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Anatomo-functional organization of the lateral part of primary motor and premotor cortices of the macaque monkey

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1. INTRODUCTION

1.1. The cortical control of movement: an historical account

From the ninetieth century, scientists believed that cerebellum and brain stem were the only parts of the nervous system directly involved in the control of movement. Cerebral cortex was considered as a whole and mainly involved in high mental processes such as perception, volition and intelligence, but not in “movement”, which was considered a mere executive automatic function.

The first scientist that suggested an involvement of the cerebral cortex in movement was John Hughlings Jackson (1835-1911), who collected a lot of information from clinical observations on pathology affecting the central nervous system such as epilepsy and hemiplegia. He described “epileptiform convulsions” without loss of consciousness, and characterized by a “march” that would later become known as “*Jacksonian march*”: the convulsions affected body parts in a somatotopically organized fashion, going from the hand, through the arm to the face and involving only unilateral parts of the body unless in cases in which a more severe form affected the whole body. Face and fingers, furthermore, were found to be the body parts more affected by these attacks. The observations on patients affected by hemiplegia brought him to notice that unilateral paralysis was not associated with sensory deficits, suggesting a distinction in coding sensory and motor information, and that severe paralysis could affect limbs but not the trunk. From these evidence, Jackson hypothesized that the cerebral cortex was somatotopically organized with different degrees of representations of the different body parts. He proposed that hand, face and foot had the greater representation in the brain. Furthermore he suggested that motor control is characterized by different levels of organization: the more automatic and primitive the movement is, the more likely it is controlled by the corpus striatum and the cerebellum, while planning of complex motor behaviour would involve the cerebral cortex.

The experimental proof of Jackson’s intuitions came from Gustav Theodore Fritsch and Eduard Hitting (1870), who did their electrical brain stimulation experiments on the frontal lobe of dogs, and David Ferrier (1876), who got the same results carrying out brain stimulation experiments in the monkey. Fritsch and Hitzig found that electrical stimulation of limited parts of the cortex could elicit movements of different controlateral body parts, while the damage of this electrically excitable cortex did not completely abolish the movements: they resulted less fluent and precise, while sensorial perception was not affected. These findings, together with the report of some recovery of the motor function after damage, let Fritsch and Hitzig to suggest that more than one brain region would control motor behaviour. Furthermore, Ferrier accepted Jackson's distinction between voluntary and involuntary activities, observing that motor functions were less affected by lesions of the cortex in organisms lower on the phylogenetic scale (Finger 1994).

Summing up, these results greatly contributed to demonstrate the cortical control of movement, and that the degree of this control depends on its voluntary nature.

1.2. Voluntary movement: the first anatomical and functional studies

In the twentieth century, once demonstrated the cortical control of movement, a great number of experiments on the cerebral cortex were carried out. New histological techniques helped to shed light on the organization of the cortical motor region. Camillo Golgi (1843-1926) developed the silver nitrate staining method, which permits to visualize the whole nerve cell, including its body, axon and branching dendrites. Santiago Ramon Y Cajal (1852-1934), using the Golgi stain, demonstrated that the nervous system is formed by discrete units (the neuronal cells) that are in contiguity and not in continuity with each other. Then, several anatomists proposed different subdivisions based on cytoarchitectonic parcellation. In 1905 Alfred Campbell proposed the existence in the frontal precentral gyrus of an "intermediate precentral zone", rostral to the "precentral motor cortex" (Weinrich, 1984). According to Campbell, Korbinian Brodmann (1909) observed that the frontal lobe, the cortical sector electrically excitable, is characterized by a not homogeneous structure. He identified two areas, one caudal (area 4) and one rostral to it (area 6), ahead of the central sulcus. The leading idea was that difference in structure (e.g. cellular size and density) could be the basis of different functions. Indeed, Fulton and co-workers (Fulton 1934, 1935) supported the idea that the precentral gyrus consists of two functional motor areas (Weinrich et al.

1984). He spoke of a “precentral motor cortex” (now defined “primary motor cortex”) and he introduced the term of “premotor cortex” to indicate area 6, which he considered as a motor association area, based on the observation of the complex motor disturbances which result from its lesion (Sessle and Wiesendanger 1982).

All these findings, together with successive anatomical and stimulation studies (Vogt and Vogt 1919; Von Bonin and Bailey 1947; Penfield, 1951; Woolsey, 1952), essentially supported the motor role of the frontal agranular cortex, and assigning a key role in sensory integration to the parietal lobe, stressed the idea that action and perception are processes controlled by different structures. For a long time, it has been considered that the information flows from the posterior part of the brain to the frontal areas, where it is integrated with those about motor plans elaborated in the prefrontal cortex. In this view, the information is unidirectional, from sensory and associative regions to those involved in executive functions. Backward information (i.e. from motor areas to associative cortices) was scarcely taken into account.

1.3. Action and perception: new concepts

In the eighties, several functional and anatomically studies added new important evidence on the frontal and parietal properties (Mountcastle et al. 1975; Hyvärinen, 1981; Rizzolatti, 1981; Matelli 1984, 1985; Gentilucci et al. 1988; Rizzolatti et al. 1988). In the premotor cortex, neurons responding also to visual and tactile stimuli were found, as well as neurons responding to arm and hand movements were recorded in the parietal areas 5 and 7. These new data were in contrast with the classical idea of a unidirectional flow of information from associative cortex to the motor one. Indeed, successive studies have revealed that a mosaic of distinct areas, reciprocally connected, forming a series of dedicated circuits, constitutes both frontal and posterior parietal cortices. These parieto-frontal circuits work in parallel performing different types of transformation for actions, thus they represent the basic elements of the cortical motor system (Rizzolatti et al. 1998; Rizzolatti and Luppino 2001). In the light of all this evidence, the idea of the ‘percept’ as a unitary representation of the sensory stimuli in the environment (e.g. objects) is not anymore supported by empirical data. Rather, several descriptions of the objects in the spatial environment are not only pictorial, but also pragmatic, that is in terms of the potential motor acts necessary to reach these objects. Altogether, these considerations indicate that the motor cortex cannot be merely confined to a role of movement execution, but it must be considered in several cognitive processes.

1.4. Movement, motor act and action

Physiologically, we can define "*movement*" the changing in space position of a joint, as a result of the activation of one or few muscles. It is a situation that rarely we observe in our daily life: usually, our motor activity involves more than one joint and has a precise goal. When several simple movements are executed synergically and smoothly in order to reach a goal, we speak of "*motor act*". For example, grasping a glass of water implies the complete extension of the fingers and their closure on the glass, in order to take possession of it. The same fingers movements could be done in order to scratch, but the motor goal is different. When several motor acts, each with its specific goal, are planned in order to reach a higher goal, we speak of an "*action*". Movements, motor acts and actions are, then, expression of different degrees of the voluntary movement.

In the ventral premotor cortex, Rizzolatti and co-wokers (1987, 1988) found motor neurons discharging during specific hand shape configurations for grasping objects, and do not activate during movements that involve the same muscles but have different goal. The discovery of a hand representation also in the ventral premotor cortex stimulates the study of the hierarchical organization of the cortical motor control, suggesting that different areas contribute differentially to movement organization. In fact, reversible inactivation of the hand field of the primary motor cortex causes a profound impairment of individual fingers force and movement execution, while inactivation of area F5 does not preclude fingers movements, but induce some impairment in assuming the appropriate hand posture (Fogassi et al. 2001).

1.5. The anatomo-functional parcellation of the frontal agranular cortex

The frontal lobe of primates is anatomically formed by two main sectors: the first, rostral and granular, is identified with prefrontal cortex and has essentially cognitive functions, the latter, caudal and without the layer of granual cells, has clearly motor control functions and coincides with agranular cortex, subdivided in areas 4 and 6 by Brodmann.

A series of anatomical studies from the half of ’80s till today (Matelli et al., 1985; Barbas e Pandya, 1987; Matelli et al., 1991; Petrides e Pandya, 1994) had revealed a lack of homogeneity of the frontal agranular cortex of the monkey, identifying seven distinct cytoarchitectonic areas: F1 corresponds to Brodmann area 4 (primary motor cortex) while areas from F2 to F7 identify the ventral (F4 and F5), the dorsal (F2 and F7) and mesial (F3 and F6) portions of Brodmann area 6 (premotor cortex). These areas have specific patterns of connections with the spinal cord (Luppino and Rizzolatti 2000) thus contribute differently to the motor control (Rizzolatti et al, 1998). The cortico-spinal projections, in fact, originate from areas F1, F2, F3 and parts of F4 and F5 (the caudal motor areas). An important difference among these areas is the terminal territory on the spinal cord: only F1 has cortico-spinal projections that terminate in lamina IX, where motor neurons are located, while fibers coming from premotor cortices end only in the intermediate region (laminae VI, VII and VIII). The indirect recruitment of motor neurons pools in part constitutes the anatomical substrate of the different excitability of these cortices: electrical stimulation of ventral and dorsal premotor areas evokes movements with higher intensity thresholds than those necessary to stimulate the primary motor cortex. Moreover, all these areas are connected with area F1, and thus are involved in movement control, both directly and via F1. It was suggested that this anatomical segregation had a functional counterpart: spinal projections from F2, F3, F4 and F5 activate preformed innate synergies in order to determine the global frame of the movement, while fibers from F1 could break these circuits and determine the fine morphology of the movement (Luppino and Rizzolatti 2000).

In contrast, the rostral cortices (F6 and F7) have descending input terminating in various parts of the brain stem, but have no direct connections with the spinal cord or with F1 (Luppino and Rizzolatti 2000). These areas cannot, therefore, control movement directly. However, their diffuse connections with the other motor cortices permit to control movement in order to determine “when” and “in which circumstances” movement could be done (Rizzolatti and Luppino 2001).

This subdivision based on cortico-spinal connections is also supported by the pattern of those with the other motor areas (“intrinsic connections”) and with the areas outside the agranular frontal cortex (“extrinsic connections”). As said above, the rostral premotor areas do not send fibers to F1, but have connections with the other premotor cortices (Luppino et al. 1993). They are defined “prefronto-dependent motor areas” because of their rich connections with the prefrontal cortex, which has executive functions. In contrast, the caudal premotor areas (“parieto-dependent motor areas”) are much more connected with the posterior parietal cortex. Furthermore, precise pattern of connections between premotor and parietal areas form circuits that play different role in the motor control.

For the objectives of the present thesis, part of area F1 (primary motor cortex) and of the areas F4 and F5 (ventral premotor cortex) will considered.

1.6. Anatomical and functional properties of the primary motor cortex (F1) and of the ventral premotor areas (F4 and F5)

The primary motor cortex F1

Several architectonic maps of the agranular cortex of the macaques monkey have been published (Brodmann 1909; Vogt and Vogt 1919; Von Bonin and Bailey 1947; Matelli et al. 1985; Barbas and Pandya 1987; Matelli et al. 1991), and most investigators agree that the primary motor cortex, unlike premotor cortices, is basically homogeneous. Some distinctive cytoarchitectonic features (Von Bonin and Bailey 1948; Geyer et al. 1998, 2000; Rivara et al. 2003; Sherwood et al. 2004; Belmalih et al. 2007) permit to recognized it in all the primates: it’s agranular, lacking the IV layer and poorly laminated; it’s characterized by prominent giant Betz cell in the layer V, by a predominance of pyramidal type cells, by low cell density and overall large cellular sizes.

The anatomical and functional studies have demonstrated its critical role in the control of voluntary movements of the body (Woolsey et al. 1952; Philips and Porter 1977; Evarts and Fromm 1980; Porter 1985; Humphrey 1986; Lemon 1988). Its functional properties were extensively studied during hand and arm movements and several studies demonstrated that F1 neurons are tuned to some kinematic parameters of movement such as hand position (Georgopoulos et al. 1984; Kettner et al. 1988), speed (Ashe and Georgopoulos 1994; Moran and Schwartz, 1999a), direction of motion (Georgopoulos et al. 1982; Ashe and Georgopoulos 1994; Fu et al. 1995) and force (Taira et al. 1996; Sergio and Kalaska 1998). Another crucial aspect of F1 motor properties is the possibility to control individual fingers (Porter and Lemon 1993) due to its direct access to spinal motor neurons: lesions of the primary motor cortex, in fact, result in severe paresis of the controlateral hand and loss of fingers fine control (Kuypers et al. 1978; Matsumura et al. 1991; Liu and Rouiller 1999).

Beyond the connections with the caudal premotor cortices that contribute to motor planning, area F1 receives projections from the primary somatosensory cortex, which, especially those from area 3a, may provide short-latency peripheral inputs important for movement feed-back (Ghosh et al. 1987).

The ventral premotor area F4

Area F4 is located in the caudal sector of area 6, just rostral to area F1. It contains a somatotopically organized representation of body movements: arm and axial movements are located medially and oro-facial laterally, although with a certain degree of overlap (Gentilucci et al. 1988). There is no representation of distal movements. As far as sensory properties are concerned, neurons of F4 are strongly responsive also to tactile stimuli (unimodal neurons), or to both tactile and visual stimuli (bimodal neurons) (Gentilucci et al. 1983, 1988; Graziano and Gross 1994; Rizzolatti et al. 1981a,b; Fogassi et al. 1996). The somatosensory receptive fields (RFs) of uni- and bimodal neurons are large and predominantly located on the face, the neck, the arm and the trunk. Visual RFs are located around the body ("peripersonal space"), in register with the tactile receptive field. In the large majority, the visual receptive fields of these neurons are independent of the eye position (Fogassi et al. 1992, 1996; Gentilucci et al.

1983; Graziano et al. 1994) remaining anchored to the tactile RF when the monkey moves the eye or the body part on which the tactile RF is located. These properties suggest that F4 neurons code space in a somatocentered and not retinocentered frame of reference (Gentilucci and Rizzolatti 1989; Graziano et al. 1994).

The somatosensory and visual properties of F4 derive very likely from the strong inputs it receives from the inferior parietal lobule (Chavis and Pandya 1976; Godshalk et al. 1984; Petrides and Pandya 1984; Matelli et al. 1986; Cavada and Goldman-rakic 1989) and, in particular, from the ventral intraparietal area (VIP) (Luppino et al. 1999). Area VIP, located in the fundus of the intraparietal sulcus (Colby et al. 1993), also contains many neurons responding to visual stimuli only when they are located in the peripersonal space. In one third of the visually-responsive neurons, the receptive field is encoded in head-centred and not in retino-centred frame of reference (Duhamel et al. 1997). This area receives visual inputs from MST and MT (Maunsell and Van Essen 1983; Ungerleider and Desimone 1986; Boussaoud et al. 1990), while the somatosensory one from areas PEC and PFG (Seltzer and Pandya 1986).

The functional properties of VIP and F4 suggest that this circuit plays a role in encoding the peripersonal space and in transforming object locations into appropriate movements toward them (Rizzolatti et al. 1998).

The ventral premotor area F5

Area F5 is located in the rostral part of the ventral premotor cortex and hosts neurons coding specific goal-directed motor acts (Rizzolatti et al. 1988; Gentilucci et al. 1988). A group of these neurons discharge during proximal motor acts ("reaching" and "bringing to the mouth" neurons), another one during hand and mouth motor acts ("grasping", "holding" and "tearing" neurons). Neurons discharging during distal motor acts, in particular during grasping, also show selectivity for the type of grip through which the object is grasped ("whole hand prehension", "precision grip", "side grip", "finger prehension"). Of these neurons, about the 40% responded to somatosensory stimuli, while about the 20% to visual stimuli. It was suggested that area F5 contains a storage ("*vocabulary"*) of motor acts, which could be accessed via somatosensory and visual inputs.

Recent anatomical studies (Belmalih et al. 2009; Gerbella et al. 2010) identified three sectors of F5 based on cytoarchitectonic and odological characteristics. One is located in the dorsal convexity (F5c) and two within the inferior postarcuate bank: the sector defined as F5p is more posterior and dorsal, while that defined as F5a is more anterior and ventral. These data, together with different functional properties, suggest that the three areas correspond to distinct aspects of motor control and cognitive motor functions (Belmalih et al. 2009).

Neurons of these sectors differ in the visual stimuli able to elicit their response. In area F5p, visuomotor neurons defined as "canonical" (Rizzolatti and Fadiga 1998) respond when the monkey grasps an object with a particular type of grip and also when it observes an object of the same size congruent with that grip (Murata et al. 1997; Raos et al. 2006). The discharge during the observation of that graspable object appears to reflect the coding of 3D object features such as size, shape and orientation (Rizzolatti et al. 1988, Murata et al. 1997, Raos et al. 2006). These visual properties are similar to those of AIP neurons, area of the intraparietal sulcus richly connected with F5 (Luppino et al. 1999; Borra et al. 2008). It was suggested that the description of intrinsic object characteristics is first visually coded in area AIP, and then transmitted to area F5 leading to the selection of distal motor programs appropriate for the hand-object interaction (Jeannerod et al. 1995).

Area F5p is the only sector of F5 displaying connections with both the hand field of the primary motor cortex and the spinal cord (Matelli et al. 1986; Borra et al. 2010). The weak direct and the strong indirect access to spinal segments suggest that F5p has a relatively role not only in the motor planning of object-oriented hand motor acts, but also in their execution (Belmalih, 2009; Borra et al. 2010). Furthermore, its spinal projections may represent the neural substrate for the F5 hand motor field's role in the recovery of manual dexterity after F1 lesions (Borra et al. 2010).

The F5 sector lying on the postarcuate convexity cortex (F5c) typically display different visual properties from those of F5p: this area, in fact, contains mirror neurons, those visuomotor neurons active both when the monkey performs a goal-related motor act and when it observes a similar motor act made by another individual (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996). From their discovery to now, different types of mirror neurons were described (see section below).

It has been proposed that functional properties of F5c neurons reflect coding, in visual modality, of motor acts made by others that are mapped onto the own motor repertoire (Rizzolatti et al. 2001). Thus, it suggests that premotor cortex could play an important cognitive role: the internal actions representation, when activated by the observation of an action performed by others, would lead to action understanding and imitation (Rizzolatti et al. 1998). This sector of F5 presents rich connections with area PFG (Rozzi et al. 2006), which is a sector of the rostral inferior parietal lobule (IPL). In both areas F5 and PFG, it has been demonstrated the existence of grasping motor neurons and mirror neurons that could be influenced by the goal of the action (i.e., grasp-to-eat or grasp-to- place), in which the coded act is embedded (Fogassi et al. 2005; Bonini et al. 2010). These data suggest a possible functional circuit, involving both PFG and F5, for action organization and intention understanding.

As far as F5a is concerned, less data are available when compared with the other two F5 sectors. In fact, because of its anterior position in the postarcuate bank, it has been only marginally involved in electrophysiological studies. Cytoarchitectonically, it displays features that appear transitional between those of ventral premotor cortex and those of frontal granular cortex. A recent fMRI study in the macaques sheds light on the possible functional properties of F5a (Nelissen et al. 2005): both F5c and F5a were found to be active during the observation of goal-directed motor acts, but their activation needed a different richness of contextual elements. In fact, F5c was active only for the observation of a person grasping objects in full view, while F5a was active also for the observation of an isolated hand grasping objects, a hand mimicking grasping and a robot arm grasping objects. It has been suggested that F5a, being less context-dependent, code motor acts at a more abstract level, possibly related to its general meaning. Future electrophysiological and odological studies will clarify whether it should be considered as an additional premotor area, possibly to be included in the group of the "rostral premotor areas" as defined by Rizzolatti and Luppino (2001) (Belmalih et al. 2009).

The mirror neurons

Mirror neurons (MNs) are a particular class of visuomotor neurons discovered in the ventral premotor area F5 (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 1996a,b) and found also in the parietal area PFG (Fogassi et al. 2005; Bonini et al. 2010). Similarly to purely motor neurons, they discharge during motor acts having a specific goal (e.g. grasping). Their peculiarity is that they discharge also during the observation of the same or of similar motor act performed by another monkey or by an experimenter. The effective observed motor acts evoking MNs discharge are grasping, manipulation, holding etc, that are the same motor acts belonging to monkey motor vocabulary. Almost all MNs show congruence between the effective observed and executed motor act (di Pellegrino et al. 1992; Gallese et al. 1996). It has been proposed that this direct matching between the observed motor act and its internal representation is a possible mechanism that enables action understanding (Rizzolatti and Arbib 1998).

Since their first discovery (di Pellegrino et al. 1992), it has been demonstrated that the majority of them show a “strict” or “broad” congruence between the effective executed and observed motor act. However, the discovery of MNs that discharge indendently from the effector used by the observed agent gives more emphasis to the hypothesis that this class of neurons code the goal of a motor act. In fact, in the lateral part of the area F5, MNs discharging during the observation of grasping performed with hand and mouth were recorded (Ferrari et al. 2003). Moreover, it has been demonstrated that they can code motor goal of acts outside monkey motor repertoire, as grasping act performed by a tool (a pliers or a stick). These data suggest that a prolonged visual exposure to motor acts made with a tool could cause an association between the tool and the experimenter hand (Ferrari et al. 2005; Ferrari et al. 2009). Because of the motor nature of MNs response, it was suggested that motor learning could affect not only motor knowledge but also the generalization on MNs visual responses. In fact, after the monkey was trained to use pliers, MNs from the area F5 were recorded during the observation and the execution of grasping performed with the hand or a tool. The results showed that almost all the MNs discharged during the observation of both hand and tool grasping, although the earliest and strongest response was during hand motor act (Rochat et al. 2010).

Another interesting factor is that some MNs discharge during the hearing of a motor act (e.g. breaking peanuts), also in absence of a visual motor act (Kohler et al. 2002). These "audiovisual mirror neurons" give a more abstract example of action coding, representing motor acts independently of whether they are performed, heard or seen (Keysers et al. 2003).

The face representation in the precentral cortex of the monkey

The first studies carried out on cortical face representation were through surface electrical stimulation. Walker and Green (1938) evoked, with prolonged stimulation, different type of movements in area 4 and area 6 of Brodmann: jaw twitches in area 4 and rhythmic masticatory movements in area 6. However, Luschei and co-workers (1970) observed that no jaw movements were evoke with the same parameters that produce discrete movements of hand, foot, lips or tongue, instead they were evoked using longer stimulation trains. These results, together with lesion experiments that produced difficulty in controlling but not producing jaw movements, suggested that the role of mouth field precentral cortex is predominantly to inhibit jaw-closing motoneurons or the system that excite them (Luschei and Goodwin 1974).

With the introduction of intracortical microstimulation, it became possible to obtain more detailed maps of the oro-facial motor region. Because of the more excitable nature of the primary motor cortex than that of the premotor cortex, the investigated face representation was often confined to the primary motor cortex.

The use of ICMS and the identification of corticobulbar projections (Huang et al. 1988) permit to extensively map the face primary motor cortex. These authors found twitch-like movements of the face, jaw and tongue, with the representation of facial muscles that partially enclosed and overlapped representation of jaw and tongue muscles. This data supported previous findings (McGuinness et al. 1980) of a rough topographic organization of facial muscles, with the tendency for adiacent muscles to occur together. Moreover, both these studies both found a greater cortical representation of the zygomaticus among all the orofacial muscles: it is a mimetic muscle, controlling the retraction of the corners of the mouth and the side of face, but is not directly involved in ingestive behaviour. These data suggested that the rough topographic organization of face motor cortex is consistent with the need of close coordination of face, jaw, and tongue muscles not only in ingestive (e.g. mastication, swallowing) but also social (communicative expressions and vocalization) behaviours (Huang et al. 1988).

Other studies (Murray and Sessle 1992) recorded neurons in the face field of the primary motor cortex during trained oro-facial tasks. Different efferent zones were found combining results from ICMS and recordings. In fact, tongue-related neurons were recorded at sites from which ICMS evoked different direction of tongue movements. In fact, neurons at these sites showed variation of their firing rates associated with different directions of tongue protrusion. Moreover, as hand field cortical lesions result in major deficits in distal digits movements, cooling of face motor cortex (Murray et al 1991) impairs the fine control of the tongue during the tongue-protrusion task (Murray and Sessle 1992). The authors of this study hypothesised that the different efferent zones are recruited to affect the appropriate change in tongue shape and position during task performance.

Although in a lower percentage than that of tongue-related neurons, also jaw-related neurons active during biting task were found. The variety of different neuronal activity patterns showed by these neurons during the biting task (Murray and Sessle 1992; Hoffman and Luschei 1980) supported the findings of lesion studies (Luschei and Goodwin 1975) that showed difficulty in controlling, but not producing, closing jaw movements, and allowed the authors to hypothesize a cortical control during biting, mastication and other jaw movements, although subcortical circuits influencing brain stem motoneurons could not be excluded.

Although the majority of studies on cortical face representation were carried out in the primary motor cortex, some evidences of face representation emerge also in other cortical areas. Physiological and electrical stimulation studies (Gentilucci et al. 1988; Graziano et al. 2007) have demonstrated the presence of mouth motor representations also in the most lateral portion of the ventral premotor cortex. Studies from Morecraft and colleagues (2001) supported the presence of more than one face representation in the cerebral cortex. In fact, they studied the corticobulbar projections from face representations of five regions, including the primary motor and the ventral premotor cortices. They found that the facial nucleus receives inputs from all the face representations, but with some topographic organization. In fact, the lateral subnucleus, which innervates the perioral musculature, received a higher percentage of projections from the primary motor, the premotor and the rostral cingulate motor cortices.

Thus, anatomical and physiological studies support the presence of a mouth representation also in the ventral premotor, as well as in the primary motor cortex. The widespread representation of a body-part movements is a general organization principle of the primate cortical motor system: as described for fingers movements, the primary motor cortex appears to break subcortical patterns of movement also for mouth control. Few information on the role of the premotor cortex in the control of oro-facial movements derives from the data collected till now.

1.7. Aims of the study

Several studies have investigated the functional properties of the primary motor and the ventral premotor cortices. Classical extracellular recording studies have revealed that F1 represents motor parameters such as force or direction (Evarts 1968; Georgopoulos et al. 1982). In turn, area F5 is responsible for visuo-motor transformations for grasping and action recognition (Rizzolatti et al. 1988; Gallese et al. 1996), while area F4 is involved in coding of peripersonal space in motor terms (Fogassi et al. 1996).

Some electrophysiological studies have revealed that ethologically relevant, complex, movements can be evoked through ICMS applied in several regions of the frontal motor cortex that form functional clusters that specialize in different common actions (Graziano et al. 2002). Furthermore, reversible inactivation of area F5 and F1 has different impact on motor performance, suggesting specific roles for each area in movement planning and execution. Several other studies have targeted specific sectors of primary motor and of premotor cortices, greatly contributing to clarify their functions (Rizzolatti et al. 2001; Graziano et al. 2007). However, a more extensive investigation of the wide cortical sector included between the inferior arcuate and the central sulcus is still lacking. This would helpful for clarifying the relative contribution of different motor, somatosensory and visual responses, in the organization of the most lateral part of the primary motor and premotor cortices.

For these purpose, we carried out an electrophysiological study on this region by means of intracortical microstimulation and extracellular recordings of multiunit activity, attempting to correlate different clusters of functional properties with cytoarchitectonic borders among different areas.

2. MATERIALS AND METHODS

The experiments were carried out on two monkeys (*Macaca nemestrina*). Before the beginning of the recording sessions, both monkeys were trained to sit on a primate chair and to interact with the experimenters in a partially restrained condition. They received their daily ration of food, liquid and solid, on the chair and directly from the experimenter's hands, in order to accustoming them to interact with the experimenter.

2.1. Surgical procedures for the implantation of the head-holding system and the recording chamber

After the habituation and training period, the monkeys were operated under general anaesthesia (ketamine hydrocloride, 5 mg/Kg intramuscular [i.m.] and medetomidine hydrocloride, 0.1 mg/kg i.m.), and a head-holding system was implanted. Upon recovery from anesthesia the animals were returned to their home cages and closely monitored. Dexamethasone and prophylactic broad-spectrum antibiotics were administered pre- and postoperatively. Analgesics were administered intra- and postoperatively (Borra et al. 2010).

The system of head-holding was constituted by four hollow titanium cylinders, fixed by mean of acrylic cement (Antibiotic Simplex©) to 'Evarts-screws' implanted on the skull near each cylinders. The cylinders were positioned in symmetric positions of both hemispheres, over the anterior fronto-temporal and posterior occipital regions. Once implanted, the head-holding system was not used for at least one month, in order to allow the bone to embed the screws and stabilize the whole system.

Then, four metallic sliding bars fixed to the head-holder were inserted into the cylinders in order to keep the monkey head fixed. For at least another month, the monkeys had to continue the habituation/training phase in daily sessions, but with the head restrained. In this way, monkeys were accustomed to receive food, somatosensory and visual stimuli with the head fixed, until they became accustomed and quiet also in this condition. Finally, a second surgery to implant the recording chamber was carried out.

The cortical region of interest was the precentral area extending from the posterior bank of the inferior arcuate sulcus to the anterior bank of the central sulcus. In the medio-lateral axis, the recording chamber was placed as laterally as possible, in order to reach the mouth field of primary motor and premotor cortices. The location of this region was identified without opening the dura, on the basis of stereotactic coordinates of the anatomical landmarks measured in previously employed monkeys.

The surgical procedures and post-surgical pain medication were the same described for the implantation of the head-holding system. In this case, a craniotomy was performed over the identified region of interest, and a titanium recording chamber was positioned on the skull and fixed to it by mean of titanium screws. The borders of the chamber lying on the skull were then sealed to the bone with acrylic antibiotic cement. Finally, the chamber was filled with sterile Vaseline oil and temporary closed by a titanium cap fixed to the chamber by mean of four screws.

At the end of the surgery, the stereotaxic coordinates of a corner of the chamber were measured, in order to use them as a reference point for the construction of the recording grid.

2.2. Recording techniques

The recording device consisted of a microdriving terminal (AlphaOmega Engineering, Nazareth, Israel) holding a tungsten, glass-coated microelectrode (impedance 0.5-1 MΩ). The MT was attached to a stereotaxic micromanipulator fixed to the monkey head-holder, with its vertical branch rotated 35° with respect to the vertical axis (0°), in order to penetrate the dura perpendicularly. The electrode was inserted through the intact dura, properly prepared prior to each recording session. A dedicated software package (EPS, Alpha Omega, Nazareth, Israel) allowed one to drive the engine controlling the electrode vertical movements.

Neuronal activity was filtered and amplified through a dedicated system (MCP*plus*, Alpha Omega, Nazareth, Israel), and then sent to an oscilloscope and an acoustic amplifier (Grass Technologies, West Warwick, USA). The signal was also sent to a PC for acquisition of raw multiunit activity and, in parallel, to a dual voltage-time window discriminator (Bak Electronics, Germantown MD), in order to isolate single neurons action potentials. Isolated spikes could also be sent to a dedicated channel of the acoustic amplifier and to the PC, for the acquisition, storage and subsequent analyses.

Contact detecting electric circuits allowed generating TTL (5V) signals in correspondence with the occurrence of the main behavioural events. These signals were sent to the PC and recorded in parallel with the multiunit and single unit activity, enabling to align neural activity to the behavioural event of interest and to construct the response histogram (by averaging at least ten trials).

In cases of the presentation of visual stimuli, the experimenter provided the TTL signal manually. In cases of active motor responses or tactile stimulations, the signal was automatically generated when the touched object entered in contact with the monkey's skin. A Lab-View based software allowed to acquire the neuronal activity and the electrical signals related to the behavioural events to which the response had to be correlated.

During each experimental session the electrode was inserted through the dura until the first neuronal activity was detected. The electrode was then deepened into the cortex in steps of 250 μm, until the border between the grey and the white matter was reached. At each site, multiunit and single unit activities were recorded and their correlation with any type of sensory (visual and tactile) stimulation and motor activity was noted on a protocol and subsequently inserted into a database.

The first recording session was performed in the anterior part of the chamber, in order to identify the eye-related activity of frontal eye field (FEF). Functional identification of FEF was based on the properties emerging from the clinical testing of the neurons, verifying their response during eye movements of different types (i.e. large-small saccades, selectivity for a certain direction of the eye movement, etc.), and on the movements evoked by electrical intracortical microstimulation (ICMS). Once this region was identified in the medio-lateral axis, the subsequent recording sessions were carried out moving the electrode caudally, in lines of 1 mm one from the other.

2.3. Intracortical microstimulation (ICMS)

The recording microelectrode was also used for delivering intracortical monopolar and monophasic trains of cathodic square wave pulses, with the following parameters: total train duration 50 ms, single pulse width 0.2 ms, and pulse frequency 330 Hz. The train of pulses was generated by a Stimulus Isolator (World Precision Instruments, Stevenage, UK). The current intensity ranged from 3 to 40 μA, and the current strength was controlled on an oscilloscope by measuring the voltage drop across a 10 KΩ resistor in series with the stimulating electrode.

In each penetration, ICMS was performed every 500 μm of depth (that is, in 1 step out of 2), starting 500 μm below the site where the first multiunit activity was detected. At each site, ICMS was delivered when the monkey was quiet and relaxed, and those episodes during which monkeys performed voluntary movements were not considered for establishing the stimulation threshold. Movements were considered evoked by ICMS when two experimenters, observing the animal during pulse delivering, independently identified the same rapid movement or muscular twitch as evoked by the stimulation. The procedure consisted of an initial stimulation with a current intensity of 40 μA. If each train delivered at this intensity reliably evoked the same movement (typically 3 out of 3 consecutive stimulation with the above described parameters), successive stimulations were delivered by progressively decreasing current intensity, in order to identify the threshold. Threshold was defined as the current intensity capable of evoking movements in 50% plus one of the ICMS delivered (typically 4 out of 6 or 5 out of 8 stimulations).

In order to study the differences among the stimulated areas, we considered for each penetration the site with the lowest threshold and used it for the comparative data analyses. If the stimulation of all the sites of a penetration did not evoke any overt movement with the above defined parameters, the penetration was defined not excitable.

Data analysis

The percentage of excitable and not excitable penetrations in the investigated region was analysed by using Chi-square Tests. As far as the stimulation thresholds are concerned, statistical comparisons between anatomo-functional subdivisions and monkeys were carried out by using a factorial ANOVA for repeated measures, followed by Newman-Keuls post-hoc tests.

2.4. Testing of the functional properties of neuronal activity

In order to characterize multiunit and single unit properties, the following testing procedures were applied.

Motor properties

Neuronal activity related to hand movements was tested by presenting the monkey graspable objects or pieces of food of different size, shape and orientation, located in different quadrants of the reaching space. When neuronal activity related to grasping was found, a particular attention was dedicated to test it with different types of grip. The types of grip tested were classified according to the posture assumed by the hand during the 'shaping' and 'actual grasping' phases (see Macfarlane et al. 2009), as follows: *precision grip,* characterized by the opposition of the pulpar surface of the last phalanx of the index finger and the thumb; *side grip*, characterized by the opposition of the radial surface of the last phalanx of the index finger and the thumb; *finger prehension,* involving all the fingers but the thumb, wrapped around the object (typically a cylindrical one), with no opposition with the hand palm; w*hole hand prehension,* characterized by all the fingers and the thumb, wrapped around the object.

Hand- and arm-related activity were dissociated by presenting graspable objects far from or near the monkey's body, so that they could be grasped either with or without arm extension, respectively. Neuronal responses were temporary attributed to *reaching* if depended on arm extension to reach the target, or to *bringing* if the response was present only during arm retraction for bringing food to the mouth. In order to distinguishing such responses from possible responses due to hand related motor activity (i.e. hand shaping or object holding, respectively), shaping/actual grasping and holding responses were also tested with the monkey's arm restrained. Objects were approached to the monkey's hand restrained, so that no movement of the arm was required nor allowed. In this way, it was possible to test hand-related activity independently from arm-related motor acts.

Different types of mouth motor acts were tested by using different types of stimuli (solid food, juice delivered from a syringe, or yogurt licked from a stick). The food was approached to the monkey's mouth by the experimenter, so that the monkey had not to use its hand/arm. *Mouth grasping* was defined as the closure of the teeth on the food; *sucking* was defined as the complex and coordinated series of movements required for maintaining the lips tighten on the syringe during suction of the juice from it; *licking* was defined as the protrusion of the tongue for licking juice or yogurt presented on a stick.

When any movement of the tongue (e.g. during protrusion or retraction) or the jaw (e.g. during chewing, yawning or threat expressions) was capable of evoking neuronal discharge independently of a specific motor goal or during a number of different types of motor acts involving that movement, the neuronal activity was classified as related to a simple *tongue* or *jaw movement,* respectively*,* rather than to a specific motor act such those listed above.

Eye-related motor activity (fixation, saccades or smooth pursuit) was studied by presenting and moving interesting stimuli, such as pieces of food, solids or light spots in a dark environment, in different parts of the extrapersonal (out of reach) visual space.

The contribution of any axio-proximal effectors is extremely difficult to test because of the limited axial mobility of head-restrained monkeys. Therefore, we could not ascertain which precise effector or group of muscles was involved in each specific case, rendering necessary to use exclusion criteria to identify activity related to axio-proximal effectors (neck, shoulder or trunk). When no motor activity related to any of the effectors cited above (hand, arm or mouth) was detected, we also tryed to evoke some axio-proximal movements by approaching food directly to the mouth of the monkey or by presenting the monkey food items which it attempted to reach with the mouth or the arms from different directions and at different speeds. If, in these conditions, some motor activity was detected it was attributed to a unitary category "axio-proximal movement".

Somatosensory properties

Somatosensory properties were characterized according to the type of stimulus effective in triggering a neuronal response: *light touch* was assessed by gently touching the skin or bending the hairs with a cotton wad, a small brush, or by blowing air puffs on the skin; *deep touch* was assessed by applying pressure to restricted cutaneous territories by touching them with a stick, by finger tapping or by squeezing muscle bellies; *joint responses* were tested by means of joints mobilization.

As far as the inner mouth is concerned, the tactile stimulations were made by keeping opened the monkey's mouth with a stick-tool and by gently touching the tongue or the teeth with tweezers or a cotton-wad.

Visual properties

Visual properties were studied by presenting the monkey with 3D-objects (e.g. food items and solids) of different shape, size and orientation, moved in various space locations, direction and distances from the monkey. Different visual responses were tested using specific sets of visual stimulations and categorized as follows: *object presentation* was tested by presenting the monkey with objects in the peri- and extrapersonal field by holding them with a tool (stick or plier) or by disclosing objects hidden behind an occluding screen, in order to avoid that the experimenter's hand was also visible; *object motion* was studied by moving objects held by a non-biological tool following linear or circular trajectories in the peri- and extrapersonal space; *peripersonal visual responses* were tested by moving objects along trajectories directed toward or away from different monkey's body parts within its peripersonal space; *biological motion* was assessed by presenting the monkey with non-object-related experimenter's movements, such as moving the hand, head, trunk or limbs; *mirror responses* were tested by presenting the monkey with hand-related motor acts (e.g. grasping) executed uni- or bimanually, or with mouth-related motor acts (e.g. mouth grasping, sucking, licking). All the motor acts were performed or mimicked by the experimenter in front of the monkey.

Note that all the motor and somatosensory properties were studied both in light and dark conditions, to evaluate the possible contribution of visual guidance.

2.5. Statistical analysis of the neurophysiological data

Single neuron analysis

Neuronal activity associated to certain properties among those defined above, was recorded in at least ten trials for each of the tested condition. The activity of each neuron was expressed as mean firing rate (spikes/second) and statistically compared between two different time epochs: 1) baseline activity (500 ms), taken from 2000 to 1500 ms before the event of interest (stimulus application/presentation or motor response) and 2) sensory stimulation/motor response (500 ms), taken from the application of the stimulus (visual/somatosensory) or centred on the active contact of monkey body part with the target (from 250 ms before to 250 ms after this event), during active movements. The neuron activity was then compared across these epochs and the tested conditions by using a two-way ANOVA (factors: Condition and Epoch), followed by Newman-Keuls post-hoc tests. All analyses were performed using a significance criterion of $p<0.05$.

2.6. Histology, identification of the recorded areas and methodology for the anatomo-functional comparisons

At the end of the neurophysiological experiments, electrolytic lesions (10 μA cathodic pulses per 10 s) were performed at known coordinates, in order to delimit the external borders of the studied region and to allow the subsequent anatomical reconstruction of the recording grid. After one week following the lesions, each animal was anaesthetized with ketamine hydrochloride (15 mg/kg intramuscularly) followed by i.v. lethal injection of sodium thiopental and perfused through the left cardiac ventricle with saline, 3.5-4% paraformaldehyde and 5% glycerol in this order. All solutions were prepared in phosphate buffer 0.1 M, pH 7.4. Then, the animal was placed in a stereotaxic apparatus, the bone and the dura removed and the stereotaxic coordinates of various landmarks were measured. The brain was then removed from the skull, photographed, and placed in 10% buffered glycerol for 3 days and 20% buffered glycerol for 4 days. Coronal serial sections (60 μm thickness) were cut on a freezing microtome and stained with Thionin. Each second and fifth section of a series of five were stained using the Nissl method. The locations of penetrations were then reconstructed on the basis of electrolytic lesions, stereotaxic coordinates, penetration tracks and their depths. More specifically, penetrations deeper than 4000 μm located inside the arcuate sulcus with properties typical of frontal eye field (FEF) were used in order to localize the rostral border of area F5 convexity. The caudal border of the recorded region was defined based on the appearance of the properties typical of the primary somatosensory cortex (SI).

Subsequently, the cytoarchitectonic features of primary motor and premotor cortices were identified based on the criteria defined by Belmalih et al. (2007, 2009). Since cytoarchitectonic features often change gradually from one region to another, the borders between adjacent areas were drawn in the middle of transitional zones (about 0.5 mm wide). The reconstructed grid was then related to the cytoarchitectonic parcellation. This allowed us to attribute the functional properties of each penetration to a specific cytoarchitectonic area.

Then the number of sites in which each property was present, in each area, was calculated and expressed as percentage of the total number of sites of that area.

Data analysis and map construction

The functional properties of neuronal activity (single neurons and multiunit activities) identified at each recoding site was noted for each penetration and used to reconstruct the functional organization of primary motor and premotor cortices. Only the sites located between the cortical surface and 4000 μm of depth were included in the database. Thus, seventeen was the maximum number of recording sites (steps of 250 μm) in each penetration. Cortical sites deeper than 4000 μm were used for establishing the location of arcuate and central sulci and to identify the cortical areas located inside the bank of the various sulci. The properties of neurons recorded from these sites will not be described in the present study.

For each penetration site, we identified the functional properties of single and multiunit activity. Responses to motor, somatosensory and visual stimuli and their characteristics were reported for each penetration on the penetrations grid in terms of percentage of sites per penetration in which each property was found.

The number of sites in which each functional property was present was then compared between distinct cytoarchitectonic areas, by using Chi-square tests: each functional property was expressed as the percentage of sites in which it was found on the total number of sites of that area.

3. RESULTS

3.1. Anatomical localization and cytoarchitectonic features of the recorded region

We carried out 231 penetrations in the two monkeys (117 in M1, 114 in M2), for a total of 1292 investigated sites in M1 (11.04 sites for penetration on average) and 1316 in M2 (11.54 sites for penetration on average).

A lateral view of the left hemispheres recorded in the two monkeys (M1 and M2) is shown in Figure 1. Figure 1A illustrates the penetrations grid in the investigated region, overlapped on the cytoarchitectonic parcellation of ventral premotor (areas F5 and F4) and primary motor (F1) cortex (according to Matelli et al. 1985; Belmalih et al. 2007; 2009). Figure 1B shows the photomicrographs of some sections representative of the investigated cytoarchitectonic subdivisions.

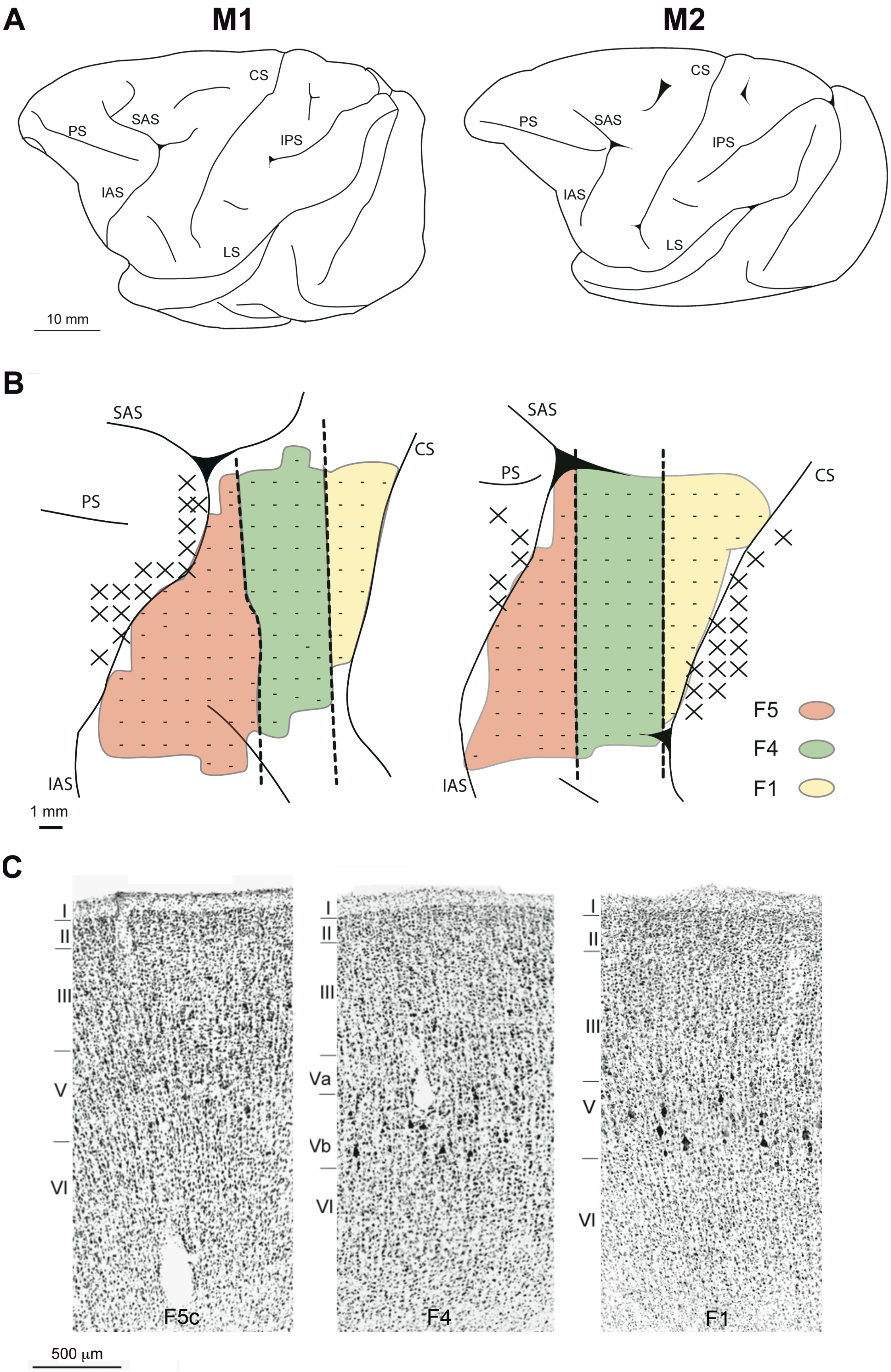
A
M1
M2
CS
PS
SAS
IPS
IAS
LS
10 mm
CS
PS
SAS
IPS
IAS
LS
B
SAS
PS
CS
IAS
1 mm
SAS
PS
CS
IAS
F5
F4
F1
C
I
II
III
V
VI
F5c
I
II
III
Va
Vb
VI
F4
I
II
III
V
VI
F1
500 μm

Figure 1. Penetrations grid of the investigated region superimposed on the cytoarchitectonic parcellation of the lateral cortex. (A) Left hemispheres of each monkey. (B) Each dot represents the location of a penetration. The yellow region corresponds to F1, the green region to F4, and the red region to F5. (C) Photomicrographs showing sections representative of the cytoarchitectonic organization of the three investigated areas (F5, F4, and F1). Note that F1 is characterized by a prominent V layer with giant pyramidal arranged in multiple rows. Relatively large pyramids are also in the layer Vb of the area F4, which also displays a few cells and an evident size gradient in the layer III with medium-sized pyramids in the lower part of it. In contrast, area F5c is characterized by poor lamination and by an overall cell smallness (Belmalih et al. 2007; 2009). PS, principal sulcus; SAS, superior arcuate sulcus; IAS, inferior arcuate sulcus; CS, central sulcus; LS, lateral sulcus; IPS, intraparietal sulcus.

3.2. Anatomo-functional organization of ventral premotor and primary motor cortical convexity

Preliminar comparative analyses of the functional properties among the cytoarchitectonically-identified areas evidenced a strong functional similarity of the different areas, apparently due to a huge non-homogeneous distribution of motor, visual and electrically-evoked responses within each area. However, specific properties are not randomly distributed, rather, they appear to be organized along the dorso-ventral axis, with properties related to hand and arm dorsally, and to face-mouth ventrally. Together with the distribution of the visual properties, almost absent in the ventral portion, these observations prompted us to trace a functional border in areas F4 (F4d and F4v) and F1 (F1d and F1v). Therefore, subsequent analyses of anatomo-functional relationships will be carry out taking into account five anatomo-functional subdivisions, namely, area F5, area F4d and F4v, area F1d and F1v.

Figure 2A shows the cortical regions related to brachio-manual (blue) and the mouth (yellow) movement as derived from extracellular multiunit recordings. It is clear that hand and mouth overlap in a large cortical region (green) encompassing area F5 and the dorsalmost sectors of cytoarchitectonic areas F4 and F1. More specifically, in both F4v and F1v there is a high and similar (M1: χ^2=0.22, ns; M2: χ^2=0.00, ns) percentage of penetrations with mouth motor activity, while in F4d and F1d brachio-manual and mouth penetrations are widely represented, similarly in the two subdivisions (M1: $\chi^2=0.18$, ns; M2: $\chi^2=0.13$, ns). Taken together, the dorsal portions of F4 and F1 have a high representation of brachio-manual associated to mouth responses as compared to that of their ventral portions (M1: $\chi^2=22.54$, $p<0.001$; M2: $\chi^2=10.30$, $p<0.001$). In contrast, mouth responses are more widely present in the ventral portions of these subdivisions as compared to their dorsal counterparts (M1: $\chi^2=35.96$, $p<0.001$; M2: $\chi^2=32.98$, $p<0.001$). Penetrations with sites showing visual properties (orange circles) have been superimposed in the same maps, revealing that visual responses are confined in area F5 and in the dorsal portion of F4 and F1 in both monkeys. Figure 2B shows similar maps for both monkeys in which the hand and mouth representations have been derived from ICMS.

Figure 2C summarizes the results of the comparisons among the five subdivisions in terms of electrical excitability and presence of sites with visual responses. The ventral subdivisions of both F1 and F4 do not differ from the dorsal counterpart in terms of relative proportion of electrically excitable penetrations (F1: M2: $\chi^2=0.29$, ns; F4: M1: $\chi^2=0.12$, ns; M2: $\chi^2=0.03$, ns). Area F5 contains the lowest number of excitable penetrations as compared to both cytoarchitectonic areas F4 (M1: $\chi^2=21.69$, $p<0.001$; M2: $\chi^2=5.64$, $p<0.05$) and F1 (M1: $\chi^2=12.99$, $p<0.001$; M2: $\chi^2=9.71$, $p<0.001$). Area F1 has a higher percentage of penetrations with excitable sites (M1: 94%; M2: 81.5%) as compared to F4 (M1: 81.5%; M2: 67.3%), although this difference does not reach statistical significance (M1: $\chi^2=0.15$, ns; M2: $\chi^2=1.10$, ns).

As far as visual responses are concerned, they are more widely represented in area F5 than in the ventral portions of F4-F1 in M1 ($\chi^2=11.36$, $p<0.001$), although in M2 this difference does not reach the statistical significance ($\chi^2=0.43$, ns). Note that visual responses are nearly absent in the ventral part of F4 and F1 in both monkeys. Thus, this distribution of visual properties fits well with the cytoarchitectonic rostro-caudal border between F5 and the ventral portion of area F4 (F4v). In contrast, the dorsal sectors of both F4 and F1 have a rich representation of visual properties, which is similar for the two sectors (M1: $\chi^2=0.01$, ns; M2: $\chi^2=0.29$, ns) and even richer than that of area F5 (F4d: M1: $\chi^2=3.80$, $p<0.05$; M2: $\chi^2=11.95$, $p<0.001$; F1d: M1: $\chi^2=4.08$, $p<0.05$; M2: $\chi^2=11.95$, $p<0.001$).

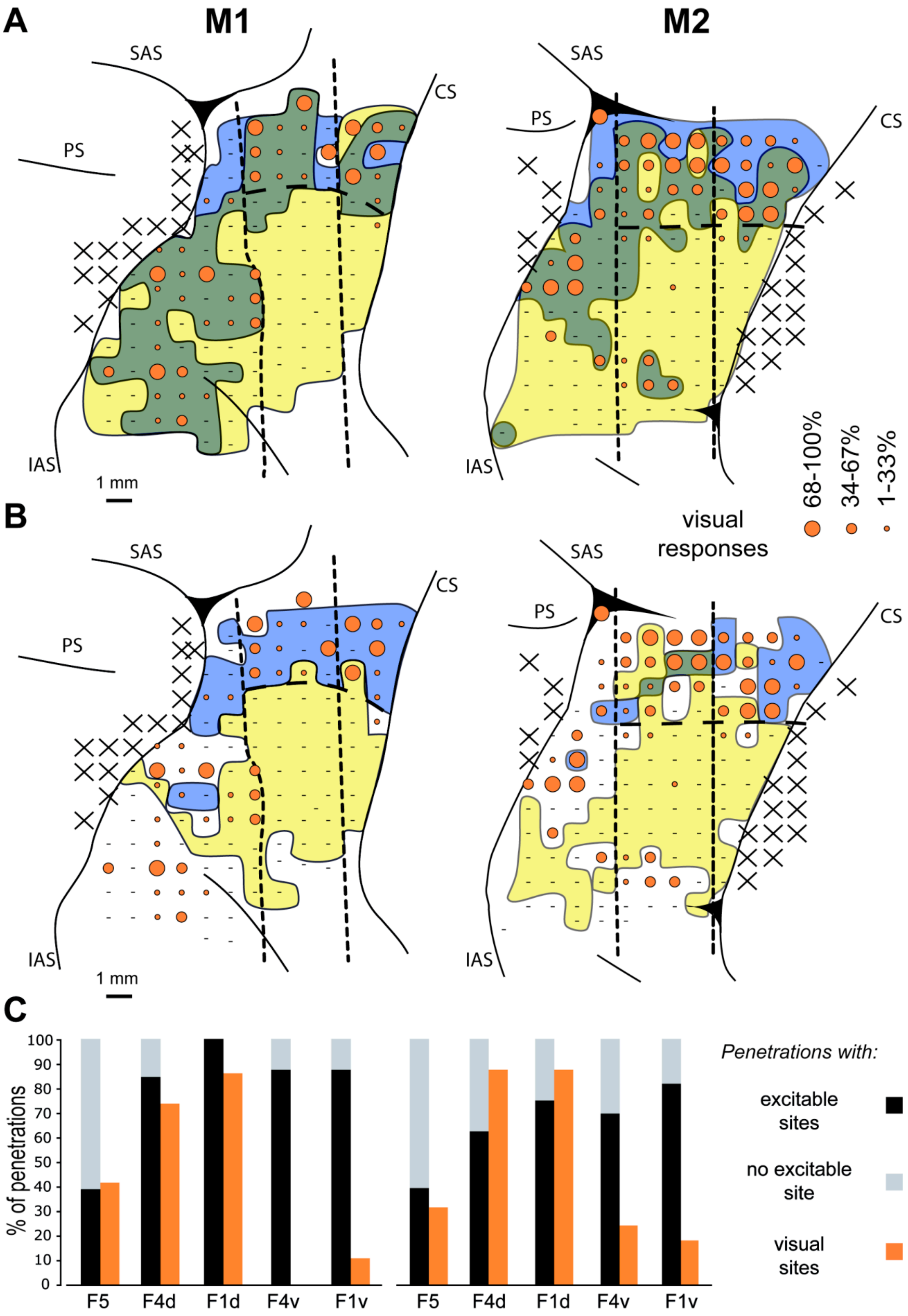

Figure 2. Representation of brachio-manual and mouth fields, and of penetrations containing sites showing visual responses. (A) Distribution of penetrations with extracellular multiunit

activity related to brachio-manual (blue), mouth (yellow) or brachio-manual and mouth responses (green). (B) Distribution of penetrations in which ICMS evoked brachio-manual (blue), mouth (yellow) or brachio-manual and mouth responses (green). Orange circles identify the penetrations characterized by the presence of visual responses. The size of each circle represents the percentage of sites in which the response was present, calculated on the total number of sites of the penetration. Vertical dashed lines indicate cytoarchitectonic borders between F5 from F4 (rostral border) and F4 from F1 (caudal border). Horizontal dashed line indicates the anatomo-functional borders defined on the basis of both the presence/absence of visual responses and the type of effector (brachio-manual vs mouth) activated by mean of ICMS. Dots indicate the location of each penetration; crosses indicate penetrations falling outside the regions of interest. (C) Histograms represent the percentage of penetrations with excitable (black) and not excitable (grey) sites, and with visual responses (orange), in each of the investigated subdivisions. Abbreviations as in Figure 1.

Movements evoked by ICMS

Intracortical microstimulation was delivered in all the 231 penetrations, every 500 μm from the beginning of the cortical activity. In 142 (61.5%) of the penetrations it has been possible to evoke movements, and the mean of the lowest thresholds among penetrations was 22.3 μA. In contrast, in the 89 remaining penetrations (38.5%), the electrical stimulation had no effect in any of the investigated sites.

Figure 3 shows the localization of excitable penetrations in terms of the effector activated by ICMS and its threshold.

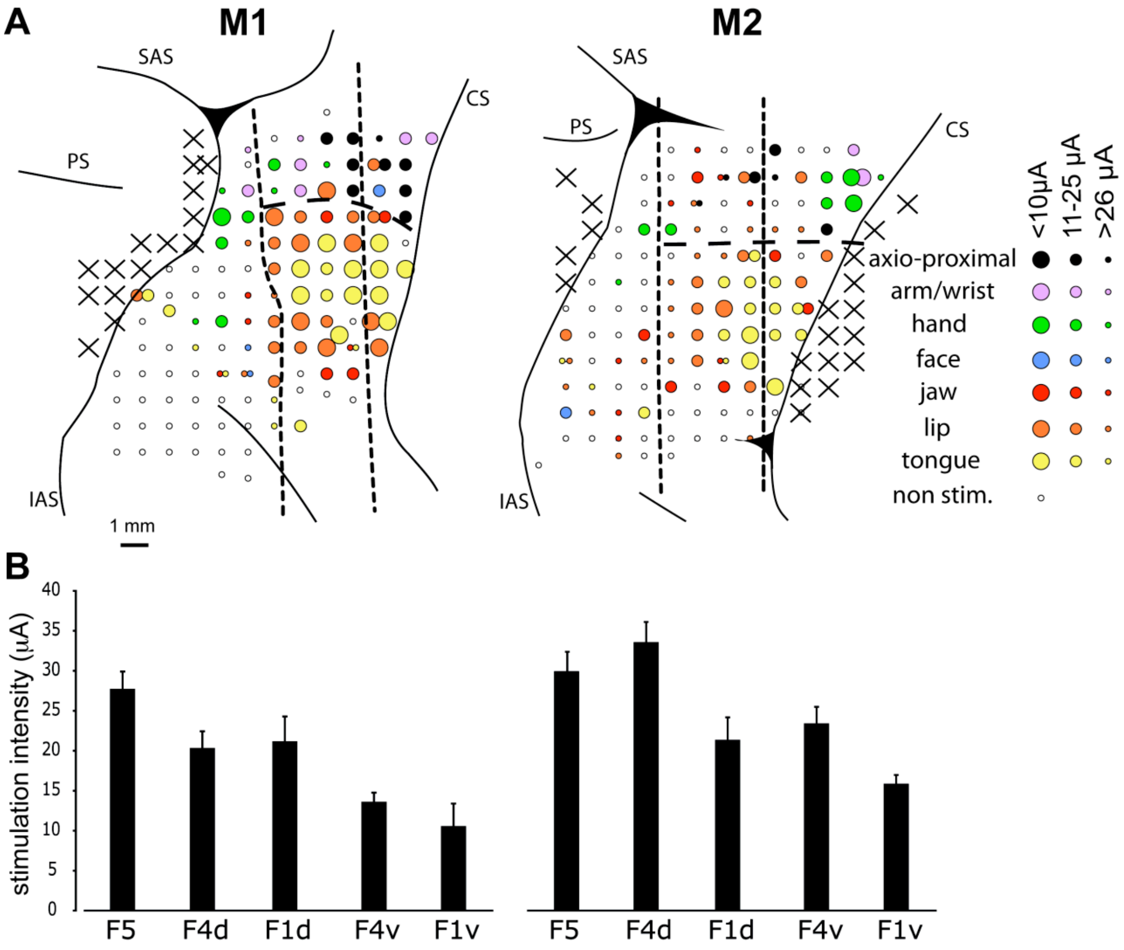

Figure 3. Distribution of movements evoked by ICMS and their thresholds in the two monkeys. (A) Localization of movements evoked by ICMS: circles of different color identify the body-part evoked by stimulation in each penetration, while the size of the circles represents the lowest threshold for that penetration. Little white circles represent penetrations in which it was not possible to evoke any movement with the used stimulation parameters. Others conventions as in figure 2. (B) Histograms represent the mean threshold for the penetrations in each investigated region. Abbreviations as in Figure 1.

The stimulation maps present a rough somatotopic organization. Brachio-manual and axial movements were evoked only in the dorsal portion of the primary motor and premotor cortices. In particular, in M1, hand movements were represented in dorsal areas F5 and F4, while in M2 they were mostly confined in a dorsal and caudal portion of area F1, although hand movements could be evoked in some penetrations in F4 and F5. In both monkeys, arm and axial movements were evoked in a region up to 4-5 mm rostrally to the central sulcus. Tongue movements occupy a wide region closed to the central sulcus and, around this core of penetrations, there are some spots characterized by lip and jaw movements.

In both monkeys there is a core of penetrations where movements of different body-parts were evoked at low threshold (less than 10 μA). This core is located near the central sulcus and includes, in M1, more than half (53.3%) of all the penetrations falling in the explored areas F4v and F1v. In M2 we found only a few penetrations (8.6%) with very low threshold, mainly localized in F1 or in the most caudal part of F4v. Moving rostrally, the electrical excitability drops dramatically and, in F5, besides a low number of penetrations in which it was possible to evoke some movements, no penetration was found with threshold lower than 10 μA.

A 2x5 factorial ANOVA (factors: Monkey and Area) was applied in order to compare in the two monkeys the stimulation thresholds of the distinct anatomo-functional subdivisions. This analysis revealed a main effect of the factor Monkey [$F(1)=18.1$, $p<0.001$], indicating that, in M1, ICMS thresholds are lower as compared to those of M2. More interestingly, a significant main effect of the factor Area [$F(4)=13.5$, $p<0.001$], followed by Newman-Keuls post-hoc tests, revealed that area F5 has stimulation thresholds similar to those of area F4d, and both these subdivisions have higher thresholds than those of all the remaining anatomo-functional sectors ($p<0.05$ for all comparisons). Furthermore, F1v shows the lowest thresholds as compared to all the others sectors ($p<0.05$ for all comparisons).

Motor responses

Motor activity was observed in association with brachio-manual and mouth movements. Particular attention was dedicated to test whether the neuronal discharge was related to simple movements of a specific effector (hand, arm, mouth or face) or to motor acts endowed with their specific goal (see Materials and Methods section for details). Results show that, as far as the mouth is concerned, in the investigated cortical sectors there are different types of mouth-related motor activities, some encoding simple movements (e.g. tongue protrusion), others related to goal directed motor acts (e.g. licking). In contrast, as far as hand and arm are concerned, only activity related to motor acts was observed.

Figure 4 shows that areas F4v and F1v have in common a lower percentage of sites with responses during motor acts as compared to their dorsal subdivisions, as well as to area F5.The data evidence a shift toward an increasing proportion of sites with activity related to motor acts rather than movements moving dorsally and rostrally, although with some differences between monkeys. In fact, in M1, F5 is the area with the highest percentage of responses during motor acts, as compared to both F4d (χ^2=11.98, p<0.001) and F1d (χ^2=82.65, p<0.001), and to both F4v (χ^2=427.93, p<0.001) and F1v (χ^2=186.36, p<0.001). In M2, responses to motor acts are less frequently found in F5 than in F4d (χ^2=43.48, p<0.001) and F1d (χ^2=23.84, p<0.001), which are similar one to the other in this respect (χ^2=2.04, ns). However, F5 has a higher percentage of sites with activity related to motor act than both areas F4v (χ^2=97.70, p<0.001) and F1v (χ^2=111.31, p<0.001). In both monkeys, areas F1v and F4v show the larger number of sites with activity related to simple movements (p<0.001 for all the comparisons).

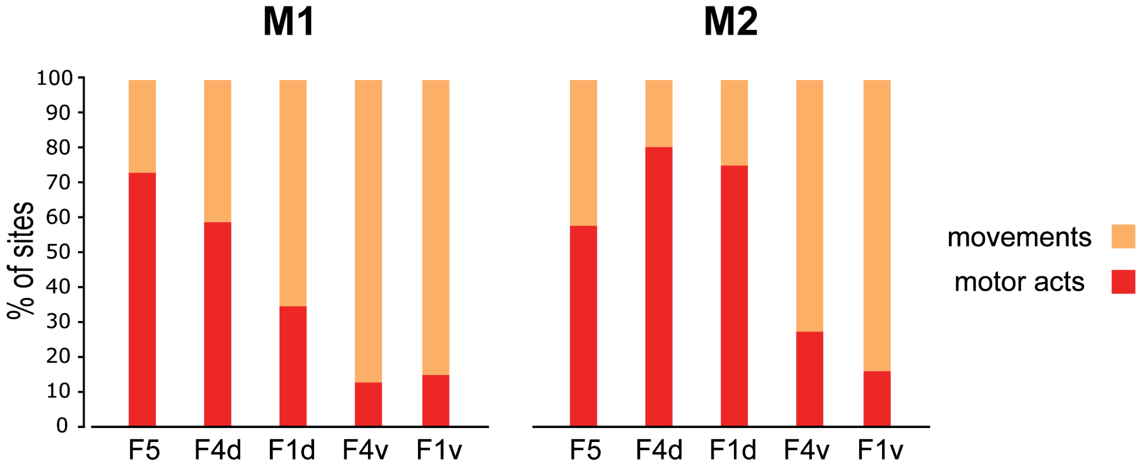

Figure 4. Relative proportion of the sites with responses during movements (orange bars) and motor acts (red bars). All the conventions as in figure 2.

Figure 5 shows the localization of brachio-manual motor acts and of axial movements in the investigated areas. These type of responses are completely absent in the ventral sector of F4 and F1 of M1, and poorly represented in the same sectors of M2, mainly confined near the anatomo-functional border with their dorsal counterparts. In contrast, brachio-manual motor acts are widely represented in both area F5 and the dorsal portion of F4 and F1. Responses related to axial movements are present in the dorsal portions of areas F4 and F1.

Considering the distribution in the investigated areas of each effector, area F1d has the highest percentage of axial responses than those in F4d for both monkeys (M1: χ^2=5.09, $p<0.05$; M2: χ^2=11.89, $p<0.001$), while some differences emerge between the two monkeys as far as responses during arm motor acts are concerned. Indeed, in M2 these responses are nearly absent in F5, while they are similarly represented in F4d and F1d (χ^2=2.84, ns). In contrast, in M1, arm motor acts are less represented and similarly distributed in the three areas.

Sites with responses during hand grasping are widely distributed in area F5 and in the dorsal sectors F4 and F1 in both monkeys. On the contrary, the ventral sectors of area F4 and F1, are the areas with the lowest percentage of sites with hand responses ($p<0.001$ for all the comparisons with the other subdivisions).

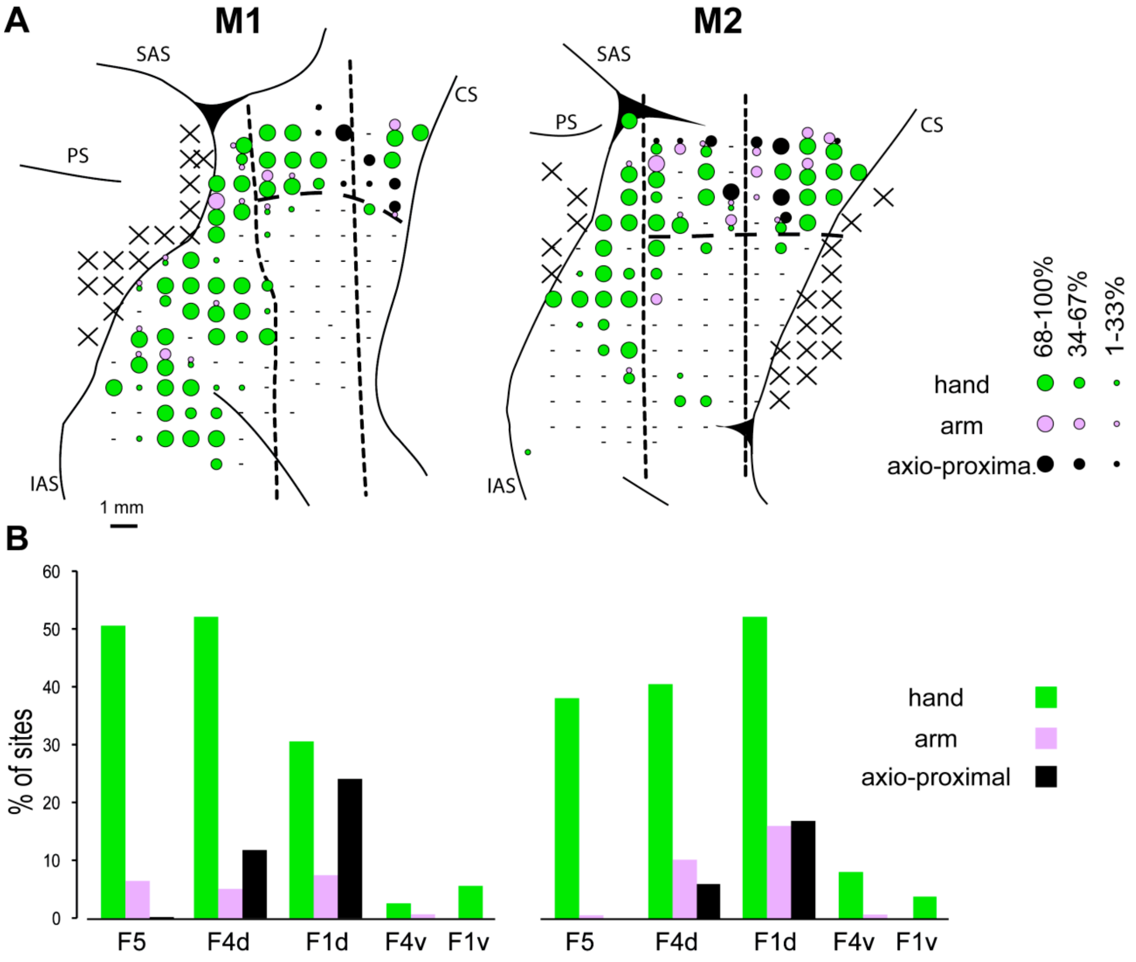

Figure 5. Distribution of hand and arm motor acts and axial movements in the investigated areas. (A) Circles of different colors represent hand grasping (green), arm bringing/reaching (pink) and axial (black) responses. The size of the circles represents the percentage of sites per penetration in which each property was found. (B) Distribution of sites with hand grasping, arm bringing/reaching and axial responses for each of the identified sectors calculated with respect to the total number of sites recorded in that sector. Other conventions as in figure 2.

By employing objects of different size and shapes for testing monkeys' grasping act (see Materials and Methods), it has been possible to evidence some selectivity for the type of grip in a considerable percentage (59.1%) of the investigated grasping-related sites.

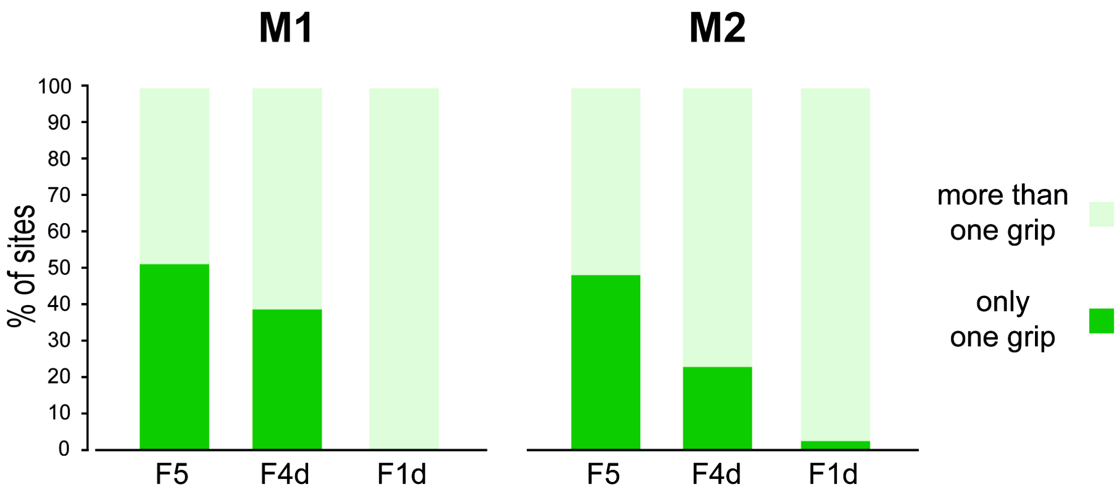

Figure 6. Distribution of sites with responses during grasping performed with only one or more than one type of grip. Note that the ventral sectors of F4 and F1 are not included since sites with hand-related motor responses are nearly absent in these regions. Dark green: sites showing activity related to hand grasping with only one grip; light green: sites showing activity related to hand grasping with more than one type of grip.

Figure 6 shows the proportion of sites, for each area, in which grasping activity was present for only one or more than one grip type. It is evident that the investigated sector differ in terms of grip selectivity. In area F1d, almost all the sites showed responses during grasping when performed with different types of grip in both monkeys. In F5, about half of the sites in both monkeys showed activity related to only one specific type of grip. By comparing F5 grip selectivity with that of the other subdivisions, it emerges that sites with responses to only one type of grip are more represented in this area than in F4d (M1: χ^2=13.55; M2: χ^2=11.92, p<0.01 for both the comparisons) and in F1d (M2: χ^2=63.88, p<0.001).

Figure 7 shows the distribution of mouth motor acts (Figure 7A) and simple movements (Figure 7B) in the investigated region. The most frequent type of mouth-related motor act was mouth grasping (18,9% of all the sites with mouth-related motor responses). Other motor acts involving the mouth, such as licking (9,1% - Figure 8A) or sucking (8,7% - Figure 8B), was also represented, although to a minor extent. Activity during mouth simple movements was related to jaw (32,6 % - Figure 8D) and tongue (30,6% - Figure 8C).

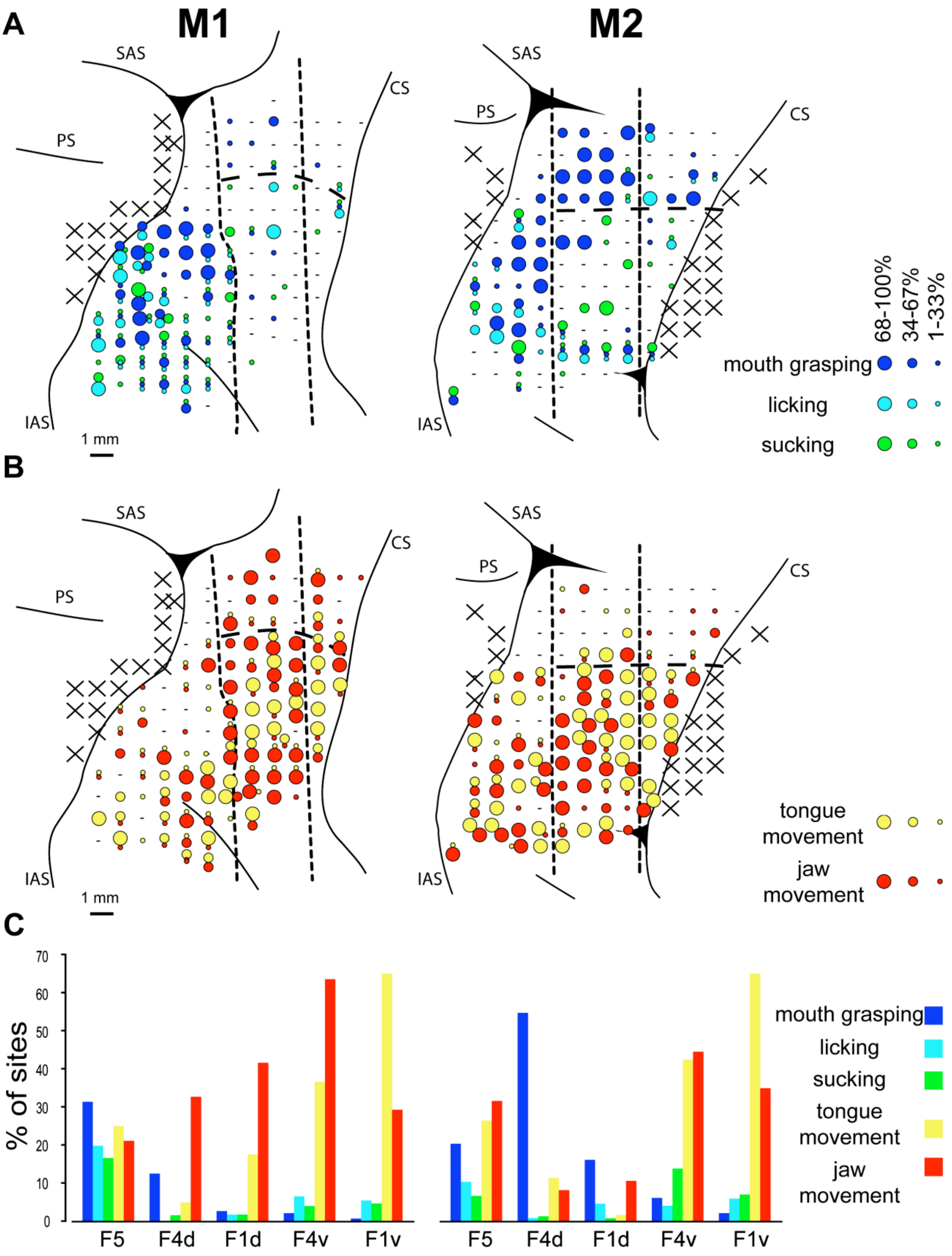

Figure 7. Distribution of mouth motor acts (A) and simple movements (B) in the investigated areas. (C) Histograms representing the relative proportion of mouth grasping (green bars), licking (light blue bars) and sucking (dark blue bars) motor acts and of tongue (yellow bars) and jaw (red bars) simple movements. Other conventions as Figure 2.

Tongue and jaw simple movements are widely represented especially in areas F4v and F1v, with a certain degree of overlapping between the two representations. However, these two regions contain a different distribution of sites responding during simple movements depending on the specific effector. In fact, sites with responses during tongue movements are prevalent in F1v than in F4v in both monkeys (M1: χ^2=8.01; $p<0.001$; M2: χ^2=28.04; $p<0.001$), while in F4v there is a higher number of sites with responses during jaw movements as compared to F1v (M1: χ^2=41.13, $p<0.001$; M2: χ^2=4.72, $p<0.05$). These two regions contain a low percentage of sites with responses during mouth motor acts (mouth grasping, licking and sucking), while these are more represented in area F4d (M2) and in area F5 (M1).

Figure 8 shows the examples of neurons discharging during mouth motor act, licking (A) and sucking (B), and during tongue (C) and jaw (D) movements.

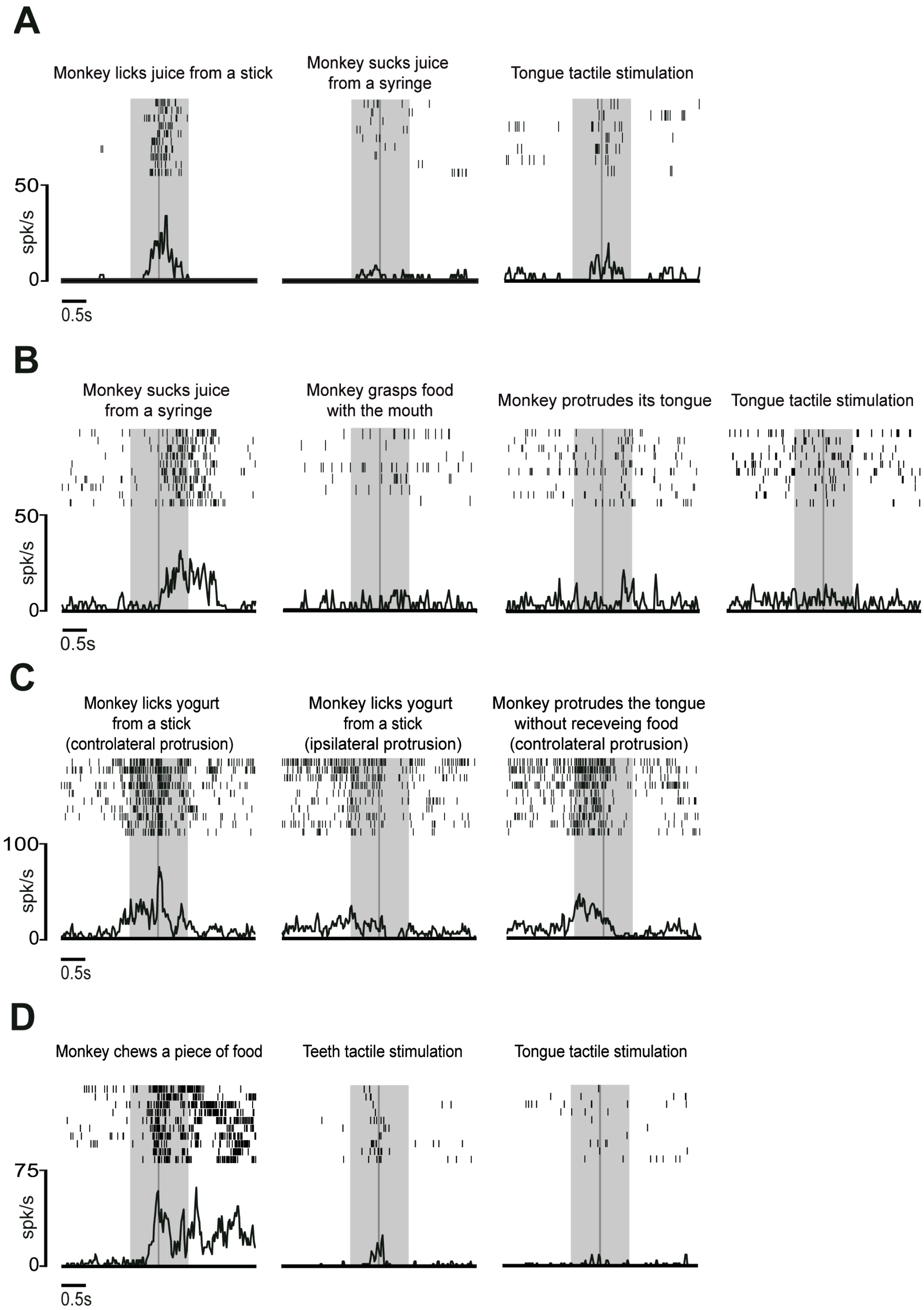

Figure 8. Examples of neurons responding during execution of different mouth motor acts. (A) Neuron shows selective discharge when monkey licks juice from a stick. (B) Neuron selectively discharges when monkey sucks juice from a syringe. (C) Neuron discharges with the same

intensity when monkey licks yogurt from a stick and when protrudes tongue only controlaterally. (D) Neuron discharges for rhythmic jaw movements during chewing. Examples of mouth motor acts were recorded in F5, while example of tongue and jaw movements were recorded in F1v and F4v, respectively. In each panel, the rasters and histograms represent the neuron response during a single experimental condition. The histogram represents the neuronal activity averaged across ten trials. The activity is aligned with the moment at which the monkey touches the target object with the tongue or the lip. Abscissae: time in ms (bin width, 20 ms). Ordinates: firing rate in spikes/s.

Somatosensory responses

Somatosensory responses are widely represented in the investigated areas, mainly in association with motor activity.

The tactile fields are, typically, large, often including more than one body parts (face and arm). The majority of them is bilateral (70.8%), while 27.9% are located on the side of the body contralateral to the recorded hemisphere. Tactile fields confined on the ipsilateral side of the body are rare (1.3%). The large majority of tactile fields are on oro-facial region (71%), followed by those on the brachio-manual one (25.5%). Because of the difficulty to discriminate among tactile responses of different structures inside the mouth, we classified these responses as “inner mouth”. Our testing procedures (see Materials and Methods) allowed us to discriminate among the different somatosensory submodalities. “Deep touch” is well represented (26.6%), while “joint” mobilization (10%) and “light touch” (3.9%) are less frequently found. Almost half (59.5%) of the sites showing responses to somatosensory stimuli activate for more than one submodality.

Figure 9 shows the distribution of somatosensory brachiomanual (A) and orofacial (B) tactile fields in the investigated areas. It clearly emerges that sites with somatosensory responses are widely distributed in all the investigated areas. It also shows that there is a somatotopic organization in the localization of the tactile fields: those localized on the hand, arm or neck/trunk are nearly absent in the ventral sectors of area F4 and F1,that are instead mainly related to inner mouth. Interestingly, we observed an association between brachiomanual and orofacial tactile fields: those located on brachiomanual and on face and lips surface clearly overlap in F4d and F1d in both monkeys, and in F5 (only in M2).

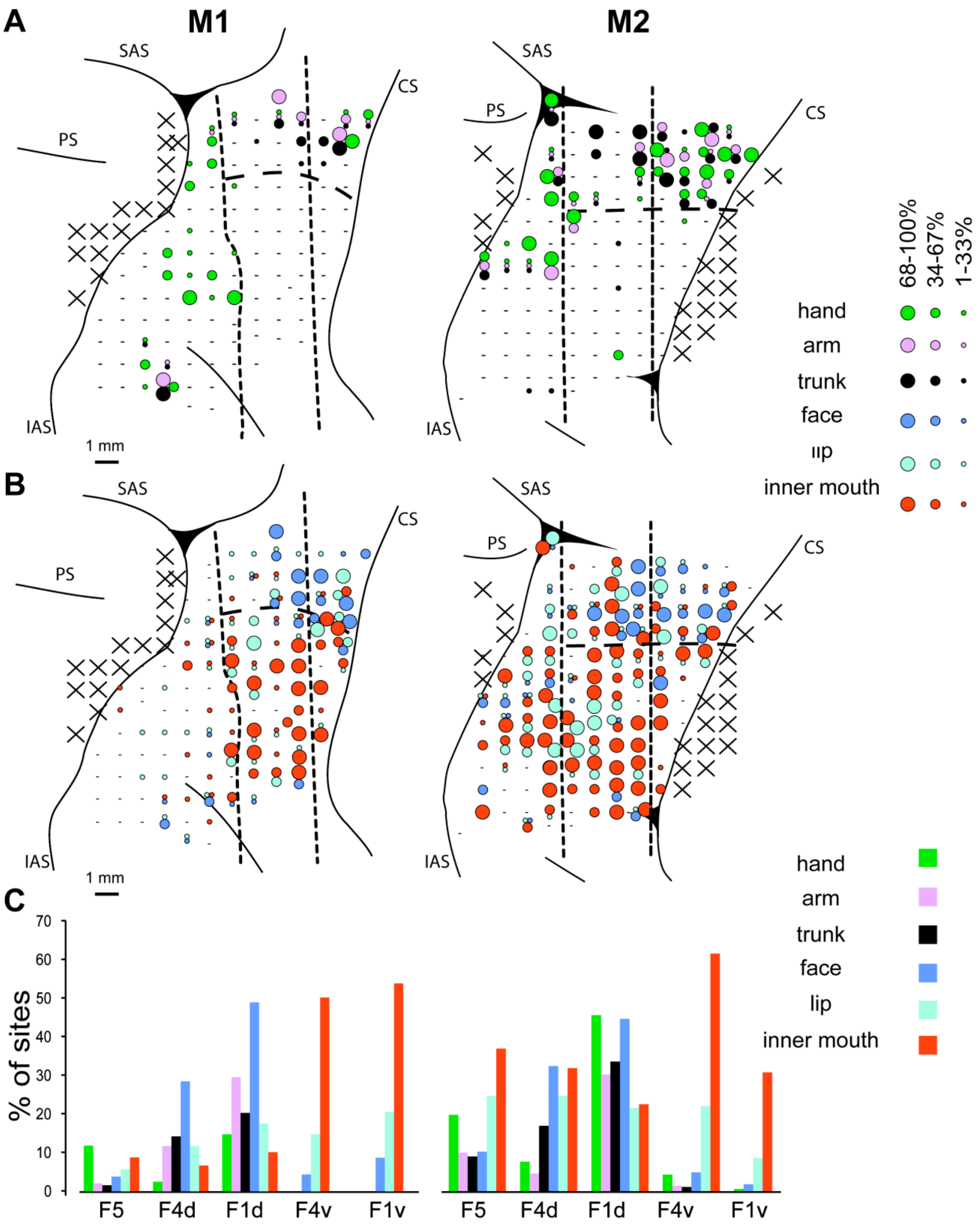

Figure 9. (A) Distribution of axial (black), arm (pink) and hand (green) tactile fields. (B) Distribution of tactile fields on face (blue), lips (light blue) and on the inner mouth (red). (C)

Proportion of the different tactile fields in the investigated areas. Other conventions as in Figure 2.

Figure 10 shows the percentage of sites showing sensory and motor responses, separately or in association. Both sites with motor (M) and with associated sensory and motor (M+S/V) responses are widely represented in the investigated region. In general, sensory-motor sites are more frequently found than those with motor responses alone (χ^2=46.27; p<0.001), despite some differences between the two monkeys. In fact, in M1, the number of purely motor sites is higher than that of sensory-motor sites (χ^2=22.76; p<0.001), while in M2 it is lower (χ^2=205.88; p<0.001). In absence of any motor response, sites with somatosensory responses, alone (M1: 5.1%; M2: 6.3%) or in association with visual responses (M1: 1.2%; M2: 1.0%) are rare.

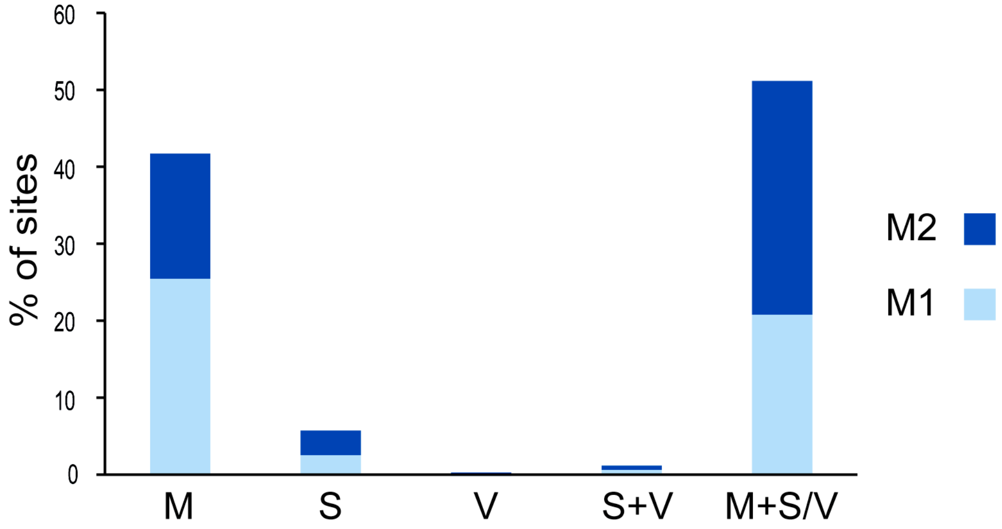

Figure 10. Distribution of sites showing motor, somatosensory or visual responses, or the combination among two or more responses. Light blue and dark blue represent the percentage of sites recorded in M1 and M2, respectively, calculated on the total number of sites recorded in both monkeys. M, motor; S, somatosensory; V, visual; S+V, somatosensory+visual; M+S/V, motor + somatosensory and/or visual.

Visual properties

A variety of visual properties were found in the 19% of the recorded sites. A really small percentage of these sites showed purely visual responses (5.7%), while the majority showed also activity related to body-parts movements.

Visual responses have been assigned to different categories according to the type of visual stimulus most effective in eliciting them. They will be described below, ordered on the basis of their relative frequency among all the visual sites included in the investigated areas.

Peripersonal (n=214; 43%). This category is formed by sites responsive to 3-D objects moved in the visual space within monkey reaching distance. Activity could be related to stimuli moved either in a tangential plane (5.9%), or along a circular trajectory (6.9%), or in depth towards (79.4%) or away from (1.5%) the monkey. In some cases (6.3%) the response could be independent of the plane and direction of the moving stimulus. The visual fields in which the presentation of the stimuli was effective in triggering neuronal response were generally large, and in most cases located near the face (70.0%) or face and forelimb/trunk together (15.0%). In a smaller number of sites the region of the visual field in which stimulus presentation was effective was located near the forelimb (11.0%), or near all body parts, both ipsi- and contralaterally (4.0%). Furthermore, in most cases the receptive visual field was controlateral (53.3%), in many other cases mostly bilateral (44.4%), while more rarely around ipsilateral body parts (2.3% - Figure 10A).

Almost all (87.4%) of the sites with peripersonal visual responses also showed tactile responses related to the body parts correspondent to the visual spatial field.

Mirror properties (n=168; 34%). The sites showing mirror properties have been studied for both their visual and motor responses. According to previous studies (Gallese et al., 1966; Rizzolatti et al. 1996), sites with mirror responses do not respond or respond very weakly to object presentation and to mimicking of motor acts in the absence of the target object. The majority of sites with mirror properties showed activity related to the observation of hand motor acts (57.9%), or of both hand and mouth motor act (33.3%- Figure 10C). A little percentage of sites with mirror responses (8.8%) was activated by the observation of mouth motor acts performed by the experimenter. Figure 10B shows an example of a mirror neuron discharging during tearing/breaking.

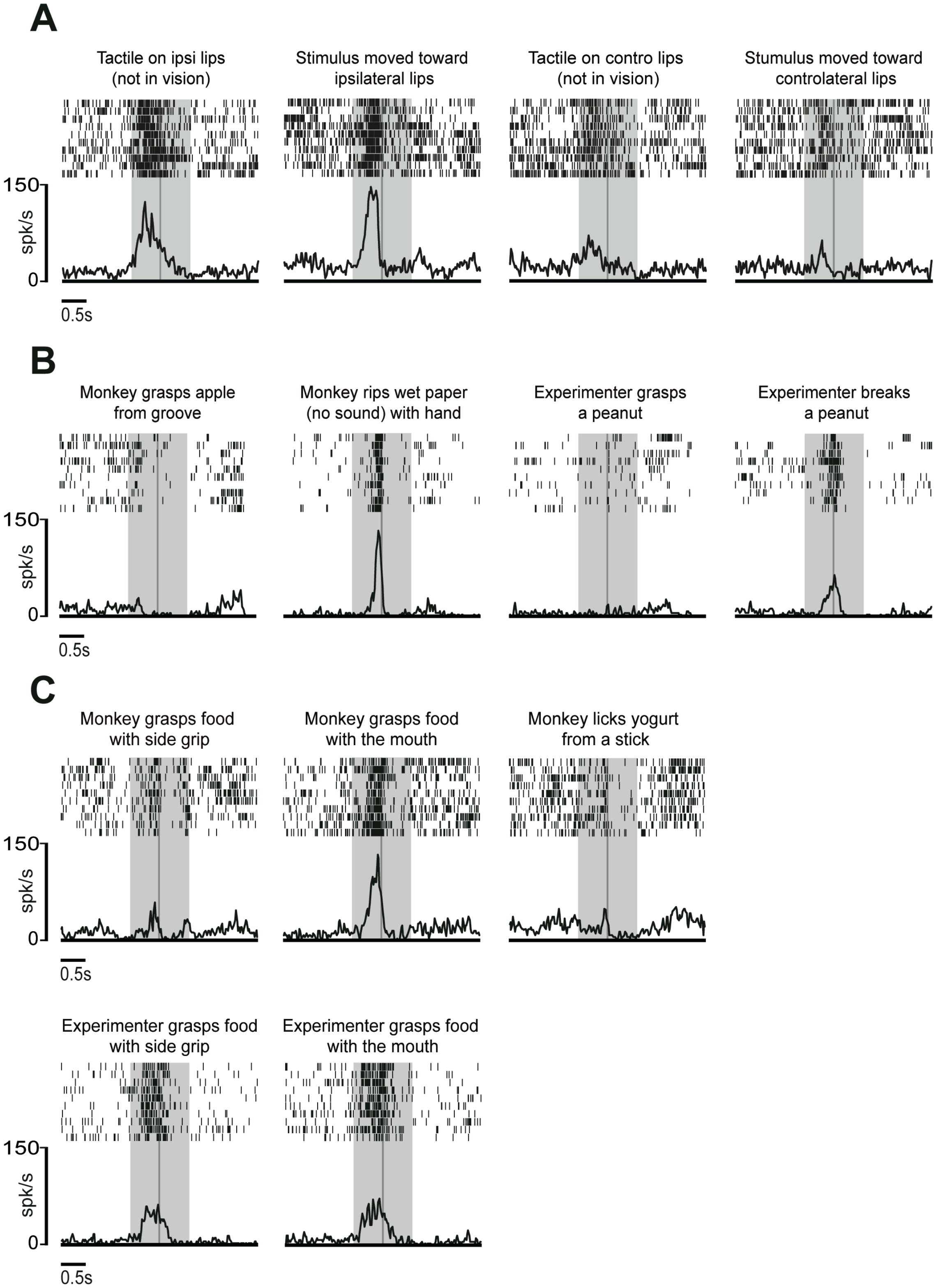

Figure 10. Examples of neurons showing different visual responses. (A) A peripersonal neuron discharging when a stimulus is approaching the ipsilateral lips, showing a visual RF in register with tactile RF. (B) A mirror neuron discharges during execution and observation of a tearing/breaking motor act. (C) A mirror neuron active during both hand and mouth grasping.

Peripersonal neuron was recorded in area F4d, while both mirror neurons were recorded in area F5. Conventions as in Figure 8.

Moving objects (n=54; 11%) and *biological motion* (n=26; 5%). These categories are formed by responses to body-parts movements. Sites responsive to moving objects also activate during motion of different types of objects in the monkey visual field. Note that these objects were held by stick or other tools, in order to prevent the monkey to see experimenter's body parts. In contrast, sites responsive to biological motion were activated specifically by the sight of the experimenter's hand/arm, head, trunk or limb moving in the monkey visual field.

Object presentation (n=34; 7%). The majority of sites with object presentation responses (87,2%) were tested also during execution of active movements: in all these cases they appear to be characterized also by motor properties.

Figure 11 shows the distribution of visual properties in the investigated region. It is evident that the different categories of visual responses are differently distributed in area F5, F4d and F1d, and poorly represented in the ventral sectors of area F4 and F1. The dorsal sectors of F4 and F1 are the areas with the highest percentage of peripersonal responses as compared to F5 ($p<0.001$ for all the comparisons), and they do not differ from each other in both monkeys (M1: $\chi^2=0.40$, ns; M2: $\chi^2=0.01$, ns). Mirror responses increase in their number moving rostrally, becoming higher the number of sites with mirror responses in area F5 than F1d (M2: $\chi^2=21.57$, $p<0.001$) and than F4d (M1: $\chi^2=6.83$, $p<0.001$; M2: $\chi^2=10.37$, $p<0.001$) in both monkeys. Sites with responses to moving objects are richly and similarly represented in F4d and F1d of only M2 ($\chi^2=2.59$, ns). The other visual properties (object presentation and biological motion) are poorly represented in F5, F4d and F1d of both monkeys.

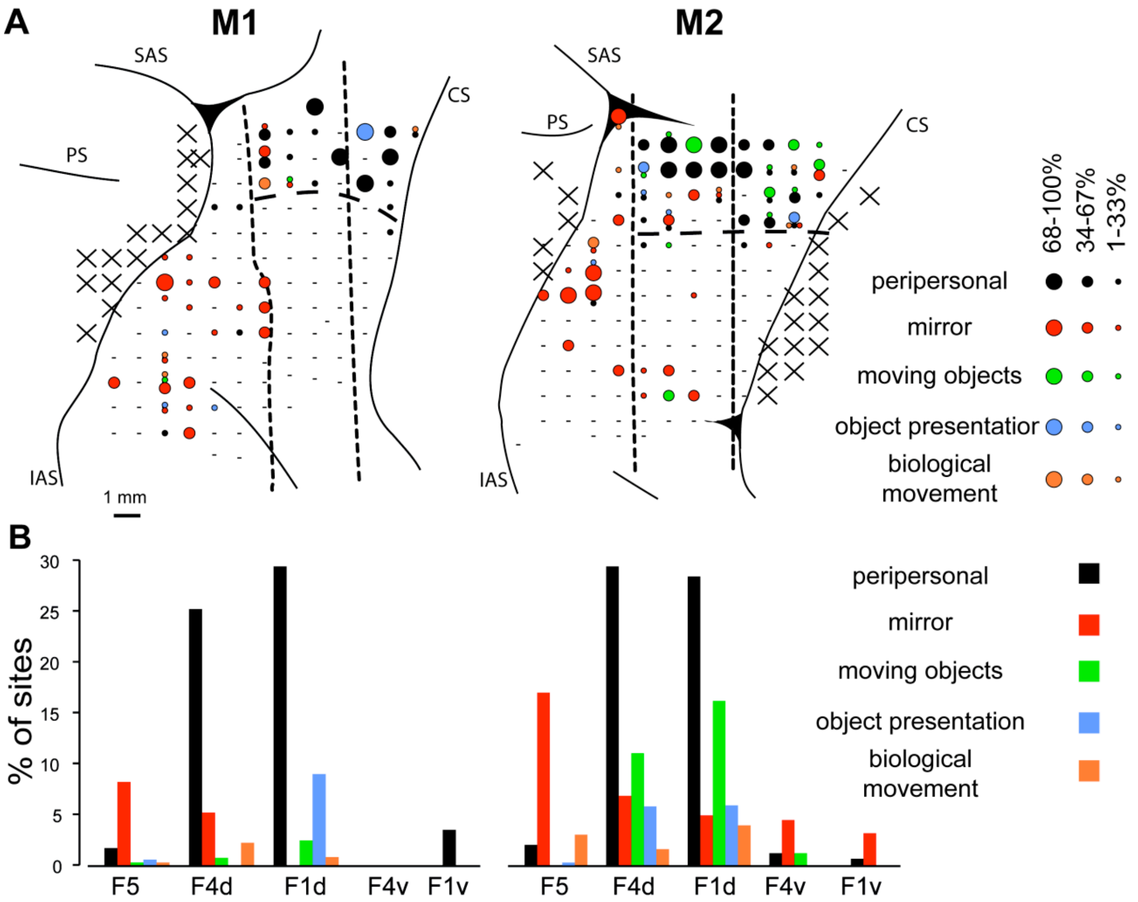

Figure 11. (A) Representation of the different types of visual responses in the investigated sectors. (B) Proportion of each type of visual responses in each of the investigated anatomo-functional subdivisions. Conventions as in Figure 2.

Relationship between visual and motor properties

The several types of visual responses are differentially located in the investigated region. Therefore, we have carried out further analyses in order to study the relationship between visual properties and motor responses derived by extracellular recordings and ICMS. Since the study of the relationship between mirror and motor responses was of particular interest, we compared the localization of mirror responses in the investigated areas with that of the other visual responses collapsed together. In fact, some visual properties (moving objects, biological motion and object presentation) are present in a low percentage and they are often found in the same penetrations of peripersonal responses. We defined this category as “non-mirror visual responses”.

Figure 12 shows the distribution of penetrations with mirror responses (red circles) and with non-mirror visual properties (green circles) superimposed to the brachio-manual and mouth motor fields (blue and yellow regions, respectively), as resulted from extracellular recordings. It emerges a certain topographic organization with mirror penetrations more concentrated in F5 than in the dorsal sectors of F4 and F1, where non-mirror visual penetrations are located. Interestingly, it emerges that penetrations with mirror responses are localized in the region of overlap between brachio-manual and mouth motor activity.

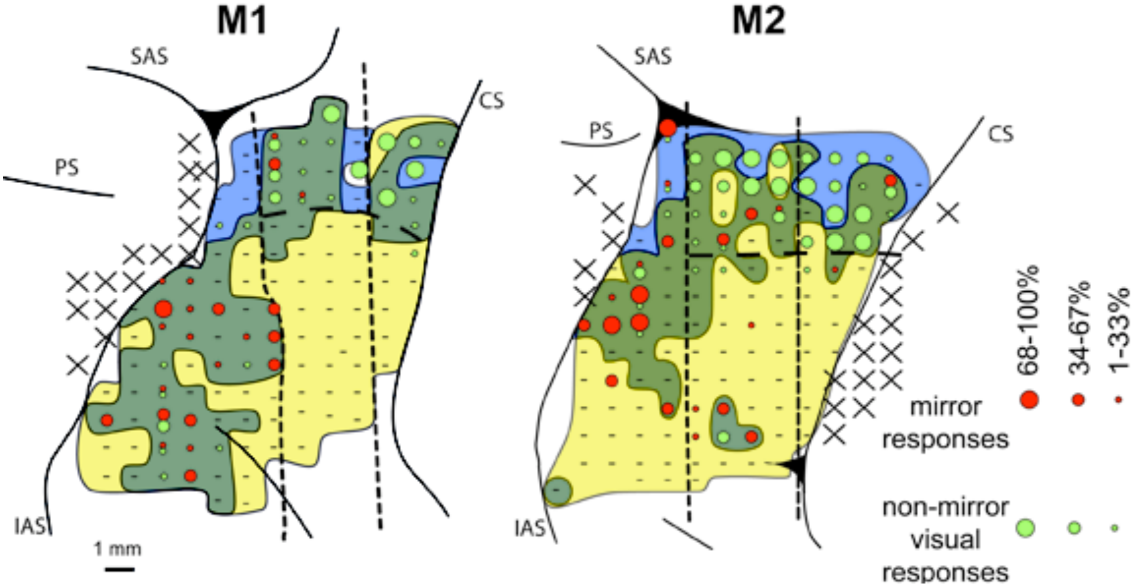

Figure 12. Distribution of penetrations with extracellular multiunit activity related to brachio-manual (blue), mouth (yellow) or brachio-manual and mouth responses (green) and localization of penetrations with mirror responses (red circles) and with non-mirror visual responses (yellow circles. Conventions as Figure 2.

In Figure 13A penetrations with mirror and non-mirror responses are superimposed to the excitable (grey region) and non-excitable (white region) parts of the investigated region. It clearly emerges that the penetrations with mirror responses were mostly found in non-excitable sector, while those with non-mirror visual responses are widely distributed also in the excitable region. Figure 13B shows the percentage of penetrations with mirror and non-mirror visual responses that show electrical excitability. It emerges that the percentage of non-excitable penetrations with mirror responses is higher than the percentage of non-excitable penetrations with non-mirror visual responses ($\chi^2=5.97$, $p<0.05$).

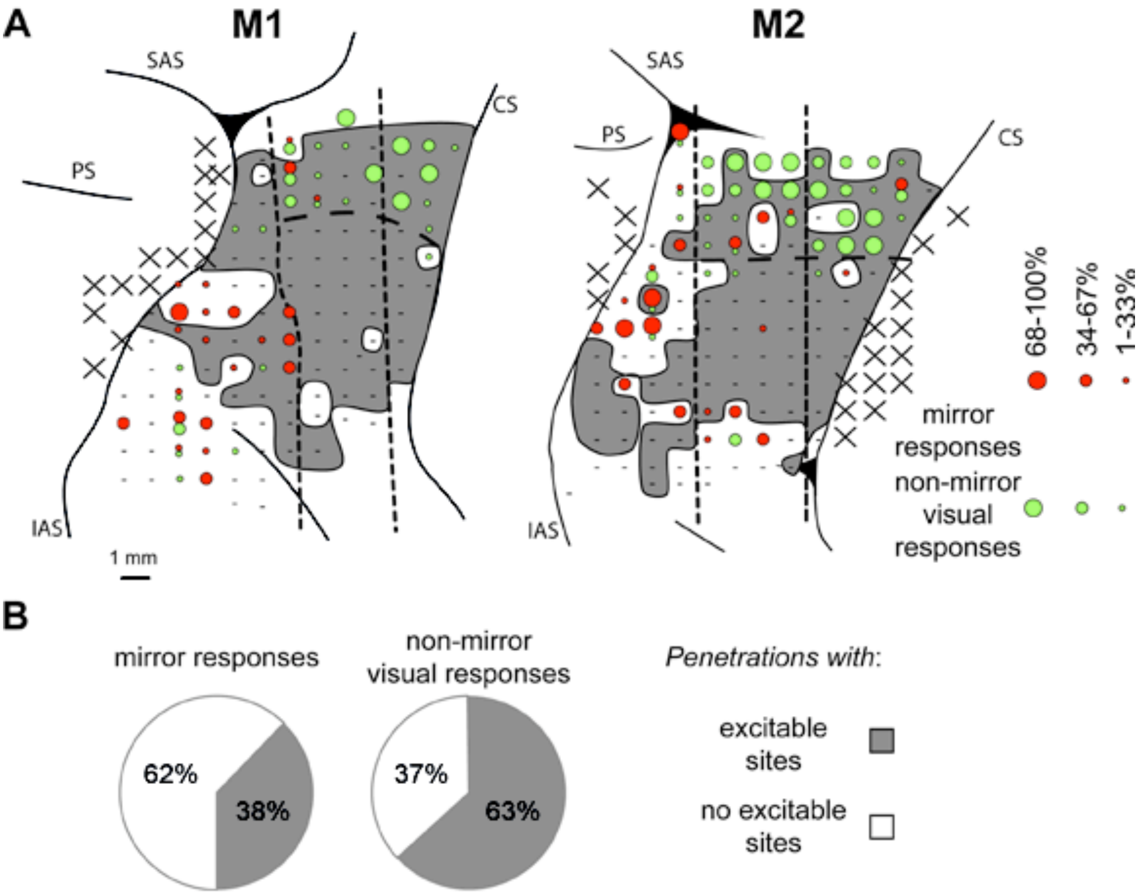

Figure 13. Representation of the excitability of the investigated region and the distribution of penetrations with visual responses. (A) Grey area represents the region in which excitable penetrations were found. Circles identify the localization of penetrations with mirror (red) and non-mirror visual (green) responses. (B) Pie charts represent the proportion of excitable (grey) and non-excitable (white) penetrations, with mirror and non-mirror visual responses. Other conventions as Figure 2.

4. DISCUSSION

One of the main findings of this study was the identification of a functional border dividing the primary motor area F1 and the premotor area F4 in a dorsal and a ventral part. Indeed, the study of motor responses and the results of intracortical microstimulation revealed that the dorsal sectors of areas F4 and F1 (F4d and F1d) contain a representation of the hand and the arm, partly overlapped with the mouth field, which, in turn, is mainly confined to the ventral sectors of the same areas (F4v and F1v). Motor and somatosensory properties are widely distributed in all the investigated areas, and usually coupled based on the body part to which neuronal activity was related. Simple movements are encoded in both F4v and F1v, while responses during motor acts increase going dorsally and rostrally. Visual responses were almost absent in the ventral sectors of F4 and F1, while they were frequently found in area F5 and in areas F4d and F1d. Interestingly, visual responses in area F5 were mostly related to action observation (mirror responses) and confined to non electrically excitable sites; on the contrary, in F4d and F1d visual responses were related to the encoding of the peripersonal visual space and were mainly found in electrically excitable penetrations.

4.1. Dorso-ventral functional subdivisions of cytoarchitectonic areas F4 and F1

From the first conceptualization of a “premotor cortex” (Fulton, 1935), an increasing amount of data have supported the idea of a hierarchical organization of the cortical motor system, in which the premotor areas are involved in sensory-motor transformations necessary for action organization, while the primary motor area F1 is deemed to play a major role in segmenting motor acts into elementary movements (Rizzolatti et al. 1998), thanks to its direct connections with the motor neurons of the spinal cord (Luppino and Rizzolatti 2001; Lemon 2008).

Based on the work of Matelli and colleagues, the lateral part of the frontal motor cortex is formed by three cytoarchitectonic areas (Matelli et al. 1985; see also Belmalih et al. 2007; 2009), namely, areas F1, F4 and F5. Area F1 corresponds to the primary motor cortex. Area F4 has been shown to play a role in coding of peripersonal space and in the organization of reaching/avoidance motor acts (Gentilucci et al. 1983; Fogassi et al. 1996; Rizzolatti et al. 1998). Finally, area F5 appears to be crucial in visuo-motor transformations for grasping and for the encoding of goal-directed motor acts (Gentilucci et al. 1988; Rizzolatti et al. 1988; Rizzolatti et al. 1998). Thus, anatomically distinct cortical areas have specific functional roles.

In our study we first identified cytoarchitectonically the three areas F1, F4 and F5. Then, we attempted to quantitatively compare the distribution of motor, visual and ICMS responses among them, and we found that, along the dorso-ventral axis within the cortical surface of F1 and F4, there was a different topographic segregation of the main functional properties. This latter finding was highly consistent across monkeys. Therefore, we adopted an integrated set of criteria which allowed us to trace a further functional border between the dorsal and ventral parts of cytoarchitectonic areas F4 and F1. As far as area F5 is concerned, it could be possible to identify in it a similar dorso-ventral subdivision, with a dorsal field encoding executed and observed hand motor acts, and a ventral field encoding mouth or hand-and-mouth motor acts, accordingly with previous neurophysiological data (Rizzolatti et al. 1981; Rizzolatti et al. 1987; Gentilucci et al. 1988; Rizzolatti et al. 1988; Ferrari et al. 2003). However, we could not directly trace this border due to the low number of penetrations falling in this restricted sector.

The existence of a dorso-ventral gradient in the ventral part of the motor and premotor cortices is also supported by anatomical evidences. For example, the size of pyramidal cells in layer V decreases moving medio-laterally (Belmalih et al. 2007), very likely depending on the distance the axons have to cover according to their role in the control of hand-arm or mouth movement. Some authors have also proposed a dorso-ventral subdivision of Broadmann's areas 4 and 6 (Barbas and Pandya 1987), but they did not find any difference moving rostro-caudally, in contrast to Matelli and colleagues (1985). As suggested by Belmalih and colleagues (2007), different techniques, methodologies and criteria for the anatomical parcellation of cortical regions produce highly different results. This could justify the lack of agreement between the above mentioned studies dealing with the cytoarchitectonic definition of motor and premotor regions. Our results show that the integration of functional data with a multiarchitectonic approach enables to reveal further discontinuities within anatomically identified cortical areas.

4.2. Intracortical microstimulation evokes brachio-manual and oro-facial movements somatotopically organized

The results of ICMS showed a rough somatotopic organization of the effectors, with brachio-manual and oro-facial movements arranged along a dorso-ventral axis, encompassing all the investigated areas, according to previous studies (Gentilucci et al. 1988).

Arm and axio-proximal movements were evoked by stimulating F1d and F4d, while hand movements have been observed following the stimulation of the dorsal portion of F5 and of some sites of F1d (only in M2). This topographic organization is in line with previous studies in which distal movements were found mostly near the central and the arcuate sulci, while proximal movements were mainly located in the cortical convexity between these sulci (Kwan et al. 1978; Gentilucci et al. 1988). However, the most dorsal portion of the F1 sector investigated in this work appears to be slightly more lateral than the location of the core of hand-digits representation reported by previous studies (Maier et al. 2002). Thus, the primary motor hand representation was largely outside our region of interest.

It is well known that the primary motor cortex forelimb representation is characterized by a nested organization of different effectors, with the fingers zone surrounded by that of wrist and elbow (Kwan et al. 1978). Such an organization could be relevant for enabling the control of a wide variety of movements biomechanically coupled (Schieber 2001). Although we could not clearly observe such an organization relatively to forelimb because of the limited hand-and arm region explored, the representation of oro-facial movements appear to be topographically organized in a similar way. In fact, we found a core of penetrations in which ICMS evoked tongue movements, surrounded by penetrations related to lips and jaw movements. Other studies, although explored a more limited portion of the lateral motor cortex, found a representation of facial movements clustered together in the posterior and anterior portions of the precentral gyrus, with a wide representation of tongue movements in the intervening region (McGuinness et at. 1980). A successive study in which the investigated region extended more medially supported these results, showing that the medial extent of face and jaw representations overlapped with the lateral extent of the forelimb representation (Huang et al. 1988). Thus, our and the previous data support the view that multiple cortical zones containing representations of similar movements for the same effector constitute a general organizational principle of the motor cortex. This may favour the emergence of ethologically relevant and smoothly organized actions (Graziano and Aflalo 2007).

As far as the stimulation thresholds are concerned, our results show that the ventral sectors of F4 and F1 are the most electrically excitable and have the lowest stimulation thresholds. In particular, F1v is the sector in which movements could be evoked with the lowest thresholds as compared to all the others investigated sectors. However, the mean threshold for F1v is higher than that found by previous studies (McGuinness et al. 1980). This can be easily explained by the fact that we limited our investigation to the cortical convexity of primary motor and the adjacent premotor areas, according with other studies that did not explore the anterior bank of the central sulcus (Clark and Luschei 1974; Huang et al. 1988). A decrease in the cortical excitability was found moving rostrally and dorsally. Indeed, area F5 has the lowest number of electrically excitable penetrations, usually requiring high current intensity to evoke movements.

Interestingly, by using pulse trains approximating the time scale of natural behaviours, previous studies were able to evoke complex postures involving multi-joint coordinated movements (Graziano et al. 2002), even in cortical regions corresponding to those we found scarcely excitable in our study. Furthermore, these electrically-evoked coordinated movements were arranged in a topographic manner (Graziano and Aflalo 2007): hand-to-mouth movements were found in a cortical sector grossly corresponding to the dorsal portion of area F5 and the rostral part of area F4d, defensive movements in a sector corresponding to our F4d (and the lateral part of the dorsal premotor cortex, not investigated in the present study), and chewing movements in a region corresponding to the ventral portion of F1 and F4 (i.e. F1v and F4v).

Taken together, these data suggest that the ventral portion of area F1 and F4, showing similar properties related to mouth movements, might form a unitary functional area, encompassing anatomical borders.

4.3. Motor properties and sensory-motor associations in the primary motor and ventral premotor cortices

Several studies have demonstrated that neurons of the ventral premotor cortex are active during the execution of goal-directed motor acts rather than during simple movements involving the same effectors (Gentilucci et al. 1988; Rizzolatti et al. 1988; Gallese et al. 1996). These observations derived from an accurate clinical testing of neuronal activities, the same we employed in this study. They demonstrated that ventral premotor cortex neurons, differently from the majority of those recorded in primary motor cortex, responded specifically when the monkey performed a certain hand (i.e. grasping, holding, tearing) or mouth (licking, grasping, sucking) motor act, but not when the same pattern of simple movements involved in that act were employed for performing intransitive gestures (yawning, threatening, lip-smacking) or for other behavioural purposes (scratching, grooming). A recent, more controlled, experimental study, conclusively demonstrated that ventral premotor neurons encode the goal of motor acts regardless of the sequence of simple movements required for performing them (Umiltà et al. 2008). These data suggest that the cortical motor system is hierarchically organized, with premotor regions mainly involved in coding goal directed motor acts, and primary motor cortex devoted to the execution of simple movements.

The results of extracellular recordings carried out in the present study have shown a different distribution of the motor properties, not only in terms of the effector involved, but also of the hierarchical level – motor act or movement - of the motor responses.

One of the main findings is that while for mouth-related motor responses we found both cortical sites related to a specific motor act and others in which the activity was better correlated to simple movements, for hand and arm motor responses we found neuronal activity specifically related to motor acts in all the sites of the investigated region. In fact, these motor acts, besides area F5, are widely represented also in the dorsal portions of areas F4 and F1. Although this finding could appear in contrast with the literature since many authors reported that the primary motor cortex encodes simple movements (Georgopoulos et al. 1982; Lemon et al. 1986), it could be explained by the fact that we limited our investigation to a restricted region of the hand representation of the primary motor cortex that we called F1d. Indeed, this sector is located laterally to the typical primary motor hand-digits motor representation (Lemon 1981), and previous recordings carried out in a sector of area F1 mainly overlapping with that investigated in the present study have shown that almost half (45%) of the recorded neurons encoded the goal of hand motor acts at a high level of motor abstraction rather than the simple movements performed by the monkey (Umiltà et al. 2008).

All the recorded subdivisions are also active during the execution of mouth motor acts, and very often single neurons have been described responding during both hand-and-mouth grasping (see for example Figure 10C), supporting an abstract coding of motor goal, regardless of the specific effector used for achieving it (Rizzolatti et al. 1988; Bonini et al. 2010). Taken together, these findings suggest that the cytoarchitectonic area F1 is not functionally homogeneous, and the sector F1d is characterized by motor properties more similar to those of the rostral premotor areas F4d and F5 than to those of its adjacent dorsal part.

In contrast, areas F4v and F1v show the highest percentage of responses during simple movements of tongue and jaw rather than during specific motor acts, according to previous studies (Murray and Sessle 1992; Huang et al. 1988). Interestingly, by combining ICMS and extracellular recordings, Murray and Sessle (1992) found that different zones of the primary motor cortex controlling tongue movements show different pattern of neuronal activity in order to produce the appropriate change in tongue shape and position for accomplishing a tongue-protrusion task. As far as jaw movement is concerned, although mastication is probably produced by other structures such as the brain stem, activity of the primary motor cortex is considered crucial to the fine control of jaw movement. The remarkable similarity of the motor properties of F4v and F1v revealed by our data fits well with the results obtained with ICMS, and reinforced our proposal of a unitary functional mouth area in the ventral primary motor and premotor cortex, encompassing the anatomical border between F4v and F1v.

The ICMS data and the motor properties discussed up to now point to a functional continuity between, on one side, the ventral portions of areas F4 and F1, mainly related to the control of tongue and jaw movement patterns and, on the other side, the dorsal portions of the same areas F4 and F1, encoding hand and arm or hand-and-mouth goal directed motor acts at a higher level of motor abstraction.

Besides showing a high number of sites encoding specific motor acts, our data revealed that area F5, as compared to hand-related motor sites in areas F4d and F1d, is characterized by a higher number of sites activated during a specific type of grip. Previous findings showed that, particularly in the dorsal part of area F5 and within the sector lying into the posterior bank of the inferior arcuate sulcus (Belmalih et al. 2009), there are neurons showing a high selectivity for the type of grip (Rizzolatti et al. 1987; 1988; Murata et al. 1997; Raos et al. 2006). Although we did not explore the bank of area F5 (area F5p), the finding of a marked grip selectivity support the role of the hand motor activity of this area in the visuo-motor transformations necessary for grasping objects.

The association between tactile and proprioceptive properties with motor responses appears to be a general feature of the motor system (Asanuma et al. 1980; Wong et al. 1978). Also in our study, we found widely distributed somatosensory properties, mainly associated to motor responses. Sensory responses alone, or visuo-somatosensory associations independent from motor activity were rarely found.

According to previous studies (Gentilucci et al. 1988; Rizzolatti et al. 1988; Fogassi et al. 1996), the majority of somatosensory and joint responses has been found in the caudal part of the investigated region, namely area F4 and F1. Our clinical testing easily allowed to identify when neuronal discharge was related to tactile stimulation applied to the skin of the trunk, forelimb or face, allowing us to define also the body region involved in the stimulation evoked response. In contrast, sensory responses related to passive stimulation of structures of the inner mouth could be identified only by employing exclusion criteria, rendering therefore quite problematic the precise localization and extension of their sensory receptive field in these awake monkeys. Therefore, we included responses related to teeth and tongue in a broader category called “inner mouth”. In line with the dorso-ventral segregation of motor responses and ICMS evoked movements, tactile fields located on the external surface of the body (trunk, arm-hand and face) are mainly represented in the dorsal portion of areas F4 and F1. These sectors also show many sites responding to the stimulation of the face and the lips, which are frequently target of forelimb motor acts (e.g. bringing to the mouth). The data on F4d confirm previous works (Gentilucci et al. 1988; Fogassi et al. 1996). Tactile fields located in the inner mouth, on the contrary, where mostly represented in areas

F4v and F1v, in which neuronal activity is enhanced during active movements of the tongue and the jaw.

These data support the motor role attributed to somatosensory properties of motor cortical neurons (Rizzolatti et al. 1981a). In fact, previous studies by Rizzolatti and co-workers carried out in anesthetized monkeys have shown that the response to tactile or joint stimulation of ventral premotor neurons was conditional upon a simultaneous stimulation of another articulation, suggesting a “praxic function” (Rizzolatti et al. 1981) of sensorimotor association within the agranular frontal cortex. More recent data confirmed this view in awake monkeys, showing that sensory-motor associations enable to organize motor acts and simple goal directed actions according to the position of the target stimuli in a somatocentered peripersonal space (Fogassi et al. 1992, 1996; Graziano, Yap and Gross, 1994; Graziano et al. 1997).

Recent studies on higher order sensory-motor functions of the ventral premotor cortex have suggested that area F5 is involved in higher order cognitive function such as decision components linking somatosensory (Romo et al. 2004) sensation with a corresponding motor response and its outcome.

4.4. Visual responses and the relationship between visual and motor properties

Similarly to somatosensory responses, also visual responses have been found mainly in association with motor properties, in about 20% of the recorded sites. However, they are not sparsely distributed among all the studied areas. In fact, visual properties are nearly absent in areas F4v and F1v, while they are widely represented in F4d and F1d, and also in the ventral portion of area F5, in line with previous studies (Gentilucci et al. 1988; di Pellegrino et al. 1992; Graziano et al. 1994; Fogassi et al. 1996; Gallese et al. 1996; Murata et al. 1997; Ferrari et al. 2003; 2005). The lack of visual responses in the ventral sectors of area F4 and F1 is congruent with their motor properties, mostly related to tongue and jaw movements, that require somatosensory feedback rather than visual input. Indeed, these areas could correspond to the region identified by previous studies as involved in the control of fine jaw and tongue movement (Murray and Sessle 1992) and, similarly to the region of the primary motor cortex controlling independent finger movements (which is outside our investigated region), do not present visual responses (Lemon 1981).

Peripersonal (Fogassi et al. 1996) and mirror (di Pellegrino et al. 1992; Gallese et al. 1996) responses are the most represented among visual properties, and present a different topographic distribution. F4d and F1d contain a similar and consistent number of sites with peripersonal responses, while mirror responses are mostly found in area F5. Interestingly, these sectors, that show the richest set of visual properties, are also characterized by motor activity related to brachio-manual motor acts, alone or in association with mouth motor acts such as licking or grasping with the mouth. This support the idea that visual properties, are matched with the representation of motor responses in order to provide the more appropriate support to the organization of action and interaction (Caggiano et al. 2009) in different working spaces.

The relationship between electrical excitability and visual properties suggests which could be the possible contribution of these latter to different motor functions. In fact, non-mirror visual responses (mainly peripersonal) are found in those areas, F4d and F1d, in which ICMS evoke mainly axio-proximal and arm movements, and which are richer of somatosensory responses during the stimulation of trunk, forelimb and face. It has been proposed that this sensory-motor matching could be involved in motor coding of the peripersonal space, thus contributing to organizing actions related to specific visual and somatosensory (body-centered) fields (Fogassi et al. 1996; Rizzolatti et al. 1997). In contrast, mirror neurons are mostly present in area F5, in correspondence with penetrations related to the coding of hand and mouth motor acts, scarcely or not at all electrically excitable with the stimulation parameters employed in this study. This very low excitability of the penetrations endowed with mirror, but not other visual properties, suggests that the output of mirror neurons may have a stronger impact on other cortical motor and non-motor structures through cortico-cortical, rather than cortico-spinal, projections. These functional properties are more related to motor encoding of those action features that are relevant for action and intention understanding (Gallese et al. 1996; Rizzolatti et al. 2004; Bonini et al. 2010), and for planning and generating appropriate behaviours during social interaction (Caggiano et al. 2009), thus providing a more abstract description of the social world based on the individual motor knowledge.

Interestingly, a recent study has also revealed that some corticospinal neurons in the bank of area F5 can exhibit mirror-like activity and can thus directly influence the activity of the spinal cord via the pyramidal tract during action observation (Kraskov et al. 2009). Interestingly, area F5 has its direct, although limited, projection to the spinal cord (Dum and Strick 1991, He et al. 1993). However, these projections seems to start only from area F5p (Borra et al. 2010). These findings suggest that area F5p can influence the overt motor output, directly, or acting through its cortico-cortical projections to area F1 (Schmidlin et al. 2008; Gerbella et al. 2009). In contrast, the convexity of area F5 (area F5c) appears to be involved in more abstract and socio-cognitive functions. Then, future studies should further investigate the role of F5 and of mirror neurons in the control of motor output.

5. CONCLUSIONS

The present data show that in the ventral part of the frontal motor cortex, beyond the anatomical borders among cytoarchitectonic areas, some functional sectors can be identified.

Results of ICMS and of recording of motor and sensory properties allowed to identify, in the caudal part of the recorded region, a ventral sector (F4v and F1v), characterized by high electrical excitability, mouth simple movements, and absence of visual properties, and a dorsal sector (F4d and F1d), characterized by higher stimulation thresholds, forelimb and mouth motor acts, and different types of visual properties. In the rostral part of the recorded region, area F5c shows a dramatic drop of cortical excitability, higher motor specificity for the type of grip, and more complex visual properties, such as mirror responses.

It could be hypothesized that the identified functional subdivisions, although heterogeneous from the cytoarchitectonic point of view insofar as they encompass anatomical borders, nevertheless they could be characterized by specific patterns of connections with other cortical areas. Indeed, these could justify their relatively functional homogeneity. Anatomical studies carried out by injections show reciprocally strong connections between the mouth field of area F1 and that of area F4 (Matelli et al. 1986), corresponding to our areas F1v and F4v, respectively. Since anatomical connections between cortical areas are usually fully reciprocal, some further information could be derived from studies based on tracers injections in other cortical areas. For example, areas F4d and F1d appear to be linked with inferior parietal area PFG, which is related to hand and arm movement, while their ventral counterparts are more tightly linked with area PF, which involves mouth movements and the representation of orofacial sensory stimuli (Rozzi et al. 2006; 2008). Area F5c is strongly connected with AIP (Borra et al. 2008), area PFG and PF (Rozzi et al. 2006), but also with prefrontal areas 12 and 46v, which are potential source of information related to the processing of visual, memorized or actual, non-spatial information, and of higher order aspects of action organization (Gerbella et al. 2010). Notably, F4 and F1 completely lack prefrontal connections. Therefore, also these anatomical data support the view that the caudal sectors of the investigated region are characterized by more execution-related motor functions, with the ventral portion playing a role in the organization and fine control of jaw and tongue movement, and the dorsal one appearing mainly involved in the organization of goal-directed motor acts and simple actions within the peripersonal space. In the rostral sector of the investigated region, area F5c does not appear to be strictly related to purely motor function, but rather to high order cognitive capacities emerging from motor representations.

From an evolutionary point of view, it has been proposed that monkey area F5 could be homologous of Broca's area (Brodmann area 44), which is considered crucial for speech production (Rizzolatti and Arbib 1998). Besides language functions, this latter area is involved in action recognition, motor imagery and imitation (Rizzolatti et al. 1996; Iacoboni et al. 1999; Binkofski et al. 2000; Buccino et al. 2001; Pulvermüller and Fadiga 2010). Experiments carried out in patients during brain surgery and with TMS (Ojemann et al. 1989; Pascual-Leone et al. 1991; Epstein et al. 1996) have shown that the stimulation of the pars opercularis of this area produces speech arrest while the patient is speaking, while the stimulation of more caudal cortical region does not produce speech arrest but alters the control of orofacial movements.

Altogether, these observations highlight some remarkable similarities between the monkey and the human frontal motor cortex. The posterior regions show more evident motor functions linked to different operational spaces, while the rostral ventral premotor cortex possesses the capacity to exploit the individual's motor knowledge at a more abstract level, enabling the emergence of socio-cognitive functions which could have been important precursors for the evolution of high order cognitive functions typical of humans, such as imitation and language.

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