Somatotopic Organization of the Lateral Part of Area F2 (Dorsal Premotor Cortex) of the Macaque Monkey

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Raos, Vassilis, Gianfranco Franchi, Vittorio Gallese, and Leonardo Fogassi. Somatotopic organization of the lateral part of area F2 (dorsal premotor cortex) of the macaque monkey. J Neurophysiol 89: 1503–1518, 2003; 10.1152/jn.00661.2002. The somatotopy of the lateral part of dorsal premotor area F2 has been studied by means of intracortical microstimulation and single neuron recording. The results show that most of this sector of F2 is excitable with low-intensity currents (3–40 µA) and that intracortical microstimulation evokes forelimb and trunk movements. Both proximal and distal forelimb movements are evoked in similar percentages. The proximal and distal forelimb representations partially overlap. However, proximal movements tend to be located more medially (laterally to the superior precentral dimple), whereas distal movements tend to be located more laterally (medially to the spur of the arcuate sulcus). The somatotopic organization demonstrated with microstimulation is confirmed by the similar somatotopic organization of active movements and of somatosensory properties revealed by single-neuron recording. The excitability and somatotopic organization of the lateral part of area F2 are discussed in relation to previous electrophysiological and anatomical findings. The involvement of the distal forelimb representation of area F2 in programming and controlling reaching to grasp movements is suggested.

Introduction

The agranular frontal cortex of the monkey is formed by architectonically different areas (Barbas and Pandya 1987; Geyer et al. 2000; Matelli et al. 1985, 1991; Vogt and Vogt 1919; von Bonin and Bailey 1947). According to Matelli et al. (1985, 1991), the dorsal part of the agranular frontal cortex is subdivided into three areas: area F1, corresponding to the primary motor cortex, and areas F2 and F7, which, altogether, correspond to the dorsal premotor cortex. Area F2 occupies the caudal two-thirds of superior area 6. It is bordered caudally by area F1 and extends, rostrally, up to the border with area F7, which is about 3 mm in front of the genu of the arcuate sulcus. Medially it is delimited by area F3 (the supplementary motor cortex) and laterally by the spur of the arcuate sulcus, which separates it from the ventral part of the agranular frontal cortex. F2 extends much more rostrally than area 6DC of Barbas and Pandya (1987) and corresponds roughly to the region referred to as the caudal part of the dorsal premotor cortex (PMdc) in the physiological studies (see Wise et al. 1997).

Area F2 directly projects to the spinal cord (Dum and Strick 1991; He et al. 1993; Kuypers 1981; Murray and Coulter 1981) and to the brain stem (Keizer and Kuypers 1989). In spite of its rich corticospinal projections, F2 has been considered as not excitable using the classical stimulation parameters introduced by Asanuma (1981; see Kurata 1993; Weinrich and Wise 1982). However, Godschalk and collaborators (1995) first reported the existence of excitable sites in area F2 using stimulation parameters different from the classical ones. These methodological differences make difficult to draw any final conclusion on the excitability of F2.

Another issue directly connected to the former is that of movement organization in F2. The topography of the corticospinal projections (Dum and Strick 1991; He et al. 1993), the data from single-neuron recording studies (Kurata 1993), and intracortical microstimulation studies (Godschalk et al. 1995) suggest that in F2 there is a gross somatotopic arrangement, with a hindlimb field located medially to the superior precentral dimple and a forelimb field located laterally to it.

 Whereas only few recording studies concentrated on the hindlimb movements, several studies investigated the properties of the forelimb field of dorsal premotor cortex, showing that neurons in this field are involved in planning and controlling arm reaching movements (for review, see Wise et al. 1997). Therefore the lateral part of F2 has been traditionally considered to be associated with the control of proximal forelimb movements. On the other hand, in a limited number of studies, it has been reported also the presence of neurons responding to wrist movements (Kurata 1993). However, the detailed somatotopy of the lateral part of area F2 is largely unknown, and the existence as well as the location of a distal forelimb movement representation is a matter of controversy.

The aims of the present study were to verify the excitability of the lateral part of area F2 and compare it with that of area F1 by means of intracortical microstimulation, using classical microstimulation parameters, to obtain a detailed somatotopic microstimulation map of the lateral part of F2, and to compare this latter with the map obtained from the passive and active properties of

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neurons recorded in the same sites in which microstimulation was performed in order to have a better knowledge of the input-output coupling within this cortical sector.

The results showed that almost the entire lateral part of area F2 is excitable with low-threshold currents and that there is a somatotopic organization along the mediolateral axis. Although there is a certain degree of overlap, proximal forelimb movements tended to be located more medially (laterally to the superior precentral dimple), whereas distal forelimb movements tended to be located more laterally (medially to the spur of the arcuate sulcus).

A preliminary account of these data were published elsewhere (Fogassi et al. 1999).

Methods

The experiments were carried out on two awake monkeys (Macaca nemestrina), weighing 5 and 4 kg, respectively. Before the beginning of the experimental sessions each monkey was trained to sit in a primate chair, to be handled by the experimenters, and to interact with them. When training was completed, a recording chamber was implanted above the region of the dorsal agranular frontal cortex. Surgical and recording procedures were the same as described in detail by Fogassi et al. (1996a). After complete recovery from the surgery, experimental sessions began.

Microelectrode penetrations were made perpendicularly to the cortical surface, spaced at 1-mm intervals in both the rostrocaudal and mediolateral axes. To make the penetrations perpendicularly to the cortical surface, the electrode was inclined of 30°. In each penetration, both intracortical recording and microstimulation were performed through the same electrode.

All 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 humane care and use of laboratory animals.

Intracortical microstimulation

Tungsten microelectrodes (impedance, 0.5–1.5 MΩ, measured at 1-kHz frequency) were used for both microstimulation and single-neuron recording. Intracortical microstimulation (ICMS) consisted of trains of cathodal pulses (pulse duration: 50 ms, pulse width: 0.2 ms, pulse frequency: 330 Hz) generated by a constant current stimulator. The used current intensity was 3–40 μA. The current strength was controlled on an oscilloscope by measuring the voltage drop across a 10-kΩ resistor in series with the stimulating electrode. Because it is known that the use of current intensities lower than 40 μA prevent tissue damage (Asanuma and Arnold 1975), in our microstimulation study currents higher than 40 μA were never employed. Furthermore, to be sure that microstimulation did not provoke any appreciable change in neuronal properties, neuronal recording was carried out before and after microstimulation. No effect was detected.

The standard stimulation procedure consisted of an initial stimulation with a current of 40 μA followed by successive stimulations with progressively decreasing current intensity. When no movement could be evoked with these stimulation parameters, the stimulus train duration was increased to 100 ms (“modified procedure”). The modified procedure was introduced to have a better idea of the movement representation in sites not excitable with the normal procedure. It is possible that this procedure recruits other circuits in addition to those activated with the standard procedure (Jankowska et al. 1975, Stoney et al. 1968). However, we noticed that the types of movement evoked by the longer stimulation train were similar to those evoked in the closest sites excitable with the standard procedure.

In each penetration, microstimulation was performed every 500 μm of depth, starting 500 μm from the site where the first action potential was recorded. Movements evoked by microstimulation were determined as follows. Two experimenters observed the animal. Movements were recorded only if both observers identified them, and the observed movements were constantly evoked from the same site. Microstimulation at each site was delivered when the monkey was relaxed. Once movements at a given stimulation site were identified, the threshold for each movement was determined. Threshold was defined as the current intensity at which movements were evoked in 50% of the trials. Threshold was determined using the standard stimulation procedure.

As far as the type of evoked movement is concerned, movements of the shoulder and the elbow were considered proximal, whereas movements of the forearm, the wrist, and the fingers were considered distal. The joint displacements evoked by microstimulation included those of the shoulder (abduction, adduction, elevation, depression, extension, flexion, internal or external rotation), elbow (flexion or extension), wrist (flexion, extension, ulnar, or radial deviation, pronation, supination), and fingers (flexion or extension of all fingers, abduction or adduction of the thumb).

Testing of single neurons properties and recording procedures

Neuronal activity was recorded through the same electrode used for microstimulation. The signal was amplified and monitored on an oscilloscope. Individual action potentials were isolated with a dual voltage-time window discriminator (Bak Electronics, Germantown, MD). The output signal from the discriminator was monitored and fed to a PC for acquisition. Once a neuron was isolated, neuronal activity was studied when the monkey performed active movements and during the application of somatosensory stimuli. Active movements consisted of forelimb movements (reaching and grasping objects of different size, shape, and orientation, presented in different space sectors) and trunk movements (orienting toward interesting stimuli or avoidance of threatening stimuli). Neurons were classified as distal only when they fired consistently during a particular distal movement regardless of whether the arm was flexed, extended, adducted, or abducted. The objects used for testing distal movements were selected to elicit different grip types. For example, a raisin placed inside a slit required a precision grip consisting of the opposition of the first phalanx of the thumb to the first phalanx of the index finger, whereas a syringe filled with juice required a whole-hand prehension consisting of a flexion of all fingers around the object. Somatosensory stimulation consisted of hair bending, superficial touch, or light pressure of the skin (tactile stimulation), muscle palpation and slow and fast rotation of the joints (proprioceptive stimulation). The active movements and/or the sensory stimuli that best correlated with neuronal discharge were identified.

Histological identification

Histological procedures and reconstruction of penetrations were performed according to Luppino et al. (1991). In brief, at the end of the experiment, electrolytic lesions (10-μA cathodal current for 10 s) were made at points of known stereotaxic coordinates forming a rectangle delimiting the external limits of the studied area. One week later, the animals were killed with a lethal dose of barbiturate [pentobarbital sodium (Nembutal), 80 mg/kg] and perfused transcardially with buffered saline followed by fixative. After the bone and the dura were removed, the animal was placed in a stereotaxic apparatus, and the stereotaxic coordinates of various landmarks such as the central and the arcuate sulci were assessed. The brain was then removed and photographed. Coronal serial sections 60 μm thick were cut on a freezing microtome, stained with thionin, and used for the identification of the electrolytic lesions and of the electrode tracks and for the definition of the cytoarchitectonic borders of the frontal agranular cortical areas according to the criteria of Matelli et al. (1991). Note
that the cytoarchitectonic borders of F2 are generally better identified on paraganglionic rather than coronal sections. However, because the inaccuracy in border recognition in coronal sections is limited to a few hundred micrometers and the electrode tracks are better identified on coronal than on paraganglionic sections, in the present study, the coronal cutting plane was preferred. The reconstructed trajectories of each penetration were plotted on drawings of sections taken at 180-μm intervals. The reconstruction was based on the recognition and the localization of penetration tracks, recording coordinates, recording data, and surface landmarks. Responses evoked from the white matter were excluded from the analysis on the basis of the penetration reconstruction. For each animal, the cytoarchitectonic borders delimiting the areas of the dorsal agranular frontal cortex were superimposed on the electrophysiological maps. This enabled us to unequivocally attribute to distinct motor areas (F1 and F2) the functional properties described in the present study.

Data analysis

On the basis of electrical microstimulation and single neuron recording, the data were analyzed using the criteria that follow. (1) Lowest current values (threshold) used for evoking movements in each penetration. These threshold values were determined considering all stimulation sites comprised between 1,500 and 2,500 μm of depth in which the output cortical layers are located (see Asanuma 1981). (2) Types of movement evoked at the lowest threshold as defined in 1) in each penetration. (3) Frequency at which a certain movement type was evoked along each penetration. This value was calculated as follows. For each stimulation site (each depth at which microstimulation was delivered) of each penetration, the type of movement evoked at the lowest threshold was noted. Then the movement types represented among all sites of each penetration were computed and expressed as a percentage of all stimulated site of that penetration. (4) Frequency of neurons responding during active, proximal and distal, forelimb movements, as well as axial movements, in each penetration of the forelimb sector. And 5) frequency of neurons responding to somatosensory stimulation of proximal and distal forelimb and trunk in each penetration of the forelimb sector.

By using these criteria, different maps were constructed for each monkey and superimposed on a drawing of the lateral brain surface of the recorded hemisphere of each monkey.

RESULTS

Location and borders of the explored area

The location of the explored cortical region for each monkey is shown in Fig. 1. One hemisphere in each monkey was studied. In both monkeys, the explored area extended from the central sulcus, caudally, to the border with area F7, rostrally, and from the superior precentral dimple, medially, to the spur of the arcuate sulcus, laterally. In Fig. 2 the cytoarchitectonic border between F1 and F2 is shown. According to the classification of Matelli et al. (1991), F1 is characterized by the presence in layer V of giant pyramidal cells arranged in multiple rows, whereas F2 contains only few, scattered giant pyramidal cells in layer V and a thin row of medium-sized pyramids in the lowest part of layer III.

Excitability

Movements were evoked from 392 of 904 stimulated sites (43%) in area F2 and from 417 of 445 stimulated sites (94%) in area F1. The percentage of the sites of areas F1 and F2 from which movements were evoked (effective sites) in both monkeys is shown in Fig. 3. The excitability of the two areas was very similar in both monkeys. It is clear from the histograms that F2 is less excitable than F1. Seventy-one percent of the effective sites of area F2 were excitable using the standard stimulation procedure and 29% using the modified train procedure, whereas in area F1 almost all the effective sites (95%) were excitable using the standard stimulation procedure.

The spatial distribution of the excitability of the studied regions is shown in Fig. 4 (low-threshold maps). As a general pattern, large part of the explored F2 is excitable. In both monkeys, a sector with unexcitable sites was found in the most anterior part of area F2, near its border with area F7. Figure 4 shows also that area F1 is excitable along its whole extent.

To visualize the change in excitability when moving from the posterior to the anterior border of F2, we plotted the percentage of effective and not excitable sites as a function of the distance between each column of the stimulation grid and the cytoarchitectonic border between F1 and F2, considered as the zero of the x axis. The bar graph presented in Fig. 5 shows that the percentage of effective sites is about 70% near the posterior border of F2, while near the anterior border of F2 it drops to 10%, approximately. It is worth to note that the percentage of effective sites in area F1 rises to 94%.

Threshold

Figure 6 shows the percent distribution of movement thresholds determined in F1 and F2 considering all sites excitable with the standard stimulation procedure. Note that there is a shift of the excitable sites of F2 toward higher threshold values when compared with the excitable sites of F1. In F1, 65% of the sites are excitable with threshold values up to 20 μA and only 35% of the sites are excitable with threshold values ranging between 21 and 40 μA. In F2 this pattern is reversed.
only 30% of the sites are excitable with threshold values below or equal to 20 μA, whereas 70% of the sites are excitable with threshold values comprised between 21 and 40 μA. The average threshold values for F1 and F2 for both monkeys are reported in Table 1. The differences in the average threshold values between F1 and F2 in both monkeys were statistically
significant (Student’s t-test, \( P < 0.0001 \)). Therefore the two areas differ in their excitability thresholds, F2 being, on average, excitable with higher thresholds than F1.

To verify whether the difference in threshold distribution between F1 and F2 could be dependent on the type of movement evoked by microstimulation, we plotted the thresholds distribution in F1 and F2, separately for distal and proximal forelimb movements (for the somatotopical arrangement of proximal and distal movements see next section). Figure 7 shows that in F1 61% of the sites from which proximal movements are evoked and 63.5% of the sites from which distal movements are evoked are excitable with threshold values up to 20 \( \mu \text{A} \). In F2, only 24.7% of the sites from which proximal movements are evoked and even fewer (17.6%) of the sites from which distal movements are evoked, are excitable with threshold values up to 20 \( \mu \text{A} \). Moreover, Table 1 shows that the average threshold of both proximal and distal movements is significantly higher in F2 than in F1 and that in F2 the average threshold for evoking proximal movements is not significantly different from that needed for evoking distal movements. These results rule out the possibility that the difference in threshold between F1 and F2 is due to the type of movement evoked by microstimulation.

**Somatotopy and characteristics of evoked movements**

Evoked movements in F2 consisted of fast, short-lasting movements. Following Mitz and Wise (1987) and Luppino et al. (1991), we classified the evoked movements into three
classes: simple, contiguous, and complex. Most of them (n = 360, 92%) consisted in the displacement of a single joint (simple movements). Displacement of two adjacent joints (contiguous movements) represented 6% (n = 24) of the total number of evoked movements, whereas displacement of more than two joints or of not contiguous joints (complex movements) represented 2% (n = 8) of the total number of evoked movements. The evoked movements always involved body parts contralateral to the stimulated hemisphere.

The number and the percentages of the various types of proximal and distal simple movements evoked by microstimulation in both F1 and F2 are presented in Table 2. In the forelimb field of area F2, proximal and distal movements were almost equally represented. A similar distribution in percentage of proximal and distal movements was observed also in F1; however, these latter data must be taken with caution because part of it was not microstimulated in particular the lateral part of the hand representation.

In Figs. 8 and 10, the types of movement evoked at the lowest threshold in each penetration are presented. Figure 8 shows the gross somatotopy and the organization of proximal and distal movements in area F1 and in area F2. In the forelimb field of F1, proximal movements are represented medially to distal movements. In the forelimb field of F2 there is a partial overlap between the representations of proximal and distal movements. However, there is a tendency for the proximal representation to be located more medially (laterally to the superior precentral dimple), and for the distal representation to be located more laterally (medially to the spur of the arcuate sulcus). This tendency was more pronounced in monkey 2.

To quantify this tendency, the percentage of sites evoking proximal or distal forelimb movements was calculated for each row of the grid, and each percentage was plotted as a function of the distance between each row and the most medial row of the grid. The bar graph presented in Fig. 9 demonstrates that proximal forelimb sites decrease and distal sites increase moving along the mediolateral direction within the forelimb field.

Movements evoked from the forelimb field of F2 included all possible shoulder, elbow and wrist displacements (for the classification of all possible joint displacements, see METHODS section). It must be noted that pronation and supination movements of the forearm have been included among wrist movements, because they are usually involved in matching hand orientation with object orientation during hand actions. Moreover, the number of sites from which simple forearm movements were evoked is low (n = 16) in respect to that of the sites from which simple wrist movements (n = 97) were evoked.

<table>
<thead>
<tr>
<th>TABLE 1. Average threshold values (µA) of evoked movements</th>
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<tbody>
<tr>
<td>Monkey 1</td>
</tr>
<tr>
<td>F1</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Forelimb field</td>
</tr>
<tr>
<td>(n: F1 = 264, F2 = 225, F2 = 66)</td>
</tr>
<tr>
<td>(n: F1 = 123, F2 = 99, F2 = 27)</td>
</tr>
<tr>
<td>(n: F1 = 141, F2 = 73, F2 = 126, F2 = 37)</td>
</tr>
</tbody>
</table>

The subscripts refer to monkeys 1 and 2, respectively. * Statistically significant differences between average threshold values of F1 and F2, as revealed by the Student’s t-test (P < 0.0001).
thus not affecting the general somatotopic organization of proximal and distal movements.

Figure 10 shows a more detailed somatotopic organization of the forelimb field. Evoked movements are described in terms of the joint around which the displacement occurred. The presence of more than one symbol for a given penetration means that two movements were evoked at the same threshold.

To give a more complete description of all movements evoked along each penetration, a map of the “most represented movements” is presented in Fig. 11, which takes into account all the movements evoked at the lowest threshold in each stimulated site along each penetration (see METHODS). It is clear that in area F2 in many instances both proximal and distal movements could be evoked in a single penetration. In general, among the penetrations in which mostly proximal movements were evoked, shoulder movements were prevalent on elbow movements, whereas among the penetrations in which mostly distal movements were evoked, wrist movements prevailed on finger movements. In addition, in those penetrations in which only distal or only proximal movements could be evoked, the stimulation could produce movements at low threshold of different joints, for example, movements of the wrist and of the fingers. These movements were, in general, evoked from different sites.

Figures 12 and 13 show some examples of the distribution in depth of the movements evoked along each of the penetrations reported on the corresponding coronal section. Figure 12D shows the threshold and movements representation in a row of penetrations extending from the bank of the central sulcus, caudally, to the level of the arcuate sulcus, rostrally.

Table 2. Movement types evoked by microstimulation

<table>
<thead>
<tr>
<th></th>
<th>F1 n</th>
<th>Percentage</th>
<th></th>
<th>F2 n</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISTAL</td>
<td></td>
<td></td>
<td>Distal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fingers</td>
<td>89</td>
<td>23.7</td>
<td>Fingers</td>
<td>55</td>
<td>16</td>
</tr>
<tr>
<td>Wrist</td>
<td>98</td>
<td>26.1</td>
<td>Wrist</td>
<td>113</td>
<td>33</td>
</tr>
<tr>
<td>PROXIMAL</td>
<td>188</td>
<td>50.2</td>
<td>Proximal</td>
<td>175</td>
<td>51</td>
</tr>
<tr>
<td>Elbow</td>
<td>23</td>
<td>6.2</td>
<td>Elbow</td>
<td>50</td>
<td>14.6</td>
</tr>
<tr>
<td>Shoulder</td>
<td>165</td>
<td>44</td>
<td>Shoulder</td>
<td>125</td>
<td>36.4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>375</td>
<td>100.0</td>
<td>Total</td>
<td>343</td>
<td>100.0</td>
</tr>
</tbody>
</table>

FIG. 8. Somatotopic map of the stimulated region of monkeys 1 and 2 based on the movement evoked at the lowest threshold in each penetration. Ellipses represent forelimb movements and diamonds represent trunk movements. □, □ represent proximal movements, ●, distal movements; and ○ represent complex, proximal, and distal movements. Combined symbols mark penetrations in which 2 different movements were evoked at the lowest threshold. ——, different agranular frontal areas. Other abbreviations as in Fig. 1.
The somatotopic organization of active movements was very similar to that obtained on the basis of microstimulation data. A comparison between the types of movement evoked by the microstimulation at a given site and the types of active movement that were most effective in triggering the neurons’ discharge at the same site, revealed a high degree of compatibility (monkey 1: 77%, monkey 2: 85%). Neurons responding during active movements of the arm (n = 401), hand (n = 242), and trunk (n = 50) were found. Figure 14, A and B, shows also clearly that in the medialmost sector of the forelimb representation almost all neurons were active during arm movements, whereas in the more lateral sector, neurons responding to either arm or hand movements tended to mix. It is interesting to note that neurons recorded in the penetrations of the most medial sector, where no excitatory sites were found, were activated mainly during arm and axial movements.

The somatotopic organization based on neurons somatosensory properties was, as in the case of active movements, very similar to that found with microstimulation. However, in some penetrations, neurons responding to passive stimuli could not be found. A comparative analysis similar to that performed for active movements was made also for the somatosensory properties. We compared the types of movements evoked by microstimulation at a given site with the body part whose proprioceptive or tactile stimulation activated the neurons recorded at the same site. As already found for active movements, also this comparison revealed a high degree of congruence (monkey 1: 75%, monkey 2: 90%). Neurons responding to passive stimulation of the arm (n = 183), hand (n = 96), and trunk (n = 55) were found. Thus neurons responding to passive stimulation of proximal body parts were more numerous than those responding to passive stimulation of distal body parts. Neurons responding to stimulation of the trunk were recorded more medially, those responding to stimulation of the hand were located more laterally, whereas neurons activated by passive stimulation of the arm were present in all the recorded area. Most of the recorded neurons responded to proprioceptive stimulation (70%), while a smaller percent responded to tactile stimulation (20%). An even smaller percentage (10%) responded to more than one somatosensory modality.

**DISCUSSION**

The present data show that most of lateral part of area F2 is excitatory with low-threshold currents and that there is a somatotopic organization along its mediolateral axis. Although a certain degree of overlap is observed, proximal forelimb movements tend to be located medially (laterally to the superior precentral dimple), whereas distal forelimb movements tend to be located laterally (medially to the spur of the arcuate sulcus).

The discussion will deal initially with the anatomical identification of area F2, then with its excitability and its somatotopic organization. At the end, an interpretation of the possible role of area F2 in movement control in the light of these and other recent data will be suggested.

**Anatomical and functional identification of area F2**

In the present work, area F2 was anatomically identified using the criteria defined by Matelli et al. (1991). The subdivision proposed by these authors, initially based on regional...
differences in the pattern of cytochrome oxidase activity (Matelli et al. 1985), was subsequently confirmed and extended using other techniques such as Nissl staining (Matelli et al. 1991) and, most recently, immunohistochemical staining of neurofilament proteins with the monoclonal antibody SMI-32 (Gabernet et al. 1999; Geyer et al. 1998, 2000; Petrides et al. 2000).

This parcellation was further validated by neuroanatomical data (Marconi et al. 2001; Matelli et al. 1998). Injections of tracers in areas F1, F2, and F7 marked different areas. In particular, two injections made in the same monkey near to the border between F2 and F7, one in the rostral part of F2, the other in the caudal part of F7, produced a completely different pattern of labeling (Matelli et al. 1998; see Figs. 11, 13, and 16).

All these histological and neuroanatomical data suggest that the dorsal part of agranular frontal cortex is formed by different identifiable areas. It must be noted that the identification of a border between different areas can be affected by many factors such as the criteria used for the parcellation or the cutting plane. In F2, for example, when Nissl staining is used, the changes of different cytoarchitectonic features do not occur exactly at the same level. Also, the caudal and rostral borders of F2 are more easily identified on parasagittal than on coronal sections. Thus a transition zone that encompasses few hundred microns is often assigned. However, note that a rostral or caudal shift of a few hundred microns in the border between F1 and F2 or between F2 and F7 would not affect the interpretation of the data of the present work.

Single-neuron recording data support the functional differentiation between the primary motor cortex and the caudal and rostral subdivisions of the dorsal premotor cortex. Weinrich et al. (1984) found that signal- and set-related units were by far more numerous in premotor cortex (47 and 34%, respectively) than in precentral motor cortex (15 and 14%, respectively). Moreover, in movement-related units, the discharge begun...
significantly earlier in premotor (150 ms before movement onset) than in precentral motor cortex (80 ms before movement onset). Mushiake et al. (1991) showed that in a task in which the monkey had to perform an externally or internally triggered motor sequence, neurons of premotor cortex were clearly dependent on visual information, whereas neurons of primary motor cortex were not.

The functional differences between the primary motor cortex and the dorsal premotor cortex are neither abrupt nor absolute. However, these differences do exist, indicating that there are distinct cortical areas. The presence of a gradual functional trend within the proximal arm representation of the dorsolateral frontal lobe has been suggested based on relative measures of direction-related activity (Johnson et al. 1996). Johnson et al. concluded that although these data do not indicate a clear-cut functional border between MI and PMd, they confirm the existence of anatomical and physiological differences between the rostralmost and caudalmost portions of the investigated region.

**Excitability of area F2**

The present data demonstrate that it is possible to evoke movements from area F2 using the classical stimulation parameters. This allows also a direct comparison with the excitability of area F1 studied with the same parameters.

It is generally accepted that excitability is correlated with the existence of corticospinal connections. The finding that most of the forelimb field of area F2 is excitable is congruent with its heavy connections with the spinal cord (Dum and Strick 1991; He et al. 1993; Keizer and Kuypers 1989; Murray and Coulter 1981; Nudo and Masterton 1990). The difference in excitabil-
FIG. 12. Examples of the types of movements evoked by microstimulation along penetration tracks in area F2 of monkey 1. A–C, left: the reconstruction of microelectrode tracks on outlines of coronal sections taken at 3 rostrocaudal levels (shown in the inset) is presented. Right: the same tracks are reported, schematized and magnified, to show, for each penetration, the movement evoked every 0.5 mm of depth as well as the range of the current threshold required to evoke it. D: types of movements evoked at different depths along penetrations belonging to a lateral row of the stimulation grid, the reconstruction of which is shown in the inset. Letters inside each symbol represent the joints involved in the evoked movement as shown in the key. The shading of each symbol indicates the range of threshold values as indicated in the key.
ity and in average threshold between F2 and F1 is most likely due to the lower percentage of corticospinal projections of the former in respect to the latter area. (Kuypers 1981; see Porter and Lemon 1993). A drop of excitability was observed moving from the posterior to the anterior border of F2. This drop correlates well with the caudorostral decrease of corticospinal density in area F2, as shown by Galea and Darian-Smith (1994) after injection of fluorescent tracers in the dorsolateral column of the spinal cord. Finally, the loss of excitability in the rostral part of F2 coincides quite well with the dramatic drop of corticospinal neurons in this sector as demonstrated by many authors (Dum and Strick 1991, 1996; Galea and Darian-Smith 1994; He et al. 1993; Kuypers 1981; Murray and Coulter 1981; Nudo and Masterton 1990).

The low excitability of the region around the superior precentral dimple could be partially explained by some anatomical findings (Galea and Darian Smith 1994; He et al. 1993). Galea and Darian Smith (1994) showed that in the region around the superior precentral dimple, there is a decrease in the number of labeled corticospinal neurons. Also in the work of He et al. (1993), who injected fluorescent dyes in the gray matter of cervical and thoracic spinal cord, the retrogradely labeled zone in the dorsal premotor cortex shows a decrease in the density of labeled neurons in the dimple region.

In the past, a criterion used for defining the transition between the primary motor cortex and the dorsal premotor cortex was that of a sharp decrease in excitability in the latter area (Kurata 1989; Weinrich and Wise 1982). However, in those studies, that were mainly devoted to the investigation of single-neurons properties, a detailed description of microstimulation data was not given.

A change in perspective on the issue of the excitability of dorsal premotor cortex was provided by the microstimulation study of Godschalk et al. (1995). These authors first demonstrated that it is possible to evoke movements by microstimulating dorsal premotor cortex. The present data on the excitability of area F2 are in general agreement with this latter study. It must be noted, however, that their data were obtained using microstimulation parameters not directly comparable with those used in our study (microelectrode impedance: 30–100 kΩ in their study vs. 0.5–1.5 MΩ in our study; type of stimulation: biphasic in their study vs. monophasic in our study; train duration: always 31 pulses in their study vs. normally 16.5 pulses in our study; current intensity: up to 60 µA in their study vs. up to 40 µA in our study).

In a recent report (Graziano et al. 2002), microstimulation was used to study primary motor and premotor cortex using very long train durations (500 ms) and high currents (up to 150 µA). The authors pointed out that they used long stimulus trains because this time scale approximates that of normal reaching and grasping movements and the time scale of neuronal activity that normally accompanies movement. The stimulation with these parameters evoked coordinated complex postures that involved many joints. The authors suggested that,

FIG. 13. Movement types evoked along penetration tracks in area F2 of monkey 2. A–C, microelectrode tracks reconstructed on outlines of coronal sections taken at 3 rostrocaudal levels (shown in the inset). Below each coronal section, the same tracks are reported, schematized, and magnified, to show, for each penetration, the movement evoked every 0.5 mm of depth as well as the range of the current threshold required for evoking it. Symbols and conventions as in Fig. 12.
the map represented in the motor cortex and the adjacent premotor cortical areas is a map of spatial locations near the body to which movements are directed and not a map of the body in terms of muscles or movements. The findings of Graziano et al. cannot be easily compared with those of the present study. In fact, the stimulation parameters used by Graziano et al. mainly reflect the activation of interneuronal circuits that can affect several motoneuronal pools, whereas those used in the present study tend to produce a more local monosynaptic and/or oligosynaptic activation of the spinal motoneurons.

Movement types and somatotopic organization of area F2

Before discussing in detail the somatotopic arrangement of area F2, we would like to briefly discuss a factor that may influence the interpretation of the data, namely the possible effects of current spread.

It is known that the theoretical current spread into the cortex is proportional to the current intensity. Current spread has been estimated to be confined within a radius of 90 μm for current intensities of 10 μA and of 400 μm for current intensity of 40 μA (Andersen et al. 1975; Cheney and Fetz 1985; Jankowska et al. 1975; Marcus et al. 1979; Ranck 1975; Stoney et al. 1968). Because the distance between each penetration of our grid was 1 mm, that is, a distance longer than the maximum current diffusion at the highest current intensity used, then current spread cannot influence the attribution of a specific movement type to each penetration. Current spread per se should not influence also the attribution of the movement type at each site of a single penetration because the two closest microstimulation sites within each penetration were spaced 500 μm in depth.

In the present study, most of the excitable sites in F2 elicited, when stimulated, simple movements, that is, displacement of a single joint. Simple movements were also typically evoked.
from area F1, confirming several previous studies (Huntley and Jones 1991; Kwan et al. 1978; see also Asanuma 1981). However, the average threshold at which movements were evoked was lower in F1 than in F2. This difference in threshold is similar to the difference in threshold found between the medial part of F1 and F3 (Luppino et al. 1991). Thus generally speaking, higher threshold currents are required for evoking movements from dorsal and mesial premotor cortex than those needed in area F1. In spite of the similarity in microstimulation threshold, F2 and F3 differ in the relative percent of simple and complex movements. Although simple movements are the majority of evoked movements in both areas, complex movements are much more represented in F3. Because both areas can reach the spinal motor neurons via a direct (Dum and Strick 1996) and an indirect (through the brain stem) (see Keizer and Kuypers 1989) pathway, it is not easy to establish why these areas show this different pattern of evoked movements. We can only hypothesize that at the spinal cord level, the distribution of the descending fibers coming from the two areas must be differently organized.

In the forelimb field of F2, the number of evoked proximal movements was almost equal to that of distal movements. This finding suggests that area F2 does not exert such a sophisticated control on discrete distal forelimb movements as F1 does and that in F2, the control of proximal and distal movements tend to assume equivalent functional importance.

In the microstimulation study of Godschalk et al. (1995), the location of proximal and distal movements is given. They show that the sectors of dorsal premotor cortex from which proximal and distal movements are evoked largely overlap (see their Fig. 3C); however, it is not clear to which degree because the movement details are not shown.

In the present study, in the forelimb field of F2, a segregation between proximal and distal movements is evident along the mediolateral axis. Proximal movements tend to be more concentrated in the medial half, distal movements in the lateral half, though with a certain degree of overlap.

The presence of both distal and proximal movements in the forelimb field of F2 is in agreement with the anatomical findings of Strick and collaborators (Dum and Strick 1991; He et al. 1993). They showed that the forelimb region projects to both the upper and lower cervical segments. They found, however, that neurons projecting to lower cervical segments had a higher density within and around the lateral bank of superior precentral dimple, whereas neurons projecting to the upper cervical segments were more concentrated in a more lateral region of the dorsal premotor cortex. This mediolateral organization of proximal and distal forelimb representations based on corticospinal projections seems to be inverted with respect to that found in the present study based on microstimulation data. To explain this apparent discrepancy, a series of considerations must be made. 1) In the work of He et al. (1993), there is a substantial overlap of neurons labeled after injections of either upper or lower cervical segments (see their Fig. 10). The difference in concentration between populations of neurons labeled after either injection is visible only when high-density peaks are considered. 2) Although motoneurons that innervate hand muscles are mainly concentrated in the lower cervical segments (C7–T1), where the retrograde tracer has been injected, in the same region, there are also motoneurons that innervate proximal muscles such as the triceps and, partially, the biceps (Jenny and Inukai 1983). Thus the injection of a retrograde tracer in the C7–T1 segments most likely involved not only distal but also proximal muscle representation. 3) A similar consideration can be made for distal muscles such as the extensor carpi radialis, which is controlled by motoneurons that are located in the C4–C6 segments of the spinal cord (Chiken et al. 2001; Jenny and Inukai 1983). These latter segments were not injected in the study of He et al. (1993). This could have caused an underestimation of the distal component of the corticospinal projection from the dorsal premotor cortex, and, in particular, of wrist movements that, in our study, are the most represented among the evoked distal movements. 4) Corticospinal neurons that end in the upper cervical segments, and therefore have been considered proximal, could influence also distal motoneurons via propriospinal connections (Alstermark et al. 1999).

Taking into account all these considerations, it is plausible that the mediolateral gradient in the organization of proximal/distal movements found in our study is not necessarily in contrast with the pattern of corticospinal projections.

Classically, dorsal premotor cortex was considered to play no role in the control of distal movements. However, the surface stimulation maps of Woolsey already showed the presence of distal movements in a region corresponding to the forelimb field of the dorsal premotor cortex (Woolsey et al. 1952). More recently, a study in which microstimulation was used for different aims, showed that wrist and finger movements can be evoked from a region that we would consider as part of area F2 (Huntley and Jones 1991).

A further direct support to the notion that distal movements are represented separately from proximal movements in the lateral part of area F2 comes from hodological studies. After injection of two different dyes in the rostrolateral part of area F2 and in the part of F2 immediately lateral to the dimple, respectively, two different areas of ventral premotor cortex were retrogradely labeled (Matelli et al. 1999). After the rostrolateral injection, neurons in area F5 were labeled, whereas after the injection lateral to the dimple, neurons in area F4 were labeled. It is known that area F5 controls mostly distal movements (Murata et al. 1997; Rizzolatti et al. 1987, 1988; see also Kurata and Tanji 1986), whereas in area F4, proximal and axial movements are represented (Fogassi et al. 1996b; Gentilucci et al. 1988). The presence of a differential connectivity of sectors of F2 with areas having different motor representations suggests that, consistent with the present data, the medial part of the forelimb field of area F2 could be involved in the control of proximal and axial movements, whereas the lateral part could be more involved in the control of distal movements. In this respect, our study shows that wrist movements are the most represented among distal movements. The contribution on wrist control of this F2 sector can be relevant for tasks in which the wrist must be oriented in relation to the orientation axis of the object to be grasped or when wrist orientation becomes conditional for subsequent hand actions.

Summing up, microstimulation and anatomical data are in support of the presence of a distal and a proximal representation in the forelimb field of area F2. This does not mean that the two representations are completely separated one from each other. The presence of a sector of overlap between proximal and distal movements could underpin their putative functional integration.
Comparison between microstimulation and neuronal recording data

Testing active and passive properties of neurons recorded at the same sites where microstimulation was delivered gives an additional insight on the somatotopic representation and the functional properties of the studied area. This testing reveals in area F2 a very good congruence between its somatotopy as shown by both passive and active neuronal properties and that obtained by electrical microstimulation. For example, in a site in which microstimulation produced wrist movements, neurons were related to movements of the hand in which wrist orientation constituted a crucial component and/or to the passive stimulation of the hand. Similarly, in a site in which microstimulation evoked a displacement of the shoulder, neurons were related to arm reaching and/or to the passive displacement of the shoulder. Overall, maps obtained with microstimulation and neuronal recording related to arm reaching and/or to the passive displacement of the proximal shoulder. Overall, maps obtained with microstimulation and neuronal recording resulted to have a very similar somatotopic organization. In addition, the location of and the partial segregation between proximal and distal movements found with microstimulation were confirmed on the basis of neuronal recording. Taken together, all these data suggest that in the forelimb field of area F2 there are two major sectors that probably subserve different functions. What may be these functions?

Since the first studies of Wise and coworkers (Weinrich and Wise 1982; Weinrich et al. 1984; Wise et al. 1983), many authors described that neurons of the dorsal premotor cortex are active during preparation and/or execution of arm reaching movements in visually instructed tasks (Caminiti et al. 1991; Crammond and Kalaska 1994, 1996; Johnson et al. 1996). Recently, Hoshi and Tanji (2000) demonstrated that in the same area, there are neurons that integrate information about the target location and the forelimb to be used to accomplish a reaching action. On the other hand, other studies suggested that in the forelimb field of area F2 there are also neurons discharging during the execution of tasks requiring mainly movements of the wrist (Kurata 1993). Finally, in recent experiments from our lab, neurons from the forelimb field of area F2 were recorded during the execution of a paradigm requiring reaching to grasp movements directed to different objects. Most of the recorded neurons located in the most lateral part of the forelimb field specifically coded the combination of wrist orientation and finger configuration (Raos et al. 1999). Thus in agreement with microstimulation data, also recording studies show that in the forelimb field of F2 both proximal and distal movements are represented.

In light of these and other functional data and of the above mentioned recent anatomical findings, it seems that in both dorsal and ventral premotor cortex, proximal and distal forelimb movements are represented at least twice: proximal movements in F2 (Caminiti et al. 1991; Crammond and Kalaska 1994, 1996; Hoshi and Tanji 2000; Johnson et al. 1996; Weinrich and Wise 1982) and in F4 (Fogassi et al. 1996a; Gentilucci et al. 1988) and distal movements in F2 (Kurata 1993, Raos et al. 1999) and in F5 (Kurata and Tanji 1986; Murata et al. 1997; see also Rizzolatti et al. 1987, 1988).

The link between F4 and the medialmost sector of the F2 forelimb field could subserve planning and control of reaching actions in space. In this “proximal” circuit, both the somato-centered space and some motor parameters, such as direction and amplitude, could be coded. Conversely, the link between F5 and the most lateral part of F2 could subserve planning and control of distal actions toward objects. In this “distal” circuit, both fingers and wrist configuration could be coded.

In this respect, it is interesting to note that Jeannerod (1981) and Arbib (1981) proposed independently a model of prehension, according to which this function would be processed by two independent channels, one for the transport (reach) and the other for the grasp component. This model was subsequently substantiated by human kinematic data (Gentilucci et al. 1991; Jeannerod 1984). The two above-mentioned links between areas of dorsal and ventral premotor cortex could provide a neural substrate for this model.

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