Differential Involvement of Neurons in the Dorsal and Ventral Premotor Cortex During Processing of Visual Signals for Action Planning

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Submitted 25 October 2005; accepted in final form 19 February 2006

INTRODUCTION

In primates, the premotor cortex is located on the lateral surface of the frontal cortex corresponding to Brodmann’s area 6 (Brodmann 1909). Studies have precisely examined the anatomical and functional organization of macaques, which have a premotor cortex located in the caudal bank of the arcuate sulcus and in the convex region rostral to the primary motor cortex (Brodmann’s area 4). The premotor cortex was first characterized functionally by its involvement in visually guided motor behavior. Premotor neurons respond to the appearance of visual signals (Godschalk et al. 1981; Kubota and Hamada 1978; Rizzolatti et al. 1981) and discharge during the preparation and execution of movements under visual guidance (Godschalk et al. 1985; Mushiake et al. 1991; Weinrich and Wise 1982). In line with these observations, lesions of the premotor cortex cause deficits in the visual guidance of motor behavior (Halsband and Passingham 1985; Moll and Kyupers 1977).

Many reports have indicated that the premotor cortex is composed of two sections: ventral (PMv) and dorsal (PMd) areas. Previous studies have described differences in neuronal activity between these two areas. PMd neurons are particularly active during a preparatory motor-set period (Weinrich and Wise 1982; Wise 1985) and in relation to visuomotor-association tasks (Kurata and Wise 1988; Mitz et al. 1991). In contrast, PMv neurons respond to somatosensory stimuli applied to either the face or the arm and to visual stimuli corresponding to peripersonal stimuli (Fogassi et al. 1996; Graziano et al. 1997). Subsequent studies revealed further properties of PMv neurons: they are selective for the three-dimensional shape of objects to be grasped (Murata et al. 1997), the direction or movement trajectory in visual/ extrinsic space (Kakei et al. 2001; Mushiake et al. 1997; Ochiai et al. 2005; Schwartz et al. 2004), attention to visuospatial stimuli (Boussaoud and Wise 1993a,b), decision-making based on somatosensory signals (Romo et al. 2004), and activity that mirrors the actions of viewed subjects (Rizzolatti and Craighero 2004).

Lesion studies have supported these findings by describing different effects resulting from ablation of either the PMv or PMd. Monkeys with PMd lesions exhibit difficulty in executing conditional visuomotor association tasks (Halsband and Passingham 1985; Kurata and Hoffman 1994; Petrides 1986), and monkeys with PMv lesions exhibit deficits in visually guided grasping movements (Fogassi et al. 2001; Rizzolatti et al. 1983), orienting responses (Rizzolatti et al. 1983; Schieber 2000), and remapping from visual to motor space (Kurata and Hoshi 1999). Cortico–cortical connections linking the parietal and premotor cortex have also revealed a difference in major input sources from the parietal cortex to the PMv and PMd: the PMd receives its major input from areas medial to the intraparietal sulcus, whereas the PMv receives major input from areas ventral and lateral to the intraparietal sulcus and its lateral surface (Caminiti et al. 1996; Johnson et al. 1996; Johnson et al. 1996; Kurata 1993; Luppino et al. 1999; Matelli et al. 1998; Rizzolatti and Luppino 2001; Tanne-Gariepy et al. 2002).

Only a few studies have directly compared PMd and PMv neuronal response properties by recording neurons from both areas in individual subjects. These studies found that set-related activity mainly occurred in the PMd, whereas movement-related activity occurred in both the PMd and PMv (Kurata 1993). A different study that used a behavioral task to dissociate attention to visual space from the intention to move an arm reported that the PMd and PMv tended to be involved in representing intention and attention, respectively (Bous-
We studied two male monkeys (*Macaca fuscata*, 8 kg) that were cared for according to National Institutes of Health guidelines. Our previous studies used the same two monkeys (Hoshi and Tanji 2000, 2002, 2004a,b; Hoshi et al. 2005). Our previous report (Hoshi et al. 2005) described in detail the experimental setup and methods used for data recording, animal surgery, and histological processes.

**Behavioral task**

The monkeys were trained to perform a target-reaching movement using two sets of instructions: one instruction indicated the target location and the other indicated which arm to use when reaching for the target (Fig. 1A). After an intertrial interval of ≥3 s, the task commenced when a monkey placed one hand on each touch pad and gazed at a fixation point (FP; 1.2° in diameter) that appeared at the center of the touch-sensitive screen. Their eye movement/position was monitored using an infrared eyecamera system (R-21C-AS, RMS, Hirosaki, Japan). If fixation continued for 1,200 ms, the monkey was given the first instruction (the 1st cue; 400-ms duration), which contained information about either the target location or which arm to use. A small, colored cue superimposed on the central FP indicated the type of instruction, i.e., whether it related to target location or arm use. For monkey 1, a green circle or red square indicated an arm-use instruction, whereas a blue circle or red cross indicated a target-location instruction. For monkey 2, a green square and a blue cross indicated instructions for arm use and target location, respectively. At the same time, a white square (8 × 8") appeared to the left or right of the FP and indicated laterality of arm use (for arm-use-related instructions) or target location (for target-related instructions). If fixation continued for 1,200 ms during the subsequent delay period (1st delay), the second instruction (the 2nd cue; 400 ms) was given to complete the information required for the subsequent action. Thereafter, if fixation continued for 1,200 ms during the second delay, squares appeared on each side of the FP (set cue ≥1,000 ms), instructing the monkey to prepare to reach for the target when the FP disappeared (the GO signal). If the monkey subsequently reached for the target with a reaction time of <1 s, it received a reward of fruit juice 600 ms after touching the screen. *Monkey 1* was required to gaze at the FP for 800–1,200 ms before the GO signal appeared. *Monkey 2*...
was also required to gaze at the fixation point until the end of the second delay but not required to do so after the onset of the set cue. The order of appearance of the target and arm instructions was alternated in a block of 20 trials, and laterality was randomized within each block. A series of five 250-Hz tones after a reward signaled reversal of instruction orders.

**Data analysis**

**DEFINITION OF TASK-RELATED NEURONS AND THE 10 TASK PERIODS.** We sampled all neurons for which activity was recorded during at least four blocks of trials (i.e., 80 trials). To apply a task-related definition to neuronal activity, we divided the behavioral task into the following six phases: control (200–700 ms after attaining fixation); prefirst cue (the 500-ms period before the appearance of the 1st cue); first cue and delay (100 ms after onset of the 1st cue to the onset of the 2nd cue); second cue and delay (100 ms after onset of the 2nd cue to the onset of the set cue); set cue (onset of the set cue to the appearance of the go signal); and movement (the 500-ms period around the time at which movement began). We defined a neuron as “task-related” if distribution of the discharge rate (spikes per second) differed significantly in at least one of eight trial types (ANOVA, \( P < 0.05 \), repeated over 8 types of trials with 8 sequences of 1st and 2nd cue presentation (as shown in 8 panels in Fig. 2). For statistical analysis and display, data for the five task events were aligned separately to onsets of first and second cues, the set cue, the go signal, and the time at which the screen was touched. These data were analyzed separately before being merged at the midpoint of the first and second delays and at the set cue phase (i.e., 600 ms after cue offset and 600 ms before the onset of the 2nd cue or the set cue phase, and 600 ms after the set cue onset and 600 ms before the go signal).

Our subsequent statistical analysis of neuronal activity properties involved dividing the entire task into one control period (200–700 ms after attaining fixation) and 10 task periods defined as follows: precue (the 500-ms period before onset of the 1st cue); first cue (100–500 ms after onset of the 1st cue); early first delay (500–1,000 ms after onset of the 1st cue); late first delay (the last 500 ms before onset of the 2nd cue); second cue (100–500 ms after onset of the 2nd cue); early second delay (500–1,000 ms after onset of the 2nd cue); late second delay (the last 500 ms before onset of the set cue); early set cue (the 500-ms period after onset of the set cue); late set cue (500 ms before the appearance of the go signal); and movement (the 500-ms period before the screen was touched).

**Statistical analysis using interspike intervals**

To analyze neuronal activity with high temporal resolution, we first calculated the instantaneous firing rate as the inverse of the interspike interval (inverse-ISI; 1-ms resolution). When a spike occurred, the firing rate was updated and stored in the subsequent 1-ms data points until the next spike occurred, which, in turn, renewed the subsequent data points. Although original inverse-ISI data were calculated with 1-ms resolution, statistical analyses were applied for data re-sampled from the original data set at every 10 ms (i.e., 10-ms bin). We did this because the firing rate generally remained <100 Hz. As the rate of neuronal discharge tended to follow a Poisson distribution, the inverse-ISI data were square root-transformed to stabilize the variance (Zar 1999).

To estimate whether neuronal activity reflected information contained in the first or second cue, or their combination, we used one-way ANOVA. We examined how well each of the following equations accounted for neuronal activity

\[
Firing \text{ rate index} = \beta_0 + \beta_1 \times (\text{first cue})
\]

\[
Firing \text{ rate index} = \beta_0 + \beta_2 \times (\text{second cue})
\]

\[
Firing \text{ rate index} = \beta_0 + \beta_3 \times (\text{combination of first and second cues})
\]

In Eqs. 1–3, the firing rate index is for inverse-ISI data that were sampled every 10 ms; \( \beta_0 \) is the intercept and \( \beta_1, \beta_2, \beta_3 \) are coefficients. Categorical factors for the first and second cues are the four instructions provided by the cues (right arm, right target, left arm, and left target). Categorical factors for the combination of the first and second cues are the four possible combinations of arm use and target location provided by the first and second cues. First, we calculated the probability (categorical factors for the combination of the first and second cues are the four possible combinations of arm use and target location provided by the first and second cues. First, we calculated the probability (P value) that the coefficient in each equation equaled zero. We calculated P values for each 10-ms time point (i.e., for each bin) by creating an algorithm that was executed using commercial software (MATLAB 6.5, MathWorks, Natick, MA). The threshold used for statistical significance was set at \( \alpha = 0.01 \). Then, we calculated the sum of squares (SS) among groups and divided this value by the total SS to obtain the SS ratio; these SS values were obtained using ANOVA tables. The SS ratio was analyzed for each 10-ms data bin. The larger the SS ratio, the better the neuronal activity reflected the equation factor (i.e., the 1st cue, the 2nd cue, or the combination of the 2 cues). Based on the analysis of probability and the SS ratio, we classified the neurons into four categories according to whether instantaneous activity was best and significantly represented by the first cue, second cue, combined information from both cues, or whether none of the regression coefficients significantly differed from zero. This classification was used for data in every 10-ms bin. We also applied multi-way ANOVA to the inverse-ISI data to categorize neuronal activity with high temporal resolution. The next section details this analysis.

**R E S U L T S**

**Neuronal database**

We recorded neuronal activity in the postarcuate premotor areas of monkeys performing the behavioral task described in...
METHODS. Success rates recorded during the behavioral task exceeded 96% for both monkeys. We delineated the PMd from the PMv using the spur of the arcuate sulcus; the area dorsal to the spur was defined as the PMd and the area ventral to the spur was defined as the PMv. We found a total of 1,016 task-related neurons in the PMd (n = 505 in monkey 1; n = 511 in monkey 2) and 358 task-related neurons in the PMv (n = 204 in monkey 1; n = 154 in monkey 2). Because previous research has examined properties of neuron activity in the PMd and PMv during the motor set period (Hoshi and Tanji 2002), we focused on neuronal activity relevant to reception of visual cues and to subsequent processing to achieve action planning. For this report, we constructed a database using neurons that exhibited significantly changed activity in at least one of the first seven task periods (i.e., from precue to late 2nd delay; see METHODS for task period definitions) compared with the control period (paired $t$-test, $P < 0.05$, corrected for 8 trial types but not corrected for 7 task periods). Consequently, this report examines the response properties of 825 PMd and 223 PMv neurons. Figure 1, B and C, shows the fraction of neurons in the database that changed activity during each task period. The neurons in both areas changed their activity by $>35\%$ during these task periods (represented by thick lines); the fraction peaked when the second cue was presented. These results suggest that both the PMd and PMv were actively involved in the earlier part of the behavioral task when the animal was required to detect visuospatial information (i.e., the position of the white square), to retrieve a specific motor instruction from the visual cue, and to combine the arm-use and target-location instructions to plan the future action.

Activity in anticipation of a cue’s appearance

Before the appearance of the first cue (i.e., during the precue period), 218 (26%) PMd and 36 (16%) PMv neurons exhibited activity significantly changed compared with the control period (Fig. 1, B and C). It is possible that anticipatory activity reflected specific expectations for the appearance of the first instruction because instructions for arm use and target location were presented in a fixed order within each block of 20 trials. For example, the neuron illustrated in Fig. 3A showed greater activity before the appearance of the arm instruction than the target instruction. To investigate this possibility, we applied a two-sample $t$-test for activity within the precue period (factor: order of instructions). Of the 218 PMd neurons that exhibited anticipatory activity, 28 neurons (12%) exhibited activity that significantly differed depending on the forthcoming first instruction (2-sample $t$-test, $P < 0.01$). Selective neurons had a median difference of 5.1 spikes per second (range: 2.6–12.6 spikes/s). In contrast, of the 36 PMv neurons that exhibited anticipatory activity, only 1 neuron (2%) exhibited a significant difference in activity (2-sample $t$-test, $P = 0.0922$, Fisher’s exact test for count data) due to the small PMv sample size, results suggest that specific expectations of a forthcoming cue tended to be represented more frequently in the PMd than in the PMv.

Differences in neuronal activity in the PMd and PMv during the first cue and first delay periods

After the first cue appeared, $>35\%$ of neurons in the PMd and PMv exhibited activity significantly changed compared with the control period (Fig. 1, B and C). We found three classes of neuronal activity in the PMd and PMv. The first class of activity reflected only the visuospatial feature of the first cue (i.e., the position of the white square). In the example shown in Fig. 2, the PMv neuron showed visual responses if a white square appeared to the left of the fixation point, regardless of
whether the cue instructed the arm use or reach target. The second class of activity responded to the instruction for the arm use. In the example shown in Fig. 3A, the PMd neuron responded preferentially to the right arm instruction. Finally, the third class of activity responded to the instruction for the target location. In the example shown in Fig. 3B, the PMd neuron responded preferentially to the right target instruction.

To analyze how PMd and PMv neuron activity represented information provided by the first cue, we examined neuronal activity using two-way ANOVA ($\alpha = 0.01$); the two categorical factors (i.e., independent variables) included the position of the white square (position: left or right) and the instruction type (instruction: arm use or target location). The square-rooted activity of each 10-ms bin obtained by inversing the ISI (see METHODS) was used as a dependent variable. We applied this analysis to every 10-ms bin of activity for all neurons in the database ($n = 825$ in the PMd; $n = 223$ in the PMv).

Figure 4, A and B, presents the fraction of neurons that exhibited significant selectivity to the cue position (i.e., the position of the white square; position $< 0.01$ or instruction*position $< 0.01$). During the first-cue period and the following delay period, we found position selectivity continuously in 30% of PMd neurons (Fig. 4A, —), and in 20% of PMv neurons (Fig. 4B, —). Although the PMv neuron illustrated in Fig. 2 did not show vigorous activity during the delay period, we found that a group of neurons in PMv showed sustained delay period activity. These results suggest that the selectivity for the spatial location of the visual cue (right or left) was reflected well in both areas during the cue and delay periods. For these cue-position selective neurons, we studied how many neurons were selective only for the position of the white square and not for the type of instruction (2-way ANOVA: position $< 0.01$, instruction $\geq 0.01$, position*instruction $\geq 0.01$). We considered these neurons to represent only a cue’s visuospatial information rather than its motor instruction. The ··· in Fig. 4, A and B, illustrates results. In the PMv (Fig. 4B), 81% of cue-position selective neurons, on average, were judged as visuospatial, whereas in the PMd, only 54% were visuospatial (Fig. 4A).

Subsequently, we analyzed selectivity for instruction (arm use or target location). Figure 4, C and D, shows the fraction of neurons in each 10-ms bin that appeared to be significantly selective for the type of instruction (2-way ANOVA: instruction $< 0.01$ or position*instruction $< 0.01$). More than 20% of neurons in the PMd were instruction-selective. Of these, an average of 50% preferred arm instruction (represented by the dotted line in Fig. 4C) and 50% preferred target instruction (represented by the difference between ··· and — in Fig. 4C). In contrast, we only rarely found instruction selectivity in the PMv (Fig. 4D), supporting our judgment that an average of 81% of position-selective PMv neurons represented only visuospatial information (Fig. 4B). A $\chi^2$ test ($\alpha = 0.01$) confirmed that the PMd exhibited more frequent target instruction-selective activity (detected in 136 bins of 160 10-ms bins during the cue and delay periods). Arm-selective activity was also more frequent in the PMd (148 bins). In ~5% of PMd neurons, instruction selectivity appeared even before the onset of the first cue (anticipatory activity, Fig. 4C; see the above section).

We analyzed the timing of the onset of activity changes in response to the first cue. We defined the onset of cue-selective

![Figure 4](http://jn.physiology.org/)

**Fig. 4.** Time course of the cue-position selectivity during the 1st-cue and delay periods. A and B: bin-by-bin plots of position selectivity expressed as the fraction of all neurons in the database ($n = 825$ in PMd; $n = 223$ in PMv). ···, when the cues appeared. —, fraction of neurons that were position-selective (2-way ANOVA, position $< 0.01$ or position*instruction $< 0.01$), calculated successively for each 10-ms bin. ····, fraction of neurons that were position-selective only (not instruction-selective; 2-way ANOVA, position $< 0.01$, instruction $\geq 0.01$, and position*instruction $\geq 0.01$). A and B are data for neurons in the PMd and PMv, respectively. The tick marks on the horizontal axis represent 400-ms intervals. ▲, onset latency, i.e., the time at which the fraction of selective neurons first exceeded 10% of the total population. The time below the ▲ indicates the actual latency (latency for cue-position selectivity, followed by the latency for visuospatial selectivity in the parenthesis). C and D: time course of instruction selectivity during the 1st-cue and delay periods. C and D: bin-by-bin plot of instruction selectivity expressed as the fraction of all neurons in the database. —, fraction of neurons that exhibited arm- or target-instruction selectivity, calculated successively for each 10-ms bin (2-way ANOVA, instruction $< 0.01$ or position*instruction $< 0.01$). ····, fraction of neurons that exhibited greater activity for the arm instruction. ▲, onset latency, i.e., the time at which the fraction of neurons selective for the instruction 1st exceeded 10% of the total population. The time below the ▲ indicates the actual latency.
activity as the time when the fraction of selective neurons first exceeded 10% of the total neuron population (i.e., all neurons in the database, \( n = 825 \) in PMd and \( n = 223 \) in PMv). The onset of position selectivity was 100 ms in the PMv and 140 ms in the PMd (Fig. 4, A and B, \(-\)). The onset of visuospatial selectivity (i.e., selective only for the position, and not for the instruction) was 110 ms in the PMv and 150 ms in the PMd (Fig. 4, A and B, \( \cdots \)). Therefore visuospatial selectivity appeared 40 ms earlier in the PMv than in the PMd. The onset of instruction selectivity appeared at 200 ms in the PMd (Fig. 4C), 50 ms later than the onset of visuospatial selectivity (150 ms) in the same area. The onset of instruction selectivity in the PMv appeared at 1,540 ms (Fig. 4D).

These results suggest a difference in neuronal response properties between the PMd and PMv. PMv neurons mainly represented a cue’s visuospatial information, whereas PMd neurons amply retrieved instruction contents along with the visuospatial information, suggesting that PMd neurons represented more processed information than PMv neurons. Data on response latencies support this view because the latency of the visuospatial activity was 40 ms longer in the PMd than in the PMv, and the latency of instruction selectivity in the PMd was 50 ms longer than that of the visuospatial selectivity.

**Laterality of responses during the first cue and first delay periods**

Because we made the task symmetrical for right–left laterality, it was possible to analyze the preferred side of the visuospace or instruction by recording neuronal activity in a single hemisphere. We studied side preference for neurons judged to be selective for visuospatial or instruction information. Figure 5, A and B, shows the results for visuospatial activity (i.e., neuronal activity selective only for the cue position, and not for the instruction). In the PMd (Fig. 5A), neuronal activity that preferred the contralateral side developed earlier than ipsilateral-prefering activity; response latency was 140 ms for contralateral-prefering activity and 170 ms for ipsilateral-prefering one. In this analysis, the threshold of the response latency was halved into 5% of the total population (i.e., all neurons in the data base, \( n = 825 \) in PMd and \( n = 223 \) in PMv) because an original group of selective neurons was subdivided into right- and left-selective groups. Fractions of neurons preferring either side reached similar levels at 350 ms and this trend continued throughout the delay period. In the PMv (Fig. 5B), contralateral-prefering visuospatial activity had a latency of 100 ms and ipsilateral one had a latency of 150 ms. Fractions of ipsilateral- and contralateral-prefering activity did not differ after 1,000 ms from when the first cue appeared. These results suggest that visuospatial information from the contralateral side arrived earlier in both the PMd and PMv and that the representation of space eventually became bilateral. We used the same method to study the laterality preference of instruction-selective neurons in the PMd (Fig. 5C). Contralateral- and ipsilateral-prefering activity had response latencies of 220 and 250 ms, respectively. Neurons preferring the contralateral target or arm tended to dominate. For arm-selective activity, 59% on average showed greater activity for the contralateral arm-use instruction. For target-selective activity, 54% on average showed greater activity for the contralateral target or arm-use instruction. However, because >40% of neurons preferred ipsilateral arm use or target location, PMd neurons in one hemisphere could be viewed as representing instructions for bilateral arm use and target location.

**Location of neurons responding to the first cue**

Figure 6A shows a cortical surface map indicating the recording site covering both the PMd and PMv where we sampled neuronal activity with electrode penetrations spaced by 1 mm (gray area). A thick dotted line indicates the border between the PM and the primary motor cortex (MI), operationally defined according to the criteria established previously.
(Fogassi et al. 2001; Kurata 1993). In this map, we plotted the locations of all task-related neurons by classifying their activity (at the end of the 1st cue period) into one of three categories: visuospatial-selective (2-way ANOVA, position < 0.01, instruction ≥ 0.01 and position*instruction ≥ 0.01), instruction-selective (instruction < 0.01 or position*instruction < 0.01), and nonselective (instruction ≥ 0.01, position ≥ 0.01, and position*instruction ≥ 0.01). In the PMd, we found both visuospatial- and instruction-selective neurons predominantly in the rostrocaudal extent. Interestingly, quantities of these neurons decreased abruptly at penetrations 3–4 mm more rostral to the genu of the arcuate sulcus. In the PMv, we found visuospatial neurons predominantly in the caudal bank and its lip region of the arcuate sulcus ventral to the spur. Seventy percent of the visuospatial PMv neurons were recorded >1,000 μm (1,893 ± 1,244, mean ± SD; range, 60–6,600 μm) below the depth where we first encountered neuronal activity. The neuron shown in Fig. 2 was found at the depth of 2.4 mm.

We performed a more precise analysis of the rostrocaudal distribution of the two classes of PMd neurons by measuring the distance in the rostral direction relative to the genu of the arcuate sulcus and counting the number of classified neurons within each 1-mm width of cortex positioned rostrocaudally. Figure 6, B and C, shows results obtained at two different times after the cue (Fig. 6B represents the end of the 1st cue period and Fig. 6C represents the end of the 1st delay period). We found visuospatial neurons (represented by blue trace) at both times, predominantly in the rostrocaudal extent of the PMd. In contrast, we found instruction-selective neurons (represented by red trace) more often rostral to the genu of the arcuate sulcus. Of these, neurons preferring arm instruction (represented by the dotted line) and target instruction (represented by the gray line) overlapped in their distribution.

**Differences in PMd and PMv neuronal activity during the second cue and second delay periods**

After the appearance of the second cue, >50% of neurons in both the PMd and PMv exhibited changes in activity compared with the control period (Fig. 1, B and C). We found that PMd neurons primarily reflected a specific combination of the two instructions, rather than the second cue itself. For example, the PMd neuron shown in Fig. 7A reflected right-arm use in the forthcoming action. On the other hand, the PMd neuron shown in Fig. 7B reflected right-target reach. In contrast, PMv neurons tended to reflect the visuospatial property of the second cue. For instance, the PMv neuron shown in Fig. 2 responded if the second cue contained a white square to the left of the fixation point. We also found that information for the forthcoming action gradually developed in the PMv during the second delay period and that the activity preferentially reflected the location of reach target. For example, the PMv neuron shown in Fig. 2 started reflecting the left target reach during the late phase of the second delay period.

**Fig. 6.** Anatomical location of visuospatial and instruction neurons. A: location of each neuron is rendered on the cortical surface. This figure shows data for the end of the first cue period. For the purpose of display, random noise of < ±0.4 mm was added to the original data, except for the data crossing the PMd–PMv border. The 2 left hemispheres from the 2 monkeys are superimposed. Red circles, instruction-selective neurons (2-way ANOVA, instruction < 0.01 or position*instruction < 0.01). Blue squares, visuospatial neurons (2-way ANOVA, position < 0.01, instruction ≥ 0.01, and position*instruction ≥ 0.01). Bar, a nonselective neuron (2-way ANOVA, position ≥ 0.01, instruction ≥ 0.01, and position*instruction ≥ 0.01). The gray area in the cortical surface indicates the recording site where neurons were sampled with electrodes inserted at 1-mm intervals. B and C: rostrocaudal distribution of instruction-selective or visuospatial neurons in the PMd. The number of neurons obtained in each 1-mm width of the PMd in the mediolateral plane is plotted in the rostrocaudal direction. Red line, instruction-selective neurons. Blue line, visuospatial neurons. Black dotted line, instruction-selective neurons with greater activity for the arm-use cue. Green solid line, instruction-selective neurons with greater activity for the target-location cue. B shows the data at the end of the 1st cue period, whereas C shows the data at the end of the 1st delay period.
We applied regression analysis to neuronal activity in 10-ms bins (see Eqs. 1–3) and were able to classify each neuron into one of four categories based on whether its activity was significant and most selective for the first cue, the second cue, a combination of cues, or was nonselective. We repeatedly calculated the fraction of neurons assigned to each of the four categories for successive 10-ms bins.

Figure 8A shows a bin-by-bin plot of the PMd neuron fractions that exhibited significantly modulated and most selective activity for the first cue (black trace), second cue (blue trace), and a combination of cues (red trace). After the first cue appeared, the PMd neuronal activity that was selective for the first cue (including both the visuospatial and instruction selectivity) developed quickly and became dominant; the first cueselective activity was most frequently found from 130 ms after the first cue presentation to 200 ms after the second cue presentation (the black tick marks at the top of the panel; $\chi^2$ test, $\alpha = 0.01$). After the second cue appeared, the fraction of first cue-selective neurons decreased rapidly. The decrease or disappearance of the first-cue selective activity, after the appearance of the second cue, is apparent in the examples of PMd neurons illustrated in Fig. 3, A and B, and also in Fig. 7, A and B. By contrast, the fraction of neurons that was selective for the second cue (blue) or combination of cues (red) quickly increased. This property is illustrated for PMd cells shown in Fig. 7, A and B. In these examples, activity began to reflect the forthcoming action, already while the second cue was still presented.

Combination-selective neurons became dominant at 220 ms after the appearance of the second cue when the second cue was still presented (the red tick marks at the top of the panel; $\chi^2$ test, $\alpha = 0.01$). Simultaneously, the fraction of neurons selective for the second cue decreased rapidly. Selectivity for the combination of cues (red trace) reached a plateau (25% of all PMd neurons) within 400 ms after onset of the second cue. Representation of the first cue, which was dominant during 200 ms after the second cue onset, turned out to represent an action (i.e., the combination of 2 instructions) at 220 ms after the second cue was presented.

Figure 8B shows a bin-by-bin plot of PMv data using the same format as in Fig. 8A. After the appearance of the first cue, the fraction of PMv neurons selective for the first cue increased quickly. The first cue-selective activity was most frequently found from 110 ms after the first cue onset to 120 ms after the second cue onset (the black tick marks at the top of the panel). After the appearance of the second cue, the fraction of first cue-selective neurons decreased rapidly, whereas the fraction of neurons selective for the second cue or combination of the cues rapidly increased. An example of the second-cue response of a PMv neuron is shown in Fig. 2, where responses were apparent if the second cue displayed a white square in the left side. The fraction of PMv neurons selective for the second cue was greater than the fraction of PMd neurons (56 10-ms bins, represented by the blue tick marks in Fig. 8C; $\chi^2$ test, $\alpha = 0.01$), suggesting that the second cue’s visual feature had a relatively greater impact in the PMv than in the PMd. During the second delay period, the fraction of neurons selective for the combination increased gradually, whereas the fraction of neurons selective for the second cue decreased; the PMv neuron illustrated in Fig. 2 started reflecting the left target reaching toward the end of the second delay period. The first 10-ms bin when the combination-selective activity became dominant was at 400 ms (the red tick marks at the top of the B), which was 180 ms later compared with the PMd. The 10-ms bins in which the combination-selective activity was found most frequently increased progressively toward the end of the delay period.

Next, we analyzed the timing of the onset of changes in PMd and PMv neuron activity in response to the first and second cues using the data shown in Fig. 8, A and B. We defined...
onset of activity” as the time when the fraction of selective neurons first exceeded 10% of the total neuron population. In this study, onset of selectivity for the first cue in the PMv was 110 ms after the appearance of the first cue, and in the PMd, it was 140 ms after the appearance of the first cue. The onset of selectivity for the second cue was 150 ms after the appearance of the second cue in both areas, and the onset of combination selectivity was 170 ms after the appearance of the second cue in the PMv and 140 ms after the appearance of the second cue in the PMd. These data reveal that while information about the cue reached the PMv earlier (110 ms in the PMv vs. 140 ms in the PMd after the 1st cue), information about action developed earlier in the PMd (170 ms in the PMv vs. 140 ms in the PMd after the appearance of the 2nd cue).

To examine the selectivity for the second cue position and for the combination of two cues, we quantitatively compared neuronal activity in the PMd and PMv. We applied the analysis to neurons selective for the first cue, second cue, or combination of two cues at the end of the second cue period (Fig. 8, A and B). To evaluate position selectivity of the second cue, we applied two-way ANOVA with two factors (the position of the white square in the 1st and 2nd cues) on the square-root transformed neuronal activity at the end of the second cue period. From the ANOVA table, we estimated spatial selectivity by calculating the sum of squares (SS) between groups (SS-bg) and dividing this value by the total SS (SS-total) to obtain the SS ratio

\[
\text{SS ratio for the position of CUE2} = \frac{\text{SS-bg for the position of CUE2}}{\text{SS-total}} \quad (4)
\]

The greater this value was, the greater the selectivity for the second-cue position; Fig. 9A shows results of the comparison of spatial selectivity in the PMd and PMv. The SS ratio for the position of the second cue was significantly greater for the PMv neurons than for the PMd neurons (Kolmogorov–Smirnov test, KS = 0.3101, \(P < 0.0001\)), indicating that spatial selectivity was represented more in the PMv than in the PMd.

To evaluate the two-cue combination selectivity, we applied the following linear regression model on activity

\[
\text{Firing rate index} = \beta_0 + \beta_1 \times (\text{CUE2}) + \beta_2 \times (\text{COMBINATION}) \quad (5)
\]

In this formula, the firing rate index is calculated at the end of the second cue period, \(\beta_0\) is the intercept, and \(\beta_1\) and \(\beta_2\) are coefficients. Categorical factors of the second cue (CUE2) are the 4 instructions that could be conveyed by the second cue (right-arm, right-target, left-arm, and left-target). Categorical factors of the two-cue combination (COMBINATION) are the 4 possible combinations of the two instructions given by the first and second cues. We calculated the sum of squares (SS) between groups (SS-bg) and divided this value by the total SS (SS-total) to obtain the SS ratio. We calculated the SS ratio as follows

\[
\text{SS ratio for COMBINATION} = \frac{\text{SS-bg for COMBINATION}}{\text{SS-total}} \quad (6)
\]

The greater this value was, the greater the selectivity for the two-cue combination; Fig. 9B shows result of the comparison of combination selectivity in the PMd and PMv. The SS ratio for the two-cue combination was significantly greater for the PMd neurons than for the PMv neurons (Kolmogorov–Smirnov test,
We analyzed the localization of the three classes of PMd neurons more precisely in the rostrocaudal direction. Figure 10, C and D present results based on the two sets of data (Fig. 10C, end of the second cue period; Fig. 10D, end of the second delay period). At the end of the second cue period, first cue- (black) or second cue- (blue) selective neurons were more likely to be located rostrally. Combination-selective neurons were found broadly in the PMd at both times.

Development of information for arm use and target location

The monkeys were required to integrate two sets of information, i.e., arm use and target location, to plan a future action. The time course of their development in the PMd and PMv was of interest, which we examined by applying a three-way ANOVA to the neurons we judged to be most selective for a combination of the two cues (the red trace shown in Fig. 8, A and B). The three factors included arm use (ARM), target location (TARGET), and the order of the two instructions (ORDER). Based on this analysis, we classified neurons into four categories: selective for arm use (ARM < 0.01 or ARM*TARGET < 0.01), selective for target location (TARGET < 0.01 or ARM*TARGET < 0.01), selective for both arm use and target location (ARM < 0.01 and TARGET < 0.01, or ARM*TARGET < 0.01), and nonselective (ARM ≥ 0.01, TARGET ≥ 0.01, and ARM*TARGET ≥ 0.01). We applied this analysis to instantaneous activity during every 10-ms bin (see the Methods section) if it had been classified as combination selective because we wanted to exclude activity primarily involved in detecting the visuospatial signal and in representing partial motor instruction provided by the first or second cue. In the PMd (Fig. 11A), the fractions of neurons selective for target location or arm use increased simultaneously in response to the second cue and remained at approximately equal levels throughout the second-cue and delay periods. In the PMv (Fig. 11B), while arm-use and target-location representations developed simultaneously, information about target location became progressively dominant toward the end of the delay period. Therefore although arm-use and target-location representations were similar and stable in the PMd population, information about target location grew progressively in the PMv during the delay period.

Laterality of arm-use and target-location representations

We analyzed side preference (i.e., ipsilateral or contralateral to the recording hemisphere) of information related to arm use or target location by examining whether the left (ipsilateral) or right (contralateral) side led to greater neuron activity related to future action (i.e., combination-selective neurons, see Fig. 8, A and B). Figure 11, C and D present the results for PMd neurons. Arm-selective neurons (Fig. 11, C) exhibited a response latency of 160 ms for contralateral arm-selective activity, versus 200 ms for the ipsilateral arm. This analysis used 5% of the total population as the response latency threshold. Therefore activity preferring the contralateral arm developed 40 ms earlier than the activity preferring the ipsilateral arm. Fractions of neurons preferring contralateral or ipsilateral arms reached similar levels while the second cue was still being presented, and this trend continued throughout the second delay period. During the second-cue and delay periods, on average, 49% of activity preferred the contralateral arm.

Location of neuronal selectivity after the appearance of the second cue

We mapped the locations of neurons classified as first-cue selective, second-cue selective, combination selective, and nonselective after onset of the second cue, using the same method shown in Fig. 6A. Figure 10, A and B present two maps based on data obtained at the end of the second cue period (Fig. 10A) and at the end of the second delay period (Fig. 10B). In the PMd, we found the three classes of neurons (first-cue selective, black circles; second-cue selective, blue squares; combination selective, red crosses) located broadly in the rostrocaudal extent at the end of the second cue period. At the end of the second delay period (Fig. 10B), we again found the combination-selective neurons located broadly in the PMd. In the PMv, we found the three classes of neurons mainly in the caudal bank and its lip region of the arcuate sulcus ventral to the spur. These neurons were located in areas of the PMd and PMv similar to the map shown in Fig. 6A.

FIG. 9. Comparison of selectivity of the 2nd cue responses for PMd and PMv neurons. A: comparison of position selectivity for the 2nd cue in PMd and PMv neurons. The cumulative fractions of the SS ratio for the position of the second cue for the PMd (n = 386, ...) and PMv (n = 67, —) are shown. B: comparison of 2-cue combination selectivity for PMd and PMv neurons. The cumulative fractions of the SS ratio for the combination, for the PMd (n = 386, ...) and PMv (n = 67, —), are shown.

KS = 0.3302, P < 0.0001), indicating that combination selectivity was represented more in the PMd than the PMv.
Response latencies for target-selective activity were 140 ms for the contralateral target and 190 ms for the ipsilateral target (Fig. 11D). The fraction of neurons preferring the contralateral target dominated slightly throughout the delay period; 61% of activity preferred the contralateral target during the second-cue and delay periods.

Figure 11, E and F present results for PMv neurons. Response latencies for the arm-selective neurons (Fig. 11E) were 180 ms for the contralateral arm and 200 ms for the ipsilateral arm. During second-cue and delay periods, on average, 50% of activity preferred the contralateral arm while the remaining 50% preferred the ipsilateral arm. When we examined target-selective activity, we found that response latencies were 180 ms for the contralateral target and 200 ms for the ipsilateral target (Fig. 11F). The fraction of neurons preferring the contralateral target dominated slightly; during the second-cue and delay periods, 60% of activity preferred the contralateral target.

Localization of arm-use and target-location selectivity in the PMd and PMv

We examined the distribution of neurons selective for arm use and/or target location. Figure 12A presents the selectivity distribution of neuronal activity measured at the end of the second delay. In the PMd, neurons selective only for target location (represented by blue squares; three-way ANOVA: ARM ≥0.01, TARGET <0.01, and ARM*TARGET ≥0.01) were more likely to be found rostrally, while neurons only selective for arm use (represented by green diamonds; three-way ANOVA: ARM <0.01, TARGET ≥0.01, and ARM*TARGET ≥0.01) were more likely to be found caudally. Interestingly, PMd neurons selective for both arm use and target location (represented by black dots; three-way ANOVA: ARM <0.01 and TARGET ≥0.01, or ARM*TARGET <0.01) were found broadly in the rostrocaudal extent of the PMd. In the PMv, neurons were mainly selective only for target location; these neurons were predominantly found in the caudal bank and its lip region of the arcuate sulcus ventral to the spur.

We performed a more precise analysis of the rostrocaudal distribution of the three classes of PMd neurons by measuring the distance of each neuron in the rostral direction relative to the genu of the arcuate sulcus and counted the number of classified neurons within each 1-mm width of the cortex positioned rostrocaudally. Figure 12, B and C show results...
measured at two different times after cue onset (Fig. 12B, at the end of the second cue period; Fig. 12C, at the end of the second delay period). At both times, we found neurons selective only for target location (blue trace), more often rostral to the genu of the arcuate sulcus. In contrast, we found neurons selective only for arm use (green trace) more often caudal to the genu of the arcuate sulcus. Using these distribution data of the PMd, we measured the Mahalanobis distances from each data point to the center of the arm-use only neurons and target-location only neurons. In Fig. 12A, we draw a line (indicated by *) where the two Mahalanobis distances were equal. Area to the left of the line indicates where the distance is shorter to the center of the target-location only neurons. In contrast, area to the right of the line indicates where the distance is shorter to the center of the arm-use only neurons.
arm-use only neurons. Thus this analysis successfully classified the PMd into the rostral and caudal part based on the distributions of target-location and arm-use selectivity of the forthcoming action. However, neurons selective for both factors (black trace in Fig. 12, B and C) were distributed widely in the rostrocaudal extent of the PMd. Taken together, these analyses revealed that the PMd rostral region preferentially represented the target location in conjunction with arm use, and that the PMd caudal region preferentially represented arm use in conjunction with target location.

**Relationship of response selectivity during the first and second task phases**

It was of interest to know how each PM neuron responding to the first cue behaved after the second cue, and how the selectivity of each neuron responsive to the second cue exhibited responses to the first cue. To answer these questions, we examined the relationship of response selectivity of each neuron at two different epochs: 1) at the end of the first cue period (using the data shown in Fig. 4) and 2) at the end of the second delay period (using the data shown in Figs. 8 and 11). The results of this analysis are summarized in Table 1 (for PMd neurons) and Table 2 (for PMv neurons). For neurons in both the PMd and PMv, a great majority of selective activity during the first task phase (i.e., at the end of the first cue period) was not carried over to the second phase (i.e., at the end of the second delay period). In the PMd (Table 1), the visuospatial selectivity or instruction selectivity in the first task phase \((n = 347)\) was replaced by nonselective activity \((n = 202, 58\%)\), the combination selective activity \((n = 117, 34\%)\), and second cue selective activity \((n = 13, 4\%)\) in the second task phase. In the PMv (Table 2), the visuospatial selectivity or instruction selectivity in the first task phase \((n = 60)\) was replaced by nonselective activity \((n = 27, 45\%)\), the combination selective activity \((n = 26, 43\%)\), or second cue selective activity \((n = 6, 1\%)\) in the second task phase.

Looking backward, we examined how the two-cue combination selective neurons in the second phase behaved in the first phase. Out of the 228 combination selective PMd neurons, 111 \((49\%)\) neurons were classified as nonselective, 51 \((22\%)\) as instruction selective, and 66 \((29\%)\) as visuospatial in the first phase (Table 1). In the PMv, out of the 45 combination selective neurons, 19 \((42\%)\) neurons were classified as nonselective, 8 \((18\%)\) as instruction selective, and 18 \((40\%)\) as visuospatial in the first phase (Table 2). We then studied the relationship between the selectivity in the first task phase and selectivity for the arm use or the target location of a forthcoming action in the second task phase. Figure 13, A and B show the results for the PMd and PMv, respectively. In PMd (Fig. 13A), neurons selective for the visuospace or instruction given by the first cue were evenly distributed among the three groups of the two-cue combination selectivity (i.e., arm-use only, target-location only, and both arm-use and target-location). In contrast, in PMv (Fig. 13B), neurons selective for the visuospatial information given by the first cue (represented with the light gray) showed a tendency to cluster in the group of neurons selective only for the forthcoming target location; this tendency was significantly different compared with the PMd \((\chi^2 \text{ test, } P = 0.0008)\).
In this study, we recorded neuronal activity in two separate areas of the premotor cortex (the PMd and PMv) while the two subject monkeys performed a behavioral task during which they were actively engaged in planning a future reaching movement based on two visual instruction signals.

The following four points summarize our main findings.

First, PMv neurons predominantly reflected the locations of visuospatial signals given with the first cue, and this visual response property also accompanied the second cue. Second, about half of PMd neuron first-cue responses reflected motor instructions retrieved from the cue (arm or target to be selected). When the second cue was given, PMd neurons began to reflect a combination of the two instructions (one for the arm and the other for the target) already reflecting a planned action. Third, the visuospatial representation and representations for arm-use or target-location information were bilateral in both the PMd and PMv. Fourth, regional differences in response properties appeared within the PMd and the PMv. When the first cue was given, responses reflecting motor instructions appeared more prominently in the rostral part of the PMd. In response to the second cue, activity reflecting a forthcoming action was distributed widely in the PMd; target location was represented more rostrally and arm use was represented more caudally. In the PMv, neurons responding to the visual signal and neurons selective for the forthcoming action were found mainly in the caudal bank of the arcuate sulcus and its posteriorly adjacent lip region. The following section presents these findings along with findings from previous reports and proposes two functional networks involved in sensorimotor processing and integration: the dorsal and ventral frontal networks encompassing the premotor and dorsolateral prefrontal cortex.

**DISCUSSION**

In this study, we recorded neuronal activity in two separate areas of the premotor cortex (the PMd and PMv) while the two subject monkeys performed a behavioral task during which they were actively engaged in planning a future reaching movement based on two visual instruction signals.

The four points summarize our main findings. First, PMv neurons predominantly reflected the locations of visuospatial signals given with the first cue, and this visual response property also accompanied the second cue. Second, about half of PMd neuron first-cue responses reflected motor instructions retrieved from the cue (arm or target to be selected). When the second cue was given, PMd neurons began to reflect a combination of the two instructions (one for the arm and the other for the target) already reflecting a planned action. Third, the visuospatial representation and representations for arm-use or target-location information were bilateral in both the PMd and PMv. Fourth, regional differences in response properties appeared within the PMd and the PMv. When the first cue was given, responses reflecting motor instructions appeared more prominently in the rostral part of the PMd. In response to the second cue, activity reflecting a forthcoming action was distributed widely in the PMd; target location was represented more rostrally and arm use was represented more caudally. In the PMv, neurons responding to the visual signal and neurons selective for the forthcoming action were found mainly in the caudal bank of the arcuate sulcus and its posteriorly adjacent lip region. The following section presents these findings along with findings from previous reports and proposes two functional networks involved in sensorimotor processing and integration: the dorsal and ventral frontal networks encompassing the premotor and dorsolateral prefrontal cortex.

**Anatomical localization of neuronal activity in PMd and PMv**

In the PMd, we found most of the task-related neurons up to the area 3–4 mm rostral to the genu of the arcuate sulcus. The rostral area where we found a cluster of neurons selective for the type of the instruction corresponds to the area possessing cortico-cortical connections with the dorsolateral prefrontal cortex (Lu et al. 1994; Takada et al. 2004), especially dorsal to the principal sulcus (Barbas and Pandya 1987; Luppino et al. 2003). Cisek and Kalaska (2005) found neuronal activity reflecting a motor planning process in a corresponding area. In the same study, they confirmed that the rostral part of the recorded area included area F7 (prePMd) by analyzing its anatomical connection with the parietal cortex (Matelli et al. 1998). Taken together, the rostral part of the PMd where task-related neurons are concentrated is a transition region between F2 and F7 (Matelli et al. 1985) and PMdc and PMdr (Barbas and Pandya 1987; see for review Picard and Strick 2001).

Conversely, in the region ventral to the arcuate spur, we found neuronal activity selective to the visual signal and selective for the forthcoming action primarily from the caudal bank of the arcuate sulcus and its posteriorly adjacent lip region. The region corresponded to the rostral part of the PMv (or area F5) (Matelli et al. 1985) connected with the dorsolateral prefrontal cortex (Lu et al. 1994; Takada et al. 2004), especially with its ventral part (Wang et al. 2002). This area is a part of the PMv reported to be related to arm movements (Kurata 1993; Kurata and Hoshi 2002) or responsive to visual signals (Boussaoud et al. 1993; Godschalk et al. 1981; Kubota and Hamada 1978; Rizzolatti et al. 1981).

**TABLE 1. Relationship of response selectivity of PMd neurons in the first and second task phases**

<table>
<thead>
<tr>
<th>First Phase Selectivity*</th>
<th>Combination Selective</th>
<th>Second Phase Selectivity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm only</td>
<td>Arm and target</td>
</tr>
<tr>
<td>Arm instruction (96)</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Target instruction (102)</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Visuospatial (149)</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>Nonselective (478)</td>
<td>28</td>
<td>43</td>
</tr>
<tr>
<td>Total (825)</td>
<td>62</td>
<td>91</td>
</tr>
</tbody>
</table>

The parentheses enclose the number of neurons classified. *Selectivity at the end of the first cue period was presented. ** Selectivity at the end of the second delay period.

**TABLE 2. Relationship of response selectivity of PMv neurons in the first and second task phases**

<table>
<thead>
<tr>
<th>First Phase Selectivity*</th>
<th>Combination Selective</th>
<th>Second Phase Selectivity**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm only</td>
<td>Arm and target</td>
</tr>
<tr>
<td>Arm instruction (7)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Target instruction (7)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Visuospatial (46)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Nonselective (163)</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Total (223)</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

The parentheses enclose the number of neurons classified. *Selectivity at the end of the first cue period was presented. ** Selectivity at the end of the second delay period.
Neuronal activity before the appearance of the first cue

Because the two instructions for arm use and target location were presented in a fixed order within a block of 20 trials, it was possible for the animals to expect the forthcoming instruction type. We found that 12% of activity-modified PMd neurons significantly reflected whether the forthcoming first cue would instruct the arm use or target location. We also found that, in contrast, only 2% of activity-modified PMv neurons showed this selectivity.

Anticipatory activity of PMd neurons before the appearance of the predictable visual signals was first revealed by Mauritz and Wise (1986). More recently, the precue activity was revealed to reflect an abstract rule of the behavior: matching or nonmatching of two successive visual signals (Wallis and Miller 2003). These previous reports and our observation suggest that PMd is an area where expectation for the appearance of events, behavioral rule, and/or specific expectation for the forthcoming signal are represented.

FIG. 13. Relationship of response selectivity in the first and second task phases. The distribution of selectivity in the first task phase is displayed in histograms in which the two-cue combination selectivity in the second task phase is divided in three categories in the abscissa. The two-cue combination selectivity was assessed at the end of the second delay period (see Figs. 11 and 12), whereas the selectivity during the first phase was assessed at the end of the first cue period (see Figs. 4 and 6). A is for PMd neurons, and B is for PMv neurons. Original data are summarized in Tables 1 and 2.

Visuospatial responses of PMv neurons compared with PMd neurons

Extensive research has been conducted on PM neuron responsiveness to visual signals. Early studies of the premotor cortex reported neuronal response to the appearance of visual signals (Godschalk et al. 1981, 1985; Kubota and Hamada 1978; Rizzolatti et al. 1981). Subsequent studies revealed that premotor neurons represent visuospatial information related to a cue (Boussaoud et al. 1993; Fogassi et al. 1996; Graziano et al. 1997), an object’s three-dimensional features (Fogassi et al. 1999; Murata et al. 1997), and attention to a spatial location (Boussaoud and Wise 1993a,b; Lebedev and Wise 2001). The neuronal activity we observed supports these previous reports because it responded to visual instruction cues in both the PMv and PMd. Our study made a novel discovery: PMv and PMd neurons respond differently to visual signals. PMv neurons were characterized by responses reflecting the visuospatial properties of visual instruction signals; most responses to the first cue and a considerable proportion of responses to the second cue were selective for the location of the white square constituting both visual signals. About half of the PMd neurons responding to the first cue reflected instructions of the first visual cue (which arm to use or the target location). In response to the second visual cue, PMd neurons reflected not only instructions of the second cue but also a combination of information given in the first and second cues.

We analyzed the onset latency of cue-position selectivity for PMd neurons exhibiting visuospatial selectivity for a visual signal in comparison with PMv neurons (Fig. 4, A and B, —). We found that the PMd had an onset latency of 140 ms, 40 ms longer than the PMv onset latency. We compared these data with data previously reported for the prefrontal cortex (Hoshi and Tanji 2004a). In the dorsolateral prefrontal cortex (DLPFC) of monkeys performing the same behavioral task, the dorsal DLPFC had a latency of 190 ms and the ventral DLPFC has a latency of 110 ms. These results indicate that visuospatial information arrived in the ventral areas of the premotor and dorsolateral prefrontal cortices earlier than in their dorsal counterparts. Because ventral and dorsal areas are linked with similar sets of parietal areas (Petrides and Pandya 1984), the difference in latency between the PMd and PMv seems to indicate that spatial information is processed more quickly in parietal areas linked to the ventral prefronto–premotor region. Further, the selective activity developed earlier in the premotor cortex than in the DLPFC by 10~50 ms. A possible interpretation might be that the premotor cortex retrieves relevant spatial information in an expedited manner to exert motor control as quickly as possible under a condition of performing a well-trained behavioral task.

Retrieval and integration of motor instructions in the PMd

Previous studies have reported that premotor neurons represent movement parameters (such as movement direction or amplitude) to be performed when visual signals are used for instruction (Boussaoud and Wise 1993b; Fu et al. 1993; Kurata and Wise 1988; Mitz et al. 1991; Wise et al. 1983). These studies indicated that PMd neurons are able to retrieve information about movement parameters and store it during a preparatory period. If instructions provide two motor parame-
ters (such as direction and amplitude) sequentially with an intervening interval, the process of motor preparation involves premotor cortex neurons first reflecting one parameter and then combining both instruction parameters (Kurata 1993, 1994; Riehle 2005; Riehle and Requin 1989, 1995). These studies clearly indicate that PMd neurons are able to retain one component of motor information before combining it with another component. Our study introduced a novel behavioral situation in which subjects were required to select an action by retrieving information about the effector to be used and information about the target to reach in extrinsic space. We found that PMd neurons were able to retrieve information about either the effector or the spatial target, retain the fragment of information given first until receiving the second fragment, and then combine the two sets of information to generate information specifying a forthcoming action; however, few PMv neurons were able to accomplish this process. Analyses of response latencies revealed that both first-cue and combination selectivity appeared as early as 140 ms after the appearance of the second cue (Fig. 8A). As a population, it seemed as if the representation of motor instruction in the PMd was transformed into the representation of action at 220 ms after the second cue onset (Fig. 8A). Further, the combination selectivity became stable while the cue was still presented. In contrast, the representation of two-cue combination in the PMv became dominant at 180 ms later, i.e., at 400 ms, and the combination selectivity gradually developed during the delay period (Fig. 8B). These findings suggest that PMd neuron responsiveness was predetermined before cue appearance, expediting processes for action planning. Combined, our findings provide new evidence about the crucial role the PMd plays in planning action by integrating information provided by visual instructional signals.

In the dorsal DLPFC of monkeys performing the same task, the response latency of the instruction selective activity after the first cue onset was 250 ms (Hoshi and Tanji 2004a), which was longer by 50 ms compared with the PMd. Further, the combination selective activity in the dorsal DLPFC after the second cue onset was 210 ms (Hoshi and Tanji 2004a), which was longer by 70 ms compared with the PMd. These results indicate that information on motor instruction and action developed earlier in the PMd than in the dorsal DLPFC. Wallis and Miller compared response latencies between the PMd and prefrontal cortex and reported a similar finding; the development of response-related neurons was earlier in the PMd by 60 ms (Wallis and Miller 2003). They suggested that the prefrontal cortex might receive “efference copy” of motor response from the PMd. They also found that the rule-selectivity developed earlier in the PMd and suggested that the familiarity of the task engaged the PMd. Another possible explanation would be that the information on action develops via separate pathways through the basal ganglia (Brasted and Wise 2004; Hadj-Bouziane and Bousaud 2003; Inase et al. 2001; Mink 1996; Nixon et al. 2004; Pasupathy and Miller 2005) because the PMd and prefrontal cortex constitute separate parallel loops (Alexander et al. 1986; Middleton and Strick 2001).

Previous studies have found that PMd neurons rarely represent the colors or shapes of objects used as instruction signals (Kurata and Wise 1988; Mitz et al. 1991; Wallis and Miller 2003). In contrast, the prefrontal cortex responds to diverse features of visual objects and processes that information to generate motor information, such as movement direction or location of a reach-target or saccade-target (Asaad et al. 1998; Genoveseio et al. 2005; Hasegawa et al. 1998; Hoshi et al. 2000; Kim and Shadlen 1999; Quintana and Fuster 1992; Quintana et al. 1988; White and Wise 1999; Yajeya et al. 1988). Therefore the PMd seems to be involved in the process of action planning in concert with the prefrontal cortex as well as by other structures including the parietal cortex (Calton et al. 2002) and basal ganglia (Brasted and Wise 2004; Inase et al. 2001; Pasupathy and Miller 2005).

Laterality of visuospatial responses and instructed motor information

This study examined the laterality of PM neuron representation with respect to visuospatial responses and from the perspective of motor information retrieval. When we examined visuospatial response to cue signals, we found that both PMv and PMd neuron activity reflected similar degrees of ipsilateral and contralateral locations of visuospatial cues (Fig. 5, A and B). We also found that response latencies for visual cues in the contralateral field were shorter by 30–50 ms, suggesting PM proximity to information from the contralateral visual field. When we examined PMd representation of motor instruction, we found that neurons in one hemisphere reflected bilateral motor instructions on arm use and target location after the first cue (Fig. 5C) and that soon after the second cue, PMd neurons integrated bilateral information on arm use and target location (Fig. 11, C and D). This bilateral representation of motor instruction and action by PMd neurons is important because it suggests that local circuits within one hemisphere perform the neural computations required for motor planning for bilateral action. Our findings of a representation of bilateral action in PMd are consistent with previous reports of bilateral movement representations in PMd from single-neuron (Brinkman and Porter 1983; Cisek et al. 2003; Kermadi et al. 2000) and imaging (Geurtsen et al. 2005; Kollias et al. 2001; Nirkko et al. 2001) studies.

Rostrocaudal functional specialization within the PMd

We found regional differences in neuronal response properties within the PMd, especially in the rostrocaudal direction. After the appearance of the first cue, we found a higher density of neurons selective for the type of instruction in the rostral part of the PMd (Fig. 6). This part of the PMd coincides with a transition area between the rostral and caudal part of PMd, reported to be rich in visuomotor information (Fogassi et al. 1999; Raos et al. 2003). Recently, Cisek and Kalaska (2005) showed that this transition area contains neurons reflecting “the potential reach direction.” Because the rostral part of the PMd receives ample input from the DLPFC (Lu et al. 1994), it is possible that regional selectivity reflects an interaction between the PMd and DLPFCd. Visuospatial neurons in the PMd had a shorter onset latency (150 ms) than instruction-selective neurons (200 ms), and visuospatial neurons were found broadly in the rostrocaudal extent of the PMd. These observations suggest that visuospatial input moves directly from the posterior parietal cortex. Anatomical tracing studies have shown the exis-
tence of posterior parietal input to the rostral and caudal parts of the PMd (Caminiti et al. 1996; Matelli et al. 1998), and researchers have suggested that this input plays a part in visually guided motor behavior (Wise et al. 1997). Although the functional importance of input from the prefrontal and parietal cortex remains to be determined, our finding that the rostral PMd exhibits preferential involvement in representing motor instructions (both for arm and target) provides evidence that this part of the PMd is crucial for retrieving information about components of future actions and retaining that information until each component is combined when planning an action.

After the appearance of the second cue, neurons selective for the target location appeared more frequently in the rostral area, whereas those selective for arm use appeared more frequently in the caudal area. Shen and Alexander (1997) reported that neurons in the rostral area of the PMd were more selective for a visible cursor’s trajectory than for actual limb movements during the instruction delay period. Lebedev and Wise (2001) found that neurons in the rostral area of the PMd preferentially reflected attended location rather than reach-target location. More recently, Cisek et al. (2003) used a reaching task involving either arm to demonstrate that rostral PMd neurons were tuned for the target (or direction) of both arms and registered the preferred direction of each arm. These reports along with our own indicate that rostral PMd neurons are involved in representing the location of a target to be reached or an item’s attended location rather than specifying a body part (effector) to be used in future actions.

Researchers have reported that neuronal activity in the caudal PMd reflects movement amplitude and direction (Fu et al. 1993, 1995; Kurata 1993), actual movement trajectory (Shen and Alexander 1997), and the use of arm or leg (Kurata et al. 1985). Neurons in the caudal PMd has also been reported to reflect shoulder rotation (Caminiti et al. 1991) or arm orientation (Scott et al. 1997). The border dividing two rostro-caudal subdivisions of the PMd, determined on the basis of distributions of target-only and arm-only neurons (Fig. 12A), coincides with the border determined with anatomical findings, connectivity to the dorsolateral prefrontal cortex and the primary motor cortex (Lu et al. 1994), and cyto- and myelo-architectonic characteristics (Barbas and Pandya 1987). These reports, combined with our finding that caudal PMd neurons were preferentially influenced by arm use, indicate that the caudal PMd, interconnected with the primary motor cortex (Dum and Strick 2005; Lu et al. 1994), is more involved in representing planned movement of a body part for use in future actions.

Despite the aforementioned regional differences in arm-use and target-location representations related to cue instructions, it is worth noting that more than a third of the two-cue combination-selective neurons in rostral and caudal areas were selective for both arm use and target location (Fig. 12), and overall, PMd neurons represented similar degrees of information about both an action’s components (Fig. 11A). These results highlight the important role the PMd plays in integrating information about target location and arm use, which is carried over to the subsequent behavioral stages of motor preparation and execution (Hoshi and Tanji 2000).

Ventral prefronto–premotor network across the premotor cortex and DLPFC

In a previous study, we proposed that the existence of a dorsal prefronto–premotor network can be inferred on anatomical and physiological bases (Hoshi and Tanji 2004a). This paper proposes another possible network between the prefrontal and premotor cortex: a ventral prefronto–premotor network. This study demonstrated that PMv neurons represent visuospatial information provided in the first cue rather than motor instructions. We also found this response property in the DLPFCv of monkeys performing the same behavioral task (Hoshi and Tanji 2004a). These results support a report by Boussaoud and Wise (1993a,b); which showed that both PMv and DLPFCv neurons preferentially represent attention to a cue’s visuospatial position. We also found that neurons reflecting visuospatial features of the second cue were more abundant in the PMv than in the PMd (Fig. 8C). The second cue had a similarly stronger impact in the ventral part of the dorsolateral prefrontal cortex than in the dorsal part (Hoshi and Tanji 2004a). These observations revealed a functional similarity between the PMv and DLPFCv.

Anatomical studies show that the part of the PMv, the caudal bank and lip region of the inferior limb of the actuate sulcus where we found visuospatial neurons, corresponds to the area interconnected chiefly with the area ventral to the principal sulcus (Wang et al. 2002). Moreover, the two areas commonly receive parietal inputs from areas PF and PFG in the inferior parietal lobule (Cavada and Goldman-Rakic 1989; Pandya and Seltzer 1982; Petrides and Pandya 1984). Thus the proposed existence of a ventral network, as well as the dorsal network (Hoshi and Tanji 2004a), encompassing the prefrontal and prefrontal cortex has a structural background (see for review, Hoshi 2006).

It has been shown that separate parietal regions project to F2/F7 (PMd) and to F4/F5 (PMv) (Kurata 1991; Matelli et al. 1998; Rozzi et al. 2005; Tanne-Gariepy et al. 2002). Rizzolatti and co-workers have proposed dorsal and ventral premotor-parietal circuits on the grounds of the anatomical connectivity patterns and differences in response properties of neurons (Rizzolatti and Luppino 2001). Taken together with these findings, our proposal of dorsal and ventral prefronto-premotor circuits suggests the existence of two, much larger distributed dorsal and ventral fronto-parietal, networks for visuomotor control.

Sensorimotor processing in the premotor cortex

Boussaoud and Wise (1993a,b) compared neuronal activity in the PMd and PMv with a behavioral task in which two successive visual cues were given. The first cue (a spatial-attentional/ mnemonic cue) indicated a spatial location to be attended, whereas the second cue (a motor instructional/conditional cue) instructed a motor direction. They found that PMv neurons showed greater activity after the first cue than the second cue, suggesting that the PMv reflects spatial aspects of the visual signal (such as spatial attention or memory). PMd neurons, by contrast, preferentially reflected motor significance given by the cues. Our findings that PMv neurons preferentially reflected visuospatial aspects of the first cue and that PMd neurons reflected motor instruction are in line with their observations.
Four subsequent studies are relevant to our present study from a standpoint of exploring neural basis of sensorimotor transformation in the premotor cortex. Among them, two studied visuomotor transformation in the premotor cortex by giving sequential signals. Ohbayashi et al. (2003) used a behavioral task in which monkeys were required to make two saccadic eye movements either in accordance with or in reversed order of two successive spatial cues. They showed that PMd neurons were involved in receiving visuospatial signals or in determining and preparing forthcoming eye movements. Cisek and Kalaska (2005) used a behavioral task in which two potential reach directions were presented first out of which a correct direction was determined after the second cue was presented. They found that PMd neurons in the rostral part represented a potential reach direction and also a final reach direction after the second cue was given. In the third study, Wallis and Miller (2003) reported that PMd neurons reflected an abstract behavioral rule, such as matching or nonmatching of two visual objects as they had shown in the prefrontal cortex (Wallis et al. 2001). In the fourth study, Romo et al. (2004) showed that PMv neurons were involved in receiving and processing two successive vibratory stimuli, and in deciding an action based on the combination of the two.

In the current study, we obtained two other findings that required discussion from a different viewpoint. First, as the task progressed, we found that the fraction of neurons exhibiting decrease of activity grew in number, most evidently after the second task progressed, we found that the fraction of neurons exhibited a decrease in activity coding a relevant motor act and suppressing activity no longer relevant. Conversely, half of neurons that showed combination-selective activity during the second task phase did not exhibit selective responses to the first cue (visuospatial or instructional) did not show any selectivity after the appearance of the second cue. Conversely, half of neurons that showed combination-selective activity during the second task phase did not exhibit selective responses to the first cue (Tables 1 and 2, Fig. 13). These findings suggest that there might be two distinct populations of neurons within the premotor cortex. One group of neurons situated closer to the sensory input (processing input-stage information) may receive and keep visual information, or tentative motor information, before a final action can be determined. The other group of neurons situated closer to the output (processing output-stage information) may code a motor command once an action is determined. On the other hand, we also found that about half of combination-selective neurons (defined in the 2nd task phase) also responded selectively to the first cue. These neurons coding both a tentative signal and a final action might work as a neural element bridging the input and output stages. It seems important to note that we did not find any correlations between the selectivity after the first cue and second cue in the PMd (Table 1, Fig. 13A).

It has been shown that PMd neurons retain partial motor instructions, such as direction or amplitude, before the responses can be determined, and they integrate multiple instruction parameters once that can be identified (Kurata 1993, 1994; Riehle 2005; Riehle and Requin 1989, 1995). More recently, Romo and co-workers found evidence for a population of cells in SMA/pre-SMA (Hernandez et al. 2002) and PMv (Romo et al. 2004) that encoded sensory information necessary to choose the appropriate motor response and a second population that becomes active only after the monkeys have processed all that sensory information and chosen their motor response. Similarly, Cisek and Kalaska (2005) described two separate populations of cells, one that they called “potential-response” cells that coded sensory information about possible motor responses and “selected-response” cells that only became active after the monkeys had enough sensory information to choose which movement to make and signaled that final chosen action.

These properties seems useful for the arbitrary sensorimotor mapping (Wise and Murray 2000) because it suggests that the association between the sensory cue and motor act is flexible. In contrast, in the PMv, the visuospatial neuron defined in the first task phase tended to reflect a location of the future reach target in the second task phase (Table 2, Fig. 13B). This aspect of neuronal activity may suggest more direct coupling between the input and output stages of information processing in the PMv. These differences seem to provide evidence in favor of direct sensorimotor integration in the PMv and indirect sensorimotor integration in PMd (see following text).

Direct and indirect sensorimotor integration in the PMv and PMd: a hypothesis

Previous studies have reported that PMv lesions cause deficits in attending to peripersonal space (Rizzolatti et al. 1983), orienting to a side contralateral to the lesion (Schieber 2000), or executing visual tracking with wrist movements (Kurata and Hoffmann 1994), whereas PMv lesions do not impair conditional motor behavior (Kurata and Hoffmann 1994) that requires arbitrary association of a sensory signal with a movement. Our present finding that PMv neurons predominantly reflected visuospatial information of the cues, rather than the motor instruction, seems to provide explanations for the previous lesion studies. Our results also accord with a recent report showing two classes of spatial representations in the PMv: one class representing the location of target in the visual space and the other representing the location of target in the motor space (Kurata and Hoshi 2002). Another relevant aspect of PMv function has been reported in connection with grasping and manipulating of objects. A lesion in the PMv leads to deficits in grasping objects without any paralysis of finger movements themselves (Fogassi et al. 2001). Prior to this lesion study, Murata et al. (1997) showed that PMv neurons reflect three dimensional features of objects and reported further that visual component and motor component of neuronal selectivity were congruent. These findings suggest that an important function of the PMv is to match the motor act directly with the sensory information, such as matching the movement of the wrist or whole body with the location of a target, or matching the
grasping movement with the three-dimensional feature of the target. The mirror neuron (Rizzolatti and Craighero 2004), which matches a viewed act with an actual self movement, might be categorized as a variant of direct matching between sensory information and motor act.

In contrast to the PMd, the PMv seems to be more involved in representing motor information instructed by an arbitrary visual signal as shown in our study. This process has been inferred to be a crucial component of the conditional motor behavior, requiring subjects to associate a sensory signal with motor act in an arbitrary manner (Wise and Murray 2000). This view is substantiated by lesion studies reporting impairments of conditional motor behavior (Halsband and Passingham 1985; Kurata and Hoffman 1994; Petrides 1986). The arbitrary sensorimotor mapping required for conditional motor behavior can be viewed as requiring indirect sensorimotor integration in which the sensory signal does not directly indicate action in contrast to the direct sensorimotor integration deemed to require participation of the PMv. In this respect, a recent report by Cisek and Kalaska (2004) is relevant. They found that PMd neurons discharged when subjects were looking at a learned, conditional visuomotor task being performed by others as well as performing the task by itself. They suggested that the knowledge of the conditional rule is necessary for the PMd neurons to be active, whereas the knowledge would not be necessary for the PMv mirror neurons. Taken together, we propose that a core aspect of functional differences between the PMd and PMv can be explained in terms of direct and indirect sensorimotor integration.

Conclusion

In this study, we found physiological properties of neurons in the PMv and PMd that indicated functional specialization of the two areas. PMv neurons primarily represented visuospatial features of visual instruction signals. In contrast, PMd neurons reflected two sets of motor information instructed with the visual signals, one for the effector to be used and the other for the target to reach. When one of the two sets of information was first given to the subject, that partial information for action was held in register as a sustained neuronal activity. Subsequently, when the second instruction was given, the two components of information were integrated to generate information specifying the forthcoming action. We propose that the PMv is involved in representing visuospatial features of the visual signals and the location of the target to reach, whereas the PMd is involved more in retrieving and combining motor instructions for action planning.

Acknowledgments

We thank K. Kurata for helping to set up experimental system and K. Shima for performing the animal surgery. We also thank M. Kurama, Y. Takahashi, K. Samejima, and S. Hoffman for technical assistance.

Grants

This work was supported by the Japan Society for the Promotion of Science to E. Hoshi, the Japanese Ministry of Education, Culture, Sports, Science and Technology, and the Japan Science and Technology Corporation to J. Tanji.

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