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Neurons related to reaching-grasping arm movements in the rostral part of area 6 (area 6aβ)

G. Rizzolatti, M. Gentilucci, R.M. Camarda, V. Gallese, G. Luppino, M. Matelli, and L. Fogassi

Istituto di Fisiologia Umana, Universita'di Parma, Via Gramsci 14, I-43100 Parma, Italy

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Summary. Single neurons were recorded from the rostral part of the agranular frontal cortex (area 6aβ) in awake, partially restrained macaque monkeys. In the medialmost and mesial sectors of this area, rostral to the supplementary motor area, neurons were found which were activated during arm reaching-grasping movements. These neurons ("reaching-grasping neurons") did not appear to be influenced by how the objects were grasped nor, with some exceptions, by where they were located. Their activity changed largely prior to the arm movement and continued until the end of it. The premovement modulation (excitatory or inhibitory) could start with stimulus presentation, with the saccade triggered by the stimulus or after stimulus fixation. The distance of the stimulus from the monkey was an important variable for activating many neurons. About half of the recorded neurons showed a modulation of the same sign during movement and premovement period. The other half showed an increase/decrease in activity which was of the opposite sign during movement and premovement period or part of it. In this last case the discharge changes were of the same sign when the stimulus was close to the monkey and when the monkey moved its arm to reach the objects, whereas they were of opposite sign when the stimulus was outside the animal's reach. Microstimulation of area 6aβ and the reconstruction of the locations of eye movement and arm movement related cells showed that the arm field was located more medially (and mesially) than the eye field described by Schlag and Schlag-Rey (1987). It is suggested that, unlike inferior area 6, which is mostly involved in selection of effectors on the basis of the physical properties of the objects and their spatial location (Rizzolatti and Gentilucci 1988), area 6aβ plays a role in the preparation of reaching-grasping arm movements and in their release when the appropriate conditions are set.

Offprint requests to: G. Rizzolatti (address see above)

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Introduction

The classical division of the agranular frontal cortex of primates into two large movement representations, the "primary motor cortex" and the "supplementary motor area" (see Woolsey et al. 1952) has been seriously challenged in the last decade. A convergent evidence from ablation experiments (Moll and Kuypers 1977; Halsband and Passingham 1982; Petrides 1982; Rizzolatti et al. 1983), single neuron recordings (Godschalk et al. 1981; Rizzolatti et al. 1981a, b, c; Weinrich and Wise 1982; see also Wise 1985) as well as from studies of cortico-cortical connections (Matsumura and Kubota 1979; Muakkassa and Strick 1979; Matelli et al. 1986) demonstrated that the rostral part of the agranular frontal cortex represents a complex, independent region involved in motor control. In addition, evidence has been provided that within the agranular frontal cortex there are several independent representations of body movements. As far as the arm is concerned there are at least three independent representations of distal movements and four of proximal movements. Distal movements are represented in area 4, in inferior area 6 (Rizzolatti et al. 1981b; 1988; Kurata and Tanji 1986) and in the supplementary motor area (Brinkman and Porter 1979; Tanji 1984). Proximal movements are found in the same areas plus superior area 6 (see Wise 1985).

There may be a further representation of the arm in the rostralmost part of the agranular frontal cortex (area 6aβ of Vogt and Vogt 1919). Although this sector of the agranular frontal region does not seem to have direct

access to the spinal cord (see Kuypers 1981), electrical stimulation with surface electrodes evokes arm reaching movements in addition to ocular saccades and head movements (see literature in Humphrey 1979). Whilst the oculomotor function of this area (or of a part of it) has been confirmed with single neuron recordings (Schlag and Schlag-Rey 1987; Mann et al. 1988), there is only limited evidence for an arm representation. Some neurons were found to discharge during arm movements in an experiment whose principal aim was to study the properties of oculomotor neurons (Mann et al. 1988).

How are these multiple arm representations in the agranular frontal cortex involved in arm movement control? In a previous series of experiments (Gentilucci et al. 1988; Rizzolatti et al. 1988) we addressed this question by studying the functional properties of neurons located in inferior area 6. We concluded that inferior area 6 contains a vocabulary of motor acts which are coded at a single neuron level. We proposed that there are other movement vocabularies at different levels of complexity located in other cortical representations of arm movements.

The observation that in the rostralmost part of area 6 (6aβ) there are neurons apparently related to arm movements (Mann et al. 1988) prompted us to study this area in a behavioral situation identical to that employed in our previous studies of inferior area 6. Our aim was to find out which aspects of arm movements were coded in this area. Our attention was soon attracted by a particular class of neurons whose activity was clearly related to reaching-grasping movements. The main purpose of this report is to describe this new type of cortical neurons. Additional, preliminary information will be given on the general organization of the mesial and medialmost part of area 6aβ. Although it is still incomplete, this information is necessary to describe precisely the anatomical location of the neurons whose characteristics are described in this paper.

Methods

Experimental situation

The experiments were carried out on two macaque monkeys (Macaca nemestrina) selected for their docility. The monkeys were seated in a primate chair and trained to respond to visual objects. Three types of objects were used: "graspable" objects, "non-graspable" objects and "unpleasant" objects. "Graspable" objects consisted of food of different size (raisins, sunflower seeds, slices of orange, pieces of apple). "Non-graspable" objects were geometric solids, objects at hand in the laboratory and a syringe filled with orange juice (see below). "Unpleasant" stimuli consisted of laboratory gloves and of a pair of forceps which were opened and closed in front of the monkey. All stimuli were presented in various space positions within the reaching distance of the monkey (peripersonal space) or outside this distance (extrapersonal space). The objects were presented by hand and, except during initial qualitative testing of the neurons, with the animal still and its arm placed on an armrest attached to the primate chair.

The monkey's behavior became very stable after several weeks of training. In the case of "graspable" objects, the monkey oriented towards the stimulus, fixated it and, if it was within its reaching distance, grasped it. In the case of "non-graspable" stimuli the monkey oriented towards them, but did not attempt to make reaching-grasping movements. The most used "non-graspable" object was a syringe filled with orange juice. This stimulus is motivationally very similar to "graspable" objects. The monkey was conditioned to fixate the syringe, but not to grasp it. If the monkey kept its arm still, the syringe was moved towards its mouth and the juice given directly to the mouth. If the animal moved its arms, the syringe was withdrawn. In the case of "unpleasant" objects, the monkey fixated them and frequently tried to push them away.

The way in which reaching and grasping arm movements were tested has been described in detail elsewhere (see Gentilucci et al. 1988; Rizzolatti et al. 1988). Briefly, stimuli of different size were presented first centrally and then peripherally in each of the four visual quadrants. Stimuli of different size evoked different types of prehension (precision grip, finger prehension, whole hand prehension). Stimuli located in different parts of the visual space evoked different proximal movements. By testing a large variety of movements and discarding the components that were ineffective, it was possible to decide which of proximal or distal movements was effective in triggering a given neuron. For discussion of this testing method see Rizzolatti et al. (1988).

Data recording

When the monkey responded stably to the various stimuli, it was prepared for the experiment. Under Ketamine anesthesia (15 mg/Kg/i.m. supplemented every 30 min.) and using methods previously described (Gentilucci et al. 1988) a chamber was implanted for single-unit recordings and microstimulation. Single neurons were recorded using tungsten microelectrodes (impedance 0.5–1.5 MΩ, measured at 1 KHz frequency) inserted through the dura which was left intact. Neuronal activity was amplified and monitored on an oscilloscope. Individual action potentials were isolated with a voltage discriminator. The output signal from the voltage discriminator was monitored and fed to a PDP 11/23 computer for analysis.

The recording microelectrodes were also used for electical intracortical microstimulation. The cortex was stimulated every 500 μm by a train of cathodal pulses generated by a constant current stimulator. Train duration = 50 ms, pulse duration = 0.2 ms, frequency = 330 Hz, current intensity 3 to 40 μA. Occasionally, train durations of 100 ms were used when the standard microstimulation was not effective. The current strength was controlled on an oscilloscope by measuring the voltage drop across a 10 KΩ resistor in series with the stimulating electrode.

Eye movements were recorded using the magnetic search coil technique (Robinson 1963; for the implanting technique see Judge et al. 1980). Eye position was calibrated at the beginning of each recording session, using five standard positions: 0°, 20° right and left on the horizontal meridian, 20° up and down on the vertical meridian. Eye position was monitored on an X-Y oscilloscope and fed to the PDP 11/23 computer that analyzed neuronal activity. The eye movement sampling frequency was 200 Hz. Muscle activity was recorded bilaterally from neck muscles by using teflon isolated stainless steel wire electrodes. The EMG records were displayed on a polygraph along with integrated single neuron activity (time constant 10 ms).

Arm movements were recorded in three dimensional coordinates using the ELITE system (Ferrigno and Pedotti 1985). Two 50 Hz TV cameras monitored the position of a small reflective marker attached to the monkey's wrist. The ELITE processor for shape recognition computed the x, y, z coordinates of the marker. The signal from the ELITE was fed to a PDP 11/53 computer for 3D reconstruction and filtering. The movements were recorded in sessions in which single neurons were not recorded, but the testing procedure was identical to that used during the single neuron experiments. The reaching-grasping movements of the monkey whose data are presented in the figures have a duration of 426 ± 75 ms for a middle size stimulus (2 $\times$ 2 cm) located at a distance of 15 cm and 554 ± 39 ms for small

stimuli (e.g. a raisin), presented at the same distance. These times remained fairly constant during the experiments.

Each neuron was first tested informally by presenting each of the different types of stimuli described above in various space position. The animal's behavior during this testing was recorded on one track of a videotape. The neuronal activity during testing was recorded simultaneously on a second track. When the general properties of a neuron were sufficiently clear, response histograms were constructed by presenting repetitively "graspable" and "non-graspable" stimuli in the most appropriate locations. During quantitative testing, food was presented on a metallic carrier connected to a contact detecting circuit. Whenever the animal touched the food, a signal was sent to the PDP 11/23 computer. This signal allowed the alignment of the histograms with the moment in which the animal grasped the food.

Many factors influenced the discharge of neurons, whilst their survival time was obviously limited, hence the following strategy was adopted. The factors which during informal testing did not appear to influence the neuron's discharge were not studied further in quantitative detail. The factors that seemed to have an influence on neuronal discharge were analyzed in a formal way. As a consequence the properties of the reaching-grasping neurons described in a) and b) (see Results) are derived from the experimental protocols and videotape while their temporal properties (c) are derived from formal testing. Although we are aware that it would be methodologically more elegant to assess all of the response properties quantitatively, we preferred to have a complete or near complete description of the functional characteristics of each neuron, rather than to have a perfect description of a fragment of its behavior without any knowledge of its global behavior.

Histological identification

About 1 week before sacrifying the animal, a series of electrolytic lesions (10 μA cathodal current for 10 s) equally spaced one from another were made at the border of the investigated area. After the last experiment the animal was anesthetized with ketamine (15 mg/Kg i.m. supplemented every 30 min.), the dura was removed and the stereotaxic coordinates of the arcuate and central sulci were assessed. After an additional dose of sodium thiopental (30–40 mg, i.v.) the animal was perfused through the left ventricle with warm buffered saline followed by fixative and the brain was removed. The brain was frozen and cut (each section 60 μm). Alternate sections were stained with the Nissl method and reacted for cytochrome oxidase histochemistry (for details see Matelli et al. 1985). The locations of the penetrations were reconstructed and related to the cytoarchitectonic and histochemical divisions of the agranular frontal cortex.

Results

Types of recorded neurons

The functional properties of 177 neurons located in area 6aβ were assessed in two monkeys. 54 of these neurons were clearly related to eye movements. These cells fired in association with ocular saccades, fixation or in association with both saccades and fixation. 35 neurons became active during orienting involving neck and trunk movements or axial movements plus eye movements. 15 neurons responded to visual stimuli and appeared not to be related to eye or body movements. 3 neurons were related to mouth movements. Finally 70 neurons were activated in association with arm movements. 44 of these arm-related cells formed the group of neurons that will be described in this paper. They will be referred to as arm reaching-grasping neurons.

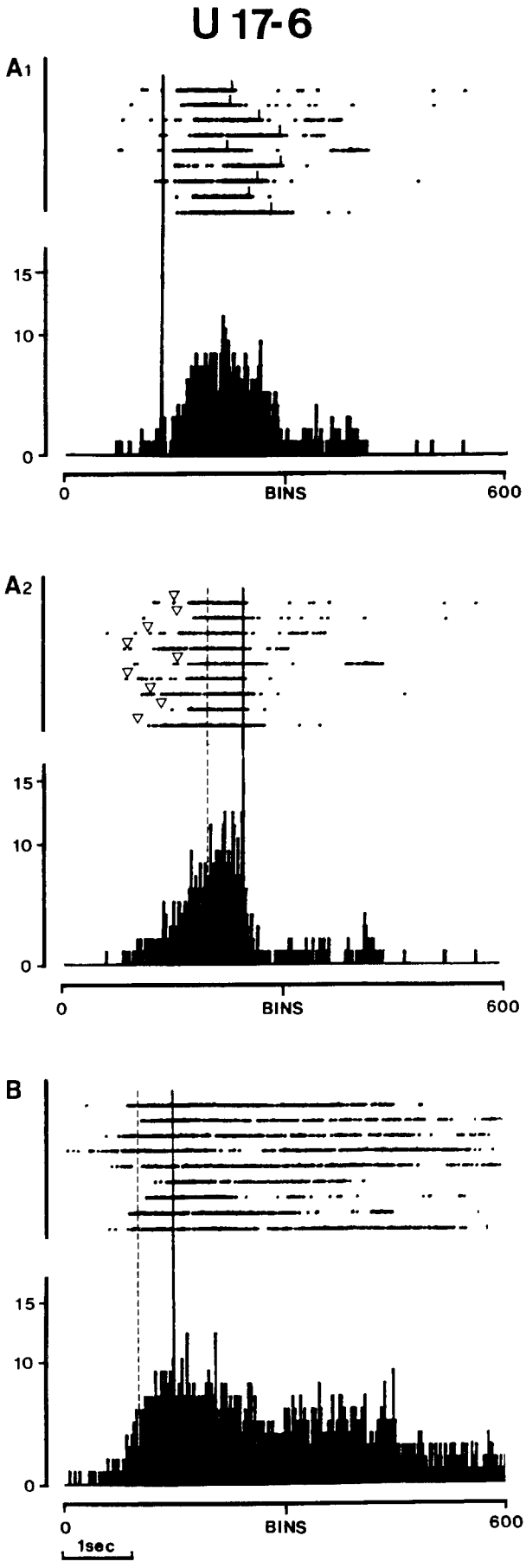
Reaching-grasping neurons

All reaching-grasping neurons shared common functional characteristics that justify their inclusion in a single class. a) The firing of these neurons correlated with arm movements performed in order to reach and grasp objects. In contrast, no discharge modifications were observed when the upper limb was moved during postural adjustments or during motor acts not related to reaching-grasping movements (e.g. pushing away, scratching). b) Reaching-grasping neurons did not appear to be influenced by how the object was grasped or, with some exceptions, where it was located. The type of grip and, with few exceptions (6 neurons out of 44), the direction of proximal movements did not influence the strength of the response. c) The neurons typically changed their firing rate before the beginning of the arm movement. This firing modulation could be associated with stimulus presentation, with the saccade triggered by the stimulus, or it could occur after stimulus fixation.

Reaching-grasping neurons fell into two major categories; twenty of the forty-four neurons showed a discharge modulation of the same sign during the movement and before it. These neurons will be referred to as reaching-grasping neurons of first category. Twenty-four neurons were modulated during movement in a way which was totally or partially not congruent with that during the premovement period. They will be referred to as reaching-grasping neurons of second category.

Figure 1 shows an example of a neuron of first category. This neuron increased its discharge during movement and premovement period. The discharge increase started after stimulus fixation. The stimulus (a piece of food) was presented to the animal within its arm reaching distance (peripersonal space). The stimulus evoked a saccadic eye movement and, subsequently, an arm reaching-grasping movement directed towards the stimulus. In A1 the responses are aligned with respect to the onset of the saccade elicited by the stimulus. In A2 the same responses are aligned with the moment when the monkey touched the stimulus. The neuron began to discharge about 150 ms after foveation (see histogram of A1) and remained active until the food was grasped. In all trials the discharge preceded considerably the beginning of the movement (interrupted line, A2), although the interval from the increase in firing rate to the movement varied.

Figure 1B illustrates that visual stimulation and visual control of arm movements were not necessary in order to activate reaching-grasping neurons. In Fig. 1B trials are shown in which the vision of the arm and food was prevented by a plastic plane attached to the primate chair. The animal knew from previous training that although not visible, the food was reachable. It is clear that the neuron discharged also during movements not triggered by visual stimuli. When the food was not directly visible, however, the premovement discharge was shorter and linked more closely to the movement initiation than when the food



could be seen (compare histograms B and A2). The activity on the right of the alignment line in B is due to the way the task was executed (see caption of the figure).

Figure 2 shows another example of a neuron of first category. Unlike the unit in Fig. 1, this neuron was inhibited during movement and premovement period. The inhibition was synchronous with the onset of the eye movement directed towards the stimulus. Stimulation procedures were the same as in Fig. 1. In A1 the histogram is aligned with the saccade elicited by stimulus presentation whereas in A2 the histogram is aligned with the moment in which the monkey touched the food.

The neuron reduced its firing rate at the same time as the onset of the saccade elicited by the stimulus presentation (A1). The inhibition reached its peak during reaching-grasping movement and continued, although markedly attenuated, after the movement. The neuron resumed its steady firing rate when the food carrier (goal for reaching-grasping movement) was withdrawn from the animal peripersonal space. This change is shown in A3 in which the responses are aligned with the saccade elicited by the withdrawing of the food carrier. This tonic effect of visual stimuli that evoke reaching-grasping movements was observed also, in various degrees, in the other neurons whose discharge modulation was triggered by eye movements.

An example of a neuron of second category is shown in Fig. 3. This neuron was excited during arm movements, but was inhibited during the initial part of the premovement period. The object (a piece of food) was presented outside the animal's reaching distance and then slowly moved towards it. In A1 the records are aligned with the saccade elicited by the stimulus, in A2 they are aligned with the moment in which the animal touched the stimulus and in A3 with the saccade following the withdrawal of the food carrier.

One can see that the neuron ceased firing when the animal fixated the object, the decrease in firing rate starting with the eye movement onset (A1). As the stimulus approached the animal the neuron became active reaching

Fig. 1A, B. Reaching-grasping neuron showing increased firing rate during premovement and movement period. **A1, A2** Reaching-grasping movements in response to a visual object; **B** same movements directed towards a hidden object. In **A1** the neuron's discharge is aligned (heavy vertical line) with the onset of the saccade elicited by the stimulus; in **A2** it is aligned with the time when the monkey touched the stimulus. Details of the stimulus presentation are described in the text. Each histogram is the sum of 9 trials. Bin width 10 ms. Individual trials are shown above the histograms. The vertical mark on the individual trial line (**A1**) indicates the time when the monkey touched the object. Inverted triangles (**A2**) indicate the onset of the saccade triggered by stimulus presentation. Interrupted line marks the estimated beginning of the movement, based on the average movement time in the test situation (426 ± 75 msec, see Methods). The activity to the right of the alignment bar in **B** is due to the fact that, without visual control, the monkey touched first the container in which the food was located and then continued the search for food with a series of reaching-grasping movements. The signal for histogram construction corresponds to the contact of the hand with the container (first reaching-grasping movement)

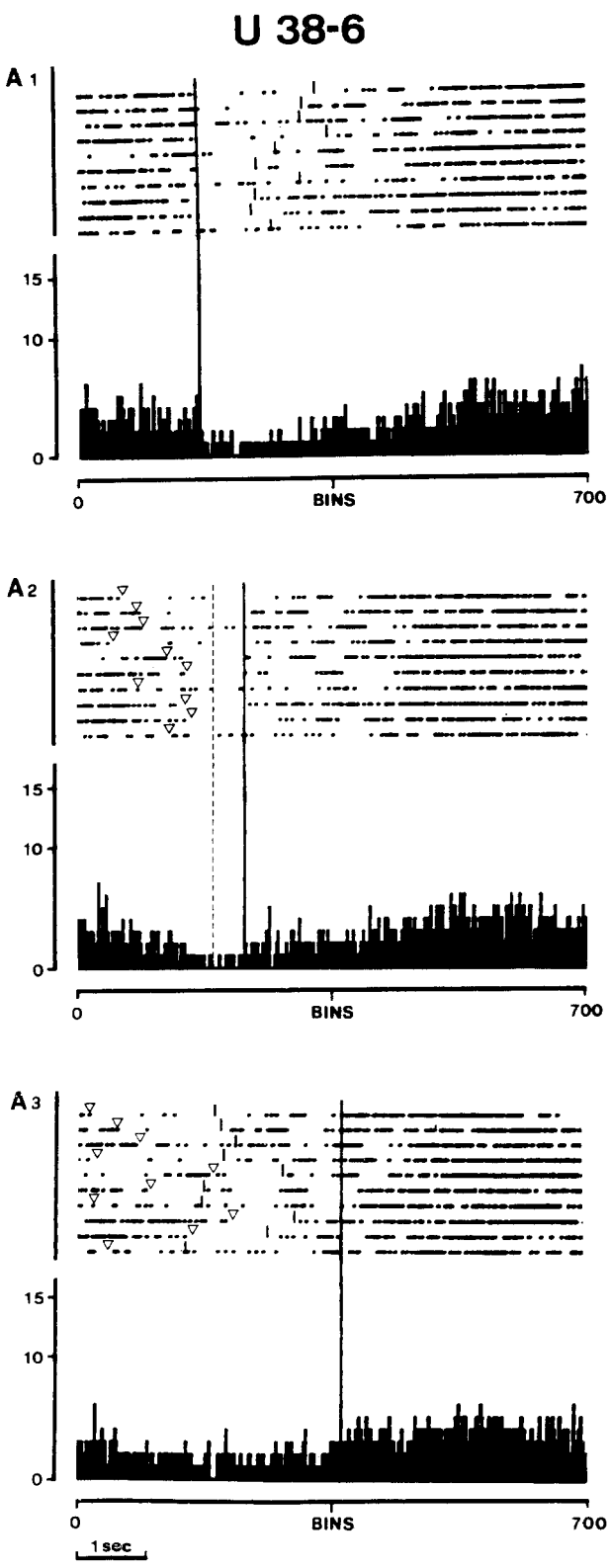


Fig. 2. Reaching-grasping neuron showing decreased firing rate during premovement and movement period. In **A1** the neuron's discharge is aligned (heavy vertical line) with the onset of the saccade elicited by the stimulus; in **A2** it is aligned with the time when the monkey touched the stimulus; in **A3** it is aligned with the saccade triggered by the withdrawing of the food carrier. Same individual trials were used to construct the three histograms. All conventions as in Fig. 1. Details of stimulus presentation are discussed in the text

its maximum firing rate during the arm movement (A2). The neuron resumed its resting firing rate when the food carrier was withdrawn (A3).

Figure 4 shows the firing rate of the same neuron illustrated in Fig. 3 in relation to eye movements during trials in which the stimulus was moved towards the monkey. It is clear that the neuron's activation was not related to a break of fixation. The firing rate increased when the stimulus was close to the monkey (peripersonal space) and reached its peak during the arm movement. Stimulus presentation per se, outside the peripersonal space, was ineffective.

The discharge modulation synchronous with the saccade onset could be either inhibitory (see Figs. 2, 3 and 4) or excitatory. An example of an excitatory effect is illustrated in Fig. 5. This neuron increased its firing rate at the same time as the onset of the saccade elicited by the stimulus presentation and continued to fire during fixation. When the stimulus was close to the animal and especially during reaching-grasping movement the discharge markedly decreased in spite of the fixation maintenance.

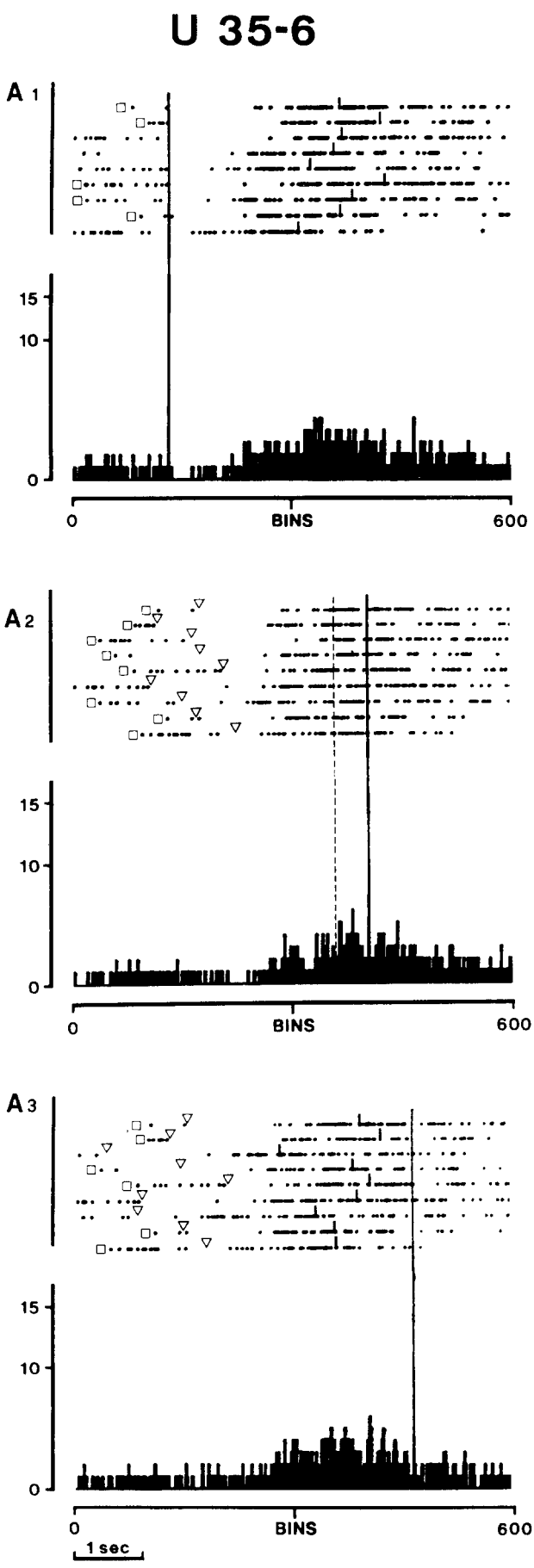
A further example of a neuron of second category is shown in Fig. 6. In this case all the effects occurred with stimuli in the peripersonal space. The stimulation was the same as for the previous neurons. In A1 the records are aligned with the saccade elicited by the stimulus, in A2 they are aligned with the moment when the monkey touched the stimulus.

The neuron was not activated by the saccade and by the following fixation. The neuron started to fire only when the fixated object was within the animal's peripersonal space. Unlike the neuron illustrated in Fig. 3 the activation was not increased further during the arm movement. On the contrary, at the beginning of the arm movement (see A2) the discharge evoked by the stimulus stopped. Note that in the last trial, in which the animal was late in moving in spite of the possibility of grasping the stimulus, the neuron remained active for more than 1.5 s. The discharge then ceased when arm movement began.

Two important properties common to both categories of reaching-grasping neurons are illustrated in Figs. 6B and 7. The first is the absence of a response when an interesting (evoking fixation) "non-graspable" stimulus was presented. The second is the occurrence of a response when a "graspable" object was presented, even if its presentation was not followed by the arm movement.

In Fig. 7, A a series of trials are shown during which a piece of food was presented which the monkey was allowed to grasp. In B, a syringe filled with orange juice was shown. As described in the methods the monkey had been trained not to grasp the syringe but to remain still until the juice was introduced into its mouth. In spite of the interest elicited by the stimulus (saccade and fixation) no discharge was evoked by the "non-graspable" stimulus. An example of this phenomenon for a unit of second category is illustrated in Fig. 6B.

Figure 7, C demonstrates that when a "graspable" object was presented, the neuron became active even when the monkey did not move the arm. In C food was presented exactly as in A, but the animal was trained to



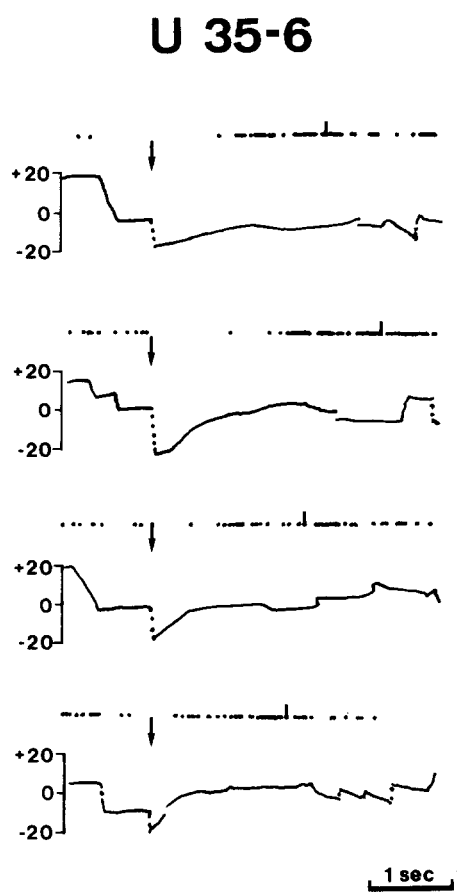


Fig. 4. Responses of a reaching-grasping neuron to a "graspable" object. Eye movements (right eye, horizontal component) and neuron's discharge are shown. Each dot corresponds to one action potential. Vertical arrows indicate the onset of the saccade triggered by stimulus presentation. Vertical marks indicate the time at which the monkey touched the object. The stimulus was presented in the extrapersonal space and slowly moved towards the monkey. The neuron was inhibited during stimulus fixation in the extrapersonal space. Fixation of the stimulus close to the animal was accompanied by excitation

wait before starting the movement. If the monkey remained still the food was either slightly advanced and the reaching-grasping movement rewarded or withdrawn; if the monkey moved the arm before the food movement towards it, the food was always withdrawn. The trials shown in figure are those in which the monkey did not move its arm. Note the prolonged "preparatory" discharge.

Fig. 3. Reaching-grasping neuron showing discharge modulation of opposite sign during the early premovement phase and movement period. The stimulus was presented in the extrapersonal space and slowly moved towards the monkey. In **A1** the neuron's discharge is aligned (heavy vertical line) with the onset of the saccade elicited by the stimulus; in **A2** it is aligned with the time when the monkey touched the stimulus; in **A3** it is aligned with the saccade elicited by the withdrawal of the food carrier. Open square: beginning of the records. Other conventions as in Fig. 1. Details of testing are discussed in the text

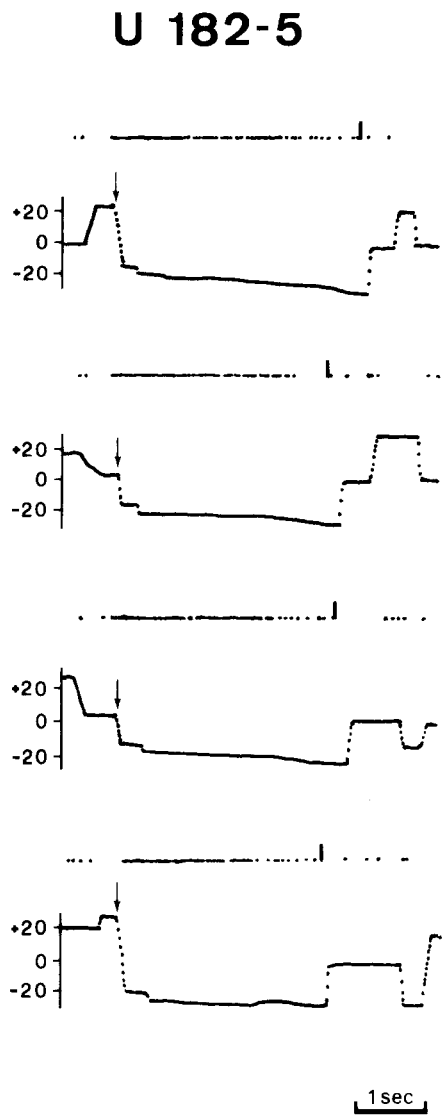


Fig. 5. Responses of a reaching-grasping neuron to a "graspable" object. Eye movements (left eye, horizontal component) and neuron's discharge are shown. The stimulus (a piece of food) was presented to the left of the monkey in the extrapersonal space. The stimulus was then moved towards the animal. The neuron started to fire with the onset of the saccade. The discharge decreased during reaching-grasping movement in spite of continuing fixation. Conventions as in Fig. 4

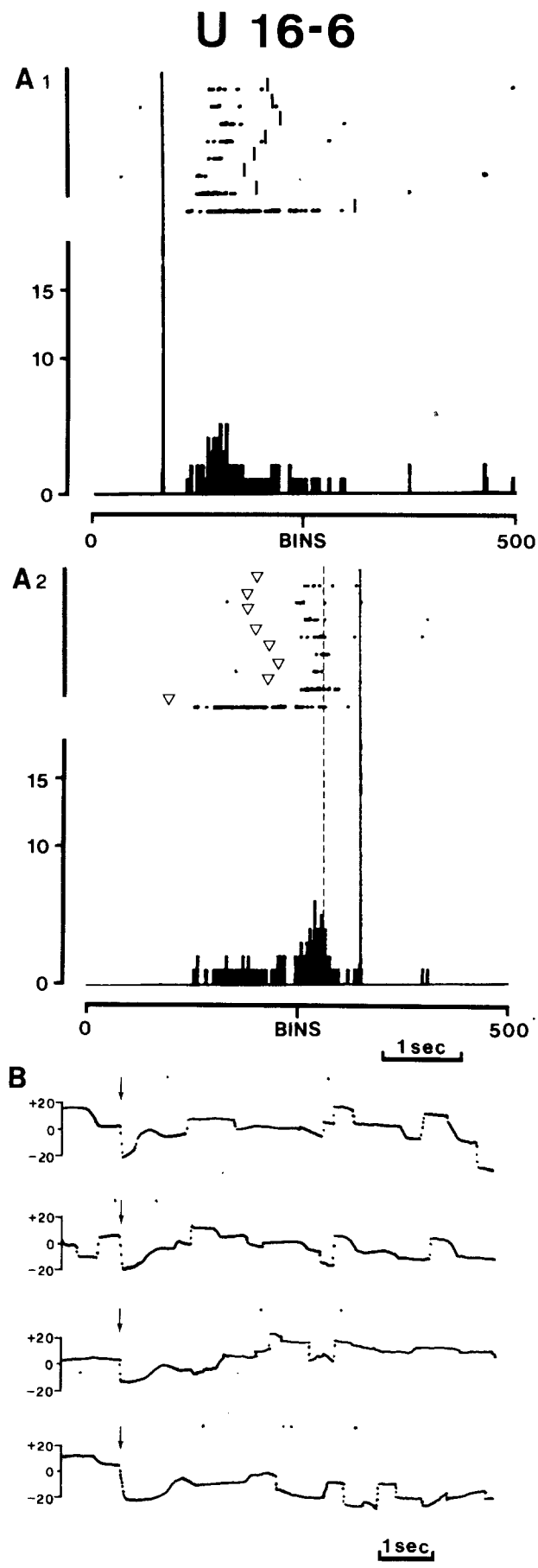


Fig. 6A, B. *Upper part* (**A1, A2**): Reaching-grasping neuron showing discharge modulation of opposite sign during the late phase of the premovement period and the movement period. The stimulus was presented in the extrapersonal space and slowly moved towards the monkey. In **A1** the neuron's discharge is aligned (heavy vertical line) with the onset of the saccade elicited by the stimulus; in **A2** it is aligned with the time when the monkey touched the stimulus. All conventions as in Fig. 1. *Lower part* (**B**) Responses of the same neuron to a "non-graspable" object (syringe filled with orange juice). Eye movements (right eye, horizontal component) and neuron's discharge are presented. Conventions as in Fig. 4

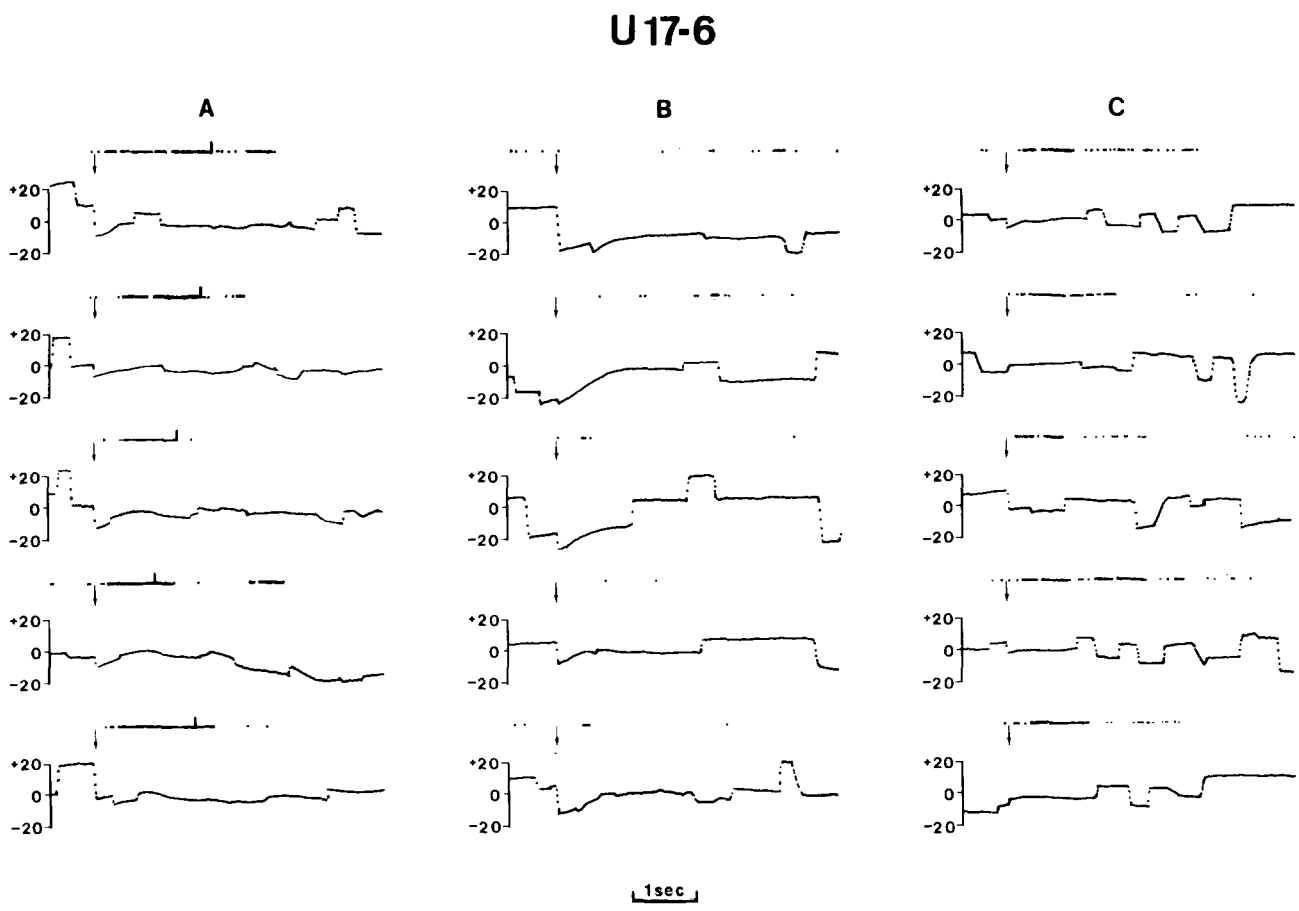


Fig. 7A–C. Responses of a reaching-grasping neuron to a "graspable" and "non-graspable" object. Eye movements (right eye, horizontal component) and the neuron's discharge are presented. In **A** a "graspable" object (a piece of food) was presented and the monkey was allowed to reach it. In **B** a "non-graspable" object (a syringe filled with orange juice) was presented. The monkey fixated the object without moving its arm. In **C** the same "graspable" object as in **A** was presented. The monkey was trained to be ready to grasp it, but was not allowed to move its arm. For detailed explanation of these testing situations see text

A synthesis of the properties of reaching-grasping neurons of first category, showing conditions and events which modified neuron discharge is presented in Table 1. The sign plus indicates an increase of the discharge, the sign minus indicates a decrease of the discharge and the sign zero indicates an absence of correlation between a given event or condition and the discharge of the neuron.

Neurons whose firing rate changed in association with an ocular saccade and those modulated by foveation are pooled together (fixation column). Fourteen neurons showed excitatory changes during both movement and premovement periods, while 6 were inhibited. 11 neurons started to fire at the presentation of a visual object (row 1 and 2). The neurons of row 1 were activated regardless of whether the stimulus was in the extrapersonal or in the peripersonal space, while the remaining were triggered only by the stimuli within the animal's reaching distance. The fixation of a graspable stimulus in the peripersonal space influenced the discharge rate of all neurons. The modification consisted of a further increase or decrease in firing rate for those neurons which were already influenced by the presentation of visual stimuli, whereas it consisted in the beginning of the premovement change for the others.

The observation that the discharge of reaching-grasping neurons was triggered only by "graspable" objects (e.g. Figs. 6 and 7) may appear in contradiction with the finding that the premovement activity was modulated in some neurons by stimulus presentation or by the onset of a saccade (e.g. Figs. 2, 3 and 5). One may argue that at this early stage of the testing, the objects could not have been recognized as "graspable" or "non graspable". Although this point has not been studied quantitatively, the analysis of the videotape recordings showed that a visual response was present at the first presentation of a "non-graspable" object following a sequence of graspable objects. The response then rapidly declined and disappeared if the presentation of the "non-graspable" object continued. The same was true for neurons which did not respond to visual stimuli but were activated by the ocular saccades towards the "graspable" objects. Thus, it appears that the expectancy of a certain type of stimulus plays an important role in determining the strength, or even the presence, of a response of the neurons.

Table 2 illustrates the behavior of neurons whose discharge was modulated differently during the premovement and movement periods (neurons of second category).

Table 1. Neurons with congruent changes during premovement and movement periods

	Visual object		Fixation		Reaching-grasping	
	Extrapersonal space	Peripersonal space	Extrapersonal space	Peripersonal space	arm-movements	N
1)	+	+	+	+	+	6
2)	0	+	0	+	+	5
3)	0	0	+	+	+	1
4)	0	0	0	+	+	2
5)	0	−	0	−	−	1
6)	0	0	−	−	−	3
7)	0	0	0	−	−	2
					Total	20

Table 2. Neurons with non-congruent changes during premovement and movement periods

	Visual object		Fixation		Reaching-grasping	
	Extrapersonal space	Peripersonal space	Extrapersonal space	Peripersonal space	arm-movements	N
1)	+	+	+	+	−	6
2)	+	+	0	0	−	2
3)	0	+	0	+	−	2
4)	+	−	+	−	−	3
5)	−	+	0	0	+	3
6)	Others, activated during reaching-grasping arm movements				+	1
7)	Others, inhibited during reaching-grasping arm movements				−	7
					Total	24

The conventions are the same as in Table 1. The occurrence of excitatory and inhibitory changes added to the other factors that may influence the neuron's discharge (stimulus presentation, fixation, peripersonal and extrapersonal space) resulted in a large variety of different types of neuronal behaviors. The most common are shown in rows 1–5, others are pooled together (row 6 and 7). Note that the majority of neurons of second category were inhibited during reaching-grasping movements (rows 1–4 and 7). About half of the neurons were excited by visual and oculomotor events preceding the movement (rows 1–3), but were inhibited at the movement onset. For the other half there was a major difference in firing rate with stimuli presented in extrapersonal and peripersonal space. The discharge during arm movement was congruent with that evoked by peripersonal stimuli. The opposite was never observed. Thus the far events appear to oppose the arm movement, whereas those in the peripersonal space may either oppose the movement, or favor it.

Anatomical location of the neurons

The upper part of Fig. 8 shows the areas forming the dorso-medial and mesial part of the agranular frontal cortex. Following Vogt and Vogt (1919) we subdivided this area into areas 6aα and 6aβ. The two areas were distinguished by using the following criteria: a) The laminar structure is more prominent in area 6aβ; b) Cells in area 6aβ are smaller than those in area 6aα; c) Layer III and V are more clearly demarcated in area 6aβ. No granular cells were observed in either area.

The lower part of Fig. 8 shows a reconstruction of the penetrations from which the neurons described in this paper have been recorded. Penetrations made in area 6aα (F2 and F3) are not presented. Each large dot indicates the beginning of a penetration. In the case of mesial penetrations the extent in depth of the penetrations is also shown. Small dots indicate the points which were electrically stimulated in the mesial penetrations. Anatomically, all recorded neurons were located in an area rostral to the SMA (6aα, F3). This conclusion was supported by physiological evidence. In both monkeys SMA was systematically stimulated. In agreement with Mitz and Wise (1987) we found that this area is formed by three somatotopic fields: a leg field, located caudally, a central arm field and a (small) mouth field located rostrally. (A detailed description of these stimulation experiments will be presented in a subsequent paper). All of the neurons described here were recorded rostral to the SMA mouth field. The cytoarchitectonic evidence and the presence of an arm area rostral to the SMA mouth field clearly indicates that area 6aβ arm representation is distinct from the SMA arm field.

Finally, it is important to note that although the population of neurons recorded from area 6aβ was heterogeneous, neurons belonging to the same functional class (eye movement neurons, orienting neurons, arm movement neurons) tended to cluster together. Most eye movement neurons were found on the dorsal convexity, whereas arm related neurons were encountered most frequently on the mesial cortical surface. Only rostrally arm movement neurons were also recorded on the dorsal convexity. Orienting neurons had an intermediate position, overlapping dorsally with eye movement cells and mesially

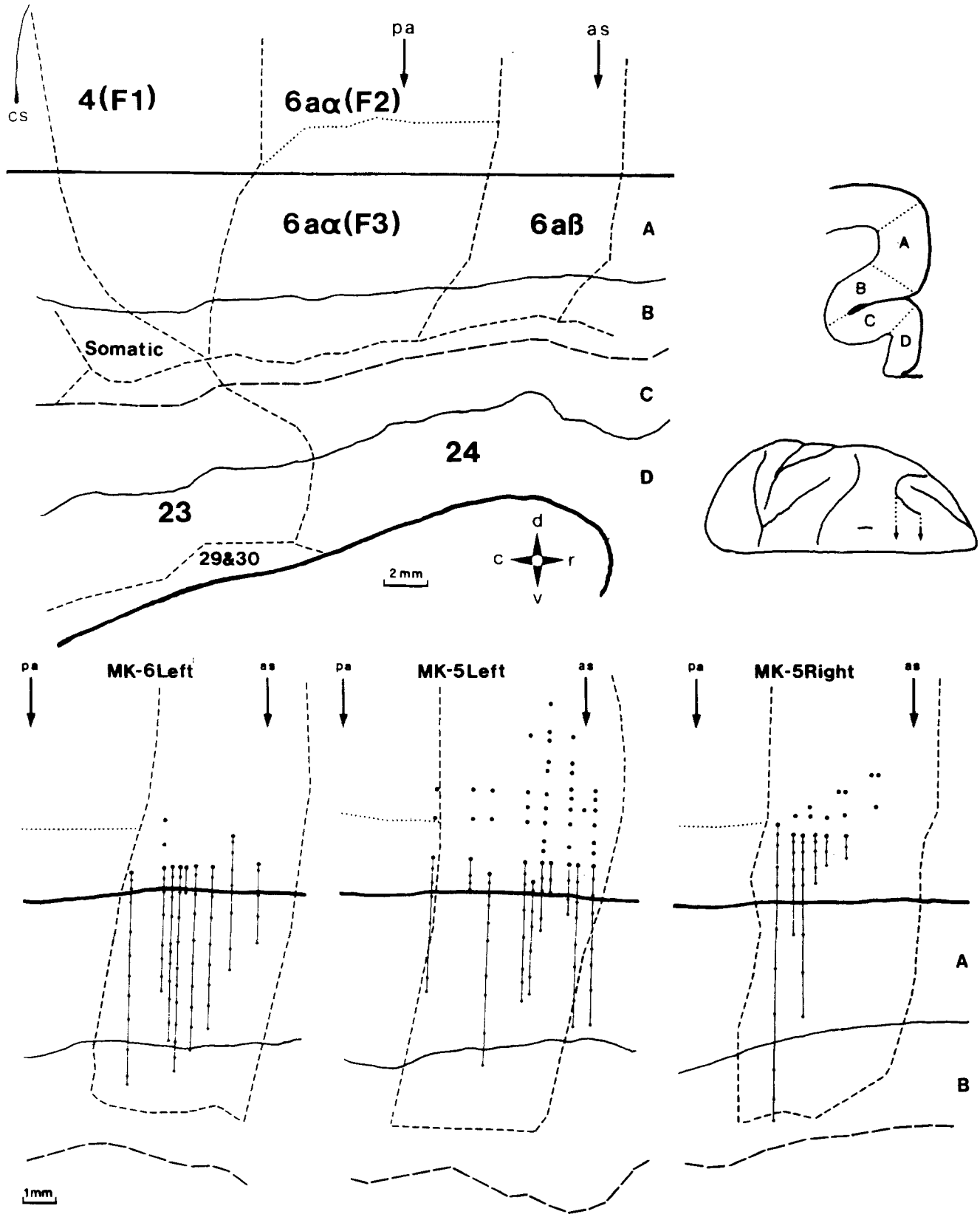


Fig. 8. *Upper part*: Unfolded view of the dorsomedial part of the agranular frontal cortex. Cytoarchitectonic areas and (in brackets) the corresponding cytochrome oxidase areas (Matelli et al. 1985) are indicated. A = mesial cortical surface, B = dorsal bank of cingulate sulcus, C = ventral bank of cingulate sulcus, D = mesial cingulate cortex. The two arrows point to the anterior (as) and to the posterior (pa) limits of the arcuate sulcus. The location of the arcuate sulcus on the cortical surface is illustrated on the right side of the figure (lower corner). *Lower part*: Reconstruction of the penetrations from which the neurons described in this paper have been recorded. For other explanations see text

with arm movement neurons. Although undoubtedly more data are necessary to specify the functional organization of the area, a global idea of it can be derived from Fig. 9. The right side of the figure shows the penetrations in which arm movement cells were recorded. These penetrations are marked by small horizontal bars. The left side of the figure shows the results of the electrical microstimulation. Note the location of the field from which eye movements were elicited. Note also that the penetrations from which arm movements were evoked with microstimulation are located mesially. It is clear that there is a rough segregation between the arm and the eye field.

Discussion

The present data are consistent with a role for area 6aβ in the control of arm movement and with the notion that this area represents a distinct premotor field (see Humphrey 1979). Neurons which became active with arm movements were encountered in the mesial part of area 6aβ and, rostrally, on the dorsomedial cortical convexity. Furthermore, microstimulation of the cortex showed that low threshold eye movements were elicited from a sector of area 6aβ outside that in which neurons related to arm movements were found. Thus, although some of the mesial neurons can be influenced by eye movements, the main motor control exerted by this sector of area 6aβ is upon arm movements.

The arm representation described in the present experiments does not belong to the supplementary motor area (SMA). 1) Unlike SMA, which is electrically excitable with relatively low currents, mesial 6aβ is weakly or not at all excitable with the standard microstimulation parameters (Macpherson et al. 1982; Mitz and Wise 1987; Matelli et al. in preparation). 2) SMA differs cytoarchitectonically and histochemically from area 6aβ. 3) SMA is somatotopically organized with a caudal leg field, a central arm field, and a rostral mouth field (see Mitz and Wise 1987; Matelli et al., in preparation). The arm field studied in the present experiments is rostral to the SMA mouth field. A change in somatotopy with a reappearance of a field already represented in a given area is evidence in favor of a new functional area. These considerations indicate that the arm representation of 6aβ is not a part of the SMA. The posterior part of the mesial agranular frontal cortex is distinct from its anterior part (see also Wiesendanger et al. 1987).

The arm representation of area 6aβ is characterized by the presence of functionally complex neurons (reaching-grasping neurons). One of their most interesting properties is the modulation (excitatory and inhibitory) of their discharge prior to arm movements. In most cases the modulation begins when a possible target for a reaching-grasping movement becomes available, and ends when the movement has been accomplished. Neurons, which tonically modify their activity prior to a movement when information is available on which movement has to be made, have been described in several cortical areas, including the motor cortex, the SMA, and superior area 6 (area F2). These neurons have been referred to as set-related neurons (Weinrich and Wise 1982; Wise and Mauritz 1985; see Wise 1985). The set-related activity of neurons in the premotor cortex reflects the motor aspect of the movement preparation rather than sensory or motivational factors (Weinrich et al. 1984; Wise and Mauritz 1985; Kurata and Wise 1988a). The strongest evidence in favor of this conclusion is the fact that most set-related neurons fire either when the monkey prepares a movement on the basis of sensory instruction stimuli or on the basis of previous experience without a sensory cue (Kurata and Wise 1988b).

The tests that we used in the present experiments are very different from the tasks employed by Wise and his coworkers, hence it is not easy to compare set-related neurons and reaching-grasping neurons. Nevertheless there are several similarities in the properties of the two types of neurons. The premovement tonic discharge of set-related cells starts with some delay after instruction stimulus presentation (pure set-related cells) or synchronously with the stimulus (signal-set related cells; Weinrich and Wise 1982). Similarly, reaching-grasping neurons fire either immediately or with delay after stimulus presentation. Set-related neurons do not discharge when the instruction is to withhold a response (Weinrich et al. 1984). Analogously, reaching-grasping neurons do not fire when a visual stimulus is presented which does not require the reaching-grasping movement, even when motivationally it is identical to the "graspable" stimulus. The duration of the tonic premovement discharge of set-related neurons varies, depending on when the monkey, prompted by a trigger stimulus (Wise and Mauritz 1985) or on the basis of an internal process (Kurata and Wise 1988b), decides to move the arm. The same is true for reaching-grasping neurons. The discharge, once started, lasts until the monkey makes the appropriate movement. Finally, the presence of an instruction stimulus is not a necessary requisite for modulating the set-related activity (Kurata and Wise 1988b). Similarly, reaching-grasping neurons fire during self-generated movements towards hidden objects.

Despite these similarities the two groups of neurons differ in some important respects. Reaching-grasping neurons are influenced by eye movements, a set of them firing synchronously with the onset of the saccade triggered by object presentation. Such arm-eye interactions have never been reported for set-related neurons. Reaching-grasping neurons are influenced by the distance of the stimulus from the monkey. Some of these neurons respond only when the stimulus is in the peripersonal space, others discharge differently depending on whether the stimulus is in the extrapersonal or peripersonal space. Set-related neurons that respond exclusively to peripersonal stimuli have not been reported. It must be said however that these neurons have not been specifically tested with near and far stimuli. Finally, set-related neurons do not appear to have the rich excitation-inhibition interactions which characterize many reaching-grasping neurons.

Conclusions concerning the differences between reaching-grasping neurons and set-related neurons are limited by the different experimental conditions in which the two types of neurons have been studied. This is not so for

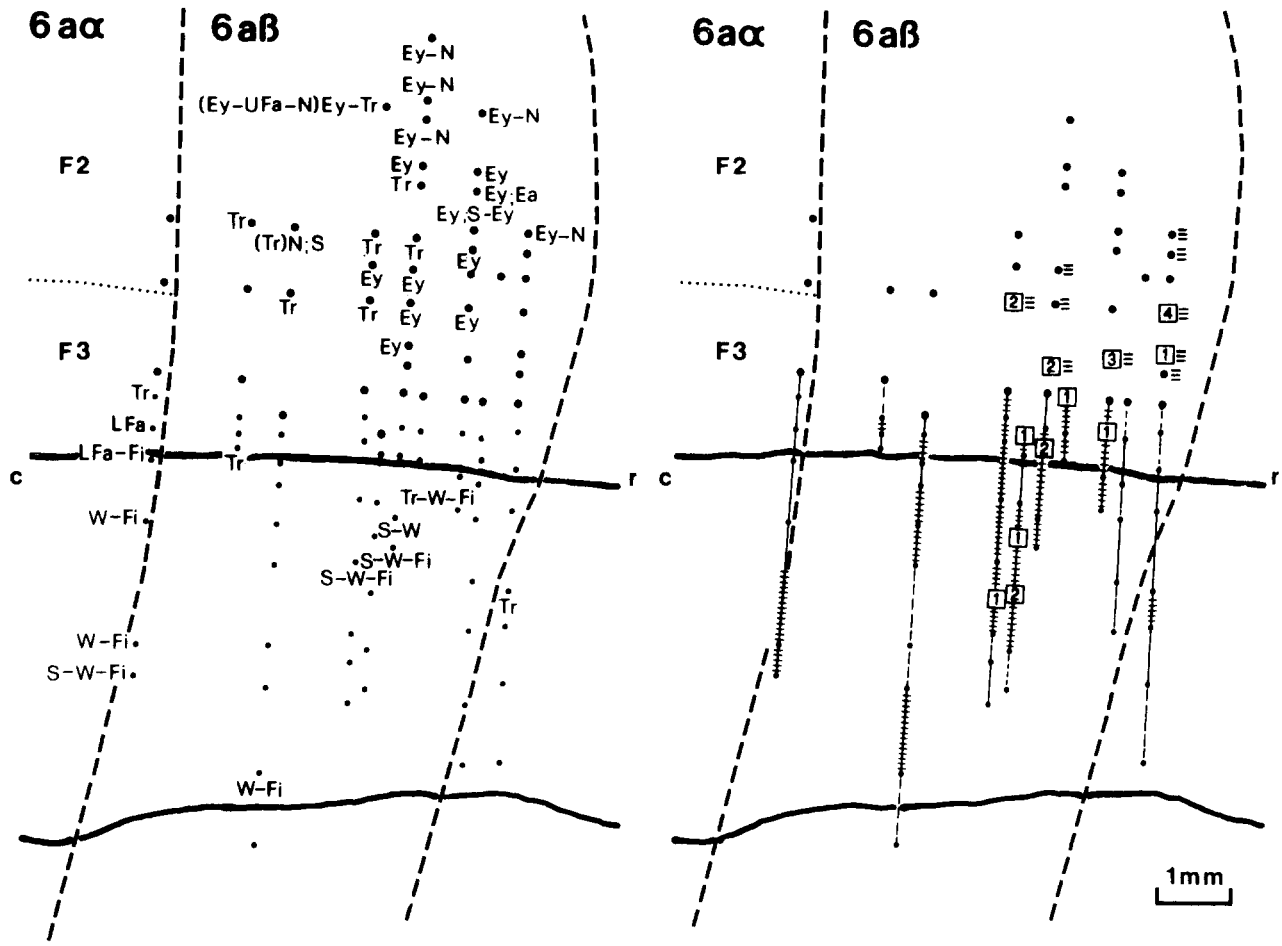


Fig. 9. Left side: Intracortical microstimulation (monkey MK-5Left). Large dots indicate the locations of the penetrations (entrance points). Small dots indicate the sites electrically stimulated in mesial cortical penetrations. The cortex is unfolded as in Fig. 7. Dots without symbols = absence of a peripheral response. Ea = ears; Ey = eye; Fi = fingers; LFa = lower face; N = neck; S = shoulder; Tr = trunk; UFa = upper face; W = wrist. Symbols linked by a bar = complex movements which could not be dissociated into their elementary components by changing the current intensity. Symbols separated by a semicolon = movements that where evoked from stimulation of different deep sites (1500–2000 μm). Symbols in brackets = movements evoked from superficial sites (< 1500 μm). Only movements different from those evoked from deep sites of the same penetration are indicated. *Right side*: Penetrations where, in addition to microstimulation, single neurons properties were analyzed. Horizontal bars show the penetrations in which neurons related to arm movements were encountered. Squares mark the penetrations in which reaching-grasping neurons were located. Numbers inside the squares indicate the number of reaching-grasping neurons recorded in a given penetration. r = rostral; c = caudal

inferior area 6 neurons which have been tested with the same experimental paradigm as reaching-grasping neurons (Gentilucci et al. 1988; Rizzolatti et al. 1988).

Inferior area 6 contains neurons which become active either during distal or proximal movements. "Distal" neurons discharge during specific motor acts like grasping, holding, tearing; they control specific types of hand grip (precision grip, finger prehension, whole hand prehension); they are not influenced by the direction of arm movements. In most distal neurons the discharge starts approximately with the movement onset. In about 20% of them, however, the activation starts earlier, as soon as the stimulus is presented. The effective visual stimulus must have a size congruent with the type of grip controlled by the stimulated neuron and should be motivationally interesting. "Proximal" neurons become active in association with arm movements directed towards a specific space sector, but they are not influenced by the type of grip. Most "proximal" neurons respond to visual stimuli and increase their firing during the arm movements. In spite of the fact that the discharge may precede considerably the arm movement, excitatory-inhibitory modulation of proximal neuron activity has not been observed.

Unlike neurons in inferior area 6, reaching-grasping neurons were not influenced by the size of the stimulus or by the type of grip. Furthermore, they did not have peripersonal visual receptive fields that (very likely) provide inferior area 6 with a body-centered reference system (see Rizzolatti and Gentilucci 1988). Thus neither the size of the object nor its location relative to the body appear to be coded by reaching-grasping neurons. They could, however, signal when an object was at a reachable distance. These distance-related responses probably do not code the position of the object in a precise reference system. The

visual response properties of reaching-grasping neurons (absence of a visual receptive field in the tangential plane, dependence of the response on the meaning of the stimulus), are inappropriate for such coding. They could trigger, however, the preparation of a reaching-grasping motor program on the basis of the distance from the animal.

The absence of a clear influence of the physical characteristics of the object on the reaching-grasping neuron discharge may appear to contradict the observation that reaching-grasping neurons differentiated between "graspable" and "non-graspable" objects. Although we cannot exclude that reaching-grasping neurons could be involved in some higher order recognition processes, it seems more parsimonious to interpret the observed difference between "graspable" and "non-graspable" object in motor terms. The premovement discharge occurred when the stimulus started the preparation of a motor program that eventually resulted in an arm reaching-grasping movement. The premovement discharge failed to occur if the stimulus did not start this motor preparation.

It has been proposed that a fundamental distinction between the function of the mesial premotor areas (defined collectively as SMA) and inferior premotor areas consists in a specialization of the former for internally-referenced behavior (Roland et al. 1980, 1982; Eccles 1982; Goldberg 1985) and in a specialization of the latter for externally-referenced acts (Rizzolatti et al. 1981, 1983; Gentilucci et al. 1983; Goldberg 1985; Passingham 1987). This distinction is difficult to accept if it implies that mesial areas are involved in "voluntary" movements, whereas the inferior areas are responsible for "reflex-like" movements (Goldberg 1985). A duplication of cortical areas based on the psychological origin of the movement seems unlikely. An external reference is obviously needed for "voluntary" movements as well as for those externally triggered (for a discussion and critique of this version of external vs. internal dichotomy see Rizzolatti 1985). Most importantly there is recent evidence that the SMA, superior area 6 and inferior area 6 all become active during self-generated and externally occasioned motor acts (Okano and Tanji 1987; Kurata and Wise 1988b). The present data indicate that the same is true for area 6aβ.

The distinction, however, between externally-referenced and internally-referenced behavior can be useful if it is meant to indicate the type of processing that occurs in the mesial areas on one hand and in inferior area 6 on the other. As discussed above an important function of inferior area 6 is that of coding the intrinsic and extrinsic properties of the objects in order to generate the appropriate motor acts. A similar set of operations has also been suggested for area 7b (Sakata et al. in preparation), an area which is anatomically connected with inferior area 6 and whose neurons share with it many properties (see Hyvärinen 1982). There is no evidence that a similar process of sensory-motor transformation takes place in the mesial premotor areas. This does not mean that external stimuli do not act on the mesial areas. There is good evidence that the SMA is influenced by visual, auditory, and somatosensory stimuli (Tanji and Kurata 1985), and the present data show that visual stimuli can affect the response of neurons in area 6aβ. The external stimuli, however, are not used to select the appropriate proximal and distal effectors. Rather these stimuli act as signals that start preparatory motor processes, which along with the sensory-motor transformation of the inferior premotor areas, allow the execution of motor acts.

The preparatory processes controlled by the mesial areas may include: postural adjustments necessary for the execution of reaching-grasping movements; inhibition of other movements such as exploratory eye movements which might be incompatible with visual control of arm movements; inhibition of reflex-like movements directed towards interesting objects and controlled by subcortical centers; potentiation of the activity of inferior premotor areas through the rich connections between inferior and mesial areas; instruction to subcortical centers to compute the quantitative parameters of the movement. Many of these suggestions have been proposed to explain the function of set-related neurons (see Kurata and Wise 1988b). Considering however the variety and diversity of the suggested functions, it is not surprising that there are neurons in several areas whose activity is modulated in advance. It is likely that some of these functions are executed in some areas, others in other areas.

A final point which deserves some comments is the richness of inhibitory modulations showed by reaching-grasping neurons. The majority of non-congruent reaching-grasping neurons were inhibited during arm reaching-grasping movements. Moreover, for many of them the inhibition started just before the arm movement, whereas the whole premovement period was characterized by activation. Since the correlation was between neuron inhibition and arm movement onset, it is reasonable to infer that the neuronal discharge inhibited arm movements, whilst its suppression allowed the arm movement. It appears, therefore, that this set of reaching-grasping neurons excited neurons responsible of some aspects of movement preparation, and inhibited other neurons controlling the arm movement initiation. Only when the preparatory process was terminated, the inhibitory action was lifted and the arm movement could start. Although obviously this interpretation is very speculative, it seems worth exploring especially considering that it could give a relatively simple answer to the rich corticocortical connections which characterize the premotor areas.

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