Behavioral/Systems/Cognitive

Hand Orientation during Reach-to-Grasp Movements Modulates Neuronal Activity in the Medial Posterior Parietal Area V6A

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Reach-to-grasp actions involve several components of forelimb movements needed to direct the hand toward the object to be grasped, and to orient and preshape the hand according to the object axis and shape. Area V6A, which represents a node of the dorsomedial frontoparietal circuits, has so far been implicated only in directing the arm toward different spatial locations. The present results confirm this finding and demonstrate, for the first time, that during reach-to-grasp, V6A neurons are also modulated by the orientation of the hand. In the present work the object to be grasped was a handle that could have different orientations. Reach-to-grasp movements were executed in complete darkness while gazing at a small fixation point. The majority of the tested cells (76/142; 54%) turned out to be sensitive to the orientation of the handle. Neurons could be modulated during preparation or execution of reach-to-grasp movements. The most represented cells were those modulated by hand orientation both during preparatory and movement periods. These data show that reaching and grasping are processed by the same population of neurons, providing evidence that the coordination of reaching and grasping takes place much earlier than previously thought, i.e., in the parieto-occipital cortex. The data here reported are in agreement with results of lesions to the medial posterior parietal cortex in both monkeys and humans, and with recent imaging data in humans, all of them indicating a functional coupling in the control of reaching and grasping by the medial parietofrontal circuit.

Key words: **neurophysiology; dorsal visual stream; grasping; hand–object interaction; monkey; superior parietal lobule; optic ataxia**

Introduction

Prehension is an act of coordinated reaching and grasping. The reaching component is concerned with bringing the hand to the object to be grasped (transport phase); the grasping component refers to the shaping of the hand according to the object features (grasping phase) (Jeannerod, 1981). According to Jeannerod et al., reaching and grasping involve different muscles (proximal and distal muscles, respectively), and are controlled by different parietofrontal circuits (Jeannerod et al., 1995): a medial circuit, involving areas of the superior parietal lobule, namely MIP/PRR, PEc, and the dorsal premotor area 6 (PMd), is mainly concerned with reaching; a lateral circuit, involving the inferior parietal lobule (in particular area AIP) and the ventral premotor area 6 (PMv), with grasping (see the brain location of these areas in Fig. 1*A*). Partially in contrast with this view is the recent finding that PMd contains proximal as well as distal representations of the arm (Raos et al., 2004). In addition, the pathways linking posterior parietal cortex with PMd and PMv, although largely segregated, partially overlap (Tanné-Gariépy et al., 2002). According to these data, the medial and lateral parietofrontal circuits are at least partially involved in both processes.

Approximately 10 years ago a new area, called V6A, was described in the caudal part of the superior parietal lobule (Galletti et al., 1996) (see Fig. 1*A*). V6A partially overlaps the area PO (Galletti et al., 2005), a cortical visual area originally described by Gattass and coworkers (Gattass et al., 1986; Colby et al., 1988). Although V6A is a visual area (Galletti et al., 1999), it also contains cells with reach-related activity (Galletti et al., 1997; Fattori et al., 2001) and is directly connected with the dorsal premotor cortex (Matelli et al., 1998; Shipp et al., 1998; Galletti et al., 2001; Marconi et al., 2001).

V6A belongs to the medial parietofrontal circuit and, as predicted by the "visuomotor channels" hypothesis recalled above (Jeannerod, 1981; Jeannerod et al., 1995), its reaching cells are modulated by the direction of reaching movement (Fattori et al., 2005). However, some preliminary results suggested that V6A reaching neurons could be modulated by other more distal components of prehension as well (Fattori et al., 2004).

To verify this hypothesis, the present work investigated whether the orientation of the hand modulates the activity of V6A cells. We used a reach-to-grasp task (see Fig. 1*B*) in which the distance and spatial location of the object to be grasped remained constant throughout the experiment (constant amplitude and direction of movement during the transport phase). The object to be grasped was a handle of constant size, so that grip aperture and finger flexion during grasping were the same in all

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trials of the task. The only variable was the orientation of the handle, which forced the animal to change the orientation of the hand during prehension according to the handle orientation.

To check whether single V6A neurons were modulated by both orientation and direction of movement of the hand, we tested single cells with the reach-to-grasp task outlined above and with a reach-to-point task with targets arranged in different spatial positions. We found that both reach direction and hand orientation are coded by the same population of neurons.

Because V6A is a visual area, all experiments were conducted in total darkness to exclude the involvement of visual feedback in cell modulation.

Materials and Methods

Experimental procedures. Experiments were approved by the Bioethical Committee of the University of Bologna and were performed in accordance with National laws on care and use of laboratory animals and with the European Communities Council Directive of 24th November 1986 (86/609/EEC), recently revised by the Council of Europe guidelines (Appendix A of Convention ETS 123: http://conventions.coe.int/Treaty/EN/Treaties/PDF/123-Arev.pdf).

Two trained *Macaca fascicularis* weighting 3 and 3.5 kg sat in a primate chair and performed reach-to-grasp and reach-to-point tasks under controlled conditions. The training to learn the tasks took 5–6 months. After training completion, the head-restraint system and the recording chamber were surgically implanted in asepsis and under general anesthesia (sodium thiopental, 8 mg/kg/h, i.v.) following the procedures reported in Galletti et al. (1995). Adequate measures were taken to minimize pain or discomfort. A full program of postoperative analgesia (ketorolac trometazyn, 1 mg/kg i.m. immediately after surgery, and 1.6 mg/kg i.m. on the following days) and antibiotic care [Ritardomicina (benzatinic benzylpenicillin plus dihydrostreptomycin plus streptomycin) 1–1.5 ml/10 kg every 5–6 d] followed the surgery.

The recording chamber provided access to the cortex hidden in the parieto-occipital sulcus. The chamber was placed on the mid-sagittal plane, and was centered at 13–15 mm posterior to the interaural line. The anterior bank of the parieto-occipital sulcus was reached by penetrating the occipital pole at an angle of 30–40° from the stereotaxic vertical. Single neurons were extracellularly recorded from the anterior bank of the parieto-occipital sulcus using glass-coated metal microelectrodes with a tip impedance of 0.8–2 MΩ at 1 KHz. Action potentials were discriminated with a window discriminator (Bak Electronics). Recording procedures used for one monkey are similar to those reported in Galletti et al. (1995). Briefly, spike times were sampled at 1 KHz, eye movements were simultaneously recorded using an infrared oculometer (Dr Bouis) and sampled at 100 Hz. Recording procedures for the second monkey were slightly different and were described in detail in Kutz et al. (2005). Briefly, spikes were sampled at 100 KHz and eye position was simultaneously recorded at 500 Hz. In both cases eye position was controlled by an electronic window (5° × 5°) centered on the fixation target. Behavioral events were recorded with a resolution of 1 ms.

Procedures to reconstruct microelectrode penetrations and to assign neurons to area V6A were as those described by Galletti et al. (1996). Briefly, electrode tracks and location of each recording site were reconstructed on histological sections of the brain (see the penetration reported in Fig. 4) on the basis of marking lesions and several other cues, such as the coordinates of penetrations within the recording chamber, the kind of cortical areas passed through before reaching the anterior bank of the parieto-occipital sulcus and the distance of recording site from the surface of the hemisphere [see Galletti et al. (1999) for a detailed description of attribution of neural recordings to V6A]. The location of each recorded cell was reported on two-dimensional maps of the cortex of the medial parieto-occipital region (Galletti et al., 1999). Then the dorsoventral and the mediolateral spatial distribution of neurons with different functional properties were compared using a χ^2 test and a Fisher exact probability test ($p < 0.05$).

The reach-to-grasp task. Animals performed a body-out reach-to-grasp task specifically designed to study the effect of hand orientations on neuronal response under controlled conditions (see Fig. 1*B*). Monkeys performed movements with the contralateral arm with the head restrained and in darkness, maintaining steady fixation of a light-emitting diode (LED) placed straight-ahead. The task was an instructed-delay reaching task in which the monkey reached and grasped a 4-cm-long handle placed on a frontal panel, 15 cm from its eyes, centered on the fixation LED. The handle could have 4 different orientations (horizontal, vertical, and halfway between them), so the monkey approached and grasped it with different hand orientations: hand pronated, half pronated, halfway pronated/half pronated, and halfway half pronated/supinated, respectively.

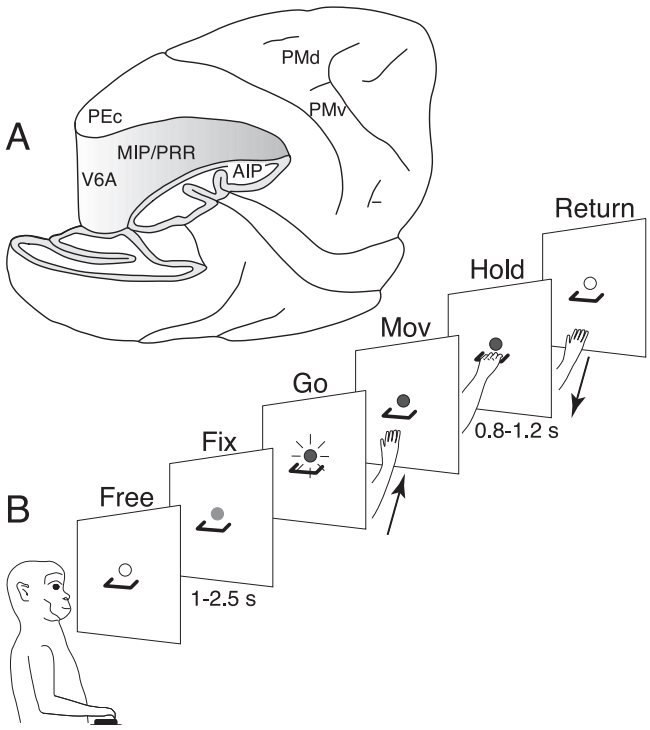

Figure 1. Recording site and schematic representation of the reach-to-grasp task. ***A***, Brain silhouette with part of the occipital pole and inferior parietal lobule cut away to show the location of recording site (area V6A) within the depth of the parieto-occipital sulcus, and of other parietal and frontal areas. ***B***, Time course and time epochs in the reach-to-grasp task. The handle could have four different orientations: horizontal, as in the figure, vertical, and halfway between the two.

Each handle orientation was tested in blocks of 15 trials, in pseudo-random sequences. After each block of trials, the background light was switched on for a few minutes to avoid dark adaptation and to allow the monkey to see the new handle orientation before the subsequent block of trials performed in darkness. In these conditions, the hand movement was memory-guided. Because it was a well practiced action that the monkey performed routinely, it became automated and it is well known that the automated actions are controlled typically by the dorsal stream (Milner and Goodale, 2008).

As shown in Figure 1*B*, reach-to-grasp 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 animal's midsagittal line. Reaching movements transported the hand from the button to the handle, positioned at eye-level at a comfortable arm-length distance. The constant position in space of the handle and fixation point excluded any possible cell modulation related to the direction of arm movement, the direction of gaze and the relative position between the two, all factors strongly affecting neural discharge in area V6A (Galletti et al., 1995; Fattori et al., 2005; Marzocchi et al., 2008). The dark environment excluded that cell's modulation could be the consequence of visual feedback evoked by the movement of the arm in the visual field. In addition, the brightness of the LED was reduced so that it was barely visible during

the task and, standing by the monkey, the experimenter could not see the handle or the monkey's hand moving in the peripersonal space, even in dark-adapted conditions.

The reach-to-grasp task, illustrated in Figure 1*B*, had the following time sequence: after some minutes of fully light environment, the light was switched off, leaving the monkey in a dark environment with no visual stimuli present. The trial began when the monkey pressed the button near its chest. After button pressing, the animal awaited instructions in complete darkness (Free). It was free to look around and was not required to perform any eye or arm movement. After 0.5–1 s the fixation LED lit up and the monkey had to wait for LED change in color without performing any eye nor arm movement (Fix). Breaking of fixation and premature button release would have interrupted the trial. After a delay period of 1–2.5 s, the LED color changed. This was the go-signal for the monkey to release the button and perform a reach-to-grasp movement (Mov) to reach and grasp the handle, pull it and maintain it pulled (Hold) till the LED switched off (after 0.8–1.2 s). The LED switch-off cued the monkey to release the handle and to press the home-button again (Return). Home-button pressing ended the trial, allowed the monkey reward, and started another trial (Free).

The reach-to-point task. The same two monkeys studied for the reach-to-grasp task were also studied with a reach-to-point task. This task elicited arm movements to precise targets arranged in different spatial locations at a distance 30° one from the other, on a panel placed on a frontal plane 15 cm in front of the eyes. Targets were very small (4 mm in diameter; 1.6° of visual angle) as they were LEDs mounted on microswitches embedded in the panel.

The monkeys reached and pressed 1 of 3 LEDs presented in pseudorandom sequence (blocks of 15 trials). The central LED was in the same spatial location as the handle in the reach-to-grasp task. Like the reach-to-grasp task, the reach-to-point task was performed in complete darkness except for the dim light of the fixation point.

The time sequence of the reach-to-point task was the same as that of the reach-to-grasp task. In the reach-to-point task, however, the change in color of fixation LED cued the monkey to perform an arm-reaching movement to reach the LED and press it (Mov). The animal then maintained the LED pressed (Hold) until it switched off (after 0.8–1.2 s). This cued the monkey to release the LED and return to the home-button (Return).

Reach-to-grasp and reach-to-point tasks were presented to the animal in separate blocks of 15 × 4 and 15 × 3 trials, respectively. The order of presentation of the two reaching tasks was reversed from neuron to neuron. Between one block of 15 trials and the next, the light in the environment was switched on for a few minutes to avoid dark adaptation during neural recording.

Data analysis. V6A neural activity during both tasks was divided into the following time epochs: Free: from home button pressing to fixation LED lit up; Fix: steady fixation of the LED during the delay period before arm movement; it was calculated on a single trial basis, starting when the gaze entered the electronic window centered on the fixation point and ending at the go signal; Mov: from 200 ms before movement onset (home-button release) to movement end (handle pulling in reach-to-grasp task or LED pressing in reach-to-point task); Hold: from handle pulling/LED pressing to 200 ms before the onset of return movement (handle or LED release); Return: from 200 ms before return movement onset (handle or LED release) to movement end (home-button pressing).

Only the units tested for at least seven trials for each orientation were taken into account. Task-related responses of single neurons were statistically assessed by repeated-measures ANOVA [time epoch (five levels) × hand orientation (four levels); significance level, $p < 0.05$]. All cells displaying a significant interaction factor 1 × 2 were further tested with a Bonferroni *post hoc* test to select neurons showing an activity significantly different in at least one of the time epochs with respect to epoch Free. The effect of hand orientation on neural activity on each of the task epochs was assessed with a one-way ANOVA (*F* test; significance level: $p < 0.05$).

One-way ANOVA was also used to compare neural activity in the same epoch in the reach-to-point task performed toward targets located in different spatial locations (*F* test; significance level: $p < 0.05$). This allowed us to identify neurons sensitive to reach direction (see Fig. 7).

A spike density function (SDF) was calculated (Gaussian kernel, half-width 40 ms) for each neuron included in the analysis, and averaged across all the trials for each tested condition. We found the peak discharge of the neuron in the behavioral epochs of interest, and used it to normalize SDF. The normalized SDFs were then averaged to derive population responses (Marzocchi et al., 2008). We statistically compared the population SDFs of the best and worst activities (see Fig. 5) with a permutation test with 10,000 iterations comparing the sum of squared errors of the actual and randomly permuted data. We considered the following time periods: during Fix, the time from 500 to 1400 ms after the appearance of the fixation LED; during Mov, from 200 ms before movement onset to 260 ms after movement onset (the weighted mean of the movement time of the two animals was 264 ms); during Hold, from the movement end to 800 ms after movement end.

Monkeys' arm movements were continuously video-monitored by means of miniature, infrared-illumination-sensitive videocameras. The orientation of hand axis during reach-to-point and reach-to-grasp tasks was estimated using video images at 25 frames/s. We analyzed the single video frames, identifying the points corresponding to radial and ulnar styloid processes. The segment joining these two points was used as an estimate of the hand/wrist axis. We calculated the difference in hand axis between the leftmost/rightmost targets in the reach-to-point task and between the horizontal and the vertical handle orientations in the reach-to-grasp task.

For neurons showing a significant effect of handle orientation, the percentage difference in discharge rate between the best and the worst orientation was quantified as follows: $(fr_{best} - fr_{worst}) \times 100/fr_{best}$, where fr_{best} and fr_{worst} are the highest and weakest average firing rates in the considered task epoch.

The resulting distributions were compared across task epochs (Kolmogorov–Smirnov test, significance level: $p < 0.05$).

All the analyses were performed using custom scripts in Matlab (Mathworks). Statistical analyses were performed using the STATISTICA software (StatSoft).

Results

A total of 142 neurons were recorded from area V6A in two monkeys while animals performed the learned reach-to-grasp task (Fig. 1*B*) with at least two handle orientations. Area V6A was identified on the basis of the functional properties of their neurons according to Galletti et al. (1996, 1999). Histological controls confirmed the location of recorded sites according to Luppino et al. (2005).

Of 142 recorded cells, 76 (54%) turned out to be task-related, as assessed by the repeated-measures ANOVA [epoch (five levels) × hand orientation (four levels), with Bonferroni correction, $p < 0.05$].

Sixty-six percent of neurons (50/76) were sensitive to the change in hand orientation that occurred when the hand approached the handle during the transport phase (epoch Mov) (Fig. 1*B*). Sixty-two percent (48/76) were sensitive to the hand orientation during handle pulling (epoch Hold) (Fig. 1*B*). Several cells (33/76; 43%) were sensitive to the wrist orientation during both transport phase and handle pulling.

Figure 2 shows examples of these neural modulations. Cell 120 (top of figure) increased its discharge rate as soon as the hand left the home-button in front of the chest of the animal and suddenly decreased it once the handle was grasped (Fig. 2, see markers on raster activity). The rate of neural discharge was dependent on the orientation of the handle (and therefore of the hand), strongly discharging when the handle was oriented vertically (hand half pronated) and weakly when it was oriented horizontally (hand pronated). Because our task did not evoke any change in grip formation, therefore, the difference in discharge in epoch Mov in

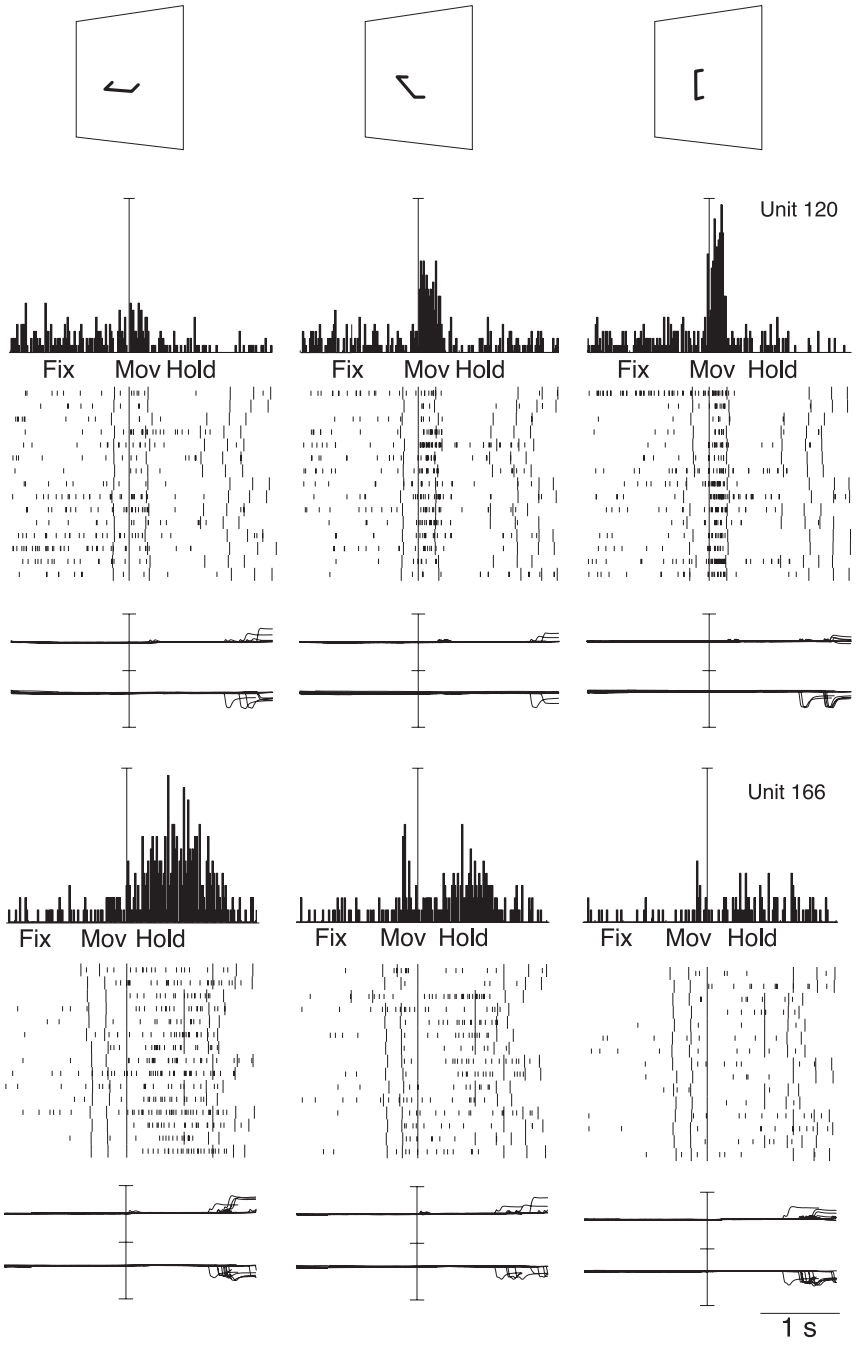

Figure 2. Effect of hand orientation on the neuronal activity of two V6A cells during the execution of reach-to-grasp movements. Top, Tested handle orientations. Middle and bottom, Peri-event time histograms, time epochs, raster displays of impulse activity, and recordings of X and Y components of eye positions. Long vertical ticks in raster displays are behavioral markers, indicating from left to right: LED change in color (go-signal), home-button release (onset of arm movement), handle-pull, LED off, handle-release, home-button press. Activity is aligned with the onset of reach-to-grasp arm movement (unit 120) and with the onset of hold (unit 166). Scale: vertical bar on histograms, 75 (unit 120) and 45 (unit 166) spikes/s; eye traces, 60°/division.

cell 120 should be ascribed only to changes in wrist orientation occurring during reaching of the handle.

Cell 166 (Fig. 2, bottom) was strongly modulated by wrist orientation during Hold. It increased its discharge rate when the hand grasped the handle and decreased it when the handle was released (Fig. 2, see markers on raster activity). Like cell 120, the rate of neural discharge was dependent on the orientation of the handle (and therefore of the hand). This time, however, the highest neural discharge was observed when the handle was horizontal (hand pronated) and the weakest one when it was vertical (hand half pronated). This cell also showed a modest activity in Mov, which was in turn modulated by the handle orientation. However, the best discharge in Mov was evoked by the oblique orientation, that is by a different orientation with respect to the preferred one in Hold.

Of the 76 cells modulated by the orientation of the hand, 42 (55%) were modulated during the preparation of reach-to-grasp movement (Fix epoch). A few of these cells ($n = 5$) were modulated only in epoch Fix, whereas the great majority were modulated in Fix as well as in Mov/Hold epochs (37/42). Figure 3 shows two examples of the latter type of cells. The first cell (unit 389) strongly increased the discharge rate as soon as the animal gazed at the fixation point, and continued to discharge while the animal was waiting to reach-to-grasp the handle that was oriented horizontally (Fix epoch in the top left panel). Note that this was not a visual response, because the task was performed in the dark and the handle had been shown to the animal only before the task began, that is, before the first trial of the block (see Materials and Methods). The discharge rate of this cell also remained high during the Mov epoch (hand approaching the handle and preparing grasping), but suddenly decreased as soon as the handle was grasped. When the handle was oriented vertically (top right panel of the figure) the cell's pattern of activity was completely different: the discharge rate remained low and unmodulated during both Fix and Mov epochs, and during Hold epoch as well.

The second cell shown in Figure 3 increased the discharge rate during reach-to-grasp movement and holding time when the animal grasped the horizontal handle (Mov and Hold epochs, respectively; see Fig. 3, bottom left). However, the cell discharged much more strongly when the animal was waiting to grasp the handle oriented vertically (Fix epoch in the bottom right panel of the figure), as well as when it actually grasped the vertical handle (Mov epoch in the bottom right panel).

During Fix epoch in both experimental conditions (horizontal/vertical handle) the animal was waiting in the darkness for the go-signal to move the hand, keeping its hand immobile on the home-button, and gazing motionless straight ahead at a small fixation spot. Because the eye, the hand position, the arm posture, and the required level of attention were the same in the two conditions, the different level of neural activity during the delay period (Fix epoch) was likely due to the preparation of a different grasping movement according to handle orientation. It is known from previous data from our laboratory that ~30% of V6A cells modulates their discharge rate during the preparation of an arm-reaching move-

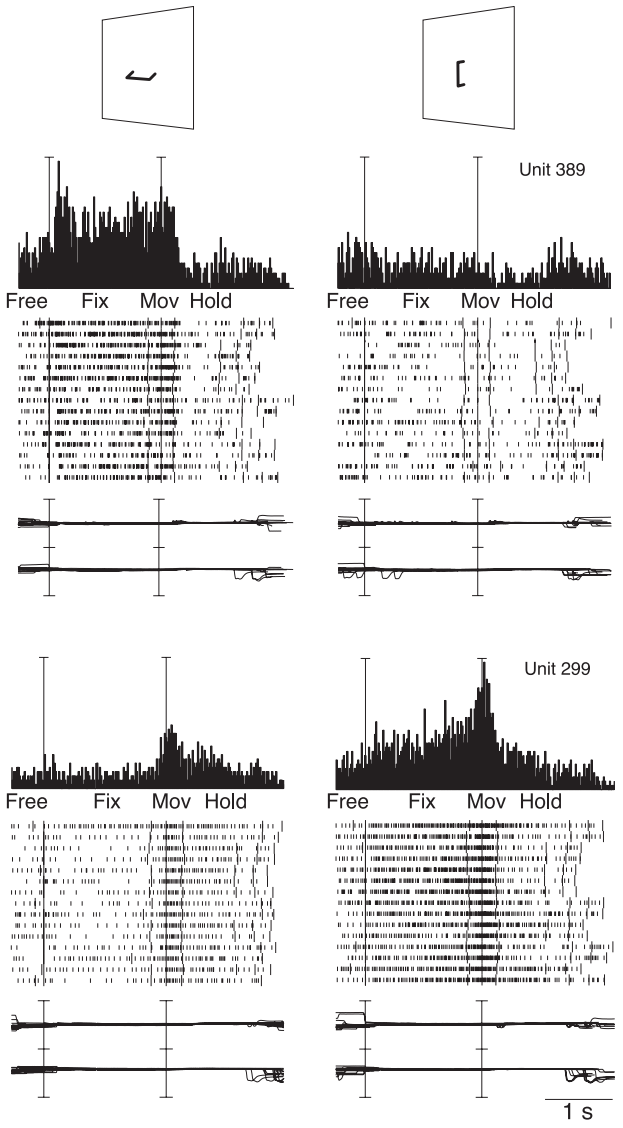

Figure 3. Effect of hand orientation on the neuronal activity of two V6A cells during preparation and execution of reach-to-grasp movements. Neuronal activity is aligned twice, with the onset of fixation LED (first marker from left) and the onset of forward arm movement. All other details are as in Figure 2. Scale: vertical bar on histograms, 85 (unit 389) and 100 (unit 299) spikes/s; eye traces, 60°/division.

ment (Fattori et al., 2001). The present results extend this finding to the preparation of more distal movements.

Population behavior

In summary, the great majority (>90%) of cells modulated by hand orientation were modulated during the execution of reach-to-grasp movements (Mov/Hold epochs). Many of these cells (>50%) were also modulated during the preparation of movement (see Fig. 3; Fix epoch). Often, the preferred orientation of a cell was the same for Fix and Mov/Hold epochs (see Fig. 3). Sometimes the preferred orientation changed according to the epoch taken into account (Fig. 2, see cell 166).

During single microelectrode penetrations the preferred hand orientation changed from cell to cell without any apparent spatial segregation of orientation preference within V6A. Figure 4 shows an example of this behavior. The two cells shown in the figure were recorded in the same penetration at a distance of 220 μm one from the other. They show brisk responses to the reach-to-

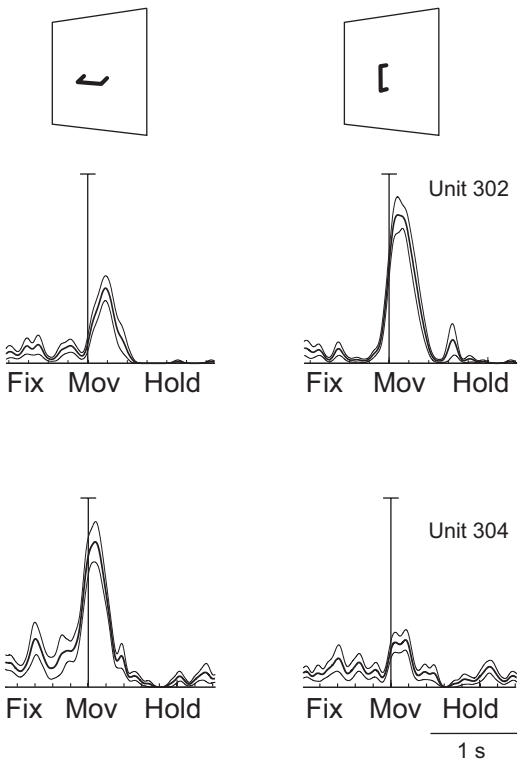

Figure 4. Opposite effect of hand orientation on the neuronal activity of two V6A task-related cells recorded in the same microelectrode penetration. Neuronal activity is expressed as SDF (thick lines) with variability band (SEM, light lines). Activity is aligned with the onset of arm movement. Scale: vertical bar on histograms, 80 (unit 302) and 40 (unit 304) spikes/s. Other details are as in Figure 2.

grasp movements, but one cell is maximally activated when the handle is vertically oriented (Fig. 4, top unit) and the other when the handle is horizontally oriented (bottom unit). We did not find any segregation of orientation preference within area V6A (χ^2 test, n.s.) and the cell population did not show any preferred hand orientation among those we tested: all orientations were almost equally represented within the recorded region. We also found that task-related cells were not spatially segregated within area V6A, being typically intermixed with neurons not influenced by the reach-to-grasp action (Fisher exact probability test, n.s.).

Figure 5 shows the cumulative spike density functions of V6A task-related cells. The continuous line represents the average mean activity of cells during the reach-to-grasp task when the animal grasped the preferred-oriented handle. The dashed line represents the average mean activity of modulated cells when the animal grasped the handle oriented in a way that evoked the weakest response in those same cells. Plots in Figure 5 refer to the best and worst activities of the same cell population as assessed in Mov epoch (Fig. 5*A*) and Hold epochs (Fig. 5*B*). All plots have been aligned twice, at the onset of LED fixation and at the onset of arm movement (Fig. 5*A*) or handle pulling (Fig. 5*B*).

It is evident a coherence of preference with Fix, when the best and worst activities in Mov were taken into account (Fig. 5*A*). The rate of discharge was significantly different in both Fix and Mov (permutation test, $p < 0.05$). This implicitly confirms that the activity of single V6A cells covaried accordingly in Fix and Mov when the hand orientation changed. Conversely, the similar activity in epoch Fix when the best and worst Hold activity was taken into account (Fig. 5*B*) indicates that the activity in Hold did

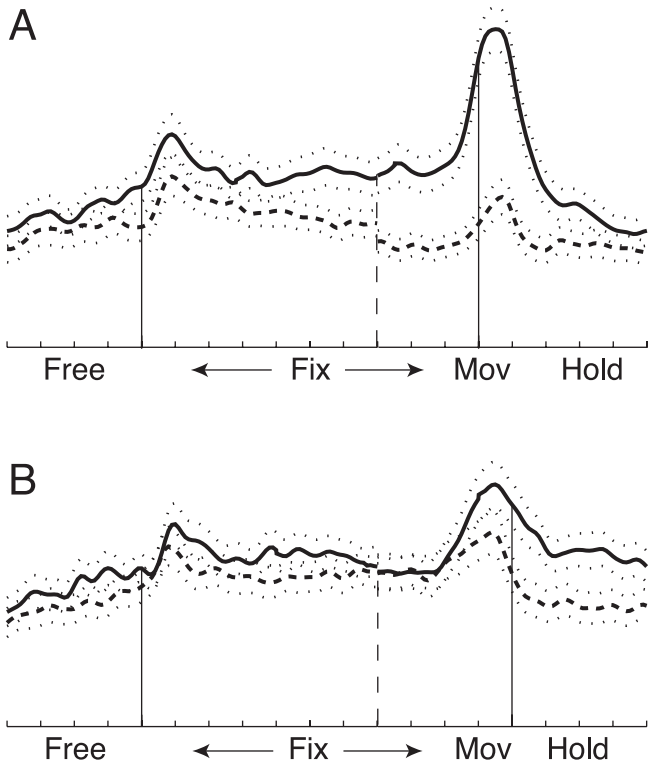

Figure 5. Effect of hand orientation on the neuronal activity of V6A task-related cells: population behavior. ***A***, The average SDF for the preferred and nonpreferred hand orientations of task-related cells are shown as continuous and dashed lines, respectively. Each cell was taken into account twice: once where it showed the highest peak activity in epoch Mov (best orientation), and a second time where it showed the lowest peak activity in that same epoch (worst orientation). Two dotted lines for each SDF indicate the variability band (SEM). The activity of cells in each population is aligned twice, on the fixation LED onset and on the onset of arm movement, respectively. The vertical dashed line marks the position in which nonaligned activity from different cells was cut away. The two curves are statistically different both in Fix and in Mov epochs (permutation test, $p < 0.05$). Scale in abscissa: 200 ms/division; vertical scale is arbitrary, being normalized responses. ***B***, SDF for the preferred (best Hold response) and nonpreferred (worst Hold response) conditions of task-related cells. The two curves were statistically different in Hold (permutation test, $p < 0.05$), but not in Fix (permutation test, n.s.). All other details are as in ***A***.

not covary according with that in Fix when the hand orientation changed (permutation test, n.s.).

The effect of handle orientation on neural activity was quantified as percentage discharge difference between preferred and nonpreferred handle orientations. The distribution of this parameter for the neurons significantly modulated in Fix, Mov, and Hold, respectively, is reported in Figure 6. The difference in discharge rate was >25% for all neurons and all epochs. In a few cases, when the neuron was completely silent for the worst orientation, the percentage difference in discharge rate reached the 100%.

The average difference in the modulated neurons was 56% for Fix, 58% for Mov and 64% for Hold epoch. The comparison between the three epochs showed that only the percentage differences of Fix and Hold were statistically different (Kolmogorov–Smirnov test, $p < 0.05$).

Are neuronal modulations during reach-to-grasp actions due to changes in reach direction, hand orientation or both?

Data presented so far demonstrate that the activity of many V6A cells is modulated by hand (wrist) orientation, a distal movement in the reach-to-grasp action. Previous data (Fattori et al., 2005) showed that several V6A cells are modulated by the direction of arm movement, a typical proximal component in the reach-to-grasp action. To establish whether single V6A cells were modulated by hand orientation or reach direction or both, we tested single cells with two tasks: the reach-to-grasp task described here and a specific reach-to-point task that allowed us to test cells' sensitivity to the direction of arm movement (Fattori et al., 2005). The reach-to-grasp task (Fig. 1*B*) required the animal to perform arm movements repeatedly in the same direction, as the handle remained in the same position in space, but with different hand orientations induced by different orientations of the handle. In contrast, in the reach-to-point task the animal reached and touched (pressed) the LED it fixated, placed in different positions on a panel in front of it (Fig. 7*A*, middle row). The task required the animal to perform arm movements in different directions but with a constant hand shaping and wrist orientation.

Whenever the recording stability allowed us to test a cell for longer periods, V6A cells underwent both the reach-to-grasp and the reach-to-point tasks. For 45 cells it was possible to test the reach-to-point task in at least 3 spatial locations and the reach-to-grasp with at least 2 handle orientations. Figure 7*A* illustrates the pattern of activity of one of these cells. When the animal reached and grasped the differently oriented handle located in a constant spatial position, the neural discharge changed dramatically according to the orientation of the handle, hence according to the orientation of the hand (upper rows of the figure). When the animal changed the direction of arm movement to reach the target in different spatial locations, the neural discharge of the same neuron changed (Fig. 7*A*, middle rows), demonstrating a sensitivity of the cell to the direction of movement. In other words, this neuron was sensitive to both the direction of arm movement and the orientation of the hand (wrist).

It could be argued that in the reach-to-point task the animal changes its wrist orientation while reaching targets in different spatial locations. If so, the observed modulation of neural activity could be ascribed to the change in wrist orientation rather than the change in arm movement direction. According to our video-based estimation, the change in hand/wrist axis when the animal reached the leftmost and the rightmost targets was ~5–10°, whereas the change when the animal grasped the vertical and the horizontal handle was ~90°. The range of variation in hand/wrist axis in the reach-to-point task is too small to explain the strong neural modulations observed in this task.

Figure 7*B* summarizes the behavior of all tested cells. Approximately 53% of neurons (24/45) were sensitive to both reach direction and wrist orientation, as the unit shown in Figure 7*A*. The 20% of tested cells (9/45) were sensitive only to reach directions and 18% (8/45) only to wrist orientation. Four of forty-five cells (9%) were not sensitive to either of the two components. The spatial distribution of neurons of the 4 categories illustrated in Figure 7*B* were not spatially segregated within area V6A (Fisher exact probability test, n.s.).

Discussion

We previously reported that many neurons in area V6A are modulated by the direction of arm movement (Fattori et al., 2005). The present data demonstrate that during reach-to-grasp actions the activity of many cells in V6A is also influenced by more distal forelimb movements like the orientation of the hand. The involvement of V6A in both reaching and grasping movements contrasts with the widely accepted view that the dorsomedial parietofrontal circuit, to which V6A belongs (Galletti et al., 2003), is concerned with the control of reaching movements,

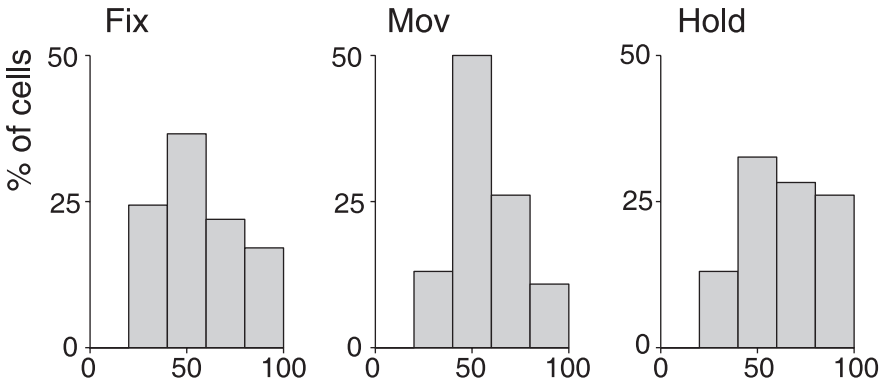

Figure 6. Strength of hand orientation effect. The three panels show the normalized distributions of percentage discharge difference between best and worst hand orientation, calculated for neurons showing a significant handle orientation effect. From left to right, the three distributions refer to Fix, Mov, and Hold epochs, respectively. Scale in abscissa: percentage of discharge difference; bin: 20% of difference.

whereas the dorsolateral parietofrontal circuit is concerned with the control of grasping (Jeannerod et al., 1995; Wise et al., 1997). Conversely, present data agree with the more recent hypothesis that medial and lateral parietofrontal circuits are both involved in reaching and grasping (Desmurget et al., 1996; Smeets and Brenner, 1999; Mon-Williams and McIntosh, 2000; Grol et al., 2007). Psychophysical studies in humans and monkeys suggest a strict interdependence of reaching and grasping. Roy and colleagues, for example, reported that the reaching component is influenced by a rapid change in object's size, suggesting a close cross-talk between reaching and grasping (Roy et al., 2002, 2006). The present data show that the orientation of the handle is processed by the same population of neurons sensitive to the direction of reaching, thereby supporting the concept that V6A is a place in which this cross-talk may take place.

The view that V6A in particular is concerned with the control of both proximal and distal movements in reach-to-grasp actions is supported by neurological studies in humans and lesion studies in monkeys. Human patients with cortical lesions that include the caudal part of the superior parietal lobule typically show misreaching (optic ataxia syndrome), but also distal deficits as failure to align their hand with the orientation of a slot (Perenin and Vighetto, 1988), abnormal finger opening while grasping an object, and failure to scale the grip aperture to the object size (Jeannerod, 1986; Jakobson et al., 1991). A reconstruction of cortical lesions in a large number of optic ataxia patients showed that the lesioned area included the medial parieto-occipital cortex, suggesting a direct involvement of area V6A (Karnath and Perenin, 2005). In monkeys, selective V6A surgical lesions produce both misreaching and misgrasping, with exaggerated finger extension while the hand approaches the object to be grasped and erroneous wrist orientation and flexion during object grasping (Battaglini et al., 2002).

Further support for a role of V6A in the control of reach-to-grasp actions comes from hodological data in monkeys and from the analysis of the functional properties of neurons of the cortical regions directly connected with V6A. Area V6A is directly connected with the dorsal premotor cortex (Shipp et al., 1998; Galletti et al., 2001; Marconi et al., 2001) in particular with area F2 (Matelli et al., 1998; Gamberini et al., 2009). Various studies (Caminiti et al., 1991; Kalaska et al., 1997; Wise et al., 1997) have demonstrated that the activity of the dorsal premotor neurons is correlated to parameters of proximal forelimb movements such as direction and amplitude of reaching, but a recent study (Raos et al., 2004) provided compelling evidence that a distal forelimb field also exists in F2: neurons in this cortical region are selective for wrist orientation and for the type of prehension required for grasping the object.

Area V6A is directly connected with the parietal areas MIP/PRR and PEc (Shipp et al., 1998; Marconi et al., 2001; Gamberini et al., 2009), both containing neurons modulated by parameters of proximal forelimb movements such as direction of reaching. But V6A is also connected with area AIP (Borra et al., 2008; Gamberini et al., 2009) whose cell activity is related to (distal) hand and finger movements performed when grasping various types of objects (Sakata et al., 1995; Murata et al., 2000).

Imaging data in normal human subjects agree with the view of an involvement of the medial posterior parietal cortex (where the putative human's V6A would reside) in both reaching and grasping activities. A PET study in which subjects performed either reaching and grasping tasks disclosed an activation of the medial parieto-occipital cortex in both tasks (Grafton et al., 1996). Some fMRI studies have shown that the medial parietal cortex is activated by the preparation and execution of pointing movements (Astafiev et al., 2003; Connolly et al., 2003) and by grip selection in imaged grasping with different hand orientations (Johnson et al., 2002). fMRI studies in which subjects were required to perform reach-to-grasp tasks showed that the medial parieto-occipital region was consistently activated (Cavina-Pratesi et al., 2007). Furthermore, Toni and coworkers (Grol et al., 2007; Verhagen et al., 2008) have recently found that the dorsomedial and dorsolateral parietofrontal circuits are both activated during reach-to-grasp tasks, the relative activation of the two pathways being related to the degree of on-line control required by the prehension movement.

In any reach-to-grasp action, proximal and distal movements partly overlap in time, because shaping of the hand starts well before the hand reaches the object to be grasped. V6A would allow a functional coupling between the different components of prehension movements, interacting continuously with other posterior parietal areas (such as MIP/PRR, PEc, AIP) and with the dorsal premotor cortex, all of them directly connected with V6A. This network of areas could monitor, and if necessary correct, the direction of arm movement, hand orientation, and grip aperture during the execution of reach-to-grasp actions. This hypothesis is strongly supported by human data showing that lesions and transcranial magnetic stimulations of the medial posterior parietal cortex disrupt the online correction of reaching movements (Desmurget et al., 1999; Pisella et al., 2000; Gréa et al., 2002).

Functional role of area V6A

The act of prehension can be a rapid ballistic movement of the hand to reach and firmly grasp an object, or a precise (and slower) arm movement that brings the hand near the object and then allows the fingers to carefully interact with it and, possibly, to manipulate it. In both cases, location in space, orientation, and size of the object are essential for successful grasping. However, in the first case a fast movement is required, accurate enough to reach out to the object, orient the hand according to object orientation, and preshape the fingers according to the size and the

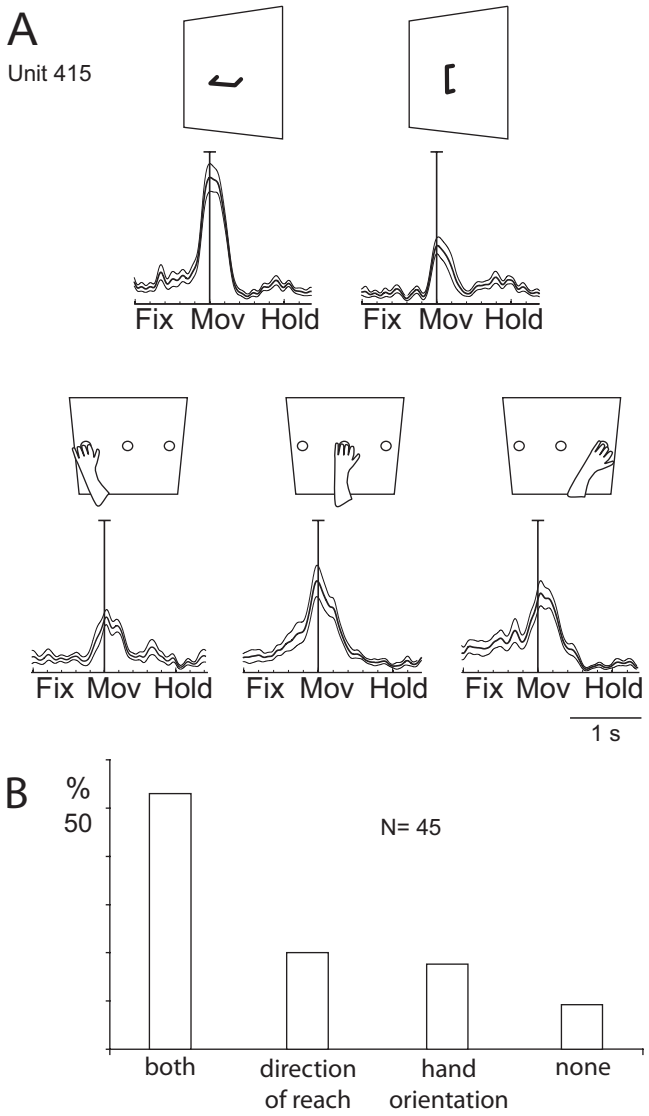

Figure 7. Coexistence in single V6A neurons of neuronal modulations for reach direction and hand orientation. ***A***, Pattern of activity of a V6A neuron to reach-to-grasp movements performed to grasp a differently oriented handle (handle orientation in the first row, neuronal activity in the second row) and to reach and press buttons located in different spatial positions (target spatial location in the central row, neuronal activity in the bottom row). Neuronal activity is expressed as SDF with variability bands. Details as in Figure 4. ***B***, Percentage incidence in V6A of neurons sensitive to reach direction, hand orientation and to both of these components of reach-to-grasp actions.

center of mass of the object. In the second case much more information about the object's intrinsic features are needed. We believe that area V6A is particularly concerned with the control of the first type of movement (Galletti et al., 2003), because it contains neurons modulated by both proximal and distal forelimb movements (present results and Fattori et al., 2005), as well as neurons that encode spatial location (Galletti et al., 1993, 1995) and orientation (Galletti et al., 1996) of visual objects, and because V6A is directly connected with the dorsal premotor cortex (Matelli et al., 1998; Shipp et al., 1998; Gamberini et al., 2009), a connection that allows a fast motor response, via the primary motor cortex or via a direct output to the spinal cord (He et al., 1993).

The present results well agree with the above reported hypothesis. V6A contains reach-to-grasp neurons whose activity is modulated during preparation/execution of prehension of differently oriented objects. Basically, we found three types of cells: (1) cells modulated only during the preparation of reach-to-grasp movement, representing <10% of the task-related population; (2) cells modulated only during the execution of reach-to-grasp movement, representing ~40% of the total population; (3) cells modulated during both preparation and execution of prehension (>50% of the total). All these types of cells fit well with the idea that V6A is engaged in the online control of reach-to-grasp movements.

As to the change in activity during the preparation of prehension, it is noteworthy that it started when the animal began to fixate (see Fig. 3; Fix epoch). However, this change in activity cannot be ascribed to a gaze signal, because the direction of gaze was the same when the animal was preparing grasping of differently oriented handles, but the rate of discharge was different in the two cases. The different rate of discharge before grasping handles with different orientations was not caused by a visual stimulation, because all trials were performed in the dark (see Materials and Methods); nor was it caused by a change in the animal's level of attention, given that this level should be the same while the animal is waiting to grasp a vertically or horizontally oriented handle. It was also not caused by the difficulty of the impending grasp movement, because the cell's activity was greater for vertical or horizontal handles depending on the cell taken into account (see Fig. 3). Although at present we do not know which is the actual signal modulating cell firing during Fix epoch, we know that it depends on the orientation the hand will have during the impending grasping. We advance the hypothesis that the activity during Fix epoch represents a "preparation" signal for the upcoming action that will require a certain wrist orientation. Future more specifically addressed experiments will hopefully verify the validity of this hypothesis.

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