

Ventral Premotor and Inferior Parietal Cortices Make Distinct Contribution to Action Organization and Intention Understanding

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It is well known that ventral premotor area F5 codes the goal of executed and observed motor acts. This area is anatomically connected with part of the inferior parietal cortex (area PFG), which has been recently shown to play a role in action organization and intention understanding. The aims of the present study were 1) to assess whether the discharge of F5 motor neurons and mirror neurons (MNs) codes action goals and 2) to clarify the relative contribution of F5 and PFG in action organization and intention understanding. To this purpose, we first recorded from F5 motor neurons and MNs of 2 monkeys while performing a motor task constituted by 2 actions ("grasp-to-eat" and "grasp-to-place") or observing the same task done by an experimenter. Results showed that some F5 neurons code grasping according to the goal of the action in which it is embedded. Subsequently, we recorded from PFG motor neurons and MNs of the same monkeys, using the same tasks. The comparison between the neuronal properties of F5 and PFG motor neurons suggests that PFG plays a major role in organizing natural actions. Furthermore, the similarities between MNs properties of the 2 areas indicate that they constitute a functional circuit underlying others' intention understanding.

Keywords: area F5, goal, mirror neurons, monkey

Introduction

Planning and executing an *action*, such as "grasping and eating an apple," implies having a final goal (e.g., "to eat the apple") that leads to the selection of an appropriate sequence of "motor acts." Reaching, grasping, bringing to the mouth, and biting represent distinct motor acts, each of which endowed with its specific motor goal (the goal of "grasping," e.g., is that of taking possession of an object). The coding of different types of motor acts relies on specific parietofrontal circuits such as, for example, the PG/VIP-F4 circuit for reaching and the AIP-F5 circuit for grasping (Rizzolatti et al. 1998; Rizzolatti and Luppino 2001; Rozzi et al. 2006; Borra et al. 2008).

Among the areas belonging to these circuits, the ventral premotor area F5 (as defined by Matelli et al. 1985) constitutes a core region for coding the goal of hand-and-mouth motor acts such as grasping, manipulating, tearing, and holding (Rizzolatti et al. 1988). A crucial property of F5 neurons is that goal coding is independent of the sequence of movements or the effector used to achieve it (Umiltà et al. 2008), thus showing a high level of motor abstraction. Area F5 is also known to contain mirror neurons (MNs) that become active during the execution of motor acts as well as during the observation of similar acts done by another individual. This property is deemed to be at the basis of primate ability to understand the goal of other

individuals' acts (Gallese et al. 1996; Rizzolatti et al. 1996; Umiltà et al. 2001; Kohler et al. 2002).

More recently, it has been shown that also motor neurons of the inferior parietal lobule (IPL) convexity code the goal of motor acts. Furthermore, in the rostral part of this lobule, MNs have been described with properties similar to those of F5 ones (Gallese et al. 2002; Fogassi et al. 2005; Rozzi et al. 2008). Moreover, it has been demonstrated that the discharge of both grasping motor neurons and MNs in the rostral part of IPL can be influenced by the goal of the action (i.e., grasp-to-eat or grasp-to-place), in which the coded act is embedded (Fogassi et al. 2005). These findings have prompted the idea that IPL neurons constitute the neural substrate for organizing motor acts into actions based on a specific action goal. During action observation, the activation of the same IPL neuronal substrate underlying one's own action organization would allow to understand the goal of others' action, that is, the "motor intention" of the agent (Fogassi et al. 2005; Rizzolatti et al. 2006).

It is well known that ventral premotor cortex (PMv) and IPL are anatomically connected (Petrides and Pandya 1984; Matelli et al. 1986; Cavada and Goldman-Rakic 1989; Rozzi et al. 2006). Notably, a recent anatomical study showed that a specific sector of rostral IPL, namely area PFG, is tightly connected with area F5 (Rozzi et al. 2006), suggesting a possible functional circuit, involving both PFG and F5, for action organization and intention understanding.

The main aim of this study was that of verifying if, and to what extent, the discharge of motor neurons and MNs of area F5 is influenced by the goal of the action in which the coded act (grasping) is embedded. Furthermore, in order to directly compare the functional properties of F5 neurons with those of PFG, we recorded a completely new set of motor neurons and MNs from the same monkeys also in area PFG, by applying the same tasks and acquisition paradigm carried out in area F5.

Materials and Methods

The experiments were carried out on 2 female macaque monkeys (*Macaca nemestrina*), which will be referred to as M1 and M2. Before recordings, the monkeys were habituated to sit in a primate chair and to interact with the experimenters. Then, they were trained to perform the motor task described below using the hand contralateral to the hemisphere to be recorded. When the training was completed, a head fixation system and a titanium recording chamber were implanted under general anesthesia (ketamine hydrochloride, 5 mg/kg intramuscular [i.m.] and medetomidine hydrochloride, 0.1 mg/kg i.m.), followed by postsurgical pain medications. These surgical procedures were the same as previously described (Fogassi et al. 1996; Rozzi et al. 2006). 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.

Motor Task

Both monkeys were trained to perform a motor task (Fig. 1*A*) consisting of 2 different conditions. The first part of the task was identical in the 2 conditions: starting from a fixed position (Fig. 1*A*, left), the monkey was required to reach and grasp a piece of food (target) using a precision grip (Fig. 1*A*, right). Then, the monkey had to bring the food to the mouth and eat it (grasp-to-eat, Fig. 1*A*—I) or place it into a container located near the mouth (grasp-to-place, Fig. 1*A*—II), in order to obtain a more palatable reward. The food morsels grasped in the 2 conditions of the task were the same. In some cases, we adopted also a modified version of the grasp-to-place condition in which the monkey placed the food or an object (a metallic cube of the same size and shape of the food morsel) in a container located near the target (Fig. 1*A*—III). In both placing conditions, in order to instruct the monkey to place the food, the reward was briefly presented before trial onset.

The apparatus used for the task consisted of a square plexiglass table (side 42 cm) attached to the primate chair. A metal cylinder (diameter 28 mm, height 25 mm) was fixed to the table, along the monkey midline at a distance of 16 cm from its chest. The monkey had to keep its hand on the metal cylinder (starting point) until the trial began. The target was located within a rectangular groove (40 × 12 mm, depth 10 mm), carved in a plastic support (5 cm height), and fixed to the table at a distance of 15 cm from the hand starting point. This apparatus forced the monkey to adopt always the same type of grip (precision grip). A plastic container (diameter 5 cm, depth 4 cm) was fixed to the monkey head holder close to its mouth or on the table near the target, on the side contralateral to the hand used to perform the task.

Before the beginning of each trial, a transparent plastic screen (40 × 23 × 0.5 cm) was interposed between the starting point and the target, in order to prevent the monkey from reaching for and grasping the food during the intertrial interval. The monkey was trained to keep its

hand on the starting point until the screen was removed (go signal), and then it had to execute one of the conditions of the task.

The trials in which the monkey detached the hand from the starting cylinder before the go signal or made an incorrect movement were discarded and not included in the data set. In particular, when the monkey ate the food in the “grasp-to-place” condition, the more palatable reward was not delivered. All conditions were run in a pseudorandom fashion, and the deleted trials were immediately repeated in order to collect at least 10 trials for each experimental condition.

Visual Task

In the visual task, the experimenter, facing the monkey, started with the hand from a fixed position (Fig. 1*B*, left), then grasped a piece of food or an object (Fig. 1*B*, right), brought the food to his mouth, and ate it (grasp-to-eat, Fig. 1*B*—I) or placed the food (or the object) into a container located near the target (grasp-to-place, Fig. 1*B*—II). The food and the object had the same size and shape. Note that the 2 conditions of the visual task were constituted by the same motor acts and were aimed at the same action goals as those performed by the monkey in the motor task. Furthermore, most MNs' motor responses were tested in the condition “place-near-the-target” (Fig. 1*A*—III), thus allowing a precise matching of the observed and executed actions not only in terms of motor acts but also in terms of movements.

The apparatus used for the task consisted of a plexiglass table (40 × 50 cm) positioned in front of the experimenter at a fixed distance of 92 cm from the monkey. A metal plate (5 × 6 cm), used as a starting point, was located on the table edge near the experimenter, along the monkey body midline, at a distance of 132 cm from its chest. The target of the experimenter's action (either the food or the object) was located

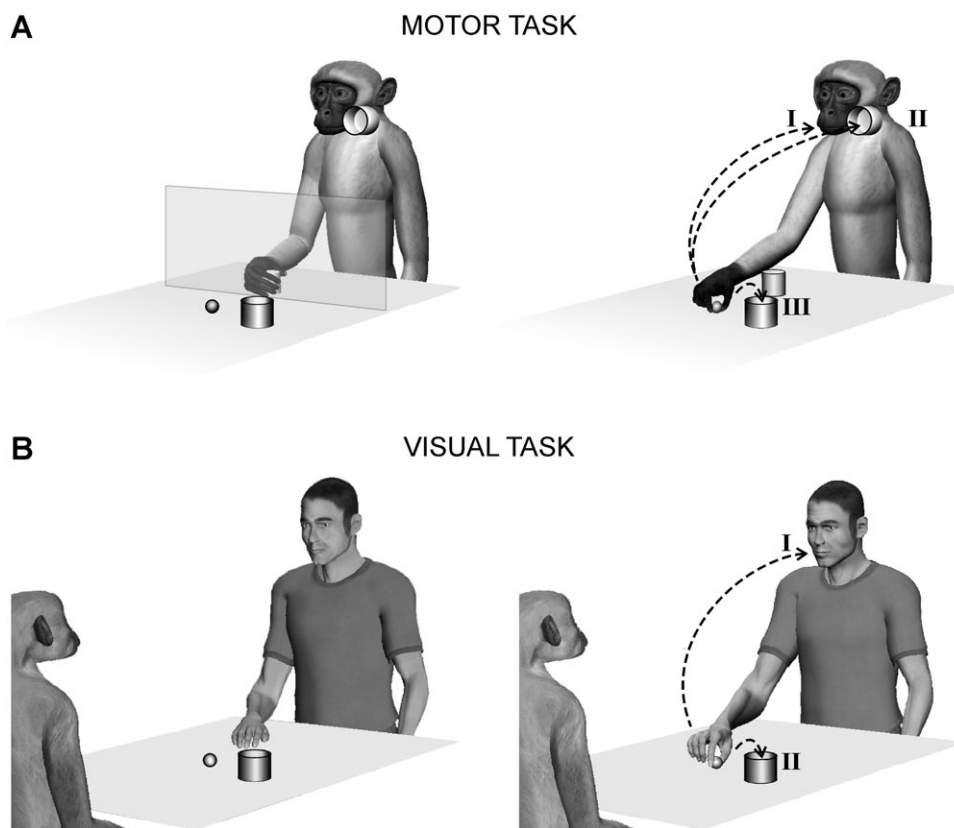


Figure 1. (*A*) Motor task. The monkey, starting with its hand from a fixed position (*A*, left), reaches and grasps a piece of food (or an object) with a precision grip (*A*, right), then it brings the food to the mouth and eats it (grasp-to-eat Condition-I) or places it (or the object) into a container (grasp-to-place Condition) located near the mouth (II) or near the target (III). (*B*) Visual task. The experimenter, starting with his hand from a fixed position (*B*, left), reaches and grasps a piece of food or an object (*B*, right), then he brings the food to the mouth and eats it (grasp-to-eat Condition-I) or places it (or the object) into a container located near the target (grasp-to-place Condition-II).

on a metal plate (3×3 cm) fixed to the table at a distance of 34 cm from the center of the starting point. A container (identical to the one used in the motor task) was fixed at a distance of 14 cm to the left or to the right of the target. The container was present only when grasp-to-place trials were run. Thus, the presence/absence of the container and the type of target to be grasped (i.e., the small cube/food) acted as contextual cues allowing the monkey to predict the experimenter's most likely motor act following grasping.

During the visual task, the monkey simply observed the scene without performing any movement and did not receive any reward. The monkey was not required to keep fixation. Eye position was monitored by means of an eye tracking system composed by a 50-Hz CCD camera provided with an infrared filter and 2 infrared spots of light. The video signal was sent to a computer equipped with dedicated home-made software in order to acquire and process in real-time the eye position along horizontal and vertical axis. This allowed discarding, immediately after their acquisition, all trials in which the monkey moved its gaze out of a 5×5 -window centered on the target location during the grasping epoch (300 ms before and 300 ms after the contact of the experimenter's hand with the target). The possible influence of monkey's active movements on neuronal discharge during action observation was avoided by discarding the trials in which the monkey performed some movements while watching the experimenter's action. All conditions were run in a pseudorandom order until 10 trials for each condition were collected.

Recording Techniques

Neuronal recording was performed by means of single glass-coated microelectrodes (impedance 0.5–1 M Ω), inserted through the intact dura. The microelectrode was mounted on an electrode holder and connected to a computer-controlled microdrive. Dedicated software (EPS; Alpha Omega, Nazareth, Israel) allowed one to control the engine for the electrode movements. The electrode holder was attached to a stereotaxic arm, mounted on the monkey head holder.

Neuronal activity was amplified and monitored on an oscilloscope. Single-neuron action potentials were isolated with a dual voltage-time window discriminator (Bak Electronics, Germantown, MD) and fed to a PC to be recorded, stored, and further analyzed.

Clinical Testing of the Recorded Neurons

Once a neuron was isolated, its motor, visual, and somatosensory properties were first tested (see Fogassi et al. 2005; Rozzi et al. 2008). Only neurons showing a motor response during hand grasping performed with a precision grip were selected for further study with the motor task described above.

Specific tests, extensively described elsewhere (see Rozzi et al. 2008), were carried out on each neuron in order to verify possible responses related to mouth or arm motor acts. In particular, neurons active during arm-related motor acts (such as arm reaching or bringing to the mouth) or selectively activated only during mouth grasping were not included in this study. Furthermore, neurons responsive to grasping with both hand and mouth were analyzed separately.

MNs are defined here by using the criteria adopted in a previous work (Gallese et al. 1996). Briefly, they respond both when the monkey performs a certain motor act (i.e., grasping) and when it observes the same act performed by another individual. In contrast, they do not respond either during the simple visual presentation of pieces of food or objects or when the monkey observes mimicked motor acts (in absence of a target). Neurons matching these criteria were first studied with the visual task and, whenever possible, further tested also with the motor task.

Recording of Behavioral Events

In both motor and visual tasks, contact detecting electric circuits were used to provide to a computer digital signals related to the main behavioral events: 1) detachment of the hand from the starting point, 2) contact of the hand with the object or food, and 3) contact of the hand with the border of the container in which the object/food had to be placed. These signals were used to align neuronal activity in different

trials and, subsequently, to construct the response histograms and data files for statistical analysis.

All the data were acquired and stored by means of Lab-View-based software, allowing us to record neuronal activity aligned with the corresponding events of the behavioral paradigm.

Definition of Grasping Epoch

We statistically analysed hand-grasping-related activity, aligned on the moment when the hand of the monkey or the experimenter touched the target object, during 2 distinct epochs of 300 ms each, before the contact (pre-contact [Pre]) and after the contact (post-contact [Post]).

The Pre epoch included the whole-hand preshaping process, till the contact of the fingers with the target. The Post epoch included the closure of the fingers for taking possession of the target and the initial period of the subsequent lifting/transport phase, in which the target was held between the fingers and moved toward its final location (mouth or container). Despite a certain degree of variability in the velocity of monkey performance, the Post epoch does not include mouth grasping or object placing.

Single Neurons and Population Analyses

The activity of each neuron, recorded during 10 trials, has been expressed as mean firing rate (spikes/s) in 3 different time epochs for both the motor and the visual task. Epoch 1600 ms long, corresponds to the time during which the hand (of the monkey or of the experimenter, depending on the task) was at rest on the starting position (baseline activity) and was calculated from the beginning of each acquisition (i.e., from 2000 to 1400 ms before the hand detached from starting position). Epochs 2 and 3 correspond to the above defined Pre and Post epochs, respectively.

In order to compare the discharge of each neuron in different conditions and epochs, a 2×3 analysis of variance (ANOVA) for repeated measures (factors: Condition and Epoch) was performed in both the motor and the visual task for all neurons, followed by Bonferroni post hoc tests. All analyses were performed using a significance criterion of $P < 0.05$. Only neurons significantly activated during at least 1 of the 2 grasping epochs with respect to baseline were included in this study. Neurons showing a significantly different discharge rate between the 2 experimental conditions during one or both grasping epochs have been defined as "action goal-related" (AGR) neurons.

In order to quantitatively assess the degree of preference expressed by single AGR neurons for grasp-to-eat or grasp-to-place, a preference index (PI) was calculated with an identical procedure for the motor and/or the visual response, as follows:

$$PI = (r_e - r_p) / (r_e + r_p),$$

where " r_e " and " r_p " are the average response of the neuron in grasp-to-eat and grasp-to-place condition, respectively, during the epoch/epochs in which statistical analysis revealed differential activation between the 2 conditions. In order to describe and compare the distribution of PIs in the F5 and PFG neuronal populations, this index was calculated also for those neurons showing no statistically significant differences between the 2 conditions. In this case, PI was calculated using the average response in the epoch/epochs in which the neuron was significantly activated with respect to baseline.

In order to explore the correlation between peak activity timing and PI for both studied areas, further analyses were carried out. The response of each neuron was expressed in terms of net-normalized mean activity, calculated as follows. First, the mean activity was calculated for each 20-ms bin through all the recorded trials of both experimental conditions. Then, for each condition, an off-set procedure was applied, subtracting the mean baseline activity from the value of each bin (net activity). The highest net activity value among those of the compared conditions was taken to divide the value of each single bin (net-normalized mean activity). Using this procedure, each neuron is characterized by a mean baseline activity equal to 0 and a peak activity value of 1. In order to reliably identify the peak of activity timing in each of the 2 conditions, a moving average (period = 60 ms) has been applied to the net-normalized mean activity, centered on each 20-ms bin. This procedure

was aimed at allowing a more reliable identification of the peak of activity (the highest value for each condition).

In order to compare the temporal pattern of the discharge of PFG and F5 AGR neurons, a different off-set procedure was performed, using as off-set value the mean baseline activity plus its standard deviation (baseline threshold). This allowed identifying the period comprised between the peak of activity and the first previous negative value as “rising” phase of neuronal activity, and the period comprised between the peak of activity and the subsequent negative value as “falling” phase of neuronal activity. The integral of rising and falling phase activity was then calculated for each neuron in each condition and compared between neurons of the 2 areas recorded in the same conditions. The comparison was carried out in each phase by using independent-samples *t*-test, with a significance criterion of $P < 0.05$ (with Bonferroni correction for multiple comparisons).

Histological Reconstruction and Identification of the Recorded Regions

In order to directly assess whether the regions containing task-related neurons are anatomically connected, at the end of neurophysiological experiments, neural tracers were injected in the PFG and F5 sectors where AGR motor neurons and MNs were recorded. Immediately before tracer injection, a recording session was performed in order to confirm the presence of reliable neural activity and properties coherent with those previously found during the electrophysiological experiment. Tracers were slowly pressure injected about 1.2–1.8 mm below the cortical surface through a Hamilton microsyringe (Reno, NV).

In M1, wheat germ agglutinin (WGA, 4% in saline; Vector Laboratories, Burlingame, CA) was injected in PFG, whereas in M2 cholera toxin B subunit, conjugated with Alexa 594 and Alexa 488 (CTB-A, 1% in phosphate-buffered saline; Molecular Probes) were injected in PFG and F5, respectively. In all cases, the volume of injected tracer was 1 μ l.

About 1 week before sacrificing the animals (7 days for M1; 10 days for M2), electrolytic lesions (10 μ A cathodic pulses per 10 s) were performed at known coordinates at the external borders of the recorded regions. After electrolytic lesions and appropriate survival period for tracers transport (14 days for CTB-A and 2 days for WGA), each animal was anesthetized and perfused as previously described (Rozzi et al. 2006).

The brain was then extracted, photographed, and cut (slice thickness 60 μ m). For M1, the third section of each 5 was processed for WGA immunohistochemistry. For both monkeys, each second and fifth section of a series of 5 were stained using the Nissl method (thionin, 0.1% in 0.1 M acetate buffer, pH 3.7). The locations of penetrations were then reconstructed on the basis of electrolytic lesions, stereotaxic coordinates, depths of penetrations, and functional properties.

Subsequently, the cytoarchitectonic features of IPL convexity and PMv were identified based on the criteria defined by Luppino and coworkers (Gregoriou et al. 2006; Belmalih et al. 2009).

Injection sites were defined according to the criteria previously described for CTB-gold and WGA-horseradish peroxidase (Luppino et al. 2001, 2003) and attributed to the different cytoarchitectonic areas. WGA-labeled neurons were identified in bright field as a black, dense and homogeneous staining in the cytoplasm. CTB-A labeling was analyzed by using standard fluorescein (for CTB-A 488) or rhodamine (for CTB-A 594) sets of filters (see Rozzi et al. 2006).

The distribution of retrograde labeling was plotted in coronal sections (600 μ m sampling) and related to the outer and inner cortical borders, by using a computer-based charting system.

Results

F5 Grasping Neurons Tested with the Motor Task

Single-unit activity was recorded from the posterior bank of the inferior arcuate sulcus and the adjacent convexity (area F5) of the right hemispheres of M1 and M2. All grasping neurons responsive during execution of precision grip were tested with the motor task ($N = 154$). The great majority of them ($N = 139$,

90.3%) discharged only during grasping with the hand, whereas a lower proportion ($N = 15$, 9.7%) discharged during grasping with the hand and the mouth. This latter class of neurons was mainly located in the most lateral part of the investigated F5 sector, intermingled with hand-grasping neurons, and it has been analyzed separately (see below).

Examples of F5 hand-grasping neurons tested with the motor task are shown in Figure 2A. Unit 125 was active during both Pre- and Post-contact epochs, discharging stronger during grasp-to-eat than during grasp-to-place. In contrast, Unit 46 discharged during Post-contact epoch and the discharge was stronger for grasp-to-place as compared with grasp-to-eat. Finally, Unit 74 showed no significant differences in discharge intensity between the 2 conditions.

Table 1 summarizes the behavior of all hand-grasping neurons recorded in the motor task. Among 139 neurons, the majority (61.9%) coded grasping act with similar discharge intensity, regardless of the action in which the coded act was embedded. More interestingly, the remaining neurons (38.1%) discharged stronger in 1 of the 2 conditions (AGR neurons), and similar proportions ($\chi^2 = 2.82$, nonsignificant [NS]) of “grasp-to-eat” (23%) and “grasp-to-place” (15.1%) neurons were found. Some ($N = 5$) of the neurons in this latter class have been further studied in a third condition in which the monkey placed the food in a container located near the target rather than near its mouth. Although placing movements differ between the 2 placing conditions, all these grasping neurons maintained the same preference for grasp-to-place. An example is shown in Supplementary Figure 1.

The great majority of AGR neurons (71.7%) were active in both Pre- and Post-contact epochs, with more than half of them (55.3%) equally active in both epochs. Concerning the epoch in which action goal preference appears, 8 (15.1%) AGR neurons were differentially activated only in the Pre-contact, 14 (26.4%) in both Pre- and Post-contact, whereas the majority ($N = 31$, 58.5%) was differentially activated only during the Post-contact epoch. Figure 2B shows the time course and intensity of discharge of all grasp-to-eat and grasp-to-place F5 recorded neurons. Population analysis has been carried out by means of paired *t*-test, showing that in the grasp-to-eat population, the discharge is higher in eating than in placing condition during both Pre- ($t = 4.0$, $P < 0.001$) and Post-contact epochs ($t = 9.1$, $P < 0.001$). Similarly, in the grasp-to-place population, the discharge is higher in placing than in eating condition during both epochs (Pre-contact: $t = 5.6$, $P < .001$; Post-contact: $t = 8.7$, $P < .001$). A 2×2 ANOVA (factors: Neuronal population and Epoch) has been applied to the differential activity between the 2 conditions of the 2 neuronal populations (grasp-to-eat and grasp-to-place) in the Pre- and Post-contact epochs. This analysis revealed (see Fig. 2C) a significant main effect only of the factor Epoch ($F_{1,51} = 11.85$, $P < 0.005$). Taken together, these results show that although the action goal preference is expressed by F5 neurons already during the Pre-contact epoch of the grasping act, it becomes higher during the Post-contact epoch, regardless of which is the Preferred condition.

As mentioned above, some F5 neurons (9.7%) discharged not only during hand but also during mouth grasping, in agreement with previous studies (Rizzolatti et al. 1988; Ferrari et al. 2003). This class of neurons has been separately analyzed because in the grasp-to-eat condition only, the discharge during hand grasping could be enhanced by a possible mouth-related motor discharge. If this were the case, hand-and-mouth neurons

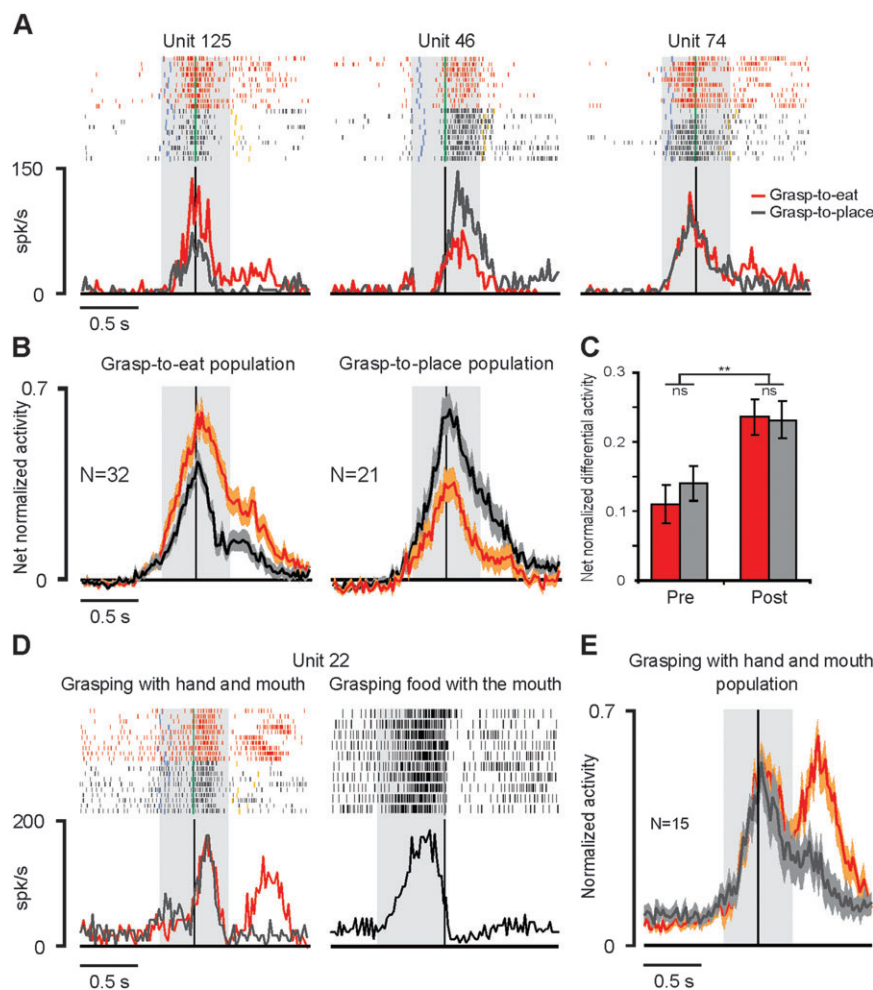


Figure 2. (A). Activity of 3 F5 neurons recorded during grasp-to-eat (red) and grasp-to-place (gray) conditions. Rasters and histograms are aligned with the moment when the monkey touched the food to be grasped. Colored bars indicate the moment when the monkey hand detached from the starting position (light blue), touched the target (green), and touched the border of the container in grasp-to-place condition (yellow). (B) Time course of the response of F5 AGR neuronal populations selective for grasp-to-eat (red line) and grasp-to-place (gray line) conditions. Orange and dark gray shading indicate 1 standard error. The net-normalized mean activity is aligned with the moment when the monkey touched the food. (C) Comparison between net-normalized differential activity of the 2 conditions in the Pre- and Post-contact epochs for F5 grasp-to-eat (red) and grasp-to-place (gray) neuronal populations. (D) Example of a grasping-with-hand-and-mouth F5 neuron. *Left part*, activity is aligned with the moment when the monkey touched the target. The second peak of discharge in the grasp-to-eat condition (red line) represents the grasping with the mouth response. Colored bars as in (A). *Right part*, activity of the same neuron when the experimenter gives a piece of food to the monkey, that grasps it with the mouth. The discharge is aligned with the end of the mouth closing phase. (E) Time course of the response of grasping-with-hand-and-mouth F5 neuronal population during grasp-to-eat and grasp-to-place conditions. Note that, as in (D), the second peak of the discharge, related to grasping with the mouth, is present only in the grasp-to-eat condition. Other conventions as in (B). In (A), (B), (D), and (E), the gray-shaded areas indicate the grasping epoch. Abscissae: time. Bin width: 20 ms. Ordinates: firing rate in spikes/s.

Table 1
F5 hand-grasping neurons recorded in the motor task

AGR		Non-AGR	Total
Eating > placing	Placing > eating	Eating = placing	
32 (23.0%)	21 (15.1%)		
53 (38.1%)		86 (61.9%)	139 (100%)

Note: Values that refer to the number of neurons appear in bold.

should discharge stronger in the grasp-to-eat condition, at least in the Post-contact epoch. An example of hand-and-mouth neuron is shown in Figure 2D. It is clear that in the discharge, there are 2 subsequent peaks of activation in grasp-to-eat but not in grasp-to-place condition. The fact that the second peak corresponds to mouth grasping is also demonstrated when this

motor act is tested separately, by letting the monkey bite directly the food without using its hand. Note, however, that this neuron does not differentiate between the 2 conditions during hand grasping. Out of the 15 hand-and-mouth-grasping neurons tested with the motor task, only one showed a preference for grasp-to-eat, during the Pre-contact epoch. Two neurons were selective for grasp-to-place, whereas the great majority ($N = 12$) did not show any selectivity in both epochs. Figure 2E shows the time course and intensity of the activity during grasp-to-eat and grasp-to-place conditions of all recorded hand-and-mouth-grasping neurons. Statistical analysis did not reveal any significant difference between the 2 conditions neither in the Pre- ($t = 0.25$, NS) nor in the Post-contact epoch ($t = 1.39$, NS). Thus, the coding of hand-and-mouth grasping by F5 neurons does not appear to be associated to a preference for grasp-to-eat actions.

F5 MNs Tested with the Visual Task

We recorded 36 MNs in F5 from both monkeys during the observation of the task performed by the experimenter.

Examples of the visual responses of F5 MNs are shown in Figure 3A. Unit 59 discharged strongly when the monkey observed the experimenter grasping a piece of food and eating it (grasp-to-eat). In contrast, the neuronal discharge was nearly absent when the monkey observed the experimenter grasping the same piece of food and placing it into a container (grasp-to-place). Unit 126 illustrates the opposite behavior, discharging stronger during grasp-to-place than during grasp-to-eat. Finally, Unit 77 did not show any significant difference in discharge intensity between the 2 conditions. Interestingly, the selectivity of AGR MNs remained the same independent of whether a piece of food or an object was used as target of the experimenter's action. This behavior is exemplified by the visual discharge of the F5 grasp-to-eat MN shown in Supplementary Figure 2. Note that the neuron, in the grasp-to-place condition, responded stronger when the target was food than when it was an object, but it still clearly maintained its preference for grasp-to-eat.

Table 2 summarizes the behavior of all F5 recorded MNs ($N = 35$) in the visual task. Results show that the great majority of them (65.7%) were differently activated during grasping observation according to the action (grasp-to-eat or grasp-to-place) in which the grasping act was embedded. In particular, all but 2 had a preference for grasp-to-eat.

Twenty-three MNs were recorded long enough to extensively test their response in both the visual and the motor task. Figure 3B shows an example of an MN having the same preference in the visual and the motor task (congruent MN).

Unit 225 discharged stronger when the monkey observed a grasping act aimed at eating than when it observed a grasping act aimed at placing, performed by the experimenter. This neuron discharged stronger also when the monkey grasped a piece of food to eat it than when it grasped the food to place it into the container. Out of the 23 MNs tested in both visual and motor tasks, 16 (70%) showed the same behavior during both observation and execution: in particular, 9 were AGR congruent MNs, whereas 7 were not AGR in both the visual and the motor task. The remaining 7 MNs (30%) were AGR in one of the task (visual or motor) but not in the other.

For each of these neurons, a preference index (PI) has been calculated for the visual and the motor response (see Materials and Methods), in order to assess the relation between the visual and the motor preference. Figure 3C shows that PI values of the visual response are positively and significantly correlated with those of the motor response ($r = 0.68$, $P < 0.001$).

Comparison between F5 and PFG Functional Properties

The demonstration of the presence of AGR neurons in area F5 raises the issue about the role played by this region in action organization with respect to that of the IPL, in which AGR neurons have been originally described (Fogassi et al. 2005). In order to address this issue, we directly compared the functional properties of the 2 regions by collecting a new data set of motor neurons and MNs from the hand region of inferior parietal area PFG, on the same monkeys and with the same version of the paradigm employed to study F5 neurons.

Motor Neurons

We recorded 120 PFG hand-grasping neurons. Hand-and-mouth-grasping neurons were virtually absent in the

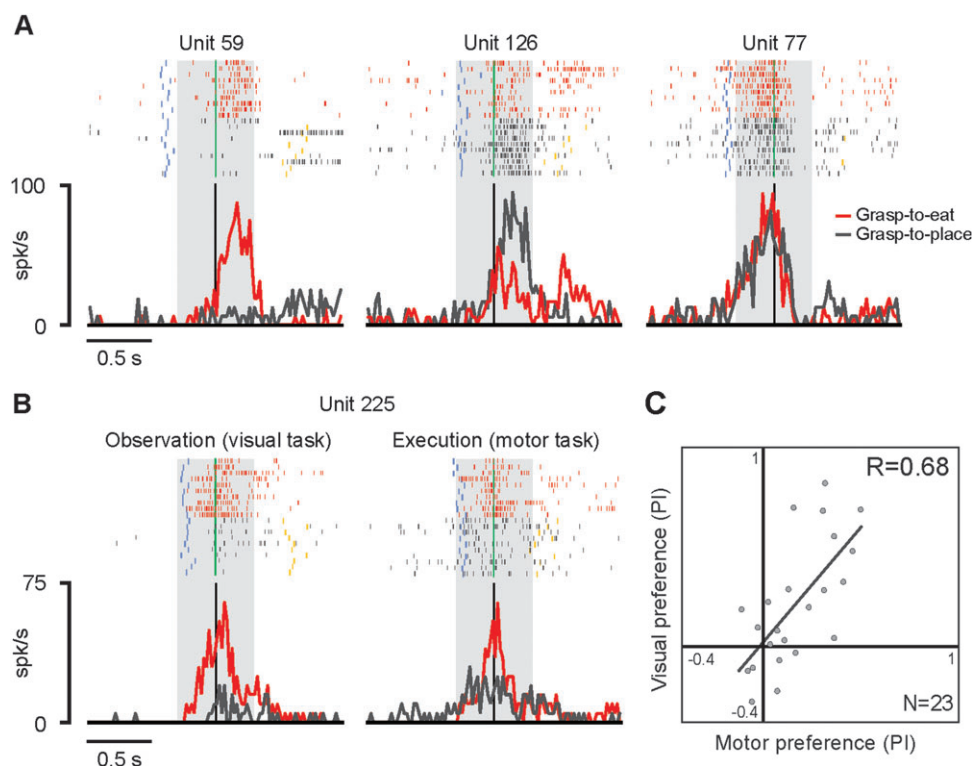


Figure 3. (A) Activity of 3 F5 MNs recorded during observation of grasp-to-eat and grasp-to-place actions performed by the experimenter. (B) Congruence between the visual and the motor responses of an F5 MN. (C) Correlation between the visual and the motor preference in all the F5 MNs recorded during both the visual and the motor task. Other conventions as in Figure 2.

investigated hand fields of PFG (less than 5% of all the recorded neurons), and therefore, they have been excluded from the present data set.

Examples of PFG hand-grasping neurons recorded with the motor task are shown in Supplementary Figure 3. Table 3 summarizes the behavior of all PFG neurons recorded in the motor task. Among 120 neurons, 45.0% coded grasping act with similar discharge intensity, regardless of the action in which the coded act was embedded. More interestingly, the majority (55.0%) discharged stronger in 1 of the 2 conditions: grasp-to-eat (43.3%) or grasp-to-place (11.7%).

Similarly to F5, the majority of PFG AGR neurons (54.5%) are active in both Pre- and Post-contact epochs, and some of them (32%) are equally active in both epochs. Concerning the epoch in which action goal preference appears, 7 (10.6%) were differentially activated only in the Pre-contact epoch, 10 (15.2%) in both Pre- and Post-contact, whereas the majority ($N = 49$, 74.2%) showed their preference only during the Post-contact epoch. The distribution of action goal preference among the epochs in F5 is not different from that of PFG (Pre-contact epoch $\chi^2 = 0.54$, NS; Pre- and Post-contact $\chi^2 = 2.32$, NS; Post-contact $\chi^2 = 3.31$, NS).

Figure 4A shows the frequency distribution of all F5 and PFG recorded neurons based on their action goal preference (in terms of PI, see Materials and Methods). It is clear that area F5 contains a greater proportion of non-AGR neurons as compared with PFG ($\chi^2 = 7.38$, $P < 0.01$). The greater proportion of AGR neurons in PFG is due to a higher number of grasp-to-eat neurons ($\chi^2 = 12.12$, $p < 0.001$) than that of F5. Furthermore, comparing the amount of preference for the action goal (PI) expressed by the 2 areas (Fig. 4B), it appears that PFG AGR neurons show a higher preference for the action goal with respect to F5 ones ($t = 2.16$, $P < 0.05$).

Figure 4C₁ shows the time course of the normalized activity of each F5 and PFG AGR neuron in its Preferred condition, aligned with the moment at which the monkey's hand contacts the target. Neurons have been ordered based on their peak of activity timing (see Materials and Methods) in order to show the variability inside each studied area. Then, a PI for the action goal has been calculated over a 120-ms period (± 60 ms with respect to peak time). Correlation analyses (Fig. 4C₂) revealed that the peak of activity timing of PFG neurons is positively correlated with their preference for the action goal ($r = 0.52$,

$P < 0.001$). That is, the later the activity reaches the peak value, the higher is the PI. This correlation is also evident when grasp-to-eat ($r = 0.53$, $P < 0.001$) and grasp-to-place ($r = 0.49$) subpopulations are separately tested, although in the case of grasp-to-place subpopulation, the statistical significance threshold is not reached ($P = 0.077$). These correlations do not occur in F5, neither for the AGR neuronal population as a whole ($r = 0.19$, $P = .17$) nor for grasp-to-eat ($r = 0.05$, $P = 0.77$) or grasp-to-place ($r = 0.30$, $P = 0.18$) subpopulations when separately tested. These findings indicate that the goal relatedness of AGR neurons in area PFG is greater and increases much more with time than that of AGR neurons in area F5. In order to clarify the possible source of these differences, we compared the amount of activity in the Preferred and Not Preferred condition, respectively, between F5 and PFG AGR neuronal populations.

Figure 4D shows the time course of F5 and PFG neuronal response in the Preferred and Not Preferred condition, aligned on the onset of neuronal activity with respect to "baseline threshold" (see Materials and Methods for definition of baseline threshold). For each neuron, the integral of the "rising" and "falling" phase of activity in the Preferred and Not Preferred condition has been calculated, in order to quantify the amount of activity associated to each phase of the 2 conditions. No differences (Bonferroni corrected P values) in the activity in their Preferred condition during "rising" ($t = 2.17$, NS) and falling phase ($t = 1.71$, NS) between PFG and F5 populations were found. On the contrary, activity in the Not Preferred condition during rising ($t = 3.39$, $P < 0.001$) and falling ($t = 2.73$, $P < 0.01$) phases are higher in F5 than in PFG populations. This finding suggests that the higher preference for the final goal expressed by PFG neurons mainly depend on their relatively lower discharge in the Not Preferred condition rather than on a higher discharge in the Preferred condition, as compared with F5 neuronal population.

The Mirror Neurons

We recorded 28 MNs from the hand field of area PFG employing the visual task. Examples of PFG MNs visual responses are shown in Supplementary Figure 4.

Table 4 summarizes the behavior of the visual response of all PFG MNs. Some of them (35.7%) discharged during the observation of a grasping act regardless of the action (grasp-to-eat or grasp-to-place) in which grasping was embedded, similarly to F5 ($\chi^2 = 0.01$, NS). The majority (64.3%) discharged stronger in 1 of the 2 conditions, with an extremely larger proportion preferring grasp-to-eat (50% of all recorded MNs) as compared with grasp-to-place (14.3%). This distribution of preferences (see Fig. 5A) is not significantly different from that of F5 MNs (grasp-to-eat: $\chi^2 = 0.63$, NS; grasp-to-place: $\chi^2 = 1.50$, NS). Furthermore, Figure 5B shows that the action goal preference calculated in terms of PI on the visual responses of F5 and PFG AGR neurons does not differ between the 2 areas ($t = 1.37$, NS).

All PFG MNs tested with the visual task discharged during both grasping observation and grasping execution, as shown for F5 MNs. For all those neurons recorded long enough to be studied in both the visual and the motor task, a PI has been calculated for the visual and the motor response (see Materials and Methods). Figure 5C shows the correlation between visual and motor preference of PFG MNs ($r = 0.56$, $P < 0.05$), overlapped with that of F5 ones. It is clear that in MNs of both

Table 2
Visual response of F5 MNs recorded in the motor task

AGR		Non-AGR	Total
Eating > placing	Placing > eating	Eating = placing	
21 (60.0%)	2 (5.7%)		
	23 (65.7%)	12 (34.3%)	35 (100%)

Note: Values that refer to the number of neurons appear in bold.

Table 3
PFG hand-grasping neurons recorded in the motor task

AGR		Non-AGR	Total
Eating > placing	Placing > eating	Eating = placing	
52 (43.3%)	14 (11.7%)		
	66 (55.0%)	54 (45.0%)	120 (100%)

Note: Values that refer to the number of neurons appear in bold.

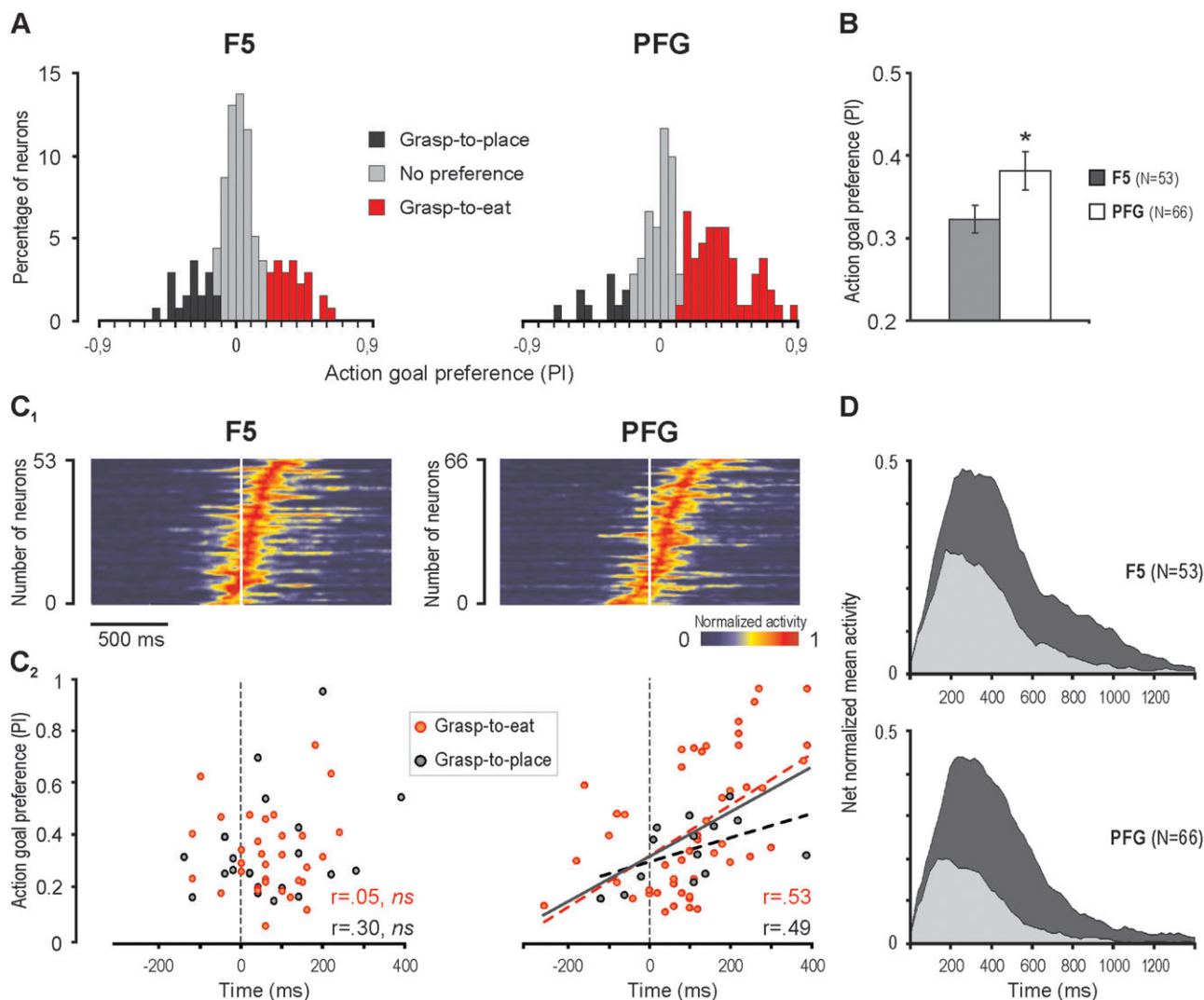


Figure 4. (A) Comparison between the distribution of F5 and PFG neurons according to their PI. (B) Comparison between average PIs (absolute values) of F5 and PFG AGR neuronal populations (C_1). Time course of the activity of F5 and PFG AGR grasping neurons in their Preferred condition, aligned with the moment when the monkey touched the food to be grasped (white line). The discharge intensity over time of each neuron is represented as a change in color along horizontal lines. Neurons have been ordered from bottom to top, based on their peak of activity timing. (C_2) Correlation between PI and peak of activity timing for F5 and PFG AGR grasping neurons. Vertical dashed line corresponds to the moment when the monkey touched the food to be grasped. Gray line represents the correlation between time and PI in the whole AGR neuronal population. (D) Temporal profile of the discharge of F5 and PFG AGR neuronal populations aligned on the activity onset, calculated with respect to baseline threshold. Responses in the Preferred and Not Preferred conditions are represented by the dark and the light gray areas, respectively.

Table 4

Visual response of PFG MNs recorded in the visual task

AGR		Non-AGR	Total
Eating > placing	Placing > eating	Eating = placing	
14 (50.0%)	4 (14.3%)	10 (35.7%)	28 (100%)

Note: Values that refer to the number of neurons appear in bold.

areas, the *motor* preference for a certain action goal (grasp-to-eat or grasp-to-place) is positively correlated with the *visual* preference for the same goal. Furthermore, the Fisher's Z procedure has been applied in order to compare the correlation coefficients obtained for PFG and F5 MN populations, revealing that they are not significantly different ($P = 0.56$).

Histological Reconstruction and Cortical Connections between F5 and PFG Recorded Regions

Figure 6 shows the reconstruction of the recorded regions in the right hemispheres of M1 and M2. The majority of penetrations were located inside the cytoarchitectonic areas PFG and F5, as defined by previous works (Gregoriou et al. 2006; Belmalih et al. 2009).

The results of tracers injection show that, in both monkeys, the sectors of PFG and F5 where motor neurons and MNs were recorded are connected. In particular, following WGA injections in PFG of M1, labelled neurons have been found in the investigated sector of F5. CTB-A 594 injection in M2 confirms this pathway (red labelling), showing in addition that following injection in the recorded sector of F5 (CTB-A 488), retrograde labelling (green) was present in PFG. This latter finding indicates that

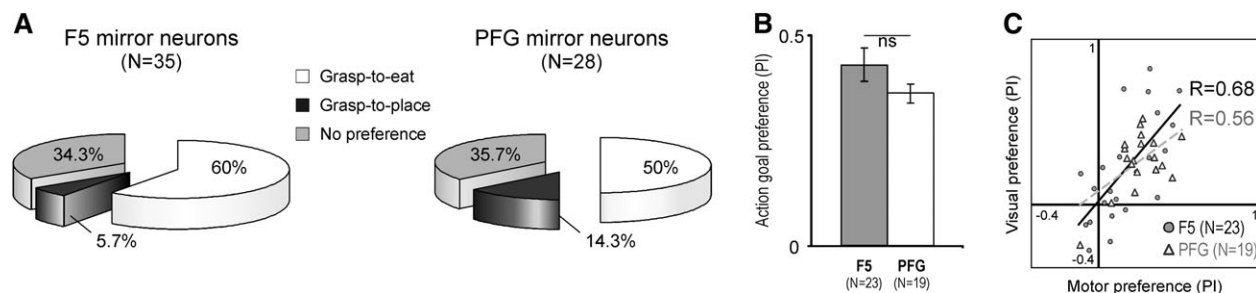


Figure 5. (A) Comparison between the percentage of F5 and PFG MNs according to their action goal preference in the visual task. (B) Comparison between average PI of the visual response of F5 and PFG AGR MNs populations. (C) Comparison between the correlations of visual and motor preference in all F5 and PFG MNs recorded during the visual and the motor task.

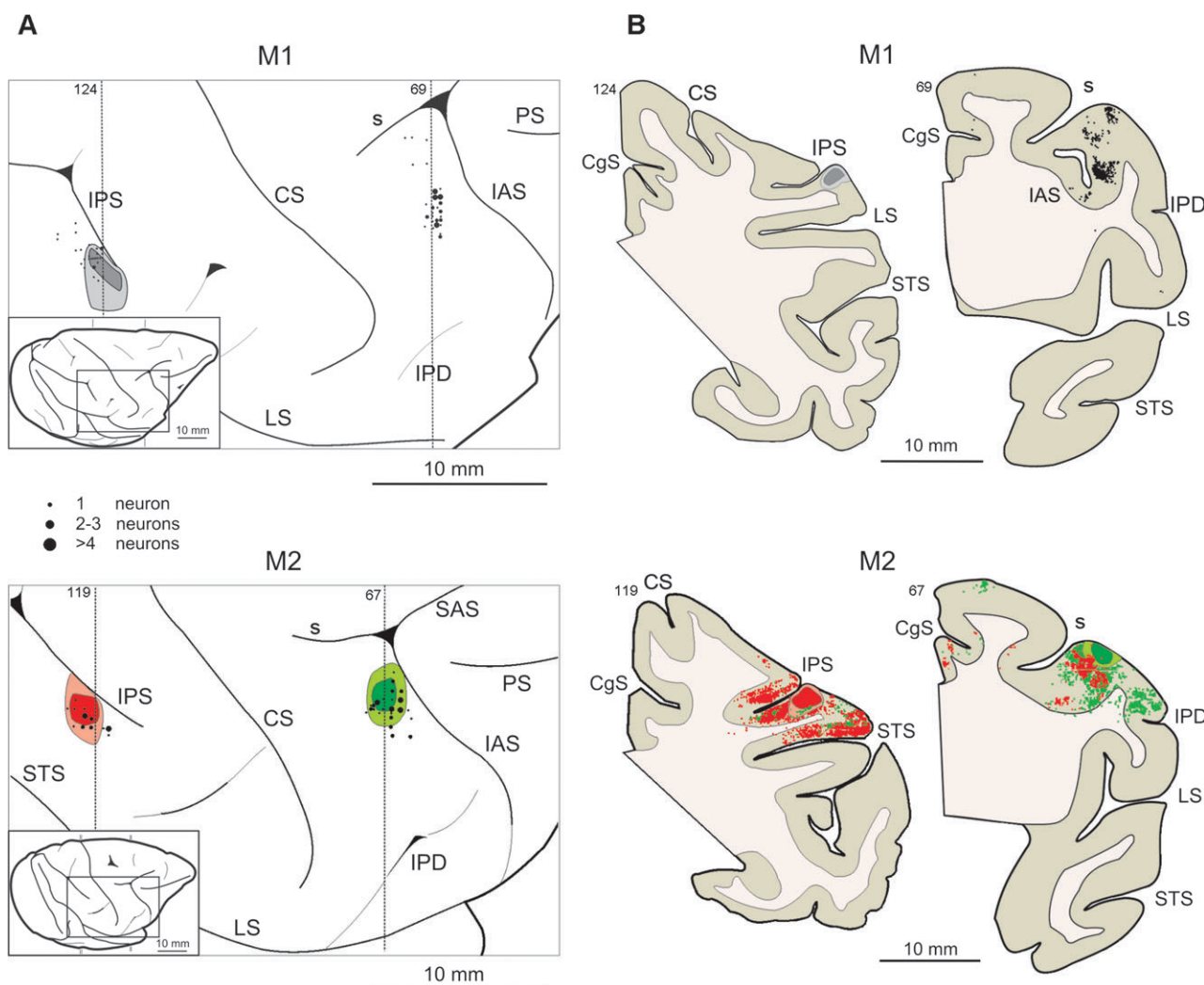


Figure 6. (A) Reconstruction of penetrations and injection sites in F5 and PFG recorded regions in the 2 monkeys. The inset depicts the dorsolateral view of 2 studied hemispheres. In the enlargement, the penetration locations and the injection sites are depicted. In each injection site, the shaded regions indicate the extension of the core (darker color) and halo (lighter color). The dotted lines indicate the levels at which the coronal sections, shown in (B), are taken. (B) Outlines of coronal sections taken at the level of the 2 recorded regions in M1 and M2. Top, black dots in section 69 indicate neurons labelled with WGA. Bottom, red and green dots indicate neurons labelled with CTB-Alexa 594 and CTB-Alexa 488, respectively. Shaded regions as in (A). CgS, cingulate sulcus; CS, central sulcus; IAS, inferior arcuate sulcus; IPD, inferior precentral dimple; IPS, intraparietal sulcus; LS, lateral sulcus; PS, principal sulcus; s, spur of the arcuate sulcus; SAS, superior arcuate sulcus; STS, superior temporal sulcus.

the connections between the 2 investigated regions are reciprocal.

A complete description of the pattern of connections of the 2 injected areas will be the focus of a separate study.

Discussion

One of the main findings of the present study is that some F5 motor neurons can code the same grasping act differently according to the goal of the action in which the coded act is

embedded. Furthermore, we demonstrated that the discharge of most F5 MNs during grasping observation is influenced by the action goal of the observed agent. Finally, the comparison of F5 and PFG neuronal properties revealed that motor neurons are more frequently and strongly influenced by action goal in area PFG, whereas no differences are evident between MNs of the 2 areas in coding the goal of observed actions.

Area F5 Motor Neurons and Action Organization

The present study employed a modified version of an experimental paradigm described in a previous work (Fogassi et al. 2005) enabling us to directly control for the possibility that spurious factors such as kinematics, type of object or force, could account for a possible differential discharge between the 2 experimental conditions (grasp-to-eat and grasp-to-place).

Results showed that one third of the F5 recorded neurons discharged differently during grasping when this act was embedded into actions aimed at different goals (AGR neurons). However, the majority of the F5 recorded neurons were not differentially activated in the 2 conditions.

Almost half of the AGR grasping neurons showed the differential activity already during the Pre-contact epoch, that is, when the hand approaches the object and preshapes with respect to its features. These findings are in line with the notion, derived from a number of psychophysical and kinematics studies, that reaching for grasping an object implies the activation of the motor processes leading to the correct action execution well ahead of the actual grasping (Jeannerod et al. 1995; Gentilucci et al. 1991, 1997; Rosenbaum et al. 2007; Ansuini et al. 2008, 2009). The neural bases of some of these processes have been previously demonstrated studying the grip selectivity of F5 motor and visuomotor neurons (Murata et al. 1997; Raos et al. 2006; Umiltà et al. 2007). In these latter studies, the selectivity for a certain grip type appeared already in an early stage of the task to be performed, and it progressively increased as the grasping execution approached. The present data suggest that, beyond coding the type of grip as shown by the above mentioned studies, the activity of some F5 neurons also reflects the goal of the action to be performed in the first phase of grasping execution.

Most of F5 AGR neurons showed their preference during Post-contact epoch. In addition, population analysis showed that the preference for a specific action goal, although already present in the Pre-contact, further increases in the Post-contact epoch. This increased impact of action goal on the latest epoch of grasping neurons activity suggests that neurons coding motor acts recruited earlier in an action sequence could facilitate those neurons coding motor acts activated later in the same action (see Fogassi et al. 2005; Fogassi and Luppino 2005). In line with this suggestion is the receptive field organization of some PMv neurons. For example, neurons have been described having both proprioceptive responses to elbow flexion and tactile responses on the face and the mouth, but their activity is conditional upon simultaneous stimulation (Rizzolatti et al. 1981). This organization could play a role in rendering smooth the most natural actions (i.e., eating or avoidance behaviors) performed by the monkey. Moreover, long-train intracortical microstimulation of this premotor region elicits hand-to-mouth movements similar to the actions naturally performed by the monkeys (Graziano et al. 2002). Thus, the action goal relatedness of some F5 neurons shown in this study could

constitute a possible mechanism underlying the organization of natural actions in this region.

The findings of the present study could also be compatible with an alternative interpretation, namely that AGR neuron's differential response could simply reflect the control of hand-mouth synergies, regardless of action goal. However, this interpretation can be discarded for many reasons. First, a large proportion of AGR neurons fire stronger during grasp-to-place, even independently of the kinematics of the action ("placing near the mouth" or "placing near the target"). Second, all neurons included in the main data set did not show any mouth-related response. Third, according to the hand-mouth synergy hypothesis, the discharge of hand-and-mouth-grasping neurons should have been stronger when the action goal was eating. This never occurred despite the subsequent discharge related to mouth grasping was present in the grasp-to-eat condition only. Thus, although we cannot exclude the existence of hand-and-mouth neurons showing a preference for grasp-to-eat during hand grasping, hand-and-mouth synergies are unlikely to represent the basic organization principle of AGR neurons.

Taken together, our data indicate that in F5 there are 2, not mutually exclusive, different levels of goal coding: the goal of "motor act" and that of "action." As far as the motor act level is concerned, here we show that the great majority of F5 neurons (non-AGR-grasping neurons) code specifically the goal of the motor act, discharging similarly regardless of the action in which the coded act is embedded and, in some instances, in an even more abstract fashion, that is independent on the effector used (e.g., hand-and-mouth-grasping neurons) (see also Rizzolatti et al. 1988; Ferrari et al. 2003; Umiltà et al. 2008). As far as the "action goal" level is concerned, the discharge of F5 AGR neurons, besides coding a specific motor act, also reflects the goal of the whole action in which the coded act is embedded.

As previously suggested for IPL neurons (Fogassi et al. 2005; Fogassi and Luppino 2005; Rizzolatti et al. 2006), here we propose that the discharge of F5 AGR-grasping neurons reflect the motor intention of the acting individual. Noteworthy, here the term "intention" does not refer to the anticipated activation of a forthcoming motor plan (Snyder et al. 1997, 2000) nor to a generic "intention for action" acting as a go signal for motor activation (Hoshi et al. 2005; see also Haggard 2008). Instead, it refers to the predictive nature of the motor knowledge one possesses about the outcome of his/her own action. This view confirms and expands our understanding on the motor cognitive functions of PMv (Rizzolatti et al. 2002; Fiebach and Schubotz 2006; Hoshi and Tanji 2007; Pardo-Vazquez et al. 2008).

Area F5 MNs and Intention Understanding

In this work, we studied the visual response of F5 MNs by means of the visual task previously applied in the investigation of IPL MNs (Fogassi et al. 2005). The results of the present study show that the great majority of these neurons discharged differently during observation of grasping according to the goal of the action in which it is embedded. Notably, almost all AGR MNs' visual responses showed a preference for grasp-to-eat with respect to grasp-to-place condition.

A spurious factor that could explain the observed preference for grasp-to-eat is the possible occurrence of subtle activation of mouth muscles during monkey observation of the hand-grasping motor act performed by the experimenter on the food item. This is unlikely because it has been previously demonstrated that there is no EMG activation of both hand (Gallese

et al. 1996) and mouth (Ferrari et al. 2003) muscles during observation of both hand-and-mouth motor acts. Other spurious factors, such as motivation, reward expectancy, or attention could in principle be responsible for the differential discharge observed between the 2 conditions. The first 2 factors appear unlikely because the observation of the visual task was not followed by any reward delivery, and the 2 conditions were run in a pseudorandom fashion. As far as attention is concerned, there are 2 reasons allowing us to rule out this interpretation. First, the trials in which the monkey did not look at the scene were excluded from the data set. Second, although, in some cases, we noticed that the discharge of grasp-to-eat neurons was slightly higher during grasp-to-place when the target was food rather than an object, the neuron maintained its preference for grasp-to-eat. This is in agreement with previous findings on IPL MNs (Fogassi et al. 2005).

The predominance of grasp-to-eat MNs could be due to the fact that this is the monkey's most natural and motorically familiar action, perfectly mastered by the animals even before any motor training. This interpretation is also supported by the largely overlapping hand and mouth representation of motor acts in this area (Rizzolatti et al. 1988), very likely constituting the anatomo-functional substrate for organizing feeding behaviors. In line with this view, the response of MNs when tested with the motor task usually showed a congruent preference for grasp-to-eat. The lowest percentage of grasp-to-place MNs could depend on the fact that monkeys were less exposed to the observation of this action in their everyday life. A longer exposure to grasp-to-place action could favor the increase of this MNs category, similarly to what previously suggested in other studies on visuomotor neurons of the premotor cortex (Cisek and Kalaska 2004; Ferrari et al. 2005).

In conclusion, the visual preference of AGR MNs appears to code the goal of the action performed by another individual. Thus, in agreement with the interpretation of the preference evidenced during the execution of the motor task, F5 MNs visual preference could contribute to the observer's understanding of others' motor intention.

Relative Contribution of Areas F5 and PFG to Action Organization and Intention Understanding

By comparing the percentage of AGR motor neurons found in area F5 in the present study with that previously reported in IPL (Fogassi et al. 2005), it emerges that area F5 contains a considerably lower proportion of AGR neurons as compared with IPL. However, this difference could be merely due to different monkeys, paradigms employed, and extension or heterogeneity of the explored regions in the 2 studies. Therefore, in order to rule out these factors, here we recorded F5 and IPL neurons in the same monkeys by adopting the same paradigm. Note also that the IPL region investigated in the present study was limited to area PFG (Gregoriou et al. 2006), which shares with F5 many functional properties, among which the presence of hand MNs (Rizzolatti et al. 1988; Gallese et al. 1996; Rozzi et al. 2008). The additional advantage of our approach has been that of allowing a direct investigation of the anatomical connectivity between the 2 studied regions (see below).

The comparison between F5 and PFG neurons recorded with the motor task revealed that F5 actually contains a lower percentage of AGR neurons and that, in addition, F5 AGR

neurons have a lower degree of preference for the action goal with respect to PFG ones. These findings suggest that the 2 areas provide different contributions to action goal coding, with PFG playing a more important role than F5 in this function. In particular, this appears to be due to a higher number of PFG neurons selective for grasp-to-eat with respect to F5, suggesting the presence of a wider neuronal system for natural actions in the parietal as compared with premotor cortex.

It has been previously hypothesized (Fogassi et al. 2005; Rizzolatti et al. 2006) that IPL neurons showing a preference for the action goal could be organized in dedicated neuronal chains, each coding a specific action. Such an organization would explain the functional relevance of having neurons coding a specific motor act (e.g., grasping) discharging preferentially when this act is included in a specific action. In order to better investigate the different contribution of PFG and F5 to action organization, we compared the temporal aspects of neuronal discharge between the 2 areas. These analyses revealed that the later the peak of activity of PFG AGR neurons, the higher the preference they express for the action goal, whereas this relation does not exist in F5 AGR neurons. Notably, this correlation exists in PFG not only when the whole AGR neuronal population is considered but also for the grasp-to-eat and grasp-to-place subpopulations tested separately, although in the case of grasp-to-place the lower number of neurons probably prevents to reach the conventional statistic threshold. This suggests a more important role of PFG as compared with F5 in organizing the action based on its specific goal. Another interesting aspects emerging from the comparison of the 2 areas is that when the Preferred conditions of all AGR neurons of the 2 areas are considered, the discharge during the rising and falling phase of neuronal activity is not different, whereas a lower discharge is present in PFG as compared with F5 neurons in their Not Preferred conditions during both the rising and falling phase of activity. This finding could depend on possible local inhibitory influences affecting stronger PFG than F5. The phenomenon of local inhibition is well framed within the hypothesized neuronal chain organization. For example, the higher discharge of a grasping neuron in eating condition could facilitate neurons coding bringing to the mouth and mouth opening and, at the same time, could inhibit neurons embedded in the alternative action chain aimed at placing.

In contrast to purely motor neurons, a substantial similarity has been reported between PFG and F5 MNs, concerning both their visual properties and visuomotor congruence. In fact, in both regions, most MNs are AGR and prefer grasp-to-eat condition in both the visual and the motor task. This indicates that representing one's own and others' motor act in relation to the goal of the action in which the coded act is embedded constitutes a general property of this class of visuomotor neurons. Thus, MNs appears to provide a more abstract level of motor representation as compared with purely motor neurons, encompassing the 2 anatomically connected areas in which MNs have been recorded.

If one accepts that action goal is the real factor influencing both the motor and the visual discharge of AGR motor neurons and MNs, it would be important to clarify the possible causes of such an influence. It is clear that during both action execution and observation, contextual information is necessary for selecting the motor representation of actions. The present study demonstrates that this selection is manifested by the existence of grasping neurons committed to specific actions in

both parietal (see also Fogassi et al. 2005) and premotor cortex. Notably, different brain regions such as ventral prefrontal (Tanji and Hoshi 2008), cingulate (Isomura and Takada 2004; Rushworth et al. 2007), and mesial premotor (Tanji 2001) cortices can exploit contextual information, previous experience, or abstract signals for representing and selecting action sequences and behavioral goals. These cortical regions are anatomically connected to both premotor (Matelli et al. 1986; Barbas and Pandya 1987; Barbas 1988; Petrides and Pandya 2002) and parietal cortices (Petrides and Pandya 1984; Cavada and Goldman-Rakic 1989; Luppino et al. 1993; Rozzi et al. 2006) in which motor neurons and MNs have been recorded. These connections would enable prefrontal and mesial cortical regions to select specific pools of neurons (AGR neurons) coding chained motor acts allowing the activation of motor representations of goal-directed actions within specific contexts. This chained organization, on the motor side, has very likely the advantage to render smooth the development and control of the coded action. On the visual side, when information about others' actions in a specific context is available, it endows the observer with a predictive motor representation and, thus, with a basic form of understanding of the motor intention underlying the observed agent's action.

Possible Network Involved in Action Organization and Intention Understanding

The results of tracers injections carried out in this study evidenced the presence of reciprocal connections between the PFG and F5 sectors in which single neurons have been recorded in the motor and the visual task.

On the one hand, this anatomical pattern very likely underlies the functional similarities between the 2 areas, such as the presence of AGR motor neurons and MNs in both of them. These functional similarities are particularly evident when MNs are concerned, strongly supporting a common involvement of PFG and F5 in action organization and intention understanding.

On the other hand, as previously shown for areas belonging to other parieto-premotor circuits (Stanton et al. 1995; Caminiti et al. 1996; Rizzolatti and Luppino 2001; Pesaran et al. 2008), PFG and F5 appear to have distinct functional specificity. These differences can be due to a stronger influence of prefrontal cortex on PFG as compared with F5 (Matelli et al. 1986) and, in turn, to a more massive anatomo-functional connection of F5 than PFG with primary motor and premotor cortices (Muakkassa and Strick 1979; Matelli et al. 1986; Belmalih et al. 2007; Prabhu et al. 2009). According to this neuroanatomical pattern, area PFG would have a more important role in organizing motor acts into an action based on its goal, whereas area F5 would be more devoted to code the goal of single motor acts in an abstract fashion, even independently of the used effector (Rizzolatti et al. 1988; Umiltà et al. 2008).

According to this proposal, it could be argued that PFG has a leading role with respect to F5 in action organization also within the temporal domain. Although the present single neurons data do not allow this latter conclusion, recent electroencephalographic and cortical field potentials studies in both humans (Wheaton et al. 2005a, 2005b) and monkeys (Gemba et al. 2004) indicate that the parietal cortex becomes active well before the premotor cortex during self-paced voluntary movements. Another monkey study (Pesaran et al.

2008) using a reaching task found that, although the information flows initially from premotor to parietal cortex, a subsequent backward propagation of activity reflects the final decision process. Hence, it is plausible that also in our study both these information flows exist, contributing to the organization and selection of intentional actions in the parietal cortex, so that the selected action could then access the premotor vocabulary of motor acts for action execution.

Of course, further experimental support to the proposed anatomo-functional specialization of parietal and premotor cortex in action organization is needed. Of outstanding interest would be, in this respect, the investigation of the outcomes of reversible inactivation or ablation experiments carried out in electrophysiologically characterized sectors of the 2 areas. The available neuropsychological data on apraxia in humans (see Leiguarda and Marsden 2000) suggest that a primitive system for the organization of transitive natural actions could have been the precursor for the development of more sophisticated praxic skills (see also Frey 2008) together with the capacity to recognize even intransitive, meaningful gestures (Pazzaglia et al. 2008).

Conclusions

Behavioral data on action organization in humans clearly show that each motor act belonging to an action, both when executed (Jeannerod 1988; Gentilucci et al. 1997; Rosenbaum et al. 2007) or simply observed (Gangitano et al. 2004), is influenced by the previous act, suggesting that planning an action requires the early programming of all the motor acts constituting it. In agreement with this proposal, a recent human study (Cattaneo et al. 2007) using the same experimental paradigm employed in the present investigation showed that in typically developing children, during eating actions, electromyographic activity of muscles involved in mouth opening increases already well before the hand grasps the food. Interestingly, this activation was found also during observation of the same action.

These findings, together with the present data, suggest that in both humans and monkeys the parieto-premotor system endows individuals with predictive representations of the next motor acts belonging to an action. This organization could contribute to the fluidity of action execution and to the emergence of a basic and automatic form of motor intention understanding.

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Supplementary Material

Supplementary Figures 1–4 can be found at: <http://www.cercor.oxfordjournals.org/>

Notes

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