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Anatomo-functional organization of the ventral primary motor and premotor cortex in the macaque monkey

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Abstract

The ventral agranular frontal cortex of the macaque monkey is formed by a mosaic of anatomically distinct areas. Although each area has been explored by several neurophysiological studies, most of them focused on small sectors of single areas, thus leaving to be clarified which is the general anatomo-functional organization of this wide region. To fill this gap, we studied the ventral convexity of the frontal cortex in two macaque monkeys (*Macaca nemestrina*) using intracortical microstimulation and extracellular recording. Functional data were then matched with the cytoarchitectonic parcellation of the recorded region. The results demonstrated the existence of a dorso-ventral functional border, encompassing the anatomical boundary between areas F4 and F1, and a rostro-caudal anatomo-functional border between areas F5 and F4. The ventral subdivision of areas F4 and F1 was highly electrically excitable, represented simple mouth movements and lacked visual properties; in contrast, their dorsal counterpart showed a higher stimulation threshold, represented forelimb and mouth motor acts and hosted different types of visual properties. The data also showed that area F5 was scarcely excitable, and displayed various motor specificity (e.g. for the type of grip) and complex visual (i.e. mirror responses) properties. Overall, the posterior areas F4 and F1 appear to be involved in organizing and controlling goal-directed mouth motor acts and simple movements within different parts of the external (dorsal sector) and internal (ventral sector) space, whereas area F5 code motor acts at a more abstract level, thus enabling the emergence of higher order socio-cognitive functions.

Introduction

In the last two decades, several studies have dealt with the anatomical and functional organization of the agranular frontal cortex showing that, beyond its role in motor control, this region can be involved in several perceptual and cognitive functions (see Rizzolatti & Luppino, 2001; Hoshi & Tanji, 2007; Cisek & Kalaska, 2010).

This functional complexity is associated with a considerable anatomical heterogeneity (Matelli *et al.*, 1985; Barbas & Pandya, 1987; Belmalih *et al.*, 2007). In particular, Matelli *et al.* (1985) subdivided the most ventral portion of the agranular frontal cortex, extending from the central sulcus to the inferior limb of the arcuate sulcus, into three different cytoarchitectonic areas: the primary motor area F1, located in the depth of the anterior bank of the central sulcus and in the convexity immediately rostral to it, and the ventral premotor areas F4 (caudal) and F5 (rostral). Subsequently, several neurophysiological studies focused on these areas, mostly investigating the

dorsal portion of F4 (Gentilucci *et al.*, 1988; Fogassi *et al.*, 1996) and the whole area F5 (Rizzolatti *et al.*, 1988; di Pellegrino *et al.*, 1992; Gallese *et al.*, 1996; Murata *et al.*, 1997; Fogassi *et al.*, 2001; Ferrari *et al.*, 2003; Raos *et al.*, 2006). Altogether, these studies demonstrated that in these cortical regions neurons discharge during the execution of goal-directed arm, hand and mouth motor acts rather than of simple movements (Gentilucci *et al.*, 1988; Rizzolatti *et al.*, 1988), thus suggesting the existence of a close match between cytoarchitectonic areas and their functional properties.

In contrast, only a few studies have investigated the anatomo-functional organization of the face and mouth representations lying in the most ventral part of this region (Hoffman & Luschei, 1980; McGuinness *et al.*, 1980; Huang *et al.*, 1988; Murray & Sessle, 1992b), corresponding to the ventral areas F4 and F1. In particular, it remains to be clarified whether these areas can be differentiated based on their encoding of simple or complex mouth–face movements and to what extent the distribution of functional properties enables distinguishing between a primary motor and a premotor mouth–face area.

In the present study we combined the recording of neuronal activity and short-train intracortical microstimulation (ICMS) in the whole

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ventral part of the agranular frontal cortex in order to (i) investigate the distribution of different functional properties and (ii) assess the type of match between this distribution and the cytoarchitectonic parcellation of the investigated region.

Materials and methods

The experiments were carried out on two captive-born adult *Macaca nemestrina* (7 years old), one male and one female. 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. A head fixation system and a titanium recording chamber were then implanted under general anaesthesia (ketamine hydrochloride, 5 mg/kg i.m. and medetomidine hydrochloride, 0.1 mg/kg i.m.), followed by postsurgical pain medications. Surgical procedures were the same as previously described (Rozzi *et al.*, 2006; Bonini *et al.*, 2010). All of the experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma and complied with the European law on the human care and use of laboratory animals.

Recording techniques

Multi-unit activity was recorded by means of single glass-coated microelectrodes (impedance 0.5–1 M Ω) inserted through the intact dura. The microelectrode was mounted on an electrode holder and connected to a computer-controlled microdrive. The electrode holder was fixed to a stereotaxic arm, mounted on the monkey head holder, in order to move the electrode over the region of interest, and a dedicated software package (EPS, Alpha Omega, Nazareth, Israel) allowed the control of the microdrives for the vertical electrode movement.

The neuronal activity was filtered and amplified through a dedicated system (MCPplus, Alpha Omega), and then sent to an oscilloscope and an acoustic amplifier (Grass Technologies, West Warwick, USA). The signal could also be sent to a dual voltage–time window discriminator (Bak Electronics, Germantown, MD, USA) in order to isolate the action potentials of single neurons. Isolated spikes could then be fed to a PC to be recorded, stored and subsequently analysed in relation to the behavioural events of interest.

Contact-detecting electric circuits were employed to generate digital signals in correspondence with the occurrence of the main behavioural events. These signals were sent to the PC and stored, enabling the alignment of the neuronal activity with the behavioural event of interest (contact with the object during motor responses or tactile stimulation, and specific phases of visual stimulation) (for further details, see Rozzi *et al.*, 2008). Response histograms were constructed by averaging 10 individual trials.

During each experimental session, the electrode was inserted through the intact dura until the first neuronal activity was detected. The electrode was then deepened into the cortex in steps of 250 μ m, until the border between the grey and the white matter was reached. At each site, two experimenters studied the relations between neuronal activity and any type of sensory (visual and tactile) stimulation delivered and monkey's motor responses. The results of these testing procedures (see 'Testing procedures of the neuronal activity') were inserted into a digital database for subsequent analyses.

The first recording sessions were performed in the anterior part of the chamber, in order to identify eye-related motor activity of the frontal eye field. The functional identification of the frontal eye field was based on the occurrence of responses during different types of eye

movements and, in particular, on the elicitation of saccadic movements with electrical ICMS. Once this region was identified along its medio-lateral axis, the subsequent recording sessions were carried out by moving the electrode caudally, in parallel rows spaced at 1 mm.

Intracortical microstimulation

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

In each penetration, ICMS was performed at every 500 μ m of depth (i.e. in one step out of two), starting 500 μ m below the site where the first multi-unit activity was detected. At each site, ICMS was delivered when the monkey was quiet and relaxed, and those cases in which monkeys performed voluntary movements were not considered for establishing the stimulation threshold. Movements were considered to be evoked by ICMS when two experimenters, observing the animal during pulse delivery, independently and repeatedly identified the same joint displacement or muscular twitch. The procedure consisted of an initial stimulation with a current intensity of 40 μ A. In the absence of any overt movement, the site was considered as 'non-excitabile'. If each stimulation delivered at this intensity reliably evoked the same movement (typically three out of three consecutive stimulations with the above-described parameters), successive stimulations were carried out by progressively decreasing the current intensity, in order to identify the threshold. The threshold was defined as the lowest current intensity capable of evoking movements in 50% plus one of the stimulations delivered (usually four out of six, or five out of eight stimulations).

We compared, by means of factorial ANOVA, the electrical excitability of the investigated areas by considering two parameters: (i) the number of penetrations with at least one electrically excitable site over the total number of penetrations in that area, and (ii) the mean threshold of each area, as obtained by averaging the threshold of each electrically excitable penetration.

Testing procedures of the neuronal activity

The ICMS enabled us to exclude sites related to eye movements from further testing. For each of the remaining sites, we employed the testing procedures described below, according to a previous report (Rozzi *et al.*, 2008), in order to verify whether neuronal activity was related to brachio-manual (hand and/or arm) movement, mouth movement or both. If the neuronal activity was related to more than one body part, all of the testing procedures described below were carried out to specify the motor and somatosensory properties of each body part. When neuronal activity was clearly related to a single body part (hand, arm or mouth) we focused only on its motor and somatosensory properties. Concerning visual responses, we systematically tested them in all investigated sites, independently from the represented body part.

According to many previous studies (Rizzolatti *et al.*, 1988; Alexander & Crutcher, 1990; Crutcher & Alexander, 1990; Kakei *et al.*, 1999, 2001; Umiltà *et al.*, 2008), we tested neuronal motor activity in order to clarify whether it was related to simple hand, arm or mouth movements or to goal-directed motor acts. Although neuronal activity related to simple movements is present whenever a

specific body part displacement occurs, regardless of the immediate motor goal (e.g. fingers flexion during scratching, grooming, grasping, etc.; see Rizzolatti *et al.*, 1988), activity related to motor acts is specifically correlated with more complex motor synergies (e.g. hand opening and closure) aimed to attain a specific motor goal (e.g. grasping and taking possession of an object).

Hand motor activity

We first tested hand grasping in the proximity of the monkey's body, with the arm corresponding to the tested hand restrained, in order to distinguish hand-related motor activity from possible responses due to arm movements; this test was also carried out by closing the monkey's eyes. We further checked whether different types of movements (i.e. scratching, grooming or spontaneous finger flexion movements) were equally effective in triggering neuronal discharge in order to establish whether the activity was related to simple movements or motor acts. Grasping-related responses were also assessed during the execution of different types of grip, classified according to the posture assumed by the hand during the 'shaping' and 'actual grasping' phases (see Macfarlane & Graziano, 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 of the fingers but not the thumb, wrapped around the object (typically a cylindrical one), without hand palm opposition; and whole hand prehension, characterized by all of the fingers and the thumb, wrapped around the object.

Arm motor activity

We tested arm responses by presenting the monkey with pieces of food in different space locations; neuronal responses were attributed to arm reaching or bringing to the mouth depending on whether they were triggered by arm projection in space or by flexion of the arm toward the mouth, respectively. Simple arm extension responses were distinguished from those related to goal-directed reaching acts by prompting the monkey to extend the arm to push away a panel held close to his body.

Mouth motor activity

Mouth-related motor responses were studied by using different types of stimuli, namely, solid food to test grasping with the mouth and chewing responses, drops of juice delivered by a syringe to test sucking responses and a stick dipped into yogurt to test licking responses. In all cases the food was brought to the monkey's mouth by the experimenter while keeping monkey's eyes closed. This enabled us to distinguish mouth motor responses from visual responses triggered by visual stimuli approaching the face. Different types of mouth motor acts were tested: mouth grasping was defined as the closure of the teeth on the food; sucking was defined as the complex and coordinated series of lips and tongue movements required for sucking juice from a syringe; and licking was defined as the protrusion of the tongue for licking yogurt presented on a stick. When a simple movement of the tongue (i.e. protrusion or retraction) or of the jaw (i.e. lifting or lowering) was sufficient for evoking neuronal discharge during several distinct motor acts, i.e. independently of a specific motor goal, the neuronal activity was classified as related to a simple tongue or jaw movement, respectively.

Axio-proximal movements

Neuronal responses related to active movements of axial and proximal body parts (trunk, neck, or shoulder) are usually extremely difficult to

test because of the limited mobility of these body parts in the head-restrained monkey, and a more detailed characterization of this type of coding was outside the aims of this study. Therefore, we attributed to a single and broad category called 'axio-proximal movements' all those neuronal responses clearly related to active movements that could not be reliably attributed to the hand-arm or the mouth using the criteria detailed above.

Somatosensory responses

Different types of somatosensory stimuli were applied to distinct body parts (hand, arm, trunk, face, lip, inner mouth), with the monkey's eyes closed. Light touch was assessed by lightly 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 skin territories by touching them with a stick, by finger tapping or by squeezing muscle bellies. Joint-related responses were tested by means of joint mobilization. Possible somatosensory responses to stimuli applied inside the mouth (i.e. to the teeth or the tongue) were tested by keeping the monkey's mouth open with a tool and by gently touching the tongue or the teeth with tweezers or a cotton wad. Due to the close proximity of many anatomical parts of the inner mouth, and the fact that some of them are soft and difficult to test independently from one another, we chose to include all of the responses clearly attributable to some of these anatomical parts as 'inner mouth'-related. Whenever neuronal activity could be triggered by both active movement and passive stimulation, we favoured the latter attribution.

Visual properties

Monkeys were presented with three-dimensional objects (i.e. food items and solids) of different shape, size and orientation, moved in various space locations, directions and distances from the monkey or with biological stimuli. Different types of visual responses were tested using specific sets of visual stimuli, as follows: object presentation was tested by presenting the monkey with objects in the reachable (peripersonal) and not-reachable (extrapersonal) space by holding them with a tool (stick or pliers) or by disclosing objects hidden behind an opaque screen; object motion was studied by moving objects held by a tool along linear or circular trajectories, in both peripersonal and extrapersonal space; peripersonal visual responses were tested by moving objects along trajectories directed toward or away from different monkey body parts within its reaching space; biological motion was assessed by presenting the monkey with non-object-related experimenter's movements, such as moving the hand, head, trunk or limb; and mirror responses were tested by presenting the monkey with hand-related motor acts (i.e. grasping) executed unimanually or bimanually, or with mouth-related motor acts (i.e. mouth grasping, sucking, licking). All of the motor acts were performed by the experimenter in front of the monkey.

Single neurons analysis

Although the primary aim of this study focused mainly on multi-unit activity, we also recorded some examples of single neuron activity that were particularly representative of the investigated properties. The activity of each neuron, recorded for 10 successive trials, was expressed as the mean firing rate (spikes/s) and statistically compared between baseline activity (500 ms), taken from 2000 to 1500 ms before the event of interest (stimulus application/presentation or motor response), and one of the following epochs of interest: (i) for motor and action observation responses, a 500 ms epoch centred on

the contact of the monkey's or the experimenter's body part with the target (from 250 ms before to 250 ms after this event); (ii) for somatosensory/visual responses, a 500 ms epoch beginning with the application or the presentation of the stimulus. The neuron activity was then compared across conditions and epochs by using a two-way ANOVA (factors – Condition and Epoch), followed by Bonferroni *post hoc* tests. All analyses were performed using a significance criterion of $P < 0.05$.

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

At the end of the neurophysiological experiments, electrolytic lesions (10 μ A 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 penetration grid. At 1 week after the lesions, each animal was anaesthetized with ketamine hydrochloride (15 mg/kg, i.m.) followed by intravenous lethal injection of pentobarbital sodium and perfused through the left cardiac ventricle with saline, 3.5–4% paraformaldehyde and 5% glycerol in this order, prepared in phosphate buffer 0.1 M, pH 7.4. The brain was then removed from the skull, photographed, cryoprotected, and then frozen and cut in coronal sections. Each second and fifth section of a series of five was stained using the Nissl method. The locations of penetrations were then reconstructed on the basis of electrolytic lesions, stereotaxic coordinates, penetration tracks and recording depths. More specifically, penetrations with properties typical of the frontal eye field were used to localize the rostral border of area F5, whereas the caudal border of the recorded region was defined based on the appearance of the properties typical of the primary somatosensory cortex.

Subsequently, the cytoarchitectonic features of the primary motor and premotor cortices were identified according to the criteria of Belmalih *et al.* (2007, 2009). In particular, low cell density, poor lamination and a prominent layer V with giant pyramidal cells arranged in multiple rows characterize area F1. Area F4 displays an evident cell size gradient in layer III, with increasing cell size moving from its upper to its lower part. The deeper part of layer V presents relatively large pyramids. The convexity of area F5c is characterized by poor lamination, by an overall small cell size of layer III and V pyramids, and by a radial organization in layers II, V and VI. As 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, thus allowing the attribution of the functional properties of each penetration to a specific cytoarchitectonic area.

Comparative data analysis and map construction

The database included the functional properties and ICMS results of all of the sites located between the cortical surface and 4 mm of depth. Cortical sites deeper than 4 mm were used for establishing the location of arcuate and central sulci, and the identification of the cortical areas located inside the banks. The properties of neurons recorded from these sites will not be described in the present study.

Chi-square tests were used to compare the frequency of penetrations with electrically excitable sites among different areas, whereas possible differences in the mean stimulation thresholds among the areas were assessed by means of factorial ANOVA, followed by Bonferroni *post hoc* test.

The frequency of sites in which a specific functional property was present, in each area, was calculated and expressed as a percentage of the total number of sites of that area and it was then compared among distinct cytoarchitectonic areas by using chi-square or Fisher's exact probability tests. Furthermore, to construct the maps of the different functional properties, each penetration was labelled with a circle, the size of which varied as a function of the percentage of sites showing that property out of the total number of sites of that penetration.

Results

Anatomical localization and cytoarchitectonic features of the recorded region

We carried out 231 penetrations in the left hemispheres of the two monkeys (117 in M1, 114 in M2), for a total of 1292 investigated sites in M1 (11.0 ± 3.51 sites for penetration) and 1316 in M2 (11.5 ± 3.76 sites for penetration).

A lateral view of the left hemisphere of M1 and M2 is shown in Fig. 1A. Figure 1B illustrates the penetrations grid, overlapped on the cytoarchitectonic map of the ventral agranular frontal cortex. Figure 1C shows the photomicrographs of some sections representative of the investigated cytoarchitectonic subdivisions. For the criterion used to identify the architectonic areas, see Materials and methods.

Anatomo-functional organization of the ventral motor cortex convexity

By observing the distribution of the functional properties in the investigated region, a clear dorso-ventral dishomogeneity, especially in its posterior part (corresponding to cytoarchitectonic areas F4 and F1), can be noted. Indeed, as shown in Fig. 2, the motor representations of different body parts [in terms of both multi-unit activity (Fig. 2A) and movements evoked through ICMS (Fig. 2B)] appeared to be relatively segregated, with the forelimb field located dorsally to the oro-facial region. Furthermore, visual properties were widely distributed in the dorsal sectors of F4 and F1, whereas they were almost absent in the ventral sectors.

Based on these early findings, we considered it appropriate to introduce a further functional border within areas F4 and F1 by subdividing both of these areas into a dorsal and a ventral sector. Concerning area F4, we called these sectors F4d (dorsal) and F4v (ventral). Concerning F1, as we did not study the whole area but only its ventral part (F1v), we called its subdivisions F1vd (dorsal) and F1vv (ventral). In order to statistically validate this functional subdivision, subsequent analyses on the distribution of motor, somatosensory and visual properties were carried out by hypothesizing the existence of five sectors: the convexity of area F5, F4d, F4v, F1vd and F1vv. It must be noted that the dorso-ventral border could also extend rostrally through area F5 to the arcuate sulcus. However, because of the low number of penetrations available in this restricted cortical sector, we could not statistically address this hypothesis. For this reason, and because we limited our investigation to the cortical convexity, we will refer to the rostral investigated region as to the F5c described by Belmalih *et al.* (2009).

As shown in Fig. 2A, sectors F4d and F1vd were characterized by a higher number of penetrations with brachio-manual activity, alone or associated with mouth responses, compared with F4v and F1vv, which in turn contained a higher number of penetrations related to the mouth only [Fisher's test for M1 – F4d vs. F4v ($n = 42$), $P = 0.000$; F1vd vs. F1vv ($n = 16$), $P = 0.01$; for M2 – F4d vs. F4v ($n = 49$), $P = 0.002$; F1vd vs. F1vv ($n = 27$), $P = 0.000$]. Figure 2B shows that hand

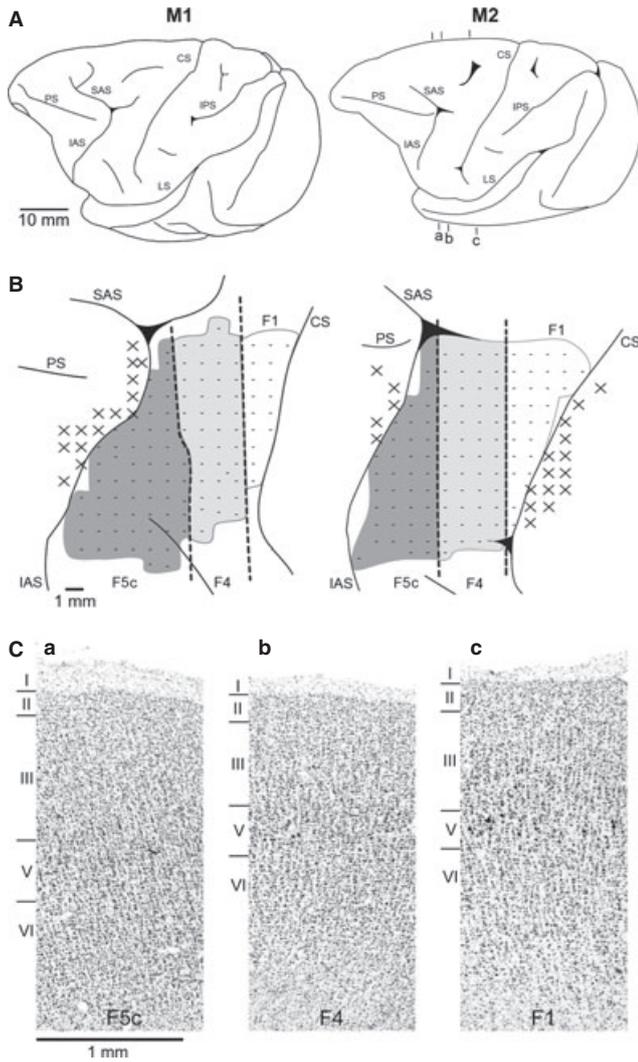


FIG. 1. (A) Lateral view of the left hemispheres of M1 and M2. In M2, lines a, b and c indicate the levels at which the coronal sections, shown in C, were taken. (B) Penetrations grid superimposed on the cytoarchitectonic borders (dashed lines) identified within the investigated region. Each dot represents the location of a single penetration. The white region corresponds to the convexity of area F1, the light grey shaded region corresponds to area F4, and the dark grey shaded region corresponds to area F5c. Crosses indicate penetrations falling outside the regions of interest. (C) Photomicrographs showing sections representative of the cytoarchitectonic organization of the three investigated areas (F5c, F4, and F1). Roman numbers correspond to the different cortical layers. CS, central sulcus; IAS, inferior arcuate sulcus; IPS, intraparietal sulcus; LS, lateral sulcus; PS, principal sulcus; SAS, superior arcuate sulcus.

movements evoked through ICMS were present only in area F5c, and in the sectors F4d and F1vd. Furthermore, considering the distribution of penetrations with sites responsive to visual stimuli (orange circles), it is clear that F4v and F1vv almost lacked visual responses, whereas these were more widely represented in their dorsal sectors [Fisher's test for M1 – F4d vs. F4v ($n = 42$), $P = 0.000$; F1vd vs. F1vv ($n = 16$), $P = 0.01$; for M2 – F4d vs. F4v ($n = 49$), $P = 0.000$; F1vd vs. F1vv ($n = 27$), $P = 0.001$].

Movements evoked by intracortical microstimulation

In 142 (61.5%) out of 231 penetrations, ICMS elicited movements in at least one of the investigated sites of each penetration, with a mean

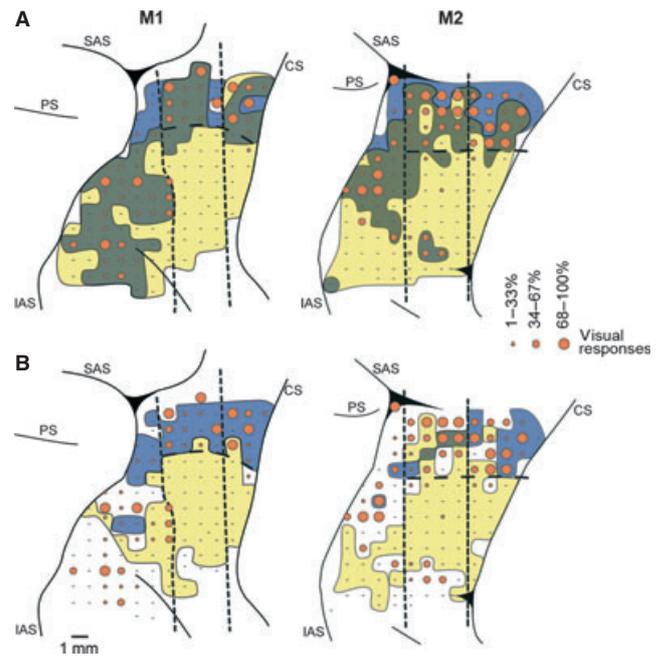


FIG. 2. (A) Cortical fields in which the extracellular multi-unit activity was related to brachio-manual (blue), mouth (yellow) or brachio-manual and mouth (green) responses. (B) Cortical fields in which ICMS evoked brachio-manual (blue), mouth (yellow) or brachio-manual and mouth (green) responses. Orange circles identify the penetrations characterized by the presence of visual responses. The size of each circle represents the percentage of sites with visual responses, out of the total number of sites of the penetration. Horizontal dashed lines indicate the anatomo-functional borders defined on the basis of both the presence/absence of visual responses and the type of represented effector (brachio-manual vs. mouth). Other conventions as in Fig. 1.

stimulation threshold of 22 μ A. In the remaining 89 penetrations (38.5%), ICMS carried out with the parameters employed in the present study (see Materials and methods) had no effect in any of the investigated sites.

Figure 3A shows the proportion of penetrations with at least one electrically excitable site and those without excitable sites. Area F5c contained the lowest percentage of excitable penetrations as compared with all other sectors [Fisher's test – F5c vs. F4d ($n = 126$), $P = 0.003$; F5c vs. F1vd ($n = 121$), $P = 0.0002$; F5c vs. F4v ($n = 159$), $P = 0.000$; F5c vs. F1vd ($n = 116$), $P = 0.000$]. In contrast, more than 70% of the penetrations in the investigated sectors of areas F4 and F1 were excitable, with no significant differences among them.

A 2 \times 5 factorial ANOVA (factors – Subject and Sector) was employed in order to compare the stimulation thresholds of the different anatomo-functional sectors in the two monkeys. This analysis revealed a main effect of the factor Subject ($F_{1,132} = 18.180$, $P = 0.000$), indicating that, in M1, the ICMS thresholds were generally lower than in M2. Most interestingly (see Fig. 3B), it also revealed a significant main effect of the factor Sector ($F_{4,132} = 13.524$, $P = 0.000$). Bonferroni *post hoc* tests showed that F5c and F4d had similar stimulation thresholds ($P = 1.00$), and both of them had thresholds higher than those of all of the remaining sectors (F5c vs. F1vd, $P = 0.033$; F5c vs. F4v, $P = 0.000$; F5c vs. F1vv, $P = 0.000$; F4d vs. F4v, $P = 0.004$; F4d vs. F1vv, $P = 0.000$) except for F4d compared with F1vd (F4d vs. F1vd, $P = 0.537$).

Figure 3C shows the distribution of excitable penetrations in terms of the effector activated by ICMS at threshold. The stimulation maps reveal a rough somatotopic organization, with brachio-manual and axio-proximal movements represented only in the dorsal portion of the

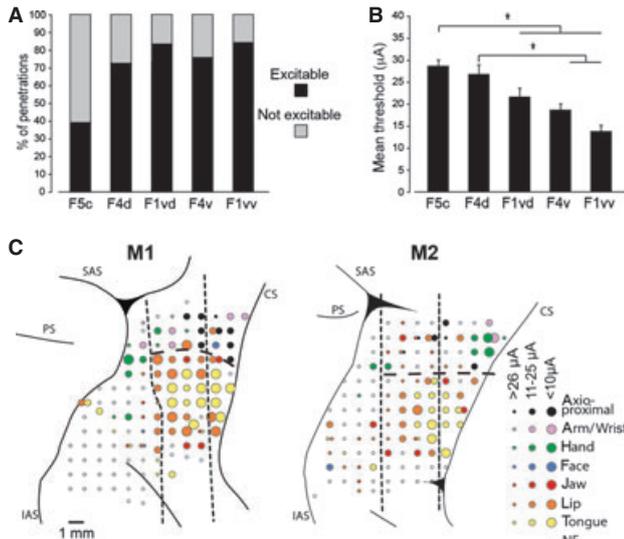


FIG. 3. (A) Histograms representing the percentage of excitable (black) and non-excitable (grey) penetrations in each of the investigated sectors. (B) Histograms representing the mean threshold for the electrically excitable penetrations within each investigated sector. $*P < 0.05$. (C) Localization of movements evoked by ICMS; circles of different colours identify the body part activated by the stimulation at threshold intensity in each penetration, whereas the size of the circles represents the threshold. Small white circles represent penetrations not electrically excitable (NE) with the stimulation parameters employed in this study. Other conventions as in Fig. 2.

whole investigated region. In particular, in M1, hand movements were represented in the most dorsal part of area F5 and in F4d, whereas in M2 they were mainly confined to a dorsal and caudal portion of F1vd. In both monkeys, arm and axial movements were evoked by stimulating a region extending up to 4–5 mm rostrally to the central sulcus. Concerning mouth movements, tongue movements occupied a wide region close to the central sulcus, forming a relatively large spot surrounded by penetrations characterized by lip and jaw movements along its dorsal, rostral, and ventral edges.

In both monkeys, a cluster of penetrations in which movements of different body parts (tongue and lip) were evoked with quite low current intensity ($< 10 \mu\text{A}$) was found. This cluster was located near the central sulcus, within the ventral sectors of areas F4 and F1. Moving rostrally, the electrical excitability dropped dramatically and, in F5c, besides a low number of penetrations in which it was possible to evoke some movement at medium or high current intensity, penetrations with threshold lower than $10 \mu\text{A}$ were virtually absent.

Motor responses

During the testing of motor responses, particular attention was paid to assessing whether they were related to simple movements of a certain effector or body part (hand, arm, mouth or face) or to goal-directed motor acts (see Materials and methods for details about this distinction).

Figure 4 shows that F4v and F1vv had a lower percentage of sites responsive during goal-directed motor acts compared with F4d, F1vd and F5c [F4v vs. F4d, $\chi^2 = 358.61$ ($n = 1284$), $P = 0.000$; F4v vs. F1vd, $\chi^2 = 256.98$ ($n = 1238$), $P = 0.000$; F4v vs. F5c, $\chi^2 = 501.31$ ($n = 2526$), $P = 0.000$; F1vv vs. F4d, $\chi^2 = 277.79$ ($n = 779$), $P = 0.000$; F1vv vs. F1vd, $\chi^2 = 205.32$ ($n = 733$), $P = 0.000$; F1vv vs. F5c, $\chi^2 = 327.64$ ($n = 2021$), $P = 0.000$]. Figure 5A shows the localization of brachio-manual motor acts and of axio-proximal

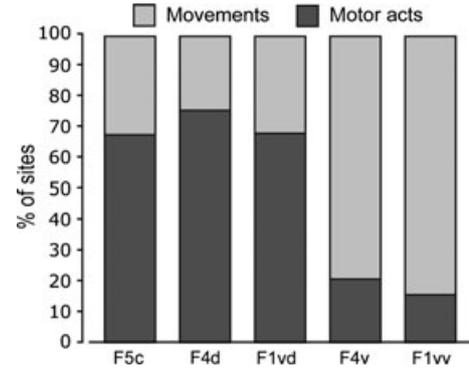


FIG. 4. Relative proportion of sites showing neuronal responses during movements (light grey) and motor acts (dark grey) in each of the investigated sectors.

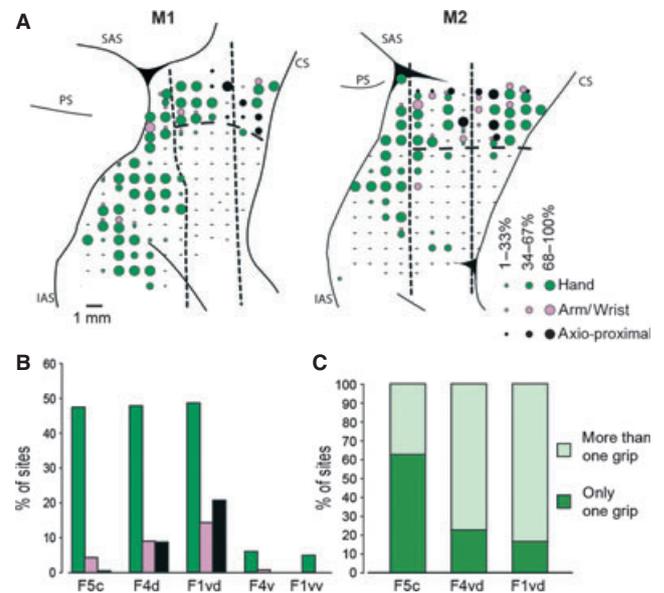


FIG. 5. (A) Representation of hand and arm motor acts and axio-proximal movements in the investigated region. Conventions as in Fig. 2B. (B) Percentage of sites with hand, arm and axial motor responses for each of the identified sectors, calculated with respect to the total number of sites recorded in that sector. Colour codes as in A. (C) Distribution of hand grasping sites responsive during only one (dark green) or more than one (light green) type of grip. The ventral sectors of F4 and F1 are not included as almost no hand grasping site was found in these regions.

movements in the investigated sectors (see Materials and methods for the definition of axio-proximal movement). These responses were nearly absent in F4v and F1vv, whereas they were widely and similarly represented in F5c, F4d and F1vd. Arm motor acts and axio-proximal movements were mostly limited to F4d and F1vd. The histograms in Fig. 5B show that sites with hand grasping-related responses were found more frequently in F5c, F4d and F1vd [with no differences among these sectors – F5c vs. F4d, $\chi^2 = 0.00$ ($n = 1344$), $P = 0.96$; F5c vs. F1vd, $\chi^2 = 0.09$ ($n = 1347$), $P = 0.761$; F4d vs. F1vd, $\chi^2 = 0.02$ ($n = 629$), $P = 0.9$] compared with F4v and F1vv [F5c vs. F4v, $\chi^2 = 318.77$ ($n = 1691$), $P = 0.000$; F5c vs. F1vv, $\chi^2 = 169.81$ ($n = 1319$), $P = 0.000$; F4d vs. F4v, $\chi^2 = 234.11$ ($n = 973$), $P = 0.000$; F4d vs. F1vv, $\chi^2 = 136.48$ ($n = 601$), $P = 0.000$; F1vd vs. F4v, $\chi^2 = 241.67$ ($n = 976$), $P = 0.000$; F1vd vs. F1vv, $\chi^2 = 140.73$ ($n = 604$), $P = 0.000$]. Sectors F4d and F1vd

also contained a relevant and similar [$\chi^2 = 3.80$ ($n = 629$), $P = 0.051$] percentage of sites activated during movement of the arm, which was higher than that of area F5c [F4d vs. F5c, $\chi^2 = 38.75$ ($n = 1195$), $P = 0.000$; F1vd vs. F5c, $\chi^2 = 107.79$ ($n = 1194$), $P = 0.000$]. Furthermore, F1vd had a higher percentage of sites responsive to axio-proximal movements than F4d [$\chi^2 = 17.02$ ($n = 629$), $P = 0.000$]. In contrast, both arm and axio-proximal motor activity was virtually absent in F1vv and F4v.

In more than half of the sites with hand grasping-related responses (59.1%), we assessed the possible neuronal grip selectivity by employing objects of different size and shape as the target for the monkeys' hand grasping. Figure 5C shows the proportion of sites, in each area, in which grasping activity was present during only one or more than one grip type. It is evident that the investigated sectors differed in terms of grip selectivity. In fact, more than half of the sites in the area F5c showed activity related to only one type of grip, and this proportion was significantly higher than that observed in both F4d [$\chi^2 = 57.42$ ($n = 393$), $P = 0.000$] and F1vd [$\chi^2 = 66.53$ ($n = 364$), $P = 0.000$], which, in turn, did not differ from each other [$\chi^2 = 1.21$ ($n = 269$), $P = 0.271$].

The most frequent type of response for mouth-related motor acts was grasping with the mouth (20.6% of all sites with mouth-related motor responses) followed by licking (9.1%) and sucking (8.7%). Figure 6 shows examples of neurons discharging during licking (Fig. 6A) and sucking (Fig. 6B). Despite the fact that the motor acts eliciting the most effective discharges of these neurons implied movements of the tongue and its tactile stimulation, their response during other tongue movements and the passive stimulation of the tongue was less or not at all effective in triggering the response of the neurons. However, the majority of mouth-related responses (61.5%) were better correlated to simple movements, such as tongue (Fig. 6C) and jaw (Fig. 6D) movements. In particular, the neuron shown in Fig. 6C responded during contralateral tongue protrusion, regardless of whether the monkey received food or not, but it did not activate during ipsilateral protrusion. Figure 6D shows a unit discharging during simple lifting of the jaw; the response was aligned on the closure of monkey's teeth on the target (biting) but subsequently it was clearly rhythmically present whenever the monkey lifted the jaw for chewing the food. Importantly, it could not be explained by simple tactile stimulation of teeth and tongue.

Figure 7 shows the distribution of mouth motor acts (Fig. 7A) and simple movements (Fig. 7B) in the investigated region. Sites with responses related to mouth motor acts rather than to mouth simple movements (see Fig. 7C) were more frequently found in area F5c compared with all of the other subdivisions, which, in turn, were more widely characterized by the encoding of simple movements [F5c vs. F4d, $\chi^2 = 38.40$ ($n = 1362$), $P = 0.000$; F5c vs. F1vd, $\chi^2 = 17.13$ ($n = 1283$), $P = 0.000$; F5c vs. F4v, $\chi^2 = 249.27$ ($n = 1922$), $P = 0.000$; F5c vs. F1vv, $\chi^2 = 174.88$ ($n = 1476$), $P = 0.000$; F4v vs. F4d, $\chi^2 = 282.82$ ($n = 1020$), $P = 0.000$; F1vv vs. F1vd, $\chi^2 = 32.27$ ($n = 495$), $P = 0.000$]. Further differences also emerged by comparing the mouth responses of F4v and F1vv; in fact, F1vv contained a higher proportion of sites with responses during tongue movements with respect to F4v [$\chi^2 = 58.34$ ($n = 948$), $P = 0.000$], whereas F4v had a higher number of sites with responses during jaw movements [$\chi^2 = 36.55$ ($n = 948$), $P = 0.000$].

Somatosensory responses

Out of the total number of investigated sites, somatosensory responses were widely represented in both monkeys (M1, 44.2%; M2, 64.5%). Among the sites with somatosensory responses, we found that a low

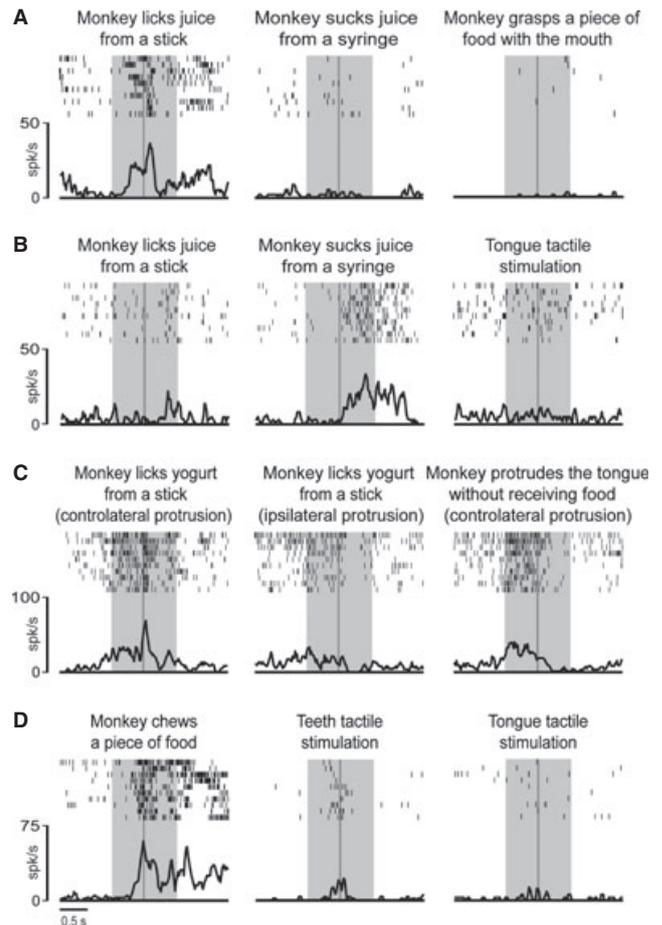


FIG. 6. Examples of neurons responding during the execution of different mouth motor acts or movements. (A) F5c neuron discharging more strongly when the monkey licks juice from a stick. (B) F5c neuron discharging selectively when the monkey sucks juice from a syringe. (C) F1vv neuron discharging similarly when the monkey licks yogurt from a stick and when it protrudes the tongue contralaterally without receiving or touching anything. (D) F4v neuron discharging during each lifting movement of the jaw, both during the initial biting of the food (alignment point) and subsequent chewing. In each panel, the rasters and histograms represent the neuron response during a specific experimental condition. Each histogram represents the neuronal activity averaged across 10 trials. The activity is aligned with the contact of the monkey tongue or mouth with the object.

percentage of them showed somatosensory responses alone (M1, 11.6%; M2, 9.8%) or in association with visual responses (M1, 2.8%; M2, 1.6%), whereas the great majority were associated with motor activity (M1, 85.6%; M2, 88.6%).

The tactile fields were typically large, often including more than one body part (e.g. the face and the arm). The majority of them were bilateral (70.8%), whereas 27.9% were limited to the contralateral body side. Tactile fields confined to the ipsilateral side of the body were only rarely found (1.3%). Among the different somatosensory submodalities, 'deep touch' was well represented (26.6%), whereas 'joint' mobilization (10%) and 'light touch' (3.9%) were less frequently found. Note that more than half (59.5%) of the sites showing responses to somatosensory stimuli were activated in more than one submodality.

Figure 8 shows the distribution, in the investigated region, of somatosensory responses evoked by the stimulation of the hand, arm and trunk (Fig. 8A) and of the face, lip and inner mouth (Fig. 8B). Note that, due to the difficulty in discriminating the exact location and

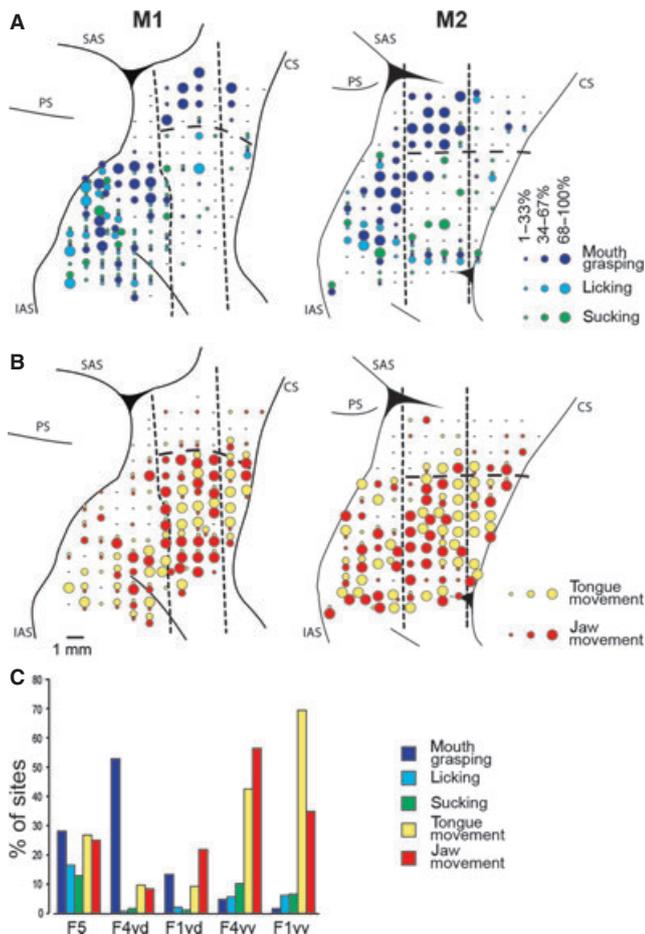


FIG. 7. Distribution of mouth motor acts (A) and simple movements (B) in the investigated areas. (C) Histogram representing the relative proportion of mouth grasping (blue), licking (light blue) and sucking (green) motor acts and of tongue (yellow) and jaw (red) simple movements. The percentage of properties is calculated as described in Fig. 5. Other conventions as in Fig. 2.

extension of tactile fields inside the mouth, we assigned these responses to a general category called ‘inner mouth’ (see Materials and methods). It is evident that sites with somatosensory responses were widely distributed in all of the investigated sectors, arranged in a rough somatotopic manner; cortical sites related to the lip and inner mouth were mainly localized in F4v and F1vv, whereas tactile fields on the hand, arm or neck/trunk were more frequently found in F1vd and F4d. Interestingly, we observed an association between brachio-manual and oro-facial tactile fields; the brachio-manual regions clearly overlapped with those linked to the face and the lips in F4d and F1vd of both monkeys, as well as in F5c (although only in M2).

Visual properties

Visual properties were found in 19% of the recorded sites. The great majority (94.3%) of the sites in which visual responses were identified also activated during the monkey’s active movements, whereas a small percentage (5.7%) showed visual responses not associated with any motor response.

Visual responses were assigned to different categories, according to the type of stimulus most effective in eliciting them. The type of visual properties most frequently found out of the total sites showing visual responses were ‘peripersonal’ ($n = 214$; 43%), followed by ‘mirror’

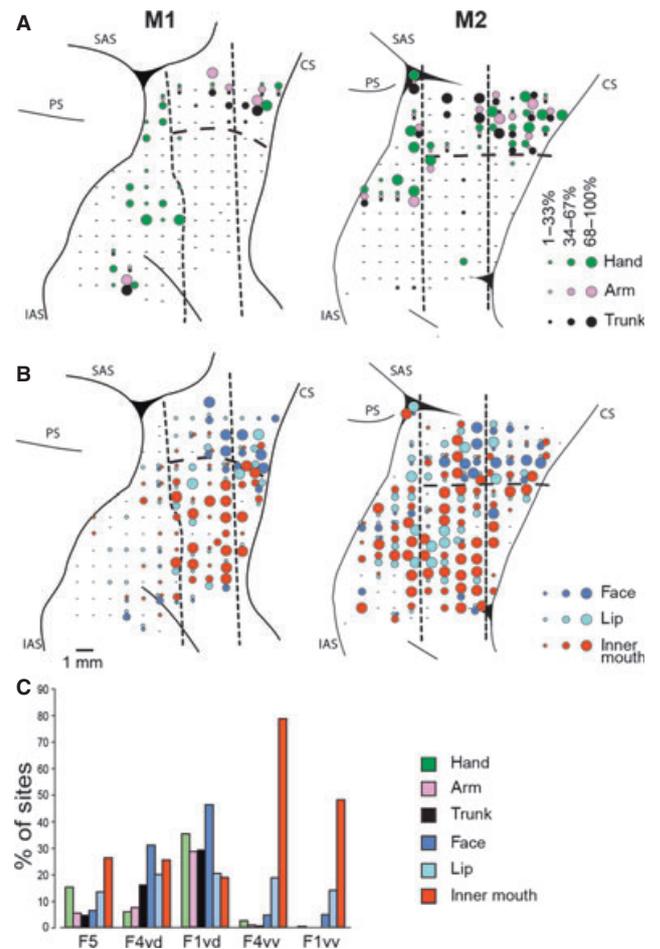


FIG. 8. (A) Distribution of hand, arm and axial tactile fields. (B) Distribution of tactile fields on the face, lip and inner mouth. (C) Proportion of the different tactile fields in the investigated areas. Colour codes as in A and B. The percentage of properties have been calculated as described in Fig. 5. Other conventions as in Fig. 2.

($n = 168$; 34%), ‘object motion’ ($n = 54$; 11%), ‘biological motion’ ($n = 26$; 5%), and ‘object presentation’ ($n = 34$; 7%).

Figure 9A and B shows the distribution of visual properties in the investigated region. It is evident that visual responses were nearly absent in F4v and F1vv, whereas they were highly represented in F5c, F4d and F1vd. Sectors F4d and F1vd contained the highest percentage of peripersonal responses compared with F5c [F4d – $\chi^2 = 233.36$ ($n = 1344$), $P = 0.000$; F1vd – $\chi^2 = 245.10$ ($n = 1347$), $P = 0.000$], and they did not differ from each other in this respect [$\chi^2 = 0.3$ ($n = 629$), $P = 0.852$]. Mirror responses were more frequently found in the most rostral part of the recorded region, particularly in area F5c compared with F4d [$\chi^2 = 7.84$ ($n = 1344$), $P = 0.005$] or F1vd [$\chi^2 = 20.87$ ($n = 1347$), $P = 0.000$]. Sites with responses to object motion were found almost exclusively in F4d and F1vd of M2, whereas the other visual properties (object presentation and biological motion) were sparsely and scarcely represented.

Among the cortical sites showing visual properties, although the majority responded during active movements of the monkeys, not all of them were excitable with ICMS. Comparing the proportion of excitable and non-excitable penetrations among those endowed with different types of visual properties (Fig. 9C), a distinction between mirror and peripersonal responses clearly emerged; in fact, whereas mirror responses co-localized with non-excitable penetrations, peri-

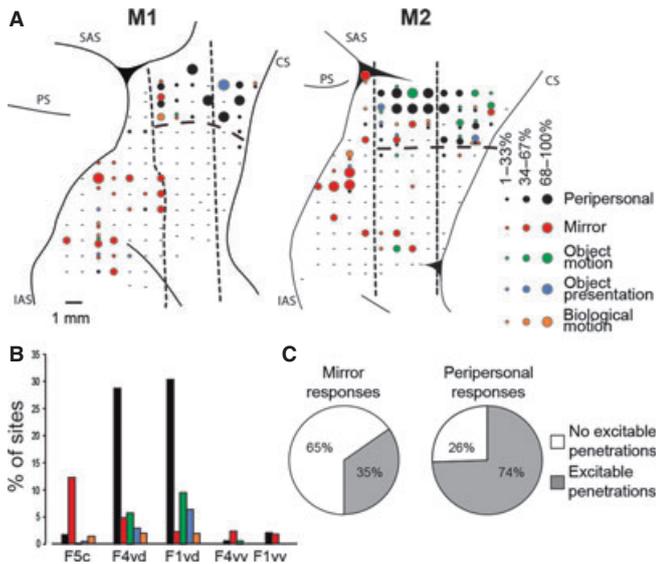


FIG. 9. (A) Distribution of the different types of visual properties in the investigated region. (B) Histograms showing the proportion of each type of visual property in each of the investigated sectors. Colour codes as in A. (C) Pie charts representing the proportion of excitable (grey) vs. non-excitable (white) penetrations among those endowed with mirror and peripersonal responses. The proportion of the different functional properties has been calculated as described in Fig. 5. Other conventions as in Fig. 2.

personal responses mainly co-localized with electrically excitable penetrations [$\chi^2 = 12.01$ ($n = 86$), $P = 0.0005$].

Discussion

In this study, we combined neurophysiological techniques in order to correlate the effects of short-train ICMS and the properties derived from the extracellular recording of multi-unit and single-unit activity, with the cytoarchitectonic parcellation of the ventral part of monkey primary motor and ventral premotor cortices.

The results showed that in both areas F1 and F4 there are two distinct functional clusters: one located dorsally, including F1vd and F4d, and devoted to the control of forelimb and mouth motor acts, and the other situated more ventrally, including F1vv and F4v, involved in the control of mouth movements. Interestingly, both of these clusters extend across the architectonic borders between areas F4 and F1. Furthermore, area F5c showed remarkably different features compared with those of the more posterior sectors.

Distinct anatomo-functional subdivisions play different roles in the control of forelimb and oro-facial movement

Areas F4d and F1vd form a functional cluster involved in the control of goal-directed motor acts in the peripersonal space

The dorsal sectors of areas F4 and F1 appear to represent a functional cluster where ICMS produced forelimb and face movements, and neuronal activity was related to forelimb and mouth goal-directed motor acts. Our data are in line with previous works showing that the dorsal part of area F4 has a role in the control of goal-directed arm movements toward different space sectors (Gentilucci *et al.*, 1988; Fogassi *et al.*, 1996). Furthermore, the finding that in F1vd neuronal activity could be related not only to simple movements (Georgopoulos *et al.*, 1982; Lemon *et al.*, 1986; Schieber, 2001) but also to goal-directed motor acts is in line with previous reports (Alexander &

Crutcher, 1990; Crutcher & Alexander, 1990; Kakei *et al.*, 1999, 2001; Umiltà *et al.*, 2008).

The functional similarity between F4d and F1vd is also supported by the distribution of the visual and somatosensory properties. In fact, a considerable number of sites in both sectors responded to visual stimuli moved within specific fields of the peripersonal space. This type of response, previously attributed solely to area F4 (Fogassi *et al.*, 1996; Rizzolatti *et al.*, 1997), has here also been found with similar frequency in the convexity of the sector of area F1 adjacent to F4d. Interestingly, in the penetrations of both F4d and F1vd in which visual peripersonal responses were found, ICMS and active motor responses were related to axio-proximal and forelimb movements.

The association between visual and somatosensory properties in a region devoted to the control of goal-directed motor acts constitutes a sensory-motor mechanism enabling individuals to represent the peripersonal space in motor terms (Fogassi *et al.*, 1996; Rizzolatti *et al.*, 1997). Taken together, these findings indicate that F4d and F1vd form a functionally unitary cluster that encompasses their cytoarchitectonic boundaries and plays a relevant role in the organization of arm and hand motor acts within specific body-centred spatial fields.

Areas F4v and F1vv form a functional cluster devoted to the control of oro-facial movements

Compared with the dorsal sectors of areas F4 and F1, F4v and F1vv are more electrically excitable. In particular, ICMS applied to these sectors produced tongue and jaw movements at relatively low current intensity, in agreement with previous studies (Huang *et al.*, 1988; see also Clark & Luschei, 1974). Moreover, the neuronal activity of these areas was mainly related to simple movements of the tongue and the jaw rather than to goal-directed motor acts, similarly to what was previously reported (Murray & Sessle, 1992a,b).

The sensory properties of the ventral sectors of F4 and F1 allow them to be further distinguished from their dorsal counterparts. In fact, in the ventral sectors, visual responses are nearly absent, whereas somatosensory responses are widely represented. In particular, somatosensory responses were more frequently found following passive stimulations of inner mouth structures (e.g. tongue, teeth and palate) rather than of the hairy and glabrous skin of the lips and the face. Taken together, these findings suggest that F4v and F1vv form a functional cluster mostly related to the control of tongue and oro-facial simple movements. By exploiting somatosensory and proprioceptive information coming from the body parts here represented, this cortical region of the monkey brain probably plays a role in the control of food-processing behaviours.

The lack of significant differences between F4v and F1vv in terms of electrical excitability, sensory responses, type of effectors and degree of movement complexity raises some doubts about the distinction between the convexity of the primary motor (F1) and premotor (F4) cortex in the motor control of the face and the mouth, in line with previous studies (Huang *et al.*, 1988). However, there is still the alternative possibility that a primary motor mouth region does exist and lies in the anterior bank of the central sulcus, which was not explored in our study. This sector could show even lower electrical thresholds (McGuinness *et al.*, 1980) than those found in the F4v–F1vv complex. A recent study (Rathelot & Strick, 2009) favoured this possibility, showing that in the forelimb field of the primary motor cortex (F1) two anatomical subdivisions can be identified, based on the type of cortico-spinal connections – a rostral region, located in the convexity of the precentral cortex, projects to spinal interneurons, thus exerting an indirect control on the target muscles, whereas a caudal region, lying in the bank of the precentral sulcus, projects monosyn-

aptically to motor neurons. If a similar anatomical substrate also characterizes the mouth region of the precentral cortex, it could justify our ICMS findings, showing higher thresholds in the precentral convexity than those expected in a primary motor cortex. However, further studies are needed to experimentally address this issue as, up to now, no data are available to allow a comparison of the functional properties of the cortex lying into the most ventral part of the bank of the central sulcus with those of the adjacent precentral convexity.

The convexity of area F5 is a functional region involved in coding goal-directed motor acts

The convexity of area F5 explored in the present study has been considered here as a unitary functional region because the low number of penetrations in its dorsal part did not allow us to compare its properties with those of F4d and F1vd. However, some differences between its dorsalmost and ventralmost parts are evident, suggesting that the restricted dorsal sector could be functionally homogeneous with the anatomical subdivision named F5p (Belmalih *et al.*, 2009), lying in the caudal bank of the inferior arcuate sulcus.

Altogether, the functional properties of F5c differ in several aspects from those of both F4d and F4v. Compared with both sectors of F4, area F5c contains a lower number of electrically excitable penetrations, and even excitable penetrations required high current intensity to elicit movements ($> 26 \mu\text{A}$).

Area F5c also differs from F4 in its motor properties. The great majority of sites in area F5c show selective responses during goal-directed motor acts, particularly hand and mouth grasping, whereas in F4v there is a wide representation of mouth simple movements. Moreover, when comparing the neuronal properties of area F5c with those of F4d, it emerges that in F4d neuronal responses are largely independent from the hand shape required for grasping, supporting the role of this area in the encoding of reaching–grasping movement in space, whereas responses in F5c frequently show strong grip selectivity. This latter finding fits well with the results of previous studies (Rizzolatti *et al.*, 1988; Murata *et al.*, 1997; Raos *et al.*, 2006; Fluet *et al.*, 2010) mainly focused on the sector of area F5 lying in the posterior bank of the arcuate sulcus (area F5p – see Belmalih *et al.*, 2009). Thus, our data extend to area F5c the role of controlling hand configuration during object grasping.

The distribution and the type of visual responses in the investigated region support the proposal of area F5c as a functional unit. In fact, in F5c there are many visually responsive neurons, the activities of which are mainly related to the observation of others' motor acts (mirror responses – see di Pellegrino *et al.*, 1992; Gallese *et al.*, 1996). Interestingly, by considering the relationship between the type of visual responses and the cortical excitability in the whole investigated region, we found that, in contrast with penetrations characterized by peripersonal responses that are frequently electrically excitable with relatively low stimulation thresholds, those endowed with mirror responses are rarely electrically excitable with the stimulation parameters employed in the present study. These differences suggest that the output of peripersonal neurons may have a stronger impact on the motor output than that of mirror neurons, as also shown by the results of previous chemical manipulation (Cooke & Graziano, 2004), short-train (Gentilucci *et al.*, 1988, 1989) and long-train (Graziano *et al.*, 2002) ICMS studies. Nevertheless, a recent study has also revealed that in area F5c there are some mirror neurons that project their axon directly to the spinal cord. Interestingly, many of these cortico-spinal mirror neurons showed complete suppression of discharge during action observation, although intensely fired during monkey grasping acts, suggesting that they could be part of a cortical mechanism for the inhibition of self movement during action observation (Kraskov *et al.*, 2009).

Taken together, these findings support the view that the convexity of area F5 constitutes a functional area playing a role in perceptuo-cognitive functions such as the recognition and understanding of others' actions (Bonini *et al.*, 2010; Rizzolatti & Sinigaglia, 2010).

Anatomo-functional considerations

The present data allowed us to identify two distinct functional regions encompassing the cytoarchitectonic boundary between areas F4 and F1, one located dorsally (F4d–F1vd) and the other more ventrally (F4v–F1vv). Furthermore, we could show that area F5c is not only anatomically but also functionally distinct from the caudal area F4.

The similarity of functional properties between adjacent, but cytoarchitectonically distinct, areas can be explained on the basis of anatomical connectivity and other physiological data.

First, the mouth fields of areas F4 and F1 (corresponding to our F4v and F1vv, respectively) are strongly interconnected (Matelli *et al.*, 1986), and share similar patterns of connections with thalamic nuclei (Matelli *et al.*, 1989) and with other cortical areas, such as the mouth fields of primary and secondary somatosensory cortices (Matelli *et al.*, 1986; Tokuno *et al.*, 1997). Furthermore, previous studies have compared the pattern of activation of primary motor and premotor regions corresponding to our F4v–F1vv complex and area F5, respectively, during jaw movements (Yoshino *et al.*, 2000). The results showed that the primary motor neurons located caudally exhibited tonic activation, very likely associated with the control and maintenance of jaw position, whereas the premotor neurons recorded more rostrally had phasic changes in their discharge during the preparation (set-related activity) and/or initiation of the movement, suggesting a more relevant role of these latter neurons in higher order aspects of the organization of mouth movement.

Second, no study is available concerning possible connections of the dorsal sector of area F1 (F1vd) with the posterior parietal cortex (possible source of visual and somatosensory inputs), although preliminary data suggest that this link could exist (Ugolotti Serventi *et al.*, 2010). On the contrary, strong connections of area F4d with the inferior parietal area PF (Rozzi *et al.*, 2006) and the ventral intraparietal area (Luppino *et al.*, 1999) are well established. These connections would explain the role of F4d in the organization of mouth behaviour (Rozzi *et al.*, 2008), in the synergism between mouth and hand movement (Yokochi *et al.*, 2003), and its involvement in visuo-motor transformations for reaching a target in the peripersonal space (Rizzolatti *et al.*, 1997). As the dorsal sectors of areas F4 and F1 (F4d and F1vd) are reciprocally and strongly connected (Matelli *et al.*, 1986), this link could justify the presence in both areas of similar functional properties. Further connectional studies based on specific functional features could better characterize the pattern of connections of these two sectors.

As far as area F5 is concerned, several studies revealed that this area is strongly connected with the anterior intraparietal area (Borra *et al.*, 2008), and with the parietal areas PFG and PF (Rozzi *et al.*, 2006; Bonini *et al.*, 2010), supporting the well-known role of area F5 in sensory–motor transformations. Furthermore, the convexity of area F5 also has strong connections with the sector of F5 lying in the anterior and ventral part of the postarcuate bank (area F5a) that, in turn, is linked with the prefrontal areas 12 and 46v. These latter pathways are potential sources of mnemonic and non-spatial sensory information, probably playing a role in the processing of abstract contextual cues for high-level functions such as action organization and planning (Gerbella *et al.*, 2011).

Notably, these patterns of cortico-cortical connections support a more direct involvement of F4d–F1vd and F4v–F1vv in motor control processes related to hand/arm motor acts and mouth movements

within different working spaces, and high-level pragmatic and perceptuo-cognitive roles for the convexity of area F5.

General considerations in a comparative perspective

From an evolutionary point of view, it has been proposed (Rizzolatti & Arbib, 1998; Fogassi & Ferrari, 2007) that monkey area F5 could be homologous to Brodmann's area 44 (posterior sector of Broca's area), which is crucial for speech production. Recently, in the lateral portion of area F5, neurons discharging selectively during monkey vocalization have been described (Coudé *et al.*, 2011), suggesting that this region could subserve the control of both oro-facial movements involved in ingestive and communicative gestures (Ferrari *et al.*, 2003) and that of sound emission. Experiments carried out in patients during brain surgery and with transcranial magnetic stimulation (Ojemann *et al.*, 1989; Pascual-Leone *et al.*, 1991; Epstein *et al.*, 1996) have shown that the stimulation of the pars opercularis of Broca's area (Brodmann area 44) produces speech arrest if the pulse is delivered while the patient is speaking, whereas the stimulation of a more caudal region does not produce speech arrest but alters the executive, motor control of oro-facial movements.

Altogether, these observations highlight some remarkable similarities between the organization of monkey and human lateral frontal motor cortex. The posterior regions show a more evident role in motor control, whereas the rostral region possesses the capacity to exploit the individual's motor knowledge at a more abstract level, enabling the emergence of socio-cognitive functions that could have been important precursors for the evolution of high-order cognitive capacities typical of humans, such as language.

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Abbreviations

ICMS, intracortical microstimulation.

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