Functional Properties of Grasping-Related Neurons in the Dorsal Premotor Area F2 of the Macaque Monkey

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The discharge of neurons in the dorsal premotor area F2 of the macaque monkey using a behavioral paradigm for studying the neuronal discharge during observation (object fixation condition) and grasping of different 3-dimensional objects with and without visual guidance of the movement (movement in light and movement in dark conditions, respectively). The main result is that almost all studied neurons were selective for both the type of prehension and the wrist orientation required for grasping an object. Three categories of neurons were found: purely motor, visually modulated, and visuomotor neurons. The discharge of purely motor neurons was not affected by either object presentation or by the visual feedback of the hand approaching to and interacting with the object. Visually modulated neurons presented a different discharge in the 2 movement conditions, this determining a decrease in selectivity for the grip and wrist orientation in the movement in dark condition. Visuomotor neurons typically discharged during the object fixation task even in the absence of any grasping movement. Nine of them also displayed a different discharge rate between the 2 movement conditions. Congruence was observed between the neuronal response during the most effective type of prehension and the neuron response during observation of the object requiring that particular prehension. These results indicate an important role of F2 in the control of goal-related hand movements.

INTRODUCTION

Area F2 is a cytoarchitectonic area (Matelli et al. 1991; see also Matelli et al. 1985) that occupies the caudal two thirds of superior area 6 (dorsal premotor cortex (PMd)). It is located anterior to area F1 (primary motor cortex), extends rostrally ≤ 3 mm in front of the genu of the arcuate sulcus, and, laterally, up to the spur of the arcuate sulcus, which separates it from inferior area 6.

Dorsal premotor cortex has been extensively studied during the last two decades. Electrophysiological studies carried out in monkeys trained to perform reaching movements toward visual targets showed that neurons in the dorsal premotor cortex display 3 types of activity: signal-related (at the appearance of the instruction stimulus), set-related (when the movement onset is withheld), and movement-related (in association with movement onset) (Wise 1985). Signal-related activity was initially thought to be visual, whereas later investigation showed it to be, at least in part, related to the motor significance of the stimulus (Boussaoud et al. 1996). A number of studies have demonstrated that the set- and movement-related discharge of the dorsal premotor neurons is correlated to parameters of reaching movements such as direction and amplitude (Caminiti et al. 1991; Fu et al. 1993; Kalaska et al. 1997; Wise et al. 1997). In these studies, however, only proximal forelimb movements were taken into account, the contribution of the distal forelimb movements to the neuronal discharge not being considered until recently.

A recent electrophysiological study demonstrated that within area F2 a distal forelimb field also exists. Wrist and finger movements can be evoked by intracortical microstimulation in this field. Furthermore, single-neuron recording revealed the presence of many neurons related to distal actions (Raos et al. 2003). In addition, neurons responding to visual and somatosensory stimuli have been reported (Fogassi et al. 1999; Raos et al. 2003).

Prompted by these findings, we investigated the properties of neurons located in the distal forelimb field by use of a behavioral paradigm that allows the study of neuronal discharge during both observation and grasping of different 3-dimensional objects with and without visual guidance. The present study demonstrates that in the distal forelimb representation of area F2 there are neurons that are selective for both the type of prehension and the wrist orientation required for grasping the object. These results indicate an important role of F2 in the control of goal-related hand movements. A preliminary account of these data was previously presented (Raos et al. 1999).

METHODS

Single-unit activity was recorded from the forelimb field of area F2 in 2 hemispheres (contralateral to the moving forelimb) of 2 awake monkeys (Macaca nemestrina). The monkeys (one male and one female, weighing 4 and 5 kg, respectively) were seated on a primate chair with the head fixed and familiarized with the experimental setup. They were trained to perform a behavioral paradigm (see following text). After completion of the training a recording chamber was implanted. Surgical and recording procedures were previously described (Fogassi et al. 1996). 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.

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Single-neuron recording was carried out using tungsten microelectrodes inserted into the dura perpendicularly to the cortical surface (impedance 0.5–1.5 MΩ, measured at 1-kHz frequency). Recording of neuronal activity and microstimulation were performed through the same electrode. The recorded 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.

**Naturalistic testing**

Naturalistic testing preceded the selection of neurons tested with the behavioral paradigm. The activity of each recorded neuron was correlated with the execution of active movements as well as with somatosensory and visual stimulation. Active movements consisted of forelimb movements, such as reaching for and grasping objects of different size, shape, and orientation, presented in all space sectors, as well as of trunk movements, such as orienting toward interesting stimuli or avoiding 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 in 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. The naturalistic approach allows the experimenter to observe the discharge of single neurons during the performance of a wide range of behavioral activities and then, on the basis of these observations, to make hypotheses that can be tested with more standardized tasks. The advantage of this approach is that the experimental design is not biased by preconceived hypotheses. The characterization obtained by the naturalistic testing was congruent with the characterization resulting from testing with the behavioral paradigm (see RESULTS).

**Behavioral apparatus and paradigm**

The grasping neurons preselected with the naturalistic test were further thoroughly studied by means of the behavioral paradigm originally devised by Sakata and coworkers (see Murata et al. 1996, 1997, 2000). The monkey was seated in front of a box containing a PC-controlled rotating turntable subdivided into 6 sectors, each containing a different object presented one at a time. A spot of light from a red/green light-emitting diode (LED) was projected onto the object through a half mirror. Neurons were tested in 4 experimental conditions run separately. Each trial in all conditions started in complete darkness. The temporal sequence of the events during the 4 conditions is illustrated schematically in Fig. 1.

1) **Movement in light condition (ML):** When the LED was turned on (red color), the monkey had to fixate it and press a key for a variable period of 1.0 to 1.2 s. When the key was pressed, the box was illuminated and the object became visible. Subsequently, the LED changed color (from red to green), and the monkey was required to release the key, reach for and grasp the object, pull and hold it. The moment the key was released, the LED color changed from green back to red. After the object was grasped, the monkey was required to keep the object pulled, until the LED changed color again from red to green, and then release it. The different objects were presented in random order.

2) **Movement in dark condition (MD):** In this condition after a first discarded trial in which the object was grasped in light, the light inside the box was turned off and all the following trials were executed in complete darkness. In this condition the objects were presented in blocks. In conditions 1 and 2, the monkey was rewarded at object release.

3) **Object fixation:** A green LED was turned on. The monkey had to fixate the LED and press the key. Key press determined the illumination of the box. The monkey had to maintain fixation for 1–1.2 s, and then release the key when the LED changed color to red. The different objects were presented in a random order.

4) **LED fixation:** The task was the same as in condition 3 but in this case key press did not lead to the illumination of the box and no object was visible. The monkey was simply required to fixate the spot of light. This condition was run to rule out a possible effect of LED fixation per se on the neural response.

In conditions 3 and 4, the monkey was rewarded at the release of the key. All the events of the 4 conditions were acquired from a PC, together with neuronal activity.

The objects were presented one at a time, always in the same central position and at the same distance from the animal’s hand (16 cm). In all conditions, the monkeys’ hand movements were continuously video-monitored by means of miniature, infrared-illumination–sensitive videocameras. One of them provided the top view, the other one the side view of the performing hand. Before the go signal in all conditions the monkey was engaged in a motor behavior (key press) that prevented any possible unwanted movement of the performing arm/hand. The initial LED color (red or green) used in the movement and fixation conditions, respectively, allowed the monkey to discriminate immediately between the movement and the fixation conditions. Trials in which the motor behavior was not correct were discarded. Eye movements were always monitored using a third infrared camera.

![FIG. 1. Top: diagrammatic representation of the time sequence of the task events in movement and fixation conditions. Upward deflection: on, downward deflection: off. Bottom left: drawing illustrating the monkey behavior during movement conditions (key press, grasping and pulling). Bottom right: drawing illustrating the monkey behavior during fixation conditions (key press and key release).](http://jn.physiology.org/Downloaded from 10.220.33.2 on May 1, 2017)
mounted inside the box. All trials in which the monkey broke fixation were discarded.

A variety of objects of different size, shape, and axis orientation was used. The types of grip evoked by the various objects varied according to their physical characteristics (Fig. 2). The objects used and the corresponding grips are as follows. Small plate (width: 45 mm, length: 40 mm, height: 3 mm): primitive precision grip performed using the thumb and the radial surface of the second and third phalanges of the index finger (the plate oriented horizontally or vertically, grasped with the hand half-pronated or pronated, respectively); small ring (diameter: 15 mm): digging out grip with the index finger inserted into a ring (horizontally or vertically oriented, grasped with the hand pronated or half-pronated, respectively); large cylinder in container (object: length: 45 mm, base diameter: 50 mm; container: length 45 mm, width: 55 mm, height: 100 mm): all the fingers were inserted in the container, with the 4 fingers in opposition to the thumb (container horizontally or vertically oriented, cylinder grasped with the hand half-pronated or pronated, respectively); small sphere (diameter: 10 mm), cone (base diameter: 10 mm, length: 15 mm), or cube (face diagonal: 10 mm) in groove (width: 12 mm, length: 12 mm, height: 50 mm): advanced precision grip performed with the pulpar surface of the last phalanx of the index finger opposed to the pulpar surface of the last phalanx of the thumb (object horizontally or vertically oriented, grasped with the hand half-pronated or pronated, respectively); small sphere, cone, cube: side grip performed using the thumb and the radial surface of the last phalanx of the index finger (grasped with the hand half-pronated); small cylinder (base diameter: 5 mm, length: 45 mm): finger prehension using the first 3 fingers (hand half-pronated). Medium and large sized plate (medium, height: 7.5 mm; large, height: 30 mm), ring (medium, diameter: 30 mm; large, diameter: 50 mm), sphere (medium, diameter: 20 mm; large, diameter: 30 mm), cone (medium, base diameter: 20 mm, length: 30 mm; large, base diameter: 30 mm, length: 45 mm), cube (medium, face diagonal: 20 mm; large, face diagonal: 30 mm), and cylinder (medium, base diameter: 15 mm, length 45 mm; large, base diameter: 30 mm, length: 45 mm) were grasped essentially in the same way as the corresponding small objects but using more fingers.

For each object in each movement condition the time between the occurrence of the key release event, signaling the beginning of the reaching movement, and the occurrence of the object pulling event, signaling the accomplishment of the grasping movement, was used for the calculation of the total reaching/grasping movement time.

**Data analyses for the behavioral paradigm**

The analysis of neuronal activity during the movement conditions was made by subdividing the discharge recorded during each trial in the following epochs: 1) spontaneous activity: time before the onset of the trial, duration 500 ms; 2) key-press: time from red LED on to key-press; 3) object presentation: from 100 to 400 ms after key press; 4) set: from 400 ms before the go signal to 100 ms before key release; 5) premovement: 100 ms before key release; 6) movement: from key release/movement onset to the beginning of object pulling; 7) holding: a period of 500 ms calculated from the moment in which the monkey began to pull the object. In the fixation conditions, each trial was subdivided into 3 epochs that corresponded to the first 3 epochs of the movement conditions.

The subdivision between a object presentation epoch and a set epoch, although arbitrary, was done to keep as separate as possible the peak of the visual response from the subsequent sustained activity preceding movement onset. The same epoch definition was used in another study in which the same behavioral paradigm was used (Murata et al. 1997). For the definition of the set-related activity we used the classical criterion introduced in the early 1980s by Wise and coworkers (Weinrich and Wise 1982; Weinrich et al. 1984) and subsequently used in many other studies (di Pellegrino and Wise 1993; Johnson et al. 1996; Kurata 1993; Kurata and Wise 1988).
Response histograms were constructed by summing 8 individual trials. In each trial, the mean discharge frequency was calculated for each epoch. The mean discharge frequency of epochs 2 to 7 for the movement conditions and of epochs 2 to 3 for the fixation conditions was compared with the mean background discharge frequency of epoch 1 (Student’s t-test, 2-tail, \(P < 0.001\)). All neurons displayed statistically significant differences in activity between epoch 1 and at least one of the movement or observation epochs, and were therefore considered task-related neurons. A 3-way ANOVA (\(P < 0.01\)) was performed (factors: condition, hand orientation, shape of the object) followed by a Newman–Keuls procedure (2-tail, \(P < 0.05\)).

A population analysis, in which all task-related recorded neurons were included, was performed taking into account the net average discharge frequency of each neuron for each grip, in each orientation, epoch, and condition. Each neuron contributed one entry in each data set constructed. The first data set contained the population response to the “best” grip (associated with the maximum discharge), the “second-best” grip, and the “worst” grip according to the net average discharge frequency in the ML condition. The resulting grip rank order was also used for the MD condition. The grips were matched in orientation to exclude the contribution of the wrist to the grip differences. The second data set contained the population response to the preferred grip in the preferred orientation (the one that evoked the maximum discharge) and the preferred grip in the nonpreferred orientation in the ML condition. In analogy with the first data set, the preference found in ML was also used in the MD condition. Depending on where the peak of the activity was, the net discharge frequency in premovement or movement epoch of the best grip/orientation in the ML condition was considered as 100. The discharge frequency of all the other epochs, conditions, and grips/orientations was expressed as percentage of the peak discharge frequency. To assess the variation of the normalized discharge frequency in relation to the condition, the grip/orientation, and the epoch, a 3-way ANOVA (\(P < 0.05\)) was performed followed by a Student–Newman–Keuls procedure (2-tail, \(P < 0.05\)). The differences between best–second-best, best–worst, second-best–worst, and preferred–nonpreferred orientation, in ML and MD respectively, were tested with the Mann–Whitney \(U\) test (2-tail, \(P < 0.05\)).

**Histology**

Histological analysis was carried out in both monkeys. The reconstruction of the electrode tracks was based on the penetration traces, recording coordinates, recording data, and surface landmarks. For each animal the cytoarchitectonic borders delimiting the areas of the dorsal agranular frontal cortex were matched with the electrophysiological maps (see Luppi et al. 1991; see also Rao et al. 2003).

**Results**

Single-unit activity was recorded from the forelimb field of area F2 in 2 hemispheres of 2 monkeys (Monkey 1 and Monkey 2). The anatomic location of the studied region, which was histologically identified, is shown by the gray area in Fig. 3A.

**Naturalistic testing of neurons**

Out of 658 neurons responding during execution of active forelimb movements, 243 (37%) were classified as reaching

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**Figure 3** A: side views of the right hemisphere of Monkey 1 and Monkey 2, respectively. Dashed lines delimit different agranular frontal areas. Shaded regions indicate the sector of F2 from which neurons were recorded. Region delimited by the rectangle is enlarged in B. B: circles on the enlarged side view of the hemisphere represent the entry points of the penetrations, which are reconstructed beside. On the left side of each penetration track (gray line) the movement evoked by microstimulation every 0.5 mm of depth as well as the range of the current threshold required to evoke it are reported. Letters inside each circle represent the joints involved in the evoked movement (W, wrist; F, fingers; S, shoulder). Small empty squares represent nonexcitable sites. Background shading of each circle is indicative of threshold values (the darker the shading, the lower the threshold; threshold range from 5 to 40 \(\mu\)A). On the right side of each penetration track are reported the types of neurons (key-legend on right) recorded with the behavioral paradigm. Abbreviations: AI, inferior limb of the arcuate sulcus; AS, superior limb of the arcuate sulcus; C, central sulcus; P, principal sulcus.
neurons and 308 (46%) were classified as grasping neurons. The remaining 17% of neurons responded during other fore-limb movements such as pushing away or arm retraction.

**Neurons tested with the behavioral paradigm**

Out of 308 neurons classified as grasping neurons using the naturalistic test, 44 were kept long enough to be fully tested with the behavioral paradigm. Forty-two neurons displayed specificity for a grip or for a combination of grip and wrist orientation. The location of neurons recorded with the behavioral paradigm from the right hemisphere of Monkey 1 is shown in Fig. 3B. Note that the location of grasping neurons correlates well with the location of the sites from which distal movements can be evoked by electrical microstimulation.

The motor selectivity of the neurons is presented in Table 1. Thirty-two neurons were activated by grasping of small objects. Among them 12 preferred the execution of the advanced precision grip, 9 the digging out with the index grip, 6 the side grip, and 5 the primitive precision grip. Ten neurons were activated by grasping of large objects, 8 of which preferred the fingers prehension with the fingers opposed to the thumb, and 2 the fingers without thumb opposition grip.

When the discharge for the best grip was compared with the discharge for the second-best grip, it appeared that 66% (n = 29) of the neurons were highly selective for a grip type, displaying, during the execution of the best grip, a response that exceeded of ≈20% that elicited during the execution of the second-best grip. The comparison of the neuronal discharge in response to the 2 possible wrist orientations showed that 72% (n = 32) of the neurons were highly selective for a specific wrist orientation, showing a difference between preferred and nonpreferred orientation of ≈20%. The number of neurons preferring grips performed with hand pronated was equal to that of neurons preferring grips performed with hand half-pronated.

On the basis of their response properties, the 42 grasping neurons were subdivided into 3 main categories: purely motor neurons (n = 16, 38%), visually modulated neurons (n = 13, 31%), and visuomotor neurons (n = 13, 31%).

The purely motor neurons did not respond during the object fixation condition, and their discharge during the set, premovement, and movement epochs did not vary between the 2 movement conditions (ML and MD).

Figure 4 illustrates an F2-selective purely motor neuron. Panels show neural activity recorded during the ML condition for grasping 6 different objects, each of 2 sizes. This neuron preferred the execution of small aperture side grip. The motor response for 3 small objects (cube, cone, sphere) grasped with the same type of grip was identical. The same preference was displayed in the MD condition. Note the reduction in discharge when the same grip with a larger aperture was executed.

Another example of an F2 purely motor neuron is presented in Fig. 5. Panels show neural activity recorded during the ML condition for grasping 4 different objects, each with 2 orientations. The objects have been set in columns according to the wrist orientation required for grasping them and not according to their axis of orientation. This neuron preferred the “digging out with the index” type of grip, performed for grasping the small ring, with the wrist half-pronated. Note the dramatic difference in the discharge when the wrist orientation changed. The same neuron displayed the same wrist orientation preference during the “advanced precision grip” performed for grasping small cube in groove, as well as during the “finger without thumb opposition” prehension performed when the large ring was grasped. The absence of wrist orientation preference, during grasping of the large plate oriented either horizontally or vertically, demonstrates that the neuron codes a combination of grip and wrist orientation rather than a simple rotation of the wrist.

The visually modulated neurons did not respond during the object fixation condition but their discharge rate during the set, premovement, and movement epochs of ML was statistically different from that in the same epochs of MD.

Figure 6 shows an example of a visually modulated F2 neuron. This neuron displayed specific set, premovement, and movement activity. It discharged maximally during grasping of the small horizontal ring in the light. The execution of the same grip with the wrist half-pronated (small vertical ring) in the same condition resulted in a decrease of the discharge during the set, premovement, and movement epochs. The difference of the discharge attributed to the change of orientation was less evident in the MD condition, suggesting that this preference depended on the availability of visual information about the object. It is noticeable that the decrease of selectivity in dark was mainly the result of an increase of activity (almost double) for the nonpreferred orientation.

The visuomotor neurons discharged during presentation epoch of the object fixation condition. Nine of them also displayed different discharge rate between the 2 movement conditions, whereas 4 of them did not show any difference.

The response during presentation epoch of the object fixation condition was considered valid only if 1) it was higher than the response in the same epoch during the LED fixation, 2) it was selective for an object or for a group of objects, 3) it was present also in the presentation epoch of ML condition, and 4) this latter was higher than in the corresponding epoch of MD condition. In 10 out of the 13 neurons a congruence was observed between the response during the most effective type of grip and the response during the presentation of the object requiring that particular grip.

Figure 7 illustrates a typical F2 visuomotor neuron, displaying high activity during the presentation epoch of the small vertical ring, both in ML and “object fixation” conditions (this latter in the absence of any grasping movement). Furthermore, this neuron also displayed a remarkable specificity during the set, premovement, and movement epochs for the same object in the ML condition. Note also the decreased discharge for the

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**TABLE 1. Motor selectivity of neurons**

<table>
<thead>
<tr>
<th>Object</th>
<th>Grip</th>
<th>Number of Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small plate</td>
<td>Primitive precision</td>
<td>5</td>
</tr>
<tr>
<td>Small ring</td>
<td>Digging out with the index</td>
<td>9</td>
</tr>
<tr>
<td>Small sphere/cone/cube</td>
<td>Side grip</td>
<td>6</td>
</tr>
<tr>
<td>Small sphere/cone/cube</td>
<td>Advanced precision</td>
<td>12</td>
</tr>
<tr>
<td>in groove</td>
<td>Finger prehension with thumb opposition</td>
<td>8</td>
</tr>
<tr>
<td>Cylinder in container</td>
<td>Finger prehension with thumb opposition</td>
<td>2</td>
</tr>
</tbody>
</table>

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FIG. 4. Example of a selective F2 purely motor neuron. For each object, in each condition, rasters of 8 trials, the resulting histogram and a bar graph, with the mean firing rate and SE during each task epoch, are presented. Left and central columns: neuron response for grasping the small objects in movement in light (ML) and movement in dark (MD) conditions, respectively. Right column: response of the same neuron for grasping the medium objects in ML condition. Small gray bars in each trial correspond to the different events of the task. First and second horizontal lines below each histogram indicate the object presentation and object holding periods, respectively, averaged across trials. Rasters and histograms are aligned (vertical bar) with the beginning of the movement. A time scale (s) is placed on the abscissa of each histogram. Bar graph abscissa: epoch (see METHODS). Bar graph ordinate: mean firing rate (spikes/s).
preferred object in MD condition during the same epochs. All visuomotor neurons displayed set-related activity.

Among the neurons that displayed different discharge rate during the movement epoch between the 2 movement conditions \((n/11005\) 22, 13 visually modulated and 9 visuomotor), 14 (63.6%) decreased their activity for the preferred grip/orientation in the MD condition, 4 (18.2%) increased their activity in the MD condition, and 4 (18.2%) did not alter their activity for the preferred grip/orientation. With respect to the activity for the nonpreferred grip/orientation, 8 neurons (36%) displayed higher activity in the MD than in the ML condition, 2 neurons (10%) displayed higher activity in the ML than in the MD condition, and 12 neurons (54%) did not display any difference. These variations in activity for the preferred and nonpreferred grips/orientations in the MD condition explain the loss of specificity showed by the ANOVA analysis \((P/110021\) 0.01) in 13 (59%) neurons in this condition. The remaining 9 neurons (41%) maintained their grip/orientation specificity in the MD condition.

Figure 8 illustrates the net normalized mean activity of the neuronal population studied with the paradigm. In A and B the population response during the execution of the best, second-best, and worst grips in ML and MD is presented. A 2 Conditions \(\times 3\) Grip \(\times 7\) Epoch ANOVA showed a significant main effect for Grip \(F(2,191) = 31.052, P < 0.0001\) and for Epoch \(F(6,1152) = 187.833, P < 0.0001\), as well as 3 significant interactions C \(\times\) G, \(F(2,191) = 5.324, P < 0.001\); G \(\times\) E, \(F(12,1152) = 10.607, P < 0.0001\); C \(\times\) G \(\times\) E, \(F(12,1152) = 1.777, P = 0.047\). The existence of a significant Condition \(\times\) Grip \(\times\) Epoch interaction indicates that, at the population level, the availability of visual information differently affects the discharge associated with different grip types depending on the task phase.

The statistically significant differences revealed by the Newman–Keuls post hoc analysis \((P < 0.05)\) are summarized in Table 2. In summary, the population discriminates between different grips during grasping execution both in light and in dark. However, this selectivity is more pronounced in light, extending also to the epochs preceding movement execution. The lack of visual feedback equally affected the best and the worst grip.

The Mann–Whitney U test revealed that the best/second-best grip difference in the movement epoch in ML was statistically different from the corresponding difference in MD \((P < 0.05)\). This explains the decrease in selectivity between the 2 grips in dark. Furthermore, the differences in best/worst grip found during the object presentation, set, premovement, and movement epochs in ML were statistically different from the corresponding ones in MD. This is likely attributable to the increase in the discharge for the worst grip in dark. This increase, however, was not enough to annul the difference between the 2 grips in the movement epoch.

In Fig. 8, C and D the population response during the execution of the best grip in the preferred and the nonpreferred orientation in ML and MD is presented. A 2 Conditions \(\times 2\) Orientations \(\times 7\) Epochs ANOVA showed a main significant
effect for Orientation [$F(1,105) = 10.540, P = 0.0016$] and for Epoch [$F(6,636) = 131.013, P < 0.0001$] and a significant O × E interaction [$F(6,636) = 3.067, P = 0.0058$].

The statistically significant differences revealed by the Newman–Keuls post hoc analysis ($P < 0.05$) are summarized in Table 3. In summary, the neuronal population in light shows a preference for a specific wrist orientation during grasping execution that disappears when the visual feedback is absent. The lack of visual feedback equally affected the preferred and the nonpreferred orientation.

The Mann–Whitney $U$ test showed that the differences in activity between preferred and nonpreferred orientation in ML are statistically different from the corresponding ones in MD during the premovement and movement epochs ($P < 0.05$), thus explaining the loss of wrist orientation specificity in dark.

It is evident from the records that different times are required for the accomplishment of the different grips. This may lead one to suggest that movement duration could be a factor affecting neurons discharge and contributing to the discharge differences among the various grips. If this was true, then the slower performed grip would be always the best one, the second faster performed grip the second best, and the faster performed grip the worst, or vice versa. The same rule would also apply to the wrist orientations. The preferred orientation would be the one performed slower than the nonpreferred one or vice versa. However, analysis of movement times showed that the best grip was slower only in 45% of the cases, the second best was the second faster only for 32% of the cases, and the worst was the faster only in 55% of the cases. Regarding the wrist orientations, the preferred one was faster only in 50% of the cases. The rank order of the movement duration for the best, second-best, and worst grip or the preferred and nonpreferred orientation observed during the ML condition was also preserved in the MD condition. A 2 Condition × 3 Grip ANOVA revealed no significant differences in the movement times between ML and MD conditions. Regression analysis performed between the movement times for the best, second-best, and worst grip and the corresponding normalized discharge rates for the ML and the MD conditions, respectively, showed that in both conditions the $r^2$ values are very low (ML: 0.028; MD: 0.002), demonstrating that only an extremely small percentage of the variance can be explained by
FIG. 7. Example of an F2 visuomotor neuron responding during object presentation. Panels show neural activity recorded during the ML condition (left column), the MD condition (central column), and the object fixation condition (right column) for 5 different objects. Rasters and histograms are aligned (vertical bar) with key press (object illumination). Bottom right corner: neuron activity during the LED fixation condition. Conventions as in Fig. 2.
the correlation between movement time and discharge frequency.

DISCUSSION

Grasping neurons in F2

The present study demonstrates that in the lateral part of the forelimb field of the dorsal premotor cortex there are neurons active during planning and execution of grasping actions. They show high selectivity for specific types of grip and wrist orientation.

The use of objects of different size, shape, and axis orientation requiring different types of prehension is a common strategy for the study of grasping. Although it is expected that different types of prehension involve different patterns of distal muscle synergies, one could also argue that proximal muscles are differently influenced during different types of grasp. A recent study investigated whether distinct patterns of EMG activity could be identified during grasping of different objects (Brochier et al. 2001). For this purpose, EMG activity was recorded from hand muscles and from anterior deltoid. It has

TABLE 2. Statistically significant differences related to the grip preference at the population level, as revealed by the Newman–Keuls paired comparisons

<table>
<thead>
<tr>
<th>Grip Preference</th>
<th>Object Presentation</th>
<th>Set</th>
<th>Premove</th>
<th>Move</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML best grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>ML second best grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>ML worst grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>MD best grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>MD second best grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>MD worst grip</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
</tbody>
</table>

✱, spontaneous vs. other epochs; ✱, ML best vs. ML worst; ✱✱, ML second best vs. ML worst; ✱✱✱, MD best vs. MD worst; ✱✱✱✱, ML best vs. MD best; ✱✱✱✱✱, ML worst vs. MD worst.

TABLE 3. Statistically significant differences related to the orientation preference at the population level, as revealed by the Newman–Keuls paired comparisons

<table>
<thead>
<tr>
<th>Orientation Preference</th>
<th>Object Presentation</th>
<th>Set</th>
<th>Premove</th>
<th>Move</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML preferred orientation</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>ML nonpreferred orientation</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>MD preferred orientation</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
<tr>
<td>MD nonpreferred orientation</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
<td>✱✱</td>
</tr>
</tbody>
</table>

✱, spontaneous vs. other epochs; ✱, ML preferred vs. ML nonpreferred.
been demonstrated that during grasping of a given object, the same basic pattern of coordinated hand muscle activity was reproduced across trials, which could be distinguished from the pattern obtained during grasping of a different object. The greater the difference in hand posture required for the grasping, the greater the scale of the pattern differences. On the contrary, the pattern of deltoid activity was indistinguishable among the different objects. Consequently, the differences of discharge during execution of different types of grip observed in the present study, cannot be attributed to the proximal muscles involved in the transport phase of movement (identical for all objects).

Only a few studies investigated the parameters coded by dorsal premotor neurons during the execution of simple wrist movements (Kurata 1993; Riehle and Requin 1989), and none during execution of hand-grasping actions. This is the first study that investigated whether neurons of the dorsal premotor cortex are also involved in grasping execution. Our findings show that there are grasping neurons in the lateral part of the forelimb representation of dorsal premotor cortex (medial to the spur of arcuate sulcus), a region where microstimulation evokes wrist and finger movements (Raos et al. 2003).

The activity of F2 grasping neurons is not related to individual finger movements, but to the grasping action as a whole. A good example is given by the neurons presented in Figs. 5 and 6. The preferred prehension of these neurons was the digging out grip, which requires the insertion and the flexion of the index finger. Although the same finger movement was also performed during grasping of the large ring, the neuron discharge in this case was substantially less. Neurons related to grasping actions were previously described in area F5 (Murata et al. 1997; Rizzolatti et al. 1988). Both areas F2 and F5 may control the execution of these actions either through their direct connections with the primary motor cortex, area F1 (see Geyer et al. 2000), or those with the spinal cord, or both (see for example He et al. 1993). Through these connections the movement details necessary for the precise accomplishment of the chosen action would be specified. Thus it appears that the CNS uses synergies to reduce the number of degrees of freedom and thereby to reduce the complexity of hand control.

Action coding seems to be a property of premotor cortex not limited to distal actions. Concerning the lateral part of dorsal premotor cortex, Hoshi and Tanji (2000) reported neurons that code both the position of the target in space, and the arm that the monkey must use to reach this spatial position. They conclude that these neurons show an action code.

Response to object presentation

F2 visuomotor neurons responded also to object presentation. The responses to the objects were specific for an object or a set of objects. Their discharge was temporally locked to stimulus presentation and was present even in the absence of any subsequent grasping movements. Given these properties, the explanation of the visual responses of F2 neurons in terms of “signal” or “preparation” seems inadequate. Neurons responding to the presentation of graspable objects in the premotor cortex, with properties very similar to those of F2 visuomotor neurons, were previously described in ventral premotor area F5 (Murata et al. 1997; Rizzolatti et al. 1988).

These responses have been interpreted as a description of the presented object in motor terms (“motor representation” interpretation). That is, every time an object is presented, the representation of the particular grip required for grasping that specific object is evoked. Depending on the instruction, this representation can be translated in overt movement execution (as in ML condition) or can remain as a kind of “motor imagery” (as in Fixation condition). Given 1) the specificity of the responses of F2 grasping-related neurons to object presentation and 2) the congruence between the neuron response during the most effective type of prehension and the neuron response during the presentation of the object requiring that particular prehension, the motor interpretation may also be valid for the response of F2 neurons to object presentation. This interpretation does not exclude that for some of these neurons the response to object presentation could be purely visual.

The presence of object-related responses in F2 raises the question of the origin of their visual input. The first candidate for supplying this kind of information is area F5 because of its connections with the lateral part of F2, F2vr (Marconi et al. 2001; Matelli et al. 1999) and its similar, well established neuronal responses to object presentation. Another possible, not mutually exclusive, hypothesis is that visual information about object characteristics is provided by parietal areas that should be endowed with object-related properties.

The parietal areas with properties related to object information, that is, AIP and CIP (Murata et al. 1996, 2000; Tsutsumi et al. 2001), are scarcely connected to F2vr (Luppino et al. 1999). The only area of the inferior parietal lobe connected, although not strongly, with F2vr, is area PFG (Rizzolatti and Luppino 2001). Up to now few neurons related to object presentation were found in this area (for review, see Hyvärinen 1982). However, recently, a more consistent number of neurons responding to object presentation has been reported in the inferior parietal lobule (Ferrari et al. 2003).

F2vr receives its major visual input from areas V6A and MIP of the superior parietal lobe (Matelli et al. 1998). Although neuronal responses to object presentation have never been investigated in area V6A, it has been proposed that through its visual properties (consistent percentage of neurons whose RF encompasses the central part of the visual field and neurons sensitive to the orientation of stationary visual stimuli) it could provide critical visual information to the motor structures controlling orientation of the hand (Gallese et al. 2003). Little is also known about the visual properties of area MIP. A recent study reports the existence of neurons responding to the presentation of 3D objects, displaying significant orientation preference (Nakamura et al. 2001). The above-mentioned evidence, although far from being conclusive, is nevertheless indicative that structures of the superior parietal lobe may also be involved in the elaboration of object-related information.

Summing up, object information to the lateral part of F2 could, in principle, be fed by different parietal areas or by premotor area F5. Depending on the source, this information could represent a visual elaboration made by high-order posterior cortical areas or a further transformation of the visual input in pragmatic terms made by areas of the premotor cortex different from F2. A thorough investigation is needed before drawing any definitive conclusion on this matter.
Set-related activity in F2

The present study shows that during the period in which the monkey withholds a grasping action, many F2 distal neurons are strongly active. This sustained activity is evident at both the single-neuron and the population level, in both ML and MD conditions, and starts well before the movement onset. The presence of the discharge also in dark indicates that it is not simply visually driven. The sustained discharge also displays a certain degree of object/grip specificity during the ML condition, which is reduced during the MD.

Since the earliest works of Wise and coworkers (Wise 1985) the presence of a discharge during the waiting phase in which the animal is preparing a movement (set-related activity) was subsequently described in many single-neuron recording studies of the dorsal premotor cortex (for a review see Wise et al. 1997) dealing with arm-reaching movements. This set-related activity of the reaching-related neurons is often directionally tuned. The present study confirms, in area F2, the presence of neurons active during the set period, and extends this finding to distal actions.

What could be the meaning of the sustained activity displayed by F2 grasping neurons? The fact that this activity starts at the moment in which the waiting period begins and not just before movement execution, suggests that it is not related only to pure movement preparation. One possibility is that this activity represents a persistence of the visual response to object presentation (ML condition). However, this interpretation cannot explain the sustained activity present also in the MD condition. Another possibility, which we favor, is that the sustained activity reflects the continuous activation of the object representation in motor terms (see above).

Visual guidance of grasping

The results of the present study suggest that many neurons are influenced not only by the vision of the object, but also by the visual input about the scene in which the action occurs and, in particular, by the vision of the hand approaching to and interacting with the object. Indeed, whereas during grasping in light F2 neurons code both grip type and wrist orientation, during grasping execution in the absence of visual guidance, they are still able to discriminate between the best and the worst grip but lose the capacity to differentiate wrist orientation. Given that 1) there are no significant differences between the movement times required for the accomplishment of the grips among the 2 movement conditions, and 2) the rank order of the movement duration for the grips or the orientations in ML condition is also preserved in MD, the decreased specificity observed in MD cannot be attributed to different movement times between the 2 movement conditions. It has been recently reported that the activity of some PMd neurons during preparation of an upcoming reach movement seems to be closely involved in processing visual information for the spatial guidance of the movement trajectory (Ochiai et al. 2002). Which areas could be involved in providing the visual information necessary to control the hand configuration with respect to object shape and orientation during ongoing movement?

As pointed out earlier, F2vr receives rich visual input from areas V6A and MIP. The visual properties of V6A neurons (selectivity for stimulus orientation, motion, and motion direction; presence of RFs encompassing the central part of the visual field) are appropriate to provide F2 with the input needed for the visual control of reaching-to-grasp movements as proposed by Galletti (2003). Indeed, it was recently reported that V6A neurons are affected by the hand–target interaction (Battaglia-Mayer et al. 2001), suggesting a role in the visual monitoring of hand position in space. Moreover, Colby and Duhamel (1991) found neurons in area MIP that yield a weaker activation when a reaching movement is performed in the dark than in the light (see their Fig. 7). Finally, recent data demonstrated that the ventrorostral sector of F2 receives direct input from area MST (Luppino et al. 2001). This area could provide directly to F2 the visual information regarding hand motion during grasping execution.

Role of lateral F2 in grasping actions

The similarities between grasping neurons of F2vr and grasping neurons of F5 suggest that areas F2 and F5 may collaborate in the control of grasping actions. Let us examine how this collaboration could possibly work, and why 2 pre-motor areas involved in grasping actions would be required. Area F5 plays a primary role in selecting the most appropriate type of grip on the basis of the object affordances provided by AIP to which it is reciprocally connected, thus activating a motor representation of the object. This motor representation is then supplied to area F2. Area F2 grasping neurons can keep in memory the motor representation of the object and combine it with visual information provided by cortical areas of the superior parietal lobe, and presumably by MST, to continuously update the configuration and orientation of the hand as it approaches the object to be grasped.

The final output for action execution most likely involves both area F5 and area F2. The strong anatomical connections of the lateral part of F2 with both F1 and the spinal cord assign to F2 a key role in the control of forelimb actions.

When grasping action is performed in the dark, the monkey is still able to perform a correct grasping, on the basis of object memory. Which area provides the specific information suitable for a correct grasping in this condition? The present study shows that visually modulated F2 neurons still discharge in the dark, but partly lose their specificity, especially during actual grasping. Very likely in the dark, other areas, such as F5, provide the animal with the memory of the specific pragmatic code needed to program the correct type of grasping. It is possible that during the final phase of grasping, F2 neurons are also driven by somesthetic information on wrist orientation or displacement of fingers.

The present results shed new light on the functional mechanisms presiding over the control of visually guided hand-grasping actions. These mechanisms appear to involve a network of areas, among which F2vr seems to play a crucial role, by monitoring the configuration of fingers and wrist orientation during planning and execution of grasping actions.

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