

I Know What You Are Doing: A Neurophysiological Study

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Summary

In the ventral premotor cortex of the macaque monkey, there are neurons that discharge both during the execution of hand actions and during the observation of the same actions made by others (mirror neurons). In the present study, we show that a subset of mirror neurons becomes active during action presentation and also when the final part of the action, crucial in triggering the response in full vision, is hidden and can therefore only be inferred. This implies that the motor representation of an action performed by others can be internally generated in the observer's premotor cortex, even when a visual description of the action is lacking. The present findings support the hypothesis that mirror neuron activation could be at the basis of action recognition.

Introduction

In the monkey ventral premotor cortex, there is a sector that controls hand and mouth movements (Rizzolatti et al., 1981, 1988; Kurata and Tanji, 1986; Hepp-Reymond et al., 1994). This sector, which has specific histochemical and cytoarchitectonic features, has been named area F5 (Matelli et al., 1985). A fundamental functional property of area F5 is that most of its neurons do not discharge in association with elementary movements but are active during actions such as grasping, tearing, holding, or manipulating objects (Rizzolatti et al., 1988).

Among other neuron types, F5 contains a striking class of neurons that discharge both when the monkey performs specific hand or mouth actions and when the monkey observes another individual making similar actions. These neurons have been called "mirror" neurons (Gallese et al., 1996; Rizzolatti et al., 1996).

The visual feature that activates mirror neurons is the observation of an interaction between the agent of the action and the object being the target of it. Mirror neurons typically do not respond to the sight of a hand miming an action. Similarly, mirror neurons do not respond to the observation of an object alone, even when of interest to the monkey (e.g., food).

The vast majority of mirror neurons shows congruence between the effective observed action and the effective executed action (Gallese et al., 1996; Rizzolatti et al., 1996). This congruence is sometimes extremely strict.

In this case, the effective motor action and the effective observed action coincide both in terms of goal (e.g., grasping) and in terms of how the goal is achieved (e.g., precision grip). For most neurons, however, the congruence is broader and is confined to the goal of the action. These broadly congruent neurons are of particular interest because they generalize the goal of the observed action across many instances of it.

What can be the functional role of mirror neurons? The hypothesis has been advanced that these neurons are part of a system that recognizes actions performed by others. This recognition is achieved by matching the observed action on neurons motorically coding the same action. By means of such a neural matching system, the observer during action observation is placed in the same "internal" situation as when actively executing the same action (Gallese et al., 1996; Rizzolatti et al., 1996, 2000).

There is, however, an intriguing issue here. In everyday life, objects move into and out of sight because of interposition of other objects. Yet, even when an object, target of the action, is not visible, an individual is still able to understand which action another individual is doing. For example, if one observes a person making a reaching movement toward a bookshelf, he/she will have little doubt that the person in question is going to pick up a book, even if the book is not visible. Full visual information about an action is not necessary to recognize its goal. Action understanding could be based on a mechanism that can trigger the internal motor representation of the action.

If mirror neurons indeed represent the neural substrate for action recognition, they (or a subset of them) should become active also during the observation of partially hidden actions. The aim of the present experiment was to test this hypothesis. The experiment consisted of two basic experimental conditions. In one, the monkey was shown a fully visible action directed toward an object ("full vision" condition). In the other, the same action was presented but with its final critical part (hand-object interaction) hidden ("hidden" condition). The results showed that the majority of mirror neurons responded also in the hidden condition. These results provide strong support for the hypothesis that mirror neurons are involved in action recognition.

Results

We recorded 220 neurons from area F5 of two monkeys (119 neurons in monkey 1 and 101 in monkey 2). All recordings were performed in the right hemispheres. Of the recorded neurons, 103 (47/119 in monkey 1 and 56/101 in monkey 2) discharged both during hand actions made by the monkey and during observation of similar actions performed by the experimenter. These neurons were therefore classified as mirror neurons. Among them, 37 (21 in monkey 1 and 16 in monkey 2) were recorded for a time sufficiently long to test them in all the experimental conditions.

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Table 1. Mirror Neurons Subdivided According to Observed Hand Actions Effective in Activating Them

Observed Hand Actions	Number of Neurons Responding in Full Vision Condition	Number of Neurons Responding in Hidden Condition
Grasping	7	3
Holding	3	1
Placing	2	2
Grasping + holding	19	8
Grasping + manipulating	2	1
Approaching + manipulating	4	4
Total	37	19

Table 1, left and central columns, shows the actions whose observation triggered the neurons and the number of neurons responsive to the observation of each of them, respectively. Twelve neurons responded to the observation of one action only, while the remainders responded to the observation of two actions. Neurons responding to grasping and holding and to grasping and manipulating typically started to discharge at the end of the hand transport phase, while grasping neurons started to discharge either at the onset of the observed action or at its end. The observation of neurons discriminating between different types of hand-object interactions confirms previous findings (Gallese et al., 1996). No quantitative analysis of this phenomenon will be reported in the present paper.

Following characterization of their functional properties, the neurons were tested as illustrated in Figures 1 and 2 (see the hand movement cartoons). The experimenter stood in front of the monkey, behind a metallic frame that allowed the vision of the experimenter's upper body and arms. In the two experimental conditions, the hand of the experimenter, starting from a stationary position within the frame space, moved toward an object placed on a plane also located within the frame space, grasped the object, and held it for about 1 s (see Figures 1A and 1B). The only difference between these two conditions resides in the fact that in Figure 1A (full vision condition) the entire action was visible to the monkey, while in Figure 1B (hidden condition) an opaque screen was slid halfway into the frame to obscure the second half of the action, hiding the hand-object interaction. In the two further conditions, the importance of the presence of an object was tested by replicating the two experimental conditions without object (Figures 1C and 1D, "miming in full vision" and "hidden miming," respectively).

In all trials, the experimenter's hand position and movements were recorded using a computerized movement recording system (see Experimental Procedures). The following events were detected: the onset of the hand movement (green bars in the rastergrams); the crossing of a stationary marker placed where the hand started to be obscured by the occluder in hidden trials (red bars); the touching of the object target of the action or, in trials without objects, the touching of the location at which the objects were otherwise placed (pale blue bars). These events (and the respective colored bars in the figures) divide each trial into four epochs: "background," period before the green bar; "early movement," period extending from the green to the red bar; "late

movement," period extending from the red to the pale blue bar; and "holding," 500 ms following the pale blue bar.

Figures 1 and 2 illustrate the main results of the experiment, as displayed by two neurons. First, in the hidden condition, neurons responded to the presented action as if there were no screen and the whole action could be seen. Second, when the object was taken away (hidden miming condition), neurons failed to respond to the presented action, although what the monkey saw was identical in the two hidden conditions—the only difference being the monkey's "knowledge" of the presence of the object. For these neurons, "out of sight" was therefore not "out of mind."

These effects were confirmed by a statistical analysis. A four epoch \times two visibilities (full vision versus hidden) \times two object presences (present versus absent) ANOVA showed a significant main effect of epoch [$F(3,144) = 27, p < 0.001$ and $F(3,144) = 24, p < 0.001$ for Figures 1 and 2, respectively] and object presence [$F(3,144) = 51, p < 0.001$, and $F(3,144) = 55, p < 0.001$ for Figures 1 and 2, respectively]. A Newman-Keuls post-hoc ($p < 0.01$) analysis revealed that these main effects were due to the firing rate in the late movement and holding period being significantly elevated compared to background only in the two conditions in which the object was present. There was, on the other hand, no significant main effect for visibility (both $p > 0.2$) nor significant epoch \times visibility or epoch \times visibility \times object interactions (all $p > 0.3$). A Newman-Keuls post-hoc analysis confirmed that the firing rate during hidden conditions did not differ significantly from that occurring in their full vision counterpart for any of the epochs.

In order to rule out the possibility that some of the observed effects were due to differences in hand movements between conditions, we analyzed the experimenter's hand kinematics for each trial of each condition. The recorded hand trajectories were aligned with the moment when the hand crossed the stationary marker. They are superimposed and displayed as pairwise comparisons in the bottom line and rightmost column of Figures 1 and 2. In Figure 1, the only kinematic differences were in the starting position of the experimenter's hand (see Figures 1A versus 1B and 1C versus 1D), the remaining trajectory being virtually identical. Note that these kinematic differences occurred between conditions that produced identical neural responses (Figures 1A versus 1B and 1C versus 1D), while, conversely, conditions that produced different neural responses showed identical kinematics. In Figure 2, no differences in kine-

matics are visible between conditions. Hand kinematics therefore cannot account for the observed neural responses.

Some intertrial variation in the firing rate of the neurons is visible in the rasters. This variability is probably due to unsystematic variations in the cognitive state of the animal, as there are no obvious differences between kinematics in trials with higher and lower firing rate.

To test whether responses to hidden actions were present overall in the population of 37 neurons studied, their responses were analyzed together as a population. The responses in full vision and hidden conditions were therefore compared. Since neurons differed in their firing rates, the firing rates of each neuron were normalized by subtracting the activity occurring during the background epoch from that occurring during the early movement, late movement, and holding epochs ("net activity"). After this normalization, the activity during the background period was always zero. This net activity was then averaged over all ten trials and divided by the highest average activity occurring for that neuron in full vision. Figure 3 illustrates the average over all 37 neurons of that activity as a function of epoch and condition. A four epoch \times two conditions ANOVA showed significant main effects for epoch [$F(3,108) = 25, p < 0.001$] and condition [$F(1,36) = 9, p < 0.01$] as well as a significant interaction [$F(3,108) = 6, p < 0.001$]. A Newman-Keuls post-hoc analysis ($p < 0.01$) revealed that the firing rate in all but the early movement epoch for the hidden condition was significantly higher than during the background activity. This indicates that the population as a whole responded to an action even while the action is hidden. The post-hoc analysis also revealed that during early movement, firing rates did not differ between the two conditions. During the late movement and holding epoch, on the other hand, the full vision condition produced a larger population response than the hidden condition. Note, finally, that the post-hoc analysis revealed that the response during the late movement and holding epoch was higher than that during the early movement and background. Hence, in the hidden condition, the response was maximal after the hand disappeared.

To isolate the contribution of individual neurons to the effect demonstrated by the population, all 37 neurons were tested using a two visibilities (full vision versus hidden) \times four epochs ANOVA with ten trials in each condition. Neurons were classified as responding significantly in a particular condition if they showed a significant main effect of epoch or an epoch \times visibility interaction and when a Newman-Keuls analysis showed that one movement epoch produced an activity higher than the background epoch (all $p < 0.01$). According to this criterion, 19/37 neurons were found to respond significantly during the hidden condition (12/21 in monkey 1, 7/16 in monkey 2), and, as expected, all 37/37 neurons responded significantly in the full vision condition (see Table 1).

For the 19 neurons responsive during the hidden condition, we then examined, epoch per epoch, how the responses in the hidden condition compared with those in the full vision condition, using the same Newman-Keuls analysis. In 7/19 neurons, there was no significant difference between the responses in the hidden and full

vision conditions (neurons shown in Figures 1 and 2 are drawn from this category). For 9/19 neurons, responses were more pronounced in the full vision condition. For 1/19 neurons, the response was significantly more pronounced in the hidden condition. Two neurons finally showed an ambivalent effect. For one of these latter two, the response was stronger during the late movement epoch in the hidden condition, while it was stronger during the holding epoch in the full vision condition. For the other neuron, the peak response occurred during the holding phase in the hidden condition and immediately after the analyzed holding phase in the full vision condition. Figure 4 illustrates examples of neurons belonging to those different categories.

Neuron 1 of Figure 4 exemplifies the behavior of a mirror neuron belonging to the nine neurons showing a significant but smaller response in the hidden compared to the full vision condition. Rasters and histograms are aligned at the moment in which the experimenter's hand touched the object (blue bars and black lines, respectively). Note the specificity of the neuron's response to the effective observed action (grasping and holding versus placing and holding) in both full vision and hidden conditions. As for all mirror neurons presented in this article, this neuron also responded while the monkey performed an action similar to the one effective when observed (Figure 4E1). When the monkey performed other types of hand actions (data not shown), no response was observed.

Neuron 2 of Figure 4 shows the behavior of one of the two ambivalent mirror neurons. As one can see, the increase in activity during grasping (late movement epoch) may be attributed to a shift of the response during hidden condition from holding to grasping. In full vision, the neuron discharged mostly during holding (Figure 4A2). In hidden condition, the discharge started as soon as the experimenter's hand disappeared from vision, peaked before grasping completion, and ceased during the early holding phase (Figure 4B2). The lack of response when the same action was mimed (Figures 4C2 and 4D2) indicates that the activity in the former trials was related to the monkey's knowledge of the object presence.

Neuron 3 of Figure 4 exemplifies the behavior of a mirror neuron belonging to the remaining 18/37 neurons not responding significantly in the hidden condition.

The characteristics of all neurons of the present study remained constant during the whole testing period. In particular, neurons that did not respond in the hidden condition continued not to respond even when arousing stimuli were delivered between trials to the monkey and despite the fact that a reward was given after every trial (see Experimental Procedures). Furthermore, neurons responding in hidden condition and those responding in full vision condition only were not segregated in different parts of F5. In electrode penetrations in which we were able to study more than one neuron, it occurred that both classes of neurons were represented.

In pilot experiments, eye movements during full vision, hidden, miming in full vision, and hidden miming conditions were measured. Analysis of the data revealed that eye tracking of the experimenter's hand was very rare in all conditions. A typical example of eye position during

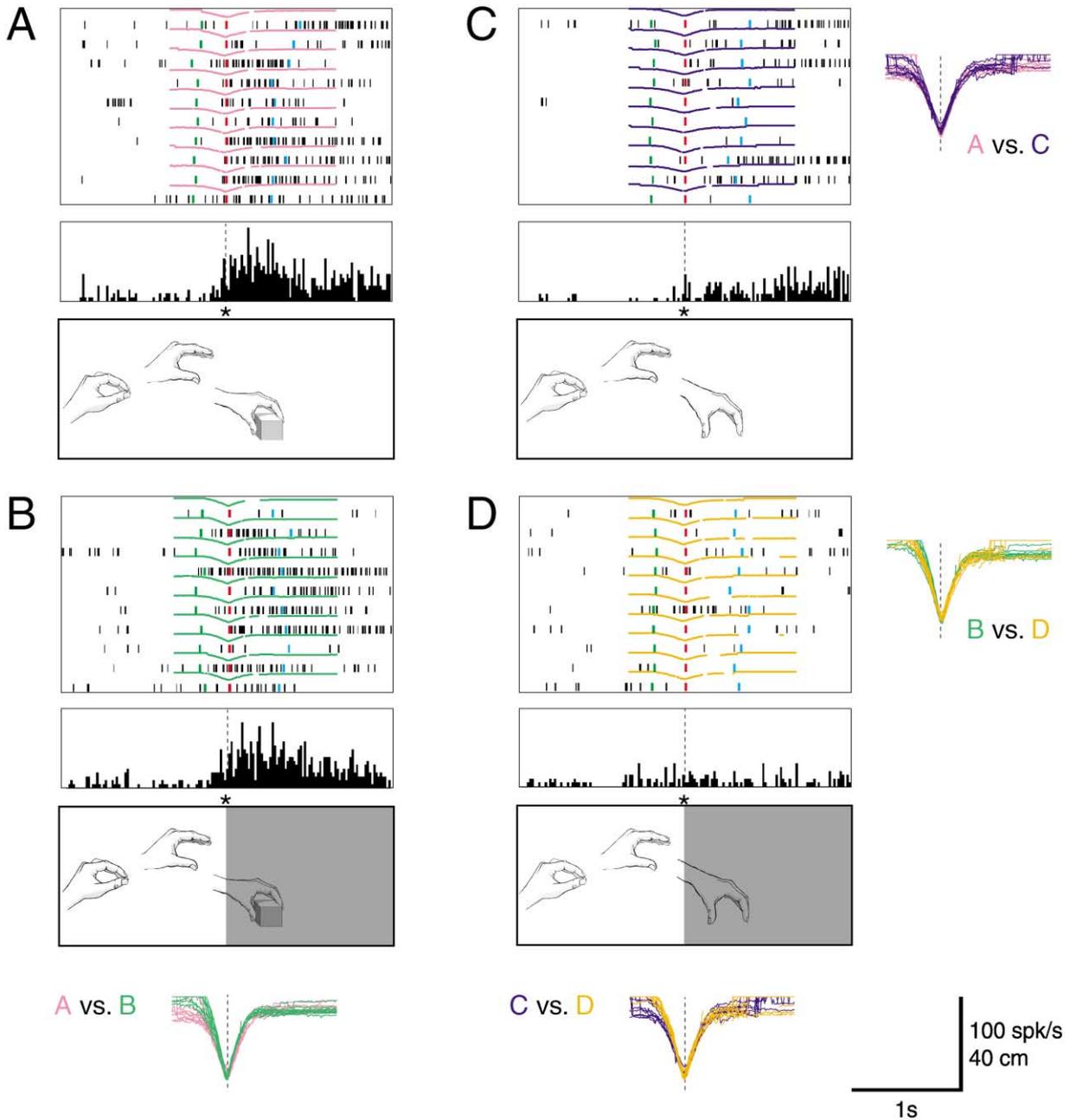


Figure 1. Example of a Neuron Responding to Action Observation in Full Vision and in Hidden Condition but Not in Mimed Conditions

The lower part of each panel illustrates schematically the experimenter's action as observed from the monkey's vantage point: the experimenter's hand started from a fixed position, moved toward an object, and grasped it (A and B) or mimed grasping (C and D). The black frame depicts the metallic frame interposed between the experimenter and the monkey in all conditions. In (B) and (D), the gray square inside the black frame represents the opaque sliding screen that prevented the monkey from seeing the action that the experimenter performed behind it. The asterisk indicates the location of a stationary marker attached to the frame. In hidden conditions, the experimenter's hand started to disappear from the monkey's vision when crossing this marker. The behavioral paradigm consisted of two basic conditions: full vision condition (A) and hidden condition (B). Two further conditions were performed: miming in full vision (C) and hidden miming (D). In these last two conditions, the monkey observed the same movements as in (A) and (B) but without a target object. In each panel above the illustration of the corresponding experimenter's hand movements, raster displays and histograms of ten consecutive trials recorded during these hand movements are shown. Above each raster, a colored line represents the kinematics of the experimenter's hand movements, expressed as the distance between the hand of the experimenter and the stationary marker over time. The colored bars in the rasters represent landmarks of the hand movement of the experimenter in that particular trial: the onset of the hand movement (green bars), the crossing of the stationary marker (red bars), the touching of the object target of the action, or, in trials without objects, the touching of the location at which the objects were otherwise placed (pale blue bars). Rasters and histograms are aligned with the moment at which the experimenter's hand was closest to the fixed marker (minimum in the kinematics, red bars in the rasters, and dashed vertical line in the histograms). To illustrate how similar the kinematics were between conditions, in the bottom line and rightmost column, the hand kinematics shown in the rasters are overlaid and magnified in the vertical dimension while preserving their temporal scale and alignment. Each trace has the same color in the raster panel

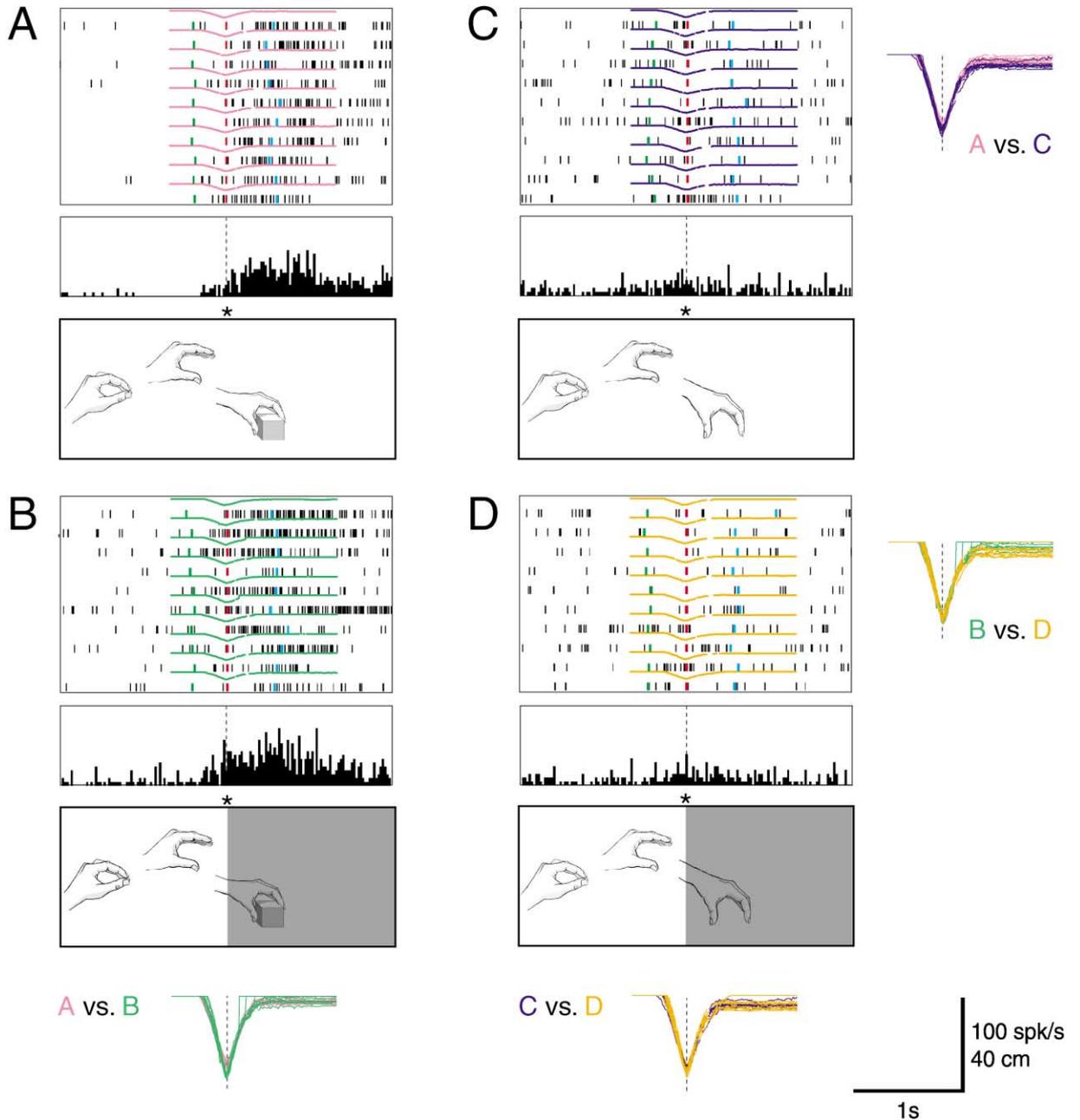


Figure 2. Further Example of a Mirror Neuron that Responded to Action Observation in Full Vision and in Hidden Condition but Not in Mimed Conditions

This neuron responded to the observation of grasping and holding in full vision (A) and in hidden condition (B). (C) and (D) show the discharge of the same neuron in miming in full vision and hidden miming conditions, respectively. All other conventions are as in Figure 1. Note that the hand kinematics saturated in the initial 250 ms but overlapped extremely well thereafter.

the four conditions is shown in Figure 5. The mean distance in degrees (\pm SEM) between the point of gaze and the object/plane location is shown at six times (-500

ms to 0 ms) relative to the moment at which the experimenter's hand touches the object/plane. While, at the moment of touching, there was no significant difference

and in the overlay panel, allowing identification of the corresponding condition. The illustrated neuron responded to the observation of grasping and holding in full vision (A) and in the hidden condition (B) in which the interaction between the experimenter's hand and the object occurred behind the opaque screen. The neuron response was virtually absent in the two conditions in which the observed action was mimed (C and D). See text for statistical results. Histograms bin width = 20 ms. The scale of the abscissa is identical for all elements of the illustration. The ordinate is in spikes/s for histograms and centimeters for the overlaid kinematics.

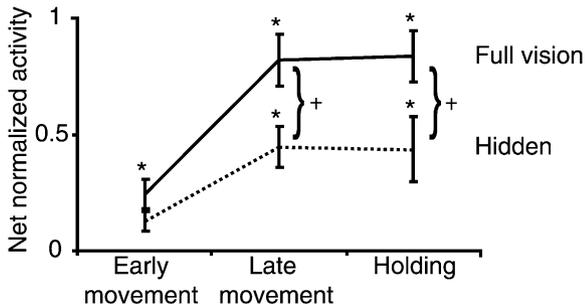


Figure 3. Population Response in Full Vision and Hidden Condition For the early movement, late movement, and holding epochs, the net normalized activity is shown, averaged (\pm SEM) over all 37 tested neurons of the population. This is done separately for the full vision and hidden condition as solid and interrupted lines, respectively. Due to the normalization (see text for details), the net activity is always 0 during the background epoch. An asterisk signifies that the activity of the population during this epoch is significantly higher than that during the background epoch of the same condition. A cross next to a bracket signifies that the activity of the population during that epoch is significantly different between the two conditions. (All $p < 0.01$ according to a Newman-Keuls post-hoc analysis, see text for details.)

between conditions, in full vision, the monkey gazed at the object before the hand touched it. This gazing was not observed in the other three conditions. Because a neural response was present both in the full vision and the hidden condition although gaze was directed at the object only in the full vision condition, an ocular movement toward the object does not appear to be a *conditio sine qua non* for mirror neurons to respond. Given these results and the fact that recording eye movements complicated experiments significantly (see Experimental Procedures), eye movements were not monitored during most physiological experiments.

Discussion

The main finding of the present study is that half of the F5 mirror neurons recorded respond selectively to the observation of specific hand actions, even when the final part of the action, i.e., the part most crucial in triggering the response in full vision, is hidden from the monkey's vision. In order to activate the neurons in the hidden condition, two requirements need to be met: the monkey must "know" that there is an object behind the occluder and must see the hand of the experimenter disappearing behind the occluder. It appears therefore that the mirror neurons responsive in hidden condition are able to generate a motor representation of an observed action, not only when the monkey sees that action, but also when it knows its outcome without seeing its most crucial part (i.e., hand-object interaction).

A possible objection to this interpretation is that the discharge in the hidden condition could be a delayed or prolonged discharge triggered only by the initial (visible) part of the action. This interpretation contrasts with the results obtained in the condition when the observed action was mimed. When the action effective in trig-

gering the neuron was repeated without an object behind the occluder, the response was absent, in spite of the fact that what the monkey saw was exactly the same movement in the two conditions. Furthermore, in many neurons, and specifically in those discharging during the late phase of grasping and during holding, the response was maximal at the end of the hidden action, and, overall in the population, activity was significantly larger when the hand was hidden compared to the early movement epoch when the hand was still visible (see Figure 3). The opposite should be expected if the responses were simply triggered by the initial arm movement.

Similarly, an interpretation of the responses in hidden condition as determined by attention that the monkey pays to the object can be excluded. First, mirror neurons do not respond to the observation of objects alone (Gallese et al., 1996; Rizzolatti et al., 1996). It is therefore implausible that such an ineffective stimulus could drive the neurons' responses when hidden. Furthermore, neurons of the type illustrated in Figure 4 neuron 1 show that having an object disappear behind the occluder in itself is not enough to trigger the neurons—placing the object behind the occluder produced no response.

Also, an interpretation of the response in hidden condition purely in terms of memory of the object hidden behind the occluder is not tenable. The "memory of the object" hypothesis would predict a maintained discharge initiated by the presentation of the object (see Fuster, 1989; Goldman-Rakic, 1987; Murata et al., 1996). The neurons presented in this study did not show this behavior: their response was time locked to the action, not to the presentation of the object. This temporal correlation indicates that all responsive neurons coded the action made by the experimenter, albeit hidden, and not the object.

In principle, the experimenter may have inadvertently revealed the presence of the object behind the occluder by moving his hand in a different way when the object was present compared to when the object was absent. To rule out this possibility, we examined the hand movements recorded during the testing of each neuron. The analysis revealed no systematic kinematic differences linked with the presence of the object. In some neurons, hand movements were extremely similar in all conditions (see Figure 2), while, even for those neurons for which subtle differences between conditions existed, these were not systematically linked to the responses (see Figure 1). Overall, hand movements can therefore not account for the observed effect.

Finally, gazing was not a deciding factor for the observed results. Neural responses were observed in hidden condition, despite the fact that, in this condition, gaze was not directed at the object location. Furthermore, the difference in responses between hidden and hidden miming conditions also cannot be explained by differences in eye position: in both cases, gaze was directed away from the object/plane location in the 500 ms preceding the touching of the object/plane. Some of the intertrial variability observed in responses, however, may be linked to the variability of the eye position within a condition.

The present findings demonstrate that a population of premotor neurons responding to the observation of actions are able to code the presented actions without

full vision of them. Although neurons endowed with such a property were never described before, visually responsive neurons discharging when the effective visual stimuli are not visible were reported before by Assad and Maunsell (1995), Graziano et al. (1997b), and by Perrett and his coworkers (Baker et al., 2001; Jellema and Perrett, 2001).

Assad and Maunsell (1995) recorded from neurons in the posterior parietal cortex (PP) responding to movements of a simple visual stimulus, even when the movement could only be inferred. In all their conditions, a stationary stimulus was first presented for 100 ms. In movement blocks, either the monkey could see the stimulus move toward the center of fixation (full vision trials) or the stimulus disappeared to reappear 500 ms later as if it had moved behind an invisible occluder (occlusion trials). These movement blocks were alternated with blocks of “blink” trials in which the stimulus, after a presentation of 100 ms, disappeared for 500 ms and then reappeared at the location where it had disappeared—as if it never moved. Interestingly, the PP neurons responded during the invisible part of occlusion trials but not during the physically identical part of blink trials. The context of the block therefore enabled PP neurons to “infer” the movement of the stimulus while it was occluded.

Graziano et al. (1997b) studied the response properties of the monkey ventral premotor cortex neurons. In agreement with previous studies (Gentilucci et al., 1983, 1988; Fogassi et al., 1996), they showed that many ventral premotor neurons are “bimodal,” responding to tactile stimuli located on the monkey’s face or arm and to visual stimuli presented in the space around the tactile receptive fields. These neurons, which are predominantly located in the ventral premotor area F4 (Gentilucci et al., 1988), code space in somatocentered coordinates (Fogassi et al., 1996; Graziano et al., 1997a). What is most interesting for the present discussion is the observation made by Graziano et al. (1997b) that a subset of F4 neurons that discharged tonically when an object was introduced within their visual receptive field continued to fire also when the lights were turned off and the monkey could not see the object anymore.

The study of Perrett and his coworkers regarded the functional properties of the neurons of the superior temporal sulcus region (STS) (for review, see Carey et al., 1997). They showed that in the anterior STS there are many neurons that discharge when the monkey observes biological movements. Among them, they described a set that selectively responds when the monkey sees an individual that moves and hides behind an occluding screen (Baker et al., 2001; Jellema and Perrett, 2001). An important feature of these STS neurons is that their response is highly dependent on how the individual hides. Their discharge is maximal when the individual gradually disappears behind a screen, while it is much weaker when he/she abruptly disappears from monkey’s sight by the brisk closure of a shutter. Interestingly enough, behavioral studies show that gradual occlusion is one of the main cues for object permanence in humans (Michotte, 1963; Gibson, 1979).

Taken together, these three studies demonstrate that the perceptual apparatus of the macaque brain is able to represent objects or visual stimuli, while their pres-

ence can only be inferred from past perceptual history, providing a form of knowledge about the presence of an object. What the findings of the present study demonstrate is that this knowledge can be used to go a step further: representing actions of which the inferred object is a target. What mirror neurons code is not the mere presence of an object but rather that a specific action is directed toward an existing object (Gallese et al., 1996; Rizzolatti et al., 1996). Hence, to selectively respond in hidden conditions, mirror neurons have to infer and represent the occluded specific action in addition to the inferred object, which is the goal of the action.

Recently, Filion et al. (1996) demonstrated that monkeys are able to infer the movement of a hidden object. These authors trained monkeys to hit a target either when it reappeared out of an occluding surface or, in a further experiment, when it was still hidden. The results showed that the monkeys were able to hit a target as soon as it reappeared, in spite of the fact that the object trajectory changed from trial to trial. Similarly, they were able to learn to hit the target during the phase in which its trajectory was hidden. These findings are in good agreement with the conclusions drawn from neurophysiological experiments on the neuronal capacity to represent the invisible stimuli and their movement.

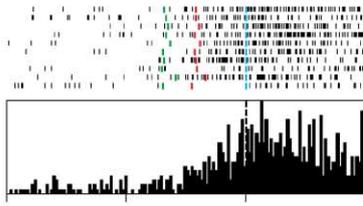
In the present study, 49% of the tested neurons did not respond to hidden action observation. There are two main possible explanations for this finding. One relates to the cognitive state of the animal. To represent the hidden action, the monkey must pay attention to the behavior of the experimenter, remember the presence of the object behind the occluder, and, most importantly, reconstruct the “missing” part of the action. It is possible therefore that some of the neurons did not respond because during testing the monkey did not make this cognitive effort.

An alternative possibility is that the neurons that did not respond in hidden condition lacked input from areas enabling mirror neurons to internally generate the action in this condition. What makes this second hypothesis more likely is the fact that, typically, when a neuron responded to the hidden action, despite minor intertrial fluctuations of its firing rate, it continued to respond throughout the period of testing. Conversely, the neurons that responded in full vision and did not show any response to hidden actions did so consistently. If the first hypothesis were true, one should expect major and consistent oscillations in the response according to the cognitive state of the monkey.

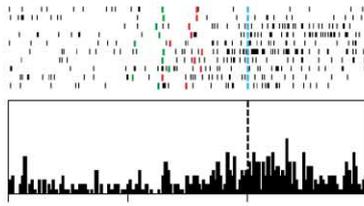
In conclusion, the results of the present study show that a population of mirror neurons is able to represent actions also when crucial parts of these actions are hidden and can only be inferred. This indicates that, even when visual cues are limited, the activation of mirror neurons can place the observer in the same internal state as when actively executing the same action. This would enable the observer to recognize the hidden action. The present findings further corroborate the previously suggested hypothesis that the mirror neurons’ matching mechanism could underpin action understanding (Gallese et al., 1996; Rizzolatti et al., 1996, 2000).

Neuron 1

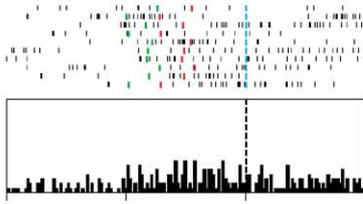
A1



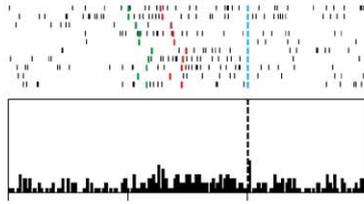
B1



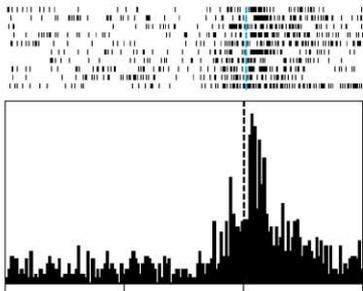
C1



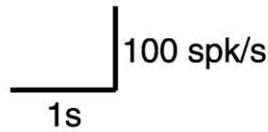
D1



E1

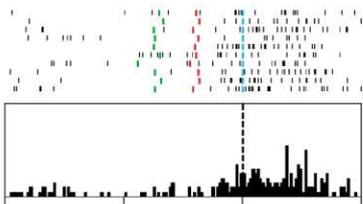


- Hand movement onset
- Crossing of the stationary marker
- Touching object/plane

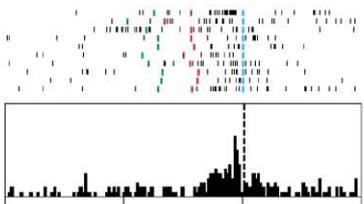


Neuron 2

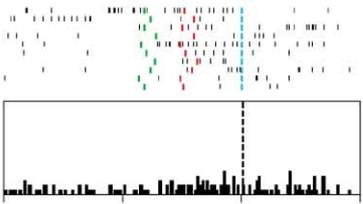
A2



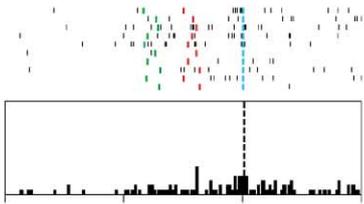
B2



C2

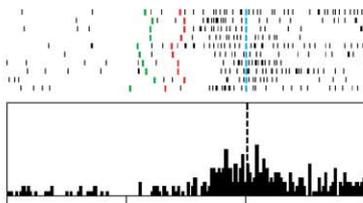


D2

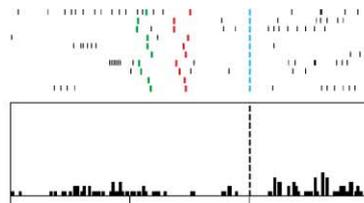


Neuron 3

A3



B3



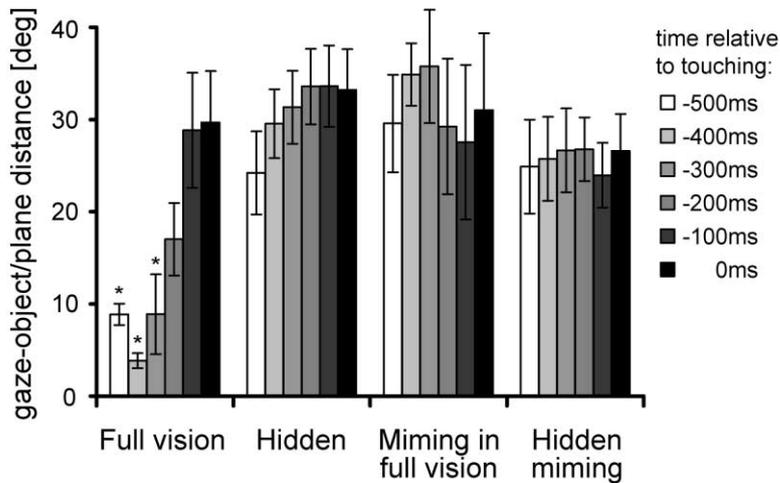


Figure 5. Eye Position during the Observation of Hand Actions in All Four Conditions of the Pilot Experiment

The mean distance in degrees (\pm SEM) between the point of gaze and the object/plane location is shown at six times (-500 ms to 0 ms) relative to the moment at which the experimenter's hand touches the object/plane. While at the moment of touching, no significant difference exists between conditions; in the 500 ms to 300 ms preceding touching, only in the full vision condition, the monkey was gazing at the object location. At each of the six points in time, a four-condition ANOVA with ten trials per condition was performed. The results indicate a significant main effect of condition at -500 ms [$F(3,36) = 4, p < 0.02$], -400 ms [$F(3,36) = 16, p < 0.01$], and -300 ms [$F(3,36) = 6, p < 0.01$]; a trend at -200 ms [$F(3,36) = 2, p = 0.13$]; and no main effect at -100 ms [$F(3,36) = 0.45, p > 0.7$] or at the moment of touching [$F(3,36) = 0.23, p > 0.8$]. Asterisks indicate significant differences ($p < 0.05$).

Experimental Procedures

Basic Procedures

The experiments were carried out on two macaque monkeys (*Macaca nemestrina*). In both monkeys, neuron activity was recorded from one hemisphere. 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.

Before starting the neurophysiological experiments, the monkeys were habituated to the experimenters. They were seated in a primate chair and trained to receive food from the experimenters. Monkeys received pieces of food of different size, located in different spatial locations. This pretraining was important for subsequent testing of the neuron's motor properties (see below) and for teaching the animal to pay attention to the experimenters.

The surgical procedures for neuron recordings were the same as previously described (see Gentilucci et al., 1988; Rizzolatti et al., 1990). The head implant included a head holder and a chamber for single-unit recordings. Neurons were recorded using tungsten microelectrodes (impedance 0.5 – 1.5 M Ω , measured at 1 kHz) inserted through the dura, which was left intact. Neuronal activity 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 voltage-time discriminator was monitored and fed to a PC for analysis.

The recording microelectrodes were also used for electrical microstimulation (train duration, 50 ms; pulse duration, 0.2 ms; frequency, 330 Hz; current intensity, 3 – 40 μ A). The current strength

was controlled on an oscilloscope by measuring the voltage drop across a 10 K Ω resistor in series with the stimulating electrode.

Recording Sites

The chamber for single-unit recordings was implanted stereotactically. The chamber rostrocaudal and mediolateral axis dimension (20 mm \times 15 mm) was such as to allow to record from the whole ventral premotor cortex from area F1 (primary motor cortex) to the caudal part of the frontal eye fields (FEF) included. The stereotactic parameters were chosen on the basis of our previous single-neuron recording experience in the ventral premotor cortex (see Gentilucci et al., 1988; Rizzolatti et al., 1988; Fogassi et al., 1996; Gallese et al., 1996). In one monkey, the location of the arcuate sulcus was assessed before chamber implantation, using magnetic resonance (MR) images.

After chamber implantation, the ventral part of the agranular frontal cortex was functionally explored (single neuron recordings and intracortical microstimulation) in order to assess the location of areas F1 (primary motor cortex), F4, and F5 (ventral premotor cortex) and to find out the sector of F5 where mirror neurons are mostly located.

The criteria used to characterize functionally the different areas were the following. Area F1: low threshold of excitability to microstimulation (typically few μ As when stimulating deep layers), vigorous discharge during active movements, response to passive somatosensory stimuli virtually from all recorded sites. Area F4: moving the electrode rostrally from F1 hand field, appearance of proximal and axial movements to electrical stimulation, increase in stimulation threshold, appearance of visual responses, presence of large tactile receptive fields located on the face and body and of visual

Figure 4. Examples of Mirror Neurons, Showing Different Types of Responses

The responses of three mirror neurons are shown to illustrate three types of responses. All rasters and histograms are aligned on the moment in which the hand of the experimenter (all except [E1]) or the monkey (E1) touched the object or, in conditions without objects, the empty plate where the object is otherwise placed. All histograms share the same scale. Neuron 1 showed a strong response to grasping and holding observation in full vision (A1). The response, although present, was markedly reduced in hidden condition (B1). In (C1) and (D1), the experimenter placed an object on a plane and held it there for 1 s, in full vision (C1) and in hidden condition (D1). Note the specificity of the neuron response manifested by the very low intensity of the discharge during placing and holding observation in both conditions. (E1) shows the response of the neuron during the monkey's active grasping of a small piece of food, with the hand contralateral to the recorded hemisphere. Neuron 2 responded to grasping and holding observation in full vision (A2) and in hidden condition (B2). In full vision (A2), the neuron started to discharge during the late grasping phase and increased its firing during holding. In hidden condition (B2), the neuron started to respond as soon as the experimenter's hand disappeared from the monkey's sight, reached the discharge peak intensity at the end of grasping, and decreased during holding. (C2) and (D2) show the response of the same neuron in the two mimed conditions. Note the almost complete absence of a response in these conditions. Neuron 3 showed a strong response during the observation of the experimenter's grasping and holding actions (A3). The same actions, when hidden, did not evoke any response (B3).

peripersonal RFs around the tactile ones. Area F5: going further rostrally, reappearance of distal movements requiring higher stimulation currents than F1, visual responses present either in response to the presentation of 3D objects or to observation of complex actions, presence of a large number of neurons discharging in association with goal-directed hand movements. The validity of identification of area F5 on the basis of its functional properties has been histologically confirmed in several previous experiments (see Rizzolatti et al., 1988, 1996; Gallese et al., 1996; Fogassi et al., 2001).

Neuron Selection

Each neuron, once isolated, was first tested "clinically" in order to ascertain its motor and visual properties. In brief, the monkey was presented with a variety of objects of different size and shape. They consisted of food items and objects at hand in the laboratory. The objects were presented within and outside the reaching distance of the monkey. The monkey was trained to fixate them and, when at reaching distance, to grasp them (for details, see Rizzolatti et al., 1988, 1990).

Mirror properties were tested by performing a series of hand actions in front of the monkey. These actions were related to object grasping (presenting the object to the monkey, putting it on a surface, grasping it, giving it to a second experimenter, or taking it away from her/him), object manipulation (breaking, tearing, folding), or were intransitive (i.e., non-object related) actions. These were with or without "emotional" content (e.g., threatening gestures or lifting the arms, waving the hand, respectively, see Gallese et al. 1996).

In order to verify whether the recorded neuron coded specifically hand-object interactions, the following actions were also performed: movements of the hand miming grasping in the absence of the object and prehension movements of food or other objects performed with tools (e.g., forceps and pincers).

Only neurons that had mirror properties related to hand actions (motor responses during monkey's actions and responses to similar actions performed by the experimenter) and that presented stable responses were selected for further testing.

Behavioral Paradigm

Once a neuron was classified as a mirror neuron and the effective observed action(s) determining its discharge assessed, it was studied as follows. A metallic frame (dimensions: 86 × 66 cm) was interposed between the experimenter and the monkey. This frame was at about 2 meters of distance from the monkey. To this frame, a plane was attached on which objects could be placed. The objects were the targets of the experimenter's actions. A sliding opaque screen (dimensions: 43 × 66 cm) was mounted on the frame and could be moved so as to enable or prevent the monkey from seeing the experimenter's action performed behind it.

The behavioral paradigm consisted of two basic experimental conditions: full vision condition and hidden condition. In both conditions, the monkey was presented with a series of actions made by the experimenter behind the frame. The experimenter's hand always moved parallel to the frame. The experimenter's hand started from a fixed position visible to the monkey within the frame. Before movement onset, the hand was kept in pinch position (without object) for touching and grasping actions and with object in the case of placing. The final position of the hand movement on the plane was constant. Thus, the hand trajectories from start to the endpoint were always approximately the same (see below).

In full vision condition (see Figure 1A), all phases of the experimenter's action from its onset to its completion were visible to the monkey. The actions were touching, grasping, manipulating, holding, releasing, or placing the object on the plane. In hidden condition (see Figure 1B), the opaque screen was interposed so as to prevent the monkey from seeing the hand-object interaction. Before the beginning of each trial, the opaque screen was briefly slid back to show the monkey the presence of the target object. The screen was then slid on again, and the hand of the experimenter, starting from a position from which it was still visible to the monkey, moved behind the opaque screen to interact with the object.

Two further conditions were also employed: miming in full vision (see Figure 1C) and hidden miming (see Figure 1D). In these further

conditions, the experimenter's actions were performed as in the basic conditions, but there was no object. Note that from the monkey's vantage point, in the hidden conditions there was no difference between object-directed and mimed action. All conditions consisted of ten consecutive trials. In all conditions, reward (a piece of food) was given to the monkey at the end of each trial.

In order to correlate neurons' discharge with the different phases of the experimenter's hand action, hand movements were recorded by means of a computerized motion analysis system (ELITE system, BTS srl, Milano, Italy) that utilizes infrared light-reflecting passive markers. Two markers were placed on the distal phalanges of the thumb and index finger of the experimenter's hand. A third stationary marker (indicated by the asterisk in Figures 1 and 2) was placed on the metallic frame in correspondence with the edge of the opaque screen, marking the position in which the experimenter's hand started to disappear to the monkey's sight in the hidden conditions. The 3D position of the three markers was continuously calculated by means of a specially designed computer program elaborating the markers' bidimensional data acquired by two infrared-sensitive cameras. After online calibration and photogrammetric procedures, the distances between the experimenter's index finger and thumb and those between the two fingers and the fixed marker were calculated every 10 ms and fed to a computer together with the neuron's discharge.

In order to measure the neuron activity during the various phases of the experimenter's hand actions, in each trial, the following events were detected and marked: (1) onset of the hand movement; (2) crossing of the stationary frame marker by the experimenter's hand; and (3) hand-object or hand-plane (in mimed actions) contact. Events (1) and (2) were computed by means of kinematic analysis with the ELITE system (for details, see Fogassi et al., 1996). Event (3) was computed by means of a contact-detecting device whose signals were fed to a PC. Rasters and histograms could be aligned with each of the three different events for further analysis.

Analysis of Neuronal Responses for Single Neurons

All physiological data reported in this paper were acquired from trials of the behavioral task described above. The neurons' activity during the behavioral task was analyzed by subdividing the neural discharge during each trial in the following epochs. (1) Background, time before the onset of the experimenter's hand movement (indicated by green bars in rasters). This activity is taken to reflect the spontaneous activity of the neuron. (2) Early movement, period from movement onset to crossing of the stationary marker (indicated by red bars in rasters). (3) Late movement, period from crossing of the stationary marker to object or plane contact (indicated by pale blue bars in rasters). (4) Holding, 500 ms following object or plane contact.

For each neuron, the mean firing rate (spikes/s) was calculated for each epoch and compared with the activity in other conditions and epochs using multiway between-subject ANOVA followed by Newman-Keuls post-hoc analyses as described in the text. All analyses were performed with $p < 0.01$ as significance criterion.

Population Analysis

There were substantial differences in the firing rates of different neurons, both in terms of spontaneous and peak firing rate activity. To compare the responses in different epochs and conditions over the entire population of neurons, a normalization procedure had to be used.

First, to account for differences in spontaneous activity, firing rates were transformed into net firing rates by subtraction of the firing rate observed during the background epoch of the same trial. For each neuron, condition, and epoch, separately, this net firing rate was then averaged over all available trials to obtain a single number.

Second, to account for differences in peak firing rate between neurons, these average net firing rates were divided by the highest net average firing rate observed in the full vision condition for that neuron. Three of the 37 neurons were inhibited during the observation of the action and showed negative net firing rates. The activity of these three neurons was therefore normalized by dividing the net firing rates of all epoch with the minimum (negative) net firing rate in the full vision condition to rectify the response of the neuron for

the population analysis, as if it had been excited by the observation of the action.

The resulting average normalized net firing rates can be interpreted as proportions of the response in full vision, with 0 standing for background activity and 1 for a response identical to the peak response in full vision. Results are shown in Figure 3. The effect of condition and epoch on the population was then assessed using one entry for each neuron, condition, and epoch, using a two condition (full vision versus hidden) \times four epochs (background, early movement, late movement, and holding) within-subject ANOVA followed by a Newman-Keuls post-hoc comparison, all at $p < 0.01$ (note that this is the only within-subject analysis of the paper, since, in this case, different neurons can be treated as different "subjects").

Analysis of Eye Movements

In pilot experiments, eye movements during full vision, hidden, miming in full vision, and hidden miming conditions were measured using an infrared oculometer with a resolution of 1–5 min arc (Dr Bouis, Germany; see Bach et al. 1983 for further details). Because this oculometer needs to be placed directly next to the eye, a monkey free to move his hands disrupts the system. Due to the necessity to test motor properties of neurons during physiological experiments and, therefore, to leave the monkey's hands free to move, we did not record eye movements any further during physiological recordings.

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