Differential involvement of neurons in the dorsal and ventral premotor cortex
during processing of visual signals for action planning

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\textbf{Running head:} Visual signal processing in the premotor cortex

\textbf{Number of figures:} 13

\textbf{Number of tables:} 2

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ABSTRACT
We examined neuronal activity in the dorsal and ventral premotor cortex (PMd and PMv, respectively) to explore the role of each motor area in processing visual signals for action planning. We recorded neuronal activity while monkeys performed a behavioral task during which two visual instruction cues were given successively with an intervening delay. One cue instructed the location of the target to be reached, and the other indicated which arm was to be used. We found that the properties of neuronal activity in the PMd and PMv differed in many respects. After the first cue was given, PMv neuron response mostly reflected the spatial position of the visual cue. In contrast, PMd neuron response also reflected what the visual cue instructed, such as which arm to be used or which target to be reached. After the second cue was given, PMv neurons initially responded to the cue’s visuospatial features and later reflected what the two visual cues instructed, progressively increasing information about the target location. In contrast, the activity of the majority of PMd neurons responded to the second cue with activity reflecting a combination of information supplied by the first and second cues. Such activity, already reflecting a forthcoming action, appeared with short latencies (<400 ms) and persisted throughout the delay period. In addition, both the PMv and PMd showed bilateral representation on visuospatial information and motor-target or effector information. These results further elucidate the functional specialization of the PMd and PMv during the processing of visual information for action planning.
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 (Kubota and Hamada, 1978; Rizzolatti et al., 1981; Godschalk et al., 1981) and discharge during the preparation and execution of movements under visual guidance (Weinrich and Wise, 1982; Godschalk et al., 1985; Mushiake et al., 1991). In line with these observations, lesions of the premotor cortex cause deficits in the visual guidance of motor behavior (Moll and Kuypers, 1977; Halsband and Passingham, 1985).

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 (Mushiake et al., 1997; Kakei et al., 2001; Schwartz et al., 2004; Ochiai et al., 2005), attention to visuospatial stimuli (Boussaoud and Wise, 1993a; Boussaoud and Wise, 1993b), decision-making based on somatosensory signals (Romvo 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; Petrides, 1986; Kurata and Hoffman, 1994), and monkeys with PMv lesions exhibit deficits in visually guided grasping movements (Rizzolatti et al., 1983; Fogassi et al., 2001), 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, while the PMv receives major input from areas ventral and lateral to the intraparietal sulcus and its lateral surface (Kurata, 1991; Caminiti et al., 1996; Johnson et al., 1996; Matelli et al., 1998; Luppino et al., 1999; 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, while 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 (Boussaoud and Wise, 1993a; Boussaoud and Wise, 1993b). Our comparison of motor set-related activity revealed that PMv neurons mainly reflected visual target-location, while PMd neurons reflected both target location and arm use (Hoshi and Tanji, 2002; Hoshi and Tanji, 2004d).

We found in a previous study that neurons in the dorsal and ventral parts of the dorsolateral prefrontal cortex (DLPFC) exhibit different response properties. During action-planning based on two sets of visual information, neuronal activity in the dorsal DLPFC (DLPFCd, dorsal to the principal sulcus) reflected specific motor instructions for arm use and target location and combined them to plan the action, while neuronal activity in the ventral DLPFC (DLPFCv, ventral to the principal sulcus) preferentially represented the cue’s visuospatial features (Hoshi and Tanji, 2004a). Cortico–cortical connections preferentially link the DLPFCd and DLPFCv with the PMd and PMv, respectively (Barbas and Pandya, 1987; Petrides and Pandya, 1999; Petrides and Pandya, 2002; Wang et al., 2002; Luppino et al., 2003). These findings made it of great interest to compare the PMd and PMv neuron response properties in the same subjects performing the same behavioral task. This paper discusses how PMd and PMv neurons exhibited distinct response properties when subjects received visual signals for two sets of instructions specifying components of forthcoming actions and combined them for action planning. A preliminary report of this study has already appeared as an Abstract (Hoshi and Tanji, 2004c).
METHODS

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; Hoshi and Tanji, 2002; Hoshi and Tanji, 2004a; Hoshi and Tanji, 2004b; 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 at least 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 eye-camera system (R-21C-AS, RMS, Hirosaki, Japan). If fixation continued for 1,200 ms, the monkey was given the first instruction (the first 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, while 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 (first delay), the second instruction (the second 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 to 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 ten 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); pre-first cue (the 500-ms period before the appearance of the first cue); first cue and delay (100 ms after onset of the first cue to the onset of the second cue); second cue and delay (100 ms after onset of the second 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 [analysis of variance (ANOVA), \( p < 0.05 \), repeated over eight types of trials with eight sequences of first and second cue presentation (as shown in eight 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 second 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 ten task periods defined as follows: pre-cue (the 500-ms period before onset of the first cue); first cue (100–500 ms after onset of the first cue); early first delay (500–1,000 ms after onset of the first cue); late first delay (the last 500 ms before onset of the second cue); second cue (100–500 ms after onset of the second cue); early second delay (500–1,000 ms after onset of the second 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 inter-spike intervals

To analyze neuronal activity with high temporal resolution, we first calculated the instantaneous firing rate as the inverse of the inter-spike 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 was 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 below 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.

\[
\text{Firing rate index} = \beta_0 + \beta_a \times (\text{first cue}) \quad (1)
\]
\[
\text{Firing rate index} = \beta_0 + \beta_b \times (\text{second cue}) \quad (2)
\]
\[
\text{Firing rate index} = \beta_0 + \beta_c \times (\text{combination of first and second cues}) \quad (3)
\]

In equations (1)–(3), the firing rate index is for inverse-ISI data that were sampled every 10 ms; \( \beta_0 \) is the intercept and \( \beta_a, \beta_b, \text{ and } \beta_c \) 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 (\( p \)-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 first cue, the second cue, or the combination of the two 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.
RESULTS

Neuronal database

We recorded neuronal activity in the post-arcuate premotor areas of monkeys performing the behavioral task described in the Methods section. 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). Since 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 pre-cue to late second delay; see the Methods section for task period definitions) compared to the control period (paired t-test, α = 0.05, corrected for eight trial types, but not corrected for seven task periods). Consequently, this report examines the response properties of 825 PMd and 223 PMv neurons. Figure 1B and C show 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 in order to plan the future action.

Activity in anticipation of a cue’s appearance

Before the appearance of the first cue (i.e., during the pre-cue period), 218 (26%) PMd and 36 (16%) PMv neurons exhibited activity significantly changed compared to the control period (Fig. 1B 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 pre-cue 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 (two-sample t-test, α = 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 (two-sample $t$-test, $\alpha = 0.01$). Although the difference in distribution between the PMd and PMv was not significant ($p = 0.0922$, 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, more than 35% of neurons in the PMd and PMv exhibited activity significantly changed compared to the control period (Fig. 1B 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 inter-spike interval (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 4A and B present 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 (represented by the solid line in Fig. 4A), and in 20% of PMv neurons (represented by the solid line in 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 (two-way ANOVA: POSITION < 0.01, INSTRUCTION $\geq 0.01$, POSITION*INSTRUCTION $\geq$
We considered these neurons to represent only a cue’s visuospatial information, rather than its motor instruction. The dotted lines in Fig. 4A and 4B illustrate 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 4C and D show the fraction of neurons in each 10-ms bin that appeared to be significantly selective for the type of instruction (two-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 dotted and solid lines 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-square test (\(\alpha = 0.01\)) confirmed that the PMd exhibited more frequent target location-selective activity (detected in 136 bins out of 160 10-ms bins during the cue and delay periods). Arm-selective activity was also more frequent in the PMd (148 bins). In about 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 activity as the time when the fraction of selective neurons first exceeded 10% of the total neuron population (i.e., all neurons in the data base, \(n = 825\) in PMd and \(n = 223\) in PMv). The onset of position selectivity was 100 ms in the PMv and 140 ms in the PMd (represented by the solid line in Fig. 4A 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 (the dotted line in Fig. 4A and B). 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, while 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

Since 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 5A and B show 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-preferring activity; response latency was 140 ms for contralateral-preferring activity and 170 ms for ipsilateral-preferring 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-selective 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-preferring visuospatial activity had a latency of 100 ms and ipsilateral one had a latency of 150 ms. Fractions of ipsilateral- and contralateral-preferring activity did not differ following 1,000 ms after the appearance of the first cue. 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-preferring 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 instruction. However, since >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 with 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 (Kurata, 1993; Fogassi et al., 2001). In this map, we plotted the locations of all task-related neurons by classifying their activity (at the end of the first cue period) into one of three categories: visuospatial-selective (two-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 ≥
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 ~ 6600 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 6B and C show results obtained at two different times after the cue (Fig. 6B represents the end of the first cue period and Fig. 6C represents the end of the first 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 to the control period (Fig. 1B, 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 left-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.

We applied regression analysis to neuronal activity in 10-ms bins [see equations (1)–(3) in the Methods section] 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 cue-selective 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-square 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. 3A and 3B, and also in Fig. 7A 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. 7A and 7B. 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-square 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 two 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, while 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-square 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, while the fraction of neurons selective for the second cue decreased; the PMv neuron illustrated in Fig. 2 started reflecting the left target reach 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 panel $B$), 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 first 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 second 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 (n = 386 in PMd and n = 67 in PMv; see 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 first and second 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.

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

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.

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

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 second-cue position; 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, KS = 0.3302, \(P < 0.0001\)), indicating that combination selectivity was represented more in the PMd than the PMv.

**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 10A 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.

We analyzed the localization of the three classes of PMd neurons more precisely in the rostrocaudal direction. Figure 10C 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. 8A and 8B). 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 \(\geq 0.01\), TARGET \(\geq 0.01\),
and ARM\*TARGET $\geq 0.01$). 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. 8A and B). Figure 11C and D present the results for PMd neurons. Arm-selective neurons (Fig. 11C) 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. 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 11E 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 12B 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 the center of the 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. 12B 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 13A 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 ant target-location). In contrast, in PMv (Fig. 13B),
eurons 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-squared test, $P =
0.0008$).
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 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.

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 to 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 (pre-PMd) 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 by Matelli et al. (1985), and PMdc and PMdr by Barbas and Pandya (1987; see for review Picard and Strick, 2001).
Conversely, in the region ventral to the arcuate spur, we found neuronal activity responding 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 (Kubota and Hamada, 1978; Rizzolatti et al., 1981; Godschalk et al., 1981; Boussaoud et al., 1993).

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 pre-cue activity was revealed to reflect an abstract rule of the behavior: matching or non-matching 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.

Visuospatial responses of PMv neurons compared to 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 (Kubota and Hamada, 1978; Rizzolatti et al., 1981; Godschalk et al., 1981; Godschalk et al., 1985). 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 (Murata et al., 1997; Fogassi et al., 1999), and attention to a spatial location (Boussaoud and Wise, 1993a; Boussaoud and Wise, 1993b; 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 cues. 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 (represented by the solid line in Fig. 4A and 4B). 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. Since 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 (Wise et al., 1983; Kurata and Wise, 1988; Mitz et al., 1991; Fu et al., 1993; Boussaoud and Wise, 1993b). 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 parameters (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 (Riehle and Requin, 1989; Kurata, 1993; Kurata, 1994; Riehle and Requin, 1995; Riehle, 2005). 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 instruction 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 (Mink, 1996; Inase et al., 2001; Hadj-Bouziane and Boussaoud, 2003; Brasted and Wise, 2004; 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 (Yajeya et al., 1988; Quintana et al., 1988; Quintana and Fuster, 1992; Hasegawa et al., 1998; Asaad et al., 1998; Kim and Shadlen, 1999; White and Wise, 1999; Hoshi et al., 2000; Genovesio et al., 2005). 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 (Inase et al., 2001; Brasted and Wise, 2004; 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. 5A and 5B). We also found that response latencies for visual cues in the contralateral field were shorter by 30 to 50 ms, suggesting PMv 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. 11C 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; Kermadi et al., 2000; Cisek et al., 2003) and imaging (Kollias et al., 2001; Nirkko et al., 2001; Geurtsen et al., 2005) 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.” Since 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 existence 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, while 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 (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 (Kurata, 1993; Fu et al., 1993; Fu et al., 1995), 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 rostrocaudal 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), 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 (Lu et al., 1994; Dum and Strick, 2005), 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. 11A), 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; 1993b), 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 (Pandya and Seltzer, 1982; Petrides and Pandya, 1984; Cavada and Goldman-Rakic, 1989). Thus, the proposed existence of a ventral network, as well as the dorsal network (Hoshi and Tanji, 2004a), encompassing the premotor 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; Tanne-Gariepy et al., 2002; Rozzi et al., 2005). 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 prefrontal-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; 1993b) 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, while 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 non-matching 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 appearance of the second cue (Fig. 1B and C). The increase and decrease of neuronal activity might reflect the process of reinforcing the final motor choice while rejecting the other remaining option. The importance of inhibitory activity eliminating irrelevant action was reported in the study by Cisek and Kalaska (2005), in which neuronal activity reflecting irrelevant potential reach direction was suppressed soon after a final motor act was determined. Further, it was shown that PM neuronal activity reflecting the abstract rule (Wallis and Miller, 2003), or reflecting the memorized frequency of vibration stimuli (Romo et al., 2004), were suppressed once the information became no longer necessary. These results suggest that two distinct processes play equally important roles for action planning: 1) enhancing activity coding relevant motor act, and 2) suppressing activity no longer relevant.

Second, we found that half of neurons that showed 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 second 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 final responses can be determined, and they integrate multiple instruction
parameters once that can be identified (Riehle and Requin, 1989; Kurata, 1993; Kurata, 1994;
Riehle and Requin, 1995; Riehle, 2005). 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 discussion
below).

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 Hoffman, 1994), whereas PMv
lesions do not impair conditional motor behavior (Kurata and Hoffman, 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 PMv, the PMd 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; Petrides, 1986; Kurata and Hoffman, 1994). 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, while the PMd is involved more in retrieving and combining motor instructions for action planning.

Acknowledgments

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

Grants

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

**Figure 1.** Temporal sequence of behavioral events and temporal changes in neuronal activity in the PMd and PMv. *A:* Temporal sequence of behavioral events. The top panel shows a trial in which two instructions were given, *i.e.*, which arm to use (“arm”) and which target to reach (“target”), in that order. The bottom panel shows a trial in which the two instructions were given in the reverse order. *B* and *C:* Temporal changes in neuronal activity during the behavioral task. The thick line indicates the fraction of neurons that exhibited a significant increase or decrease in activity compared to the control period. The thin solid and dotted lines represent the fractions of neurons that exhibited a significant increase or decrease in activity compared to the control period, respectively. The gray areas indicate task periods during which visual cues were presented (from left to right: the first and second cues). The numbers at the top are the actual numbers of neurons that exhibited a significant change in activity during each behavioral period. *B:* Data for the PMd. *C:* Data for the PMv. Note that an individual neuron could exhibit an increase in activity during one sequence of first and second cues and a decrease in activity during another sequence, *i.e.*, the sum of the fractions could exceed the total number of neurons.

**Figure 2.** An example of PMv neuronal activity presented with raster displays and plots of spike density functions (SDFs). Gray areas (from left to right) represent when the first, second, and set cues were presented. Tic marks on the abscissa are at 400-ms intervals. First and second instructions are shown on top of each panel (RA, right arm; LA, left arm; RT, right target; LT, left target). SDFs (Gaussian kernel, \( \sigma = 20 \text{ ms} \), mean \( \pm \) SE) appear below each raster display. Raster plots and SDFs were aligned to the onset of the first and second instructions, and the onset of the set cue, and were merged at the midpoint of each delay period (600 ms after the disappearance of the cue and 600 ms before the onset of the second or set cue). Ordinate represents the instantaneous firing rate, the degree of which is indicated on the ordinate. This neuron mainly reflected the left position of the white square appearing in instruction cues, soon after the appearance of the first and second cues. In addition, in the latter part of the second delay period, the activity started reflecting the future reach target location (left) regardless of arm use.

**Figure 3.** Two examples of PMd neuronal activity. Display formats are as in Fig. 3. *A:* This neuron showed vigorous activity if the first cue, but not the second cue, instructed use of the right arm. The activity preceding the appearance of the first cue was greater before the appearance of the arm instruction (left column) than the target instruction (right column). *B:* This neuron showed vigorous activity if the first cue, but not the second cue, instructed reach to the right target.
Figure 4. Time course of the cue-position selectivity during the first-cue and delay periods. A-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). Gray areas indicate when the cues appeared. Solid lines represent the fraction of neurons that were position-selective (two-way ANOVA, POSITION < 0.01 or POSITION*INSTRUCTION < 0.01), calculated successively for each 10-ms bin. Dotted lines represent the fraction of neurons that were position-selective only (not instruction-selective; two-way ANOVA, POSITION < 0.01, INSTRUCTION ≥ 0.01, and POSITION*INSTRUCTION ≥ 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. The triangle marks the onset latency, i.e., the time at which the fraction of selective neurons first exceeded 10% of the total population. The time below the triangle indicate 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 first-cue and delay periods. C and D: Bin-by-bin plot of instruction selectivity expressed as the fraction of all neurons in the database. Solid lines represent the fraction of neurons that exhibited arm- or target-instruction selectivity, calculated successively for each 10-ms bin (two-way ANOVA, INSTRUCTION < 0.01 or POSITION*INSTRUCTION < 0.01). Dotted lines represent the fraction of neurons that exhibited greater activity for the arm instruction. The triangle at the bottom of the panel marks the onset latency, i.e., the time at which the fraction of neurons selective for the instruction first exceeded 10% of the total population. The time below the triangle indicate the actual latency.

Figure 5. Time course of ipsilateral or contralateral selectivity during the first-cue and delay periods. A: Bin-by-bin plot of laterality selectivity for visuospatial PMd neurons (selective only for the cue position, but not for the instruction) as the fraction of all neurons in the database. The gray line represents the total fraction of visuospatial neurons, reproduced from the dotted line in Fig. 4A. Please note that the scale of the y-axis is different from that in Fig. 4A. The solid black line represents the fraction of neurons that exhibited greater activity for the contralateral white-square position. The dotted line represents the fraction of neurons that exhibited greater activity for the ipsilateral white-square position. The triangles at the bottom of the panel indicate the response latency (i.e., when the fraction exceeded 5% of the total) for neurons whose activity was greater for the contralateral (black triangle) or ipsilateral (gray triangle) side. The actual latencies are indicated in parentheses using the format (latency for the contralateral, latency for the ipsilateral). B: Bin-by-bin plot of laterality selectivity for visuospatial PMv neurons displayed using the same format as in A. C: Bin-by-bin plot of laterality selectivity for instruction-selective PMd neurons as a fraction of all the neurons in the database. The gray line represents the total fraction of
instruction-selective neurons, reproduced from the solid line in Fig. 4C. The black line represents the fraction of neurons that exhibited greater activity for the contralateral instruction (arm-use or target-location). The dotted line represents the fraction of neurons that exhibited greater activity for the ipsilateral instruction.

**Figure 6.** Anatomical location of visuospatial and instruction neurons. A: The 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 two left hemispheres from the two monkeys are superimposed. Red circles, instruction-selective neurons (two-way ANOVA, INSTRUCTION < 0.01 or POSITION*INSTRUCTION < 0.01). Blue squares, visuospatial neurons (two-way ANOVA, POSITION < 0.01, INSTRUCTION ≥ 0.01, and POSITION*INSTRUCTION < 0.01). Bar, a nonselective neuron (two-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. Gray solid line, instruction-selective neurons with greater activity for the target-location cue. B shows the data at the end of the first cue period, while C shows the data at the end of the first delay period.

**Figure 7.** Two examples of PMd neuronal activity. Display formats are as in Fig. 3. A: This neuron showed greater activity if a white square of the first cue appeared to the left of the fixation point. After the appearance of the second cue, however, the same neuron reflected left arm use of the future movement. B: This neuron showed brief visual responses if the first cue indicated right target reach. During the first delay period, the activity was greater if the first cue indicate the use of the left arm. After the appearance of the second cue, this neuron showed greater activity if the animal was required to reach the right target.

**Figure 8.** Time course of neuronal activity that selectively represented the first cue, second cue, or two-cue combination. A and B: Bin-by-bin plot of neurons selective for the first cue (black trace), second cue (blue trace), or combination of two cues (combination selectivity, red trace) expressed as the fraction of all neurons. A: Data for PMd neurons. B: Data for PMv neurons. At the top of both panels, tick marks indicate 10-ms bins in which selectivity for the first cue (black) or combination of cues (red) was significantly frequent (chi-square test, α = 0.01) than others. C: Direct
comparison of neurons in the PMd and PMv that were selective for the second cue. Downward tick marks indicate 10-ms bins in which the frequency of the second cue-selective neurons out of all selective neurons was greater in the PMv than in the PMd.

**Figure 9.** Comparison of selectivity of the second cue responses for PMd and PMv neurons. A: Comparison of position selectivity for the second cues in PMd and PMv neurons. The cumulative fractions of the SS ratio for the position of the second cue for the PMd (n = 386, dotted line) and PMv (n = 67, solid line) are shown. B: Comparison of two-cue combination selectivity for PMd and PMv neurons. The cumulative fractions of the SS ratio for the combination, for the PMd (n = 386, dotted line) and PMv (n = 67, solid line), are shown.

**Figure 10.** Anatomical location of neurons selective for the first cue, second cue, or combination of the two cues. The location of each neuron was rendered on the cortical surface. 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. A: Data at the end of the second cue period. B: Data at the end of the second delay period. The two left hemispheres from the two monkeys are superimposed. Black circles, first cue-selective neurons. Blue squares, second cue-selective neurons. Red crosses, combination-selective neurons. Bar, a nonselective neuron. C and D: Rostrocaudal distribution of three classes of 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. Black line, the first cue-selective neurons. Blue line, the second cue-selective neurons. Red line, combination-selective neurons. C: Data at the end of the second cue period. D: Data at the end of the second delay period.

**Figure 11.** Time course of neuronal activity representing arm-use and target-location for neurons defined as selective for the combination of the two cues. A and B: Bin-by-bin plot of selective activity expressed as the fraction of neurons that were selective for target-location (blue lines; three-way ANOVA, TARGET < 0.01 or ARM*TARGET < 0.01), arm-use (red lines; three-way ANOVA, ARM < 0.01 or ARM*TARGET < 0.01), and both target-location and arm-use (thick black lines; three-way ANOVA, ARM < 0.01 and TARGET < 0.01, or ARM*TARGET < 0.01). The gray traces in A and B correspond to the red traces in Fig. 8A and B (i.e., the two-cue combination selective neurons), respectively. A: Data for PMd neurons. B: Data for PMv neurons. C-F: Time course of ipsilateral or contralateral selectivity for arm-use and target-location during the second cue and delay periods. C: Bin-by-bin plot of laterality selectivity for arm-use-selective PMd neurons as the fraction of all neurons. The red line represents the fraction of arm-use-selective neurons, reproduced from the red line in A. Note that the scale of the y-axis differs from that in A. The solid line represents the fraction of neurons that exhibited greater activity for contralateral
arm-use. The dotted line represents the fraction of neurons that exhibited greater activity for ipsilateral arm-use. The triangles at the bottom of the panel indicate the response latency (i.e., when the fraction exceeded 5% of the total population) for the contralateral-preferring (black triangle) and ipsilateral-preferring (gray triangle) neurons. The actual latencies are indicated in parentheses using the format (latency for the contralateral, latency for the ipsilateral). 

D: Bin-by-bin plot of laterality selectivity for target location-selective PMd neurons displayed using the same format as in B. The blue line, representing the fraction of target-location selectivity, was reproduced from the blue line in A. The solid line represents the fraction of neurons that exhibited greater activity for the contralateral target-location. The dotted line represents the fraction of neurons that exhibited greater activity for the ipsilateral target-location. 

E: Bin-by-bin plot of laterality selectivity for arm use-selective PMv neurons as the fraction of all neurons. The display format is the same as in C. The red line, representing the fraction of arm use-selective PMv neurons, was reproduced from the red line in B. 

D: Laterality selectivity for target location-selective PMv neurons. The display format is the same as in B. The blue line, representing the fraction of target location-selective neurons, was reproduced from the blue line in B.

**Figure 12.** Anatomical location of neurons selective for arm-use, target-location, or both. A: The location of each neuron was rendered on the cortical surface. 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. This figure shows data at the end of the second delay. The two left hemispheres from the two monkeys are superimposed. Blue squares, neurons selective for target-location only (three-way ANOVA, ARM ≥ 0.01, TARGET < 0.01, and ARM*TARGET ≥ 0.01). Green diamonds, neurons selective for arm-use only (three-way ANOVA, ARM < 0.01, TARGET ≥ 0.01, and ARM*TARGET ≥ 0.01). Black dots, neurons selective for both target-location and arm-use (three-way ANOVA, ARM < 0.01 and TARGET < 0.01, or ARM*TARGET < 0.01). The line indicated by * connects points where Mahalanobis distance to the center of the target-location only neurons and to the center of arm-use only neurons was equal. B and C: Rostrocaudal distribution of the three classes of PMd neurons after onset of the second cue. The number of neurons obtained in each 1-mm width of the PMd in the mediolateral plane, plotted in the rostrocaudal direction. Blue line, neurons selective for target-location only. Green line, neurons selective for arm-use only. Black line, neurons selective for both target-location and arm-use. B: Data at the end of the second cue period. C: Data at the end of the second delay period.

**Figure 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.
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 &amp; 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>

* Selectivity at the end of the first cue period was presented. The parentheses enclose the number of neurons classified. ** 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 &amp; Target</td>
</tr>
<tr>
<td>Arm Instruction (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target Instruction (7)</td>
<td>1</td>
<td>3</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>

* Selectivity at the end of the first cue period was presented. The parentheses enclose the number of neurons classified. ** Selectivity at the end of the second delay period.
Fig. 2
Fig. 3
Fig. 4

A. **Cue-Position Selectivity in the PMd**

- PMd (N = 825)
- Fraction of Neurons
- CUE-POSITION (+)
- INSTRUCTION (-)
- 1st Cue: 140 ms (150 ms)
- 2nd Cue

B. **Cue-Position Selectivity in the PMv**

- PMv (N = 223)
- Fraction of Neurons
- 1st Cue: 100 ms (110 ms)
- 2nd Cue: 1540 ms

C. **Instruction Selectivity in the PMd**

- PMd (N = 825)
- Fraction of Neurons
- INSTRUCTION (+)
- Target Inst. > Arm Inst.
- Arm Inst. > Target Inst.
- 1st Cue: 200 ms
- 2nd Cue

D. **Instruction Selectivity in the PMv**

- PMv (N = 223)
- Fraction of Neurons
Fig. 5

A. Visuospinal neurons in the PMd
- PMd (N = 825)
- Contra > Ipsilateral
- Ipsilateral > Contralateral
- (140 ms, 170 ms)

B. Visuospinal neurons in the PMv
- PMv (N = 223)
- (100 ms, 150 ms)

C. Instruction-selective neurons in the PMd
- PMd (N = 825)
- Contra > Ipsilateral
- Ipsilateral > Contralateral
- (220 ms, 250 ms)
Fig. 6

A. End of the 1st cue

B. End of the 1st cue

C. End of the 1st delay

- PMd
- PMv
- Arcuate Sulcus (AS)
- Spur of AS
- Dorsal
- Anterior

Graphs show the number of neurons at different rostrocaudal distances from the genu of the AS.
Fig. 7

A

B
Fig. 8

A

PMd
(N = 825)

1st Cue
130 ms
2nd Cue
220 ms
Set Cue

Fraction of Neurons

B

PMv
(N = 223)

110 ms
400 ms

Fraction of Neurons

C

Cue2

[Graph showing neural activity with time points and neuron counts]
Fig. 9

A 2nd Cue Position Selectivity

Cumulative Fractions

SS-ratio for Position of 2nd Cue

P < 0.0001
KS = 0.3101

B Two-cue combination Selectivity

Cumulative Fractions

SS-ratio for combination of cues

P < 0.0001
KS = 0.3302
Fig. 10
Fig. 11

A Two-cue combination selectivity

2nd Cue

Set Cue

PMD (N = 825)

Fraction of Neurons

0.0

0.1

0.2

0.3

0.4

0.5

400 ms

B Two-cue combination selectivity

PMv (N = 223)

- Two-cue combination
- Target-location selective
- Arm-use selective
- Both Arm and Target

C Arm selectivity

PMD (N = 825)

Fraction of Neurons

0.0

0.1

0.2

0.3

0.4

160 ms, 200 ms

D Target selectivity

PMD (N = 825)

Fraction of Neurons

0.0

0.1

0.2

0.3

0.4

140 ms, 190 ms

E Arm selectivity

PMv (N = 223)

Fraction of Neurons

0.0

0.1

0.2

0.3

0.4

180 ms, 200 ms

F Target selectivity

PMv (N = 223)

Fraction of Neurons

0.0

0.1

0.2

0.3

0.4

180 ms, 200 ms
Fig. 13

Two-cue combination selectivity in 2nd phase

A

PMD

Number of Neurons

0 20 40 60 80 100

Arm only Arm & Target Target only

B

PMv

Number of Neurons

0 5 10 15 20 25 30

Arm only Arm & Target Target only

Selectivity in 1st phase
- Nonselective
- Visuospatial
- Target Instruction
- Arm Instruction