

Spatial tuning of reaching activity in the medial parieto-occipital cortex (area V6A) of macaque monkey

Patrizia Fattori, Dieter F. Kutz, Rossella Breveglieri, Nicoletta Marzocchi and Claudio Galletti

Dipartimento di Fisiologia Umana e Generale, Università di Bologna, I-40126 Bologna, Italy

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Abstract

We recorded neural activity from the medial parieto-occipital area V6A while three monkeys performed an instructed-delay reaching task in the dark. Targets to be reached were in different spatial positions. Neural discharges were recorded during reaching movements directed outward from the body (towards visual objects), during the holding phase (when the hand was on the target) and during inward movements of the hand towards the home button (which was near the body and outside the field of view). Reach-related activity was observed in the majority of 207 V6A cells, during outward (78%) and inward (65%) movements as well as during the holding phase (62%). Most V6A reaching neurons (84%) were modulated in more than one phase of the task. The reach-related activity in V6A could depend on somatosensory inputs and/or on corollary discharges from the dorsal premotor cortex. Although visual and oculomotor inputs are known to have a strong influence on V6A activity, we excluded the possibility that the reach-related activity which we observed was due to visual stimulation and/or oculomotor activity. Reach-related activity for movements towards different locations was spatially modulated during outward (40%) and inward (47%) reaching movements. The position of the hand/arm in space modulated about 40% of V6A cells. Preferred reach directions and spatial locations were represented uniformly across the workspace. These data suggest that V6A reach-related neurons are able to code the direction of movement of the arm and the position of the hand/arm in space. We suggest that the V6A reach-related neurons are involved in the guidance of goal-directed arm movements, whether these actions are visually guided or not.

Introduction

Reaching out for objects is an action that primates commonly perform in their everyday life. In the action of reaching, the hand is transported from a starting position, generally near the body, to the location of the object to be reached in the peripersonal space. We usually perform outward reaching movements towards objects placed in the field of view and, unless forced to behave differently, we accompany the reaching action with an eye movement that ‘catches’ the target of reaching, bringing its image onto the fovea. We also frequently perform inward reaching movements to bring the hand towards the body, to peruse the grasped object, eat it or put it in our pockets. In some of these cases, we direct the hand towards targets located outside the field of view. In any case, regardless of whether the arm movement is directed towards a visual or non-visual object, the action is under careful control in order to be correctly performed.

It is well known that the posterior parietal cortex (PPC) is involved in the visual control of reaching movements, housing reaching activity and neural representations of space which can guide the actions needed for interacting with objects present in the environment (see Hyvarinen, 1982; Sakata & Kusunoki, 1992; Rizzolatti *et al.*, 1997; Colby & Goldberg, 1999; Galletti & Fattori, 2002).

Many researchers have investigated the directional tuning of reaching activity in single cells of monkey PPC using center-out tasks on a plane or arm-movement tasks to reach positions in three-dimensional space. When both tasks were used, different results were

observed (MacKay, 1992). In the caudal part of the superior parietal lobule (SPL), reaching activity has so far been studied only using center-out tasks that required two-dimensional hand translations on a plane (Ferraina *et al.*, 1997, 2001; Snyder *et al.*, 1997; Battaglia-Mayer *et al.*, 2000, 2003). The present work is the first to study reach-related activity in the caudal SPL in a task performed in three-dimensional space. This research is focused on area V6A, known to contain arm-movement-related activity (Galletti *et al.*, 1997; Fattori *et al.*, 2001, 2004).

The aim of this work was to study V6A neural modulations for movements of the arm aimed at reaching small visual targets located in different positions in the peripersonal space (outward movements) as well as movements aimed at reaching locations near the body, outside the animal’s field of view (inward movements). To our knowledge, this is the first study in the PPC analysing neural modulations during inward movements. We used a task able to reproduce the reaching movements performed in everyday life but in the controlled conditions offered by an instructed-delay reaching task. Our results shed light on the functional role played by this cortical sector in the control of aimed arm movements.

Some of the results have appeared in preliminary form (Fattori *et al.*, 1999, 2001).

Materials and methods

Experiments were carried out in accordance with national laws on the care and use of laboratory animals and with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and

Correspondence: Professor P. Fattori, as above.

E-mail: pfattori@biocfarm.unibo.it

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were approved by the Bioethical Committee of the University of Bologna.

Three trained *Macaca fascicularis* sat in primate chairs with their heads restrained, to perform arm movements under controlled conditions. Single cell activity was extracellularly recorded from the anterior bank of the parieto-occipital sulcus using glass-coated metal microelectrodes with a tip impedance of 0.8–2 M Ω at 1 kHz. Action potentials were sampled at 1 kHz for two monkeys (Galletti *et al.*, 1995) and at 100 kHz for the third (Kutz *et al.*, 2005). Eye movements were simultaneously recorded using an infrared oculometer (Dr Bouis, Karlsruhe, Germany) and sampled at 100 Hz for two monkeys (Galletti *et al.*, 1995) and at 500 Hz for the third (Kutz *et al.*, 2005). Eye position was controlled by an electronic window (5 \times 5 $^\circ$) centred on the reaching target in two of the monkeys. Surgery to implant the recording apparatus was performed in asepsis and under general anesthesia (sodium thiopental, 8 mg/kg/h, i.v.) and analgesics were used post-operatively (ketorolac trometazyn, 1 mg/kg i.m. immediately after surgery and 1.6 mg/kg i.m. on the following days). During the last 2 weeks of recording, electrolytic lesions (30–40 μ A cathodal current for 30 s) were made at different depths along single penetrations carried out at different coordinates within the recording chamber. After the last recording session, the animals were anaesthetized with ketamine hydrochloride (15 mg/kg i.m.) followed by an i.v. lethal injection of sodium thiopental and perfused through the left cardiac ventricle with saline, and then with 4% paraformaldehyde followed by 5% glycerol. Electrode tracks of the last microelectrode penetrations were identified on brain sections on the basis of marking lesions. Unmarked penetrations were first roughly located by interpolation with respect to the electrolytic lesions and pin tracks. See Galletti *et al.* (1995, 1996) for a detailed description of electrode track reconstruction. V6A was recognized on functional grounds following the criteria described in Galletti *et al.* (1999).

While the electrode was advanced through the V6A cortex trying to isolate a neuron, the monkey was typically in light and at rest. In these conditions, the animal spontaneously performed eye movements and different kinds of arm movements, i.e. movements directed towards the objects around it or towards its nose or face. Sometimes, we let it perform the reaching task so that cells silent at rest could be recruited in this condition. In this case, the sequence of tested positions during the reaching task was frequently changed to avoid any involuntary bias in our searching procedure (see the following section). Each neuron isolated in V6A was tested with the reaching task, whether or not it was modulated by arm movements.

Reaching task

The monkeys performed arm movements with the contralateral limb, with the head restrained, in darkness, maintaining steady fixation.

In our task, we used a planar array of targets but an out-of-plane start-point so as to evoke 'radial' reach movements directed outward from the body and towards the body, similar to what happens in natural reaches. The experimental set-up is sketched in Fig. 1A. Reaching movements started from a button (home button, 2.5 cm in diameter) placed outside the animal's field of view, 5 cm in front of the chest on the midsagittal line. Reaching movements transported the hand from the home button to targets positioned in different spatial locations on a fronto-parallel panel. Targets were very small (4 mm in diameter; 1.6 $^\circ$ of visual angle) as they were light emitting diodes (LEDs) mounted on microswitches embedded in the panel. We used two different arrangements of target positions, both located 14 cm in front of the animal with the central target placed straight ahead at eye

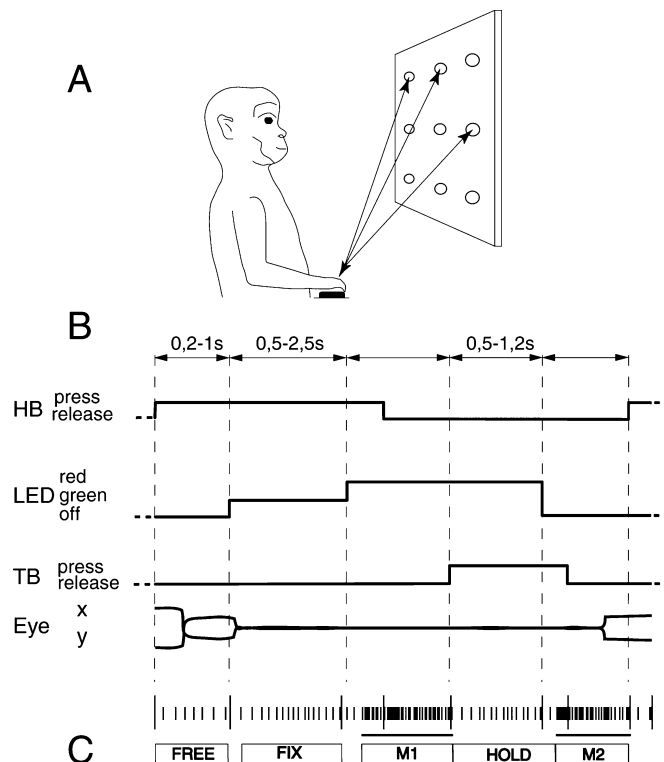


FIG. 1. Experimental set-up and time course of reaching task. (A) Scheme of experimental set-up in our reaching task. Reaching movements were performed in darkness, from a home button (black rectangle) towards one of nine targets (open circles) located on a panel in front of the animal. (B) Time course of the task; the sequences of status of the home button (HB), target button (TB) and color of the target button (light-emitting diode LED) are shown. Lower and upper limits of time intervals are indicated above the scheme. Below the scheme, typical examples of eye traces and neural activity during a single trial are shown. Short vertical ticks are spikes. Long vertical ticks among spikes indicate the occurrence of behavioral events (markers). From left to right, the markers indicate: trial start (HB press), target appearance (LED light-on green), go signal for outward movement (green to red change of LED light), start and end of outward movement (HB release and TB press, respectively), go signal for inward movement (LED switching off), start and end of the inward movement (TB release and HB press, respectively) and end of data acquisition. Horizontal lines below spikes are neural response durations calculated with the change-point algorithm (see text). (C) Time epochs during a typical trial. Rectangles below spikes indicate the time epochs used to calculate the neural discharge (see text).

level. In one animal, targets were mounted on a panel in a 3 \times 3 grid (see Fig. 1A) 7 cm (28 $^\circ$ visual angle) apart. In two animals, we used a panel with three targets in line, each 7.4 cm (30.8 $^\circ$) apart. This panel could be rotated around the central target in steps of 45 $^\circ$ to obtain more target positions on a frontal plane.

The time sequence of the reaching task is shown in Fig. 1B. A trial began when the monkey pressed the button near its chest. The animal was free to look around and was not required to perform any eye or arm movement (epoch FREE, see Fig. 1C). After 200–1000 ms, one of the LEDs lit up (LED green). The monkey had to wait for the LED to change in color, without performing any eye or arm movement (epoch FIX). After a delay period of 500–2500 ms, the LED color changed from green to red. This was the go signal for the monkey to release the button and perform an arm-reaching movement to reach the LED and press it (epoch M1); the animal then kept its hand on the LED (epoch HOLD) until it switched off (after 500–1200 ms). This cued the monkey to release the LED and return to the home button (epoch M2). The task ended with the button pressing, which allowed

monkey reward and started another trial (epoch FREE again). Trials were self-paced and no external time constraints were imposed on animal behavior.

During most of the task, monkeys fixated the LED which was also the target of the outward reaching movements. The two monkeys with an electronic window control on eye movements were forced to fixate the LED within 500–1200 ms from LED appearance (i.e. well before the go signal for the outward reach) until its disappearance (which cued the return reach). If that fixation was broken during this interval, trials were interrupted on-line and discarded. One monkey was trained to perform the reaching task without ocular constraints; it spontaneously chose to fixate the reaching target while waiting for the go signal and executing reaching movements. Trials were visually inspected off-line and those with poor fixations were discarded.

The correct performance of reaching movements was detected by press/release of microswitches (monopolar microswitches, RS Components, UK) mounted under the home button and the LEDs. Button presses/releases were recorded with 1 ms resolution. For a detailed description of the behavioral control of trial execution see Kutz *et al.* (2005).

Great care was taken to check for changes in cell activity over time during recording of reaching activity in different spatial positions. In one animal, each LED position was tested in blocks of 15 trials and LED positions were tested in pseudo-random order. To rule out changes in neural discharge among LED positions being due to variations in time of cell response properties independent of task manipulations, cell responsiveness was checked periodically. One or more target positions were routinely repeated along the sequence. If cell responsiveness in a certain target location was significantly different from that previously recorded in the same position, data were discarded off-line and no longer considered in the analysis. For the other two animals, different LED positions were typically tested (15 trials) as a sequence of randomized triplets of target locations (45 trials in total) to collect trials in one position intermingled (randomly) with the other two. After having tested three locations, the panel was oriented differently around the central target and the procedure repeated. In this way, the central LED was retested after each change in panel orientation. If cell responsiveness had changed from that previously recorded, the sequence was interrupted and data collected after the previous control were discarded.

The background light was switched on for a few minutes after each block of trials or triplets of reach locations to avoid dark adaptation. To further minimize the role of vision during reaching, the brightness of the LED was reduced so that it was barely visible during the task. Indeed, the experimenter could not see the monkey hand approaching the target, even in dark-adapted conditions.

Data analysis

Data analysis was performed trial by trial. In each trial, we carefully looked for a correlation between the neural discharge and the arm and eye movements and positions. As shown in Fig. 1C, each trial was divided into time epochs primarily based on neural or ocular events rather than on physical events as is usual in these cases. These ‘functional’ epochs were defined as follows.

- (i) FREE. From the beginning of the trial to the light up of the LED.
- (ii) FIX. Steady fixation of the LED during the delay period; this epoch was calculated on a single trial basis and therefore varied in duration according to the variable duration of fixation periods.
- (iii) M1. Outward reach movement, i.e. movement towards the visual target in the peripersonal space.

- (iv) M2. Inward reach movement, i.e. movement towards the memorized target outside the field of view.

M1 and M2 periods of neural modulation related to arm movements towards the LED and towards the home button, respectively; both epochs could start before the onset of arm movement in case the neural modulation preceded the movement onset. M1 and M2 duration changed trial by trial according to the duration of the neural discharge.

- (v) HOLD. From the end of the forward reach (LED pressing) to the onset of neural response related to the backward arm movement.

When neural activity did not change significantly during M1, M2 or HOLD, fixed durations were set for these three epochs as follows.

- (a) M1. From 200 ms before movement onset (home button release) to movement end (LED pressing).

- (b) M2. From 200 ms before movement onset (LED release) to movement end (home button pressing).

- (c) HOLD. From the end of the forward reach (LED pressing) to the onset of M2.

All these epochs were calculated trial by trial according to button and target-switch presses and releases.

For brevity, M1, M2 and HOLD will be collectively referred to as ‘action epochs’ from now on.

Cell responses in each epoch were assessed, trial by trial, by estimating the onset of neural response using the maximum likelihood estimator of a change point in a sequence of random variables (Seal *et al.*, 1983; Commenges *et al.*, 1986; see also Kutz *et al.*, 2003 for a detailed description of the change-point algorithm). For each neuron, the mean firing rate was calculated for each trial in each of the above-described ‘functional’ epochs and statistically compared with the mean firing rate in epoch FREE (two-tailed Student’s *t*-test; significance level, $P < 0.05$). This comparison was performed for each spatial location. FREE was chosen as a reference because in this epoch no visual stimuli were present and the monkey was free to look around and was not required to gaze at a fixed position; in addition, it was not executing or preparing any arm movement.

We considered acceptable for the analysis only those neurons in which we could perform the quantitative analysis in at least seven trials for each target position, following the suggestions of Snedecor & Cochran (1989) as detailed in a recent study (Kutz *et al.*, 2003). As described above, we tried to record 15 trials per target position per neuron (see Materials and methods) but sometimes isolation was lost during data collection, thus reducing the number of trials available for the analysis. In addition, the monkey performing the reaching task without eye movement constraints sometimes broke target fixation during delay preceding arm movement. As a result, it was not possible to calculate the epoch FIX in these trials and they were discarded from the analysis, further reducing the number of units and trials accepted for the analysis.

The spatial tuning of reaching activity was analysed for each unit modulated in the reaching task and tested for two or more target positions in at least seven trials for each position. We statistically compared the mean firing rate in each target position (one-way ANOVA, *F*-test; significance level, $P < 0.05$) for each of the functional epochs described above. A neuron was defined as ‘spatially tuned’ when it showed a statistically significant difference in mean firing rate in the same action epoch among different spatial locations.

As many neurons in V6A are modulated by the direction of gaze (Galletti *et al.*, 1995; Nakamura *et al.*, 1999) and because during the reaching task the monkey maintained fixation of the LED in different spatial locations during M1 and HOLD (during M2 the animal was free to move its eyes and thus usually broke fixation), one cannot exclude *a priori* that significant differences in activity among target positions in these epochs only reflect a gaze effect. To remove a

possible linearly additive effect of gaze in M1 and HOLD, we subtracted, trial by trial, the activity during FIX from the activity during M1 and HOLD, thereby obtaining a value of M1 and HOLD activity virtually not influenced by the gaze effect. We labeled these epochs as 'proper M1' and 'proper HOLD', whereas 'raw M1' and 'raw HOLD' are the terms used to indicate the total mean activity in these epochs, inclusive of any possible effect of gaze.

We tried to verify the distribution of cell preferences for directions of reaching movements, and for spatial locations of the hand during holding time, by calculating a mean vector of discharge rate for each neuron modulated in a given action epoch (Mardia, 1972). We weighted the coordinates of each tested position (x_i, y_i) with the mean activity recorded in that position (A_i) normalized by the sum of all activities in that epoch ($A_{\text{tot}} = \sum_i A_i$) as follows.

$$x_{\text{centre}} = \sum_i x_i \times (A_i/A_{\text{tot}})$$

$$y_{\text{centre}} = \sum_i y_i \times (A_i/A_{\text{tot}})$$

We then calculated the distribution of vector endpoints along the ipsilateral/contralateral axis and the top/bottom axis and plotted the distribution of preferred positions for the top/bottom and ipsilateral/contralateral parts of the workspaces.

Somatosensory stimulation

Whenever possible, V6A neurons were tested to check their sensitivity to passive somatosensory stimulation. Manual soft touching, palpation of deep tissue and joint rotation at different velocities were carried out in the three animals in this study. The experimenter stood behind the animal and delivered stimuli on both sides of the whole body. As the experiments were performed with the monkey's head fixed, responses to neck rotation could not be tested. At the beginning of training, the monkeys tried to withdraw their limbs whenever touched but later they were quiet and compliant so that it was possible to test the effect of passive somatic stimulation on the activity of recorded neurons.

When a neuron responded to a somatosensory stimulation, it was classified as follows: 'joint', when activated by joint rotation; 'deep', when activated by deep pressure of subcutaneous tissues and 'skin', when activated by light tactile stimulation of the skin but not by joint or deep pressure stimulation.

When a cell responded to joint rotation, we carefully checked whether skin stimulation around the joint was responsible for the neural response. A cell was classified as 'joint' only when the tactile stimulation did not modulate its neural activity. When there was no evidence that the responses to light tactile stimuli were due to deep tissue stimulation or joint rotations, we assumed that they were cutaneous in origin. However, the criteria which we used were operational and therefore do not exclude the participation of other somatosensory afferences, including muscle proprioception.

During somatosensory stimulation, care was taken to rule out possible influences of visual, arm and eye movements on cell discharge. Visual influences were excluded by performing somatosensory stimulation in complete darkness, turning on the background light between batteries of stimuli to avoid dark adaptation. Arm and eye movements were continuously monitored during somatosensory stimulation to check whether they could be responsible for neural discharges.

Electromyographic recordings

We recorded electromyographic (EMG) activity in separate sessions. EMG activity was monopolarly recorded with surface electrodes from

a total of 12 muscles of the upper limbs, shoulder, neck and trunk in different sessions, while one monkey performed our reaching task. Six electrodes were placed on the right side and six on the left. The raw EMG signals were filtered, recorded digitally at 500 Hz and rectified off-line. Peri-event time histograms (binwidth, 10 ms) from EMG signals were aligned with the onset of the first reaching movement (M1), as assessed by the release of the microswitch mounted under the home button.

The onset of EMG activity was compared with that of the neural response. Onsets of neural responses were determined using the change-point algorithm (as explained above) for M1 movements performed in the cell's preferred direction. Onset of EMG activity was defined as the point where the peri-event time histogram was continuously above/below the upper/lower 95% confidence band level for five consecutive bins. Confidence levels were calculated during FIX, from 700 to 200 ms before movement onset.

Results

Reach-related activity in V6A

We recorded the activity of 323 V6A neurons in three monkeys performing the reaching task in darkness. We did not pre-select arm movement-related neurons but we performed the task in all V6A neurons that we recorded from. Our recording site was within the limits of the physiologically defined V6A (Galletti *et al.*, 1999). Figure 2A and B shows the reconstruction of four microelectrode penetrations through V6A from two different monkeys. All penetrations reached V6A on the anterior bank of the parieto-occipital sulcus, where reach-related cells were recorded. Figure 2C–F shows the distribution of V6A cells recorded in three animals. Cells are reported on three-dimensional views of a macaque surface-based atlas brain reconstructed with the software CARET (Computerized Anatomical Reconstruction and Editing Toolkit, Van Essen *et al.*, 2001; <http://brainmap.wustl.edu/caret>), as described in Galletti *et al.* (2005). As shown in Fig. 2C–F, V6A cells extend from the medial surface of the brain through the anterior bank of the parieto-occipital sulcus to the most lateral part and fundus of the same sulcus (see also Galletti *et al.*, 1999, 2005).

A total of 207 V6A cells which underwent the reaching task fulfilled all the requirements to perform the quantitative analysis described in Materials and methods. When the task was performed in the central position of the panel, we found that 50% (97/195) of neurons were significantly modulated during M1 (outward reaching movement) with respect to FREE (free eye movements, no arm movements), 43% (83/195) during HOLD (hand holding) and 36% (71/195) during M2 (return movement towards the home button) (*t*-test, $P < 0.05$). If we consider V6A reaching activity in more than one position, we found that the percentage of cells modulated during action epochs increased considerably, being 78% (161/207), 72% (150/207) and 65% (134/207) for M1, HOLD and M2, respectively (*t*-test, $P < 0.05$). Considering the limited number of panel positions tested in our reaching task, and the fact that effectiveness of modulation increased when taking into account more positions, we conclude that our estimate of the incidence of reach-related activity in V6A is a conservative one.

Two examples of V6A neurons strongly modulated during arm movements are shown in Fig. 3. Outward reaches strongly inhibited the unit in Fig. 3A and strongly excited that in Fig. 3B. Inward reaches weakly excited the cell in Fig. 3A and inhibited that in Fig. 3B. Figure 4 shows two V6A cells almost completely unaffected by outward reaches but strongly excited by inward reaches.

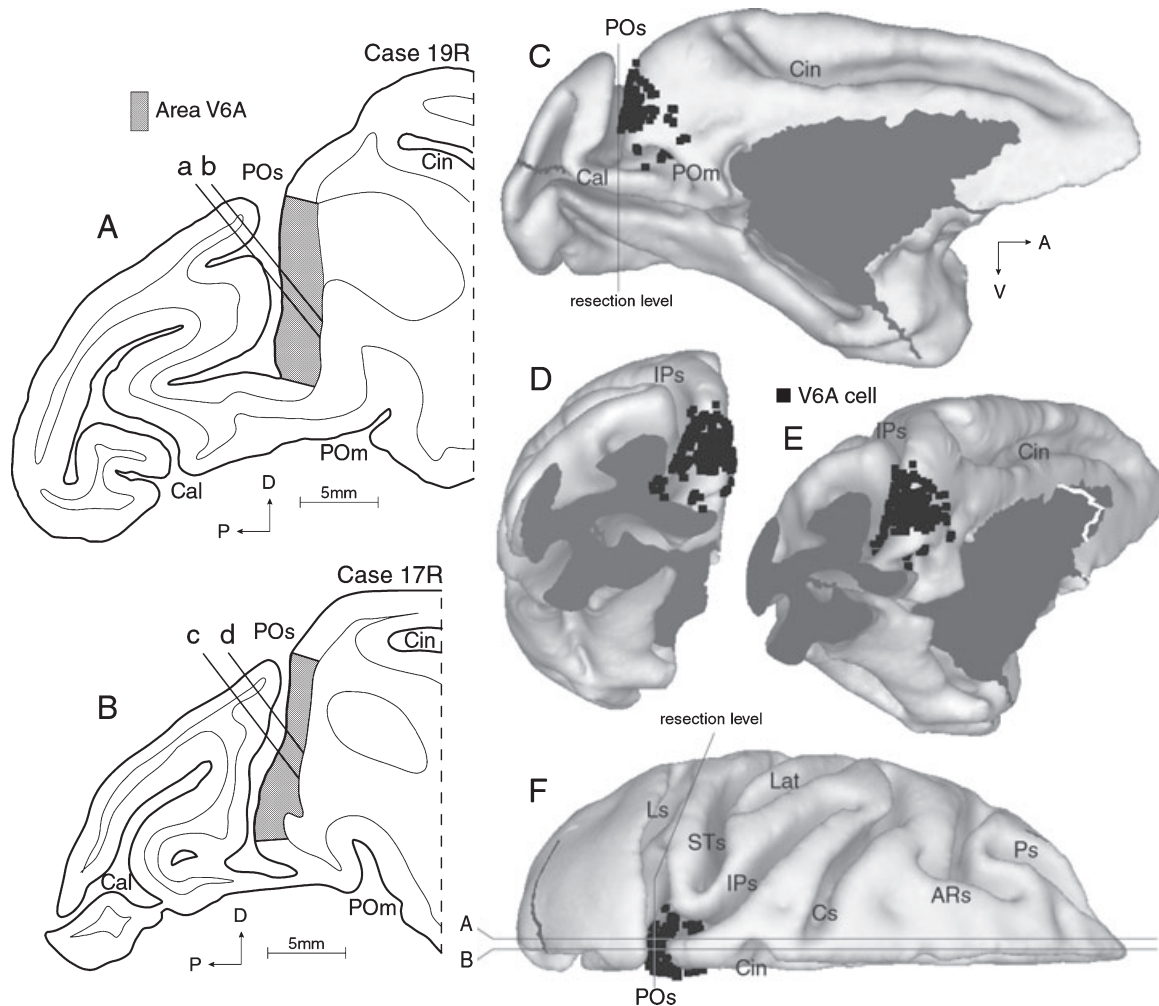


FIG. 2. Brain location of area V6A. (A and B) Posterior parts of two parasagittal sections of the monkey brain, with a reconstruction of four microelectrode penetrations (a–d) reaching deep into V6A (gray area). Sections were taken at the level shown in F. (C–F) Medial, posterior, postero-medial and dorsal views of the three-dimensional surface reconstruction of the left hemisphere of the atlas brain made with the software CARET (<http://brainmap.wustl.edu/caret>). Locations of cells recorded from V6A are shown as black squares on the three-dimensional brain reconstruction (see also Galletti *et al.*, 2005). In D and E, the occipital pole was cut away to show the entire anterior bank of the parieto-occipital sulcus (POs); the resection level is indicated in C and F. ARs, arcuate sulcus; Cal, calcarine sulcus; Cin, cingulate sulcus; Cs, central sulcus; IPs, intraparietal sulcus; Ls, lunette sulcus; Lat, lateral fissure; POm, medial parieto-occipital sulcus; STs, superior temporal sulcus; Ps, principal sulcus; A, anterior; P, posterior; V, ventral; D, dorsal.

Figure 5 shows other examples of reaching modulation, to stress the fact that a typical modulation did not exist in V6A. Almost every V6A reaching cell showed its own characteristic pattern of modulation in our task. The two units in Fig. 5A and B, for instance, were activated by both outward and inward reaching movements. On the contrary, the two units in Fig. 5C and D showed a modulation of opposite sign in M1 and M2. In addition, the neuron in Fig. 5C was inhibited during both M1 and the first part of HOLD; its activity increased in the last part of HOLD and the cell then maintained a high rate of discharge until the end of M2. The neuron in Fig. 5D showed the reverse behaviour, being excited during M1 and almost completely silent during HOLD and M2.

Like the neurons shown in Figs 3–5, most of the V6A reach-related cells (84%; 153/183) were modulated in more than one action epoch (see the larger pie diagram in Fig. 6). The great majority of these multi-action neurons (71%, 108/153) were modulated in all three action epochs, as shown in the smaller pie diagram in Fig. 6.

The hypothesis could be advanced that neurons showing different responses to different directions of movement are able to code the direction of movement. The cells reported in Figs 3, 4, 5C and D, for

example, could have this ability as they discharge differently for opposite directions of movement (outward and inward reaches differ 180° in direction). By comparing the neural discharges for reaches towards and away from the central position of the panel (the most studied location), we found that 67% of V6A neurons (91/136) were sensitive to the direction of reaching (*t*-test, $P < 0.05$).

Spatial tuning of reach-related activity

Following the hypothesis that reaching activity in V6A could encode the direction of movement, we compared the neural discharge of the same neuron in a given action epoch for movements towards or away from different spatial locations. We also compared the activity of the cell while the animal held its hand in different positions on the panel to look for a possible effect on the cell discharge of the arm position in space. As described in Materials and methods, we analysed the spatial tuning of the action epochs M1, HOLD and M2 only in those neurons in which we were able to study at least seven repetitions and in at least two target positions ($n = 169$).

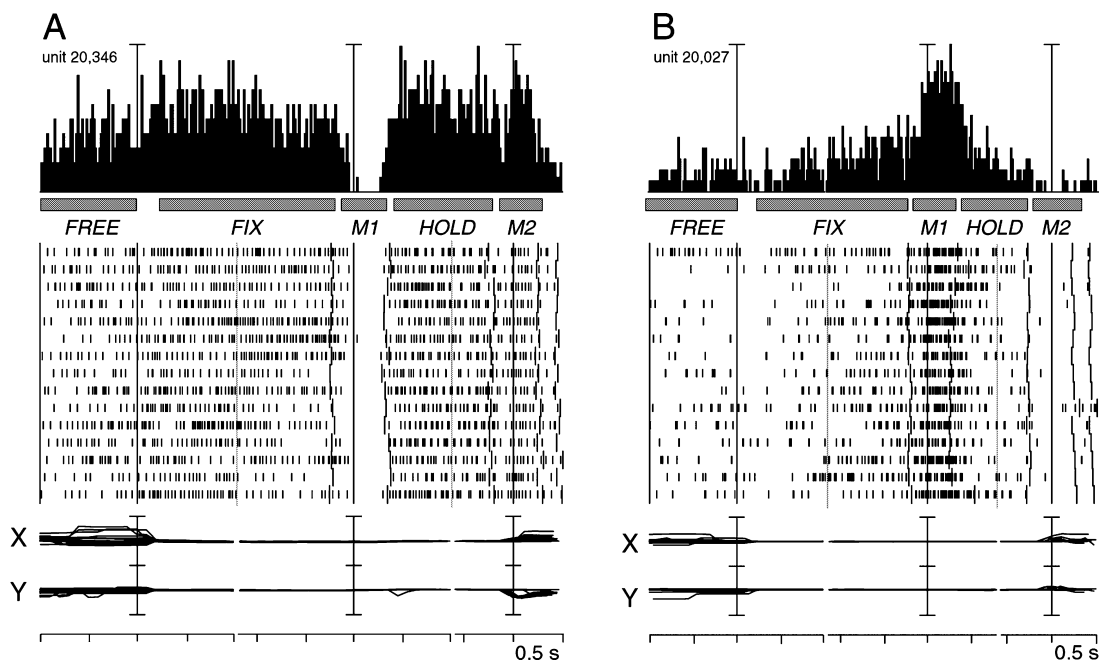


FIG. 3. Neurons modulated by outward reaches. (A and B) From top to bottom: peri-event time histograms, time epochs, raster displays of impulse activity and recordings of X and Y components of eye positions. Neural activity and eye traces are aligned three times for each neuron: with the light-emitting diode appearance (first), with the onset of outward (second) and with the onset of inward (third) movements. Peri-event time histograms: binwidth, 15 ms; scalebars, 45 spikes/s (A), 60 spikes/s (B). Eyetraces: scalebar, 60°. Other details as in Fig. 1.

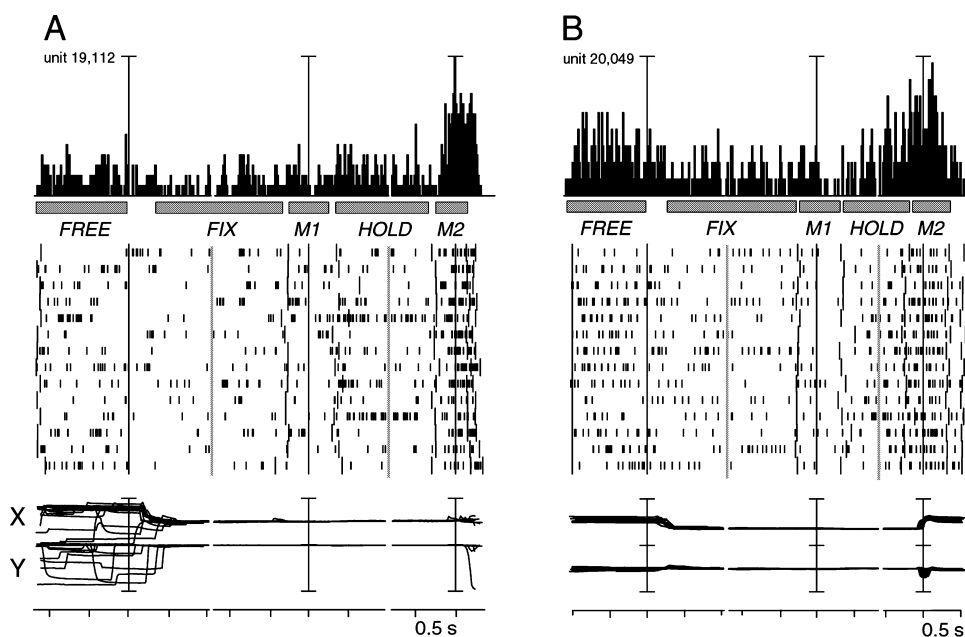


FIG. 4. Neurons modulated by inward reaches. Scalebars in peri-event time histograms: 65 spikes/s (A), 40 spikes/s (B). Other details as in Figs 1 and 3.

The spatial tuning of action epochs in V6A reaching cells differed from cell to cell. Figure 7 shows three examples of cells spatially modulated in at least one action epoch. The unit in Fig. 7A discharged more vigorously for reaches (M1 epoch) directed rightward. In this unit, a strong effect of gaze on neural activity was also evident as the discharge rate during FIX epoch increased when fixations were directed towards more rightward locations. The cell in Fig. 7B was more excited for reaches directed towards the central position of the panel with respect to reaches directed leftward or rightward. The unit in Fig. 7C was modulated only during inward reaching movements

(M2 epoch) and only when the movement started from the right part of the panel.

Figure 8 shows the response of a V6A neuron tested for movements towards and away from nine target positions. The neuron showed different discharges in all epochs (FIX, M1, HOLD and M2) according to the different target positions. During FIX, the cell discharged strongly when the animal looked leftward and downward and weakly, or not at all, when it looked rightward and upward. The differences in discharge rate among positions were statistically significant (ANOVA, $P < 0.01$). During arm movements, the cell discharged moderately or

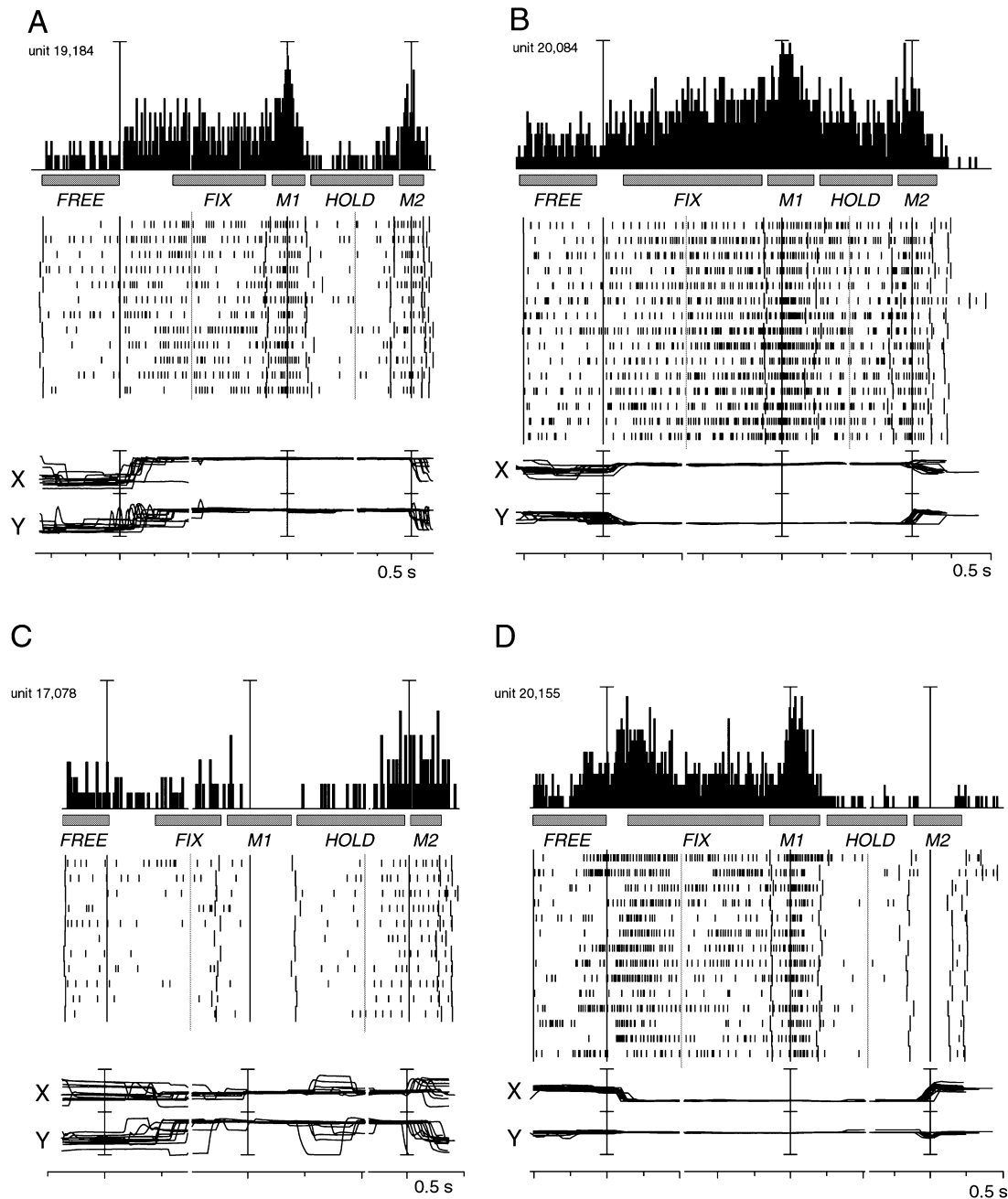


FIG. 5. Multi-action neurons. (A and B) Examples of neurons activated by outward and inward reaching movements. (C and D) Examples of neurons activated in one reaching movement and inhibited in the opposite reaching movement. Scalebars in peri-event time histograms, 50 spikes/s. Other details as in Figs 1 and 3.

not at all during outward reaching movements (M1) but it discharged strongly during inward reaching movements (M2). Both movement activities were significantly spatially modulated (ANOVA, $P < 0.01$), M1 preferring leftward directions of reaches and M2 reaches coming from the right part of the peripersonal space. The activity of the cell was also significantly spatially modulated during the HOLD epoch (ANOVA, $P < 0.01$). It is noteworthy that the spatial tuning was similar for FIX, M1 and HOLD (higher activity in the left and bottom part of the panel), whereas for M2 the highest activity was observed when the animal worked in the right part of the panel, in which the activity in FIX, M1 and HOLD was very low. Observing the examples of V6A spatially tuned neurons shown in Figs 7 and 8, it is evident that spatial tuning of neural activity during the different action epochs could coexist in the same cell or not and, when present in the same cell, the

spatial tuning of neural activity could vary differently from one action epoch to another.

Like the cells reported in Figs 7A and 8, many other V6A neurons (91/169; 54%) showed a statistically significant spatial modulation during FIX. In our reaching task, FIX modulations could be due to the preparation of reaching movement and/or to the modulating effect of the direction of gaze. We have no means of disentangling the two phenomena in our experimental conditions and new experiments would be needed for this purpose. However, as V6A is rich in neurons modulated by gaze (Galletti *et al.*, 1995; Nakamura *et al.*, 1999), and during FIX the animal maintained fixation of the visual target to be reached, it is likely that the spatial tuning observed in FIX is at least partially due to a gaze modulation on cell activity.

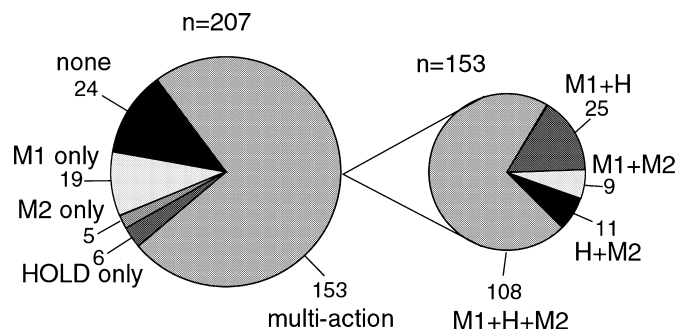


FIG. 6. Incidence of the different types of neural modulations observed during the reaching task. The bigger pie diagram shows fractions of area V6A cells with and without significant differences in neural discharge in the action epochs of the task: none, no significant modulation during reaching; M1 only, neurons significantly modulated only during outward reaching movements; M2 only, neurons significantly modulated only during inward reaching movements; HOLD only, neurons significantly modulated only during arm holding in the peripersonal space; multi-action, neurons modulated in more than one action epoch. The smaller pie diagram represents the incidence of the different types of multi-action modulations. H, HOLD epoch.

The hypothesis could also be advanced that the spatial tuning which we observed in M1 and HOLD was due to the gaze effect, because during both of these epochs the animal maintained fixation of the visual target. Although we do not know whether or not the gaze effect adds linearly to the activity in action epochs, to reduce its possible effect on action discharges we subtracted the activity during FIX from that in M1 and HOLD in all tested cells, obtaining an activity virtually devoid of the gaze effect which we called 'proper M1' and 'proper HOLD', respectively. After this correction for gaze effect, the neurons reported in Figs 7A and 8 remained significantly spatially tuned in M1 and HOLD (ANOVA, $P < 0.01$). Many other (although not all) V6A cells whose activity was affected by the direction of gaze turned out to be significantly spatially modulated even after subtraction of the gaze effect. Figure 9 compares the percentages of V6A neurons that were spatially modulated in epochs M1 and HOLD without and with subtraction of gaze effect ('raw' and 'proper' M1 and HOLD, respectively). As expected, the percentage of neurons modulated during the 'proper' epochs is lower than that found for 'raw' epochs. This means that in a number (about one-third) of V6A neurons, the apparent spatial modulation during movement and hand holding times could actually be due to the direction of gaze. However, data in Fig. 9 show that 40% of V6A cells remained spatially modulated during M1 and HOLD even after subtraction of gaze effect (68/169; 40% for either epoch), indicating that many cells in V6A were really modulated by the direction of reaching or by the arm position in space.

From now on, we will use the term 'spatially tuned' for a cell in a given action epoch only when the 'proper M1' and 'proper HOLD' were significantly modulated (even if no longer specified in the text).

Like several of the examples shown in Figs 7 and 8, many V6A neurons in our population (79/169; 47%) were spatially tuned in epoch M2. We did not calculate a 'proper M2' (subtracting the activity of FIX from that of M2) because very often during M2 the animal broke fixation (see Materials and methods). As V6A contains neurons modulated by saccadic eye movements (Kutz *et al.*, 2003), we took into account the possibility that the neural discharge during M2 could actually be due to the occurrence of the saccadic eye displacements that intervene at the end of trials. To disentangle this point, we performed a supplementary analysis in all cells with M2 discharge by

correlating neural activity with eye traces on a trial-by-trial basis. An example of this single trial analysis is shown in Fig. 10.

Both units reported in Fig. 10 showed a consistent activation during M2 without any other significant modulation. The trial-by-trial analysis reported below the cumulative data in Fig. 10 demonstrates that the discharges in epoch M2 were present in all trials for both cells, whether or not the animal maintained fixation (asterisks mark trials without fixation breaks during M2). We classified as modulated in M2 only those units in which neural discharge during this epoch was not dependent on oculomotor activity, as for the cells reported in Fig. 10.

Preferred reach directions and spatial locations

In an attempt to find the preferred direction of outward (M1) and inward (M2) reaching movements, as well as the preferred spatial location during HOLD, we determined for each action epoch the resultant mean vector of discharge for each tested neuron (see Materials and methods). We calculated the distribution of vector endpoints along the ipsilateral/contralateral axis and top/bottom axis, obtaining two histograms for each epoch (see Fig. 11) representing the distribution of preferred positions for the top/bottom and ipsilateral/contralateral subspaces, respectively. We tested whether there was an over-representation of vector endpoints with respect to the number of tested positions in each part of the workspace (chi-squared test, $P < 0.05$).

The distributions of vector endpoints for our cell population are plotted in Fig. 11. Zero represents the sagittal plane in ipsilateral/contralateral plots (left column of Fig. 11) and the horizontal plane passing at eye level in top/bottom plots (right column of Fig. 11). Ipsilateral/contralateral refers to the recording side. Apart from the peak of data around zero, due to oversampling of central positions, no laterality effects were evident for either epoch M1 or M2. Considering the distribution of vector endpoints in each sector of the workspace, there was no skewing of reach directions in a sector of the workspace explored (chi-squared test, n.s. for all plots).

Preferred spatial locations of hand holding are shown in the plots of the bottom part of Fig. 11. Although a bias towards the ipsilateral bottom parts of the working space seems to be present in these plots, it does not reach any statistical significance (chi-squared test, n.s. for ipsilateral/contralateral and top/bottom plots). Preferred spatial locations of hand holding seem to be uniformly represented across the workspace.

In conclusion, each single V6A cell shows a clear preference for movement direction and location of the arm in space but the population of V6A spatially tuned neurons as a whole does not code preferentially top, bottom, ipsilateral or contralateral directions of reaching or holding positions.

Somatosensory modulations of reach-related cells

Some of the neurons modulated during the reaching task were also tested with passive somatosensory stimulation ($n = 44$). About 30% of them (13/44) were driven by somatosensory stimuli. As shown in the histogram of Fig. 12, about half of them (7/13) were modulated by proprioceptive inputs ('joint') and the others (6/13) by passive tactile stimulation ('skin' or 'deep'); none of them responded to both tactile and proprioceptive stimuli. The great majority of V6A reach-related cells (31/44, 70%) did not respond to any of our somatosensory stimuli.

The bottom part of Fig. 12 shows the distribution of passive somatosensory receptive fields on the monkey soma; the seven 'joint' neurons were all driven by passive rotation of the shoulder and the

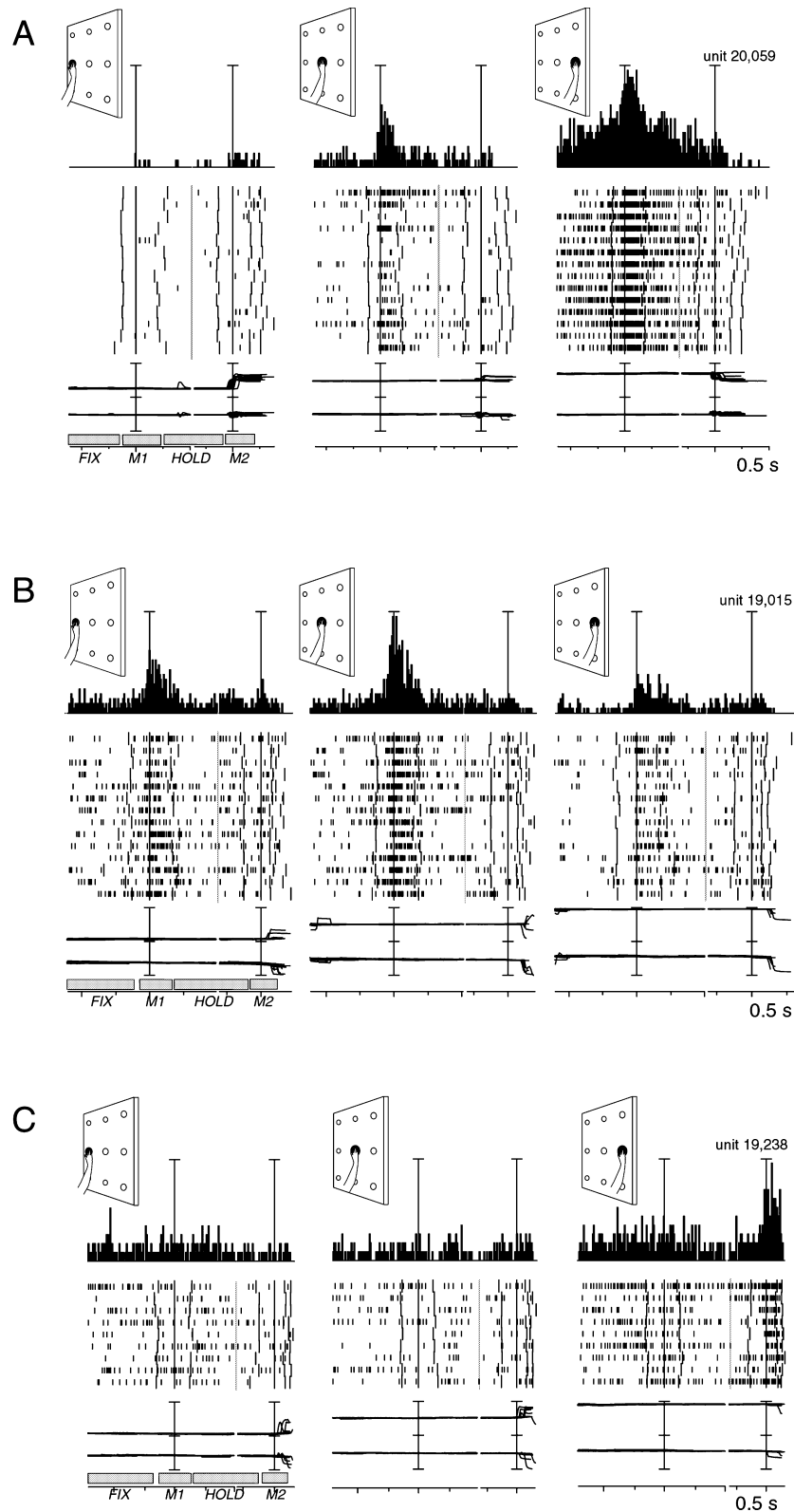


FIG. 7. Examples of spatially tuned modulations of reach-related activity. (A) Neuron spatially tuned in M1, preferring rightward M1 movements. Each inset contains the peri-event time histogram, raster plots and eye position signals and is positioned in the same relative position as the target on the panel, as sketched in the top left corner of each inset. Neural activity and eye traces have been aligned twice in each inset, with the onsets of outward (first) and inward (second) reach movements. The mean duration of epochs FIX, M1, HOLD and M2 is indicated in the bottom left inset. Scalebar in peri-event time histograms, 70 spikes/s. (B) Neuron spatially tuned in M1, preferring reaches directed to the central position. All conventions are as in A. Scalebar in peri-event time histograms, 100 spikes/s. (C) Neuron spatially tuned in M2, preferring inward movements from the right-most position. All conventions are as in A. Scalebar in peri-event time histograms, 85 spikes/s. Other details as in Figs 1 and 3.

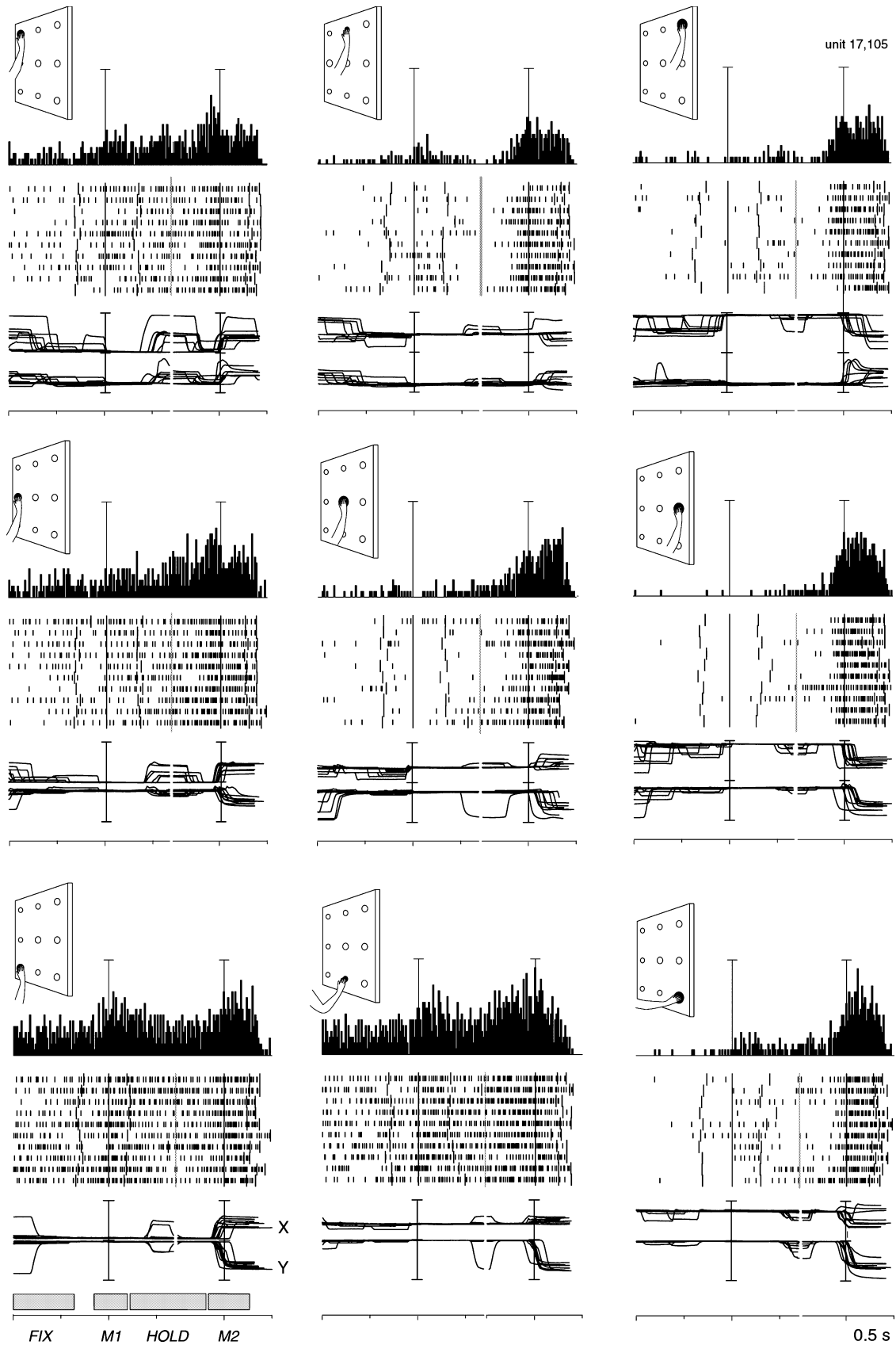


FIG. 8. Spatially tuned modulation of reach-related activity. Scalebar in peri-event time histograms, 110 spikes/s. Other details as in Figs 1, 3 and 7.

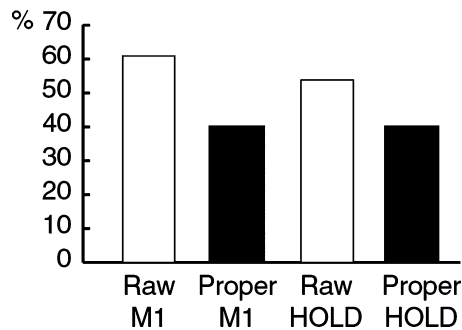


FIG. 9. Incidence of area V6A cells spatially modulated in the reaching task. Columns indicate the percentages of spatially modulated V6A cells during outward reaching movements (M1) and static position of the arm in the peripersonal space (HOLD). 'Proper' activity = 'raw' activity - FIX activity (see text). Other details as in Fig. 1.

receptive fields of the tactile neurons (three 'skin' and three 'deep') were predominantly located on the upper limb and shoulder. The distribution of somatosensory receptive fields on and near the arm is typical for V6A (Breveglieri *et al.*, 2002).

Passive somatosensory stimulation could evoke neural modulations during active arm movements like those performed in the reaching task. For example, the cell shown in Fig. 8 was a neuron modulated by proprioceptive inputs ('joint'), specifically by passive adduction and abduction of the contralateral shoulder. It could be that at least part of the neural modulation observed during the reaching task was due to the joint stimulation.

Figure 13 shows an example of spatially tuned modulation in a cell provided with a tactile receptive field ('skin') on the contralateral shoulder. In the reaching task, this neuron discharged only during outward reaches directed upward and during hand holding in the central and uppermost positions on the panel. As the tactile receptive field was located on the arm performing the reaching task (contralateral shoulder), the spatial modulation observed in the reaching task could be due to an auto-stimulation of this tactile receptive field produced by arm movement. Interestingly, the neural response was stronger when the hand reached the upper part of the panel, hence when the skin of the shoulder was maximally deformed, and arose late in the M1 epoch, as expected for a modulation driven by a somatosensory stimulation.

As reported above, the great majority of V6A reach-related cells were not activated by passive somatosensory stimuli. However, we cannot discard the hypothesis that all the modulations observed in the action epochs during the reaching task could be due to passive somatosensory stimulation, even when we were not able to find any tactile receptive field or sensitivity to joint stimulation. To investigate this possibility, we assessed the latency of the neural response during M1 to check whether it was compatible with the timing of somatosensory afferent signals.

We assessed the neural latency with respect to the movement onset in 60 V6A cells and the result of this work is shown in Fig. 14. Reach-modulated neurons showed a latency in their neural response ranging from 180 ms before movement onset to 100 ms after it, with a mean of -47 ms. In about 28% of tested neurons (17/60) the neural discharge started after the onset of movement, as was the case for the cell in Fig. 13. It is thus conceivable that the neural response of this 28% of V6A cells is the result of a somatosensory input.

For the remaining 72% of reach-related cells (43/60), the neural discharge began before the onset of reaching movement. Somatosensory inputs could arise before the onset of movement if they are

elicited by the initial muscular contraction, which precedes the actual movement of the limb. Figure 14 reports the cumulative distribution of the earliest EMG activity recorded from neck and upper limb muscles during the performance of the reaching task. Among the 43 cells discharging before the movement onset, 31 started to discharge after the earliest EMG activity. Thus, in principle, their discharge during reaching movements could be explained as an effect of a somatosensory input. However, because tens of milliseconds are needed for proprioceptive signals to reach V6A, we estimate that the responses of no more than half of these cells could be explained by proprioceptive signals or tactile stimulation. In any case, about 20% (12/60) of reach-related cells started to discharge before the earliest EMG activity. For these neurons, at least for the earliest part of their discharge during reaching, it is unlikely that the neural discharge can be ascribed to somatosensory stimulation.

Discussion

In everyday life, we reach for objects all around us, usually gazing at the object we want to grasp. Here, we tried to reproduce this natural situation by leaving the monkey to gaze at the target to be reached and by asking it to reach the target with a direct, ballistic arm movement.

We found that reaching movements modulate the neural activity of about 70% of V6A cells. Strong modulations (excitation or inhibition) were observed either when arm movements were directed outward, towards visual objects in the field of view, or inward, towards targets located near the body and outside the field of view. Most V6A reaching neurons (84%) were modulated in more than one phase of the task and nearly every possible pattern of neural modulation was found.

The reach-related activity was not the result of visual stimulation, as the task was performed in circumstances that minimize the role of vision in reaching; outward and inward reaching movements were performed in darkness, the first towards a very dim small visual target and the second towards an invisible target located outside the field of view. The presence of significant reach-related discharges when the reaching task was performed in darkness indicates the existence of a relationship between V6A cell activity and arm movement which was independent of visual feedback. Considering this fact, and the fact that we carefully checked trial by trial the correlation of neural activity with eye movements, we are confident that the observed modulation is not dependent on visual input, or on eye-movement signals, although we know that both types of input can modulate V6A cells (Galletti *et al.*, 1995, 1999; Nakamura *et al.*, 1999; Kutz *et al.*, 2003).

We found that about 40% of V6A reaching neurons show reach-related activity that was spatially tuned, in that different directions of movement evoked different neural discharges, regardless of whether the arm movement was directed towards visual or non-visual targets. We are aware that our estimate of the incidence of spatially tuned neurons is conservative because of the limited number of positions (i.e. directions) which we tested. Had we tested a higher number of positions and explored a larger part of the workspace, we would probably have disclosed a further increase in the percentage of spatially tuned neurons.

Do V6A reach-related neurons really encode the direction of movement?

In our task, during the outward reaching movements (M1), the monkey gazed at different spatial locations because it fixated the target of reaching, which was placed in different positions. Therefore, it is plausible that a gaze effect, known to have strong modulating

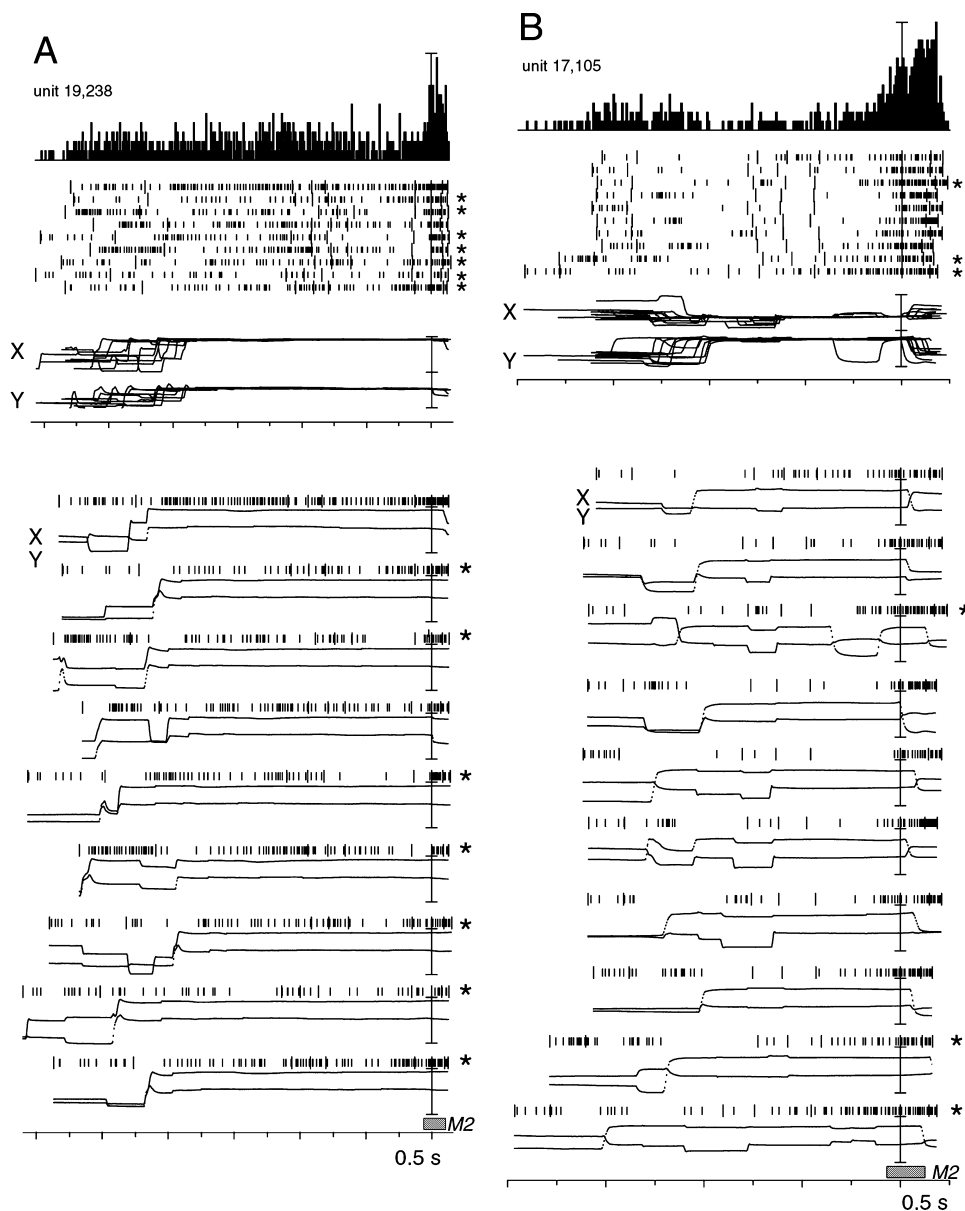


FIG. 10. Single-trial analysis of the correlation between ocular movements and neural discharge. Neural activity and eye traces are aligned with the onset of inward reaching movement. Top: peri-event time histograms, raster displays and eye traces, as explained in Figs 1 and 3. Bottom: raster plots and eye-movement recordings of each trial are shown separately; below each raster plot of spike train, the concurrently recorded eye position signals are reported. Trials without changes in gaze direction during M2 have been marked on the right with an asterisk. The neurons shown in A and B are the same as those reported in Figs 7 and 8. Scalebars in peri-event time histograms: 85 spikes/s (A), 80 spikes/s (B).

influences in V6A (Galletti *et al.*, 1995; Nakamura *et al.*, 1999), was responsible for the modulation of neural discharge during M1 epoch. For this reason, we subtracted the mean activity during fixation periods (FIX) from the mean discharge during M1 epoch to obtain a movement-related activity virtually devoid of the gaze effect. We found that most cells with a spatially tuned reaching activity remained spatially tuned even after subtracting FIX from arm movement discharges.

We are aware that the gaze could have a non-linear multiplicative effect on arm movement-related discharges, so the possibility that at least part of the spatial tuning of reach activity is due to a non-linear interaction with the gaze effect remains open. We are also aware that neural activity during FIX could reflect reach preparation, or intention to move, known to modulate the neural activity in areas neighboring V6A (Snyder *et al.*, 1997; Calton *et al.*, 2002) as well as in V6A itself

(Fattori *et al.*, 2001). Thus, when we subtract FIX from reach-related discharges we also eliminate the effect of a possible modulation due to the movement planning. We are currently devising specific experiments to separate the gaze effect from the effect of movement preparation (or intention to move) during FIX period.

The spatial tuning of reach-related activity could reflect the coding of spatial coordinates of target location, instead of the coding of direction of movement. We are inclined to discard this hypothesis because the spatial tuning was also observed in M2 (inward movements), and in M2 the target of reaching (home button) was always in the same spatial location, the different arm trajectories being due to differences in spatial location of the starting points. The presence of spatial tuning in M2 agrees with the view that these neurons encode the direction of reaching.

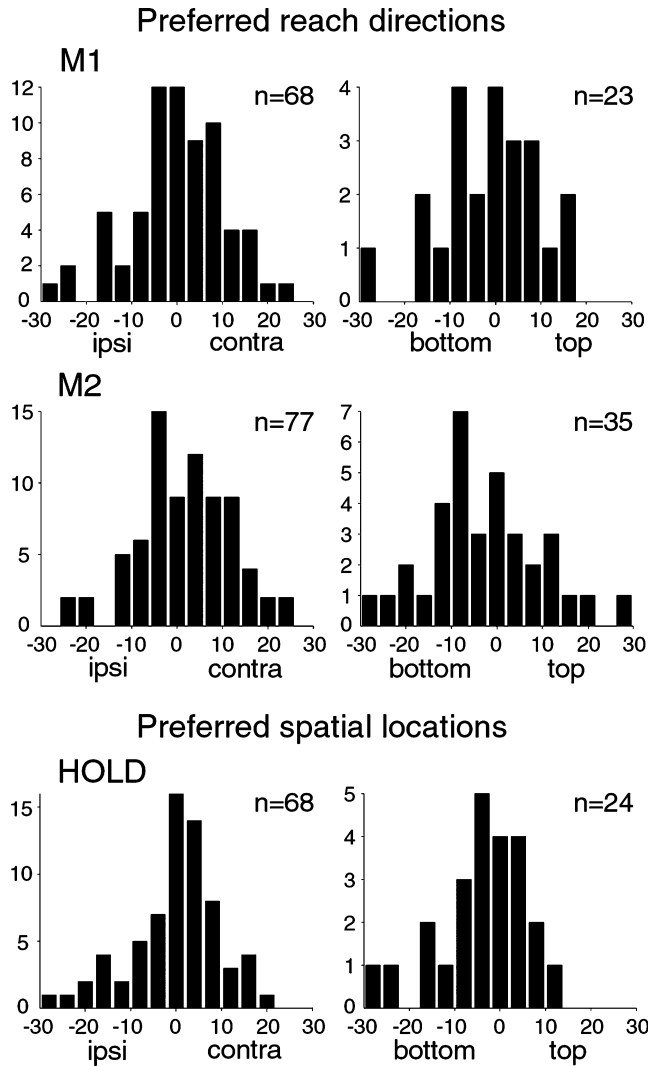


FIG. 11. Preferred reach directions and spatial locations in area V6A. Each histogram reports the frequency distribution of the resultant mean vector of discharge rate for each neuron modulated in a given action epoch (see text). Preferred reach directions for outward and inward reaches and preferred spatial locations for HOLD have been plotted twice, ipsilateral/contralateral (ipsi/contra) with respect to the recording side and bottom/top with respect to eye level. Zero in the plot represents the sagittal plane in ipsi/contra plots and a horizontal plane at eye level in bottom/top plots. For all histograms: horizontal axis shows degrees of visual angle (binwidth, 4°), and vertical axis shows the number of cells.

Movements in M1 and M2 differed in many aspects. They are directed away (M1) or towards (M2) the body and towards visual (M1) or non-visual (M2) targets. During M1 the animal maintained fixation, whereas during M2 it often made saccades. Moreover, in M1 the monkey gazed at the target of reaching while in M2 it did not, the target being outside its field of view. In spite of all these differences, the patterns of spatial tuning of M1 and M2 activities were qualitatively and quantitatively similar in V6A both at single cell and population level, suggesting again a directional coding for V6A reach-related cells.

At the population level, we did not see a particular sector of the workspace preferred with respect to others (see Fig. 11). This suggests that the population of V6A spatially tuned neurons is able to code the entire set of directions which we tested. This lack of laterality effect in

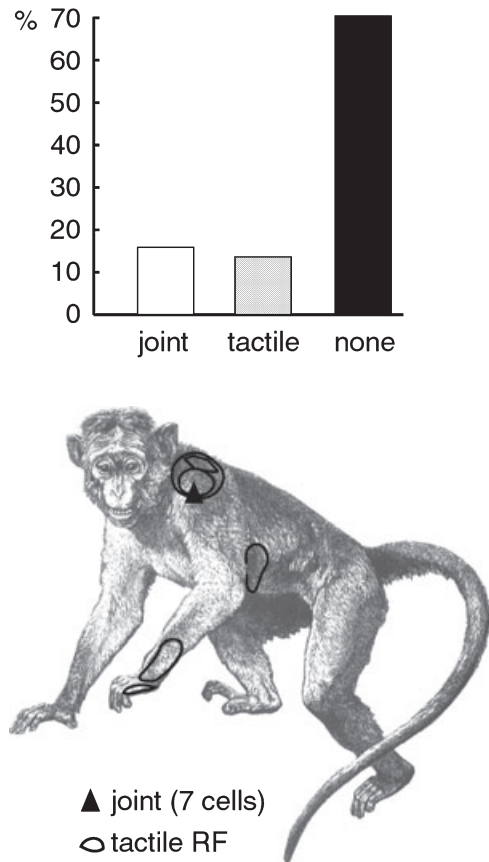


FIG. 12. Incidence of passive somatosensory modulations in reach-related cells. Top: frequency distribution of area V6A reach-related cells with responses to somatosensory stimulation. Bottom: location and types of somatosensory receptive fields of reach-related neurons. All somatosensory receptive fields have been reported on the left side of the body. RF, receptive field.

spatial tuning seems to be characteristic of SPL areas (see also Battaglia-Mayer *et al.*, 2000). On the contrary, in the inferior parietal lobule (area 7a), the contralateral space is over-represented (MacKay, 1992; Battaglia-Mayer *et al.*, 2005).

Not all the reaching cells showed an opposite behavior for opposite directions of movement, as would be predicted for cells which strongly encoded movement direction. As shown in Fig. 5A and B, some cells were almost equally activated by M1 and M2, i.e. for opposite directions of movement. It could be that the best and worst directions for those cells were orthogonal to the tested direction but this is a speculation and has yet to be demonstrated. At present, we also do not know whether this coding process refers to mechanical parameters, as we did not vary the load applied to the monkey arm, or to kinematic parameters, as we did not vary the spatial configuration of the arm during reaching. Tackling this problem will be the object of future studies.

Several reaching neurons are influenced by the position in space of target, gaze, limb and possibly other factors, alone or in conjunction. We conclude that, although the direction of movement strongly modulates the activity of reach-related cells in V6A, direction tuning alone is not a complete paradigm for understanding the behavior of these cells. Their activity probably reflects a combination of many retinal and extraretinal parameters that together qualify the whole act of prehension.

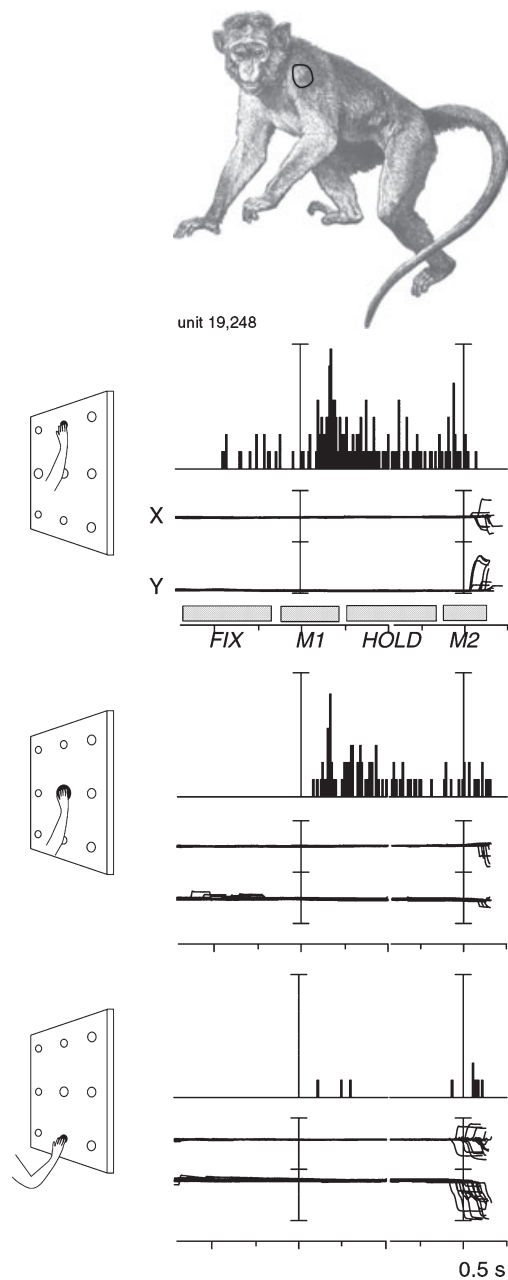


FIG. 13. Spatial tuning of reaching activity in a neuron sensitive to somatosensory stimulation. Top: location, on the contralateral shoulder, of the cell's tactile receptive field. Bottom: spatial tuning during M1 and HOLD while the animal reached targets in different positions. Scalebar in the peri-event time histogram, 30 spikes/s. Other details as in Figs 1, 3 and 7.

Spatial coding of hand/arm position in V6A

We found significant differences in neural activity during arm holding in different spatial locations (HOLD epochs). The spatial tuning of HOLD activity did not reflect a gaze effect, as spatial tuning in HOLD was still present in 40% of our population after subtraction of FIX activity from HOLD activity, and nor did it reflect the vision of the hand in the field of view, as the task was performed in darkness.

The HOLD activity could code different spatial locations of the hand in the peripersonal space, as suggested for neurons in several cortical regions in the caudal part of the SPL (Ferraina *et al.*, 1997, 2001; Battaglia-Mayer *et al.*, 2000, 2001), or the arm posture, as

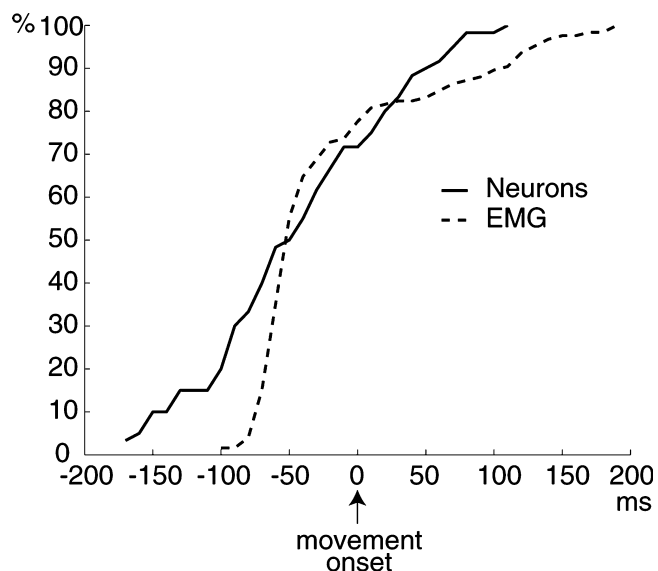


FIG. 14. Comparison between the latencies of area V6A reach-related activity and of electromyographic (EMG) activity in the reaching task. Plots are cumulative frequency distributions of the latencies of the neural responses to outward reaching movements and of the EMG activity recorded during outward reaching movements. The horizontal axis shows time in ms, and the vertical axis the percentage of V6A tested cells ($n = 60$) or of muscle EMG activation ($n = 12$).

suggested for neurons of area 5, in a more anterior sector of the PPC (MacKay, 1992; Lacquaniti *et al.*, 1995). In area 5, almost all arm-movement-related neurons are modulated by the intrinsic features of the movement, like different arm orientations during reaching (Scott *et al.*, 1997). Although we did not attempt this study in V6A, the changes in neural activity during hand holding in different spatial positions, i.e. during the static posture of arm in space without any transport component, suggest a possible involvement of V6A in coding arm geometry.

Sources of reach-related responses in V6A

As V6A contains neurons sensitive to somatosensory stimuli (Breveglieri *et al.*, 2002), the observed spatial tuning of reaching activity may be due to somatosensory inputs, i.e. proprioceptive or tactile signals from the moving limb. To assess the possible role of afferent inputs in the reach-related discharges observed in V6A, we analysed the latency of the neural response to reaching movement. We found a consistent number of units discharging before the onset of reaching movement, some of them even before the earliest EMG activity that we recorded. For these latter neurons, it is unlikely that the somatosensory input is the only source of reaching responses. It is, however, possible that there was muscular activity starting well before trial onset that we were not aware of and that could be responsible for the neural modulations which we observed.

The fact that in V6A active arm movements are often more effective than passive movements in activating the cells (Galletti *et al.*, 1997) agrees with the view of reach-related discharges in V6A as more related to active signals than passive somatosensory signals, suggesting that copies of efferent signals delivered from motor structures commanding the arm movements impinge upon V6A reaching cells. Areas F2 and F7 of the dorsal premotor cortex, reciprocally connected to V6A (Matelli *et al.*, 1998; Shipp *et al.*, 1998), seem good candidates for this job.

Comparison with other studies in the superior parietal lobule

The present report is the first extensive and systematic study reporting spatially tuned reach activity in V6A.

Some previous studies referring to V6A (Battaglia-Mayer *et al.*, 2000, 2001) did not record neural activity in the anterior bank of the parieto-occipital sulcus and pre-cuneate cortex, where V6A is located (see Fig. 2, see also Galletti *et al.*, 1996, 1999). They recorded single unit activity from the dorsal surface of the caudal SPL (see Fig. 2 of Battaglia-Mayer *et al.*, 2000), hence putatively from area caudal area PE (PEc) (Pandya & Seltzer, 1982). Nakamura *et al.* (1999) reported neurons spatially tuned in a reaching task carried out in darkness, in the most dorsal part of V6A. Their study, however, was not exhaustive, reporting data from only four cells.

The spatial tuning of reaching activities is not a new finding for the caudal SPL. Anterior and lateral to V6A is the parietal reach region, involved in coding the intention of arm movements (Snyder *et al.*, 1997). Intention activity is clearly spatially modulated but it is not known whether a spatial modulation also occurs during ongoing movements.

Dorsal and medial to V6A are areas PEc and medial area PG (PGm) (Pandya & Seltzer, 1982); spatial tuning of reach-related activity has been found in the majority of neurons in these two areas, with characteristics similar to those reported here for V6A (Ferraina *et al.*, 1997, 2001; Battaglia-Mayer *et al.*, 2000, 2001). In areas PEc and PGm, gaze-related responses were also reported but the influence of gaze on reach-related activity was analysed only for reaching movements in light, not when the reaching task was performed in darkness. Anteriorly and laterally to V6A is area medial intraparietal area (MIP) located in the caudal half of the medial bank of the intraparietal sulcus (Colby *et al.*, 1988). In the anterior part of the MIP, Johnson *et al.* (1996) found that the majority of cells showed activity modulations related to the direction of arm movements, or to the position in space of the hand, similar to our findings in V6A. However, as eye movements were not recorded in the MIP study, it is impossible to know how many of the spatially tuned cells in the MIP were modulated by the direction of gaze rather than, or together with, the direction of arm movements.

A key difference in our study with respect to the others carried out in the caudal SPL is the use of a different reaching task, where an outward reaching movement brings the hand from the body to a target in the peripersonal space, and an inward reaching movement brings the hand back towards the body. The task used in all the above-mentioned studies, on the contrary, required a translation of the hand on a frontal plane (the so-called center-out task). In the past, reaching tasks similar to that used here have been used to study directional tuning of reaches in anterior sectors of the PPC (Mountcastle *et al.*, 1975; MacKay, 1992; Lacquaniti *et al.*, 1995). In their pioneer studies, Vernon Mountcastle and coworkers found that cell activity in area 5 did not appreciably differ when the animal reached to various points in the space in front of it. In contrast, a later study by Kalaska *et al.* (1983) concerning the same cortical region, but using a planar reaching task, found a high number of neurons sensitive to the direction of reaching. The difference between the two results was suggested to be due to the different tasks. This view received strong support from the work of MacKay (1992), who directly demonstrated that center-out and body-out reaching tasks differently affected neural activity in parietal areas. All together, these data suggest that different neural mechanisms are probably involved in executing hand translations in center-out tasks and hand movements in body-out tasks. Accordingly, conclusions about differences observed in neural modulations during these two tasks must take into account the differences between the tasks.

The present study, using a body-out/in reaching task, disclosed that about 40% of V6A reach-related neurons were spatially tuned, a value that increases up to about 60% if we do not subtract the gaze effect. Compared with the data of Mountcastle *et al.* (1975), this suggests that V6A is more sensitive than area 5 to the direction of movement. However, it could be that the difference between results of the two studies is due to differences in the task, our task being more demanding in terms of arm geometry than that of Mountcastle *et al.* (1975) (where targets of reaching were horizontally aligned along a semicircle). Task demand in terms of arm geometry could be a critical factor for activating V6A reach-related cells, as it is for activating reach-related cells in area 5 (Scott *et al.*, 1997). Further experiments are needed to study this issue.

Our task is also more demanding than the classic center-out task in term of precision of the motor act, in that monkeys were required to direct their hand towards small targets. This was particularly true in the outward reaches, where the animal had to reach out and press an LED placed at arm's distance. In the inward reaches, the target was larger (home button, 2.5 cm in diameter) but reaching movements needed to be accurate because the target was located outside the visual field and had to be localized relying only on memorized somatosensory coordinates. In all the other studies carried out in the caudal SPL (Ferraina *et al.*, 1997, 2001; Battaglia-Mayer *et al.*, 2000), the hand was moved from one position to another on a touch screen and the monkey had to touch inside the limits of a defined electronic window, whose size (about 10°) was much larger than that of the target in our task (about 1.5°). It could be that different demands in the two tasks did influence the neural sensitivity to arm movement in a different way.

It is difficult to compare with others our results on neural modulations during inward reaching movements. To our knowledge, there are no other studies carried out in the SPL investigating the directional tuning in hand movements from a target in the field of view to a target outside the field of view. Neural discharges to such a type of hand movements were actually collected in area 5 but the spatial tuning of neural activity was not analysed in that study (MacKay, 1992).

Role of V6A in visuomotor processes

It has been suggested that internal representations of the world and of one's own body are needed to relate ourselves to the external world (Wolpert *et al.*, 1998). These representations derive from the concurrent computation of sensory inputs and motor outputs. The hypothesis is that we continuously estimate both the configuration of body parts (i.e. joint angles and arm position) and their interaction with the peripersonal space (i.e. contact with objects) and update this representation over time (Kalaska *et al.*, 1983; Kalaska & Crammond, 1992; Johnson & Ferraina, 1996). There is considerable evidence that the SPL plays a key role in maintaining an internal estimate of both the external world and one's own body (Wolpert *et al.*, 1998; MacDonald & Paus, 2003). We believe that V6A plays a crucial role in this job.

The convergence on V6A of both motor signals related to arm movement (from dorsal premotor cortex, Shipp *et al.*, 1998; Galletti *et al.*, 2001) and sensory signals (visual and somatosensory inputs, Galletti *et al.*, 1996, 1999; Breveglieri *et al.*, 2002) suggests a function of this area in monitoring the arm-object interaction. Actually, V6A has visual neurons appropriate to perform the visual control of prehension movements (see Galletti *et al.*, 2003 for a review); it contains cells sensitive to stimulus orientation and direction of motion (Galletti *et al.*, 1996), neurons whose receptive fields are centred on the fovea (Galletti *et al.*, 1999) and visual neurons able to code directly and indirectly the position in space of visual objects (Galletti

et al., 1993, 1995; Galletti & Fattori, 2002 for a review). We here demonstrate that somatosensory and corollary motor signals allow V6A cells to monitor the direction of reaching movements needed to interact with objects in peripersonal space, within and outside the field of view, as well as the position of the arm in space. In other words, V6A neurons might participate not only in the analysis of visual space, as previously suggested (Galletti & Fattori, 2002), but also in the on-line control of arm movement (see Galletti *et al.*, 2003) elaborating sensory inputs and motor outputs to represent the internal body state for the purpose of sensorimotor integration.

Recent data have suggested that V6A reach-related cells could contribute to coding the entire act of prehension, including the distal aspects of prehension movements (Fattori *et al.*, 2004). Reach-related cells in V6A could be used for the on-line control of the direction of prehension movements. This control of arm movement execution could be based on a representation of the goal of the movement, thanks to a continuous comparison between the represented goal of the action and the instantaneous state of the effector. V6A could play a role in both the hand transport performed during reaching and the grip formation performed during grasping (Galletti *et al.*, 2003). This hypothesis is supported by the results of lesion studies in monkeys where V6A had been selectively removed surgically. After V6A lesions, monkeys misreached food placed at specific egocentric distances, mistaking both the amplitude and direction of prehension movements and were impaired in grasping the food, showing abnormal grip aperture and anomalous wrist rotations (Battaglini *et al.*, 2002).

In this line of thought, the output signals from V6A could continuously adjust the motor command which guides the occurring arm movement and could continuously update the internal representations of the body (relative positions of the body parts) and the target of prehension. In humans, it is well known that the caudal part of the SPL plays a crucial role in the control of aimed reaching movements (optic ataxia, Perenin & Vighetto, 1988). Using new tools for anatomical localization of lesions, it was recently shown that optic ataxia would result from brain damage centred on two foci, one of which is in the medial parieto-occipital cortex (Karnath & Perenin, 2005). It seems likely that this cortical region can include a human homolog of V6A, supporting the view that the medial parieto-occipital cortex is a crucial node in the control of aimed reaching movements in both humans and monkeys.

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Abbreviations

EMG, electromyographic; HOLD, static position of the hand on the light-emitting diodes; LED, light-emitting diode; M1, reaching movement directed from the home button to the light-emitting diode on the panel; M2, reaching movement directed from the panel to the home button; MIP, medial intraparietal area; PEc, caudal area PE; PGm, medial area PG; PPC, posterior parietal cortex; SPL, superior parietal lobule; V6A, area V6A.

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