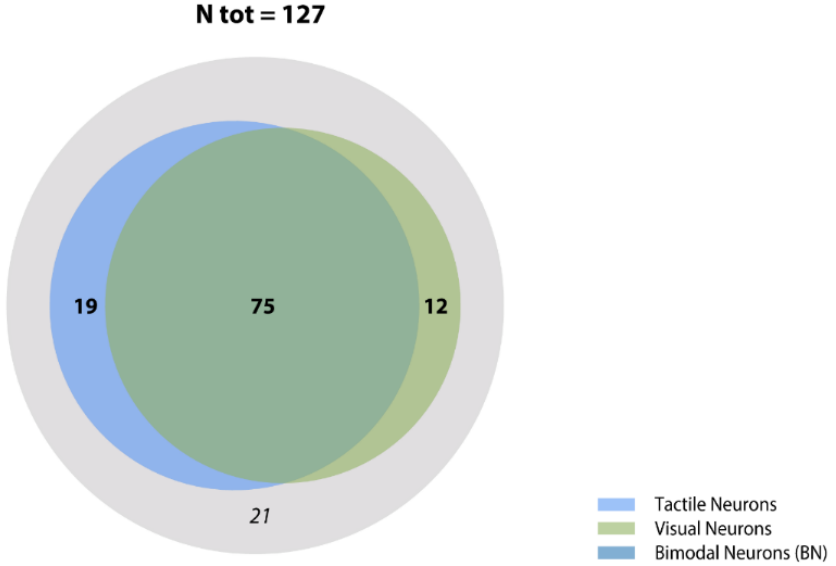


Figure 3.4 *– Example neuron with tactile and visual place fields (M2).* ***A.*** *Firing rate map revealing an excited and a suppressed body place field; Color code: red, excitatory PFs; blue, inhibitory PF.* ***B.*** *Firing rate map maps derived from the visual stimulation of the peripersonal space along different trajectories and directions. Color code as in A.*

definition of Peripersonal neurons (PPNs, n = 16, see Methods), usually showed only one tactile PF (93.7%) and 9 (56.2%) also exhibited only one visual PF (see *Figure 3.5* ***B***). The 7 PPNs (43.7%) exhibiting 2 or 4 visual PFs respond to multiple directions of movement, and were defined by Graziano and co-workers as broadly tuned (Graziano et al., 1997).

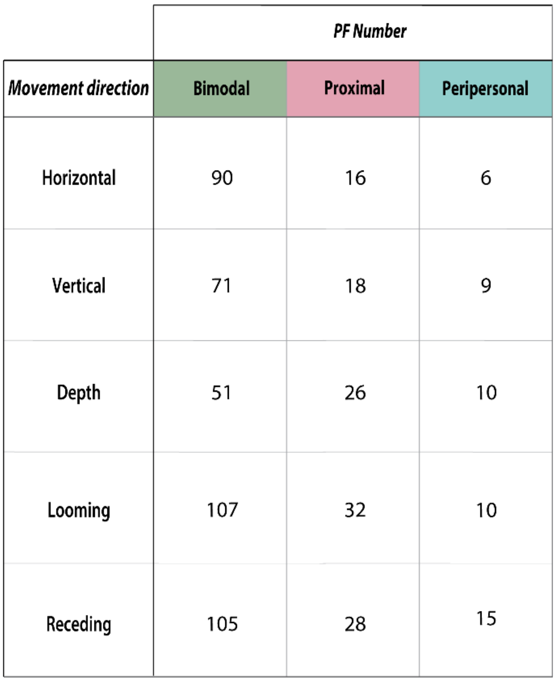
A)



B)

		1 PF	2 PF	3 PF	4 PF	5 PF	6 PF	7 PF	TOT PF	TOT Neurons
Bimodal	Tactile	57	16	2	/	/	/	/	= 95	**= 75**
	Visual	16	22	13	12	8	3	1	= 212	
Proximal	Tactile	27	7	1	/	/	/	/	= 41	**= 35**
	Visual	16	15	2	2	/	/	/	= 60	
Peripersonal	Tactile	15	1	/	/	/	/	/	= 17	**= 16**
	Visual	9	6	0	1	/	/	/	= 25	

Figure 3.5 *–* ***A.*** *Venn diagram plotting the numbers of neurons with, in grey (N=21 – 16.5%) neither tactile or visual PFs; in blue 19 neurons with only at least one tactile PF (14.9%), in green 15 neurons with only at least one visual PF (11.8%) and in teal 72 neurons (56.7%) with at least one tactile PF and at least one visual PF, that is Bimodal Neurons (BN).* ***B.*** *Table indicating the number of neurons, belonging to the three categories of Bimodal (BN), Proximal (PXN) and Peripersonal Neurons (PPN), with one or more tactile and visual PF.*



	PF Number		
Movement direction	**Bimodal**	**Proximal**	**Peripersonal**
Horizontal	90	16	6
Vertical	71	18	9
Depth	51	26	10
Looming	107	32	10
Receding	105	28	15

Table 3.1 *– Table shows the visual PF number coming from the six different movement directions (Horizontal, Vertical, Depth, Looming, Receding). The three columns represent the three different categories of Bimodal (BN), Proximal (PXN) and Peripersonal Neurons (PPS).*

Regarding the visual PFs provenances, *Table 3.1* summarizes the movement direction of the stick from which the visual PFs originated. The results we found are in line with those found by Graziano (Graziano et al., 1997): in all the categories most of the visual PFs came from the movement in the fronto-parallel plane (Horizonal and Vertical), while the depth stimulation always elicited less than half of the visual PFs in all the categories (24.0% - BN, 43.3% - PXN, 40% - PPN). However, if the directions in the fronto-parallel plane are considered separately, no constant preference can be identified that is maintained in the three groups. Also, for inward and outward direction, in our results approaching and receding stimulations produced different results based on different categories: in general, the percentages seem to be similar for Bimodal and Proximal but in the Peripersonal the 60% of the visual PFs responded to the receding stimulation.

Finally, concerning the PPNs we found 16 neurons (M1 N=9, M2 N=7) with properties compliant with the inclusion criterions (see Materials and Methods 2.3.3.3). Due to the sparsity of the sample, we cannot perform statistical analyses to understand whether there is a significant trend ipsilateral or contralateral to the implanted hemisphere, but we can describe a trend by observing the distribution of the centroids of the visual and tactile PFs. *Figure 3.6* shows that most of the visual centroids result in the space contralateral to the implanted hemisphere (Visual – N=17, 68%), while the tactile centroids are distributed more in the centre than the periphery without a clear preference for contralateral or ipsilateral space (despite a slight majority of ipsilateral centroids – 52.9%).

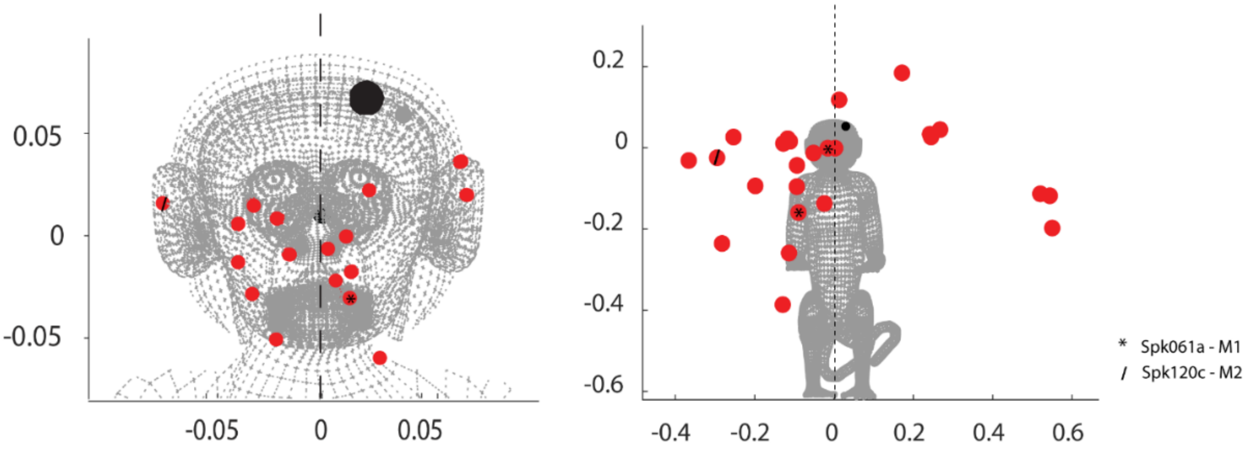


Figure 3.6 *– Peripersonal PF centroids distribution: on the right tactile PF centroids distribution (red dots), and on the left visual PF centroids distribution (red dots). Black dots represent the recorded hemisphere. The zero point (dashed line) divide the ipsilateral from the contralateral space. The dots signed with the asterisk (*) represent the neuron PFs depicted in Figure 3.3., instead the dots signed with the bar (/) represent the neuron PFs depicted in Figure 3.4. To visualize approximately the head position and shape of the monkey's head during the task, we used a monkey avatar designed by DAZ (https://www.daz3d.com/).*

3.3 Peripersonal neurons (PPNs) coding in freely moving condition

When the monkey moves freely into the cage it can react to the stick visual stimulation. Therefore, we decided to evaluate the type of reaction in response to the movement direction of the stick. The analyses in the freely moving condition were carried out only by submitting all the coordinates to a roto-translation in order to reset the monkey's head in fixed coordinates, as if it was in the chair. In this way, we calculated the angle between the fixated vector of the head and the stick vector – taking the stick coordinates x-y at the moment t0 and at the moment t1. All these angles were divided in two types of paths: those in which the stick approached the monkey (looming) and those in which the stick moved away from the monkey (receding). To identify these movements of the stick (looming or receding), we calculated the first distance derivative, if it was positive the trial was a receding trial, while if it was negative the trial was a looming trial (see Materials and Methods 2.3.3.4). Then, we mediated between all the angles of the looming trials, and we plotted it with its standard error (*Figure 3.7*). *Figure 3.7* shows that on average the stick and the monkey tend to have a right-angle conformation, i.e. the stick tends to point laterally to the monkey; but

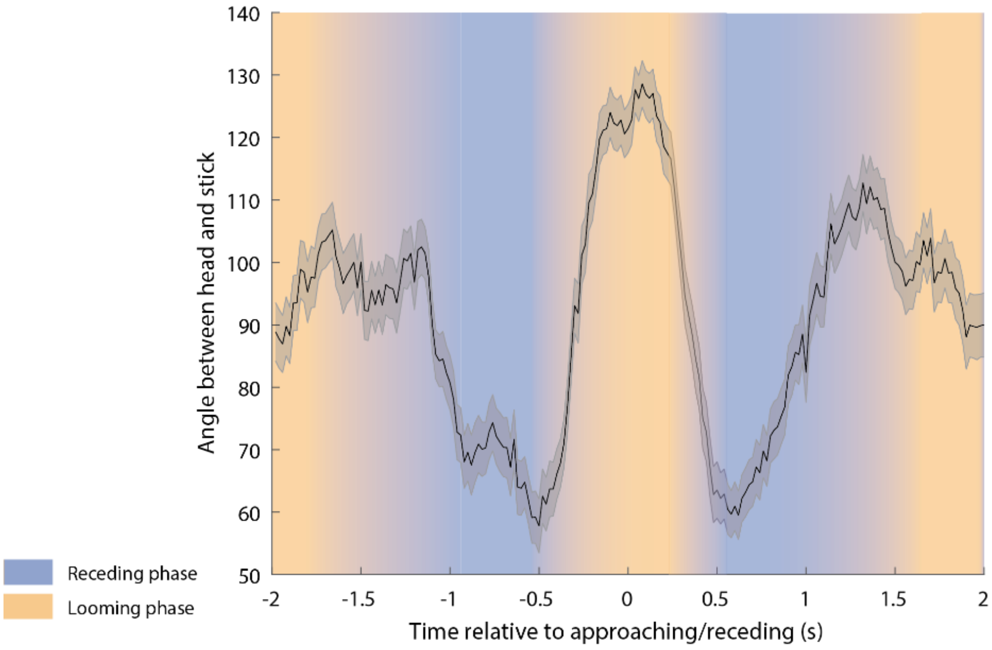


***Figure 3.7** – Behavioral analysis: average trend of the angles calculated between the stick and the monkey's head in the unconstrained condition, in the looming phases (yellow bands) and in the receding phases (blue bands).*

when the stick gets closer, the animal tends to move away by turning its head, thus increasing the angle, whereas when the stick moves away, the monkey tends to turn towards it, decreasing the angle.

Next, we wanted to investigate the visual PFs of the peripersonal neurons classified in the constrained condition during the freely moving condition, where the spatial coverage is unavoidably limited: in fact, voxels are stimulated less evenly and the stick rarely can get as close to the monkey as in the chair condition (see *Figure 3.8*). Therefore, considering the definition of PPN and the type of visual stimulation carried out in the freely moving condition, it turned out to be impossible so far to identify peripersonal PFs in the unconstrained condition using a spatial point of view; in fact, we defined a neuron as peripersonal if it satisfied, at least but not only, the proximity criterion (Material and Methods 2.3.3.3) and the stimulation in the unconstrained condition always remain further than 15 cm (see *Figure 3.8* ***B***).

Therefore, to verify possible similarities or differences in the visual PF of PPNs between the constrained and freely moving conditions, we tested the hypothesis that the sector of space constituting the PF in the constrained movement condition retains its relevance in the free movement condition; to do so, we selected the block of trials (as defined in Materials and Methods 2. 3.3.4) from which the visual PF in the constrained motion condition emerged (e.g., Looming Depth) and further divided them between those

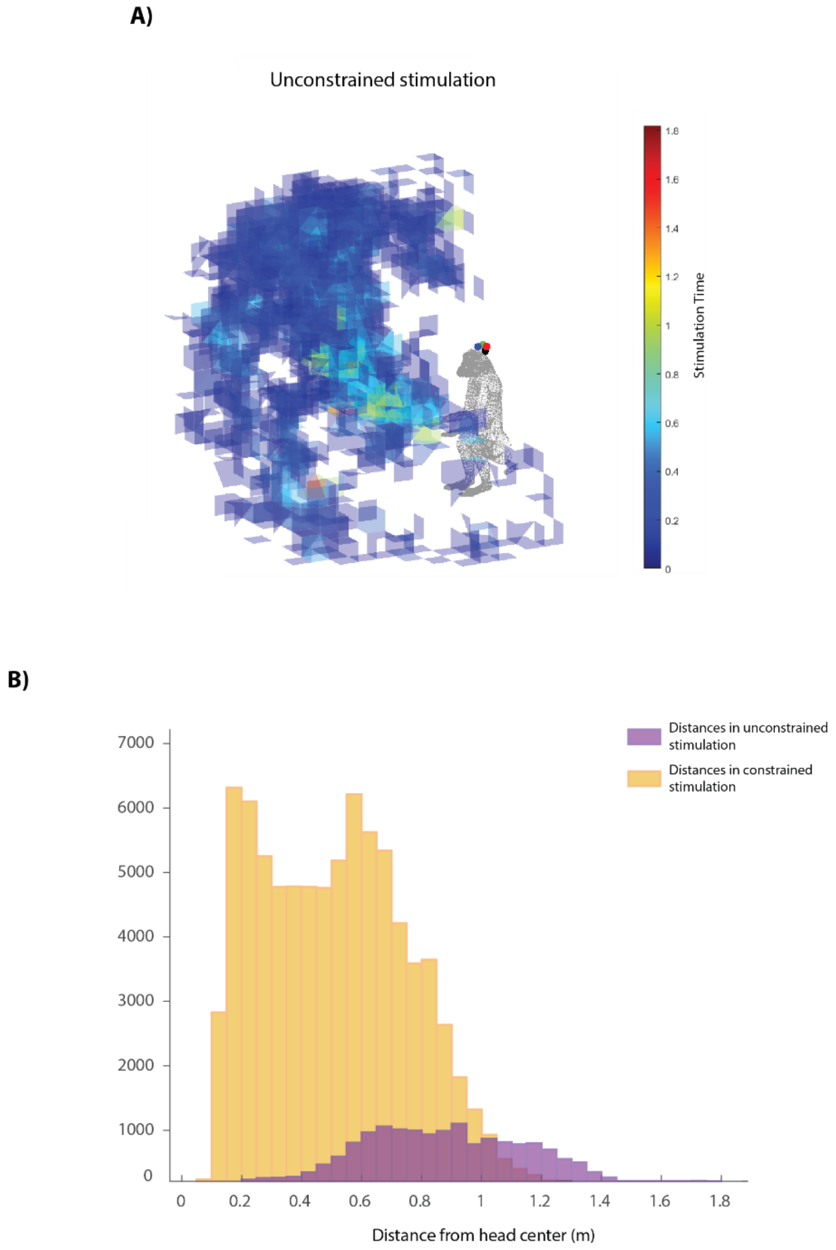


Figure 3.8 *– Characteristics of the stimulation in the feely moving condition.* ***A)*** *Real time spent by the stick in the voxels and visualization of the stimulated space during the freely moving stimulation. In all graphs the color bar tends towards blue in the less stimulated voxels and tends towards red in the more stimulated ones;* ***B)*** *Distance from the center of the monkey's head (zero point) in constrained (yellow bars) and unconstrained visual stimulation (violet bars).*

that crossed the space included in the visual PFs (In trials) and those that passed outside (Out trials). Trajectories and location were selected in the unconstrained condition based on the criterion obtained from the chair condition, and the neuron firing rate compared between the two data set (see *Figure 3.9*). The example plots shown in *Figure 3.9* show that there are some PPNs that maintain a similar firing pattern in the chair condition and in the freely moving condition, if we consider the space defining the PF in the constrained condition (**A**); on the other hand, other neurons (**B**) show an oscillatory pattern influenced by the direction of stimulus movement inside the PF in the constrained condition, while in the freely moving condition the neuronal modulation is evident only outside the PF.

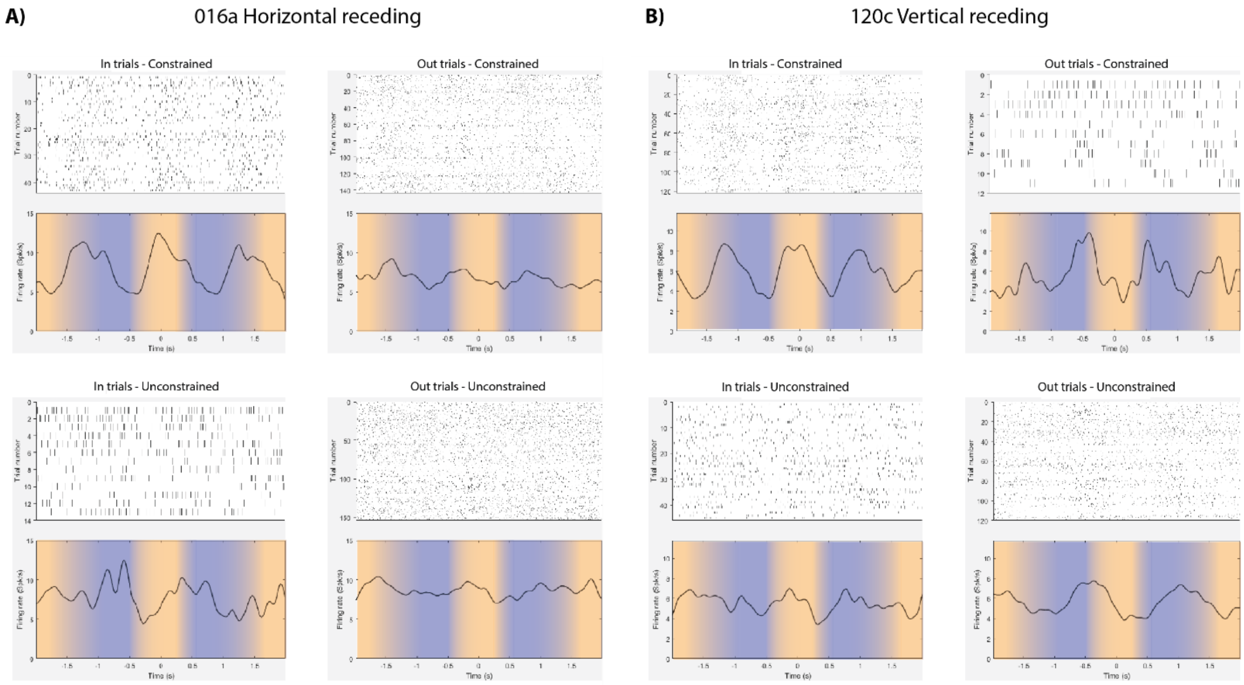


Figure 3.9 – *Raster plot of three neurons (M2): comparison between the firing of neurons in the constrained and unconstrained condition inside and outside the PF-defining space in the chair condition. In each panel, the first line represents the firing of the neuron in the constrained condition, while the second represents the firing in the unconstrained condition. As in Figure 3.2.1, the yellow bands represent the looming phases and the blue bands the receding phases. The zero point, for In trials, is at the entrance of the stick into one of the neuron's PFs, while in Out trials the zero point corresponds to the point where the first derivative of the distance is zero (approximately the point of maximum velocity).*

4. DISCUSSION AND CONCLUSION

The present study shows a new way of looking at and thinking about research on peripersonal neurons. Classically, in the early studies of the 1990s, the approach typically used involved the acute recording with single electrodes of individual neural units during stimulations to which the animal was passively exposed. These pioneering studies demonstrated the existence of specific premotor neurons responding to visual stimulation in the peripersonal space, suggesting they may be the result of the action of potential motor plans triggered by visual stimuli close to the head-restrained monkey's body.

Classical studies mostly involved single electrode recordings in which neurons were selected for further studies based on online testing performed by the experimenter, leaving unclear the relative impact of individual neurons on the overall properties and functions of the investigated area, and limiting the interpretation of the results to the highly constrained and artificial experimental setting. Instead, in the present study we used an unbiased chronic multi-electrode neural recording approach, in which no pre-selection is possible. As a consequence, the stimulation during the recording phase is fixed and identical for all the recorded units, as it cannot be subject to the changes suggested by the neuronal response. Moreover, having information about many neurons simultaneously tested in a standard way, allows to explore neural properties that classically would have been impossible to observe. With our approach, despite methodological differences, the percentages of neurons found and exhibiting specific properties are within the ranges known in the literature for constrained conditions (*Table 4.1*).

	Fogassi et al. 1996	*Graziano et al. 1997*	*Our study*
Somatosensory neurons	25.6%	18.9%	15%
Visual neurons	12.2%	3.6%	9.4%
Bimodal neurons	48.8%	32.8%	59%
Unresponsive neurons	13.3%	18%	16.5%

***Table 4.1** – Summary table useful to compare the results obtained from two classic studies, the first by Fogassi et al. from 1996 (Fogassi et al., 1996) and the second by Graziano et al. from 1997 (Graziano et al., 1997), and the present study. As the motor response analysis was only performed by Graziano and co-workers, the categories reported also include neurons with possible motor properties.*

The way in which we observed the data was initially closely related to the space; this depended on the previous information offered in the literature, which defined the peripersonal neuron as intrinsically linked to the animal's peripersonal space. However, rather than stimulating only peripersonal space, in order to also include in the analysis the way in which this space is modified, expanded and modelled as the properties of the stimulus change (e.g. direction of movement), we used two expedients: firstly, the stimulation was kept as general as possible, encompassing space around the animal of up to more than one metre; furthermore, the analysis used, taken, with appropriate adjustments, from Grieves's study on place cells (Grieves et al., 2020), allowed an objective observation of the neuron's response for each stimulated voxel without distinction between near and far space. This brought out data that more accurately investigate what had already been pointed out in the literature (Graziano et al., 1997), i.e. there are neurons that not only respond to visual stimulation within the peripersonal space but also further away. The results produced by our experimental paradigm on this subject have not been reported in the current study (only extra-personal fields are shown in the *Figure 3.2* **B***; Figure 3.3* **B***; Figure 3.4* **B**), but they open the way to multiple questions and subsequent interpretations that could also help us to clarify the role of the classically peripersonal neurons we have described here.

The stimulation of different space field and directions is of particular importance in the present study where it clearly emerges that the spatial tuning of a neuron (place field) changes significantly as the direction of movement of the stimulus varies, thus creating for a single neuron multiple PFs that change in size allowing or not to classify a neuron as peripersonal depending on the spatial and dynamic properties of the stimulus. In fact, of the 75 visuo-tactile neurons described, most have very different PFs for several directions of movement and only a few of these fulfil the inclusion criteria chosen to classify peripersonal neurons. These criteria were chosen in order to compare as cleanly as possible with the literature where peripersonal neurons are defined as being located in continuity with the tactile receptive field. The picture that emerges from our observations is very complex, with neurons that can be considered peripersonal, yet retain extra-personal sensitivities in specific conditions. Our study thus shows that exclusively near-space selective neurons, as had been classically described (Fogassi et al., 1996; Gentilucci et al., 1988; Graziano et al., 1997; Rizzolatti et al., 1981), have not been found, but rather that peripersonal spatial preference results only in combination with other stimulus characteristics, such as the movement direction.

Another interesting finding is that, with our approach, many recorded neurons exhibit multiple visual receptive fields, often depending on different directions of the moving stimulus. This finding may be due to the fact that a single neuron could have additional tactile receptive fields in other, non-investigated, part of the body (Gentilucci et al., 1988; Maranesi et al., 2012; Rizzolatti et al., 1981), and some of the visual PFs could be linked to them; this hypothesis could be disambiguated by the observation of tactile stimulation in the upper body. Another explanation might simply have to do with the fact that the neuron represents multiple zones of space based on other qualities that characterise the stimulus or the subject's internal state.

In addition to the direction of movement, it is already known that other factors modify the visual PF of the PPNs, including the practical knowledge of one’s motor abilities (Iriki et al., 1996) and the possibility of interact with the stimulus (Sambo et al., 2012). From the very first studies, the fact that neurons with visuo-tactile properties were present in praxis-related areas was not considered a coincidence (Rizzolatti et al., 1981, p. 19), and the idea that the perceptive properties of the peripersonal neurons were involved in some way in the coordination of actions has always been proposed. For this reason, the possibility of recording in an unconstrained condition, thanks to the implementation of wireless technologies, opens up the way to many new discoveries that aim to understand how neurons function in ecological contexts. In fact, this is the only way to make a real contribution to the philosophical and empirical debate on the relationship between perceptual experience and action. So far, what emerges from the literature leads us to think that there may not be a strict functional distinction between perception and action in the one’s surrounding environment, i.e., it suggests that the visual response may actually be a preparation for action and that the visual PF coincides with the representational space of potential action. Iriki and co-workers in 1996 (Iriki et al., 1996) showed for the first time how the use of tools causes an expansion of the visual PF of neurons with peripersonal properties, which they claim is due to the expansion of the body schema. This means that the increase in motor capacity leads to a change in the perceptual processing of objects next to the tool; that is, after the tool is used, far objects are processed as they were into the peripersonal space. To confirm this, another study was conducted by Farnè et al. (Farnè & Làdavas, 2000), in which it was shown that even in humans the use of tools causes an expansion of the peri-hand visual space. In sum, the expansion of the visual PF links the visual perceptual component to the subject's possibility of action. Another study that showed that the size of the visual response of peripersonal neurons changes according to the subject's action possibilities is that of Bassolino et al. (Bassolino et al., 2015); this study showed that the immobilisation of the right arm of healthy human subjects for 10 hours results in a contraction of the peripersonal space, calculated as the distance at which an auditory stimulus is able to influence the processing of a tactile stimulus (Bassolino et al., 2015). Also in humans, it has been shown that the amplitude of the hand blink reflex (a measure classically used to measure PPS) increases as the stimulus approaches, but only when there is no barrier between the subject and the stimulus (Sambo et al., 2012). The action space and the possibility of acting on the stimulus thus seem to be determining factors for the definition of the visual PF of the PPNs and we tried to verify this also in our study by giving the monkey the possibility of moving freely in space. In this new condition, the first interesting fact we showed concerns its behaviour: the fact that the animal reacts to stimulation with active behaviour suggests, first of all, that the stimulus is not ignored and also that the stimulus is perceived as aversive, since it induces an avoidance reaction. It would also be interesting to understand how and whether with positive valence stimuli (e.g. hooks with food hanging) the behaviour and thus the visual PF changes.

The possibility of recording wirelessly while the monkey is free to move has obvious advantages but also considerable complexities: in retrospect, it was indeed shown that the marker never got close enough to the monkey to mimic the stimulation in the peripersonal space done in chair (*Figure 3.8*). Considering this limitation, the only thing that can be brought out from this study regarding the constrained-unconstrained condition comparison is that the space modulating neuron in chair does not seem to retain its relevance in the freely movement condition. The datum thus described is weak and needs further verification, but if this initial observation were to be confirmed, the implications could be important: it could in fact mean that passing from a constrained condition to an unconstrained one results in a re-mapping of the space or a contraction of the PFs. This eventuality may have multiple explanations but to present them in this discussion is premature; further studies are therefore awaited to clarify this point.

Another point to be assessed concerns the link between the neuronal response (observed in *Figure 3.9*) and the trajectory of approach/retreat of the stick. Indeed, to sustain a cause-and-effect relationship verifications are required, which were not carried out in this study. Above all, it would be important to verify that the neuron's response is not related to head movement rather than stimulus movement. Graziano et al. in their 1997 study described bimodal neurons with head-related receptive fields that respond to head rotation (Graziano et al., 1997). In our case, it was described how in the cage stimulation phase the animal has head rotation responses precisely in response to the trajectories of the stimulus. This means that it is not possible to disambiguate what causes the oscillatory activation of the neuron, whether the movement of the stick or that of the head. This verification can be done by considering to replicate the experimental condition used by Graziano and colleagues: i.e., evaluating the activation of the neuron during the rotation movement of the head in the chair phase while leaving the animal with its head free.

In addition to this, it would be important for future studies to break free from the expository limitation of dividing neurons into categories; this was indeed useful in this case to have a comparison with the literature, but it is necessary for future studies to open the gaze to a different complexity that considers multiple neuronal properties, peripersonal and extra-personal, as a whole, to also check which of these properties is or is not maintained in the free movement condition. According to the framework proposed by Bufacchi and Iannetti (Bufacchi & Iannetti, 2018), the concept of peripersonal space can be reconsidered as a motor field that changes its properties depending on the potential actions triggered by a stimulus in a given context. Subsequent investigations could try to clarify which path is best pursued by making the best use of our tools. Mainly, I consider it of great interest to try to settle the debate regarding the interpretation of the visual response of the bimodal neurons by having the opportunity for the first time to test how the free movement of the monkey affects visual PFs.

REFERENCES

Bassolino, M., Finisguerra, A., Canzoneri, E., Serino, A., & Pozzo, T. (2015). Dissociating effect of upper limb non-use and overuse on space and body representations. *Neuropsychologia*, *70*, 385–392. https://doi.org/10.1016/j.neuropsychologia.2014.11.028

Belmalih, A., Borra, E., Contini, M., Gerbella, M., Rozzi, S., & Luppino, G. (2009). Multimodal architectonic subdivision of the rostral part (area F5) of the macaque ventral premotor cortex. *Journal of Comparative Neurology*, *512*(2), 183–217. https://doi.org/10.1002/cne.21892

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

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. *Journal of Neuroscience*, *34*(11), 4108–4119. https://doi.org/10.1523/JNEUROSCI.4187-13.2014

Bufacchi, R. J., & Iannetti, G. D. (2018). An Action Field Theory of Peripersonal Space. *Trends in Cognitive Sciences*, *22*(12), 1076–1090. https://doi.org/10.1016/j.tics.2018.09.004

Caggiano, V., Fogassi, L., Rizzolatti, G., Thier, P., & Casile, A. (2009). Mirror Neurons Differentially Encode the Peripersonal and Extrapersonal Space of Monkeys. *Science*, *324*(5925), 403–406. https://doi.org/10.1126/science.1166818

Cisek, P., & Kalaska, J. F. (2010). Neural Mechanisms for Interacting with a World Full of Action Choices. *Annual Review of Neuroscience*, *33*(1), 269–298. https://doi.org/10.1146/annurev.neuro.051508.135409

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., & Goldberg, M. E. (1993). Ventral intraparietal area of the macaque: Anatomic location and visual response properties. *Journal of Neurophysiology*, *69*(3), 902–914. https://doi.org/10.1152/jn.1993.69.3.902

Colombo, M., & Seriès, P. (2012). Bayes in the Brain—On Bayesian Modelling in Neuroscience. *The British Journal for the Philosophy of Science*, *63*(3), 697–723. https://doi.org/10.1093/bjps/axr043

Cooke, D. F., & Graziano, M. S. A. (2004). Super-Flinchers and Nerves of Steel: Defensive Movements Altered by Chemical Manipulation of a Cortical Motor Area. *Neuron*, *43*(4), 585–593. https://doi.org/10.1016/j.neuron.2004.07.029

Courellis, H. S., Nummela, S. U., Metke, M., Diehl, G. W., Bussell, R., Cauwenberghs, G., & Miller, C. T. (2019). Spatial encoding in primate hippocampus during free navigation. *PLOS Biology*, *17*(12), e3000546. https://doi.org/10.1371/journal.pbio.3000546

Dijkerman, H. C., & Medendorp, W. P. (2021). Visuo-tactile predictive mechanisms of peripersonal space. In *The world at our fingertips: A multidisciplinary exploration of peripersonal space* (pp. 81–100). Oxford University Press. https://doi.org/10.1093/oso/9780198851738.003.0005

Dosey, M. A., & Meisels, M. (1969). Personal space and self-protection. *Journal of Personality and Social Psychology*, *11*, 93–97. https://doi.org/10.1037/h0027040

Farnè, A., & Làdavas, E. (2000). Dynamic size-change of hand peripersonal space following tool use. *NeuroReport*, *11*(8), 1645.

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Matelli, M., & Rizzolatti, G. (1996). 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

FULTON, J. F. (1935). A NOTE ON THE DEFINITION OF THE “MOTOR” AND “PREMOTOR” AREAS. *Brain*, *58*(2), 311–316. https://doi.org/10.1093/brain/58.2.311

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

Goodale, M. A., & Milner, A. D. (1992). Separate visual pathways for perception and action. *Trends in Neurosciences*, *15*(1), 20–25. https://doi.org/10.1016/0166-2236(92)90344-8

Graziano, M. S. A., & 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. A., & Gross, C. G. (1993). A bimodal map of space: Somatosensory receptive fields in the macaque putamen with corresponding visual receptive fields. *Experimental Brain Research*, *97*(1), 96–109. https://doi.org/10.1007/BF00228820

Graziano, M. S. A., Hu, X. T., & Gross, C. G. (1997). Visuospatial Properties of Ventral Premotor Cortex. *Journal of Neurophysiology*, *77*(5), 2268–2292. https://doi.org/10.1152/jn.1997.77.5.2268

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

Graziano, M. S. A., Taylor, C. S. R., & 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

Graziano, M. S. A., Yap, G. S., & Gross, C. G. (1994). Coding of Visual Space by Premotor Neurons. *Science*, *266*(5187), 1054–1057. https://doi.org/10.1126/science.7973661

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), Article 1. https://doi.org/10.1038/s41467-020-14611-7

Hediger, H. (1955). *Studies of the psychology and behavior of captive animals in zoos and circuses* (pp. vii, 166). Criterion Books, Inc.

Hyvärinen, J., & Poranen, A. (1974). Function of the Parietal Associative Area 7 as Revealed from Cellular Discharges in Alert Monkeys. *Brain*, *97*(4), 673–692. https://doi.org/10.1093/brain/97.4.673

Iriki, A., Tanaka, M., & Iwamura, Y. (1996). Coding of modified body schema during tool use by macaque postcentral neurones. *Neuroreport*, *7*(14), 2325–2330. https://doi.org/10.1097/00001756-199610020-00010

Jackson, A., Mavoori, J., & Fetz, 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

Kandula, M., Hofman, D., & Dijkerman, H. C. (2015). Visuo-tactile interactions are dependent on the predictive value of the visual stimulus. *Neuropsychologia*, *70*, 358–366. https://doi.org/10.1016/j.neuropsychologia.2014.12.008

Kurata, K. (2018). Hierarchical Organization Within the Ventral Premotor Cortex of the Macaque Monkey. *Neuroscience*, *382*, 127–143. https://doi.org/10.1016/j.neuroscience.2018.04.033

Leinonen, L., Hyvärinen, J., Nyman, G., & Linnankoski, I. (1979). I. Functional properties of neurons in lateral part of associative area 7 in awake monkeys. *Experimental Brain Research*, *34*(2), 299–320. https://doi.org/10.1007/BF00235675

Ludvig, N., Tang, H. M., Gohil, B. C., & Botero, J. M. (2004). Detecting location-specific neuronal firing rate increases in the hippocampus of freely-moving monkeys. *Brain Research*, *1014*(1), 97–109. https://doi.org/10.1016/j.brainres.2004.03.071

Maranesi, M., Rodà, F., Bonini, L., Rozzi, S., Ferrari, P. F., Fogassi, L., & Coudé, G. (2012). Anatomo-functional organization of the ventral primary motor and premotor cortex in the macaque monkey. *European Journal of Neuroscience*, *36*(10), 3376–3387. https://doi.org/10.1111/j.1460-9568.2012.08252.x

Matelli, M., & Luppino, G. (2001). Parietofrontal Circuits for Action and Space Perception in the Macaque Monkey. *NeuroImage*, *14*(1), S27–S32. https://doi.org/10.1006/nimg.2001.0835

Matelli, M., Luppino, G., & Rizzolatti, G. (1985). *Patterns of cytochrome oxidase activity in the frontal agranular cortex of the macaque monkey.* 18(2), 125–136. https://doi.org/10.1016/0166-4328(85)90068-3

Matelli, M., Luppino, G., & Rizzolatti, G. (1991). Architecture of superior and mesial area 6 and the adjacent cingulate cortex in the macaque monkey. *Journal of Comparative Neurology*, *311*(4), 445–462. https://doi.org/10.1002/cne.903110402

McMullin, E. (2002). The Origins of the Field Concept in Physics. *Physics in Perspective*, *4*(1), 13–39. https://doi.org/10.1007/s00016-002-8357-5

Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., Weiskopf, N., Seymour, B., Dolan, R. J., & Frith, C. D. (2007). When Fear Is Near: Threat Imminence Elicits Prefrontal-Periaqueductal Gray Shifts in Humans. *Science*, *317*(5841), 1079–1083. https://doi.org/10.1126/science.1144298

Murata, A., Fadiga, L., Fogassi, L., Gallese, V., Raos, V., & Rizzolatti, G. (1997). Object Representation in the Ventral Premotor Cortex (Area F5) of the Monkey. *Journal of Neurophysiology*, *78*(4), 2226–2230. https://doi.org/10.1152/jn.1997.78.4.2226

Noel, J.-P., Bertoni, T., & Serino, A. (2021). Peri-personal space as an interface for self-environment interaction: A critical evaluation and look ahead. In *The world at our fingertips: A multidisciplinary exploration of peripersonal space* (pp. 17–46). Oxford University Press. https://doi.org/10.1093/oso/9780198851738.003.0002

Nummela, S. U., Jovanovic, V., Mothe, L. de la, & Miller, C. T. (2017). Social Context-Dependent Activity in Marmoset Frontal Cortex Populations during Natural Conversations. *Journal of Neuroscience*, *37*(29), 7036–7047. https://doi.org/10.1523/JNEUROSCI.0702-17.2017

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

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

Rizzolatti, G., Scandolara, C., Matelli, M., & Gentilucci, M. (1981). Afferent properties of periarcuate neurons in macaque monkeys. II. Visual responses. *Behavioural Brain Research*, *2*(2), 147–163. https://doi.org/10.1016/0166-4328(81)90053-X

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

Ruggiero, G., Frassinetti, F., Coello, Y., Rapuano, M., di Cola, A. S., & Iachini, T. (2017). The effect of facial expressions on peripersonal and interpersonal spaces. *Psychological Research*, *81*(6), 1232–1240. https://doi.org/10.1007/s00426-016-0806-x

Russell, W. M. S., & Burch, R. L. (1959). *The principles of humane experimental technique.*

Sambo, C. F., Forster, B., Williams, S. C., & Iannetti, G. D. (2012). To blink or not to blink: Fine cognitive tuning of the defensive peripersonal space. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *32*(37), 12921–12927. https://doi.org/10.1523/JNEUROSCI.0607-12.2012

Sambo, C. F., & Iannetti, G. D. (2013). Better Safe Than Sorry? The Safety Margin Surrounding the Body Is Increased by Anxiety. *Journal of Neuroscience*, *33*(35), 14225–14230. https://doi.org/10.1523/JNEUROSCI.0706-13.2013

Schieber, M. H. (2001). Constraints on Somatotopic Organization in the Primary Motor Cortex. *Journal of Neurophysiology*, *86*(5), 2125–2143. https://doi.org/10.1152/jn.2001.86.5.2125

Schutter, D. J. L. G., Enter, D., & Hoppenbrouwers, S. S. (2009). High-frequency repetitive transcranial magnetic stimulation to the cerebellum and implicit processing of happy facial expressions. *Journal of Psychiatry and Neuroscience*, *34*(1), 60–65.

Straka, Z., Noel, J.-P., & Hoffmann, M. (2022). A normative model of peripersonal space encoding as performing impact prediction. *PLOS Computational Biology*, *18*(9), e1010464. https://doi.org/10.1371/journal.pcbi.1010464

Vieira, J. B., Pierzchajlo, S. R., & Mitchell, D. G. V. (2020). Neural correlates of social and non-social personal space intrusions: Role of defensive and peripersonal space systems in interpersonal distance regulation. *Social Neuroscience*, *15*(1), 36–51. https://doi.org/10.1080/17470919.2019.1626763

Vignemont, F. de, Vignemont, édérique de V. de, Serino, A., Wong, H. Y., & Farnè, A. (2020). *The World at Our Fingertips: A Multidisciplinary Exploration of Peripersonal Space*. Oxford University Press.

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

Zald, D. H. (2003). The human amygdala and the emotional evaluation of sensory stimuli. *Brain Research Reviews*, *41*(1), 88–123. https://doi.org/10.1016/S0165-0173(02)00248-5